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Flowering responses to seasonal cues: what's new?

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Seasonal cues of day length or winter cold trigger flowering of many species. Forward and reverse genetic approaches are revealing the mechanisms by which these responses are conferred. Homologues of the *Arabidopsis thaliana* protein FLOWERING LOCUS T (FT) are widely used to mediate seasonal responses to day length and act as graft-transmissible promoters or repressors of flowering. Winter cold in *A. thaliana* promotes flowering by repressing transcription of the MADS box gene *FLOWERING LOCUS C* (*FLC*). The mechanism by which this occurs involves a complex interplay of different forms of long noncoding RNAs induced at the *FLC* locus during cold and changes in the chromatin of *FLC*. In perennial relatives of *A. thaliana*, flowering also requires the age-dependent downregulation of miRNA156 before winter.

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Seasonal responses in the regulation of flowering

Flowering is precisely controlled in many species by seasonal cues of day length (photoperiod) and winter temperatures (vernalization). These responses often exhibit quantitative variation among individuals of a single species, ensuring that flowering occurs at the optimal time to maximize seed production in specific environments. Such natural genetic variation supplemented with induced mutations in model species has allowed isolation of genes controlling these complex responses. Such studies have provided regulatory frameworks for photoperiod and vernalization responses and suggested the underlying regulatory logic. They have also identified conserved mechanisms between distantly related species such as *Arabidopsis thaliana* and cereal crops, however perhaps as expected for such fast-evolving adaptive traits important differences are found even between closely related species. The inherent fascination of these seasonal patterns as well as their importance in adaptation to local environments and to yield in agriculture have led to an extensive literature including several recent comprehensive reviews [1–5]. Here we focus on highlights of the recent literature mainly from the last two years that have deepened our understanding of photoperiodic and vernalization responses and the open questions that these publications pose.

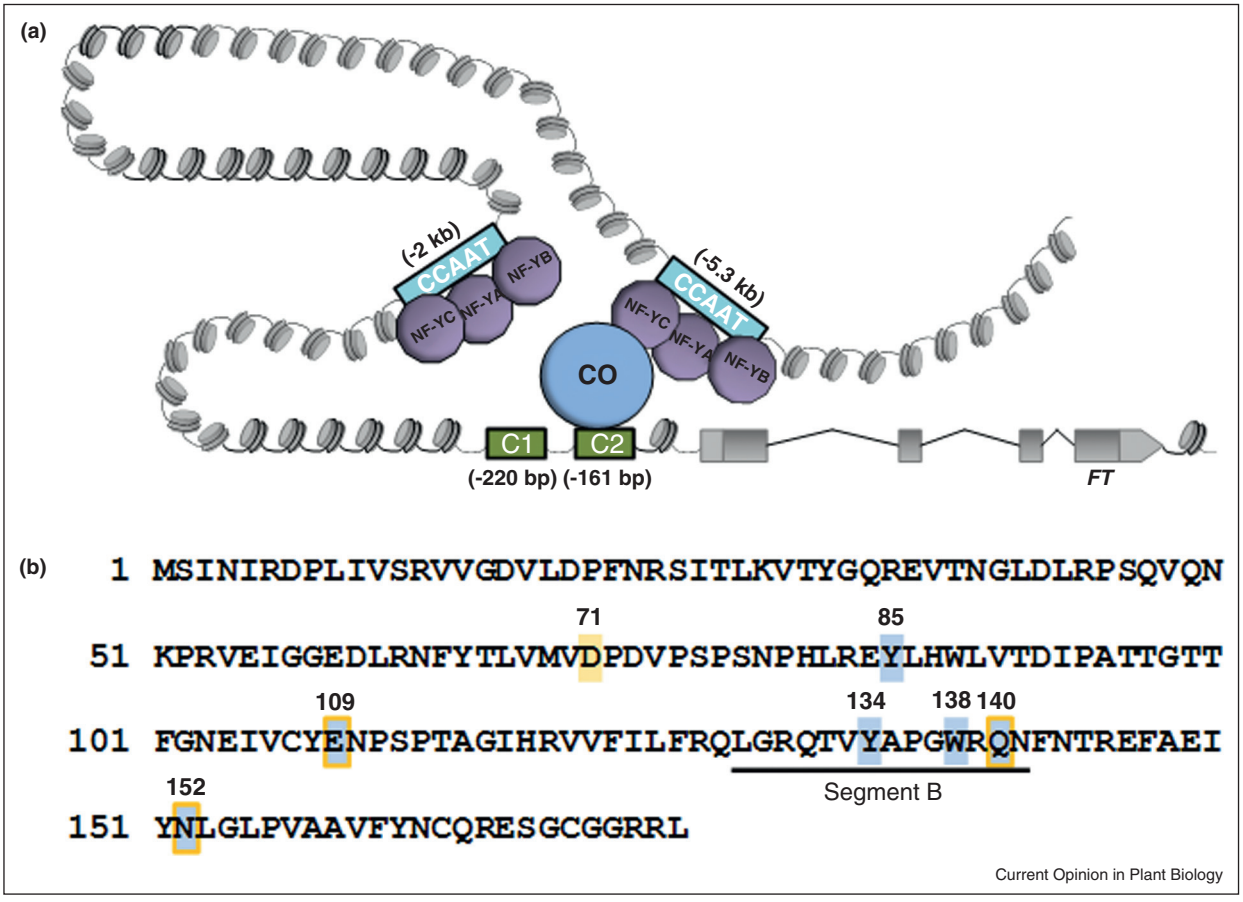
Photoperiodic response: transcriptional regulation of *FT* homologues

The canonical photoperiodic flowering pathway of *A. thaliana* promotes early flowering under long days (LDs) of spring and early summer, but not under short days (SDs) of winter [1,4]. Two genes, *FLOWERING LOCUS T* and *TWIN SISTER OF FT* are increased in transcription in the phloem companion cells under LDs. These proteins, related in sequence to phosphatidylethanolamine binding proteins (PEBPs) of animals, move to the apex where they induce flowering. Transcriptional activation of *FT*, which is expressed at higher levels than *TSF*, in response to LDs is the limiting step in photoperiodic induction of *A. thaliana*, and similar data have been obtained for *FT* homologues in many other species. In *A. thaliana* this activation of *FT* occurs through the CONSTANS (CO) transcription factor, which accumulates specifically under LDs and promotes *FT* and *TSF* transcription. The CO protein contains B-box zinc fingers at the amino terminus and a CO COL TOC1 (CCT) domain at the C-terminus. The CCT domain has sequence similarities to the NUCLEAR FACTOR-YA (NF-YA) protein and acts as a DNA binding domain [6–8]. CO binds to the proximal region of the *FT* promoter recognizing two CO Responsive Elements (CORE) that are required for *FT* activation [7,9]. Deletion analysis of the *FT* promoter showed that a distal region is also required for CO response [9]. Recently this region was shown to be recognized by NF-Y that binds to a CCAAT element around 5.3 kb upstream of the transcriptional start site [10•]. Previous genetic analysis showed that certain NF-Y subunit paralogues are required for CO to activate *FT* transcription [11], and mutational analysis demonstrated that this CCAAT box is required for CO-mediated activation of *FT* [10•]. Looping of the *FT* promoter has now been detected between the distal site to which NF-Y binds and the proximal site at which CO binds [10•], and since CO and NF-Y were previously

shown to interact [6,12], this looping is proposed to be caused by interactions between CO and NF-Y bound to DNA [10•] (Figure 1). As both complexes and their binding sites are required for *FT* activation, and loops are only detected at the times of day at which CO and *FT* are expressed, this looping may be required for the photoperiodic dependent activation of *FT* transcription. These data help clarify the structure of the *FT* promoter and the mechanism of its transcriptional activation in *A. thaliana* in response to photoperiod. The significance and basis of promoter looping must now be tested using precise mutations in *FT* promoter motifs.

Genetic analysis in a range of species using naturally occurring alleles identified proteins containing CCT domains that are important in *FT* photoperiodic regulation [13–17], suggesting that the role of these proteins in photoperiodic flowering is strongly conserved. However, typically these contain at their N-terminus a pseudo-response regulator receiver domain rather than the B-boxes found in CO [13,15–17]. These PSEUDO RESPONSE REGULATOR (PRR) proteins are members of small gene families and encode components of the circadian clock in *A. thaliana*, but members of the family have been shown to have specific roles in *FT* activation and flowering in several other species. Most recently, the *BOLTING* gene from sugar beet was added to this list [16]. This species includes annual accessions that flower rapidly without a requirement for winter cold, and others that are biennial and flower only if vernalized. These varieties differ at the *BOLTING* (*B*) locus, so that dominant alleles at *B* confer the early flowering annual growth habit, whereas biennials carry the recessive allele. Isolation of this gene showed that it encodes a PRR protein

Figure 1



Features of FT transcriptional activation and protein structure. (a) Model of recruitment of CO to the CO Response Elements (CORE, labeled C1 and C2) (adapted from [10•]). NF-Y complexes bind to two CCAAT sequences located at −2 and −5.5 kb away from the *FT* transcription start site (TSS). A chromatin loop, whose formation is favored at the end of a long day, maintains the NF-Y complex in close proximity to the CORE sequences (at −220 and −161 bp away from the TSS). This allows NF-Y complex to interact with and stabilize CO when it binds to the CORE sequences and thereby to activate *FT* transcription. (b) Mutations introduced in certain amino acids (colored blue in the sequence) convert FT into a TFL1-like floral repressor. Some of these changes are predicted to change also the protein surface charge (blue with orange boxes). Mutations in Asp-71 (in yellow), which is embedded in the binding pocket, do not result in activity changes. Residues in Segment B also differ in sugar beet BvFT2 and cause the protein to act as a repressor of flowering.