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Regulation of gene expression by light

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ABSTRACT Light signals perceived mainly by phytochromes and cryptochromes regulate plant growth and development by driving dramatic shifts of the transcriptome. Early light-responsive genes include a large proportion of transcripton factors of different DNA binding motifs. Mutations at loci encoding several transcriptional regulators, including some of those showing rapid changes in transcript levels, impair responses to light in *Arabidopsis thaliana*. Proteasome-mediated degradation involving the E3 ligase COP1 provides an additional layer of control of the levels of transcription factors. Some transcriptional regulators are shared by light, circadian and/or hormonal signalling circuits creating complex networks that interactively integrate environmental and endogenous cues.

KEY WORDS: *phytochrome, cryptochrome, transcriptome, transcription factors, light (Arabidopsis)*

Introduction

Light signals adjust plant growth and development to the prevailing environmental conditions (Casal *et al.*, 2004). One of the key events in the life cycle of plants is the acquisition of photosynthetic capacity and the re-direction of growth that take place when dark-grown tissues emerge the soil and become exposed to the daily light cycles. The developmental pattern followed in darkness is called skotomorphogenesis, where plants are etiolated, while that followed in the light is called photomorphogenesis (Fig. 1). Once in the light, if the proportion of red compared to far-red light is reduced by the presence of nearby neighbours, which selectively reflect and transmit far-red light, plants adopt a more competitive vegetative body form. Finally, neighbour signals themselves or the seasonal cues provided by the photoperiod (number of illuminated hours per day) can initiate the transition to the reproductive development (flowering) or the formation of vegetative reserve organs. The morphological changes and the photoreceptors involved in the aforementioned processes are described in further detail in another paper of this Special issue (Whitelam). Here we concentrate on light effects on the patterns of expression of nuclear-encoded genes that emerge from microarray experiments and on the mechanisms of these responses as revealed by the combination of molecular and genetic approaches in *Arabidopsis thaliana*. Excellent earlier reviews describe previous findings (Simpson and Herrera-Estrella, 1990; Thompson and White, 1991; Bowler and Chua, 1994; Terzaghi and Cashmore, 1995).

Light control of transcriptome patterns

The first use of microarrays prepared by high speed robotic printing of complementary DNA on glass substrates in combination with two-colour fluorescent labelling of the samples and laser scanning of the fluorescence intensity was applied to *Arabidopsis* plants overexpressing a homeodomain transcription factor involved in the control of photomorphogenesis (Schena *et al.*,

Abbreviations used in this paper: AP, apetala; BRI, brassinosteroid-insensitive; CDA1, CAB2 DET1-associated factor 1; CHS, chalcone synthase; CCA1, circadian clock associated 1; CIP7, cop1-interacting protein 7; CGF, CAB gata factor; CO, constans; COP, constitutive photomorphogenesis; CRY, cryptochrome; CUF-1, CAB upstream factor-1; DDB1, uv-damaged DNA binding protein 1; DET, de-etiolated; DTRE, DET1 dark response element; ELF3, early flowering 3; EST, expressed sequence tag; FKF, flavind binding, kelch repeat, f box; FHY, far-red elongated hypocotyl; FT, flowering locus t; GAI, gibberellin insensitive; HAT, homeobox from arabidopsis thaliana; HFR1, long hypocotyl under far-red; HIR, high-irradiance response; HY5, hypocotyl 5; HYH, HY5 homolog; IAA, indoleacetic acid-induced protein; LHC, light harvesting chlorophyll a/b-binding; LAF1, long after far-red light; LFHY, leafy; miRNAs, micro RNAs; PETe, planstocyanin; PFR, active form of phytochrome; PHOT, phototropin; PHY, phytochrome; PIF, phytochrome interacting factor; PIL, PIF3 like; PIN, pin-formed; RBCL; rubisco carboxylase-oxygenase large subunit; RBCS, rubisco carboxylase-oxygenase small subunit; siRNAs, short interfering RNAs; SMZ, schlafmütze; SNZ, schnarchzapfen; SOC, suppressor of constans; SPA1, suppressor of PHYA; TOC, timing of cab expression; XTR7, xyloglucan endotransglucosylate;

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1995). Since those early days microarrays have added a new dimension to the understanding of the regulation of plant gene expression by light.

Different body form, different transcriptomes in dark- compared to light-grown seedlings

Ma *et al.*, (2001) used an expressed sequence tag (EST)-based microarray to investigate the differences in gene expression patterns in 6-day-old *Arabidopsis* seedlings grown either in complete darkness or under white light. Of the 9216 ESTs included in the array, 32 % showed at least two-fold differences between white light and darkness, 18 % ESTs showed higher expression in light-grown seedlings and 14 % showed the opposite pattern. More than 3/4 of these ESTs were also affected in dark-grown seedlings exposed to 36 h white light or light-grown seedlings transferred to darkness for 36 h, indicating a relatively rapid shift in transcriptome patterns. Light can coherently affect genes with products involved in a given pathway. Figure 1 illustrates some of these processes, which are obviously related to the morphological and physiological responses to light: the synthesis of the photosynthetic machinery, including the structures and enzymes necessary for photosynthesis and the enhanced foliage growth and metabolism in the light, as well as the reduced extension growth of the stem. Interestingly, brasinosteroids are required to maintain the skotomorphogenic pattern in darkness (Li *et al.*, 1996) and brassinosteroid biosynthesis enzymes are down regulated in 6-day-old light-grown compared to dark-grown seedlings. Seedlings grown for 6 days under red (600-700 nm), blue (400-700 nm) or far-red light (700-800 nm) display largely similar transcriptome patterns when compared to darkness (Ma *et al.*, 2001). Since these wavebands activate different sets of pigments (phytochromes, cryptochromes, phototropins, chlorophylls, see below), these results suggest convergence of their signal transduction pathways onto a similar set of target genes. Again, this is consistent with the similar overall pattern of development under these conditions and its strong contrast with the developmental pattern in darkness. However, red, blue of far-red light induce quantitative differences. Most metabolic pathways are more sensitive to red and blue light than to far-red. Conversely, genes involved in phenylpropanoid biosynthesis, ethylene biosynthesis, brassinosteroid biosynthesis and glyoxylate cycle are more sensitive to far-red than red or blue light (Ma *et al.*, 2001).

Multiple transcription factors are early targets of phytochrome action during de-etiolation

Arabidopsis bears five phytochromes (phyA through phyE) (Quail *et al.*, 1995), the photoreceptors that perceive red and far-red light. The proportion of phyrochromes in their active form (Pfr) is large under red and small under far-red light. The amount of Pfr under far-red light is biologically negligible for most members of the phytochrome family with the exception of phyA. In the case of phyA, far-red light activates the so-called high-irradiance response (HIR) mode, which requires specific domains of the phyA molecule (Yanovsky *et al.*, 2002) and of the promoter of target genes (Cerdán *et al.*, 2000). The seedlings that emerge from the soil under a dense plant canopy become exposed to an environment rich in far-red light and require phyA to de-etiolate. In seedlings grown in darkness for 4 days and then transferred to continuous far-red light for several hours, transcriptome responses are largely mediated by phyA. During the first day of irradiation, approximately 10 % of the genes present in the Affymetrix *Arabidopsis* 8 K microarray are affected by far-red light and the number of induced genes doubles that of repressed genes (Tepperman *et al.*, 2001). These observations are consistent with the strong developmental shift initiated by the treatment and the higher functional complexity observed in de-etiolated seedlings. Most genes respond within the first 3 h and reach a maximum response within the first 12 h of far-red light. Following the maximum, some of the far-red light-induced genes decay in their expression levels within the first 24 h. In general, far-red light-repressed genes remain low after the initial change. More than 30 % of the genes responding to far-red light have not been assigned a cellular function but the largest functional classes correspond to the genes involved in photosynthesis / chloroplast development and cellular metabolism. Although the development of full photosynthetic capacity can take several days, some photo-

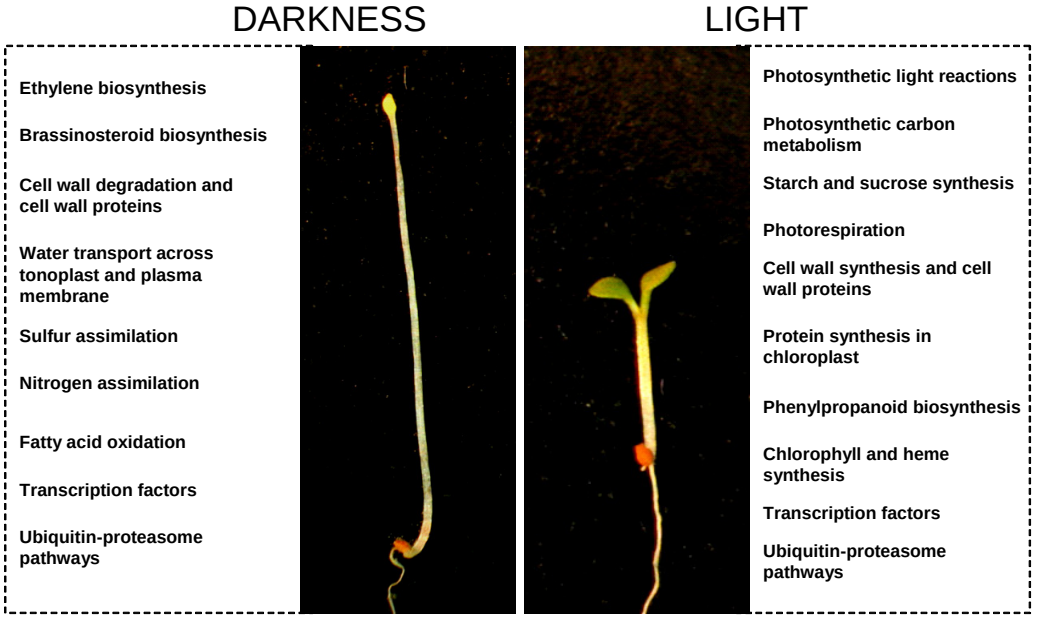

Fig. 1. Dark-grown and light-grown seedlings show large differences in transcriptome patterns that accompany the different morphology and degree of development of the photosynthetic apparatus. *The gene groups showing differential expression are shown under the condition of higher expression. Based on Wang* et al. *(2001).*

synthetic genes respond to the far-red light signal within the first hour of treatment. Transcription factors belonging to diverse classes, including zinc-finger, bZIP, homeodomain, MYB, APETALA 2 (AP2)-domain, WRKY and bHLH proteins, dominate the group of early genes (Tepperman *et al.*, 2001).

Differential contribution of phyB to transcriptome and morphological responses to red light

Red light activates all members of the phytochrome family and chlorophylls. Although phyA is the most abundant phytochrome in dark-grown seedlings, red light rapidly establishes a high proportion of Pfr, which in the case of phyA is degraded in the 26 proteasome (Seo *et al.*, 2004). Thus, phyB, the second most abundant phytochrome takes the lead in the control of seedling morphology (Quail *et al.*, 1995). At least during the first 24 h of irradiation, continuous red light induces changes in transcript level that largely overlap with those induced by continuous far-red light (Tepperman *et al.*, 2004). The similarity is particularly striking when the effects on early responsive transcription factors are considered. Although the *phyB* mutant has a clear morphological phenotype under red light its transcriptome shows little difference with the wild type. Only 14 % of the genes that respond to red light exhibit a relatively robust dependence on phyB. The residual effect of red light observed in the *phyA phyB* double mutant is at least partially mediated by other phytochromes (phyC, phyD and/or phyE) (Hamazato *et al.*, 1997). Clearly, different processes simultaneously controlled by red light have a differential contribution of the various members of the phytochrome family.

Cryptochrome 1 is the key photoreceptor in transcriptome responses to blue light

Blue light activates multiple photoreceptors. Cryptochromes (cry1, cry2) (Cashmore *et al.*, 1999; Lin and Shalitin, 2003) and phototropins (phot1, phot2) (Kasahara *et al.*, 2002) are specific blue light photoreceptors. Phytochromes also absorb blue light, which establishes an intermediate proportion of Pfr. Detailed kinetics studies have shown that the rate of hypocotyl growth of dark-grown *Arabidopsis* declines with a lag of only 30 seconds in response to blue light perceived by phot1 (Folta *et al.,* 2001). With continued exposure to blue light, sustained inhibition of hypocotyl growth beyond 30 min blue light requires cry1. The comparison of the transcriptome in wild-type and *cry1* -mutant seedlings exposed to blue light for 45 min reveals differences in genes involved in the metabolism of gibberellins, genes involved in auxin signalling and genes of cell-wall (apoplastic) proteins that could be involved in cell-wall loosening and cell-wall deposition required for growth (Folta *et al.*, 2003). Different hormones (gibberellins and auxin versus brassinosteroids) could be important in early (45 min) versus late (6 days) effects of cry1 (Ma *et al.*, 2001; Folta *et al.*, 2003). Many transcription factors are induced or repressed by blue light compared to darkness and, as observed for far-red or red light, the response is detectable within the first hour of exposure to blue light (Jiao *et al.*, 2003). This response is reduced in *cry1* and *cry1 cry2* mutants and to a lesser degree in *phyA* and *phot1 phot2* mutants (Folta *et al.*, 2003; Jiao *et al.*, 2003), indicating some degree of redundancy.

To investigate the effects of the specific blue light photoreceptors (i.e. those effects of blue light that are not induced by phytochromes), Ohgishi *et al.* (2004) used red-light grown seedlings (2 week-old) exposed for 1 h to blue light added to the red light background and compared these seedlings with those that remained under red light alone. These experiments were conducted with seedlings of the wild type, the quadruple *cry1 cry2 phot1 phot2* mutant and the corresponding triple mutants in combination with the 8K Affymetrix microarray. Although the age of the seedlings and the light protocols used by Ohgishi *et al.*, (2004) differed from those used by Folta *et al.* (2003), the results converge to underscore the dominant role of cry1, followed by cry2 and the minor effects of phot1 and phot2 in rapid responses of the transcriptome to blue light. Since phototropins localise to the plasma membrane, a contribution of phototropins to changes in gene expression could eventually be important under prolonged blue light treatments, which would allow putative phototropin-derived signals to migrate to the nucleus.

Despite the major role of phyA, phyB, cry1 and cry2 in the control of gene expression by light, several genes respond normally to the dark to light transition even in the quadruple *phyA phyB cry1 cry2* mutant (Perelman *et al.*, 2003). Genes involved in photosynthesis and electron transfer are among those promoted by light in the quadruple mutant and genes involved in cell wall loosening are among those inhibited by light in the quadruple mutant.

The response to low red to far-red ratios

At the early stages of a crop, when the foliage is poorly developed, plants are exposed to a slightly higher proportion of red than far-red light (the ratio is approx. 1.1). With increased canopy growth, selective transmission and/or reflection of light by green leaves lower the red to far-red ratio, which can be reduced to less than 0.1 in dense canopies. In response to this signal plants maximize their chances of capturing sunrays by growing taller and towards open spots, a group of responses collectively known as shade avoidance syndrome (Smith, 2000). Large changes in transcripome occur in seedlings grown under white light with a high red to far-red ratio and transferred to white light plus supplementary far-red, i.e. white light with a low red to far-red ratio (Devlin *et al.*, 2003). Similarly to the situation observed in etiolated seedlings, several of the genes up-regulated by 1 hour of supplementary far-red in light-grown plants encode transcription factors. These transcription factor genes include *HOMEOBOX FROM ARABIDOPSIS THALIANA 4* (*HAT4*), which encodes a homeobox-leucine zipper protein and *PIF3-LIKE 1* (*PIL1*), which encodes a bHLH. The expression of these genes is enhanced by low red to far-red ratios even in a *phyA phyB* mutant background, indicating that other phytochromes play an important role in the control of their expression (Carabelli *et al.*, 1996; Devlin *et al.*, 2003; Salter *et al.*, 2003)

The promotion of hypocotyl elongation by far-red light added to the white light background is largely mediated by phyB and antagonised by phyA. Far-red light reduces the proportion of phyB in its active form but activates the HIR mode of phyA. A large number of genes whose expression is up-regulated by far-red light through phyB and down-regulated through phyA encode auxin-related proteins (Devlin *et al.*, 2003). This includes several auxin regulated transcription factors such as INDOLEACETIC ACID-INDUCED PROTEIN 1 (IAA1), IAA3, IAA19, as well as proteins involved in auxin transport such as PIN-FORMED 3 (PIN3) and PIN7. Interestingly, the core-binding motif recognized

by homeodomain proteins, TAATTA, is over-represented in the promoters of the group of genes antagonistically regulated by phyB and phyA (Devlin *et al.*, 2003). Although this result would be consistent with the key role of the homeodomain protein HAT4 in the induction of shade-avoidance responses through regulation of auxin transport (Morelli and Ruberti, 2002), the expression of *HAT4* is not antagonized by phyA in response to supplementary far-red light; i.e. it does not fully match the pattern of expression of auxin related factors (Devlin *et al.*, 2003). *HAT2* is up-regulated by far-red light via phyB and down-regulated by far-red light via phyA, suggesting that it could mediate between the perception of low red to far-red ratios by phyB and the induction of the morphological responses triggered by the up-regulation of auxin-related factors.

In addition to auxin, other hormones such as gibberellins, brassinosteroids and ethylene have been proposed to mediate and/or modulate the response to low red to far-red ratios (Luccioni *et al.*, 2002; Pierik *et al.*, 2004). This hypothesis is consistent with the observation that genes encoding proteins involved in gibberellin and ethylene biosynthesis (*GIBBERELLIN 20 OXIDASE*, *1-AMINOCYCLOPROPANE-1-CARBOXYL ACID SYNTHASE*), gibberellin signalling (*GIBBERELLIN INSENSITIVE*, *GAI*), as well as the brassiosteroid receptor *BRASSINOSTEROID-INSENSITIVE 1* (*BRI1*), are also regulated by reductions in the red to far-red ratio (Devlin *et al.*, 2003). Changes in hormone levels or signalling activity ultimately modulate cell elongation through changes in cell wall extensibility leading to elongated stems and petioles in low red to far-red treated plants. In agreement with this, several genes encoding proteins that mediate cell wall loosening, such as pectin-esterases and expansins, are up-regulated by reductions in the red to far-red ratio (Devlin *et al.*, 2003).

The response to photoperiod

Photoperiod is one of the most critical environmental cues in the regulation of the time when the apical meristem enters the reproductive phase of development. *Arabidopsis* is a quantitative long day plant, i.e. it flowers earlier under long compared to short day conditions. During the first 3 to 7 days after the transition from short to long days, 101 of the genes present in the 22K Affymetrix microarrays are up-regulated in the apical meristem whereas 231 genes are down-regulated (Schmid *et al.*, 2003). Genes showing enhanced expression under long days include *SUPPRESSOR OF CONSTANS 1 (SOC1)*, *LEAFY (LFY)*, *FRUITFULL*, *AP1*, *SQUAMOSA PROMOTER BINDING PROTEIN LIKE 3*, *4* and *5*, *PISTILLATA*, *AP3* and *AGAMOUS*. The sequential induction of these genes corresponds broadly to their time of action during flower induction or development. The microarray approach also revealed several previously unknown transcription factors where those belonging to the MADS box and *SQUAMOSA BINDING PROTEIN* gene families were over-represented. Expression of all these genes up-regulated during floral induction is impaired to some extent in two late flowering mutants, *constans (co)* and *flowering locus T (ft)*, indicating that CO and FT play a regulatory role at a very early step of this inductive process (Schmid *et al.*, 2003). In contrast, only induction of a limited number of genes was impaired in *lfy* mutants, indicating that LFY acts further downstream in the floral induction cascade. Two of the down-regulated genes, *SCHLAFMÜTZE* (*SMZ*) and *SCHNARCHZAPFEN* (*SNZ*), encode AP2-domain proteins that repress flowering when over-expressed under the control of a constitutive promoter (Schmid *et al.*, 2003). These two genes are the only known repressors of flowering whose expression is reduced by long days.

It is important to note that the photoperiod-regulated genes identified by Schmid *et al.* (2003) represent only a fraction of the genes regulated by changes in day-length. This is because the authors only analyse expression patterns in one particular tissue (the shoot apex) and at one particular time point during the day (1 hour after dawn). It is known that some floral inductive genes, such as *FT,* are only up-regulated by long days at the end of the day, because their expression depends on a tight interaction between light and circadian signalling pathways (see below).

Transcriptional regulators in light signalling

One of the main conclusions of the analysis of rapid responses of the transcriptome to light signals is that many of the genes in this category encode proteins bearing DNA-binding domains. For some of these genes there is evidence for their role in the control of gene expression by light.

bZip transcription factors

The *hypocotyl 5 (hy5)* mutant shows a long hypocotyl under red, far-red, blue or white light, suggesting a role in signalling downstream diverse photoreceptors (Koornneef *et al.*, 1980). The *HY5* gene encodes a bZIP protein (Oyama *et al.*, 1997). *HY5* mRNA levels increase in response to far-red, red or blue light (Tepperman *et al.*, 2001; 2004; Jiao *et al.*, 2003). Light also increases HY5 protein stability (see below). HY5 interacts specifically with the G-box (CACGTG) and is required for normal control by light of promoters bearing this sequence (Chattopadhyay *et al.*, 1998). The analysis of HY5 binding to DNA *in vitro* by using an *Arabidopsis* promoter microarry revealed a set of promoter fragments enriched in the G-box (Gao *et al.*, 2004). G-box containing promoters are over-represented among the genes controlled by far-red light during phyA-mediated de-etiolation and interestingly the region flanking the G-box core motif shows differences between induced and repressed genes (Hudson and Quail, 2003).

HY5 HOMOLOG (HYH) is another nuclear-localised bZip protein involved in photomorphogenesis. *HYY* mRNA levels increase in response to red or blue light compared to darkness but not in response to far-red light and the abundance of HYH protein decreases in darkness (Holm *et al.*, 2002). The morphological phenotype of the *hyh* mutant is observed only under blue light. Genomic analysis reveals a substantial overlapping of the genes controlled by *hy5* and *hyh* mutations under blue light and a stronger effect in the *hy5 hyh* double mutant. HYH binds to the G-box of the light-controlled *RBCS* promoter, apparently as a heterodimer with HY5 (Holm *et al.*, 2002). The abundance of HYH protein, but not the mRNA levels of *HYH*, is positively regulated by HY5 demonstrating another level of interaction between these bZip proteins (Holm *et al.*, 2002).

The role of HY5 is not restricted to visible / far-red light. Seedlings become exposed to variable levels of UV-B radiation upon emergence from the soil. Seedlings grown under white light / dark cycles for 7 d show significant changes in gene expression when transiently exposed to supplementary UV-B radiation under the white light background (Ulm *et al.*, 2004). These effects are

specifically induced by UV-B as the background radiation is predicted to induce even activation of phytochromes, cryptochromes and phototropins under control and UV-B treatment conditions. Some of these genes are transcription factors, including HY5 and HYH. The *hy5* mutant shows impaired gene-expression responses to UV-B (Ulm *et al.*, 2004).

bHLH transcription factors

Several bHLH transcription factors that bind the G box have been implicated in phytochrome-mediated responses in genetic experiments. PHYTOCHROME INTERACTING FACTOR 3 (PIF3) is a nuclear-localised bHLH protein (Ni *et al.*, 1998). Upon irradiation, part of the phytochrome pool migrates in its Pfr form from the cytosol to the nucleus (Yamaguchi *et al.*, 1999) where it binds DNA-bound PIF3 (Martinez-Garcia *et al.*, 2000). PIF3 provides a short-cut between phytochrome and gene expression as the *pif3* mutant shows impaired induction by red light of a small group of nuclear-encoded genes of chloroplast proteins as well as impaired accumulation of chlorophyll (Monte *et al.*, 2004) (Figure 2). Five zinc finger transcription factors show reduced responses to red light in the *pif3* mutant and could be involved in the control of expression of the photosynthetic genes regulated by PIF3. Other genes, particularly some genes involved in the responses to abiotic stress or pathogens are hypersensitive to red light in *pif3* indicating a complex role as positive and negative player in phytochrome signalling (Monte *et al.*, 2004). Light perceived by phytochromes induces rapid degradation of PIF3 but leaves a residual pool (Bauer*et al.*, 2004; Monte *et al.*, 2004).

LONG HYPOCOTYL IN FAR-RED 1 (HFR1), another bHLH protein, is required for several physiological outputs of phyA and cry1 signalling (Fairchild *et al.*, 2000; Duek and Fankhauser, 2003). *HFR1* is expressed in darkness and their mRNA levels increase under far-red or blue and decrease under red light (Duek and Fankhauser, 2003), a pattern of expression that is likely to contribute to its wavelength specificity. In contrast to PIF3, HFR1 does not bind phytochromes but it binds PIF3, forming a heterodimer that in turn binds the Pfr form of phytochromes (Fairchild *et al.*, 2000). The mechanisms of phytochrome control of *HFR1* expression / activity and the role of HFR1 at the genomic level remain to be elucidated.

Mutants at a third bHLH factor, PIF4, cause hypersensitivity of the hypocotyl-growth inhibition response to red light (Huq and Quail, 2002). This response requires phyB and PIF4 actually interacts with phyB. PIF4 is therefore considered a negative regulator of phyB signalling. *PIF4* mRNA levels are very low in darkness and increase in response to red or far-red light. PIF4 is nuclear localised and binds to the G-box DNA motif but DNA bound PIF4 does not bind phyB (Huq and Quail, 2002). The expression of selected photosynthetic genes does not appear to be affected by PIF4 (Huq and Quail, 2002), whether PIF4 controls the expression of hypocotyl-growth related genes remains to be tested.

PIF1 is a negative regulator of the synthesis of protochlorophyllide, the immediate precursor of chlorophyll (Huq *et al.*, 2004). Dark-grown seedlings of the *pif1* mutant exhibit excessive accumulation of free protochlorophyllide that upon illumination causes photooxidative damage. PIF1 is a bHLH factor that interacts with the Pfr form of both phyA and phyB. PIF1 localises to the nucleus where it binds the G-box DNA motif. However, DNA-bound PIF1 fails to interact with phytochromes in gel-shift assays (Huq *et al.*, 2004). PIF1 is a transcriptional activator in transient expression assays in transgenic *Arabidopsis* seedlings grown in darkness but this activity is down regulated by light perceived by phyA or phyB (Huq *et al.*, 2004). Phytochrome promotion of chlorophyll biosynthesis could involve interference with the ability of PIF1 to bind DNA or PIF1 degradation among other possibilities.

PIF3 LIKE 1 (PIL1) was identified by its ability to interact with the clock component TIMING OF CAB EXPRESSION (TOC1) in a yeast two hybrid assay (Makino *et al.*, 2002). The circadian clock modulates *PIL1* expression, peaking at dusk and the effects of low red to far-red ratios on *PIL1* expression and stem growth, also peaking at dusk (Salter*et al.*, 2003). PIL1 is required for normal responses to low red / far-red ratios (Salter *et al.*, 2003).

Homeodomain transcription factors

One of the earliest responses to low red /far-red is the acute up-regulation of the homeodomain transcritpion factor HAT4 (also known as Athb-2), which is observed 15 min after the change in light conditions (Carabelli *et al.*, 1996). This increase in *HAT4* expression occurs even in the *phyA phyB* double mutant (Carabelli *et al.*, 1996; Devlin *et al.*, 2003) and it has been shown that phyE acts redundantly with phyA and phyB to mediate this molecular response (Franklin *et al.*, 2003). Overexpression of *HAT4* from a constitutive promoter enhances longitudinal stem growth (Schena *et al.*, 1993; Steindler *et al.*, 1999) indicating that the regulation of *HAT4* expression by changes in red / far-red ratio plays an important role mediating shade avoidance responses. This appears to be the result of an effect of HAT4 on auxin transport, as both the promotion of elongation by low red to far-red ratios or by

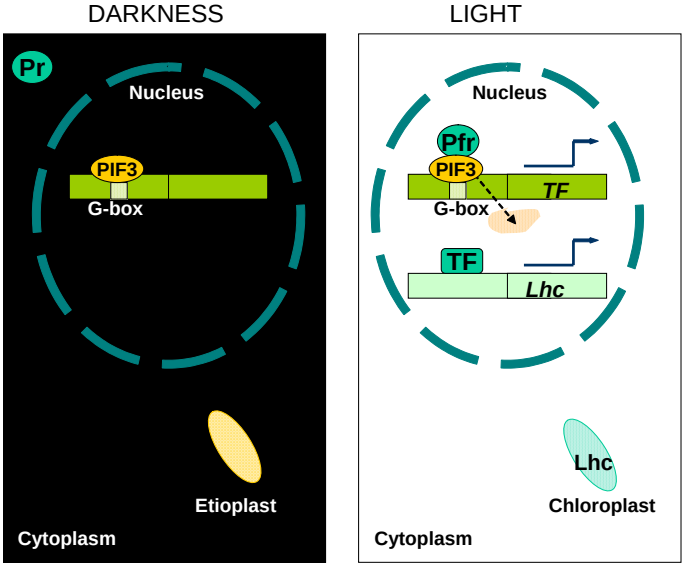

Fig. 2. Control of gene expression by the bHLH factor PIF3. *Light transforms phytochrome from its inactive (Pr) to its active (Pfr) form. Part of the Pfr population migrates from the cytoplasm to the nucleus were it binds DNA-bound PIF3. Pfr causes degradation of PIF3 and presumably activates PIF3 by unknown molecular mechanisms. In the presence of Pfr, PIF3 is required for normal expression of several photosynthetic genes and chloroplast development.*

HAT4 overexpression can be attenuated by application of auxin transport inhibitors (Steindler *et al.*, 1999). Downstream genes with expression controlled by HAT4 remain to be identified.

Myb transcription factors

CIRCADIAN CLOCK ASSOCIATED 1 (CCA1) is a Myb-related transcription factor that interacts with AATCT repeats of the promoter of the *Light harvesting chlorophyll a/b-binding (Lhc,* also known as *CAB)* protein gene *Lhcb1*3* (Wang *et al.*, 1997). The AATCT motif is necessary for normal regulation of *Lhcb1*3* expression by phytochrome and highly conserved in promoters of *Lhc* genes but not among *RUBISCO CARBOXYLASE-OXYGENASE SMALL SUBUNIT* (*RBCS*) genes, which are also strongly regulated by light. Antisense expression of the *CCA1* gene reduces the induction of *Lhcb1*3* expression by a pulse of red light without affecting *RBCS* expression (Wang *et al.*, 1997). The expression of *CCA1* oscillates following a circadian rhythm and is rapidly induced by light (Wang *et al.*, 1997; Wang and Tobin, 1998), suggesting that phytochrome could induce *Lhcb1*3* expression at least partially via the promotion of *CCA1* expression. Constitutive expression of *CCA1* disrupts the rhythm of expression of several genes with different phases (Wang and Tobin, 1998) and this is one of the arguments in favour of a role of *CCA1* in the central oscillator (see review by Más in this issue). CCA1 appears to be part of the network connecting light and clock signals to the control of gene expression.

LONG AFTER FAR-RED LIGHT (*LAF1*) is an R2R3-Myb protein that constitutively localises to the nucleus (Ballesteros *et al.*, 2001). The */af1* mutant is specifically impaired in phyA-mediated responses and shows no obvious phenotype under red or white light. The expression of genes like *Lhc, PLANSTOCYANIN (PET E), CHALCONE SYNTHASE (CHS)* and *XYLOGLUCAN ENDOTRANSGLUCOSYLATE (XTR7)* are impaired in the mutant under prolonged far-red light. LAF1 can transactivate a reporter gene when fused the DNA binding domain of GAL4 (Ballesteros *et al.*, 2001). The transcript levels of *LAF1* are low and show no obvious response to light (Ballesteros *et al.*, 2001).

Other transcriptional regulators

FAR-RED ELONGATED HYPOCOTYL (FHY3, Wang and Deng, 2002) and its closely related FAR-RED IMPAIRED RESPONSE (FAR1, Hudson *et al.*, 1999) are nuclear localised factors uncovered by forward genetics based on a mutant screening protocol under continuous far-red light. In seedlings exposed to continuous far-red light for 5 days the transcriptome of *fhy3 and* to a lesser degree *far1,* resembles that of the *phyA* mutant (Wang *et al.*, 2002). However, during the first 12 h of treatment, *fhy3* or—*far1* mutations affect light induction of only some of the transcription factors and target genes and none of the genes showing the earliest responses to far-red in the wild type were strongly affected by these mutations (Hudson *et al.,* 2003). Furthermore, a number of transcription factors show altered expression in *fhy3* and *far1* irrespective of the light treatment. These differences in transcriptome between wild type and *fhy3* or *far1* dark-grown seedlings contrast with the lack of any obvious morphological phenotype. Therefore, FHY3 and FAR1 could operate upstream the action of phyA itself (Hudson *et al.*, 2003). The *FHY3* transcript levels are downregulated by far-red light (Wang and Deng, 2002).

FAR1 and FHY3 are closely related to Mutator-like element transposases and could operate as transcriptional activators (Hudson *et al.*, 2003). When fused to the Gal4 DNA-binding domain, FAR1 and FHY3 activate transcription of reporter genes downstream the Gal4 regulatory sequence in yeast (Wang and Deng, 2002; Hudson *et al.*, 2003). Furthermore, in transgenic *Arabidopsis*, the activity of a reporter fused downstream the *lac* operator sequence is enhanced in lines carrying a second transgene with *FAR1* fused to the lac DNA-binding domain (Hudson *et al.*, 2003). FHY3 and FAR1 interact *in planta* as indicated by co-immunoprecipitation studies (Wang and Deng, 2002).

FHY1 is another nuclear-localised protein involved in phyA signalling, which bears no known functional motifs with the exception of nuclear localization and nuclear exclusion motifs and a short motif also found in mammalian septins (Desnos *et al.*, 2001; Zeider *et al.*, 2001; 2004). FHY1 activates transcription at least in yeast (Zeider *et al.*, 2001). Microarray studies have revealed a number genes affected by the *fhy1* mutation (Wang *et al.*, 2002; Zeider *et al.*, 2004).

Chromatin re-modelling

Recent observations suggest a role of chromatin remodelling in light control of gene expression in plants. The *de-etiolated 1 (det1)* mutant was discovered in a screening for seedlings showing photomorphogenesis in the dark, i.e. in the absence of the inductive signal. Light-induced genes encoded by nuclear or chloroplast genomes are expressed in dark-grown seedlings of *det1* to the same level observed in light-grown seedlings of the wild type, i.e. 20- to 100-fold more than in dark-grown wild-type seedlings (Chory *et al.*, 1989). In 8 K Affymetrix microarray experiments, nearly half of the genes induced by light in wild type show enhanced expression in the *det1* mutant (Schroeder *et al.*, 2002). This effect is not the mere consequence of altered seedling morphology because heterozygous *DET1 / det1* seedlings are morphologically similar to the wild type in darkness and show enhanced *Lhc* expression (Maxwell *et al.*, 2003). In addition to its role as repressor of light-induced genes in darkness, DET1 down-regulates inappropriate temporal or spatial expression of light-regulated nuclear and chloroplast encoded genes (Chory *et al.*, 1989). The roots of the *det1* mutant develop chloroplasts and express photosynthetic genes whose expression levels are very low or undetectable in wild-type roots. The promoter of the *CHS* gene, which is involved in flavonoid biosynthesis, is active in every leaf cell type of the *det1* mutant but restricted to epidermal and vascular tissues in the wild type (Chory *et al.*, 1989). In contrast to the enhanced expression observed in dark-grown seedlings and roots, the *det1* mutant shows reduced expression of *Lhc* genes in light-grown leaves, which are pale when compared to the wild type. Repression of *Lhcb1*1* expression in darkness requires a 40 bp DET1 dark response element (DtRE) (Maxwell *et al.*, 2003). This element binds two activities, one called CAB2 DET1-associated factor 1 (CDA1) and CCA1. Binding of CCA1 is necessary but not sufficient as the *cca1 det1* double mutant shows only a 10 fold increase of *Lhcb1*1* promoter activity (compared to the 100-fold increase observed in the *det1* single mutant) but over-expression of CCA1 does not increase *Lhcb1*1* expression in either the *DET1* or *det1* background (Maxwell *et al.*, 2003). The promotion of *Lhcb1*1* promoter activity by DET1 in light-grown seedlings requires a CAB UPSTREAM FACTOR-1

(CUF-1) element containing the ACGT G-box core. HY5 binds this motif and is required for DET1-mediated promotion of *Lhcb1*1* activity in the light. Finally, DET1-mediated repression of *Lhcb1*1* expression in the roots requires both DtRE and the CUF-1 element (Maxwell *et al.*, 2003).

In vitro, DET1 binds to the nonacetylated amino-terminal tail of histone H2B in the context of the nucleosome (Benvenuto *et al.*, 2002). The results of FRET experiments are consistent with the occurrence of this interaction *in vivo*. The *Arabidopsis* homolog of UV-Damaged DNA binding protein 1 (DDB1) co-purifies with DET1. *Arabidopsis* has two DDB1 functional copies. The *ddb1b* T-DNA mutant appears to be lethal while *ddb1a* has no obvious morphological phenotype as a single mutant but enhances the phenotype of *det1* in the *ddb1a det1* double mutant (Schroeder *et al.*, 2002). DDB1 could interact with the histone acetyltransferase in *Arabidopsis* as it does in other systems. Thus, DET1 could bind H2B and DDB1 in darkness and repress transcription. The DET1 / DDB1 complex could recruit histone acetyltransferase in response to light, causing acetylation of H2B by the latter enzyme and the release of the DET1 / DDB1 complex to promote transcription (Benvenuto *et al.*, 2002; Schroeder *et al.*, 2002). This putative dual role of DET1 (repression of transcription and promotion of transcription via recruitment of histone acetyltransferase) would be consistent with its contrasting effects on gene expression.

Post-transcriptional regulation

RNA silencing constitutes a novel layer of genetic regulation. It involves 21-26 nucleotide non-coding RNAs known as short interfering RNAs (siRNAs) or micro RNAs (miRNAs), that arise from cleavage of exogenous or endogenous double stranded RNA precursors respectively (Baulcombe, 2004). miRNAs are known to regulate gene expression in a sequence specific manner, by targeting fully complementary mRNAs for degradation, or blocking translation of partially complementary mRNAs (Baulcombe, 2004). Several miRNA have recently been described in plants and some of them were shown to regulate plant growth and development. The expression of at least one precursor of miRNA172, which is complementary to the mRNAs of the AP2 proteins SMZ and SNZ whose levels decrease under long days (see above), is up-regulated after floral induction in a *CO* and *FT* dependent manner (Schmid *et al.*, 2003). This strongly suggests that the photoperiodic regulation of gene expression is mediated, at least in part, through RNA silencing.

In seedlings exposed to a pulse of blue light, the stability of nuclear-encoded *Lhc* transcripts and of the chloroplast-encoded *RUBISCO CARBOXYLASE-OXYGENASE LARGE SUBUNIT* (*RBCL)* transcripts is reduced via an effect mediated by phot1 and NHP3 (Folta and Kaufman, 2003). The transcripts of other chloroplast-encoded genes are unaffected, indicating the selective nature of the effect.

Another layer of regulation has been reported for *LATE ELONGATED HYPOCOTYL* (*LHY,* Kim *et al.*, 2003). Expression of *LHY* driven by the strong and constitutive 35S promoter causes arrhythmic expression of *Lhc* and several other clock-regulated genes under constant light conditions. However, rhythmic expression of *Lhc* is observed under light-dark cycles in these plants. These rhythms correlate with high amplitude oscillation in LHY protein levels caused by light induction of its translation. Thus, the acute induction of *Lhc* expression in the morning is likely to be due, at least in part, to light promotion of LHY translation (Kim *et al.*, 2003).

Post-translational regulation in light signalling

Constitutive photomorphogenesis (cop) mutants were also obtained in screenings for seedlings showing photomorphogenesis even when grown in full darkness. The COP proteins can be grouped in three functional units: COP1, the COP9 signalosome and COP10. COP1 is a ring-finger-type ubiquitin E3 ligase. In darkness, COP1 acts as E3 ligase in the nucleus, targeting transcription factors like HY5 and LAF1 to degradation via the 26S proteasome (Osterlund *et al.*, 2000; Saijo *et al.*, 2003; Seo *et al.*, 2003). The pattern of the transcriptome in dark-grown *cop1* mutant seedlings is qualitatively similar to that of wild-type seedlings grown in the light (Ma *et al.*, 2002; 2003). This result is consistent with a major role of COP1 in the repression of photomorphogenic genes in darkness. Upon exposure to light, COP1 migrates from the nucleus to the citosol (Osterlund and Deng, 1998). This allows the HY5 pool to build up and photomorphogenesis proceeds. Most genes with impaired response to light in the *hy5* mutant are also regulated by COP1 in darkness (Ma *et al.*, 2002). The light-induced shift in sub-cellular partitioning of COP1 is mediated by phytochromes and cryptochromes (Osterlund and Deng, 1998). These photoreceptors physically interact with COP1 and this interaction could be important in de-activation of COP1 by light (Wang, *et al.*, 2001; Yang *et al.*, 2001; Seo *et al.*, 2004).

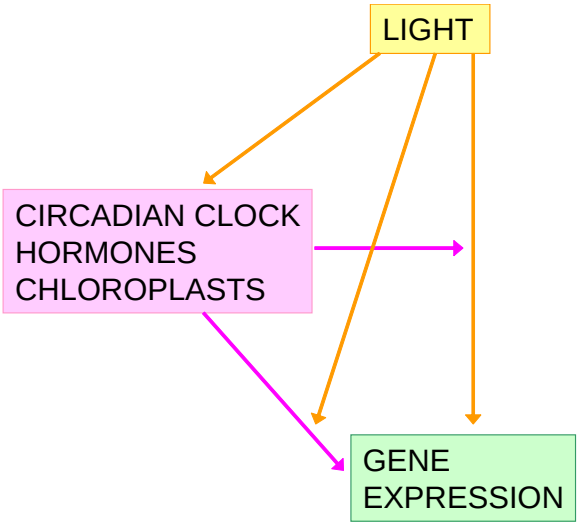

Fig. 3. Light signalling integrates an interactive network with endogenous signals (circadian clock, hormones, chloroplast signals). *Light and endogenous signals converge to control gene expression and mutually regulate each other's signalling cascades.*

COP1-INTERACTING PROTEIN 7 (CIP7) is a nuclear-localised protein without an obvious DNA-binding domain, which exhibits transcriptional activation activity in yeast and in plant cells (Yamamoto *et al.*, 1998). The transcript levels of *CIP7* are increased by light and repressed by COP1. Antisense *CIP7* transgenic seedlings show reduced expression of light-induced genes (Yamamoto *et al.*, 1998). The role of COP1 is regulated by light-quality specific factors. The effects of a weak allele of *cop1*

on seedling morphology and gene expression under far-red light are enhanced by the *spa1* mutation (Saijo *et al.*, 2003). SUPPRESSOR OF PHYA (SPA1) is a repressor of phyA-mediated responses that interacts with COP1 via their coiled-coil regions both *in vitro* and *in vivo* (Hoecker and Quail, 2001; Saijo *et al.*, 2003). This interaction is stronger in darkness than in the light. The *spa1* mutation increases HY5 abundance under far-red light but SPA1 inhibits HY5 ubiquitination *in vitro* (Saijo *et al.*, 2003). Contrary to the effects on HY5, SPA1 enhances *in vitro* ubiquitination of LAF1 (Seo *et al.*, 2003). There are four *SPA* genes in *Arabidopsis* and their products have partially redundant functions. While the *spa1* has no obvious phenotype in darkness, triple mutants with *spa2* and *spa3* or *spa4* show enhanced expression of light-induced genes in darkness (Laubinger *et al.*, 2004).

The COP9 signalosome is a nuclear-enriched complex with homology to the lid subcomplex of the 26S proteasome that interacts with the 26S proteasome, with the complex containing COP10 and possibly with the complex containing COP1 and regulates their assembly and /or activity (Yanagawa *et al.*, 2004). COP10 enhances the activity of ubiquitin conjugating enzymes that bind the COP1 complex and forms a complex with DET1 and DDB1 that has ubiquitin E2 enhancement activity (Yanagawa *et al.*, 2004). While the transcriptome of *cop1* and *det1* seedlings grown in darkness resembles that of light-grown wild-type seedlings, the transcriptome of *cop9* and *cop10* mutants is more divergent and includes alterations in other pathways (Ma *et al.*, 2003).

Interaction between light and other signals

Signalling downstream the photoreceptors bears intricate connections to other signalling networks, including those derived from the circadian clock, the hormonal status and the developmental status of chloroplasts (Figure 3). Light induction of gene expression may be affected by the phase of the circadian rhythm of sensitivity to light (Millar and Kay, 1996;Yanovsky and Kay, 2002); brassinosteroids (Li *et al.*, 1996), auxin (Gil *et al.*, 2001) and gibberellins (Alabadí *et al.*, 2004) are required to maintain a normal repression of light-induced genes in darkness while addition of cytokinins promotes the expression of these genes in darkness (Chory *et al.*, 1994). Blocking chloroplast development severely impairs light induction of transcription of nuclear genes that encode proteins targeted to the chloroplasts (Surpin *et al.*, 2002). Light in turn affects the circadian clock (Devlin and Kay, 2000), the hormonal status (Halliday and Fankhauser, 2003) and the developmental status of the chloroplasts. Light also modulates the responses to hormones. A deep analysis of all these interactions is beyond the scope of this article and we will only give a more detailed description of the interactions between light and circadian clock signalling.

Convergence of light and circadian clock signals to the control of transcription

The kinetics of light-induced gene expression has been investigated in detail in transgenic *Arabidopsis* seedlings bearing a fusion of the *Lhcb1*1* promoter to the firely luciferase reporter. The bioluminiscense signal generated by the reporter shows an acute increase in response to a brief pulse of red light (Millar and Kay, 1996; Anderson *et al.*, 1997). The induction peaks 1.5-2 h after the pulse and is followed by a decrease in expression that leads to a trough 4 to 8 h after the pulse. Then, expression levels cycle with peaks of decreasing intensity at 22 and 56 h or 15.5 and 50 h after the red light pulse (depending on the *Arabidopsis* accession). This reveals a rhythm with a period of approximately 34 h that is initiated or revealed by light (Millar and Kay, 1996; Anderson *et al.*, 1997).

Two tobacco nuclear factors, CUF-1 and CAB GATA FACTOR 1 (CGF-1) interact with the *Lhcb1*1* gene promoter. CUF-1 has affinity for the G-box and TGACGT/C motifs whereas CGF-1 binds a repeated GATA motif (Anderson *et al.*, 1994). The *Lhcb1*1* promoter mutated in the binding site of CGF-1/GT-1 and fused to the luciferase reporter shows no acute response to a pulse of red light but retains the second peak of response related to the circadian regulation. Thus, phytochrome and circadian-clock regulation can be at least partially dissected at the level of the *Lhcb1*1* promoter (Anderson *et al.*, 1997).

In the *phyA* mutant background the acute response of *Lhcb1*1* to a 2-min red-light pulse is attenuated but the subsequent peaks caused by the clock regulation are unaffected (Anderson *et al.*, 1997). In the *phyB* mutant background both the acute and circadian-clock regulated responses are reduced. The *phyA phyB* double mutant retains a weak response to the red light pulse. Interestingly, the *hy5* mutant retains a normal acute response and reduced clock-regulated response. The *early flowering 3 (elf3)* mutant is arrhythmic under continuous light conditions. In the *elf3* mutant background the acute response occurs earlier than in the wild type (0.5 compared to 1.5 h, respectively) (Anderson *et al.*, 1997). In summary, while the acute and rhythmic components of the *Lhcb1*1* response can be dissected at the level of the promoter, photoreceptors are required for the expression of the circadian-clock regulated effect and molecular players like ELF3 that operate close to the clock modulate the acute response. Clock and phytochrome signaling appear to form a network upstream the promoter rather than converge only at the promoter level. In addition, light is the primary environmental input that sets the pace of the circadian clock. In plants grown under continuous light, phytochromes and cryptochromes reduce the length of the period of *Lhcb1*3* expression (Devlin and Kay, 2000). Therefore these photoreceptors are predicted to modulate the temporal pattern of expression of numerous genes via their effect on the clock.

The interaction between light and circadian clock controls the expression of flowering genes

Long days accelerate the transition from vegetative to reproductive development in *Arabidopsis thaliana* by enhancing the expression of the floral integrator gene *FT* (Kardailsky *et al.*, 1999; Kobayashi *et al.*, 1999). Above certain threshold *FT* promotes the expression of *AP1*, a meristem-identity gene that triggers the initiation of flowers at the shoot apical meristem (Kardailsky *et al.*, 1999). cry2 and phyA activate *FT* expression under long days but not under short days (Yanovsky and Kay, 2002). These photoreceptors require high levels of *CO* expression to affect *FT* expression (Yanovsky and Kay, 2002). CO is a transcriptional regulator tightly regulated by the circadian clock (Suarez-Lopez *et al.*, 2001). During short days, *CO* expression is low during daytime and increases only after sunset, whereas during long days, *CO* mRNA levels start accumulating towards dusk (Suarez-Lopez *et al.*, 2001). This ensures that the overlap between high levels of *CO* mRNA and the illuminated part of the day is minimal during short

days and maximal during long days. This coincidence is critical, since the CO protein is unstable in the dark and it only accumulates to levels enough to promote *FT* expression when stabilized by active cry2 and phyA photoreceptors (Valverde *et al.*, 2003). The distinction between long and short days is therefore based on the interaction between light and circadian signalling.

Light not only regulates CO protein levels through post-transcriptional mechanisms but also through an effect on *CO* expression. The accumulation of *CO* mRNA levels during the afternoon of a long day requires FLAVIND BINDING KELCH REPEAT F BOX 1 (FKF1), a clock regulated protein whose levels peak at dusk (Imaizumi *et al.*, 2003). Interestingly, FKF1 has an amino-terminal PAS/LOV domain similar to that present in phot1 and phot2 (Nelson *et al.*, 2000). This PAS/LOV domain binds the chromophore flavin mononucleotide and undergoes light induced photochemistry, which is consistent with the observation that the effect of FKF1 on *CO* expression requires blue light (Imaizumi *et al.*, 2003). In addition FKF1 also contains an F-box domain, that targets proteins for proteasome-mediated degradation and six kelch repeats that may mediate protein-protein interactions (Nelson *et al.*, 2000). Thus, FKF1 could work as a novel photoreceptor recruiting a transcription factor that represses *CO* expression for ubiquitylation and subsequent degradation in a blue light dependent manner.

Conclusions and future perspectives

In recent years the list of plant genes controlled by light has grown from a few dozen to thousands thanks to microarray experiments. The functional significance of a large proportion of these genes is still unknown, offering a fertile field for a reverse genetics approach. Light signals typically trigger rapid changes in the mRNA levels of transcription factors, but the position that they occupy in a putative transcriptional cascade and the steps interposed between the photoreceptors and the first raw of transcription factors have not been fully established. Genetic modifications at some of these loci severely impair light-induced responses, evidencing a key role of transcriptional regulators in light signalling. Light also regulates the protein levels of several of these transcription factors by reducing their rate of proteasome-mediated degradation. This pathway involves COP1, a nuclear E3 ligase that migrates to the cytosol in response to light. The roles for chromatin re-modelling and RNA silencing in light-controlled gene expression have recently been proposed, suggesting multiple pathways between photoreceptors and target genes. One of the future challenges will be to account for this complex signalling network in dynamic terms. Achieving this goal will require the identification of rate-limiting steps in signalling under specific contexts.

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