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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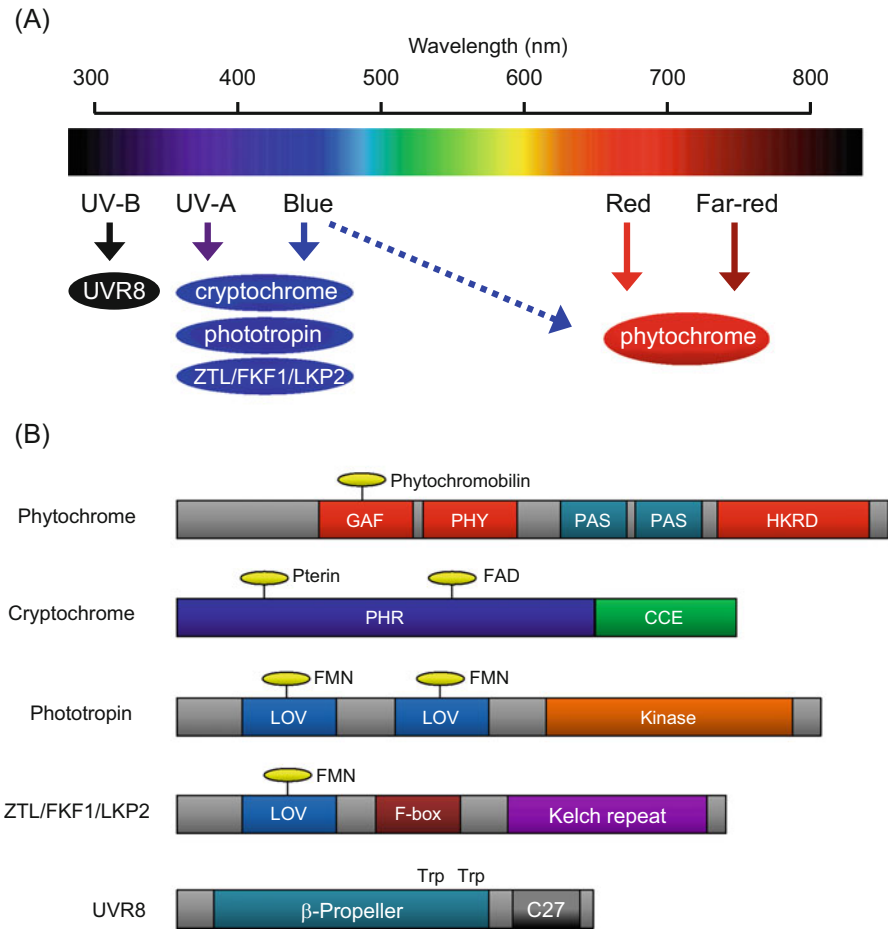

Fig. 5.1 Photoreceptors in higher plants. (**a**) Photoreceptors perceiving different parts of the light spectrum. (**b**) Structure of photoreceptor proteins. Domain structure and binding chromophores are shown. *GAF* cGMP-stimulated phosphodiesterase; *Anabaena* adenylate cyclases and *Escherichia 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

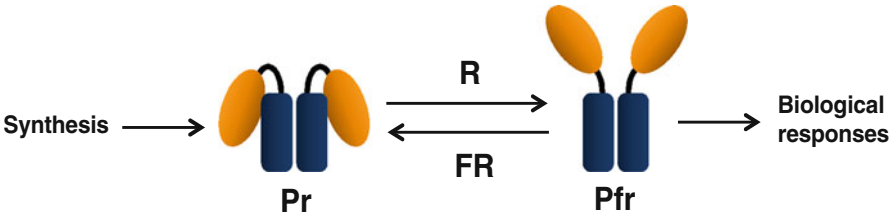

Fig. 5.2 Photoconversion of phytochrome. Phytochromes are synthesised in the Pr form. Pr absorbs R light and is converted to its biologically active form, Pfr, which induces various physiological responses. Pfr absorbs FR light and is converted it its inactive Pr form

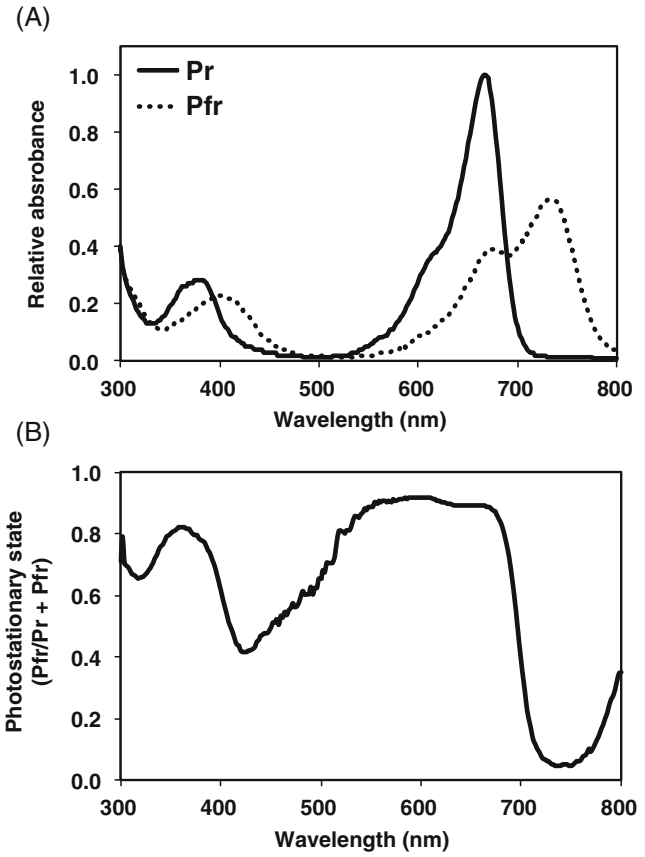

Fig. 5.3 Relative absorption spectra of purified rye phytochrome in its Pr and Pfr forms (**a**) and calculated photostationary state (**b**) (Data derived from Sager et al. 1988)

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

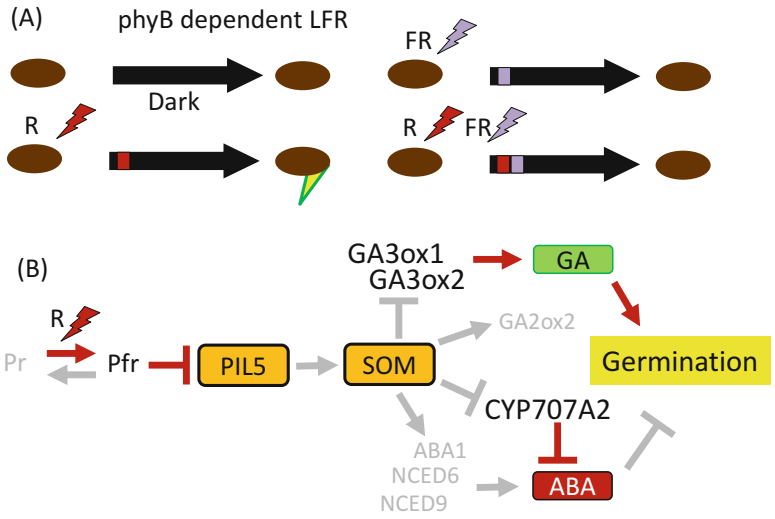

Fig. 5.4 Phytochrome B-mediated seed germination in *Arabidopsis*. (**a**) PhyB-mediated seed germination shows typical R:FR photoreversible response (LFR). (**b**) PhyB Pfr form regulates GA and ABA levels through negative regulators of seed germination, PIL5 and SOM. Active regulations after R-light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

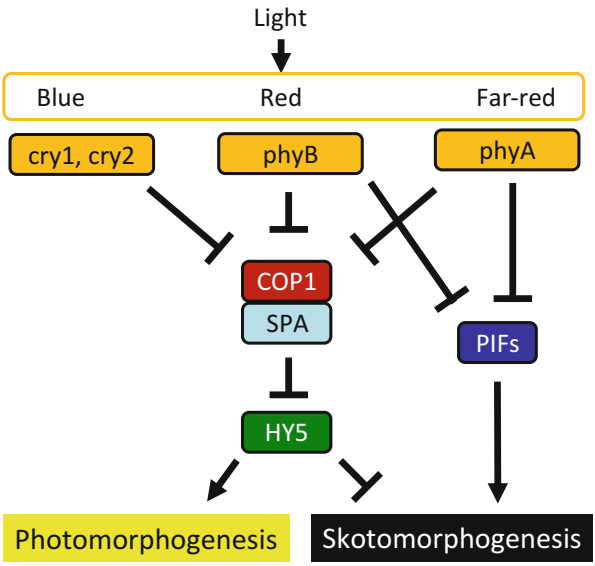

Fig. 5.5 A simplified model of the light regulation. Light sensed by photoreceptors acts to suppress two main light signalling pathways, through COP1/SPA-HY5 and PIFs

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

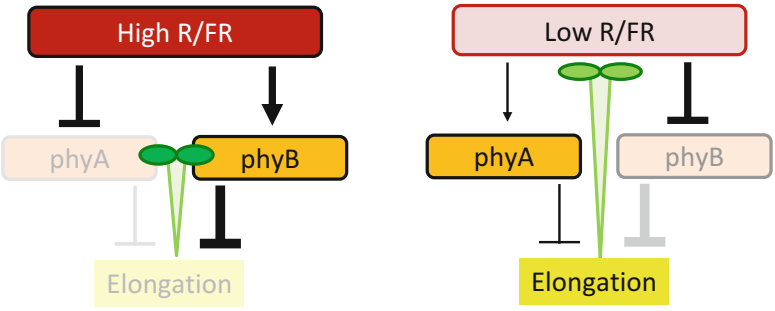

Fig. 5.6 A simplified model of the shade-avoidance response (SAR) on hypocotyl elongation. PhyB is the dominant phytochrome involved in the SAR. Light-activated phyB suppresses elongation. SAR induced by phyB deactivation is gradually antagonised by phyA, an HIR-FR response

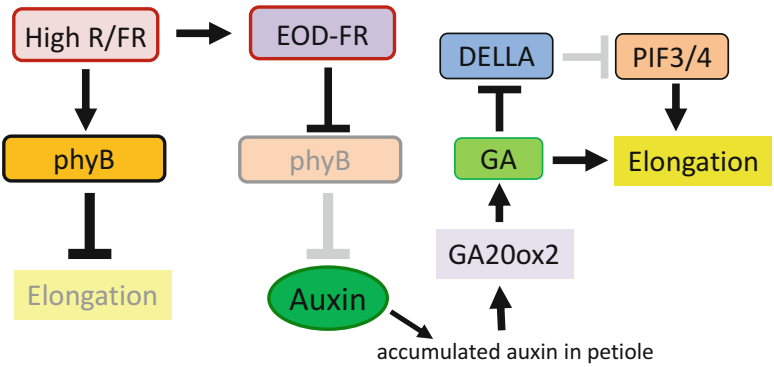

Fig. 5.7 A simplified model of the EOD-FR-induced auxin/GA cooperative petiole elongation in *Arabidopsis*. PhyB is the dominant phytochrome involved in EOD-FR. The synthesised auxin in the leaf blade by EOD-FR acts on the petiole. The *GA20ox2* transcript is upregulated in the petiole by the accumulated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

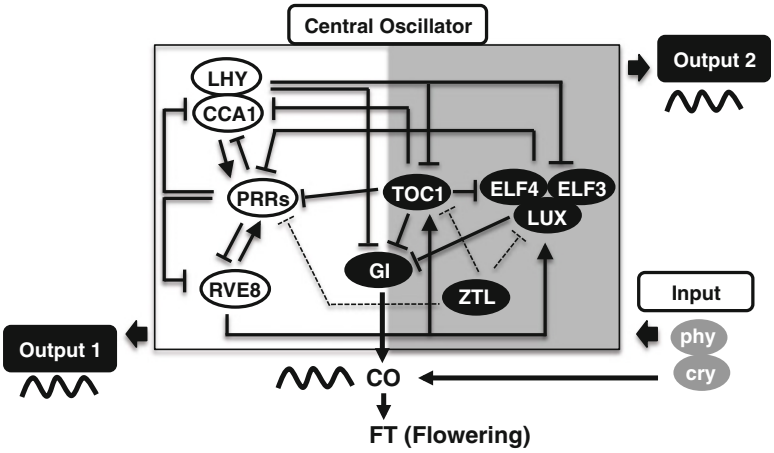

Fig. 5.8 A model for the circadian clock in *Arabidopsis*. The circadian clock is composed of multiple interlocked transcriptional and post-transcriptional feedback loops (The model was adapted from Greenham and McClung (2015))

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

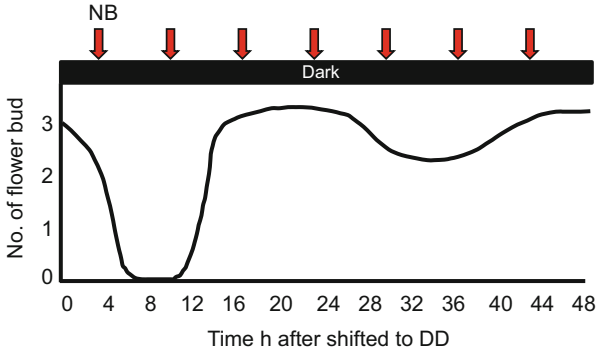

Fig. 5.9 The gated NB response in SDP. The effect of a short light pulse given at different times of night on the flowering response of *Pharbitis*. The inhibitory effect of NB on flowering occurred periodically at 8–10 h and 32–36 h after dusk under continuous darkness (Redrawn from Hamner and Takimoto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

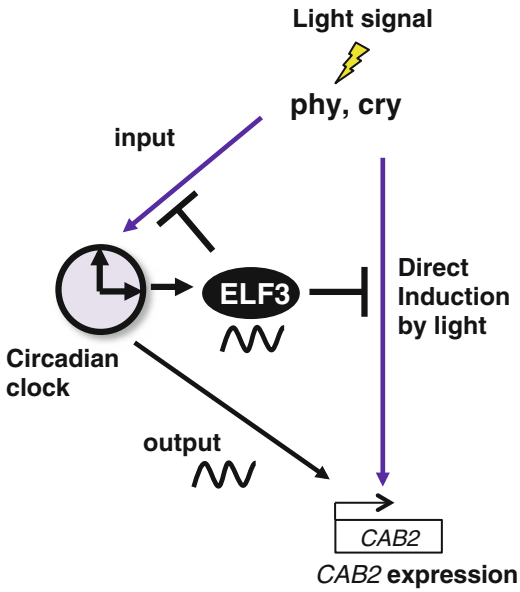

Fig. 5.10 The gating mechanism of *CAB2* expression by ELF3 and the circadian clock. *CAB2* expression shows robust diurnal rhythms peaking during the day. ELF3 acts to suppress the light input to the clock and inhibits the acute induction of *CAB2* by light at subjective night

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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