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Photoperiodic Regulation of Florigen Function in *Arabidopsis thaliana*

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One mechanism through which flowering in response to seasonal change is brought about is by sensing the fluctuation in day-length; the photoperiod. Flowering induction occurs through the production of the florigenic protein FLOWERING LOCUS T (FT) and its movement from the phloem companion cells in the leaf vasculature into the shoot apex, where meristematic reprogramming occurs. *FT* activation in response to photoperiod condition is accomplished largely through the activity of the transcription factor CONSTANS (CO). Regulation of *CO* expression and protein stability, as well as the timing of other components via the circadian clock, is a critical mechanism by which plants are able to respond to photoperiod to initiate the floral transition. Modulation of *FT* expression in response to external and internal stimuli via components of the flowering network is crucial to mediate a fluid flowering response to a variety of environmental parameters. In addition, the regulated movement of FT protein from the phloem to the shoot apex, and interactions that determine floral meristem cell fate, constitute novel mechanisms through which photoperiodic information is translated into flowering time.

INTRODUCTION

In plants, entirely new structures which house the germline of an individual must be formed post-embryonically. Once the formation of reproductive organs is specified, the process is largely irreversible (Evans, 1971). This necessitates that the phase transition between the vegetative and reproductive periods of plant development is regulated so that optimal endogenous and exogenous factors are synchronized for maximal reproductive fitness (Kinmonth-Schultz et al., 2013). Changes in season preclude many environmental conditions that plants must prepare or react to. Anticipation of seasonal change is perceived through changes in day length (the photoperiod), that are the causal agent of seasonal climate (Thomas and Vince-Prue, 1996). Season, then, and its associated changes in day-length (photoperiod) is thus an excellent integrated variable through which many other environmental conditions can be interpreted to determine the timing of the floral transition (Kinmonth-Schultz et al., 2013). For this reason, photoperiodic change is a common cue which many plant species utilize to coordinate their flowering time (Song et al., 2010).

A great deal is now known about the molecular mechanisms by which flowering in response to photoperiod in *Arabidopsis thaliana* is accomplished. At the basic level, photoperiod regulates the expression and activity of CONSTANS (CO: At5g15840), a key transcriptional activator of the gene that encodes the "florigen" protein FLOWERING LOCUS T (FT: At1g65480) (Kardailsky et al., 1999; Kobayashi et al., 1999; Abe et al., 2005; Wigge et al., 2005; Corbesier et al., 2007), which is crucial in specifying the conversion of vegetative cell fate at the shoot apex into a reproductive identity (Putterill et al., 1995; An et al., 2004; Abe et al., 2005; Mathieu et al., 2007; Tiwari et al., 2010; Andrés and Coupland, 2012) (Figure 1). For over a decade, uncovering how *CO* and *FT* are regulated by photoperiod has been a key goal in the understanding of how photoperiodic information determines flowering output, and considerable advancement has been made in understanding the photoperiodic regulatory network in Arabidopsis (Song et al., 2013). In this review, we will discuss our current understanding of photoperiodic input sensing, ambient temperature and light quality effects on photoperiodic flowering, the chromatin landscape at flowering component loci, florigen movement from the leaf to the shoot apex, and the influence of carbohydrate status on flowering in response to photoperiod.

OVERVIEW OF THE FLOWERING TIME PATHWAYS

The regulation of the timing of the floral transition is an intricate one. Multiple pathways are able to regulate the expression of "floral integrator genes" (Moon et al., 2005). These integrators are network hubs that link the pathways that measure environmental and developmental competence to the downstream targets that cause the patterning of reproductive structures and floral organs (Song

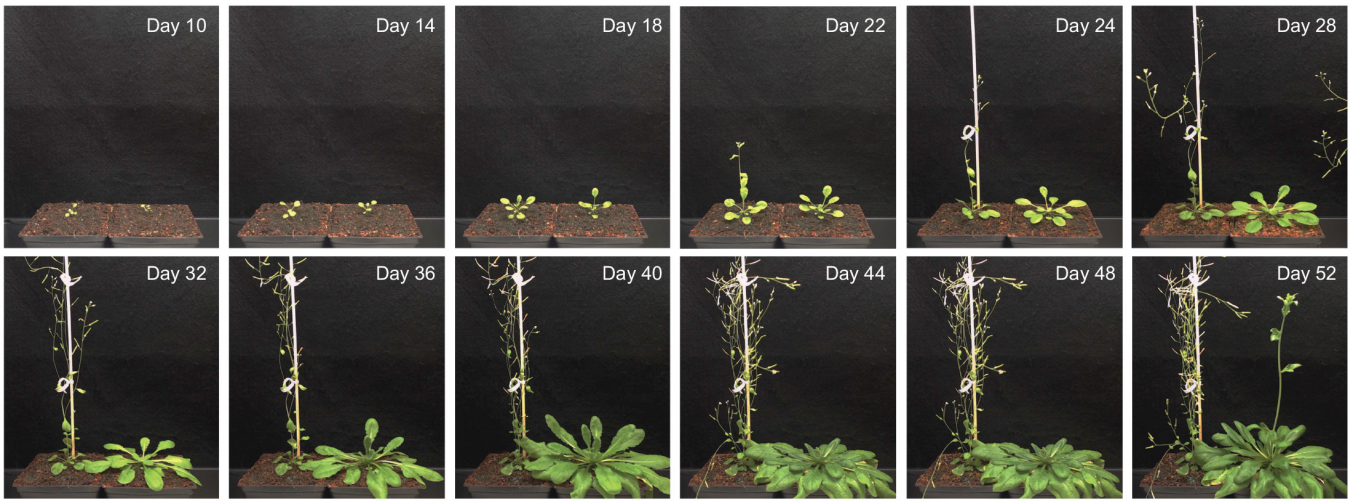

Figure 1. Flowering in response to photoperiod is mediated by *CONSTANS.*

Time series of WT (Col-0) plant (left in frame) and *co-101* mutant plant (right in frame) grown under long-day conditions (16hr L/8hr D) from 10 day-old seedlings until flowering of *co-101* mutants. Flowering in response to the inductive photoperiod is severely delayed in *co-101* mutants.

et al., 2013). Because of their unique placement within these networks, they are able to filter multiple inputs into a single output. Commonly described floral integrator genes in Arabidopsis are *FT, SUPRESSOR OF OVEREXPRESSION OF CONSTANS 1* (*SOC1:* At2g45660)*, SQUAMOSA PROMOTER BINDING LIKE* (*SPLs*), *AGAMOUS LIKE 24* (*AGL24:* At4g24540). Additionally, the meristem identity genes *FRUITFULL* (*FUL:* At5g60910)*, LEAFY* (*LFY*: At5g61850), and *APETALA 1* (*AP1*: At1g69120) show similar qualities to *FT*, *SOC1*, *SPL*s and *AGL24* in that their network placement and floral activation role; incremental expression of these integrators strongly increases competency to flower (Simon et al., 1996; Ruiz-Garcia et al., 1997; Samach et al., 2000; Yanovsky and Kay, 2002; Michaels et al., 2003b; Teper-Bamnolker and Samach, 2005). Of particular interest is *FT*, as *FT* is the main component that links the environmental sensory machinery that is present within the leaf vasculature, to the shoot apex where reproductive structures eventually form (Turck et al., 2008). Since the 1930s, it has been known that a mobile signal present within the leaves of many plant species is able to induce flowering (Chailakhyan, 1937; Chailakhyan, 1968; Chailakhian, 1970; Evans, 1971). Experimental evidence determined that FT protein is the long sought "florigenic" signal that moves through the phloem stream and initiates flowering at the shoot apex. The *FT* gene is the primary target of several of these pathways, including photoperiod, vernalization, hormone signaling, temperature and plant age (Song et al., 2013). Vernalization is a crucial sensory mechanism to prevent flowering in the fall and increase flowering competence in spring after prolonged cold, and will not be discussed in detail here [see (Kim and Sung, 2014) for review]. Among the summer annual accessions of Arabidopsis from which many of the common laboratory strains are derived, photoperiod is the major factor in dictating the timing of the floral transition, although higher temperatures can accelerate flowering even under short day conditions (Balasubramanian et al., 2006; Kumar et al., 2012; Song et al., 2013). It should be noted, however, that for the vast majority of winter annual Arabidopsis accessions, variation in flowering time predominantly is explained by variation in vernalization pathway components (Johanson et al., 2000; Michaels et al., 2003a; Lempe et al., 2005; Strange et al., 2011). While the concept of graded floral integrator expression and the input of multiple pathways in this response is useful, the discrete nature of many of these pathways has been called into question in recent years, and significant cross-talk between pathways is now known (Amasino, 2010). Throughout this review we will discuss the implications of the links between pathways on the architecture of the flowering time network.

PHOTOPERIODIC SENSORY MECHANISM

In Arabidopsis, photoperiodic information is specified through the interaction of the circadian clock and light, and both of these factors converge to regulate the expression and the activity of the CO transcription factor. The CO protein contains the B-box and CCT (CO, COL, and TOC1) domains, and is the core activator of *FT* in response to photoperiod (Strayer et al., 2000; Robson et al., 2001; Suárez-López et al., 2001; Khanna et al., 2009) (Figure 1). *CO* mRNA has a unique daily expression pattern that has a global minima in the morning, and has a maxima at night (Suárez-López et al., 2001). The control of the daily oscillation is a direct output of the circadian clock (Suárez-López et al., 2001). Additionally, under long day conditions (16h light/8h dark), there is a local maximum

point of gene expression at Zeitgeber time (ZT) 16 (16 hours from the onset of stimulus, such as light, which entrains the circadian clock= Zeitgeber). It is this maxima that is responsible for a corresponding increase in *FT* activation, as CO protein activity is nullified in the dark (Laubinger et al., 2006). It is the coincidence of light with the local maximum in gene expression of *CO* in the late afternoon that is critical for the sensing of optimal long day conditions in Arabidopsis (Imaizumi and Kay, 2006). For this reason, the system of *CO* activity can be seen as a poised response in which the circadian clock determines the window in which a time dependent light signal can activate flowering. This system follows closely to the external coincidence model for photoperiodic phenomena, and remains a paragon example of a clock regulated developmental output (Pittendrigh, 1972; Kinmonth-Schultz et al., 2013).

CO TRANSCRIPTIONAL REGULATION

As mentioned, *CO* transcriptional regulation is highly dependent upon the circadian clock in order to set the pace for its oscillation. Repression of *CO* expression during the morning, which results in the daily global minimum, is mediated by the CYCLING DOF FACTOR (CDF) family of DOF domain transcription factors (Imaizumi et al., 2005). *CDF1* (At5g62430)*, CDF2* (At5g39660)*, CDF3* (At3g47500)*, CDF5* (At1g69570) expression is tightly regulated by the circadian clock, and show concurrent peaks of expression during the late night and morning (Fornara et al., 2009). *CDF* expression is a direct output of the circadian clock, and is activated through the action of the core clock component CIRCADIAN CLOCK ASSOCIATED 1 (CCA1: At2g46830) and LATE ELONGATED HYPOCOTYL (LHY: At1g01060) MYB domain transcription factors (Schaffer et al., 1998; Wang and Tobin, 1998; Seaton et al., 2015). *CDF* expression is repressed in the afternoon and evening through the action of PSUEDO RESPONSE REGULATORs (PRRs): PRR5 (At5g24470), PRR7 (At5g02810), and PRR9 (At2g46790) (Nakamichi et al., 2007; Ito et al., 2008). The functions of individual CDF proteins, CDF1, CDF2, CDF3 and CDF5, is largely additive and redundant in repression of the *CO* transcription (Fornara et al., 2009). DOF binding sites near the transcriptional start site in the *CO* promoter are composed of a short repetitive element, the copy number of which varies by ecotype (Rosas et al., 2014). The greater the number of CDF binding sites, the greater the sensitivity to photoperiodic inputs, as the peak and trough difference of *CO* expression is highest with additional copies of CDF binding sites (Rosas et al., 2014). CDF repression of *CO* is released in the afternoon through the action of a blue light induced complex composed of the GIGANTEA (GI: At1g22770) protein and the LOV domain blue-light photoreceptor E3 ubiquitin ligase FLAVIN BINDING, KELCH REPEAT, F-BOX 1 (FKF1: At1g68050) (Sawa et al., 2007). Both *GI* and *FKF1* mRNA expression is regulated by the circadian clock (Fowler et al., 1999; Mizoguchi and Coupland, 2000; Imaizumi et al., 2003; Imaizumi et al., 2005; Song et al., 2012a). The protein expression profiles of FKF1 and GI largely overlap in long days but not in short days (Sawa et al., 2007). This complex targets CDF proteins for ubiquitin mediated proteasomal degradation through the F-box domain of FKF1, which removes the repressor from the *CO* promoter (Imaizumi et al., 2005). Photoperiod dependent formation of this GI-FKF1 complex and subsequent CDF degradation occurs through two potential mechanisms: firstly, light activated FKF1 in the afternoon can degrade CDFs in long days, but not short days since light is absent at the peak of FKF1 expression in short days; secondly, FKF1 and GI expression differs between photoperiods, and can only degrade CDFs in long days when their expression overlaps. Both mechanisms highlight the potential for both external and internal coincidence mechanisms to explain the photoperiod dependent degradation of CDFs. In addition to direct evidence, the combination of clock output and the activator potential of FKF1 through CDF degradation are sufficient to mathematically replicate the *CO* expression pattern (Salazar et al., 2009; Song et al., 2012a; Seaton et al., 2015). In the future, additional modeling of *CO* transcription may be necessary for determining individual component contributions to specific transcriptional outputs.

FKF1 homologs, ZEITLUPE (ZTL: At5g57360) and LOV KELCH PROTEIN 2 (LKP2: At2g18915), degrade CDF2 protein, and also interact with other CDFs in yeast, although a comprehensive survey as to their role in CDF protein destabilization remains to be performed (Fornara et al., 2009). CDF2 protein, in addition to negative regulation through ZTL/FKF1/LKP2 family proteins, is also post-translationally modified with the SUMO ubiquitin-like peptide that is deposited by the SUMO-targeted ubiquitin ligase 4 (STUBL4: At1g66650) protein (Elrouby et al., 2013). It is currently unknown how the sumoylation of CDF2 or other CDFs might affect *CO* transcription in a time dependent fashion or whether CDFs are the target of other post translational modifications which might affect their activity.

Upon CDF protein removal, the FLOWERING BHLH (FBH1: At1g35460, FBH2: At4g09180, FBH3: At1g51140, and FBH4: At2g42280) group of bHLH transcription factors promotes the activation of *CO* transcription. FBH family members are able to bind to E-box elements within 5' region of the *CO* promoter (Ito et al., 2012b). The combination of CDF removal and FBH activation allows for the local maxima of *CO* expression in the afternoon of long days important for *FT* activation and the promotion of flowering under inductive conditions (Ito et al., 2012a). To date, FBHs are the only known direct activators of *CO* transcription, it remains to be examined whether other factors are critical for *CO* transcriptional activation or what factors are responsible for time dependent activation of *CO* expression during the night of long and short days (Ito et al., 2012b).

CO POSTTRANSLATIONAL REGULATION

Light quality effects on CO protein

In addition to the requisite accumulation of an abundance of *CO* transcripts and CO protein to the afternoon of long days, the relative activity and stability of CO protein is critical for its function in the afternoon induction of *FT*. CO protein stability changes under a variety of external light conditions, and external light quality inputs are important factors through which CO protein activity is regulated (Valverde et al., 2004) (Figure 2). Generally, CO is stabilized under blue and far-red light, and destabilized under red light and darkness (Valverde et al., 2004). Under blue light conditions, FKF1, in addition to its described role in CDF degradation,

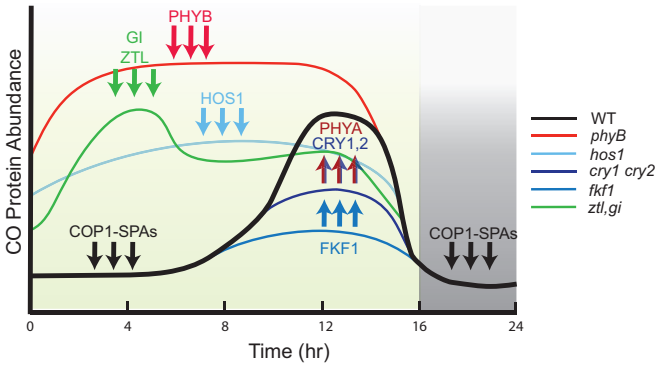

Figure 2. Post-translational control of CO determines the strength and temporal domain of CO activity to activate flowering.

CO protein activity is regulated by various factors. Action of COP1-SPA complexes during both the day and the night degrades CO protein through ubiquitin mediated proteasomal degradation. During the daytime, CRY1 and CRY2 alleviate COP1-SPA activity on CO through protein-protein interactions. CO protein is also stabilized by PHYA through an unknown mechanism. PHYA and CRY1/CRY2 activity in the late afternoon increases CO protein abundance. Correspondingly, CO protein abundance is reduced in the afternoon in *cry1 cry2* and *phyA* mutants. HOS1 protein activity destabilizes CO protein, but only during the morning of long days. Mutations in *hos1* shift the peak of CO abundance earlier in the day. FKF1 also stabilizes CO protein through an unknown mechanism. Concomitant with its daily expression in the afternoon of long days, FKF1 protects CO protein from degradation. Opposite of FKF1, ZTL is able to interact with GI during the morning to destabilize CO protein. PHYB acts antagonistically with CO, and destabilizes CO protein through an unknown mechanism. PHYB destabilization effects both the amplitude and temporal domain of CO abundance, as CO protein is highly abundant throughout the day in *phyB* mutants. Thus, CRY1, CRY2, PHYA, and PHYB contribute to CO amplitude, while HOS1 and FKF1 contribute to the temporal domain of CO protein abundance. Note: Absolute CO abundance in *phyB* vs. *hos1* mutants are extrapolated from different genetic backgrounds and therefore may be different from the above representation.

also stabilizes CO protein (Song et al., 2012a). Exactly how FKF1 stabilizes CO protein in the afternoon remains unknown. In addition to FKF1, ZTL has a role in the regulation of CO protein stability, but opposite to that of FKF1. Together with GI, ZTL directly destabilizes CO protein in the morning of long days (Song et al., 2014). The opposite effects of FKF1 and ZTL in this instance may act as a means to restrict the timing of CO protein to the late afternoon of long days.

In darkness, the CONSTITUTIVE PHOTOMORPHOGENESIS 1 (COP1: At2g32950) and SUPPRESSOR OF PHYA-105s (SPA1: At2g46340, SPA2: At4g11110, SPA3: At3g15354, and SPA4: At1g53090) form a protein complex which actively degrades CO through ubiquitin mediated proteasomal degradation (Jang et al., 2008) (Figure 2). Inhibition of CO protein by COP1/SPAs prevents CO protein produced at night from activating *FT* expression, further constraining the window of *FT* expression to the late afternoon (Laubinger et al., 2006; Jang et al., 2008). In order to limit the inhibitory effect of COP1/SPAs complex on CO protein during the day time, blue light activated and phosphorylated CRYPTOCHROME 2 (CRY2: At1g04400) photoreceptors bind to SPA1 protein and abolish their antagonistic activity on CO; thus, blue light can stabilize CO through two independent mechanisms, late afternoon stabilization by FKF1 and prevention of COP1/SPAs interference by CRY2 (Pokhilko et al., 2011; Zuo et al., 2011). The phytochrome photoreceptor PHYA (At1g09570) stabilizes CO protein under far-red light enriched conditions (Valverde et al., 2004) (Figure 2). PHYB (At2g18790) has an antagonistic role to PHYA in CO protein stabilization, as CO protein is stabilized in *phyB* mutants (Valverde et al., 2004) (Figure 2). PHYTOCHROME DEPENDENT LATE FLOWERING (PHL: At1g72390), an unknown domain protein, is also involved in stabilization of CO protein, but the stabilizing effect it mediates is absent in *phyB* mutants (Endo et al., 2013). Molecular evidence suggests that PHL may be involved in sequestration of CO away from the inhibitory effects of PHYB dependent destabilization (Endo et al., 2013) (Figure 3). Taken as a whole, light dependent regulation of CO protein stability is critical for its function, but there is little evidence yet as to how light quality effects that are found in nature might affect the flowering time response; i.e. whether spectral qualities of long days or short days found under natural conditions have real, quantitative effects on flowering time. This is an important aspect of the photoperiodic response that remains to be analyzed in greater detail, in particular to determine the sum of mutual effects of photoreceptors on flowering time regulation.

Temperature effects on CO protein stability

In addition to light dependent regulation of CO protein, ambient temperature can also affect its stability. A RING-finger E3 ubiquitin ligase HIGH EXPRESSION OF OSMOTICALLY RESPONSIVE GENE 1 (HOS1: At2g39810) negatively regulates CO protein accumulation during the morning, through binding of CO and targeting it for degradation by the proteasome (Lazaro et al., 2012). CO degradation by HOS1 is both time and temperature dependent, as HOS1 preferentially degrades CO during the morning, and CO degradation by HOS1 is increased under low temperature conditions (4°C) (Lazaro et al., 2012; Lee et al., 2012). Due to the genetic relationship between HOS1 and PHYB signaling, it is possible that PHYB connects both low temperature and red light signaling in order to negatively regulate CO protein stability (Lee et al., 2012). At increased temperature, *co* mutants flower appreciably earlier compared to normal growth conditions, which suggests that *FT* activation under higher temperatures is likely not caused by increased stability of CO protein, but through other mechanisms (Kumar et al., 2012). Thus control of CO protein stability is broadly regulated by a combination of photoperiod, light signaling and ambient temperature (Figure 2).

FT TRANSCRIPTION

FT is critical in signaling for the transition into flowering, as a floral integrator and the primary signal through which the sensory machinery in the leaf vasculature is linked with the shoot apex (Andrés and Coupland, 2012). Many factors have been found to regulate its expression; it is through the action of these many factors that an incremental response to optimal environmental conditions can push or delay the floral transition (Schwartz et al., 2009).

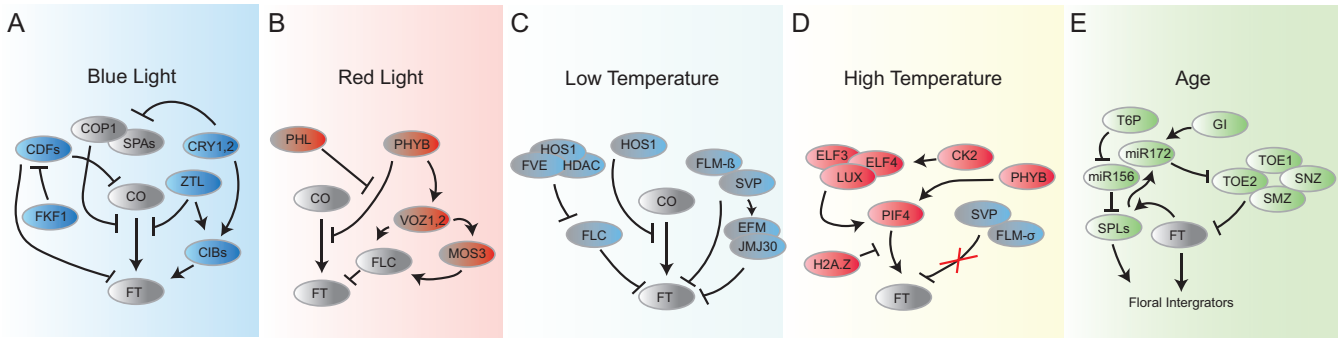

Figure 3. Regulation of CO and FT in response to environmental and endogenous cues.

(A) Under blue-light enriched conditions, *CO* and *FT* transcription is increased due to increased degradation of CDFs by FKF1. CO protein is stabilized through inhibition of COP1/SPAs activity by CRY1 and CRY2. CRY1 and CRY2 also are able to increase *FT* expression directly through activation of CIB transcription factors. CO protein is also stabilized through FKF1 activity in a blue light dependent manner. ZTL stabilizes CIB1, but destabilizes CO.

(B) Under red light conditions, PHYB is able to destabilize CO protein. PHYB activity on CO is hampered through the action of PHL. PHYB is also able to indirectly repress *FT* expression through an increase in FLC transcription, either through direct activation of FLC by VOZ1 and VOZ2, or through and indirect effect whereby VOZ1/2 increase *FLC* transcripts through regulation of the nuclear pore component MOS3.

(C) Under low ambient temperature conditions, HOS1 is able to facilitate degradation of CO protein during the morning. HOS1 is also able to effect *FT* transcription through protein complex formation with FVE and HDAC, which represses FLC transcription. This reduction in FLC indirectly increases *FT* expression. SVP is able to form functional heterodimers with a splice variant of the transcription factor FLM (FLM- ß) which represses *FT* expression. FLM- ß is preferentially spliced under low temperature conditions. SVP upregulates *EFM;* an EFM and JMJ30 complex represses *FT* transcription. SVP expression is regulated by CLF and BRM chromatin remodeling processes.

(D) Under high temperature conditions, PIF4 protein is able to upregulate *FT* expression in short day conditions, but its binding site is inhibited by the deposition of the histone variant H2A.Z to the *FT* 5' proximal region. PIF4 is also activated in response to high temperature by PHYB. Under high temperatures, SVP-FLM repression of *FT* is impaired by the preferential splicing of FLM-δ, which forms a non-functional complex with SVP. ELF3, ELF4, and LUX are circadian clock components which regulate hypocotyl elongation through PIF4 activity in the evening, and may be involved in PIF4 regulation of flowering in response to high temperature. CK2 is involved in adjusting the pace of the circadian clock in response to temperature and may be involved in temperature dependent changes in clock output that indirectly effect *FT* expression through PIF4.

(E) Relative abundance of *miR172* and *miR156* changes with age, as miR172 decreases with age and *miR156* increases. *miR172* activity reduces transcription of AP2 related repressors of *FT* expression, TOE1,2, SMZ and SNZ, and is positively regulated by GI activity. Conversely, miR156 targets SPLs transcripts, which reduces the activity of SPLs to activate other floral integrator genes at the shoot apex. These pathways enable the prevention of flowering before developmental competency, and improve sensitivity to environmental conditions as the plants further develop.

Activation of *FT* transcription in response to photoperiod

Primary activation of *FT* in the late afternoon of long day conditions is accomplished through the action of CO protein (Kardailsky et al., 1999; Kobayashi et al., 1999; Onouchi et al., 2000; Yanovsky and Kay, 2002; Yoo et al., 2005). This activation occurs through two mechanisms of CO, the first being direct DNA binding to CO-responsive elements (CORE) in the *FT* promoter (Tiwari et al., 2010); and the second being the recruitment of additional proteins that compose a CO activator complex to assist in transcriptional activation. CO is able to recruit and form a protein complex with ASYMETTRIC LEAVES 1 (AS1: At2g37630) (through its B-box domain), as well as members of the transcriptional co-activator family NUCLEAR FACTOR Y (NF-YA1: At5g12840, NF-YB1: At2g38880, NF-YC1: At3g48590) (through its CCT domain) to CCAAT elements within the *FT* promoter (Ben-Naim et al., 2006; Kumimoto et al., 2008; Song et al., 2012b). Incremental increases in *CO* expression directly correlate with a corresponding increase in *FT* expression and an acceleration of flowering time (Putterill et al., 1995). *CO* is the founding member of the plant specific B-box transcription factor family and is the primary activator of flowering among the homologues. *CO* is closely related to the CONSTANS LIKE 1 (COL1: At5g15850) COL1-5 subclade and recent domain replacement experiments have determined that the B-box domain differences between the proteins is essential for CO function (Hassidim et al., 2009; Khanna et al., 2009; Kim et al., 2013). Replacement of the CO B-box domain into the COL1 and COL2 (At3g02380) proteins caused earlier flowering than wild type COL1 or COL2 protein resulting from increased *FT* transcription, which suggests that it is the B-box domain that is critical for CO dependent flowering function (Kim et al., 2013). Several *COL* genes have been implicated in other environmental responses such as cold acclimation and light signaling so it could also be that COL proteins have regulatory roles that have not been activated under standard laboratory conditions (Hannah et al., 2005; Datta et al., 2006). Another B-box domain transcription factor B-BOX DOMAIN PROTEIN 19 (BBX19: At4g38960), which is regulated by the circadian clock, is able to interact with CO during the morning when its own expression is high, and to nullify CO activation of *FT* through interactions with the B-box domain of CO. BBX19 thus is able to further constrain the window of CO activity through depletion of any morning expressed CO protein activity (Wang et al., 2014). Under short day conditions the activity of CO protein is largely attenuated, likely through the action of BBX19

and the diminished presence of FKF1 during the light period. The instability of CO protein in the absence of light activated FKF1 and the presence of active COP1/SPAs abolishes CO protein activity and prevents *FT* transcription.

Clock dependent repression of *FT* transcription

In addition to clock regulation of *CO* transcription and protein stability, several clock-regulated factors contribute to repression of *FT* transcription. CDF transcription factors, in addition to their binding to the *CO* promoter during the morning, are able to bind to the *FT* promoter at the 5' proximal region around the transcription start site (Song et al., 2012a). Presumably, the same regulatory mechanism for CDF removal by light activated GI-FKF1 complex is able to free the *FT* promoter of repressive activity so that CO activation can be completed under inductive long day conditions. Two members of the APETALA 2 (AP2) / ETHYLENE RESPONSE FACTOR (ERF) family of transcription factors TEMPRANILLO 1 (TEM1: At1g25560) and TEM2 (At1g68840) are able to repress *FT* transcription (Castillejo and Pelaz, 2008); TEM1 binds directly to the proximal 5' region of the *FT* promoter to repress its transcription. *TEM1* transcript abundance peaks around dusk in long day conditions concurrently with *CO* expression, and the circadian clock regulates the oscillation of its expression, as its oscillation perpetuates upon transfer in continuous light (Castillejo and Pelaz, 2008). Due to its daily timing, TEM1 and TEM2 may represent a brake on the activity of *CO* activation, where the pendulum shift between both can balance the *FT* transcriptional response. In addition to their regulation of *FT* transcription, TEMs are able to regulate flowering time through the gibberellin hormone signaling pathway through the repression of *GA-3 OXIDASE1* (*GA3OX1*: At1g15550) and *GA3OX2* (At1g80340), both of which encode biosynthetic enzymes that convert inactive GA hormone in to biologically active GA_4 (Osnato et al., 2012). GI also interacts with TEMs in tobacco, and this interaction may change TEM activity (Sawa and Kay, 2011). Thus TEM proteins serve as a point of integration between the day length dependent and independent regulation to modify the flowering output.

In addition to clock-regulated outputs, many basic questions about the direct regulation of circadian clock components also remain undetermined due to the enmeshed nature of the circadian network. *CCA1* and *LHY* overexpression, for instance, results in a late flowering phenotype, yet it is unknown whether the effect of the overexpression is due to corresponding increase in CDF expression, reduced GI expression, a combination of both, and/or the regulation of other clock components (such as PRRs) or other outputs (Wang and Tobin, 1998; Park et al., 1999; Nakamichi et al., 2007). In short, genetic analyses of phenotypic effects on circadian clock outputs could be the result of many parallel interactions, and may require modeling approaches in order to disentangle the effects of multiple inputs.

Light dependent regulation of *FT* transcription

Photoperiod and clock dependent inputs are critical for mediating the time dependent regulation of *FT* transcription, and maintaining a window for *FT* activation so that long days can successfully initiate the floral transition. Similar to CO protein regulation, input of light quality through the action of photoreceptor proteins is important for modulation of *FT* transcription (Figure 3). Members of the CRYPTOCHROME-INTERACTING BASIC-HELIX-LOOP HELIX (CIB) family of bHLH transcription factors activate *FT* expression (Liu et al., 2008). CIB1 (At4g34530), CIB2 (At5g48560) and CIB5 (At1g26260) form functional complexes with CRY2 *in vivo* and at least CIB1 directly binds to the *FT* promoter via E-box elements in the 5' proximal region to activate *FT* transcription (Liu et al., 2008; Liu et al., 2013). In addition to activation by CRY2, CIB protein abundance is positively affected by ZTL and LKP2, as CIB1 protein is unstable in *ztl* mutants (Liu et al., 2013). This suggests that an integration of positive blue light activating signals occurs to affect flowering time response (Figure 3). Thus, blue light inputs on *FT* are doubly activating through the degradation of CDF proteins by FKF1 and subsequent activation by CIB proteins (Imaizumi et al., 2005; Liu et al., 2013) (Figure 3). At present it is unclear if CIB transcription or its activity are regulated by the circadian clock; indirect regulation through ZTL and LKP2 covers most of the daytime of long and short days, so the time dependent nature of CIB activity in relation to other regulatory mechanisms still needs to be determined. Presumably due to CDF repressive activity, the activation of *FT* through CIBs occurs mainly in the afternoon of long days, as CIB1 overexpressing plants have a greatly enhanced peak of *FT* expression in the late afternoon, but similar levels of *FT* expression to wild type in the morning (Liu et al., 2008).

Contrary to red light's role in CO protein stabilization, VASCULAR PLANT ONE ZINC FINGER 1 (VOZ1: At1g28520) and VOZ2 (At2g42400), two NAC (NAM, ATAF1/2 and CUC2) domain transcription factors, activate *FT* expression under long day conditions (Yasui et al., 2012). VOZ proteins interact with PHYB, and like PHYB, their movement between the cytoplasm and the nucleus is tightly regulated. As *voz1,2* double mutants have elevated expression of the MADS-BOX *FT* repressor FLOWERING LOCUS C (FLC: At5g10140), it remains to be determined whether activation of *FT* by VOZ proteins is direct, indirect, or both (Yasui et al., 2012) (Figure 3). VOZ1 also regulates the expression of the nuclear pore protein MODIFIER OF SNC1 (MOS3/SAR3: At1g80680) a protein known to be involved in flowering time regulation and which is required for the movement of mRNA out of the nucleus (Celesnik et al., 2013). This suggests that control of transcript export of flowering components may be important in the regulation of flowering time or a mechanism through which photoreceptors can post-transcriptionally regulate the flowering response.

Temperature dependent regulation of *FT* expression

Regulation of flowering time through vernalization in winter annual Arabidopsis coordinates strict control of flowering, directly shutting down *FT* expression until long periods of cold have been attained. In contrast to the temperature changes sensed through vernalization during the winter, ambient temperature change during spring can modulate the flowering time response. Regulation of *FT* expression in response to shorter-term temperature change has become apparent. Several members of the MADS-box binding transcription factor family are critical in mediating this response:

FLC and its homologues FLOWERING LOCUS M/ MADS AFFECTING FLOWERING 1 (FLM/MAF1: At1g77080), MAF2-5 (MAF2:At5g65050, MAF3: At5g65060, MAF4: At5g65070, MAF5: At5g65080), and another MADS-box protein SHORT VEGETATIVE PHASE (SVP: At2g22540) are floral repressors which bind to CArG motifs in the *FT* and *SOC1* promoters and repress transcription (Michaels and Amasino, 1999; Scortecci et al., 2003; Helliwell et al., 2006). As MADS box DNA binding TFs form homo or heterodimeric complexes, posttranslational regulation through the formation of different complexes has emerged as an important thermosensory mechanism (Song et al., 2013). Interestingly, *FLM* and *MAF2* transcripts are alternatively spliced in response to temperature (Lee et al., 2013; Pose et al., 2013; Rosloski et al., 2013). Two splice variants are produced FLM-ß and FLM-δ under low and high temperatures, respectively (Figure 3). Under low ambient temperature conditions (16 °C), the FLM- ß splice variant and SVP form a functional complex that can inhibit *FT* and *SOC1* expression (Lee et al., 2013; Pose et al., 2013). Under high ambient temperatures above 22 °C degrees, FLM-δ and SVP complexes have impaired DNA binding potential and are not able to repress *FT* and *SOC1* transcription, leading to an increase in expression and an acceleration of flowering under higher temperatures (Lee et al., 2013; Pose et al., 2013). In terms of the daily timing of this regulation, though a comprehensive survey has not been performed, an increase in *FT* transcription at only ZT16 in *flm maf3* double mutants suggests that their primary effect on *FT* expression occurs in the afternoon (Gu et al., 2013). Regulation by these complexes is likely also developmentally specific, as complex formation between SVP and FLC occurs earlier in development (7 day old vs. 11 day old) (Li et al., 2008). This reinforces the idea that these MADS-box proteins are functioning as a modulator to try to stop the floral transition from happening too early in development, but to allow for flowering if thermal conditions are optimal.

How does low ambient temperature induction of MADS domain transcription factors occur? At least at *FLC*, the autonomous pathway protein FVE (At2g19520) (a retinoblastoma related protein) is able to physically associate with the histone deacetylase complex component HISTONE DEACETYLASE 6 (HDA6: At5g63110) and confer repressive activity upon the *FLC* chromatin (Ausin et al., 2004; Kim et al., 2004; Jeon and Kim, 2011; Pazhouhandeh et al., 2011). Thus, *fve* mutants are late flowering due to the increase in *FLC* transcription. In response to lower temperatures HOS1 is able to form a protein complex with FVE and HDA6 and antagonize their repressive effect at the *FLC* genomic locus, causing an increase in *FLC* transcription and later flowering (Gu et al., 2011). Correspondingly, *hos1* mutants show a decrease in *FLC* transcription, which increases *FT*. HOS1 represents a dual factor in inhibiting flowering under lower temperatures both through increasing *FLC* transcription and through CO protein degradation (Lazaro et al., 2012; Lee et al., 2012). HOS1 also has a role in the regulation of cold responses, so its position in the network may be closest to cold input independent of the vernalization dependent sensing of cold temperatures (Figure 3).

In addition to its regulation of *FT* and *SOC1*, SVP binds to the promoters of several upstream components in multiple flowering pathways. Among the photoperiod pathway, SVP binds to the *GI* and *PRR7* promoters (Gregis et al., 2013). SVP also binds to several pathway components in the autonomous pathway and in members of the polycomb repressive complex 2 (PRC2), which suggests that SVP confers regulatory adjustments in response to temperature at multiple intersecting points in the flowering time pathways (Gregis et al., 2013). SVP also positively regulates the expression of the EARLY FLOWERING MYB PROTEIN (EFM: At2g03500) transcription factor, which is able to form a protein complex with JUMANJI 30 (JMJ30: At3g20810) to coordinate demethylation of H3K36me2 marks, the net result of which is a repression of *FT* transcription (Yan et al., 2014). SVP regulation in these instances may be context dependent; SVP negatively regulates the ability of *GI* to accelerate flowering in response to drought through an ABA-mediated response (Riboni et al., 2013). Examples such as this suggest that SVP or other regulators may preferentially regulate flowering at different points in the pathway under different environmental conditions, which implies that untested environmental parameters that shape flowering time may change the flux of the flowering network at different points (through both upstream and downstream effects). Further work needs to be done to further clarify SVP's role in this regard.

Under high ambient temperature conditions, another important regulator of the acceleration of flowering is the bHLH transcription factor PHYTOCHROME INTERACTING FACTOR 4 (PIF4: At2g43010) (Kumar et al., 2012) (Figure 3). Under short day conditions, ambient temperature increase to 27 °C causes early flowering. In *pif4* mutants, flowering under 22 °C and 27 °C conditions occurs at the same time, as the plants are unable to respond to higher temperatures (Kumar et al., 2012). PIF4 directly binds to the *FT* promoter to activate transcription, but its binding site accessibility is strongly inhibited in the presence of the nucleosomes containing the H2A.Z histone variant (Kumar et al., 2012) (Figure 4). H2A.Z deposition is also critical in mediating *FLC* repression, so the addition of H2A.Z histone variants is capable of regulating *FT* in through direct (*FT* through PIF4) and indirect means (*FT* though FLC) (Kumar et al., 2012). As *PIF4* expression is also regulated by the GA hormone pathway, PIF4 may serve as an important integrator between temperature dependent PHYB mediated responses as well as the developmental competency of the plants to flower (Nomoto et al., 2012b; Nomoto et al., 2012a).

Ambient temperature regulation through the circadian clock is another input point that may affect the floral transition. In particular *early flowering 3 (elf3*: At2g25930*)* mutants are insensitive to the delay of flowering times that takes place at 16 °C degrees (Strasser et al., 2009). ELF3, along with EARLY FLOWERING 4 (ELF4: At2g40080) and LUX ARRYTHMO (LUX: At3g46640), forms the "evening complex" (EC), a protein complex that regulates the expression of *PIF4* and *PIF5* to constrain the hypocotyl elongation response to the early evening (Nusinow et al., 2011). This circadian gating mechanism additionally serves to regulate *PIF4* expression in response to changes in ambient temperature (Figure 3) (Mizuno et al., 2014). The EC is normally able to repress *PIF4* expression in lower ambient temperatures, but transcription of *PIF4* increases as EC activity is attenuated under higher temperature conditions (Mizuno et al., 2014). ELF3 also acts as a repressor of *PRR9* expression, and thus could act in a temperature dependent manner by increasing CDF expression to repress flowering under low ambient temperature conditions (Dixon et al., 2011). Additionally, modulation of the core clock by temperature may also affect the flowering time response. CASEIN KINASE II BETA CHAIN 4 (CKB4: At2g44680) is involved in temperature dependent modulation of the circadian clock period (the process referred to as tem-

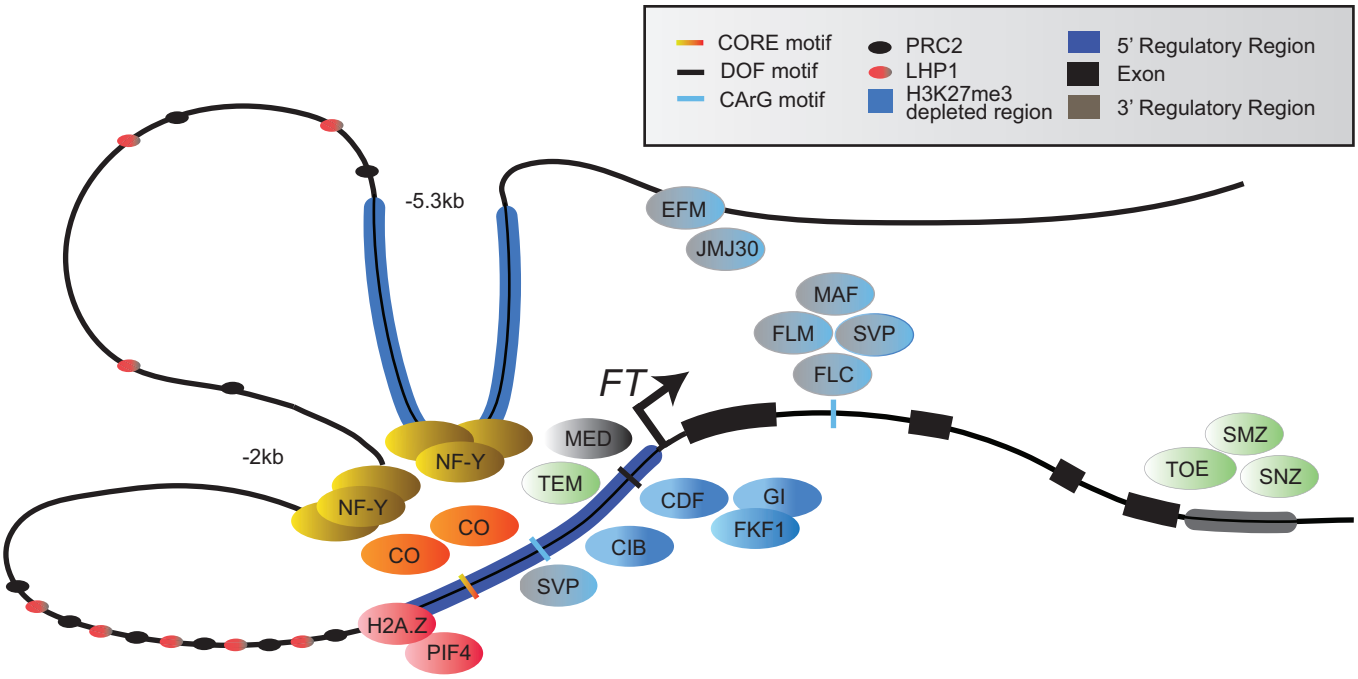

Figure 4. Transcriptional regulation of the *FT* gene.

The proximal 5' region of the *FT* promoter is the binding site for many transcription factors which can repress or activate *FT* in response to external parameters. CDFs and TEMs are able to repress *FT* expression and are regulated by the circadian clock. CIB activators are able to induce *FT* under blue light enriched conditions. SVP is able to repress *FT* expression under low temperature, and PIF4 increases it under high temperature. This regulatory region however, is normally not accessible to transcription factors through the activity of LHP1, which is enriched in this region, and PRC2, which is able to trimethylate lysine 27 residues on histones in this region. Only the -5.3kb CCAAT box motif is free from the actions of LHP1-PRC2. Once NF-Y factors are able to bind to the upstream CCAAT box sites and recruit CO to the *FT* locus, CO activity is able to remove the LHP1 presence in the 5' proximal region, which enables the activity of other *FT* regulators. EFM and JMJ30 form a complex that regulates *FT* through demethylation of histones in the *FT* locus. MADS domain factors FLC, SVP, FLM, and MAFs, are able to bind to the first *FT* intron to repression transcription, both in response to low ambient temperature as well as prior to vernalization. AP2 repressors TOE1,2, SMZ and SNZ are able to bind to the 3' regulatory region near the *FT* 3' UTR to regulate *FT* expression.

perature compensation), and preferentially phosphorylates CCA1 at 27 °C than at 16 °C (Portolés et al., 2010). Overexpression of two regulatory β-subunits, CKB3 (At3g60250) and CKB4, of *CK2* shortened the period length of clock and induced earlier flowering in both long days and short days (Sugano et al., 1999; Portolés et al., 2010). This temperature dependent circadian effect on flowering could be through multiple points of clock output, and more testing will be needed to tease apart clock dependent effects on ambient temperature signaling.

Endogenous factors affecting *FT* expression

In addition to exogenous, environmental inputs in the modulation of flowering time through *FT* transcription, several endogenous cues are also important in determination of the floral transition, to sense the developmental competency and resources of the plant to be able to fully commit to flowering. This explains why flowering occurring directly subsequent to photomorphogenesis is a rare phenomenon.

microRNA regulation of flowering and plant age

A separate pathway that is primarily regulated through the actions of microRNAs (miRNAs) brings upon sensing of juvenility and its influence on *FT* and other floral integrator expression (Figure 3). Concomitant with GI's position within many parts of the flowering pathway, GI positively regulates the expression of a miRNA *miR172* under long day conditions, while SVP negatively regulates its expression (Jung et al., 2007; Cho et al., 2012). *miR172* reduces the transcript abundance of the AP2/ERF family regulators *SCHAFLMÜTZE* (*SMZ*: At3g54990), *SCHNARCHZAPFEN* (*SNZ*: At2g39250), *TARGET OF EAT 1* (*TOE1*: At2g28550), and *TOE2* (At5g60120), which are upregulated in juvenility to prevent the floral transition from moving onward too quickly (Aukerman and Sakai, 2003; Schmid et al., 2003; Mathieu et al., 2009). These AP2 family transcription factors bind to the 3' region of the *FT* gene in order to repress transcription (Mathieu et al., 2009) (Figure 4). *miR156* has an opposite role in the flowering time response. *miR156* targets members of the *SPL* family of transcription factors *SPL3* (At2g33810) and *SPL9* (At2g42200),

which positively regulate the expression of other floral integrator genes such as *SOC1*, *AGL24, FUL*, *AP1* and *LFY* at the shoot apex (Wang et al., 2009; Jung et al., 2011; Yu et al., 2012; Wang et al., 2014). *miR172* levels, unlike *miR156,* are regulated by SPL9 and SPL10, the effect of which modifies *TOE1* and *TOE2*. This has interesting implications for the opposite roles of both miRNAs, but since TOE1 and TOE2 repress *FT* in leaves, there is a discrepancy between the *miR156* and *miR172* dependent regulation of flowering in the shoot apex versus the leaf which has not been investigated in detail yet (Wu et al., 2009). *miR156* transcripts decrease in abundance as developmental time progresses, opposite that of *miR172* (Wu et al., 2009). How the development-dependent expression of *miR156* is regulated is currently unknown, but several lines of evidence suggest it may be linked to photosynthetic status, as *miRNA156* transcription is decreased when plants are grown in the presence of several different sugars applied through the media (Yang et al., 2013). The combined action of these two miRNA dependent pathways allows for the increase of expression of *FT* in leaves, and increase in other floral integrators at the shoot apex as plants go through development, while acting as a stopgap to prevent flowering in the absence of resources required to form floral organs and an inflorescence. Interestingly, the action of this pathway appears critical in several species for the maintenance of longer juvenile periods in perennials, and adds an additional layer through which modulation of flowering can occur within optimal seasonal conditions (Bergonzi and Albani, 2011).

PHOTOSYNTHETIC STATUS

In addition to developmental age, carbohydrate status has long been implicated in the flowering time response (Evans, 1971). Though this area has remained largely unexplored, recent evidence has implicated photosynthetic output as an effect on flowering time (Wahl et al., 2013). Changes in light intensity or the application of DCMU [3-(3,4-dichlorophenyl)-1,1-dimethylurea] (a chemical inhibitor of photosynthesis) affects both *FT* transcription as well as the pace of the circadian clock (Corbesier et al., 1998; Corbesier et al., 2002; King et al., 2008; Haydon et al., 2013). Mutations in the gene that encodes the biosynthetic enzyme TREHALOSE-6-PHOSPHATE SYNTHASE 1 (TPS1: At1g78580), which produces Trehalose 6-phosphate (T6P), a photosynthetic byproduct, have greatly impaired *FT* expression (Wahl et al., 2013). Although the mechanism for *FT* activation by T6P remains unknown, it appears that its activator activity may act in a photoperiod dependent manner to sense optimal carbohydrate status in long days prior to flowering (Wahl et al., 2013) Presumably, the signal for T6P must be transduced or translocated from mesophyll cells into the phloem companion cells, where *FT* transcription occurs, but how this occurs is also unknown. In addition to *FT* transcriptional changes, transcription of *SPL* family genes is reduced in the *tps1* mutant background, partially due to an increase in *miR156* levels early in development (Wahl et al., 2013). While it has been not been demonstrated experimentally, T6P levels, sugar, and *miRNA156* transcription are inversely correlated; at least at the shoot apex this probably explains the photosynthetic link to flowering through *SPLs.* What factors connect the two pathways or what differentiates the pathways in the leaves or the shoot apex, however, remains to be investigated. This new information implies that photosynthetic status may act at several levels of the flowering response to provide endogenous input as to whether or not to commit to flowering. Though it has not been determined in detail, it would be interesting to see if new leaves acting as carbon sink in relation to mature leaves are able to contribute to *FT* production equally compared to each other, as this has implications for growth effects on the competency to flower.

FT CHROMATIN STRUCTURE

Due to the large array of factors that serve as inputs into *FT* expression, an intriguing question has been how all of these diverse factors interact and recruit or repel basal transcriptional machinery within the space of the *FT* promoter region. While the pace of the field is quickly speeding up, we are currently limited in scope with the ability to fully answer these questions. Still we have gained several clues into the structural constraints on *FT* transcription. Firstly, we know that components of the Polycomb repressive complex 2 (PRC2), whose role in floral regulation has already been well characterized in the vernalization response, are involved in *FT* transcriptional regulation (Turck et al., 2007; Farrona et al., 2011). Mutations in several components, including the SET domain protein CURLY LEAF (CLF: At2g23380), EMBRYONIC FLOWER 2 (EMF2: At5g51230),) and FERTILIZATION INDEPENDENT ENDOSPERM 1 (FIE1: At3g20740), as well as the PRC1 component LIKE HETEROCHROMATIN PROTEIN 1/ TERMINAL FLOWER 2 (LPH1/TFL2: At5g17690) result in a pronounced decrease in the accumulation of the repressive histone mark H3K27me3 throughout the *FT* promoter region, and several of these components physically associate with *FT* chromatin in ChIP assays (Takada and Goto, 2003; Saleh et al., 2008; Adrian et al., 2010; Farrona et al., 2011; Shafiq et al., 2014). Corresponding to their role as repressors of *FT* transcription through interactions with chromatin, many of the mutants of this complex are early flowering. Loss of function mutations in *clf* in particular are coupled with an enormous increase in *FT* transcription (Shafiq et al., 2014). This evidence, coupled with the fact that the required minimal promoter fragment to replicate wild type *pFT:GUS* expression is a large 5.7kb fragment, suggests that stable repression and or maintenance of a section of condensed chromatin around the *FT* locus is important for proper expression (Adrian et al., 2010). ChIP experiments have begun to elucidate changes in histone marks that occur in the *FT* promoter region, but these experiments have been performed on whole plant extracts; future experiments which enrich vascular tissue input may increase the detection of histone mark changes that occur at *FT* in the tissues where it is expressed. Currently, it has been hypothesized that H3K27me3 deposition occurs throughout much of the *FT* promoter, with accessible regions to transcription factors to enable proper *FT* induction (Andrés and Coupland, 2012). There also may be a developmental component to *FT* expression, however, as *pFT:GUS* expression in *lhp1* mutant plants greatly expanded the range of *FT* expression in vascular tissues (Adrian et al., 2010). This evidence suggests that the domain of *FT* expression in the phloem companion cells to the distal part of the leaf may be controlled through

LHP1 (Adrian et al., 2010; Farrona et al., 2011). The functional relevance of the domain of *FT* expression compared to the total amount of *FT*, however, has yet to be looked at in great detail. PRC2 components also appear to be involved in the regulation of several other key flowering regulators, including the floral repressor *SVP*. Antagonistic effects of the SWI/SNF chromatin remodeling complex and its ATPase component subunit BRAHMA (BRM: At2g46020) and the PRC2 components are involved in SVP transcriptional control. BRM, which has previously been shown to be involved in flowering repression, thus largely accomplishes its repressive activity through upregulation of *SVP* (Li et al., 2015). SVP also appears to be partially involved in both direct repression of *FT* transcription as well as indirectly through *FT* chromatin interactions; SVP upregulates the expression of EFM which interacts with the JMJ30 protein to coordinate the demethylation of H3K36me2 to repress *FT* transcription (Yan et al., 2014).

In addition to the repressive machinery which affects *FT* chromatin status, components of the mediator complex, which acts in transcription initiation, are important for control of flowering time (Figure 4). PHYTOCHROME AND FLOWERING TIME 1 (PFT1: At1g25540), the MEDIATOR 25 (MED25) subunit of the mediator complex, is critical for transcription of several photoperiodic flowering components and acts downstream of phytochrome photoreceptors (Elfving et al., 2011; Inigo et al., 2012b). Genetic evidence suggests that PFT1 serves an activating role both in *CO* transcription as well as *FT* transcription, through independent mechanisms (Inigo et al., 2012b). At least at *FT*, PFT1 is degraded by the proteasome through the action of two interacting proteins MED25-BINDING RING-H2 PROTEIN1 (MBR1: At2g15530) and MRB2 (At4g34040) (Inigo et al., 2012a). Degradation of PFT1 is important for activation of *FT*, as inhibition of PFT1 degradation prevents *FT* activation. MED18 (At2g22370) as well as the synergistic action of CRYPTIC PRECOCIOUS (CRP) / MED12 (At4g00450) and MACCHI-BOU2 (MAB2) / MED13 (At1g55325) are also involved as activators of *FT* expression, although the possibility of a regulatory mechanism similar to MED25 has not been investigated in either case (Imura et al., 2012; Zheng et al., 2013). The tailoring of specific effects of *FT* expression modulated by the Pol II holoenzyme suggests that the basal transcriptional machinery and/or the structure of the activator complexes required for *FT* transcription are important in determining the flowering output. At this point whether or not repressive machinery at the chromatin level inhibits the conformation of the larger *FT* activation complex is still a largely open question, but it will be interesting to try to piece together how the activation machinery might conform around the *FT* genomic locus.

Presently lacking, in addition, is whether or not time dependent (within a day) or photoperiod dependent (long day vs. short day) changes in *FT* locus structure occur. At least in long days, recent work has further elucidated the role of CO activation within the chromatin structure of the *FT* promoter (Figure 4). CO, together with its transcriptional cofactors NF-YA, NF-YB and NF-YC, associates with the *FT* promoter regions that contains 2 CCAAT sequences, one at -5.3kb position and a second at the -2kb position upstream of the *FT* transcriptional start site (Ben-Naim et al., 2006; Adrian et al., 2010). Using chromatin conformational capture experiments to investigate whether loops in chromatin structure form in order to bring CO activator complexes directly to the transcriptional start sites, a double loop in the *FT* chromatin structure forms (Cao et al., 2014). This conformation is analogous to the chromatin organization that can typically be found between transcriptional start sites and distal enhancer elements. Because CO protein is unlikely to be found on the *FT* promoter under short day conditions, this conformation is likely to only take place under inductive long day conditions (Cao et al., 2014). Overexpression of CO also removes LHP1 binding to the 5' proximal region, so CO recruitment may serve as an additional mechanism with which to allow other factors to exert influence over *FT* transcription (Adrian et al., 2010). Natural variation adds another layer of complexity in the *FT* promoter, as the length of the spacer region between the CCAAT box binding sites and the proximal 5' region varies by ecotype (Liu et al., 2014). The spacer region is not required for *FT* spacial expression, as a construct with the fragment missing can recue spacial expression and flowering time, but appears to be important in other climatological variables. Field trials with strains containing different sized spacer regions point to the spacer's potential role in increasing fitness in overwintering conditions, as is also suggested by the fact that populations along latitudinal gradients largely are enriched for longer *FT* fragments at higher latitudes and shorter fragments at lower latitudes (Liu et al., 2014). Additional promoter variation may explain phenotypic diversity under natural conditions that cannot be parsed through under controlled laboratory conditions. Still also unresolved is how other proteins, either activators or repressors, interact within this structural framework either at the transcriptional start site or in the 3' region (SMZ, SNZ, TOE1 and TOE2), and particularly how these change under different environmental parameters.

FT MOVEMENT

FT protein, once synthesized in the phloem companion cells of leaves, acts as a mobile signal that translocates to the shoot apex where downstream interactions are able to specify the transformation of the shoot apical meristem into an inflorescence meristem (Figure 5). As such, FT represents the long sought florigenic signal that links the leaf sensory mechanism with the floral conversion, and is a conserved mechanism for floral initiation throughout the angiosperm lineage (Andrés and Coupland, 2012). FT movement has been difficult to characterize, and indeed considerable effort has been taken to prove that it is indeed FT protein and not *FT* mRNA that constitutes the mobile signal after conflicting early reports (Corbesier et al., 2007; Jaeger and Wigge, 2007; Lin et al., 2007; Mathieu et al., 2007; Tamaki et al., 2007). Although it is controversial, *FT* mRNA might potentially be mobile as well, as it was shown that a segment at the 5' part of the mRNA is critical for its movement (Li et al., 2009). Because of technical issues surrounding the size exclusion limit of tagged FT protein leaving through the plasmodesmata or through active transport into the phloem stream and the difficulty in obtaining enough phloem sap for molecular analysis, some aspects of FT movement have been studied in *Cucurbita moschata* (Lin et al., 2007). Based on these experiments, mutations of *FT* which impair FT movement to the shoot apex do not appear to affect loading into the phloem from the companion cells. Its uptake, however, into the shoot apical meristem appears to require an active transport mechanism, the components of which are unknown. In phloem companion

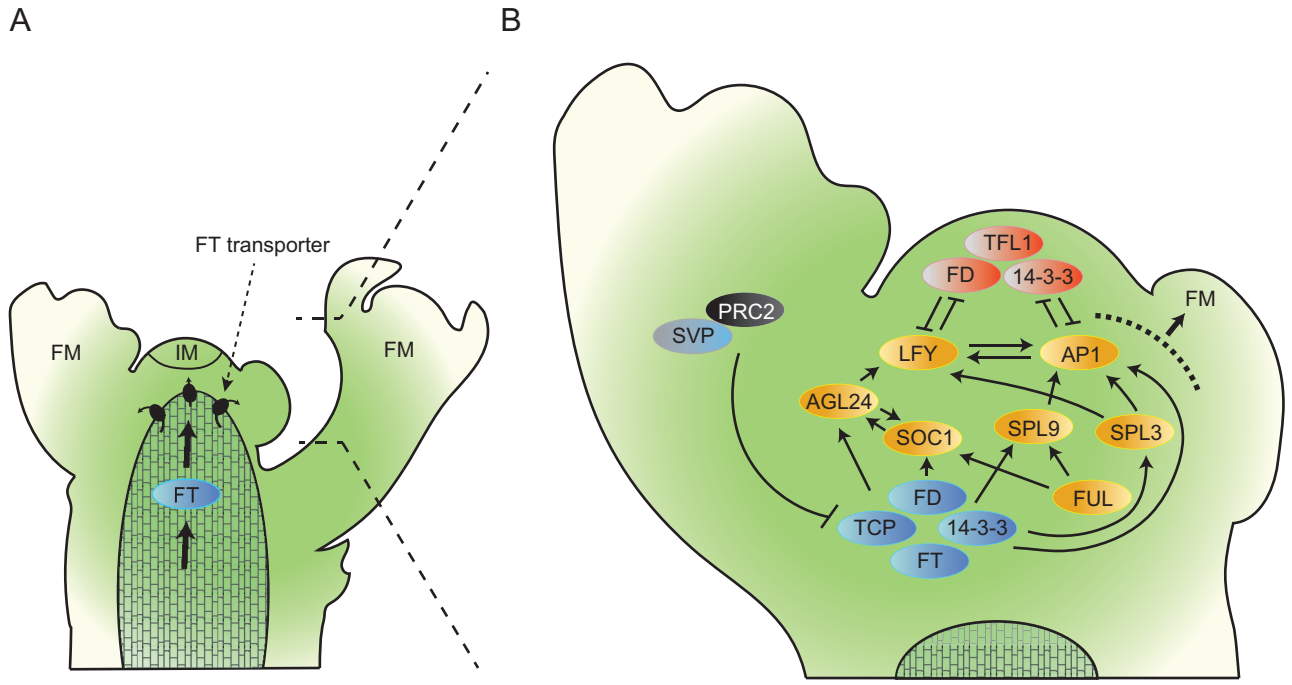

Figure 5. Specification of an inflorescence meristem and floral meristems at the shoot apex.

(A) FT protein, after being synthesized in the leaf phloem companion cells, enters into the phloem stream through plasmodesmata and move to the shoot apex. Upon arrival at the shoot apex, an active transport mechanism uptakes FT protein into the cells surrounding the shoot apical meristem.

(B) FT protein, along with TCP transcription factors, binds to the 14-3-3 adapter protein and to the transcription factor FD. FT competes for interaction with 14-3-3 and FD with TFL, which prevents FD from transcriptional activity. Active FT-14-3-3-FD complex activates LFY and AP1, which feed forward to increase each other's expression. The action of this feed forward loop initiates commitment to inflorescence cell fate. As the meristem continues in development, TFL activity is required to maintain the indeterminacy of the inflorescence, and a gradient is set whereby FT and other floral integrators are able to specify the placement of floral meristems (FM) on the flanks of the inflorescence meristem (IM). FT-14-3-3-FD complex activates expression of the floral integrator genes *SOC1*, *FUL*, and *SPL3*, *SPL4*, and *SPL5*. These, in turn, induce *AGL24* and *SPL9*, resulting in downstream activation of *AP1* and *LFY*. After floral meristem specification, FT is still required to maintain floral meristem identify; repression of *FT* is carried out by SVP and the PRC2 complex. If sufficient repression of *FT* is maintained, floral meristem identity can be converted back into a vegetative cell fate.

cells, FT protein interacts with FT INTERACTING PROTEIN 1 (FTIP1: At5g06850). Mutations in FTIP1 appear to prevent FT loading from the companion cells into the sieve tube elements (Liu et al., 2012). Presumably, multiple parts of the FT protein are required for interaction with a separate set of factors which are involved in FT loading and unloading at the phloem companion and shoot apex, respectively (Liu et al., 2012; Yoo et al., 2013a; Yoo et al., 2013b). Several candidates for involvement in the active transport of FT into the shoot apex have been identified, but the order and operations at the molecular level that is required for FT movement remain unresolved (Liu et al., 2012; Yoo et al., 2013a). Structural analysis of mutagenized protein identified two residues in close proximity near the N-terminal part of the FT protein which appear critical for mediating FT protein import into the shoot apex, as mutations at these sites expressed under the vascular specific *SUCROSE SYMPORTER2* (*SUC2*: At1g22710) promoter have opposite effects of those expressed under TFL1 promoter fragments, suggesting that the mutated protein cannot be moved out of the vasculature and into the shoot apex (Ho and Weigel, 2014).

FT is an phosphatidylethanolamine (PE)-binding protein (PEBP) similar to those found in mammals, and binds to phospholipids as has been anticipated due to its similarity to animal orthologues of the protein (Nakamura et al., 2014). Many phospholipids present within cells at the shoot apex show a diurnal oscillation of phospholipid abundance, several of which correlate with the peak of daily expression of *FT*. Phospholipid binding to residues on parts of the FT protein surface may also be important in the transport of FT into the nucleus of shoot apical cells once it arrives there, possibly through vesicular trafficking (Nakamura et al., 2014). In total, this now suggests that both active transport into the shoot apex as well as subcellular trafficking into the nucleus is an important part of FT protein regulation in the initiation of flowering.

INTERACTIONS AT THE SHOOT APEX

A balance of factors ensures that during juvenile development in Arabidopsis, the shoot apical meristem is committed only towards vegetative production. This vegetative cell fate is specified

through the action of the FT related protein TERMINAL FLOWER 1 (TFL1: At5g03840), which acts antagonistically to FT protein specifying reproductive development (Ruiz-Garcia et al., 1997; Abe et al., 2005; Taoka et al., 2011; Jaeger et al., 2013). When FT protein arrives at the shoot apex, it forms of protein complex with the bZIP transcription factor FD (At4g35900), which is facilitated through docking with the arms of the 14-3-3/GENERAL REGULATORY FACTOR 3 (GRF3: At5g38480) adapter protein at least in rice and yeast (Abe et al., 2005; Taoka et al., 2011). FD-FT-14-3-3 complex formation in Arabidopsis remains to be studied in detail, but presumably the same mechanisms apply. This FT-FD-14-3-3 activator complex then interacts with the promoters of *LFY* and *AP1* whose expression locks the shoot apical meristem into an inflorescence cell fate (Jaeger et al., 2013) (Figure 5).

TFL1 and FT proteins are highly conserved throughout flowering plant evolution and still retain very closely related protein structure. In contrast to *FT*, *TFL1* is only expressed in flanks of the shoot apex and young axillary meristems, where the protein then moves to the center of the inflorescence meristem (Bradley et al., 1997; Conti and Bradley, 2007). Because of their similar structure, the mechanism through which TFL1 acts as an inhibitor of FT mediated floral conversion is as a competitor for FD-14-3-3 docking, which is rendered non-functional upon complex formation with TFL1 (Hanano and Goto, 2011; Ho and Weigel, 2014). Structural analysis through randomized mutagenesis of FT protein found several regions of critical importance for FT function, as well as the conversion of FT protein into one with TFL1-like function in the regulation of flowering time (Hanzawa et al., 2005; Ahn et al.,2006; Ho and Weigel, 2014). It appears that two regions in particular are crucial, one is a potential ligand-binding pocket which is now thought to mediate protein-protein interactions with a members of the TEOSINTE BRANCHED 1/CYCLOIDEA/ PCF transcription factor family. In particular the residues that control the surface charge of the area around the outside of the ligand-binding pocket appear important for function (Ho and Weigel, 2014). Mutations at these sites specifically affected the binding to a subset of TCP transcription factors, as FD and 14-3-3 *in vivo* binding is unaffected by these mutations (Ho and Weigel, 2014). In addition, mutations in an area outside of the ligand-binding pocket on FT, in an external loop of an adjacent alpha helix, are also sufficient to convert FT into a TFL1-like protein. This effect is likely due to interference of these new residues that are normally required for additional protein-protein interactions (Ho and Weigel, 2014).

Another important series of interactions that occurs at the shoot apex is the balance between FT, TFL1, and LFY and AP1 transcription factors. FT and FD together activate the expression of *AP1* and *LFY*, and also LFY and AP1 positively regulate the expression of each other (Liljegren et al., 1999) (Figure 5). LFY additionally activates *FD* expression in the shoot apex. The TFL1 and FD complex acts antagonistically to all of these interactions by repressing the expression of *LFY* and *AP1* (Figure 5). Together, these floral activators constitute a positive feed back loop that reinforces commitment to floral organ specification. Modeling of these complex interactions at the shoot apex has determined that accumulation of FT via transport from the leaves is a key initialization that enables this highly buffered positive feed back loop to push the network into a net floral committed one through reinforcing expression of *AP1* and *LFY* (Jaeger et al., 2013). Because the inflorescence of Arabidopsis is indeterminate, the inflorescence meristem must maintain some vegetative cell-like properties and for this purpose, *TFL1* expression in the center of the inflorescence meristem is still required (Andrés and Coupland, 2012). On the flanks of the new meristem, the effort of the AP1-LFY feed forward loop is able to initiate the formation of floral primordia from which sexual organs will form, thus patterning the new floral tissues of the inflorescence (Figure 5).

In addition to the FT-TFL-AP1-LFY module, SPL3, SPL4 (At1g53160), and SPL5 (At3g15270) act as activators of flowering at the shoot apex. *SPL*s expression is induced by photoperiod, and they are negatively regulated through the actions of *miR156* (Wang et al., 2009; Wu et al., 2009). In addition to age dependent regulation by *miR156*, the net effect of which is increasing *SPL* expression with plant age, *SPL*s are negatively regulated by the DELLA proteins REPRESSOR OF GA1-3 (RGA: At2g01570), GIBBERELLIC ACID INSENSITIVE (GAI: At1g14920), RGA-LIKE 1 (RGL1: At1g66350), and RGL2 (At3g03450) (Galvao et al., 2012; Yu et al., 2012). This convergence point serves to integrate age and GA pathways to regulate the floral transition. In addition to regulation of inflorescence meristem (IM) specification, SPLs and GA also play a role in the IM to floral meristem (FM) transition, as mutations in either pathway show aberrations in the development of the IM during bolting (Yamaguchi et al., 2014). Feedback between these pathways, both the floral specification genes of AP1, LFY, SPLs, and their downstream targets integrate all of the factors including photoperiod, age, GA, light quality, and temperature to bring about the formation of the inflorescence.

MAINTENANCE OF INFLORESCENCE ORGAN IDENTITY?

Even after the inflorescence has become specified, additional inputs from flowering pathway components are required to prevent the reversion of the inflorescence back into a vegetative structure. Mutations in members of the PRC2 complex *CLF, EMF2,* and *SWINGER* (*SWN*: At4g02020) result in an increase in *FLC* expression in the inflorescence (Muller-Xing et al., 2014). This increase in *FLC* expression occurs concomitantly with a rise in *SVP* expression, the complex of which facilitates repression of *FT* in newly formed tissues in the inflorescence. The loss of *FT* in these tissues appears to be enough to prevent the continued production of floral organ identity and reverts the inflorescence meristem back into a vegetative state (Muller-Xing et al., 2014). *ft* mutants, however, do not show aberrations in inflorescence development, suggesting that compensation from other pathways or shared PRC2 targets required for this phenotype also contribute. Interestingly, floral reversion occurs in a variety of other plant species after floral development proceeds (Figure 6); it is possible that similar mechanisms to those found in Arabidopsis are responsible for the capacity to undergo reversion from an inflorescence back into a vegetative state in a developmentally programmed manner.

Many of the aforementioned regulators of *FT* expression have effects on inflorescence architecture, branching, and phyllotaxy, and relatively few of them have been characterized in detail. For example TCP transcription factors often have branching phenotypes in Arabidopsis. BRANCHED 1 (BRC1: At3g18550) for instance is involved in the suppression of axillary meristems at the shoot apex, through interactions with FT that suppress the

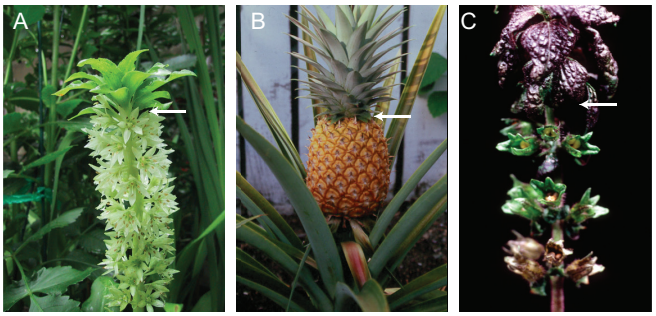

Figure 6. Reversion of the inflorescence meristem to a vegetative meristem in flowering plants.

Once inflorescence meristematic identity is specified, plants are usually committed irreversibly to the flowering response. **(A)** *Eucomis autumnalis* **(B)** *Ananas comosus* and **(C)** *Perilla fructescens* illustrate an inflorescence meristem with the capability to revert into a vegetative meristem after floral specification is complete. White arrows indicate the point at which the inflorescence reverts to vegetative identity.

ability of FT to promote axillary meristem formation (Niwa et al., 2013). Because FT and TCP transcription factors interact at the shoot apex in inflorescence meristem specification, their interaction may have important consequences for phyllotaxy of the new inflorescence (Ho and Weigel, 2014). *CO* was characterized as being a major mapped QTL for branching phenotype in Arabidopsis (Ungerer et al., 2002; Ho and Weigel, 2014). Likely many of the factors involved in specifying flowering time in the flower transition may behave in a similar manner to regulate the production of reproductive organs even after the floral transition has already been specified.

PERSPECTIVE

The regulatory network that controls the floral transition in Arabidopsis has expanded greatly over the past decade, and indeed the connectedness between what were very discrete pathways has made the genetic analysis of different components difficult to interpret. While this has led to a wealth of very sophisticated analyses, we are still limited phenotypically in determining the role of individual factors in flowering time regulation (Andrés and Coupland, 2012; Kinmonth-Schultz et al., 2013). In addition to the complexity of the network, the number of environmental and endogenous inputs that can modify the flowering output should be a constant reminder that consistency in environmental conditions must be a continual goal, as the interpretation of relatively subtle phenotypes can be confounded by numerous environmental factors that relate to ambient temperature fluctuations, light quality, biotic and abiotic stresses, and others. In addition to the role of environmental effects on the floral transition, developmental considerations also appear to be at play in many areas. Once the floral transition is initiated, many recent studies point the idea that inputs such as photoperiod are still important in mediating inflorescence architecture, maintenance of the inflorescence cell identity, and the propagation of the floral signal (Smith et al., 2010; Jaeger et al., 2013; Muller-Xing et al., 2014). This suggests that the same components also could be interacting with new partners in different tissues or under different environmental parameters.

While we are beginning to understand how different flowering regulatory factors change their activity or override other parts of the pathway under specific environmental conditions, the upstream control of these well characterized flowering components by environmental signals is relatively unknown. For instance, if temperature dependent splicing is an important factor in determining the abundance of active proteins or protein complexes, how is this splicing guided in response to temperature? What factors are responsible for their function? Likewise for factors in which temperature affects protein activity, such as the HOS1-FVE module, are the temperature based effects intrinsic properties of the proteins themselves or are other unknown cold-regulatory machinery required for their function?

Many factors have been identified which can modulate the transcription of floral integrator genes, but how these factors interact within the structure of each genomic locus has not yet been determined in great detail. In particular, we know that many factors are required to facilitate the proper *FT* expression pattern both in terms of tissues specificity as well as in a temporal fashion. How do these factors work for and against each other at the spatial level? Because of the large size of the *FT* promoter, and the implication that chromatin remodeling is critical for *FT* expression, studying the effects of modified T-DNA *FT* promoter fragments may lose some of the contextual information that may be conferred upon the original genomic locus. The advent and streamlining of genomic editing techniques such as CRISPR/CAS9 may make studying changes in transcription factor binding sites or important structural pieces of the DNA more feasible. In addition to studying these changes, additional insight into natural variation in the promoter sequences of *FT* or other floral integrator genes may shed light on differences in flowering time output that do not directly correlate with SNPs in coding regions of flowering time regulatory factors (Schwartz et al., 2009).

A next major step in the study of the photoperiod pathway and the flowering pathways in general will be determination of the signaling outputs as a system, the robustness of the network and the changes that happen in connections and strength of interactions under changes in environment and photoperiod. Modeling of parts of the pathway has already been completed for *CO* expression and for the FT-TFL-AP1-LFY regulatory pathway at the shoot apex. While these types of studies lose analytical power when many factors are included, more comprehensive analyses which seek to understand network dynamics under temperature or light quality fluctuations may shed additional light on to the phenotypes and genetic analyses which have already been performed. This knowledge will be critical to the pursuit of modification of flowering time in crop species, and in particular in trying to design ways in which to allow plants to compensate to changes in temperature and precipitation in agronomically important ways. While this goal is currently a very distant one, manipulation of flowering time is likely one that will greatly contribute to crop yields through tailoring of cultivars to specific climates or to changes in climate we anticipate will occur.

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