Chapter 6
Factors Affecting Flowering Seasonality

Yohei Higuchi and Tamotsu Hisamatsu

Abstract Environmental regulation of flowering seasonality and set seed is critical for this survival as it allows seeds to develop in the most favourable conditions. Recent genetic and molecular approaches provide a basis for understanding how plants use seasonal changes in natural daylight duration and temperature to achieve reproducible timing of flowering. Recent studies have led to the identification of members of the FLOWERING LOCUS T (FT) in *Arabidopsis*, and its orthologs in several plant species act as florigen. In addition to the floral inducer florigen, the systemic floral inhibitor anti-florigen, anti-florigenic FT/TFL1 family protein (AFT), has been identified from a wild chrysanthemum and plays a predominant role in the obligate photoperiodic response. In *Arabidopsis*, the molecular basis for vernalization process has been revealed. The key factor in the vernalization pathway is a repressor of flowering, FLOWERING LOCUS C (FLC). In temperate cereals that require vernalization to flower, three genes possibly participate in a regulatory loop to control the timing of flowering, namely, VRN1, VRN2, and VRN3. VRN2 is a key factor for flowering repression in winter varieties.

Keywords Anti-florigen • Florigen • Flowering • Seasonality • Photoperiodism • Vernalization

6.1 Photoperiodic Flowering

Many plants sense gradual change in day length (photoperiod), which is the most reliable seasonal cue at high latitude, to determine when to produce flowers. This phenomenon, photoperiodism, anticipates environmental conditions and enables plants to maximise their survival and reproduction at a suitable time of the year.

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Photoperiodism was first described in detail by Garner and Allard (1920). They demonstrated that several plant species flower in response to changes in day length, not light intensity or temperature. Flowering plants are classified into three categories based on their photoperiodic responses: short-day plants (SDP), in which flowering occurs when the night length is longer than a critical minimum; long-day plants (LDP), in which flowering occurs when the day becomes longer; and photoperiod-insensitive day-neutral plants (DNP) (Thomas and Vince-Prue 1997). Photoperiodism has had a considerable impact on the agricultural and horticultural industries, because it has enabled plant breeders and growers to control flowering time by manipulating day length. Photoperiod is perceived in the leaves, where the flower-inducing signal is synthesised under appropriate photoperiods and transmitted to the shoot apex to initiate the flower bud. In 1936, based on the grafting experiment in light-sensitive plants, Chailakhyan proposed the concept of the flowering hormone "florigen" (flower former), which is produced in the leaves and transmitted to the shoot apex to induce flowering (Chailakhyan 1936).

6.2 Florigen and Anti-florigen

Despite numerous attempts to extract florigen, the molecular structure has remained unknown for almost 70 years (Zeevaart 2008). Recently, molecular-genetic studies have demonstrated that FLOWERING LOCUS T (FT) in *Arabidopsis* and its orthologs in several plant species act as florigen (Lifschitz et al. 2006; Corbesier et al. 2007; Tamaki et al. 2007; Lin et al. 2007). *FT* was first identified as a gene responsible for the late flowering mutant of *Arabidopsis*, a facultative LDP (Kardailsky et al. 1999; Kobayashi et al. 1999). FT is expressed in the vasculature tissues of leaves under a flower-promoting LD photoperiod and forms a complex with a bZIP-type transcription factor, FD, to induce floral-meristem identity genes, such as *APETALA1* (*AP1*) and *FRUITFULL* (*FUL*) (Abe et al. 2005; Wigge et al. 2005) (Fig. 6.1). Interestingly, although *FT* is induced in leaves, *FD* expression was limited to the shoot apical meristem. In 2007, the long-distance transmission of the FT protein and its rice homolog Heading date 3a (Hd3a) from the leaves to shoot apex was determined (Corbesier et al. 2007; Tamaki et al. 2007), and the FT/Hd3a protein was demonstrated to be a molecular entity of the systemic floral stimulus florigen. *FT/Hd3a* encodes a small globular protein similar to phosphatidylethanolamine-binding protein (PEBP). Hd3a forms a complex with 14-3-3 adaptor proteins and OsFD1, which is called florigen activation complex (FAC), and then induces *OsMADS15*, a rice *AP1* homolog, transcription to induce flowering (Taoka et al. 2011). The FT/Hd3a family protein acts as universal flowering hormone "florigen" in many plant species (Wickland and Hanzawa 2015; Matsoukas 2015).

In addition to the floral inducer florigen, the systemic floral inhibitor produced in non-induced leaves can inhibit flowering. The concept of a floral inhibitor (anti-florigen) was proposed almost as early as that of florigen (Lang and Melchers

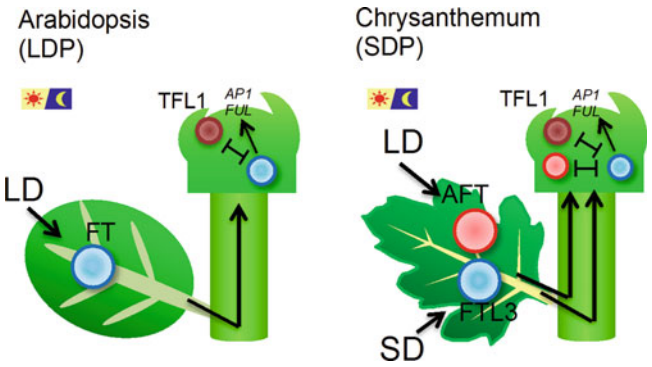

Fig. 6.1 Flowering time regulation by florigen and anti-florigen. In *Arabidopsis*, FT is synthesised in leaves under a flower-inducing LD photoperiod that moves to the shoot apex to induce floral-meristem identity genes. TFL1 is expressed in the shoot apex and suppresses flowering by antagonising FT function. In chrysanthemums, FTL3 is synthesised in the leaves under flower-inducing SD and TFL1 antagonises FTL3 function at the shoot apex. In addition, a systemic floral inhibitor (*AFT*) is synthesised in leaves under flower-inhibiting LD or NB, which antagonise florigenic activity of FTL3 at the shoot apex

1943); the appropriate photoperiod leads to the removal of the floral inhibitor, and consequently, flowering occurs. Many physiological observations such as defoliation, grafting, and localised photoperiodic treatment in *Hyoscyamus*, strawberry, *Lolium*, chrysanthemum, tobacco, and *Pharbitis* (Lang and Melchers 1943; Guttridge 1959; Evans 1960; Tanaka 1967; Lang et al. 1977; Ogawa and King 1990) suggested the existence of the systemic floral inhibitor, anti-florigen. A grafting experiment of tobacco plants with different photoperiodic responses strongly supported this hypothesis. The day-neutral (DN) tobacco normally flowered, even under an SD photoperiod, but when the LD flowering *Nicotiana sylvestris* was grafted, flowering of DN tobacco was delayed under SD (Lang et al. 1977). This result clearly indicates that a floral inhibitor produced in the leaves of *N. sylvestris* under SD systemically inhibited the flowering of DN tobacco plants. In the 1990s, molecular-genetic studies in *Arabidopsis* revealed that TERMINAL FLOWER 1 (TFL1), a member of the PEBP family protein, suppressed flowering (Bradley et al. 1997). *TFL1* is exclusively expressed in the shoot apex and maintains indeterminate inflorescence (Ratcliffe et al. 1999; Conti and Bradley 2007; Jaeger et al. 2013). Since TFL1 also formed a complex with FD, an interacting partner of FT, TFL1 suppressed flowering by antagonising florigenic activity of the FT-FD complex (Abe et al. 2005) (Fig. 6.1). Although TFL1 acts as a floral inhibitor, it possibly acts over short (cell-to-cell) distances within the meristematic zone (Conti and Bradley 2007). A recent study in a wild diploid chrysanthemum (*C. seticuspe*) identified a floral inhibitor, anti-florigenic FT/TFL1 family protein (CsAFT), which moves long distances (Higuchi et al. 2013). *CsAFT* was induced in leaves under flower-non-inductive LD or night-break (NB) photoperiods and was suppressed at very low levels under inductive SD. CsAFT proteins move long distances from leaves to the shoot apex and inhibit flowering by directly

antagonising the flower-inductive activity of the FT-FD complex of *C. seticuspe* (CsFTL3-CsFDL1) (Fig. 6.1). These findings suggest that the balance between floral inducers (florigens) and inhibitors (anti-florigens) determines flowering time variations of many plant species.

6.3 Flowering and Seasonal Time Measurement

A major factor in the seasonal control of flowering time is the photoperiod. Plants flower in response to changing photoperiod, but how do they measure the length of day and night? Classical studies in plants and animals have provided several physiological models for explaining photoperiodic responses (Nelson et al. 2010). The hourglass model proposes that day length is measured simply through some regulatory product, the accumulation of which is light dependent (Lees 1973). In this model, photoperiodic responses are triggered when the amount of this product exceeds a certain threshold level (e.g. the amount of phy-Pfr has been a candidate for the sand of an hourglass). However, the external coincidence model proposes that day length is measured through a circadian oscillator that controls the expression of some regulatory product, the activity of which is modulated by light. Photoperiodic responses are triggered only when external (light) signals coincide with the light-sensitive phase of circadian rhythms (Pittendrigh and Minis 1964). The internal coincidence model proposes that light signals set two different circadian rhythms, and a response is triggered only when these rhythms are synchronised under certain photoperiods (Pittendrigh 1972). Recent studies in *Arabidopsis* and rice demonstrated that both external and internal coincidence models are consistent with the physiological and molecular-genetic evidence in plants (Greenham and McClung 2015; Song et al. 2015) (Fig. 6.2).

The external coincidence model was proposed by Pittendrigh in the 1960s based on Bunning's hypothesis (Pittendrigh and Minis 1964; Büning 1936). In this model, light has two different roles. One is to reset the circadian clock, which is a set phase of the clock. The other is to simply transfer the presence or absence of external light that triggers photoperiodic reactions. The circadian clock entrained by the light/dark cycle sets a photosensitive phase to occur at particular time of the day. The photoperiodic reaction is triggered only when the photosensitive phase "coincided" with the external light signal. In a facultative LDP *Arabidopsis*, circadian rhythms entrained by a light signal at dawn set the expression of *CONSTANS* (*CO*), a positive regulator of *FT*. Under an LD photoperiod, the peak phase of CO in the evening interacted with light signals mediated by phyA or cry2 and stabilised the CO protein. However, the peak phase of CO expression occurred after dusk under SD, and the CO protein was degraded during darkness. Therefore, the CO protein was stabilised and activated only under LD evening conditions, when it induced *FT* expression to promote flowering (Yanovsky and Kay 2002; Valverde et al. 2004). Light signals mediated by phytochromes and cryptochromes act in the input to the circadian clock and as an external light signal that directly activates the induction of

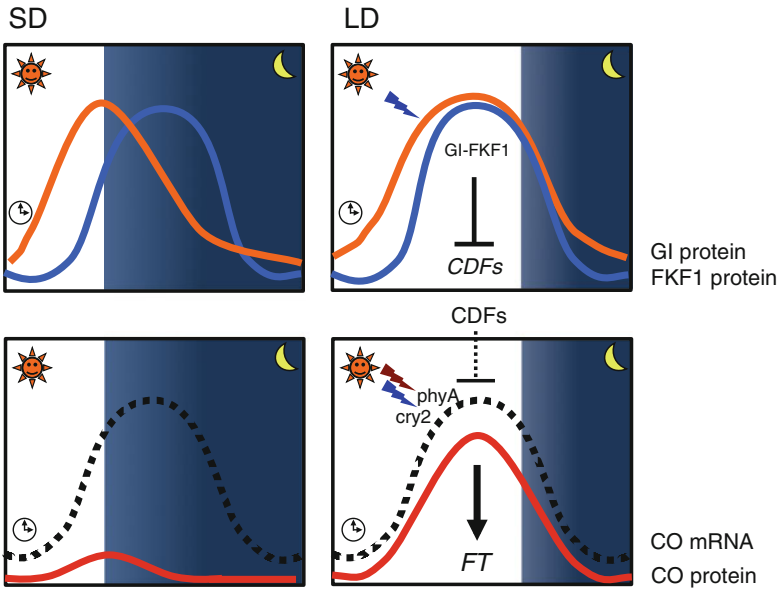

Fig. 6.2 Photoperiodic regulation of *FT* by internal and external coincidence. Accumulation of GI and FKF1 proteins synchronises in the late afternoon under LD and forms a complex (GI-FKF1) in a *blue* light-dependent manner. The activated GI-FKF1 then degrades CDF proteins, negative regulators of *CO* transcription. *CO* mRNA expression is regulated by a circadian clock to peak in the evening. Under LD, light signals mediated phyA, and cry2 stabilised CO proteins in the evening that activated *FT* transcription

florigen genes (Fig. 7.2). The evening-phased expression of *CO* under LD photoperiods is regulated by the coordinated action of a blue light receptor FLAVIN-BINDING, KELCH REPEAT, F-BOX 1 (FKF1), and a clock component GIGANTEA (GI). Transcription of both FKF1 and GI is regulated by the circadian clock. Under SD conditions, the peak phase of FKF1 and GI protein accumulation occurred at different times of the day. However, under LD, the peak phase of these proteins coincided in late afternoon and formed a complex (FKF1-GI) in a blue light-dependent manner. The FKF1-GI complex then degraded CYCLING DOF FACTORs (CDFs), negative regulators of *CO* transcription (Imaizumi et al. 2005; Sawa et al. 2007; Fornara et al. 2009). The LD-specific interaction of two different rhythms (FKF1 and GI) fitted well with the internal coincidence model (Fig. 6.2). In a facultative SDP rice, expression of *Heading date 1* (*Hd1*), an ortholog of *CO*, was regulated by a circadian clock peaking in the evening. The coincidence of Hd1 with the phytochrome signal suppressed flowering by negatively regulating the expression of the *FT* ortholog, *Heading date 3a* (*Hd3a*) (Izawa et al. 2002; Hayama et al. 2003).

6.4 Flowering Time Regulation in Chrysanthemum

Chrysanthemum (*Chrysanthemum morifolium*) is one of the most important horticultural crops worldwide. It is an obligate SDP, which flowers when the nights are longer than a critical minimum, and flowering is strictly inhibited under LD or NB. Chrysanthemum growers use blackouts or artificial lighting (day-length extension or NB) to meet the demand for marketable flowers throughout the year. Recently, molecular-genetic studies in a wild diploid *C. seticuspe* identified *FLOWERING LOCUS T-like 3* (*CsFTL3*), which encodes a systemic floral inducer in chrysanthemum (Oda et al. 2012). Unlike *Arabidopsis* and *Pharbitis*, chrysanthemums require repeated cycles of SD for successful anthesis (Corbesier et al. 2007; Hayama et al. 2007; Oda et al. 2012). Consistent with this requirement, *CsFTL3* expression is gradually increased by repeating the SD cycles (Nakano et al. 2013). However, *CsAFT* expression, which encodes systemic anti-florigen, is induced in leaves under non-inductive LD or NB, and it rapidly decreased after a shift to SD (Higuchi et al. 2013). Under non-inductive photoperiods, CsAFT produced in the leaves moved to the shoot apex and inhibited flowering by directly antagonising the florigen complex activity (CsFTL3-CsFDL1). In addition, a TFL1 homolog (CsTFL1) is constitutively expressed in shoot tips regardless of the photoperiodic conditions and shows strong floral inhibitor activity (Higuchi et al. 2013). In chrysanthemums, strict maintenance of a vegetative state under non-inductive photoperiod is achieved by a dual regulatory system: one is AFT, a systemic floral inhibitor produced in non-inductive leaves, and another is TFL1, a local inhibitor constitutively expressed at the shoot apex (Higuchi and Hisamatsu 2015; Fig. 6.3).

Light quality during NB and daytime affects chrysanthemum flowering. NB with red light effectively inhibits flowering, which is partially reversed by subsequent exposure to FR light, suggesting the involvement of light-stable-type phys in this response (Cathey and Borthwick 1957; Sumitomo et al. 2012). Interestingly, NB with blue and FR light effectively inhibit chrysanthemum flowering when grown under a daily photoperiod with monochromatic blue light, but not white (blue + red) light (Higuchi et al. 2012). This suggested that light quality during the daily photoperiod affects the sensitivity to NB at midnight, and at least two distinct phy-mediated regulation systems might exist. The knock-down of *CsPHYB* by RNAi resulted in some insensitivity to NB with red light and developed capitulum (Fig. 6.4). In *CsPHYB*-RNAi plants, *CsFTL3* was up-regulated, whereas *CsAFT* was down-regulated under NB. These results indicated that CsPHYB acts as primary photoreceptor mediating NB response and inhibits flowering by repressing *CsFTL3* and inducing *CsAFT* (Higuchi et al. 2013). Interestingly, *CsAFT* expression was strongly induced by red light given at 8–10 h after dusk under both SD and LD. Thus, induction of *CsAFT* by phy signalling is gated by the clock system, and the gate for maximal induction of *CsAFT* opens at a constant time after dusk, regardless of the entrained photoperiod (Fig. 6.4b). Moreover, if long nights (14 h) were given, flowering was successfully induced, even under non-24-h light/dark

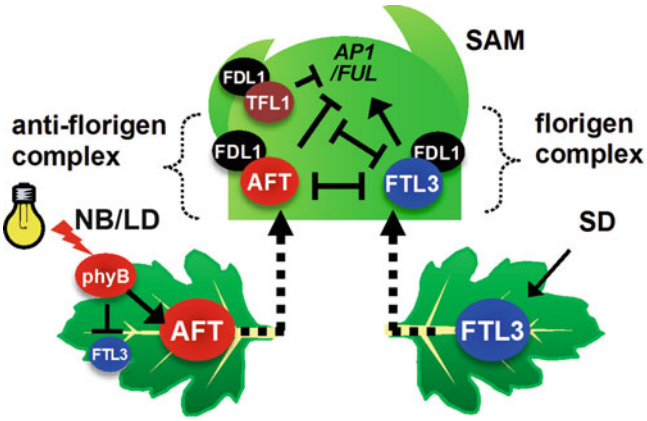

Fig. 6.3 Photoperiodic regulation of flowering in chrysanthemums. Under flower-inductive SD, FTL3 is produced in leaves to systemically induce flowering. Under non-inductive NB or LD, AFT is synthesised in leaves to systemically inhibit flowering. NB with *red* light was perceived by phyB that induces *AFT* but suppressed *FTL3* expression. *TFL1* is constantly expressed in shoot tips regardless of the photoperiodic conditions. Both AFT and TFL1 suppressed flowering by directly competing with FTL3 for binding to FDL1

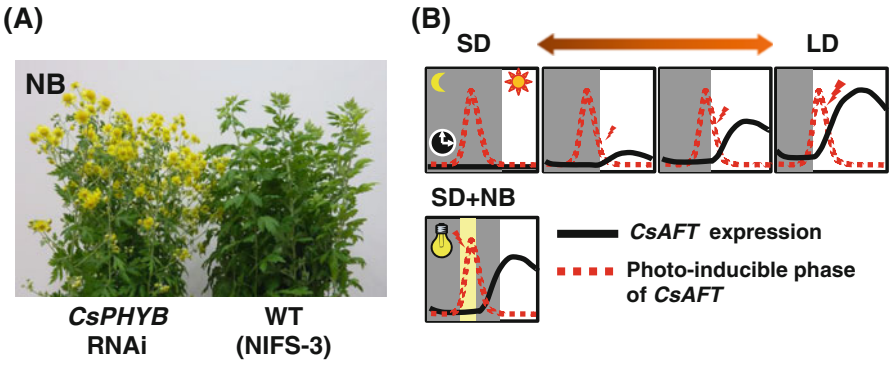

Fig. 6.4 PHYB-mediated and gated induction of *AFT*. (**a**) Flowering response of WT and *PHYB*-RNAi *Chrysanthemum seticuspe* plants under NB with red light. *PHYB*-RNAi plants are almost insensitive to NB. (**b**) Model for the induction of *AFT* in response to natural day-length extension and artificial lighting. The gate for *AFT* induction opens at a constant time after dusk regardless of the day length. As the night becomes shorter, the photo-inducible phase of *AFT* interacts with *red* light in the morning and inhibits flowering. Under NB, midnight illumination coincides with the photo-inducible phase of *AFT*

cycles. Therefore, as in the case of *Pharbitis*, day-length recognition of chrysanthemums relies on the absolute duration of darkness rather than on the photoperiodic response rhythm set by the dawn signal. Chrysanthemums measure the length of night by a timekeeping component, which is initiated from the dusk signal.

6.5 Molecular Mechanisms of Photoperiodic Flowering in Rice

Rice (*Oryza sativa*) is a facultative short-day plant that accelerates flowering under SD. Loss of function of all phytochromes (*se5* or *phyABC* triple mutant) resulted in a photoperiod-insensitive early flowering phenotype, indicating that phytochromes are required for photoperiodic flowering in rice (Izawa et al. 2000, 2002; Takano et al. 2009). In addition, phyB acts as a primary photoreceptor mediating light-induced inhibition of flowering by NB (Ishikawa et al. 2005). Compared to phys, little is known about the significance of blue light receptors such as crys and ZTL/FKF1 on the flowering time regulation in SDPs, including rice. A circadian clock output *GI-CO-FT* pathway in *Arabidopsis* is also conserved in rice (*OsGI-Hd1-Hd3a*), but the regulation of *FT* (*Hd3a*) by *CO* (*Hd1*) is reversed (Hayama et al. 2003). Rice contains an alternative and unique pathway that functions independently of *Hd1*. *Early heading date 1* (*Ehd1*) encoding a B-type response regulator promotes flowering by up-regulating *Hd3a* expression independently of *Hd1* (Doi et al. 2004). *Grain number*, *plant height*, *and heading date 7* (*Ghd7*), a CCT domain protein is induced under LD and suppressed flowering by down-regulating *Ehd1* expression (Xue et al. 2008). Interestingly, induction of both *Ehd1* and *Ghd7* by light was gated by a circadian clock. The gate for *Ehd1* induction by blue light always opened around dawn, but the gate for *Ghd7* induction with red light had different openings, depending on day length. Acute induction of *Hd3a* in response to critical day length was achieved by the interaction of these two gating mechanisms (Itoh et al. 2010). In addition to *Hd3a*, rice has another florigen gene *RICE FLOWERING LOCUS T1* (*RFT1*) that functions under LD photoperiods (Komiya et al. 2009; Fig. 6.5). Loss of function of *RFT1* results in extremely late flowering under LD, which is similar to the flowering response of absolute SDPs (Ogiso-Tanaka et al. 2013).

6.6 Flowering Time Regulation in Other Plant Species

Tomato (*Solanum lycopersicum*) is one of the most important horticultural crops worldwide and is a DNP that flowers independently of photoperiod. *SINGLE-FLOWER TRUSS* (*SFT*), a tomato homolog of *FT*, is expressed in expanded mature leaves and systemically promotes flowering (Lifschitz et al. 2006; Shalit et al. 2009). In contrast, *SELF PRUNING* (*SP*, homolog of *TFL1*) is expressed in young leaves and the shoot apex and suppresses flowering (Shalit et al. 2009). The balance between SFT and SP regulates flowering time and determinate or indeterminate shoot architecture. Weak alleles of *SFT* and mutations in *SUPPRESSOR OF SP* (*SSP*, *FD* homolog) weakened the activity of the florigen activation complex (FAC), resulting in partially determinate architecture that provided maximum yields (Park et al. 2014).

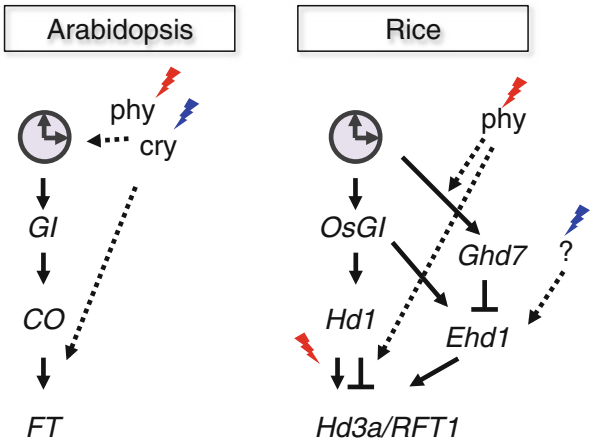

Fig. 6.5 Comparison of photoperiodic flowering pathways in *Arabidopsis* and rice. The circadian clock output *GI-CO-FT* pathway is conserved in *Arabidopsis* and rice, but the regulation of *FT* (*Hd3a*) by *CO* (*Hd1*) is reversed. Rice has an alternative pathway (*Ghd7-Ehd1*) that functions independently of *Hd1*. Rice has two florigen genes, *Hd3a* and *RFT1*. Hd3a induces flowering under inductive SD photoperiod, and RFT1 functions under non-inductive LD photoperiod

Strawberry (*Fragaria* x *ananassa*) is a perennial plant, and flowering is induced by low temperature and SD photoperiod (Heide et al. 2013). Recent studies in rose and woodland strawberry (*F. vesca*) revealed that a mutation in the *TFL1* ortholog is the principle cause of the continuous flowering phenotype in these species (Iwata et al. 2012; Koskela et al. 2012). *FvTFL1*expression is induced in shoot tips under LD but suppressed under SD. In the continuous flowering cultivar, loss of function of a strong floral repressor *FvTFL1* resulted in the derepression of flowering under LD (Koskela et al. 2012). Unlike *Arabidopsis*, *F. vesca* homologs of *FT* (*FvFT1*) and *SOC1* (*FvSOC1*) acted as floral repressors in SD flowering cultivars, because they were up-regulated under LD to activate expression of *FvTFL1* (Mouhu et al. 2013; Rantanen et al. 2014). Moreover, *FvTFL1* was regulated by a temperature-dependent pathway, independently of the regulation of *FvFT1-FvSOC1* by photoperiod (Rantanen et al. 2015) As in *F. vesca*, *F.* x *ananassa* floral inhibition pathways depend on *FaTFL1* regulation by day length via *FaFT1* and temperature, whereas the factors involved in its promotion remain unclear. A putative floral promoter, *FaFT3*, was up-regulated in the shoot tip under SD and/or low growth temperature, in accordance with the promotion of flowering in *F.* x *ananassa* (Nakano et al. 2015).

Pharbitis [*Pharbitis* (*Ipomoea*) *nil*] is an obligate SDP that initiates flowering by a single exposure to a long night (Imamura 1967). To date, *Pharbitis* homologs of *GI*, *CO*, and *FT* (*PnGI*, *PnCO*, *PnFT1/2*) have been identified (Liu et al. 2001; Hayama et al. 2007; Higuchi et al. 2011). PnFT1 has strong florigenic activity, and its expression is induced by a single SD treatment but is completely suppressed under LD or NB (Hayama et al. 2007). However, *PnCO* expression shows diurnal

rhythms, but is not affected by NB (Liu et al. 2001). Interestingly, *PnFT1* induction occurs at a constant time (12–16 h) after lights off, regardless of the day length preceding the dark period, and its expression showed circadian rhythms under continuous darkness (Hayama et al. 2007). In addition, the constitutive expression of *PnGI* resulted in a longer period length and reduced the amplitude in *PnFT1* rhythmic expression and suppressed flowering (Higuchi et al. 2011). Therefore, *Pharbitis* measured the absolute duration of night through circadian clocks that were initiated on light-to-dark transition at dusk. The dark-dominant flowering of *Pharbitis* is very similar to that of chrysanthemums.

6.7 Vernalization

Temperature is also a major seasonal cue. Plants have evolved the ability to measure a complete winter season and to remember the prior cold exposure in the spring. Winter annuals and biennials typically require prolonged exposure to the cold of winter to flower rapidly in the spring. This process where flowering is promoted by cold exposure is known as vernalization.

In *Arabidopsis*, the molecular basis for this memory has been revealed. The key factor in the vernalization pathway is a repressor of flowering, FLOWERING LOCUS C (FLC), a MADS-box transcription factor (Hepworth and Dean 2015). FLC directly represses *FT* expression (Fig. 6.6a). FLC expression is high before winter but is repressed during the cold. *FLC* expression is down-regulated within 2 weeks of experiencing cold and is epigenetically silenced by polycomb repressive complex2 (PRC2) complex that contains VERNALIZATION2 (VRN2). VRN2 is constitutively expressed; its activity is boosted through the association with plant-homeodomain zinc-finger (PHD) proteins, a VRN5/VIN3-like family. Epigenetic silencing is dependent on the cold-induced *VERNALIZATION INSENSITIVE 3* (*VIN3*), a PHD gene (Sung and Amasino 2004). The VIN3-PRC2 complex, a protein complex possessing H3K27 methyltransferase activity, established the enrichment of a series of repressive chromatin modifications at the *FLC* locus to

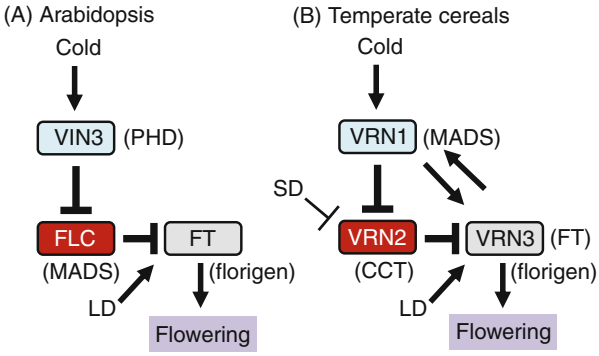

Fig. 6.6 A simplified model of the vernalization response on the florigen production in *Arabidopsis* (**a**) and temperate cereals (**b**)

keep it in a repressed state. Marking the chromatin in this way is what provides the cellular memory of winter.

In the winter, in varieties of wheat and barley that require vernalization to flower, three genes possibly participate in a regulatory loop to control the timing of flowering, namely, VRN1, VRN2, and VRN3 (Trevaskis et al. 2007; Fig. 6.6b). VRN2 is a key factor for flowering repression in winter wheat and barley. *VRN2* encodes a protein containing a putative zinc-finger and a CCT domain protein (Yan et al. 2004). Prior to cold exposure, high levels of *VRN2* act as a repressor of *VRN3* (an ortholog of *FT*) to prevent flowering (Dubcovsky et al. 2006). During cold exposure in wheat and barley, *VRN1*, a MADS-box transcription factor homologous to the floral-meristem identity gene *AP1* of *Arabidopsis*, is induced by vernalization, with the level of expression dependent on the length of cold exposure (Trevaskis et al. 2006). The induction of *VRN1* in the leaves during winter prevents the up-regulation of *VRN2* (Chen and Dubcovsky 2012). In the absence of VRN2, *VRN3* is up-regulated by LD, further enhancing an increasing *VRN1* expression and closing a positive feedback loop that leads to an acceleration of flowering. At the shoot apical meristem, *VRN1* activation by vernalization (Oliver et al. 2009) or by *VRN3* (Li and Dubcovsky 2008) accelerates the transition to the reproductive phase. GA application can substitute for vernalization in a number of biennial species as reported by Lang (1957). The substitution depends on the species. In the cold-requiring LD grass species *Lolium perenne*, exogenous GA allowed flowering in non-inductive SD conditions only in vernalized plants, whereas non-vernalized plants were unable to respond to GA either by stem elongation or flowering (MacMillan et al. 2005). The LD/GA inductive pathway is blocked unless plants are vernalized.

In sugar beet, two *FT*-like genes, *BvFT1* and *BvFT2*, have important roles in the vernalization-induced bolting and flowering (Pin et al. 2010). BvFT1 contributes to the vernalization response as a repressor. BvFT2 is essential for flowering as a promoter of flowering, whereas BvFT1 acts antagonistically and represses flowering, partly through the transcriptional repression of BvFT2.

Although VRN2 of temperate cereals and BvFT1 of sugar beet act similarly to FLC, in that it is a floral repressor, they are unrelated to the *FLC* gene. The different genes are involved in establishing vernalization in these species, indicating that vernalization systems possibly evolved after these groups of plants diverged.

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