CURRENT PERSPECTIVE ESSAY

Florigen Coming of Age after 70 Years

The report that FT mRNA is the long-sought florigen, or at least part of it (Huang et al., 2005), has attracted much attention and was ranked the number three breakthrough of 2005 by the journal Science (Anonymous, 2005). This exciting discovery has brought to center stage one of the major outstanding questions in plant biology: What is the nature of florigen? In this essay, I summarize the classical experiments that led to the florigen hypothesis and how molecular-genetic approaches combined with physiological methods have advanced our understanding of florigen. I also discuss the possible universality of florigen and some of the remaining questions regarding flowering and other photoperiod-controlled phenomena involving long-distance signaling in plants.

FLORIGEN AS A PHYSIOLOGICAL CONCEPT

Julius Sachs (1865) may be considered the father of the flower hormone concept. From his well-known experiments with partially darkened Tropaeolum majus and Ipomoea purpurea plants, he concluded that leaves in the light produce flower-forming substances in very small amounts, which direct the assimilates to form flowers in darkened shoots. However, more convincing evidence in support of flower-forming substances did not appear until after the discovery of photoperiodism, when Knott (1934) provided evidence for a long-distance signal moving from an induced leaf to the shoot apex. Expression from meristem-specific promoters of CONSTANS (CO) and FLOWERING LOCUS T (FT) will restrict the discussion mainly to the photoperiod pathway.

MOLECULAR-GENETIC STUDIES OF FLOWERING

As the physiological-biochemical approaches to flowering had begun to stagnate, along came molecular genetics with a new approach to the study of flowering. Isolation and characterization of mutants with respect to their flowering response, mainly in the facultative LD plant Arabidopsis thaliana, became the mainstay of flowering research. Mutants flowering later than wild-type plants involve positive regulators of flowering, and early flowering mutants have lost repressors of flowering. Studies of epistatic relationships among the flowering genes have resulted in a network of four response pathways that control flowering in Arabidopsis: the photoperiod, vernalization, autonomous, and gibberellin (GA) flowering response pathways (Mouradov et al., 2004; Ayre and Turgeon, 2004). An et al. (2004) speculated that FT protein might be the mobile signal or, alternatively, that FT controls the synthesis of a mobile, small substance that in some way effects flowering. Isolation and characterization of mutants with respect to their flowering response, mainly in the facultative LD plant Arabidopsis thaliana, became the mainstay of flowering research. Mutants flowering later than wild-type plants involve positive regulators of flowering, and early flowering mutants have lost repressors of flowering. Studies of epistatic relationships among the flowering genes have resulted in a network of four response pathways that control flowering in Arabidopsis: the photoperiod, vernalization, autonomous, and gibberellin (GA) flowering response pathways (Mouradov et al., 2004; Ayre and Turgeon, 2004). An et al. (2004) speculated that FT protein might be the mobile signal or, alternatively, that FT controls the synthesis of a mobile, small substance that induces flowering.

In the vernalization pathway, flowering is promoted in response to a prolonged exposure to low temperature (vernalization). In cold-requiring accessions of Arabidopsis, the MADS
In each case, the stock (below the graft union) is the donor, and the scion (above the graft union) is the receptor. Arrows point toward the graft unions. None of the control grafts with noninduced donors caused flowering in the receptors (data not shown).

(A) The SDP Kalanchoe blossfeldiana as donor for the LDP Sedum spectabile as receptor (Zeevaart, 1958).
(B) The LDP S. spectabile as donor for the SLDP Echeveria harmsii (my unpublished data).
(C) The LSDP Bryophyllum crenatum as donor for the LDP S. spectabile (my unpublished data).
(D) The LSDP B. daigremontianum as donor for the SLDP E. harmsii (Zeevaart, 1982).

Figure 1. Four Examples from the Crassulaceae in Which Flowering Is Induced in a Noninduced Scion by Transmission of Florigen from a Florally Induced Stock.

The LSDP B. daigremontianum and the SDP Sedum spectabile serve as donors for the LDP Echeveria harmsii. These worker conducted a set of elegant experiments using induction of a single Arabidopsis leaf combined with sensitive molecular techniques and microdissection of shoot apices to show that FT mRNA is the limiting factor for flowering; it is produced in the leaf and moves to the apical meristem, where its arrival is correlated with flower formation. Thus, FT mRNA fulfills the definition of florigen (at least in Arabidopsis). The objection can be raised that FT itself is not the final stimulus, but only induces another factor essential for flowering that moves along with FT transcripts from leaf to shoot apex (An et al., 2004; Huang et al., 2005; Wigge et al., 2005). However, it is unlikely that FT plays such a role in the leaf phloem. FT acts in the shoot apex by forming a complex with the basic domain/leucine zipper protein FLOWERING LOCUS D (FD). This FD heterodimer then activates the downstream floral meristem identity gene APETALA1 (AP1) (Abe et al., 2005; Wigge et al., 2005). Moreover, expression of FT from a meristem-specific promoter will induce early flowering in SD, indicating that in such transgenic plants no signal from the leaf is required for early flowering. This is strong evidence that FT mRNA is the only essential factor for floral initiation that moves from leaf to shoot apex. It is, of course, possible that FT protein also moves from the induced leaf to the shoot apex. In fact, FT protein has recently been identified in phloem exudate from inflorescence stems of Brassica napus (Giavalisco et al., 2006). If both mRNA and protein move from an induced leaf to the shoot apex, the question is: Which one is necessary for flowering, or are perhaps both required?

In some species, production of florigen appears to continue after the plants are no longer exposed to the inductive photoperiod. This phenomenon is illustrated by induced leaves of the SDP red Perilla, which were still effective donors in grafting experiments 3 months after they had been moved from SD to LD (Zeevaart, 1958). There are also species (e.g., Xanthium strumarium and Bryophyllum daigremontianum) in which flowering receptor shoots become effective donors themselves. This phenomenon, called indirect induction or non-localized induction, suggests that florigen has self-perpetuating properties (for review, see Zeevaart, 1976). The results by Huang et al. (2005) provide further insight into these phenomena. These workers reported that a few hours after the heat shock–inducible FT transgene was induced, native FT mRNA also started to accumulate both in the induced leaf and in the shoot apex. This
finding suggests that there is positive feedback whereby FT, once induced, further enhances its own expression, both in the donor leaf and in the apical meristem.

MOVEMENT OF FLORIGEN/FT mRNA

Florigen moves in the phloem along with photoassimilates (e.g., King and Zeevaart, 1973). The velocity calculated for the movement of FT mRNA in Arabidopsis was 1.2 to 3.5 mm/h (Huang et al., 2005), which is in the same range as measured for export of florigen from cotyledons of the SDP Pharbitis nil induced by a single dark period (Imamura and Takimoto, 1955; Zeevaart, 1962a). This rate is much slower than the movement of sugars in the phloem (50 to 100 cm/h). However, with adult plants of P. nil and much longer distances between donor leaves and receptor buds than in seedlings, velocities of florigen movement were much to the values for assimilate movement (Takeba and Takimoto, 1966; King et al., 1968).

A priori, it would be expected that mRNA molecules, probably forming a complex with a protein, would move more slowly than assimilates. The earlier values for velocities of florigen were based on the time it took for florigen to move out of an induced leaf and initiate a flowering response. This approach would obviously underestimate the velocity because it is based on flowering response, which presumably requires a threshold value of florigen and does not measure the first molecules arriving at the shoot apex. It is surprising, therefore, that with the direct measurement of FT mRNA arriving at the apex (Huang et al., 2005) no higher velocities were found than with the physiological approach.

Movement of RNAs and proteins in the phloem is now well established (Lucas et al., 2001). FT mRNA is produced in the companion cells and then has to move through the sieve elements to the shoot apex to induce flowering. From the termination of the protophloem strands in the shoot apical meristem, it then has to traverse, presumably symplastically, a series of meristematic cells to reach its target, the shoot apex. However, movement of FT mRNA all the way from source leaf to the shoot apex proper may not be necessary. As discussed above, FT mRNA, once produced, induces production of more FT mRNA via an autoregulatory feedback loop (Huang et al., 2005). So, it is conceivable that FT mRNA that exits from the protophloem induces expression of FT throughout the apex, thus making it superfluous for RNA molecules to move from the protophloem ends across many cells to the apex.

IS THE CO—FT SIGNALING PATHWAY UNIVERSAL FOR CONTROLLING FLOWERING?

The tenet of the florigen hypothesis is that florigen is the same in SDPs, LDPs, day-neutral plants (DNPs), LSDPs, and SLDPs. Grafting experiments can be performed only between closely related species, but results of interspecific and intergeneric grafts between different photoperiodic response types support this idea (see above). Thus, regardless of which environmental cues are required for floral induction, the end product, florigen, is the same and, by implication, regulation of CO and FT expression is central to flowering in all plant species. Indeed, the CO→FT combination in the flowering response pathway appears to be highly conserved, regardless of response type. For example, in SDP rice (Oryza sativa), the ortholog of FT, Hd3a, promotes flowering downstream of Hd1, the ortholog of CO (Kojima et al., 2002). Increased expression of Hd3a occurs in darkness; suppression by night interruption inhibits flower initiation (Ishikawa et al., 2005). Thus, the photoperiod pathway for flowering is conserved between SDP rice and LDP Arabidopsis and most likely in other species as well (Hayama and Coupland, 2004). Therefore, the differences between SDPs and LDPs appear to reside in how the genes in the flowering pathways function and are regulated. It remains to be shown, of course, that FT is the universal systemic transmissible signal (mRNA or protein) that is required for flowering.

Little work on flowering has been performed with DNPs because their flowering cannot be controlled at will. However, recent work with tomato (Solanum lycopersicum) demonstrates that flowering in this DNP is also induced by a transmissible signal, generated by the ortholog of FT, SINGLE-FLOWER TRUSS (SFT) (Lifschitz et al., 2006). Overexpression of FT or SFT in day-neutral tobacco (Nicotiana tabacum) or tomato induced early flowering in both species. Moreover, overexpression of SFT induced flowering in the SDP Maryland Mammoth tobacco in LD and in Arabidopsis under SD. Transmission of florigen via grafts was obtained from tomato overexpressing SFT (donor) to sft mutant plants, to Maryland Mammoth tobacco in LD, and to a tomato mutant u that does not flower under low irradiance. SFT was expressed in the leaves, and its protein was mainly localized in the nuclei of leaf cells. No evidence was obtained for movement of SFT mRNA from donor leaves to receptor shoots, so that it was proposed that in tomato, florigen is a signal downstream of SFT (Lifschitz et al., 2006). Removal of SFT donor shoots promptly reverted sft receptors to mutant phenotype, indicating that SFT mRNA is very short-lived in the receptors (if it crosses the graft union at all) and also that, unlike in Arabidopsis (see above), an SFT autoregulatory loop does not function in tomato. So, although there may be differences between different species and photoperiodic response types, all have in common that either FT, or a product of FT, is the flower-inducing signal.

There are many examples of successful transmission of florigen between different species (see above), but there are also many examples in which the receptor shoots did not flower (Zeevaart, 1976). Does this mean that in the latter case florigen is not functionally conserved? The work with tomato provides an answer to this question. Transgenic plants overexpressing SFT under control of the 3SS promoter were strong donors, but wild-type tomato could not complement sft mutant plants in grafting
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experiments (Lifschitz et al., 2006). This result makes it clear that the level of florigen in wild-type plants is too low to induce flowering in the receptor plants. Thus, the failure to induce flowering in receptor shoots is not due to nonidentity of florigen but due to insufficient production of florigen in the donor and/or rapid decay of florigen in the receptor.

Unlike herbaceous plants, trees flower only after a long juvenile phase that may last many years. A recent report shows that expression of FT is also a prerequisite for flowering in trees. Ectopic expression of an FT ortholog in aspen (Populus spp) resulted in early flowering and thus drastically shortened the juvenile phase. Moreover, expression of the FT ortholog increased with age of the trees (Böhlenius et al., 2006). Work by Hsu et al. (2006), reported in this issue of The Plant Cell, also shows that in juvenile Populus deltoides a critical level of FT2 expression is necessary before flowering will occur. In addition, LD-induced transcription of FT2 in spring is closely associated with floral initiation in mature trees. These results from trees provide further evidence that the CO→FT system for control of flowering time is widespread and not restricted to herbaceous plants.

GA S AND FLOWERING

GA can induce or promote flowering in many LDPs that grow as rosettes in SD. However, not all rosette plants can be induced to flower by GA, although applied GA always causes stem elongation. By contrast, GA does not induce flowering in SDPs grown in noninductive LD conditions. Because results of grafting experiments indicate that florigen is exchangeable between LDPs and SDPs, it was concluded early on in work on the role of GA in flowering that GA cannot be florigen (see Zeevaart, 1983). In the LDP Lolium temulentum, GA causes floral initiation without first causing stem elongation, and GAs, especially GAs5 and GAs6, are endogenous signals transmitted from an induced leaf to the shoot apex. These GAs have been assigned a role as florigen in grasses (King and Evans, 2003), but this role appears to be restricted to a certain group of plants, temperate grasses, just as the flower-inducing effect of ethylene is limited to the family of the Bromeliaceae (see Zeevaart, 1976, 1978). Florigen was meant to indicate a universal flower hormone. At present, FT-regulated flowering appears to be widespread, and it would be preferable, therefore, to restrict the term florigen to the FT-induced transmissible signal(s).

The effect of GA on flowering raises the question about the relationship between GA and FT expression. In Arabidopsis, GA activates the floral meristem identity gene LEAFY (LFY) (Blázquez et al., 1998) but does not regulate expression of FT (Moon et al., 2003). In support of separate GA and FT flowering pathways, King et al. (2006) also found that an increase in FT mRNA in L. temulentum in LD occurred independently of GA. LFY is conserved in plants (Maizel et al., 2005), so that with respect to the GA response pathway the question is: What is the effect of GA on expression of LFY in LDPs and SDPs that do not flower in response to applied GA?

A TRANSMISSIBLE FLOWER-INHIBITING SIGNAL OF FLOWERING

In addition to flower-promoting florigen, there is also evidence that noninduced leaves can inhibit flowering. Some of these inhibiting effects can be explained in terms of source-sink relationships between induced leaves and receptor buds. Noninduced leaves between donor leaves and receptor buds can prevent florigen from reaching the target receptor buds, as demonstrated by correlating transmission of florigen with 14C-photoassimilate translocation in Perilla (King and Zeevaart, 1973). One may call this phenomenon nonspecific inhibition due to interference with florigen movement. However, there is also evidence for specific inhibition of flowering by a mobile signal. In grafting experiments with various tobaccos, both the flowering SDP Maryland Mammoth and LDP Nicotiana sylvestris promoted early flowering in day-neutral tobacco. But when the donors were kept in noninductive daylengths, Maryland Mammoth had only a slight flower-delaying effect in the day-neutral tobacco, whereas N. sylvestris suppressed its flower formation. These responses indicate that the LDP N. sylvestris in SD produces a transmissible flower-inhibiting signal that is absent (or present at a much lower level) in the SDP Maryland Mammoth tobacco (Lang et al., 1977).

Can this physiological evidence for a flower inhibitor now be interpreted in molecular-genetic terms? Loss-of-function mutants that flower earlier than wild-type plants have lost a repressor of flowering. One such mutant in Arabidopsis is tfl1, which flowers very early with a terminal flower. Interestingly, TFL1 has homology with FT, and change of a single amino acid can convert TFL1 as a repressor of flowering to an activator of flowering (Hanzawa et al., 2005). This raises the question: Does TFL1 mRNA, like FT mRNA, also move in the phloem as a signal counteracting FT? Although TFL1 may be moving in the phloem, it is probably not a flower-regulatory signal because TFL1 is already highly expressed in the shoot apical meristem, where it interacts antagonistically with the floral meristem identity genes LFY and AP1/AP2 (Shannon and Meeks-Wagner, 1993; Ratcliffe et al., 1999). Thus, at present, there is no known gene function that is specifically associated with a transmissible flower inhibitor.

OTHER TRANSMISSIBLE PHOTOPERIODIC SIGNALS

There are other phenomena in plants besides flowering that are under photoperiodic control and involve long-distance signaling. Tuberization in potato (Solanum tuberosum) is induced by SD. Gregory (1956) showed transmission of a tuber-inducing stimulus from an induced to a noninduced shoot. When Nicotiana spp of different photoperiodic response types were grafted on...
tuberless Solanum andigenum, the SDP Maryland Mammoth tobacco induced tubers in SD only, the LDP N. sylvestris in LD only, and the DNP Trapezond tobacco in both SD and LD (Chaliahkyan et al., 1981). It is clear from these results that only flowering donors could induce tuber formation in S. andigenum, raising the possibility that florigen and the tuber-forming stimulus are interchangeable. Thus, it is not too far-fetched to propose that tuber formation is also under control of the CO→FT pathway. In more recent work, overexpression of Arabidopsis CO in potato inhibited tuber formation, and this inhibitory effect was perceived in the leaves of transgenic plants (Martínez-García et al., 2002). Results with overexpression of FT should further clarify the possible role of the CO→FT signaling pathway in tuber formation.

Several phenomena in woody species, such as cessation of apical growth, bud dormancy, cambial activity, cold acclimation, and leaf fall in deciduous species, occur in the fall under shortening photoperiods. In Betula pendula, a northern ecotype had a longer critical photoperiod and greater photoperiodic sensitivity for growth cessation than a southern ecotype, resulting in earlier dormancy and cold acclimation (Li et al., 2003). As demonstrated with actively growing seedlings of certain woody species, the locus of perception for dormancy is the leaves, whereas the buds respond with dormancy, a situation reminiscent of photoperiodic induction of flowering (see Wareing, 1957). Therefore, it is not surprising that CO is the mediator between the shortening daylength and low expression of the ortholog of FT in aspen trees, resulting in growth cessation and bud dormancy (Böhlenius et al., 2006). This shows that the CO→FT combination not only plays a critical role in flowering but can mediate vegetative growth as well.

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Jan A.D. Zeevaart

*Plant Cell* 2006;18;1783-1789
DOI 10.1105/tpc.106.043513

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