Recent advances in regulation of flowering

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Abstract

The control of flowering time in plants is critical for plant fitness and for agriculture. The genetic pathways governing this developmental transition are reasonably well understood in Arabidopsis, although substantial new gains are still being made in this system. Much new work is focusing on how the genetic networks governing flowering function in other species.

Introduction and context

The switch from vegetative to reproductive growth in angiosperms is a major developmental transition, an important determinant of life history, and a critical component of fitness (e.g., [1-3]). To maximize seed production, flowering must be coordinated with internal physiological and external environmental factors. Reproduction is energy-intensive and so should begin only when sufficient resources (stored starch or photosynthetic capacity) have accumulated and when environmental conditions will support continued growth through fruit production. Outcrossing plants will be successful only if neighbors flower at the same time, and animal-pollinated plants must time flowering to coincide with pollinator presence.

Given these constraints, it is no surprise that flowering time is tightly regulated and that there are many different strategies to control reproductive timing. Many plants are photoperiodic, with short-day plants (SDPs) and long-day plants (LDPs) flowering when days are shorter or longer than a critical photoperiod, respectively. Daylength is determined by an external coincidence system in which an external cue is compared with an internal oscillation. For example, in the LDP Arabidopsis, the circadian clock causes expression of *CONSTANS* (*CO*) mRNA to begin increasing approximately 12 hours after dawn. If there is a coincidence between light and high *CO* transcripts, then CO protein is stabilized, leading to activation of *FLOWERING LOCUS T* (*FT*) transcription and synthesis of FT

protein that acts as a mobile signal (a florigen) to induce flowering [4-6]. *CO* and *FT* also are important for photoperiod sensing and response in the SDP rice, but *CO* action has been modified so that it functions as a repressor of flowering in the presence of light (there are a number of other important differences as well) [7,8].

While the daylength may exceed the critical LDP photoperiod from spring through fall, fall may not be an appropriate period to flower. For example, if Arabidopsis plants germinating in early fall were induced to flower by the still relatively long days, they might not have time to produce mature seed before winter. Many plants have an additional system to help distinguish spring from fall: they require exposure to the prolonged periods of cold in winter before being responsive to inductive photoperiods. In Arabidopsis accessions that require vernalization, the MADS (MCM1, AGAMOUS, DEFICIENS, and SERUM RESPONSE FACTOR) domain transcription factor FLC (FLOWERING LOCUS C) is expressed strongly and represses flowering [9]. Prolonged exposure to cold causes stable downregulation of *FLC* through chromatin modification, and the plants are competent to respond to inductive photoperiods in the spring. Such vernalization-requiring accessions have been thought to be 'winter annuals', germinating in the fall, overwintering as rosettes, and flowering in the spring. Accessions with mutations in *FLC* or its activator *FRI* (*FRIGADA*) are rapid cyclers not requiring vernalization. Surprisingly, the mechanisms controlling vernalization in winter wheat and barley

appear very different from those of Arabidopsis. In these cereals, homologs of the Arabidopsis floral meristem identity gene *APETALA 1* (*AP1*) and the florigen *FT* are critical for vernalization [10]. Finally, the gibberellin and autonomous pathways are important for promoting Arabidopsis flowering in the absence of photoperiodic induction [11,12].

Major recent advances

I will focus on recent advances in two general areas: (a) refinement and surprises in Arabidopsis flowering control and (b) conservation and modification of the Arabidopsis pathway in other species. Given the extent of research on Arabidopsis flowering time, one might have thought that the major modes of regulation were known; however, recent research has revealed a new autonomous pathway. The *SQUAMOSA PROMOTER-BINDING-LIKE* (*SPL*) genes are known to affect flowering, with expression studies suggesting that they act downstream of CO and FT in photoperiodic flowering induction [13,14]. Recently, it was found that these genes are targeted by microRNA miR156 and that *SPL* expression increases over time concomitantly with a gradual decrease in miR156 expression [15,16]. Wang and colleagues [16] investigated miR156 expression and found that it was independent of gibberellins, vernalization, and *CO* and *FT*. While *SPL* expression can be induced by the photoperiodic pathway, the gradual increase is independent of *FT*, its homolog *TSF* (*TWIN SISTER OF FT*), and photoperiodic induction. This suggested the possibility that miR156 regulation of *SPL* could function as an autonomous inducer of flowering. Indeed, overexpression of miR156 delayed flowering in the absence of photoperiodic induction. This and subsequent experiments led to the conclusion that in addition to their role in photoperiodism, *SPL* genes define an autonomous pathway that can induce flowering through activation of the floral promoting MADS box genes *SOC1* (*SUPPRESSOR OF OVEREXPRESSION OF CONSTANS 1*) and *FUL* (*FRUITFULL*) [16]. Additional studies place *SPL3* upstream of floral meristem identity genes *LFY* (*LEAFY*) and *AP1* [14].

MicroRNAs have also been shown to be important for regulation of the photoperiodic pathway [17]. Mathieu and colleagues [18] found that SCHLAFMÜTZE (SMZ), an AP2-like transcription factor, is a repressor of flowering in long days. *SMZ* function appears to overlap with that of other family members, so the effects of *smz* knockout mutants are most readily seen in multiple-mutant combinations. Chromatin immunoprecipitation suggests that SMZ functions by direct repression of FT. *SMZ* is targeted by miR172, and overexpression of a cleavage-resistant *SMZ* gene resulted in a complete failure of flowering.

Additional refinement to the photoperiodic pathway comes from Fornara and colleagues [19], who found that multiple DOF (DNA-binding with one finger) transcription factors redundantly repress *CO* expression. Previous work had shown that one DOF, CYCLING DOF FACTOR 1 (CDF1), represses *CO* expression and is targeted for degradation in long days by the FKF1 (Flavin-binding, Kelch repeat, F-box 1) photoreceptor complexed with GIGANTEA (GI) [20,21]. However, *CDF1* knockdowns have only a small effect on flowering, suggesting redundancy. When the activity of four CDFs is reduced or eliminated, plants are aphotoperiodic and flower early in both short and long days [19]. Surprisingly, although *gi* mutants are late-flowering in long days, combining *gi* with the four *cdf* mutations restores near wild-type photoperiodism and cycling of *CO* transcript [19]. This result illustrates the multiple layers of regulation that exist, presumably to ensure proper flowering in the complex natural environment.

Most of what is known about the control of flowering in Arabidopsis comes from plants grown in controlled environment chambers. One of the most important papers published in the last year examined known Arabidopsis flowering mutants in the field [22]. Representative mutants from the photoperiodic, vernalization, and *FVE/LD* autonomous pathway were planted across the native European range of Arabidopsis in spring, summer, and fall cohorts timed to match germination of local populations. Surprisingly, in many environments/cohort combinations, lines expected to require vernalization did not exhibit winter annual behavior; instead, they flowered only marginally later than plants without an active vernalization pathway. This contrasts with expectations from growth chamber experiments that vernalization-sensitive plants would flower much later than 'rapid cyclers' in most circumstances. Thus, vernalization requirements can be met even in summer, perhaps by repeated exposure to cool evening temperatures. Wilczek and colleagues [22] developed a predictive model based on their results. In their modified photothermal model, plants are predicted to flower after accumulating a threshold amount of photothermal units. While such models have been used for years, the model here was extended by using prior information about the genetic differences between lines to individually scale the photothermal accumulation rate. Excitingly, this model was able to accurately predict flowering behavior in both new field and chamber plantings. Thus, the combination of detailed reductionist characterization of mutants in chambers followed by the field evaluation and modeling led to a new understanding of flowering behavior.

Several recent papers have examined variation in the flowering pathway in different species. While *Arabidopsis thaliana* is an annual plant that flowers once and then senesces, the related species *Arabidopsis alpina* is a vernalization-requiring perennial that flowers yearly after exposure to cold [23]. How does vernalization work in this perennial? In *A. thaliana*, epigenetic modification stably represses *FLC* after cold exposure for the duration of the plant's life [9]; clearly, this system must work differently in *A. alpina* to allow it to respond to vernalization yearly. Wang and colleagues [23] screened mutagenized *A. alpina* and looked for mutants that no longer required vernalization. The *perpetual flowering 1* (*pep1*) mutant was found to flower without vernalization, to flower continuously instead of reverting to vegetative growth, and to have fewer vegetative branches. Cloning revealed *PEP1* to be an ortholog of *FLC*. What has changed to allow *PEP1/FLC* to function differently in this perennial plant? Unlike *FLC*, *PEP1* is only transiently repressed in response to cold temperatures, allowing it to promote vegetative development until the following year.

To examine the genetic architecture and enable cloning loci for flowering variation (and other traits) in maize, Buckler and colleagues [24,25] created a large NAM (nested association mapping) population that captures much of the maize genetic diversity. Analysis of flowering revealed that in spite of large differences in parental flowering times, there are no large-effect QTLs (quantitative trait loci) segregating [26]. Instead, variation arises from the combined action of many small-effect loci with relatively little epistasis or gene-environment (G × E) effects. These findings differ substantially from the genetic architecture of flowering in Arabidopsis and rice, both of which harbor large-effect, environmentally sensitive loci [27-31]. An interesting possibility is that that these differences are due to different selective constraints in the outcrossing maize – where flowering within populations must occur simultaneously as compared with selfing Arabidopsis and rice, which (with regard to mating) would be tolerant of large changes in flowering time.

Future directions
A major future direction in understanding flowering will be increased comparative analyses. The current revolution in high-throughput sequencing is enabling experiments not possible 5 years ago and is opening up many more species to detailed comparative analyses – a theme in evidence at a past Keystone Symposium [32]. Specific to the studies discussed above, miR156 is present in monocots as well as dicots, but is its function in flowering conserved? Are changes in *FLC* regulation behind annual/perennial differences in many species? Is stable epigenetic modification of *FLC* common or specific to *A. thaliana*? Finally, to what extent do mating and life history strategies influence standing genetic variation present for these traits? We are lucky to be doing research at a time when answering these questions is within our grasp!

Abbreviations
AP1, APETALA 1; CDF1, CYCLING DOF FACTOR 1; CO, CONSTANS; DOF, DNA-binding with one finger; FLC, FLOWERING LOCUS C; FT, FLOWERING LOCUS T; GI, GIGANTEA; LDP, long-day plant; MADS, MCM1, AGAMOUS, DEFICIENS, and SERUM RESPONSE FACTOR; PEP1, PERPETUAL FLOWERING 1; SDP, short-day plant; SMZ, SCHLAFMÜTZE; SPL, SQUAMOSA PROMOTER-BINDING-LIKE.

Competing interests
The author declares that he has no competing interests.

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