Chapter 5
Light Acts as a Signal for Regulation of Growth and Development

Yohei Higuchi and Tamotsu Hisamatsu

Abstract Plants utilise light not only for photosynthesis but also as a signal to regulate optimal growth and development throughout their life cycle. The light quality (spectral composition), amount, direction and duration change depending on the season, latitude and local conditions. Therefore, to adapt to diverse light conditions, plants have evolved unique photoreceptor systems to mediate light responses to a broad range of wavelengths from ultraviolet-B to far-red light. Light signals can regulate changes in structure and form, such as seed germination, de-etiolation, leaf expansion, phototropism, neighbour avoidance, stem elongation, flower initiation and pigment synthesis. Plant hormones and transcriptional factors play an important role in the internal signalling that mediates light-regulated processes of development. Plants rely on their circadian clock to modify their growth and development in anticipation of predictable changes in environmental light and temperature conditions. The light signals perceived by photoreceptors affect the circadian clock and directly activate the induction of the light responses.

Keywords Circadian rhythm • De-etiolation • Gating effect • Photoreceptor • Phototropism • Seed germination • Shade avoidance response

5.1 Photoreceptors and Their Function

As sessile and photosynthetic organisms, plants monitor ambient light conditions and regulate numerous developmental switches to adapt to continually changing environments. A recent molecular genetic approach in the model plant *Arabidopsis* revealed that multiple photoreceptors act as light sensors for perceiving different light wavelengths (Fig. 5.1). These include phytochromes (phy), cryptochromes

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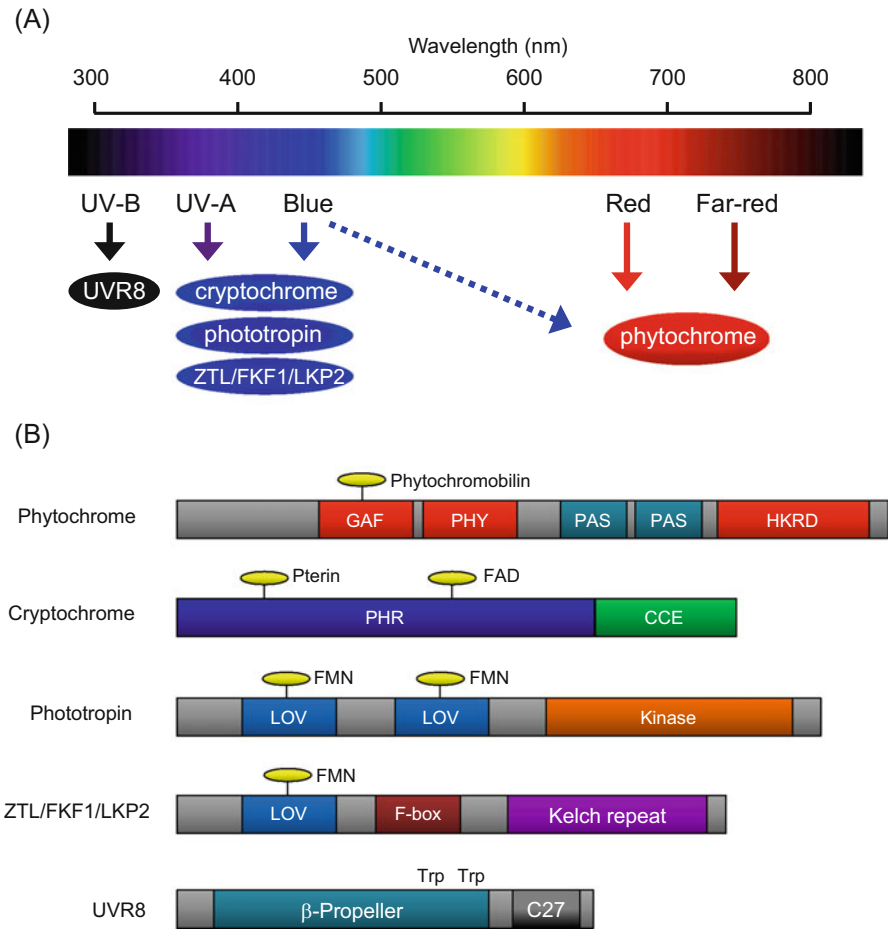

Fig. 5.1 Photoreceptors in higher plants. (**a**) Photoreceptors perceiving different parts of the light spectrum. (**b**) Structure of photoreceptor proteins. Domain structure and binding chromophores are shown. *GAF* cGMP-stimulated phosphodiesterase; *Anabaena* adenylate cyclases and *Escherichia coli* FhlA; *PAS* Per (period circadian protein), Arn (Ah receptor nuclear translocator protein) and Sim (single-minded protein); *HKRD* histidine kinase-related domain; *PHR* photolyase-homologous region; *CCE* cry C-terminal extension; *LOV* light, oxygen and voltage; *FAD* flavin adenine dinucleotide; *FMN* flavin mononucleotide

(cry), phototropins (phot), ZEITLUPE (ZTL)/FLAVIN-BINDING, KELCH REPEAT, F-BOX 1 (FKF1)/LOV KELCH PROTEIN2 (LKP2) family proteins and UV RESISTANCE LOCUS 8 (UVR8). Here, we summarise the physiological responses, light perception mechanisms and light signal transduction mechanisms regulated by multiple photoreceptors.

5.1.1 *Phytochromes (Phy)*

In the 1950s, the effect of exposure to different spectra of light on seed germination in lettuce was examined; the red/far-red (R:FR) reversibility was analysed, where R light exposure induced lettuce seed germination, but subsequent FR light exposure reversed the effect of R light (Borthwick et al. 1952). The photoreversible proteinous pigment, phytochrome, was extracted and analysed (Butler et al. 1959). Phytochromes are soluble proteins that bind phytochromobilin as chromophores and convert between two different photoreversible forms in vivo: R light (650–670 nm)-absorbing (Pr) and FR light (705–740 nm)-absorbing (Pfr) forms. In general, Pr absorbs R light and is converted to its biologically active form, Pfr, which induces various physiological responses; Pfr absorbs FR light and is converted to an inactive form of Pr (Fig. 5.2). This R:FR reversible response, which is a typical phytochrome reaction, is classified as a low-fluence response (LFR) that occurs in seed germination and night break (NB) responses with short light pulses. In addition to LFR, phytochrome responses include high-irradiance responses (HIR) and very-low-fluence responses (VLFR) (Casal et al. 1998). HIR include de-etiolation (inhibition of hypocotyl elongation and promotion of cotyledon expansion) and anthocyanin accumulation responses. VLFR is triggered by extremely low light intensities of all wavelengths, which is observed in light-induced seed germination. In contrast to LFR, HIR and VLFR do not show R:FR reversibility. It should be noted that in addition to R and FR regions of the spectrum, phy can also weakly absorb blue light (Figs. 5.1a and 5.3).

Since the absorption spectrum between Pr and Pfr partially overlaps (Fig. 5.3a), the phytochrome photoequilibrium (Pfr/P; where P = Pr + Pfr) under saturated light intensity changes depending on the light quality (Sager et al. 1988). A high R:FR ratio establishes a high Pfr/P, whereas low R:FR ratio creates a low Pfr/P (Fig. 5.3b). Under a vegetation canopy, shading by other plants creates a low Pfr/P that induces stem/petiole elongation and early flowering, which is a shade-avoidance response (Casal 2013). It is possible to estimate the effectiveness of light treatment by calculating Pfr/P under different light wavelengths; however, screening by other pigments such as chlorophylls, flavonoids and carotenoids could occur. For example, flowering inhibition by NB in chrysanthemum is mediated by

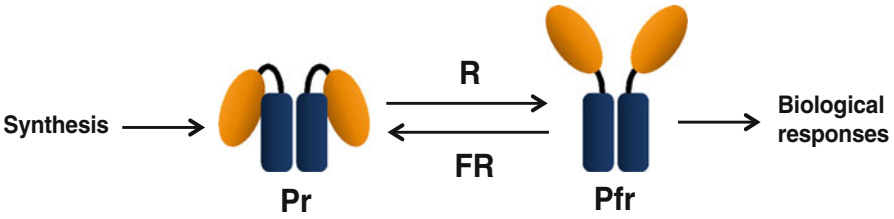

Fig. 5.2 Photoconversion of phytochrome. Phytochromes are synthesised in the Pr form. Pr absorbs R light and is converted to its biologically active form, Pfr, which induces various physiological responses. Pfr absorbs FR light and is converted it its inactive Pr form

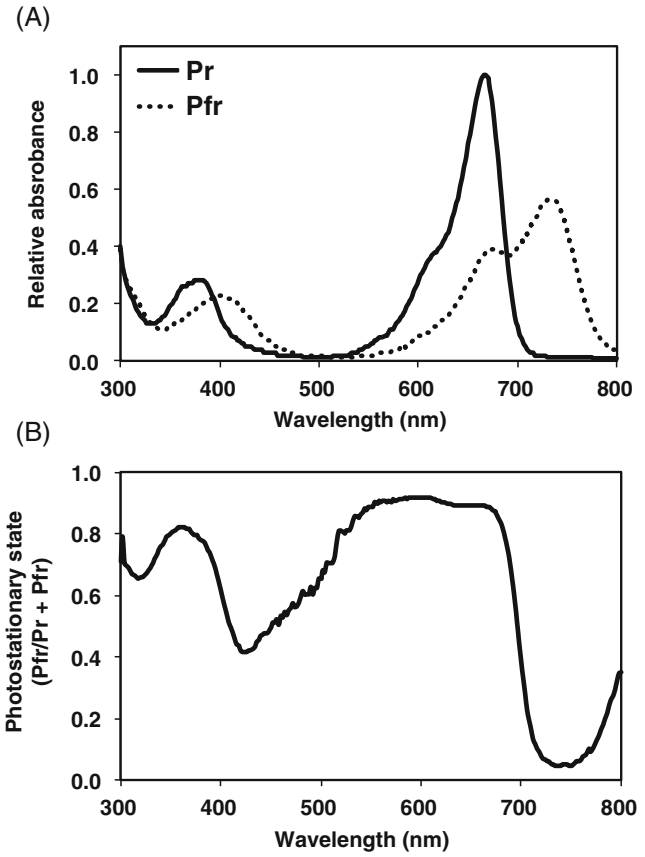

Fig. 5.3 Relative absorption spectra of purified rye phytochrome in its Pr and Pfr forms (**a**) and calculated photostationary state (**b**) (Data derived from Sager et al. 1988)

phytochromes (Higuchi et al. 2013), but spectral sensitivity to NB shifted towards shorter wavelengths (around 600 nm) than expected (Sumitomo et al. 2012). Similarly, distortion in the spectral sensitivity to flowering has been reported in *Lemna* (Ohtani and Kumagai 1980). In these cases, effects of yellow to red light have been distorted by the screening effect of chlorophyll in green leaves.

In the 1980s, molecular genetic studies identified five phytochrome genes (*PHYA*, *B*, *C*, *D* and *E*) in *Arabidopsis* (Clack et al. 1994). Phytochromes are classified into two groups (type I and II) according to their protein stability in light. PhyA is classified into type I, which accumulates under dark conditions and rapidly degrades when exposed to light. phyA mediates VLFR with a broad range of light and HIR with FR light. PhyB to phyE are light stable type II phytochromes that accumulate relatively constantly under light or dark conditions (Sharrock and Clack 2002). PhyB, phyD and phyE mediate R:FR reversible LFR and/or R:FR ratio response, which is the shade-avoidance response (Li et al. 2011). phyC

mediates R light-induced HIR in seedling de-etiolation (Franklin et al. 2003; Monte et al. 2003). In the photoperiodic control of flowering, phyA mediates the blue- and FR-light promotion of flowering, whereas phyB mediates R-light inhibition of flowering (Goto et al. 1991; Johnson et al. 1994; Mockler et al. 2003; Franklin and Quail 2010).

5.1.2 Cryptochromes (Crys)

Cryptochromes are FAD- and pterin-containing chromoproteins that share considerable homology with DNA photolyases but lack photolyase activity (Ahmad and Cashmore 1993). Cryptochromes have two domains, the N-terminal phytolyase homology region (PHR) domain that binds chromophore and C-terminal cryptochrome C-terminus (CCT) domain, which is necessary for signal transduction (Fig. 5.1). In *Arabidopsis*, two cryptochromes (cry1 and cry2) are present as blue (B)/UV-A photoreceptors that are involved in many biological responses such as inhibition of hypocotyl elongation, entrainment of the circadian clock, stomata opening, pigment biosynthesis and photoperiodic flowering (Yu et al. 2010). Cry1 protein is light stable, but cry2 is light labile. The cry2 protein is accumulated in the dark and degraded upon exposure to B light, showing diurnal rhythms (Lin et al. 1998; Mockler et al. 2003). Cry2 promotes flowering by stabilising the CONSTANS (CO) protein, a positive regulator of florigen in the long day (LD) evening (Valverde et al. 2004).

5.1.3 Phototropins (Phots)

Phototropin was first identified as a photoreceptor mediating a blue-light-induced phototropic response in *Arabidopsis*, but its structure is different from that of cryptochromes (Huala et al. 1997). Phototropins harbour two LOV domains (LOV1 and LOV2) at their N-terminus that bind FMN as chromophores and the Ser/Thr kinase domain at their C-terminus (Fig. 5.1). *Arabidopsis* contains two phototropins (phot1 and phot2) (Huala et al. 1997; Kagawa et al. 2001) that regulate numerous blue/UV-A-induced responses, maximising photosynthetic activity such as phototropism, chloroplast relocation, leaf flattening and stomatal opening (Briggs and Christie 2002; Christie 2007). Phot1 acts over a wide range of light intensities, whereas phot2 functions predominantly at high light intensities (Christie et al. 2015).

5.1.4 Zeitlupe Family Proteins (ZTL/FKF1/LKP2)

ZEITLUPE (ZTL), FLAVIN-BINDING KELCH REPEAT F-BOX 1 (FKF1) and LOV KELCH REPEAT PROTEIN2 (LKP2) are a recently identified novel class of blue-light receptor proteins. They regulate circadian rhythms and photoperiodic flowering. The ZTL family proteins possess one LOV domain at the N-terminus, which binds FMN as chromophores. They possess an F-box and six kelch repeats at their C-terminus (Fig. 5.1) and regulate target protein degradation via the ubiquitin-proteasome system (Ito et al. 2012). ZTL forms a complex with a clock-related protein GIGANTEA (GI) in a blue-light-dependent manner and regulate protein degradation of TIMING OF CAB EXPRESSION I (TOC1), a core clock component factor, to generate circadian rhythms (Más et al. 2003; Kim et al. 2007). FKF1 also interacts with GI in a blue-light-dependent manner and controls protein degradation of CYCLING DOF FACTOR 1 (CDF1), a negative regulator of flowering, to promote flowering (Sawa et al. 2007).

5.1.5 UV-B Receptor (UVR8)

More recently, the ultraviolet-B radiation (UV-B: 280–315 nm) photoreceptor UV RESISTANCE LOCUS 8 (UVR8) has been identified in *Arabidopsis* (Rizzini et al. 2011). UVR8 is a 440 amino acid protein that has beta-propeller structures (Fig. 5.1). UVR8 exists as an inactive homodimer under UV-B-deficient light conditions, but rapidly monomerises upon UV-B irradiation, which triggers numerous UV-B responses. Unlike other photoreceptors, UVR8 does not bind subsidiary chromophores, but specific intrinsic tryptophans function as chromophores for UV-B perception (Rizzini et al. 2011). The monomerised active UVR8 forms a complex with CONSTITUTIVE PHOTOMORPHOGENIC 1 (COP1) and acts as a positive regulator of UV-B signalling via the regulation of downstream gene expressions. UVR8 mediates a number of UV-B-induced responses such as photomorphogenesis, pigment biosynthesis and pathogen resistance induction (Tilbrook et al. 2013).

5.2 Light-Dependent Seed Germination

Seed germination is the first step for seed plants to initiate a new life cycle. In several species, such as lettuce, tobacco and *Arabidopsis*, light is an important regulator of seed germination. Borthwick et al. (1952) demonstrated that red light (R; 600–700 nm) induced germination in a lettuce seed variety (cv. Grand Rapids) that was pre-soaked in water in darkness and showed that far-red light (FR; 700–800 nm) could reverse this induction (Fig. 5.4a). This photomorphogenic

response ultimately led to the identification and purification of the R:FR-absorbing photoreceptors and phytochromes. Phytochromes are the major class of photoreceptors responsible for germination. Classical physiological studies have suggested the involvement of plant hormones, gibberellin (GA) and abscisic acid (ABA) as critical regulators of seed germination. R light that induces germination can be substituted by application of GA to lettuce seeds, whereas an application of ABA inhibits germination. Thus, endogenous levels of GA and ABA might be controlled by light. In fact, the endogenous levels of GA and ABA are oppositely modulated in a light-dependent manner (Seo et al. 2009). Phytochromes regulate GA biosynthesis in germinating lettuce and *Arabidopsis* seeds. Using the *phyA* and *phyB* mutants of *Arabidopsis*, phyB is the dominant phytochrome involved in the light-induced germination with the typical R:FR photoreversible response. In *Arabidopsis*, upregulation of two biosynthetic genes, *GA3ox1* and *GA3ox2*,catalyses the conversion of precursor GAs to their bioactive forms, and expression in the hypocotyl of embryos following exposure to R-light, in a phyB-mediated process, is associated with germination, whereas a GA catabolic gene, *GA2ox2*, is repressed (Fig. 5.4b). After a long period of imbibition in the dark, phyA plays a role in the irreversible response to extremely low levels of light over a wide range of wavelengths (Shinomura et al. 1996). PHYTOCHROME-INTERACTING FACTOR 3-LIKE 5 (PIL5) regulates seed germination negatively through GA (Oh et al. 2006). Expression analysis revealed that PIL5 represses the expression of *GA3ox1* and *GA3ox2* and activates the expression of *GA2ox* in both PHYA and PHYB dependent. ABA accumulates in seeds to promote dormancy and prevent premature

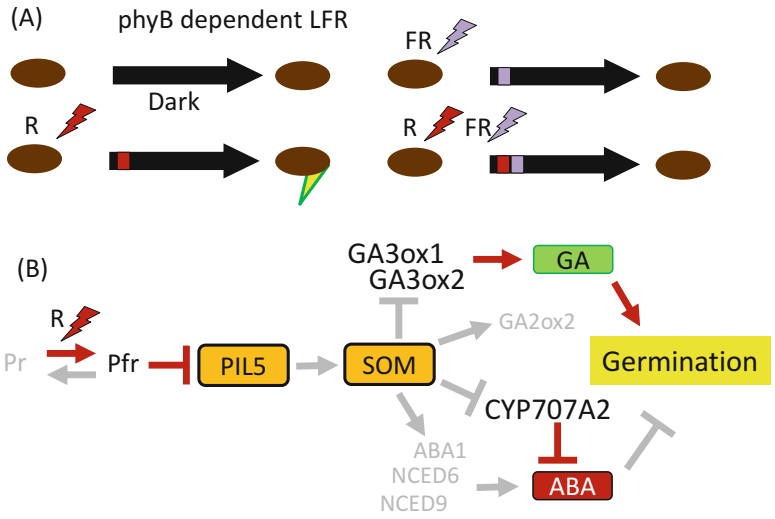

Fig. 5.4 Phytochrome B-mediated seed germination in *Arabidopsis*. (**a**) PhyB-mediated seed germination shows typical R:FR photoreversible response (LFR). (**b**) PhyB Pfr form regulates GA and ABA levels through negative regulators of seed germination, PIL5 and SOM. Active regulations after R-light exposures are shown in *Red arrows* or T-bars

germination. Consistent with the change in ABA levels, the ABA biosynthetic genes, *ABA-DEFICIENT 1* (*ABA1*), *NINE-CIS-EPOXYCAROTENOID DEOXYGENASE 6* (*NCED6*) and *NCED9*, are repressed, whereas an ABA catabolic gene, *CYP707A2*, which encodes an ABA 8′-hydroxylase, is induced by R-light exposure. PIL5 regulates both GA and ABA metabolic genes partly through SOMNUS (SOM) (Kim et al. 2008).

5.3 De-etiolation

In the dark, a seedling adopts skotomorphogenesis where it develops a long hypocotyl, an apical hook and closed cotyledons. Skotomorphogenesis is achieved by the active repression of genes that would lead to photomorphogenic development. When exposed to light, the seedling starts the de-etiolation process and switches rapidly to photomorphogenesis and inhibition of the hypocotyl elongation, promoting cotyledon development, opening the apical hook and cotyledons and initiating chlorophyll and anthocyanin biosynthesis, and true leaves begin to develop. Several classes of photoreceptors, phytochromes, cryptochromes and phototropins are involved in the photomorphogenic development (Fig. 5.5). The COP1-SUPPRESSOR OF PHYA-105 (SPA) complexes function as an E3 ubiquitin ligase and repress photomorphogenesis. COP1-SPA complexes control the light-regulated abundance of LONG HYPOCOTYL5 (HY5). HY5 is a basic leucine zipper transcription factor that binds to the promoters of numerous light-regulated genes to regulate photomorphogenic development. In the dark, COP1-SPA

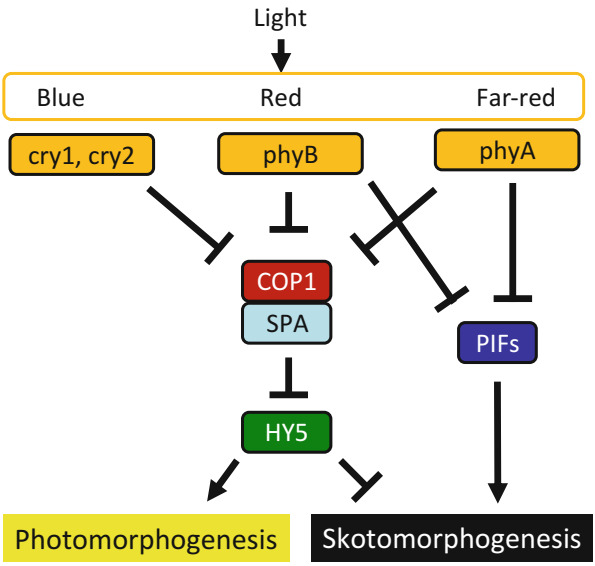

Fig. 5.5 A simplified model of the light regulation. Light sensed by photoreceptors acts to suppress two main light signalling pathways, through COP1/SPA-HY5 and PIFs

complexes target HY5 for ubiquitination, inducing proteasomal degradation. Light inactivates COP1-SPA complexes, so that HY5 accumulates. In addition to the COP1, a group of PHYTOCHROME-INTERACTING FACTORs (PIFs), basic helix-loop-helix transcription factors act to promote skotomorphogenesis (Leivar and Quail 2011). In the dark, PIFs are active and regulate gene expression to promote skotomorphogenesis. In the light, a nuclear-localised phytochrome (light-activated Pfr form) binds to PIFs and results in phosphorylation and subsequent degradation. The degradation of PIFs induces photomorphogenic development.

5.4 Phototropism

The growth of a plant towards any stimulus is called tropism, and the growth of a plant towards a light stimulus is called phototropism (Liscum et al. 2014). Phototropism is an important adaptive response where plants optimise their exposure to light. Blue wavelengths of light are more effective at orienting plant growth, which involves blue-light perception and asymmetric distribution of a plant hormone, auxin. The shoot bends towards the light because of differences in cell elongation on the two sides of the shoot. The side of the shoot that is in the shade has more auxin, and its cells therefore elongate more than those on the lighted side. The phototropic movement of plants is initiated by a blue-light receptor, phototropin (Whippo and Hangarter 2006). In *Arabidopsis*, two phototropins, phot1 and phot2, exhibit overlapping functions. The central importance of polar auxin transport and auxin signalling in phototropism has been demonstrated. Auxin efflux carrier PIN-FORMED (PIN) proteins possibly have central roles in regulating asymmetrical auxin translocation during tropic responses, including gravitropism and phototropism, in plants. When several of the PIN and kinase components were missing, plant growth was completely unresponsive to the light signals that trigger phototropism. A recent detailed analysis of various PIN gene mutants found that the contributions of PIN1, PIN3 and PIN7 to phototropic hypocotyl bending become relatively obvious when dark-grown seedlings are exposed to a short blue-light pulse (pulse-induced first positive phototropism). Strikingly, these phototropism defects become much weaker when seedlings are exposed to long-term blue-light treatments (second positive phototropism) (Haga and Sakai 2012). Blue light perceived by phototropin contributes the polar relocation of PIN proteins. Auxin streams and asymmetric growth are also regulated by AGCVIII kinases that are able to phosphorylate PINs (Barbosa et al. 2014). D6 PROTEIN KINASE (D6PK) subfamily of AGCVIII kinase-dependent PIN regulation promotes auxin transport in the hypocotyl that is a prerequisite for phot1-dependent hypocotyl bending (Willige et al. 2013). Since phytochrome-cryptochrome double mutants show a reduced phototropic response, the phototropins are not the only photoreceptors involved in phototropism (Whippo and Hangarter 2006).

5.5 Shade-Avoidance Response

The shade-avoidance response (SAR), which allows plants to escape from neighbour competitors, is an adopted response to the optimal acquisition of light energy to drive photosynthesis. The SAR is characterised by increased extension growth of the hypocotyl, stem and petiole, a more erect leaf position, increased apical dominance and early flowering (Franklin 2008). Photosynthetic pigments, such as chlorophylls and carotenoids, in the leaves absorb light over the 400–700 nm spectrum. The FR region (700–800 nm) of the spectrum is poorly absorbed by the photosynthetic pigments; consequently, sunlight reflected from or transmitted through leaves is enriched with FR light. Changes in light quality, low R:FR, are sensed by multiple light-stable phytochromes (phyB, phyD, phyE). A particular R:FR ratio is reflected in the Pfr:Pr ratio of phytochromes, thus determining the relative activity of phytochromes. Of these, phyB is the dominant phytochrome involved in the SAR (Fig. 5.6). The unique properties of phyA, a light-labile phytochrome, as an effective FR sensor in the HIR are important in natural light environments by 'antagonising' shade avoidance (Martinez-Garcia et al. 2014).

The end-of-day FR light (EOD-FR) treatment consists of a pulse of FR given at subjective dusk (Kasperbauer 1971). EOD-FR treatments result in a minimal pool of active Pfr during the dark period (Fankhauser and Casal 2004). In plants grown under day/night cycles, EOD-FR treatment mimics growth in low R:FR light conditions. The treatment is a useful method for experimentally inducing the SAR. Involvement of plant hormones, such as GA, auxin, ABA, cytokinin, ethylene and brassinosteroid, in light-regulated developments has been suggested. The perception of shade (EOD-FR) by the leaf blade induces petiole elongation in *Arabidopsis* (Kozuka et al. 2010), where it is speculated that newly synthesised auxin in the leaf blade accumulates in the petiole to induce responses. The cotyledons perceived shade (low R:FR) signal and generate auxin to regulate hypocotyl

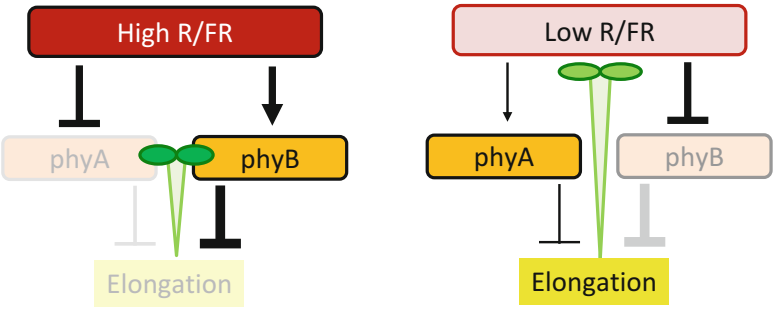

Fig. 5.6 A simplified model of the shade-avoidance response (SAR) on hypocotyl elongation. PhyB is the dominant phytochrome involved in the SAR. Light-activated phyB suppresses elongation. SAR induced by phyB deactivation is gradually antagonised by phyA, an HIR-FR response

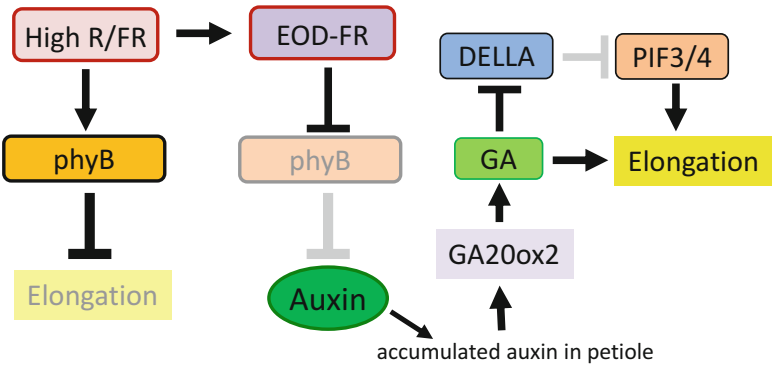

Fig. 5.7 A simplified model of the EOD-FR-induced auxin/GA cooperative petiole elongation in *Arabidopsis*. PhyB is the dominant phytochrome involved in EOD-FR. The synthesised auxin in the leaf blade by EOD-FR acts on the petiole. The *GA20ox2* transcript is upregulated in the petiole by the accumulated auxin. Increased GA promotes petiole elongation

elongation in *Brassica rapa* (Procko et al. 2014). These studies demonstrate the importance of inter tissue/organ communication in the SAR.

Transcriptomic analyses revealed that the expression of many genes related to plant hormones is regulated in response to EOD-FR (Kozuka et al. 2010). In *Arabidopsis*, upregulation of *GA20ox2* expression in the petiole following exposure to EOD-FR or low R:FR light, in a phyB-mediated process, is associated with enhanced petiole elongation (Hisamatsu et al. 2005) and floral induction (Hisamatsu and King 2008). Auxins act on the GA biosynthesis by specifically regulating the expression of two genes, *GA20ox1* and *GA20ox2* (Frigerio et al. 2006). Together, the auxin/GA cooperative response induced by EOD-FR that synthesised auxin in the leaf blade accumulated in the petiole and induced the expression of *GA20ox2*, and the increased GA induced petiole elongation (Fig. 5.7). PIFs, key transcription factors for photomorphogenesis, are growth-promoting factors (Leivar and Quail 2011), which integrate GA signalling and phytochrome-mediated SAR. The DELLA family proteins that repress GA-regulated growth are mediators of GA signalling. GA promotes DELLA degradation by binding to a GA receptor, GID1 (Hedden and Thomas 2012). DELLAs interact with PIF3/4, which blocks transcriptional activity and inhibits PIF function (Sun 2011). When plants are subjected to low R:FR from high R:FR conditions, GA levels may increase. The increased GA would promote DELLA degradation, relieving DELLA-mediated inhibition of PIF function and enhancing extension growth (Lorrain et al. 2008).

5.6 Circadian Rhythms and Biological Responses

Many circadian rhythm-related genes have been identified during flowering time mutant analyses. The time measurement mechanism is composed of an input pathway, central oscillator and output pathway. The light signals perceived by

photoreceptors such as phytochromes and cryptochromes entrain the clock. The central oscillator is composed of an interlocked transcriptional and post-transcriptional feedback loop that generates an approximately 24-h free running rhythm. The clock components include CIRCADIAN CLOCK-ASSOCIATED 1 (CCA1), LATE ELONGATED HYPOCOTYL (LHY), TIMING OF CAB EXPRESSION 1 [TOC1, also called PSEUDO-RESPONSE REGULATOR 1 (PRR1)], PRR5/7/9, REVEILLE 8 (RVE8), EARLY FLOWERING 3 (ELF3), ELF4, LUXARRHYTHMO [LUX, also known as PHYTOCLOCK 1 (PCL1)], GIGANTEA (GI) and ZEITLUPE (ZTL) (Hsu and Harmer 2014; Greenham and McClung 2015) (Fig. 5.8). The loss or gain of function of these factors results in aberrant rhythmic phenotypes. The output pathway includes regulatory factors that directly involve biological processes, such as extension growth and flowering.

PIFs are important as growth-promoting factors. PIF4 and PIF5 rhythmically express over a diurnal cycle with maximal mRNA abundance either at dawn or early morning, regardless of photoperiod conditions. The circadian clock directly controls this transcript oscillation pattern. The evening clock components ELF3, ELF4 and LUX functionally repress PIF transcription at dusk. During the day, PIFs are degraded at the post-translational level by interacting with light-activated phytochromes. Therefore, PIFs accumulate during the night, when plant growth rate is highest (Nozue et al. 2007; Nusinow et al. 2011).

Correct entrainment of circadian clocks is essential because the phase of circadian rhythms relative to the day/night cycle affects flowering time. The *toc1-1* mutant, a short-period clock mutant, shows an early flowering phenotype under SD conditions. The early flowering phenotype of the *toc1-1* mutant can be explained by their short-period phenotype and phase advance in *CONSTANS* (*CO*) mRNA expression (Yanovsky and Kay 2002). *CO* rhythmically expresses over a diurnal

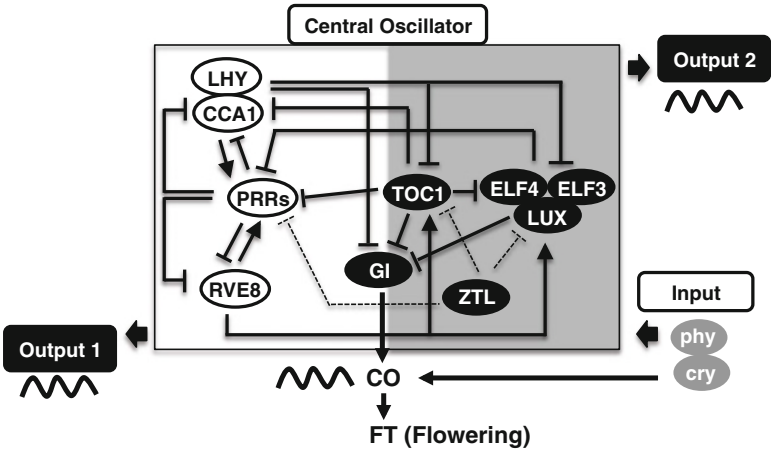

Fig. 5.8 A model for the circadian clock in *Arabidopsis*. The circadian clock is composed of multiple interlocked transcriptional and post-transcriptional feedback loops (The model was adapted from Greenham and McClung (2015))

cycle under controlling circadian clock, only when *CO* is expressed at high levels in the light phase, and the CO protein is stabilised by interacting with light signals and induces *FLOWERING LOCUS T* (*FT*) transcription, a gene encoding florigen. In wild-type plants, the rhythm of *CO* expression creates a light-sensitive phase starting from about 8 h after dawn, so there is little *CO* expression during the day in 8-h SD conditions. In *toc1-1* mutants grown in 8-h SD conditions, the phase of *CO* expression was significantly advanced, leading to a coincidence between relatively high levels of *CO* transcription during the day at dusk. The interaction between transcriptional regulation of CO by endogenous circadian clocks and external light signals at a particular phase is essential for day-length recognition for flowering.

5.7 The Gating Effects of Circadian Clocks

The phenomenon where any effects of external stimuli are limited to certain phase of the circadian clock is referred to as 'the gating effect'. For example, the inhibitory effect of NB on the floral initiation of SDP is restricted to certain time of night, and if plants were kept in continuous darkness, the photosensitive phase would appear every 24 h (Hamner and Takimoto 1964, Fig. 5.9). In *Arabidopsis*, *CHLOROPHYLL a/b-BINDING PROTEIN* (*CAB*) expression showed diurnal rhythms peaking during the day. Although a light pulse given during the day strongly induced *CAB* expression, the light given at night did not (Millar and Kay 1996), indicating that the induction of *CAB* expression by light is gated by a circadian clock. ELF3 mediates circadian gating of light responses. In the *elf3* mutant, which shows photoperiod-insensitive early flowering, the circadian

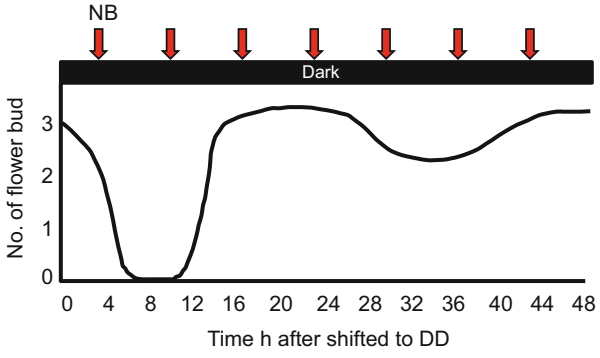

Fig. 5.9 The gated NB response in SDP. The effect of a short light pulse given at different times of night on the flowering response of *Pharbitis*. The inhibitory effect of NB on flowering occurred periodically at 8–10 h and 32–36 h after dusk under continuous darkness (Redrawn from Hamner and Takimoto (1964))

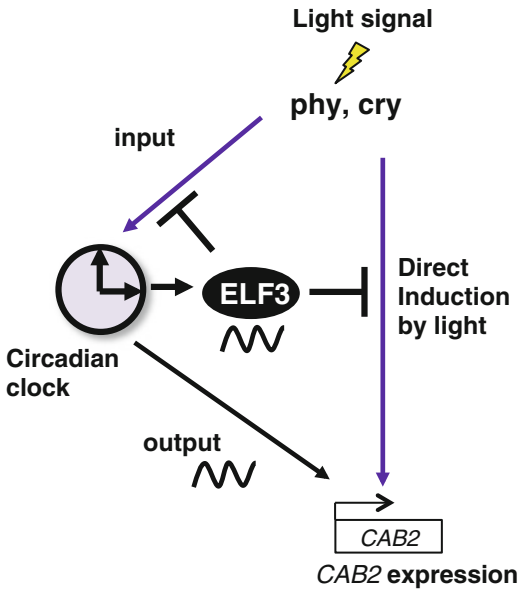

Fig. 5.10 The gating mechanism of *CAB2* expression by ELF3 and the circadian clock. *CAB2* expression shows robust diurnal rhythms peaking during the day. ELF3 acts to suppress the light input to the clock and inhibits the acute induction of *CAB2* by light at subjective night

rhythmicity of *CAB* expression disappeared under continuous light (LL), but not continuous dark (DD) (Hicks et al. 1996;). ELF3 suppressed light input to the circadian clock at a particular time of day (McWatters et al. 2000, Fig. 5.10).

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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

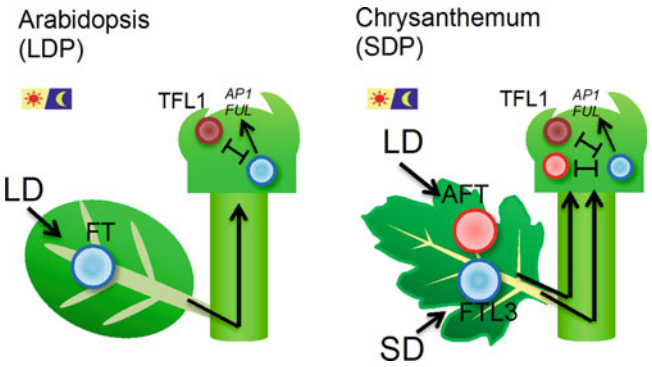

Fig. 6.1 Flowering time regulation by florigen and anti-florigen. In *Arabidopsis*, FT is synthesised in leaves under a flower-inducing LD photoperiod that moves to the shoot apex to induce floral-meristem identity genes. TFL1 is expressed in the shoot apex and suppresses flowering by antagonising FT 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

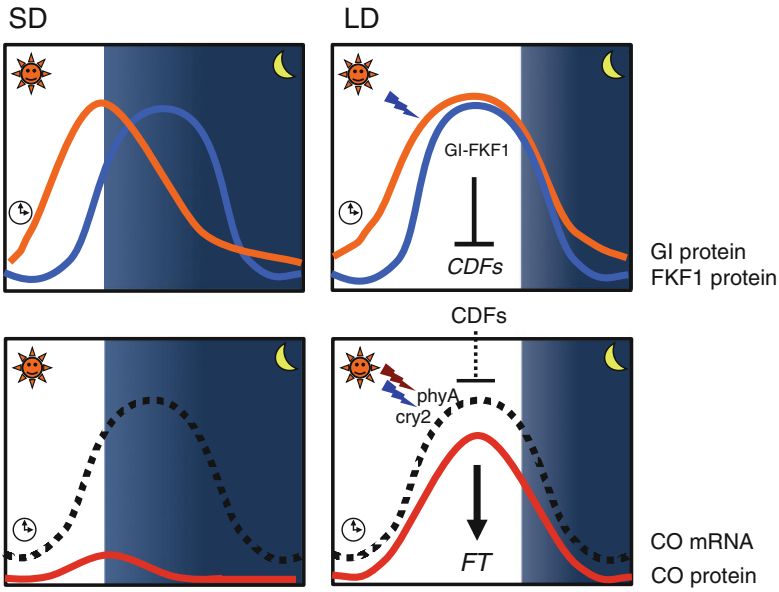

Fig. 6.2 Photoperiodic regulation of *FT* by internal and external coincidence. Accumulation of GI and FKF1 proteins synchronises in the late afternoon under LD and forms a complex (GI-FKF1) in a *blue* light-dependent manner. The activated GI-FKF1 then degrades CDF proteins, negative regulators of *CO* 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

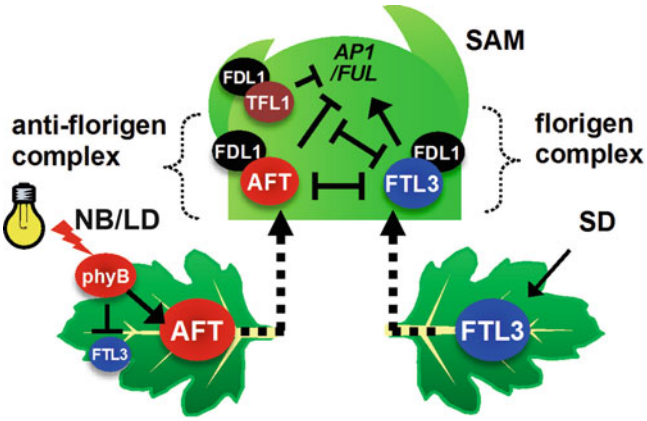

Fig. 6.3 Photoperiodic regulation of flowering in chrysanthemums. Under flower-inductive SD, FTL3 is produced in leaves to systemically induce flowering. Under non-inductive NB or LD, AFT is synthesised in leaves to systemically inhibit flowering. NB with *red* light was perceived by phyB that induces *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

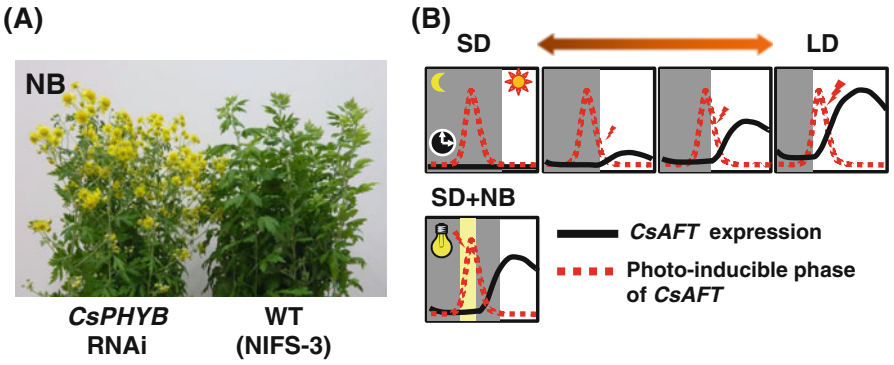

Fig. 6.4 PHYB-mediated and gated induction of *AFT*. (**a**) Flowering response of WT and *PHYB*-RNAi *Chrysanthemum seticuspe* plants under NB with red light. *PHYB*-RNAi plants are almost insensitive to NB. (**b**) Model for the induction of *AFT* in response to natural day-length extension and artificial 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).

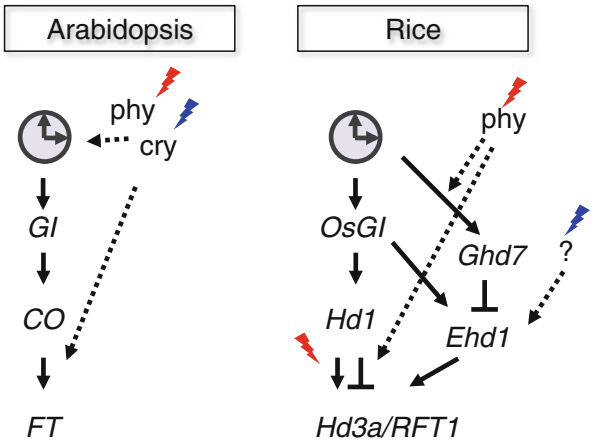

Fig. 6.5 Comparison of photoperiodic flowering pathways in *Arabidopsis* and rice. The circadian clock output *GI-CO-FT* pathway is conserved in *Arabidopsis* and rice, but the regulation of *FT* (*Hd3a*) by *CO* (*Hd1*) is reversed. Rice has an alternative pathway (*Ghd7-Ehd1*) that functions independently 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

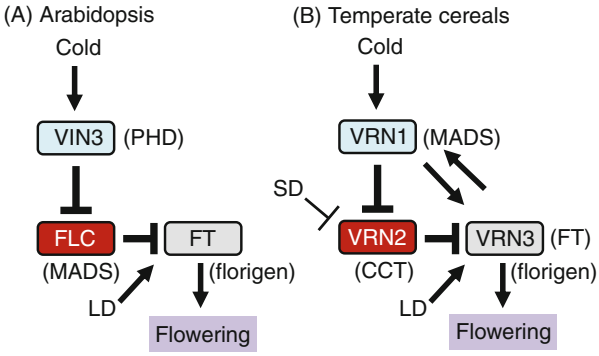

Fig. 6.6 A simplified model of the vernalization response on the florigen production in *Arabidopsis* (**a**) and temperate cereals (**b**)

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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