Minireview

The Effect of Fluctuations in Photoperiod and Ambient Temperature on the Timing of Flowering: Time to Move on Natural Environmental Conditions

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Plants have become physiologically adapted to a seasonally shifting environment by evolving many sensory mechanisms. Seasonal flowering is a good example of adaptation to local environmental demands and is crucial for maximizing reproductive fitness. Photoperiod and temperature are major environmental stimuli that control flowering through expression of a floral inducer, FLOWERING LOCUS T (FT) protein. Recent discoveries made using the model plant *Arabidopsis thaliana* have shown that the functions of photoreceptors are essential for the timing of *FT* gene induction, via modulation of the transcriptional activator CONSTANS (CO) at transcriptional and post-translational levels in response to seasonal variations. The activation of *FT* transcription by the fine-tuned CO protein enables plants to switch from vegetative growth to flowering under inductive environmental conditions. The present review briefly summarizes our current understanding of the molecular mechanisms by which the information of environmental stimuli is sensed and transduced to trigger *FT* induction in leaves.

INTRODUCTION

Day length (= photoperiod) and ambient temperature undergo continual daily and seasonal changes. These fluctuations cause repeated environmental perturbation from year to year. Plants have developed sensory mechanisms to synchronize many biological events with their surroundings. In leaves, differences in photoperiod and temperature are perceived and used for the generation of a floral cue that is transmitted into the shoot apical meristem, where floral organs form (Song et al., 2013). Flowering time is coordinated with external environmental circumstances, including seasonal availability of animal pollinators, in order to maximize seed production (Hegland et al., 2009). Photoperiod and temperature are the major environmen-

tal factors that determine the timing of flowering (Andres and Coupland, 2012; Kim et al., 2009; Song et al., 2015). In crop plants, flowering during an appropriate season is a crucial aspect for food production. The molecular mechanisms underlying the influences of changes in photoperiod and temperature on flowering have been best characterized in the model plant *Arabidopsis thaliana*. Recent advances have revealed numerous regulatory components involved in the induction of a transmittable flowering signal in response to changes in photoperiod and temperature (Andres and Coupland, 2012; Song et al., 2013; 2015). Mechanistic regulation of the signal induction by photoperiod is relatively well defined (Andres and Coupland, 2012; Song et al., 2015). However, as most laboratories grow plants under constant temperature conditions, the effect of temperature fluctuations on flowering regulation is often underestimated or ignored.

THE REGULATION OF FLOWERING TIME BY PHOTOPERIOD AND TEMPERATURE IN *ARABIDOPSIS*

Because changes in day length occur in a predictable manner, plants mainly use photoperiodic information in order to anticipate upcoming seasonal variations (Thomas and Vince-Prue, 1996). *Arabidopsis* is a facultative long day plant that flowers early in long photoperiods compared to short photoperiods (Kobayashi and Weigel, 2007). In this phenomenon, the induction of *FT* expression plays the key role. Under constant warm temperature ($\approx 22°C$) conditions, the abundance of *FT* transcripts is extremely low in short days (i.e., 8 h of light and 16 hours of dark), whereas it highly accumulates in long days (i.e., 16 h of light and 8 h of dark). The high abundance of *FT* transcripts subsequently accelerates the timing of flowering during inductive photoperiods (Suárez-López et al., 2001). This day length-dependent regulation of *FT* gene expression largely relies on the function of the CONSTANS (CO) protein, which acts as a transcriptional activator of the *FT* gene (Samach et al., 2000). The circadian clock regulation of *CO* transcription and the multiple effects of various photoreceptors on CO protein stability changes are crucial for the accumulation of CO protein under favorable conditions (Lazaro et al., 2015; Song et al., 2012b; 2015; Suárez-López et al., 2001; Valverde et al., 2004). Not only photoperiod but also ambient temperature changes largely contribute to *FT* transcription via the action of a large number of floral regulators, most of which are repressors (Andres and Coupland, 2012; Song et al., 2013). The amount of

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FT transcripts is reduced at low temperature but increased at high temperature even in short days (Fernandez et al., 2016; Kumar et al., 2012). This temperature-dependent *FT* expression can override the effect of photoperiod on the timing of flowering, preferentially in short days (Fernandez et al., 2016; Kumar et al., 2012). The present review mainly focuses on how the photoperiodic information is integrated into the induction of *FT* expression through the spatiotemporal manipulation of *CO* transcription and its protein abundance, and briefly discusses the possible effects of interactions between photoperiod and temperature on CO-mediated *FT* expression in *Arabidopsis*. Although a considerable number of floral regulators are involved in the processes described, not all will be considered.

RHYTHMIC EXPRESSION PATTERNS OF THE *CO* GENE BY DAY LENGTH

The interaction between the circadian clock and light specifies photoperiod information (Golembeski et al., 2014). Expression of the *CO* gene is controlled by the circadian clock that results in oscillation of its mRNA abundance throughout a day (Suárez-López et al., 2001). The mRNA abundance peaks at night and troughs in the morning under both short and long day conditions. The levels of *CO* transcripts remain very low during the day in short days; in contrast, these levels are high at the end of the day in long days, when light is still available (Suárez-López et al., 2001). Differences in day length-dependent *CO* transcript profiles during the light period are largely determined by amounts of the FLAVIN-BINDING, KELCH REPEAT, F-BOX 1 (FKF1)–GIGANTEA (GI) complex (Sawa et al., 2007). The formation of the FKF1-GI complex is light dependent and is important for relieving the repression of *CO* transcription mediated by the CYCLING DOF FACTOR (CDF) family of DOF domain transcription factors (Fornara et al., 2009; Sawa et al., 2007) (Fig. 1).

The CDF family members (CDF1-CDF5) function as floral repressors (Fornara et al., 2009; Imaizumi et al., 2005). Among these, the expression of *CDF1*, *CDF2*, *CDF3,* and *CDF5* genes, driven by the circadian clock, shows a similar diurnal profile, with high transcript levels during the late night and morning. The morning-enriched expression of these repressors strongly represses *CO* transcription in the morning via their association with the *CO* locus (Fornara et al., 2009; Sawa et al., 2007). The transcriptional repressor activity of the individual gene products is largely redundant and additive in the expression of *CO* (Fornara et al., 2009). Quadruple mutations in the *CDF1*, *CDF2*, *CDF3*, and *CDF5* genes lead to high accumulation of *CO* transcripts in the morning as well as the rest of day, regardless of photoperiod, which makes *Arabidopsis* insensitive to changes in day length (Fornara et al., 2009). Hence, the *cdf1 cdf2 cdf3 cdf5* quadruple mutant always flowers early under both long and short day conditions. Because the timing of changes in CDF abundance is a crucial aspect for day length sensing, daily fluctuations of their gene expressions are tightly regulated by multiple core clock proteins (Huang et al., 2012; Nakamichi et al., 2007; 2010). In the morning, *CDF* expression is probably induced by two morning components CIRCADIAN CLOCK ASSOCIATED 1 (CCA1) and LATE ELONGATED HYPOCOTYL (LHY) that are related Myb transcription factors (Nakamichi et al., 2007; Schaffer et al., 1998; Wang and Tobin, 1998). Conversely, the expression appears to be inhibited by the action of PSEUDO-RESPONSE REGULATOR (PRR) transcriptional repressor proteins, PRR5, PRR7, and PRR9, in the afternoon (Nakamichi et al., 2007). In addition to the transcriptional repression by
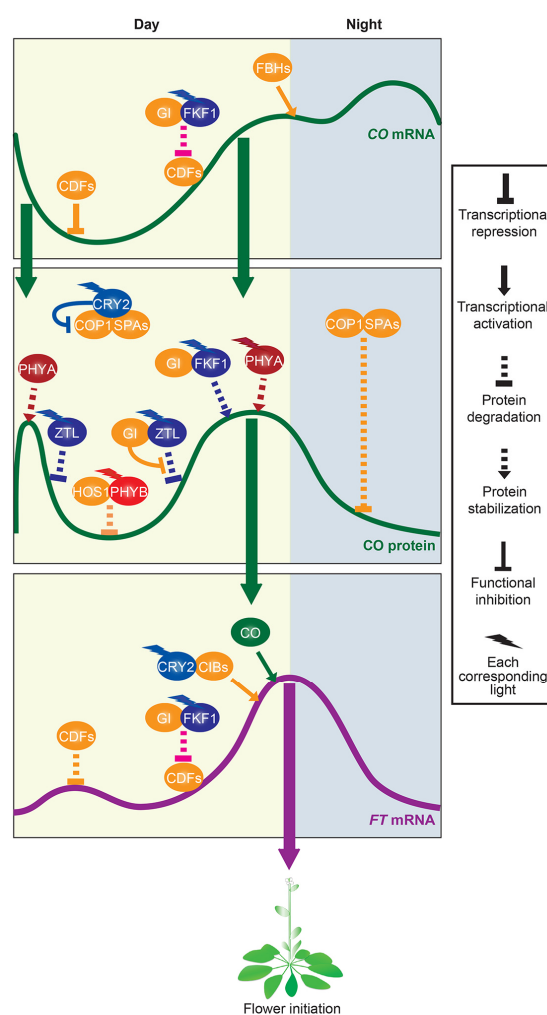

Fig. 1. Photoperiodic flowering regulation by the induction of *FT* expression under long-day conditions. In *Arabidopsis*, a high abundance of CDF proteins accumulates and represses the expression *CO* and *FT* genes simultaneously by binding to promoter regions of these genes in the morning. FKF1 protein, the expression of which coincides with that of GI in the afternoon, absorbs blue light and forms a protein complex with GI. The protein complex stimulates the degradation of CDF proteins on *CO* and *FT* promoters. Removal of CDF repression enables FBH transcription factors to gain access to the *CO* promoter. FBH proteins activate *CO* transcription throughout the rest of day. CO protein is post-translationally modulated by light quality. The protein is degraded by the COP1–SPAs complex in the dark, but is sequestered from the complex by the inhibitory function of blue light-activated CRY2. In the morning, CO is stabilized by the far-red light photoreceptor PHYA and immediately destabilized by the blue light photoreceptor ZTL and the formation of a protein complex between HOS1 and red light-absorbed PHYB. In the afternoon, CO accumulates as a consequence of the activities of FKF1, GI, and PHYA. Together with GI, blue light activation of FKF1 increases CO stability. Thus, GI indirectly regulates CO stability via the inhibition of ZTL function. In turn, *FT* expression is induced by CO and CIBs. CO binds to the *FT* promoter directly as well as indirectly with other transcription factors. In addition, CIB proteins interact with blue light-activated CRY2 and directly bind to the *FT* promoter. These CO and CIBs activate *FT* transcription at dusk, leading to the promotion of reproductive transition in long days.

PRR proteins, post-translational regulation is crucial for the day length-dependent rhythmic expression of the *CO* gene (Imaizumi et al., 2005).

The CDF proteins are targeted for degradation by the role of the E3 ubiquitin ligase FKF1 protein that contains three functional domains, LOV (light, oxygen, or voltage), F-box, and KELCH repeats (Imaizumi et al., 2005; Song et al., 2013). The LOV domain is responsible for absorbing blue light and activating the FKF1 protein. The light-activated FKF1 interacts with GI through the LOV domain in a blue light-dependent manner (Sawa et al., 2007). The expression of *FKF1* and *GI* genes are controlled by the circadian clock, and the abundance of the FKF1-GI complex differs between photoperiods (Fowler et al., 1999; Imaizumi et al., 2003; Sawa et al., 2007). GI protein abundance peaks at the end of the day, whereas FKF1 protein is mainly expressed in the dark in short days. This out-of-phase expression pattern of FKF1 and GI proteins causes a significant reduction in the complex formation between the two proteins (Sawa et al., 2007). No contribution of the FKF1-GI complex to the removal of CDF repression on *CO* transcription results in little CO protein accumulation and subsequently in extremely low *FT* transcript levels under light during short days (Sawa et al., 2007). In contrast, the diurnal protein profiles of FKF1 and GI largely overlap in long days. The peaks of FKF1 and GI abundance coincide in the late afternoon, enabling FKF1 to form a protein complex with GI and, simultaneously, to recognize the proteolytic substrates, the CDF proteins, through physical bindings via the KELCH repeat domain. The FKF1-GI complex then mediates the proteasome-dependent degradation of the CDF proteins through the F-box domain of FKF1, which results in the release of transcriptional repression of the *CO* locus (Sawa et al., 2007) (Fig. 1). This allows the *CO* gene to be expressed under these conditions, leading to the accumulation of CO proteins when daylight remains. Therefore, high *FT* transcription occurs at dusk and promotes flowering in long days (Fig. 1).

The E3 ubiquitin ligase activity of FKF1 in long days restricts CDF repression on *CO* transcription to the morning (Imaizumi et al., 2005; Song et al., 2013). Upon the removal of CDF proteins from the *CO* promoter, transcriptional activators are able to gain access to the promoter. In the activation of *CO* transcription, four basic helix-loop-helix (bHLH) transcription factors, FLOWERING BHLH1 (FBH1), FBH2, FBH3, and FBH4, redundantly induce *CO* expression from afternoon to night (Fig. 1). FBH proteins bind directly to the *CO* locus, and binding activity is higher in the afternoon than in the morning (Ito et al., 2012).

The fine-tuned temporal balance among repressors, activators, and the E3 ubiquitin ligase enables the pattern of *CO* gene expression to be repeated in a day length-dependent manner. Accordingly, plants can utilize the expression information to differentiate photoperiods for flowering time regulation.

MODULATION OF CO PROTEIN STABILITY CHANGES BY LIGHT QUALITY

The abundance of *CO* transcripts peaks at night in both long and short days but the maximum *FT* expression occurs at dusk in long days (Suárez-López et al., 2001) (Fig. 1). These observations indicate that the post-translational regulation of CO protein plays another key role in the timing of *FT* activation under inductive conditions. CO protein abundance is very low throughout the entire day in short days, but dynamically fluctuates during the daytime and darkness in long days (Song et al.,

2012b; Valverde et al., 2004). Light quality is an essential element for CO protein stability changes (Song et al., 2012b; Valverde et al., 2004). Blue and far-red light stabilize the CO protein, whereas red light destabilizes it (Valverde et al., 2004). In addition to red light, darkness manipulates CO stability through ubiquitin-dependent mechanisms (Jang et al., 2008) (Fig. 1).

During the night, the RING-finger E3 ubiquitin ligase CONSTITUTIVE PHOTOMORPHOGENIC 1 (COP1) mediates the degradation of CO by forming protein complexes with the SUPPRESSOR OF PHYA-105 (SPA1) family members, SPA1, SPA3, and SPA4, regardless of photoperiod (Jang et al., 2008; Laubinger et al., 2006; Liu et al., 2008b). This COP1-SPA complex-mediated degradation of CO is restricted only at nighttime by the functions of the blue light photoreceptors cryptochromes (CRYs), mainly CRY2 (Zuo et al., 2011). Light-absorbed CRY2 binds to SPA1, and this binding enhances the interaction between CRY2 and COP1 in response to blue light, which in turn leads to suppression of COP1/SPA1 activity (Zuo et al., 2011). Since the abundance of *CO* transcripts in short days is low throughout the day and peaks in the middle of night, this COP1-SPA complex-mediated degradation of CO is a key feature for preventing flower initiation under non-inductive conditions.

In long days, the multi-layered functions of photoreceptors generate a unique daily profile of CO protein by directly and indirectly regulating its stability at different times of the day (Lazaro et al., 2015; Song et al., 2012b; Valverde et al., 2004) (Fig. 1). The maxima of CO stabilization occur immediately after dawn with a narrow peak and around dusk with a relatively broad peak (Song et al., 2012b; Valverde et al., 2004). At least four types of photoreceptors are involved in the generation of the early morning peak. The function of the blue light photoreceptor CRY2 contributes to CO accumulation, which might be caused by the maximum induction of *CO* transcripts at the end of the night, by inhibiting COP1/SPA1 activity during the daytime (Valverde et al., 2004; Zuo et al., 2011). Furthermore, the far-red light photoreceptor Phytochrome A (PHYA) stabilizes CO protein via a currently undetermined mechanism under a low red to far-red (R:FR) ratio, around dawn and dusk (Kim et al., 2008; Valverde et al., 2004).

CO protein abundance is rapidly and markedly decreased with the onset of daylight. The red light photoreceptor PHYB appears to trigger the degradation of CO protein under a high R:FR ratio, during the daytime period (Valverde et al., 2004). PHYB forms protein complexes with HIGH EXPRESSION OF OSMOTICALLY RESPONSIVE GENES 1 (HOS1), an E3 ubiquitin ligase, and CO. The red light-activated PHYB probably promotes the binding of HOS1 to CO, leading to the ubiquitin-dependent degradation of CO (Lazaro et al., 2012; 2015). Together with the concerted action of PHYB and HOS1, FKF1 relatives, ZEITLUPE (ZTL), and potentially LOV KELCH PROTEIN 2 (LKP2), interact with CO and destabilize it in a proteasome-dependent manner (Kim et al., 2007; Song et al., 2014). These destabilization mechanisms ensure that CO abundance is continually lowered until the afternoon of long days (Fig. 1). In contrast, CO proteins are highly accumulated near the end of the day via the activity of PHYA and FKF1. PHYA increases CO stability in response to a low R:FR ratio around dusk (Valverde et al., 2004). In addition to the regulation of *CO* transcription, FKF1 also enhances CO stability (Song et al., 2012b). FKF1 binds directly to CO protein through the LOV domain, and this binding is enhanced by blue light. Because, with the exception of FKF1 and ZTL, the protein expression of photoreceptors involved in CO stability changes is constant

during the day, the FKF1-CO interaction is important for timely stabilization of CO in the late afternoon (Song et al., 2012b; 2014). Although the mechanism of CO stabilization mediated by FKF1 has yet to be fully elucidated, it appears that the GI protein plays a key role in this process. Two relative proteins, FKF1 and ZTL, play antagonistic roles in the modulation of CO stability (Song et al., 2014). These proteins bind to GI, and the formation of these protein complexes is most likely to be crucial for their functional status (Kim et al., 2007; Sawa et al., 2007). In the morning, the functionally active ZTL captures and destabilizes CO (Song et al., 2014). ZTL-mediated CO degradation is terminated when GI and FKF1 proteins are expressed. In the afternoon, ZTL interacts with GI in a blue light-dependent manner (Kim et al., 2007). The binding to GI renders ZTL nonfunctional, and in turn, CO is sequestered from ZTL. Thus, FKF1 forms a protein complex with GI at the same time. However, unlike ZTL, GI-bound FKF1 is functionally active and can still interact with other proteins such as CO and CDFs for stabilization and degradation, respectively (Sawa et al., 2007; Song et al., 2012b; 2014). Therefore, CO protein accumulation as a consequence of the activities of the FKF1-GI complex, CRY2, and PHYA largely contributes to the peak expression of the *FT* gene at dusk in long days, which stimulates floral initiation (Fig. 1).

The roles of ZTL and LKP2 proteins in photoperiodic flowering regulation are complicated and need to be further investigated. Overexpression of *ZTL* or *LKP2*, unlike *FKF1*, delays flowering, and this could be attributable to the capture of FKF1 in the cytosol by the interactions of ZTL and LKP2 with FKF1 (Takase et al., 2011). However, the proteins simultaneously destabilize CO as well as, synergistically with FKF1, CDFs and, inversely, increase the stability of CIB (CRY-interacting basic helix-loop-helix) transcription factors (Fornara et al., 2009; Liu et al., 2013a), which are *FT* activators.

TRANSCRIPTIONAL REGULATION OF *FT* UNDER INDUCTIVE CONDITIONS

Diverse external environmental signals and internal cues, age and hormones, converge on the regulation of *FT* transcription (Song et al., 2013). Many transcriptional repressors bind directly to specific *cis*-elements in the *FT* locus and hamper precocious flowering under non-inductive conditions (Andres and Coupland, 2012; Song et al., 2015). Among the repressors, the activity of CDF family members strongly prevents *FT* expression and counteracts functioning of the CO protein (Song et al., 2012b). CDF1 and probably other CDF proteins associate with *FT* promoter regions near the transcriptional start site, which overlap with the CO-responsive element (CORE) and the CIB-binding *cis*-element (Liu et al., 2013b; Song et al., 2012b; Tiwari et al., 2010). Similar to *CO* transcriptional regulation, the binding of CDFs to the *FT* promoter occurs in the morning under long days (Song et al., 2012b) (Fig. 1). Given that the induction of *FT* expression is CO protein abundance-dependent, a linear relationship is assumed to exist between CO stability and *FT* transcript levels. However, the repression activity of CDFs appears to ensure that *FT* expression is time-dependently regulated, leading to a unique daily expression profile. Accordingly, the abundance of *FT* transcripts in the morning remains low, even though the amount of CO protein peaks in wild-type plants and is substantially increased in the *ztl* mutant under these conditions (Song et al., 2012b; 2014; Valverde et al., 2004). The *FT* repression mediated by CDFs is alleviated in the afternoon. FKF1, most likely together with GI, stimulates the degradation of CDFs on the *FT* locus (Sawa and Kay, 2011;

Song et al., 2012b) (Fig. 1). The binding activity of CDFs to the *FT* promoter region is antagonized by the presence of FKF1 (Song et al., 2012b). Thus, the action of GI on the microRNA pathway is implicated in the regulation of *FT* expression independently of *CO* transcription (Jung et al., 2007).

Once CDFs are removed in the afternoon, two classes of activators of *FT* transcription are then able to gain access to the promoter (Fig. 1). A strong *FT* activator, CO protein, interacts with DNA-binding transcription factors, ASYMMETRIC LEAVES 1 (AS1) protein and CCAAT-box-binding nuclear factor Y (NF-Y) proteins (Cao et al., 2014; Kumimoto et al., 2010; Song et al., 2012a; Wenkel et al., 2006). Despite directly binding to the CORE in the *FT* promoter through the C-terminal CCT (CONSTANS, CONSTANS-like, and TOC1) domain, CO appears to be recruited to the *FT* promoter by the formation of protein complexes with the transcription factors (Song et al., 2015; Tiwari et al., 2010). Interestingly, the complex formed between CO and NF-Y proteins leads to the formation of long-distance chromatin loops, which connect distal enhancer elements to the proximal promoter elements. The timing of loop formation resembles the expression profile of the *FT* gene in long days (Cao et al., 2014). In addition, members of another activator family, comprising CIB1, CIB2, CIB4, and CIB5, redundantly and positively regulate *FT* transcription through binding to CRY2 in a blue light-dependent manner (Liu et al., 2008a; 2013b). The formation of heterodimer complexes between CIB1 and the other CIBs is important for the association with the non-canonical E-box element in the *FT* promoter (Liu et al., 2013b).

In the leaf companion cells of *Arabidopsis*, after the completion of *FT* induction, FT protein, a long-sought flowering inducing substrate, is synthesized. The protein then travels from the leaf to the shoot apical meristem and triggers a transcriptional signaling cascade leading to flower initiation in long days (Corbesier et al., 2007; Pin and Nilsson, 2012).

THE EFFECT OF TEMPERATURE FLUCTUATIONS ON *FT* INDUCTION

Temperature change independently acts as a strong flowering modulator and manipulates *FT* transcription in leaves (Fernandez et al., 2016; Kim et al., 2009; Kumar et al., 2012; Lee et al., 2007). In *Arabidopsis*, increased ambient temperature above the optimal range in short days causes the accumulation of *FT* transcripts late in the day and results in acceleration of flowering time under these conditions (Fernandez et al., 2016; Kumar et al., 2012). In contrast, decreased ambient temperature in long days down-regulates *FT* expression and delays flowering (Lee et al., 2007; 2013). In addition, a prolonged period of cold, like winter, enhances the de-repression of *FT* transcription, which is known as the vernalization response (Kim et al., 2009). Research on temperature-mediated flowering regulation has mainly focused on vernalization. However, the effect of ambient temperature on flowering (i.e., thermosensory flowering) has recently attracted attention due to the potential effects of global warming. In the thermosensory flowering pathway, the SHORT VEGETATIVE PHASE (SVP) protein functions to negatively control flowering (Lee et al., 2007; 2013). The protein associates with the *FT* locus and directly represses its transcription in response to low temperature (Fig. 2). Moreover, the role of the histone H2A variant H2A.Z is crucial for temperature-dependent *FT* expression (Kumar et al., 2012). The occupation of H2A.Z nucleosomes on the *FT* locus occurs under low temperature conditions, which results in *FT* repression. This repression is
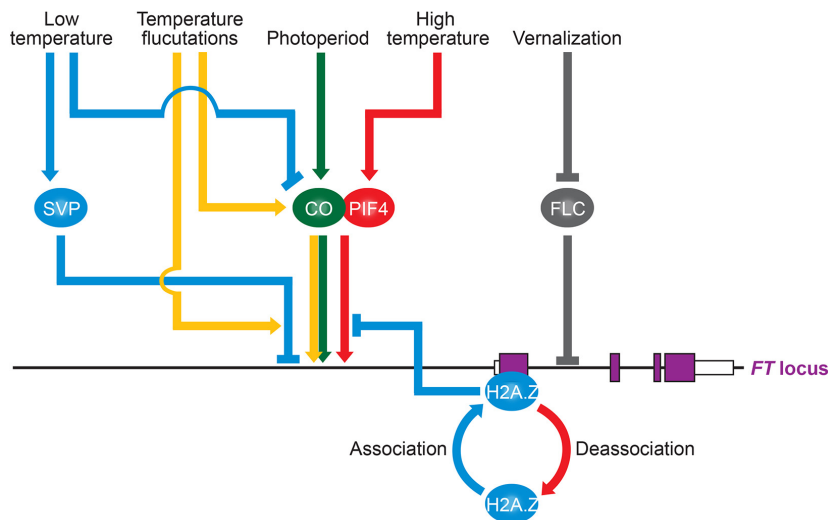
Fig. 2. The effect of temperature changes on *FT* induction. FLC, a strong floral regulator, associates with the first intron of the *FT* gene and represses the expression of the gene. Vernalization removes FLC repression of the *FT* locus. In short days, low temperature ($\approx 16°C$) increases the occupancy of H2A.Z nucleosomes on the *FT* locus, resulting in the inhibition of PIF4 binding to the *FT* promoter under this condition. However, high temperature ($\approx 27°C$) stimulates the de-association of H2A.Z from the *FT* locus and induces CO accumulation in the afternoon. These cause the formation of a CO-PIF4 complex that binds to the *FT* promoter and activates its transcription. SVP represses *FT* expression by binding to the *FT* promoter in response to low temperature in long days. Temperature fluctuations increase SVP activity at dusk and CO stability, which are also regulated by photoperiod, at night in long days, leading to changes in the gene expression profiles of *FT* in a day length-dependent manner.

relieved at high temperature, which enhances the accessibility of transcription factors to the *FT* promoter (Fig. 2). Indeed, the binding of the PHYTOCHROME INTERACTING FACTOR 4 (PIF4) transcription factor to the *FT* promoter is increased in response to rising temperatures in short days (Kumar et al., 2012). PIF4 protein induces *FT* expression late in the day at high temperature in short days. The PIF4-mediated induction of *FT* transcription requires the activity of CO (Fernandez et al., 2016). CO abundance is very low in short days, and the peak expressions of PIF4 and CO proteins synchronize at dusk under these conditions (Fernandez et al., 2016; Song et al., 2012b; Valverde et al., 2004). High temperature under short days elevates CO abundance but not PIF4 amount. This allows CO and PIF4 to form a protein complex that contributes to the activation of *FT* transcription (Fernandez et al., 2016) (Fig. 2). In contrast, high temperature in long days up-regulates *FT* expression by increasing PIF4 protein levels, but without changing CO (Seaton et al., 2015).

FLC protein plays the key role in the vernalization pathway. FLC is a transcriptional repressor and strongly reduces *FT* expression under non-inductive conditions (Andres and Coupland, 2012; Kim et al., 2009). Vernalization regulates chromatin states at the *FLC* locus and facilitates the removal of FLC repression on *FT* transcription in the spring (Fig. 2). In contrast to vernalization, a short-term cold treatment seems to repress *FT* expression. HOS1 mediates cold temperature-dependent *FT* repression through destabilization of the CO protein (Jung et al., 2012).

Discrepancies in flowering time between field experiments and corresponding laboratory experiments indicate the importance of daily temperature fluctuations (Wilczek et al., 2009). In particular, the repression activity of FLC is considerably weakened in fluctuating temperature environments (Burghardt et al., 2016). Thus, importantly, the effect of temperature fluctuations on flowering appears to be substantially photoperiod-dependent (Fig. 2) (Burghardt et al., 2016; Kinmonth-Schultz et al., 2016). The gene expression profile of *FT* is markedly influenced by warmer day/cooler night cycles in long days but not in short days (Kinmonth-Schultz et al., 2016). *FT* abundance is reduced at dusk but increases at night. This alteration is closely correlated with the robust enhancement of *CO* transcription by FBH proteins and a subsequent increase in CO protein abundance during the cooler night. Moreover, the SVP protein functions to repress *FT* expression at dusk under these conditions, without changing the protein amount (Kinmonth-Schultz et al., 2016) (Fig. 2).

The mechanism underlying ambient temperature-mediated flowering regulation remains to be elucidated. Nevertheless, given the importance of the effects of global temperature changes on plant development, including flowering (Craufurd and Wheeler, 2009; Fitter and Fitter, 2002), we have to consider employing natural temperature conditions in the laboratory in order to more precisely predict future scenarios of flowering time under conditions of changing environments. Since photoperiod sensitivity is an important feature for crop adaptation to climate changes (Craufurd and Wheeler, 2009), understanding the combinational effect of photoperiod and temperature on flowering is critical.

CONCLUSION

The transition from vegetative phase to reproductive phase is the most dramatic change in the life span of many organisms and is an irreversible process in most plant species (Kobayashi and Weigel, 2007). Therefore, the timing of this phase transition must be precisely controlled. The sophisticated networks underlying photoperiod- and temperature-mediated signaling enable plants to align the transition with favorable conditions. This review discusses the regulation of *FT* expression by the activity of CO protein as a useful output tool to measure the effect of photoperiod and temperature fluctuations on the timing of flowering in *Arabidopsis*. Because the CO-FT regulon is highly conserved across the plant kingdom, including major crop species (rice, wheat, and barley) (Song et al., 2010; 2015), understanding the molecular mechanisms by which *Arabidopsis* incorporates the information of photoperiod and temperature fluctuations to generate floral signals might give us new insights into agricultural applications. Supposedly, agricultural traits obtained under environmentally controlled growth conditions are often overestimated and disappear in outdoor fields. In

addition, the rising ambient temperature and increased environmental variability that are inherent in global climate change have already altered the reproductive phenology of plants (Craufurd and Wheeler, 2009; Fitter and Fitter, 2002). As the output of temperature-mediated flowering is gated reasonably well by the modulation of photoperiodic signaling components, studies on the interactive effects of these natural environmental factors are necessary for future crop security.

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