

Photosensory perception and signalling in plant cells: new paradigms?

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Plants monitor informational light signals using three sensory photoreceptor families: the phototropins, cryptochromes and phytochromes. Recent advances suggest that the phytochromes act transcriptionally by targeting light signals directly to photoresponsive promoters through binding to a transcriptional regulator. By contrast, the cryptochromes appear to act post-translationally, by disrupting extant proteasome-mediated degradation of a key transcriptional activator through direct binding to a putative E3 ubiquitin ligase, thereby elevating levels of the activator and consequently of target gene expression.

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Abbreviations

bHLH	basic helix-loop-helix
B/UV-A	blue/ultraviolet-A
CNS	COP9 signalosome
cry	cryptochrome
FR	far-red
FMN	flavin mononucleotide
FRc	continuous FR
PAS	Per–Arnt–Sim
Pfr	FR-absorbing conformer of phytochrome
phot	phototropin
phy	phytochrome
PIF3	phytochrome-interacting factor 3
Pr	R-absorbing conformer of phytochrome
R	red
Rc	continuous R

Introduction

The photosensory system that plants use to monitor their surrounding light environment encompasses three known classes of informational photoreceptors: the cryptochromes (cry), the phototropins (phot) and the phytochromes (phy) [1–4]. The combined activities of these molecules enable the plant to detect and respond to the presence, absence, colour, intensity, directionality and diurnal duration of impinging light signals. Defining the molecular and cellular mechanisms by which these photoreceptors perceive, transduce and integrate these signals is currently the focus of considerable research effort. This will be the focus of my review.

The photoreceptors

The three classes of photoreceptors perform distinctive photosensory and/or physiological functions in the plant [2,3,5]. The cryptochromes and phototropins monitor the blue/ultraviolet-A (B/UV-A) region of the spectrum, whereas the phytochromes monitor primarily the red (R) and far-red (FR) wavelengths. The cryptochromes and phytochromes control growth and developmental responses to variations in the wavelength, intensity and diurnal duration of the irradiation [2,3], whereas the phototropins function primarily in controlling directional (phototropic) growth in response to directional light and/or intracellular chloroplast movement in response to light intensity [5–8]. Each of these classes consists of a small family of related chromoproteins. In *Arabidopsis*, the most extensively characterised plant system, there are two cryptochromes (cry1 and cry2) [2], two phototropins (phot1 and phot2) [9] and five phytochromes (phyA–E) [10]. Individual members within each family have differential photosensory and/or physiological functions to varying extents; thereby, providing an additional layer of specialisation superimposed on a foundation of partially overlapping functions [3,4,8,9,11–13].

The cryptochromes are flavoproteins, each carrying two chromophores, a pterin or a deazaflavin at one site and FAD at another, in an amino-terminal domain related to the DNA photolyases (Figure 1a; [2,4]). Both cry1 and cry2 also contain a carboxy-terminal extension that is different in the two photoreceptors and not found in the photolyases. Both cryptochromes appear to be constitutively nuclear localised (Figure 1b; [2,14,15]). The photochemical mechanism of signal capture and transfer is, as yet, undefined, but is likely to involve a redox reaction.

The phototropins are also flavoproteins, which carry two flavin mononucleotide (FMN) chromophores associated with two Per–Arnt–Sim (PAS) subdomains in the amino-terminal domain of the molecule (Figure 1a; [4,6,8,9]). The carboxy-terminal domain contains a classical serine/threonine protein kinase whose activity is regulated by B/UV-A light absorbed by the amino-terminal domain [5,8]. Phot1 is considered to be associated with the plasma membrane as a peripheral protein (Figure 1b). The subcellular location of phot2 is yet to be defined.

The phytochromes are dimeric chromoproteins consisting of polypeptide subunits that carry a tetrapyrrole chromophore in the amino-terminal domain (Figure 1a; [16]). The carboxy-terminal domain functions in dimerisation and contains a region with sequence similarity to prokaryotic two-component histidine kinases. phyA-associated serine/threonine kinase activity has been reported [17,18], but it

remains to be determined whether this is directly involved in phytochrome signalling. The photosensory activity of the phytochrome molecule resides in its capacity to undergo reversible, light-induced interconversion between two conformers: the biologically inactive, R-absorbing Pr form and the biologically active R-absorbing Pfr form [3]. The phytochromes are cytosolically localised in their Pr form, but are triggered to translocate into the nucleus upon photoconversion to their Pfr form (Figure 1b; [19–22]).

Signalling intermediates

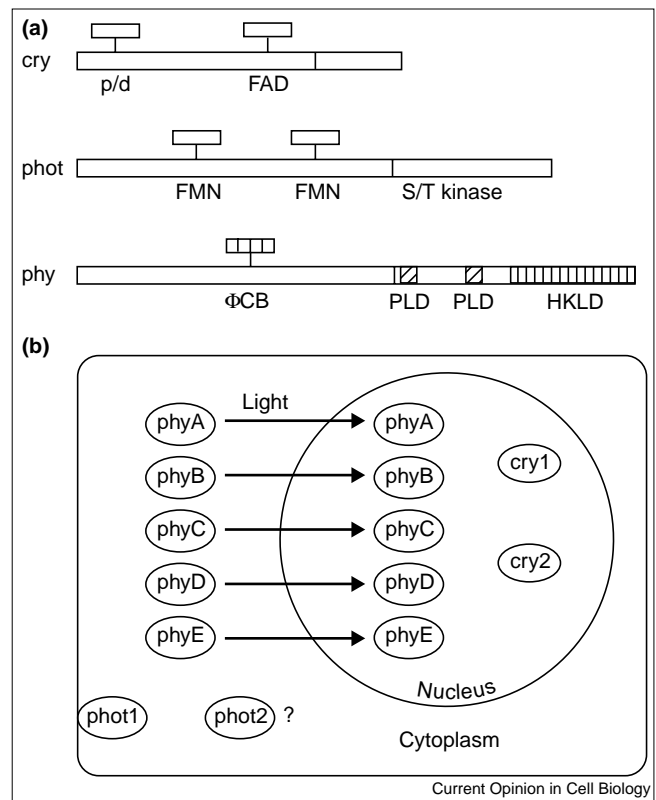
Photoperception by each of the three classes of receptors triggers specific intracellular signalling pathways that induce the selective changes in gene expression that drive the various growth and developmental responses to the light signal [23*,24]. Efforts to define these pathways have resulted in the identification of a considerable number of putative or established signalling intermediates. A simplified schematic representation of these pathways and their components, based on reported data, is presented in Figure 2a. These data are primarily from studies on young *Arabidopsis* seedlings, with much of the information having come from investigations of the de-etiolation process. This process represents the initial major developmental transition in plants, from skotomorphogenesis to photomorphogenesis, induced when dark-grown seedlings are first exposed to light.

Investigations with mutants null for the individual photoreceptors have established that four of these receptors dominate in regulating de-etiolation: cry1 and cry2 mediate B/UV-A signals [2,5], phyA mediates continuous FR (FRc) light signals and phyB is the predominant receptor of continuous R (Rc) light signals ([3,11,13]; Figure 2a).

Conventional genetic screens for defects in signalling intermediates have yielded two principal classes of mutants: the *cop/det/fus* class where seedlings deetiolate in total darkness as if they had perceived a light signal and a photodeficient class that develop normally in darkness, but have reduced or enhanced responsiveness to light signals [25–30]. The latter class includes components that appear to be specific to either phyA or phyB, or to phytochrome versus cryptochrome, signalling. These mutants suggest that early steps in each pathway involve intermediates dedicated to the individual photoreceptors and that the separate pathways converge downstream in a ‘signal integration’ process that drives later common events in the de-etiolation response (Figure 2a). By contrast, because the *cop/det/fus* class of mutations acts more or less pleiotropically, these components have generally been postulated to function downstream of the convergence of the cryptochrome and phytochrome pathways (Figure 2a).

Attempts to identify primary phytochrome signalling partners using yeast two-hybrid screens of cDNA libraries have identified three proteins, PIF3 (phytochrome-interacting factor 3) [31,32,33*], PKS1 [18] and NDPK2 [34],

Figure 1



Structure and subcellular localisation of higher plant sensory photoreceptors. (a) Schematic structures of cryptochrome (cry), phototropin (phot) and phytochrome (phy) chromoproteins. The locations and identities of the respective chromophores (small elevated rectangles) attached to the polypeptide subunits are indicated in each case. (b) Subcellular localisation of the photoreceptors. Φ CB, phytochromobilin; d, deazaflavin; FAD, flavin adenine dinucleotide; FMN, flavin mononucleotide; HKLD, histidine kinase-like domain; P, pterin; PLD, PAS-like domain; S/T, serine/threonine.

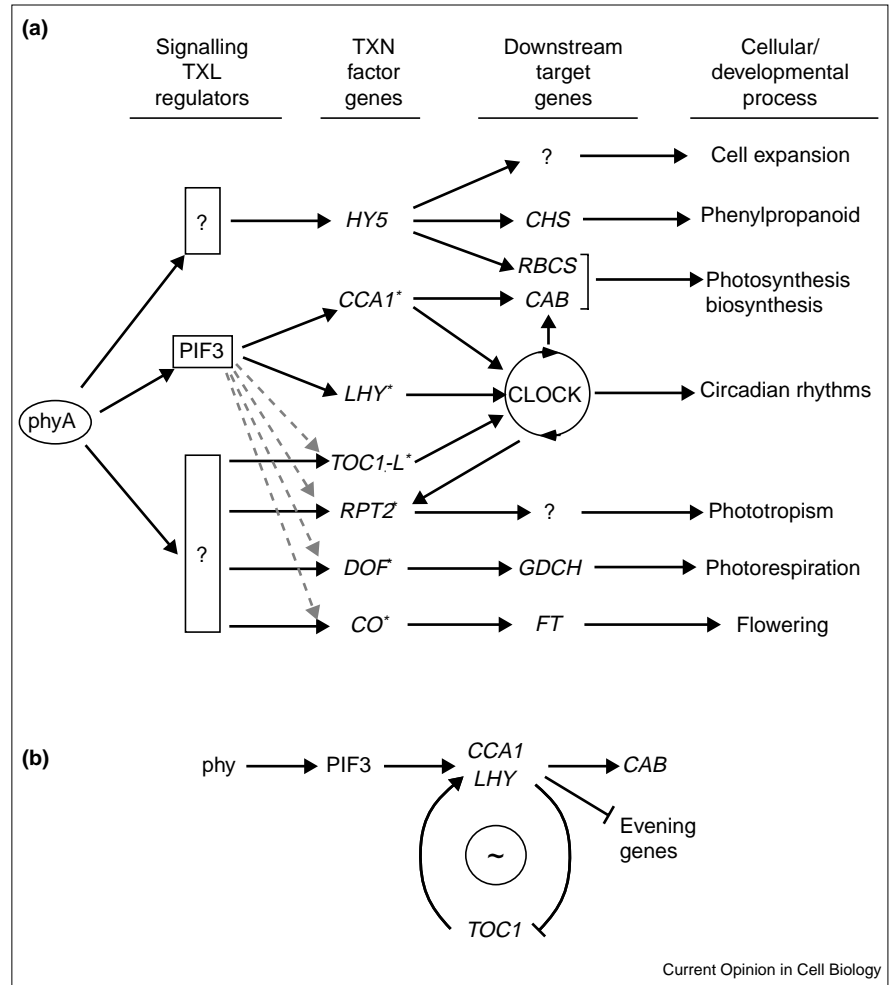
which are capable of direct binding to phytochrome molecules [35*]. Based on these binding studies and evidence of involvement in both phyA and phyB signalling *in vivo*, it is postulated that these components may be indicative of one or more shared upstream pathways, in addition to the apparent photoreceptor-specific pathway segments (Figure 2a).

Little is currently known regarding phyC, phyD or phyE signalling pathways and, until recently, no early intermediates in the cryptochrome signalling pathway had been identified (see below). However, the recent identification of a phot1-interacting factor, NPH3 [36], and a related protein, RPT2 [37], has provided the first evidence of potential signalling intermediates in the phototropin pathway (Figure 2a).

In addition to the non-targeted yeast two-hybrid screens for phytochrome-interacting factors mentioned above, several targeted molecular interaction studies involving pre-selected proteins have been reported [38,39**,40**,41–45].

Figure 3

phy-regulated transcriptional network and the circadian clock. **(a)** Simplified schematic of postulated phyA-regulated transcriptional network. It is proposed that rapidly responding phyA-regulated transcription (TXN)-factor genes are primary targets of phyA signalling through 'signalling transcriptional (TXL) regulators' constitutively present before light signal perception, and that these TXN-factor genes encode a master set of regulators, each of which regulates one or more major branches of cellular or developmental activity by controlling the expression of specific downstream target genes. PIF3, a bHLH factor, is proposed to function as one such 'signalling TXL regulator' based on its capacity to bind to G-box sequence elements in the promoters of *CCA1* and *LHY* and to bind specifically to the active Pfr form of phyA; thereby, targeting light signals directly to these genes. The promoters of several other of the transcription-factor genes also carry G-box motifs (asterisks) making them potential PIF3 targets (dashed arrows). For genes lacking functionally relevant PIF3-binding sites, such as *HY5*, which appears to lack a G-box, we postulate that other yet to be identified 'signalling TXL regulators' (question marks in boxes) may fulfil this role. Some of the key downstream genes in the different pathways, known or proposed to be targets of the TXN-factor gene-products listed, are indicated. From [23*]. **(b)** Simplified schematic of the circadian clock in *Arabidopsis*. The reciprocal feedback loop between *CCA1/LHY* and *TOC1*, which is postulated to constitute the basic framework of the oscillator mechanism, is indicated (wavy line). Phytochrome is postulated to initiate and reset the circadian oscillations upon light-signal perception by direct enhancement of *CCA1/LHY* transcription through promoter-bound PIF3. Oscillations in the levels of the MYB-like factors *CCA1* and *LHY* are



then also postulated to provide dual output signals from the clock: inducing expression of genes such as *CAB* and repressing cycling

'evening genes' (in addition to *TOC1*) through binding to target DNA elements in the promoters of those genes.

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Dual signalling mechanisms?

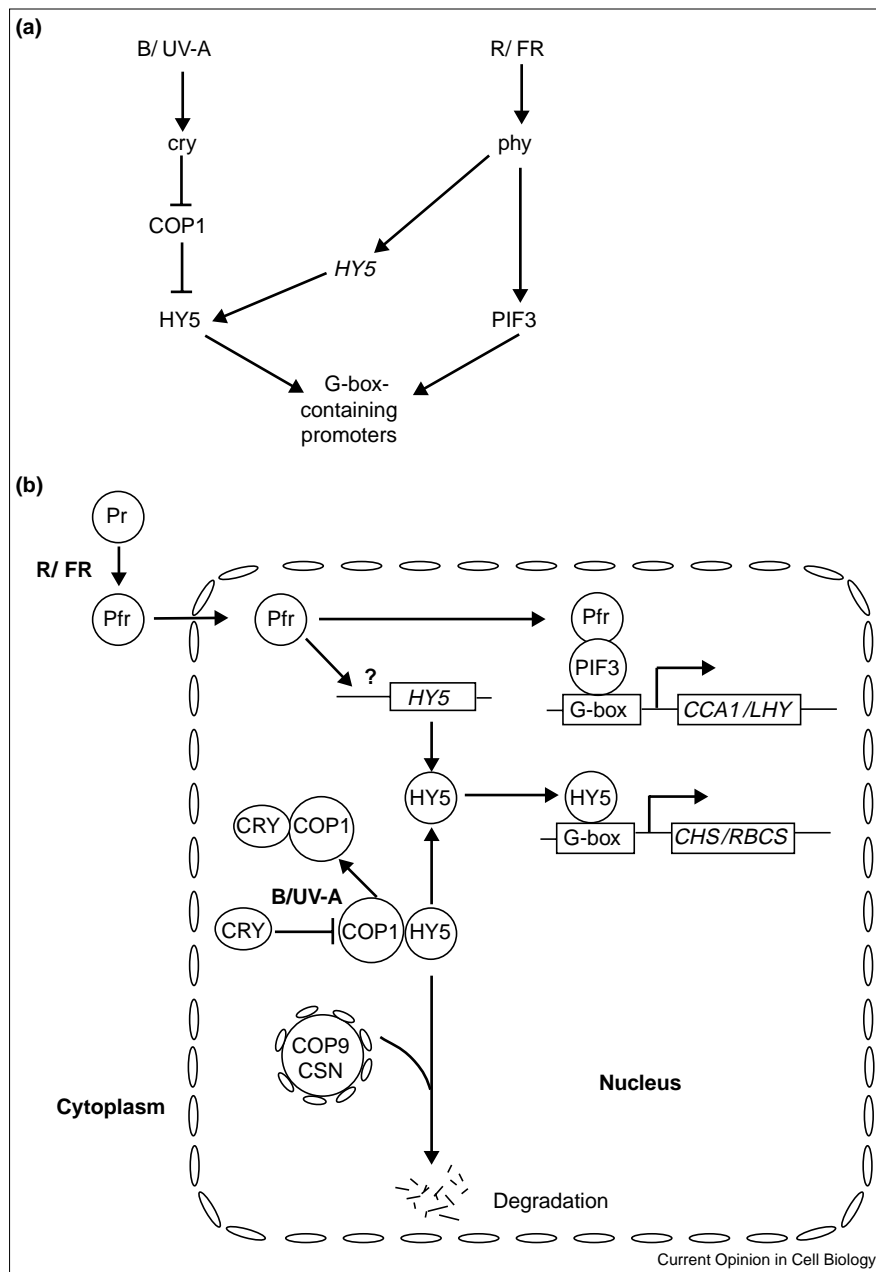
How do these various components implicated in photosignal transduction function? For the majority, although many have now been cloned (Figures 2a and b), the answer remains unknown; however, recent advances have begun to provide intriguing insight into this question. First, it is notable that many of the cloned factors localise to the nucleus [31,43,46–58]. Together with the constitutive nuclear localisation of cry1 and cry2 [2,14], and the induced nuclear translocation of the phytochromes ([21,22]; Figure 1b), these data suggest that early light signalling events are nuclear-localised. Second, evidence from several studies has converged to suggest that there may be dual mechanisms of signalling to photoresponsive genes, as outlined below.

Direct transcriptional regulation

The first phytochrome-interacting factor reported from yeast two-hybrid screens for phytochrome-signalling partners was PIF3, a constitutively nuclear member of the

basic helix-loop-helix (bHLH) class of transcriptional regulators [31,32,59]. PIF3 binds in sequence-specific fashion to a G-box, DNA-sequence motif present in various light-regulated promoters, and phyB binds to DNA-bound PIF3 specifically and reversibly upon light-induced conversion to its biologically active Pfr conformer [33**]. Seedlings with reduced PIF3 expression levels display a reduced de-etiolation phenotype in response to Rc and FRc [31] and reduced expression of two key genes, *CCA1* and *LHY*, which have G-box motifs in their promoters [33**]. These genes themselves encode MYB-like transcription factors known to be involved in regulating light- and clock-related responses [60–63]. These and other data are consistent with the overall proposal that light induces the phytochrome molecule to translocate into the nucleus, where it binds in its active Pfr form to promoter-bound PIF3 and facilitates transcriptional activation of specific target genes [33**,35*]. The biochemical mechanism by which this regulation might occur is undetermined;

Figure 4



Postulated dual signalling pathways to photoresponsive genes. (a) Simplified schematic of proposed separate signalling mechanisms utilised by phytochrome and cryptochrome pathways. It is proposed that both pathways act to regulate the abundance of the key bZIP transcriptional activator, HY5, but by different mechanisms. Photoactivated cryptochromes are postulated to act post-translationally to block constitutive proteasome-mediated HY5 degradation by binding to and inactivating COP1, a putative E3 ubiquitin ligase that potentially targets HY5 for proteolysis. By contrast, photoactivated phytochromes are postulated to act at the transcriptional level, enhancing *HY5* gene expression by an undetermined mechanism. The increased levels of constitutively nuclear HY5 protein generated by either mechanism are then proposed to activate transcription of downstream, G-box-containing genes involved in photomorphogenesis. The phytochromes are also postulated to directly regulate the expression of G-box-containing genes by physical interaction with PIF3, a bHLH protein, bound to G-box motifs in target promoters. (b) Cellular context of proposed separate cryptochrome and phytochrome signalling mechanisms. R/FR light signals perceived by the phytochromes produce the Pfr conformer, which translocates into the nucleus where it is postulated to activate transcription of G-box-containing genes either directly through binding to PIF3 (*CCA1*, *LHY*), or more indirectly by enhancing *HY5* gene transcription through an unknown mechanism (query), which leads to induced transcription of G-box-containing genes (*CHS*, *RBCS*) through elevated HY5 protein levels. B/UV-A light signals perceived by the constitutively nuclear cryptochromes are proposed to interrupt otherwise constitutive HY5 protein degradation mediated by the COP9 CNS-associated proteasome pathway by inactivating COP1 and thereby leading to elevated HY5 levels.

however, in principle, the photoreceptor molecule could function directly as a light-switchable transcriptional co-regulator [33••] and/or as a photoregulated enzyme such as a protein kinase [17,64], whereby signal transfer would involve catalysed covalent modification of one or more components of the transcriptional machinery [35]. Regardless, the data support the proposition that the phytochromes function, at least in part, by directly targeting light signals to the promoters of photoresponsive genes. This proposition is a radical departure from previously established concepts, which postulated that the phytochromes remain cytoplasmic after photoactivation and signal to nuclear genes through a second messenger system [35•,65].

Recent microarray-based expression profile analysis of phyA-regulated genes in *Arabidopsis* supports and expands upon this emerging paradigm [23•]. The data show that 44% of genes responding most rapidly to a light signal encode a diversity of putative or established transcriptional regulators. Because several of these genes already have established or putative roles in regulating the various major cellular and developmental processes underlying photomorphogenesis (Figures 2a and 3a), the data suggest that the encoded proteins represent a master-set of transcriptional regulators that coordinate the expression of the array of downstream genes that implement the photomorphogenic programme. Moreover, because several members of this

group, including *CCA1*, *LHY*, *TOC1-L*, *RPT2*, *DOF* and *CO*, have G-box sequences in their promoters, the data suggest that one pathway of phytochrome signalling may involve simultaneous direct targeting, through PIF3, of multiple members of this proposed master-set of transcription factor genes (Figure 3a). A notable exception to this specific proposal is HY5, a nuclear-localised bZIP protein, which has a well-documented major role in regulating light-induced de-etiolation [66–68]. Although *HY5* transcript levels are rapidly induced by phyA in response to a FRc light signal [23•], the *HY5* gene promoter does not appear to contain a G-box motif (Figure 3a). This observation suggests that *HY5* transcription may be regulated by phyA through a second, PIF3-independent pathway.

The centrally important role of *CCA1* and *LHY* in photomorphogenesis and the plant circadian clock has been solidified recently by exciting new contributions to our understanding of the clock mechanism. *CCA1* was originally identified as a DNA-binding protein involved in regulating light-induced expression of a *CAB* gene through sequence-specific binding to a motif in the *CAB* promoter [60,62,63]. Subsequently, both *CCA1* and the closely related *LHY* were also found to have a role in circadian clock function [61,63]. Recently, Alabadi *et al.* [69••] have reported that *TOC1*, another previously identified clock-related gene [70], positively regulates *CCA1/LHY* expression and, conversely, that *CCA1/LHY* negatively regulates *TOC1* expression. Evidence is presented that this feedback loop is likely to constitute a central component of the circadian oscillator. The model implies that perturbation of the steady-state levels of either *CCA1/LHY* or *TOC1* will initiate an oscillatory feedback loop through their mutually reciprocal regulatory activities [69••]. Because phyA and phyB rapidly and transiently induce *CCA1* and *LHY* expression, presumably through PIF3, in response to light, it can be proposed that this direct targeting of light signals to the *CCA1* and *LHY* promoters represents the mechanism of light input into the plant circadian clock (Figure 3b). According to this proposal, the presumptive phytochrome-induced spike in *CCA1/LHY* expression upon initial light exposure at dawn would then function to reset the clock each day.

Understanding of the mechanisms by which *CCA1/LHY* and *TOC1* regulate each other's expression is incomplete; however, Alabadi *et al.* [69••] have shown that the *CCA1* and *LHY* proteins can bind to a sequence element in the *TOC1* promoter. This element is highly similar to the *CCA1*-binding site originally identified in the *CAB* promoter [60] and to an 'evening element' subsequently identified in the promoters of 31 cycling genes [71••]; therefore, it seems likely that oscillations in *CCA1/LHY* levels constitute an output signal from the clock that determines the expression profiles of clock-regulated genes (Figure 3b). It is intriguing that the presumptive binding of *CCA1* and *LHY* to the *TOC1* and *CAB* promoters appears to have opposite effects on the transcriptional activities of these

two genes: enhancement of light-induced *CAB* expression and repression of *TOC1* expression (Figure 3b). Alabadi *et al.* [69••] predict that the other 'evening element' genes may also be negatively regulated by *CCA1/LHY* in a manner similar to *TOC1* (Figure 3b).

Degradative regulation of transcription factor protein abundance

Eleven recessive mutant loci define the pleiotropic *cop/det/fus* class of *Arabidopsis* mutants that exhibit almost complete photomorphogenic seedling development in darkness [55–58]. Early studies indicated that the encoded wild-type components act negatively to suppress photomorphogenesis in darkness and that this activity is reversed by light. Subsequent studies have uncovered an intriguing new pathway through which this negative activity is imposed and have provided the first insights into how light blocks this activity.

Eight of the 11 pleiotropic *cop/det/fus* components have been shown to form a large, nuclear-localised multiprotein complex, termed the COP9 signalosome (CNS; [56,72]). Significantly, the CNS complex has striking similarity to the lid complex of the 26S proteasome — the principal cellular locus of ubiquitin-targeted protein degradation in eukaryotes. This similarity suggests that the CNS may function in nuclear protein degradation, possibly as a lid complex of a novel COP9 proteasome. Support for this proposal has come recently from evidence that the CNS interacts with the E3 ubiquitin ligase, SCF^{TIR1}, considered to be involved in targeting auxin-signalling proteins for degradation [73•].

COP1, a non-CNS component containing ring-finger and WD-40-repeat domains, was identified early as a key repressor of photomorphogenesis [74]. Subsequent studies showed that COP1 acts to antagonise the positively acting constitutively nuclear, bZIP factor, HY5, which can bind to G-box motifs in the promoters of light-inducible genes, promoting their expression, and evidence for physical interaction between COP1 and HY5 in the nucleus was obtained [68,75,76]. Examination of HY5 mRNA and protein levels showed that HY5 protein abundance is suppressed in darkness by a post-translational process requiring both COP1 and the CNS complex [77•]. Based on these data and *in vitro* inhibitor experiments, it is proposed that HY5 levels are maintained at low levels in darkness through proteasome-mediated degradation involving the CNS complex and that COP1 may specifically target HY5 for ubiquitination and proteolysis by functioning as an E3 ubiquitin ligase [55,56,77•]. It is further proposed that light blocks this process, leading to enhanced accumulation of HY5 protein, based on observed light-induced increases in HY5 abundance to levels in excess of those predicted from the increased mRNA levels [77•].

Recent evidence has revealed a possible mechanism for this light-induced reversal of HY5 degradation. In a pivotal

study, Yang *et al.* [78**] demonstrated that expressing the carboxy-terminal domain of either cry1 or cry2 in transgenic *Arabidopsis* induced a constitutively photomorphogenic phenotype in dark-grown seedlings strikingly similar to that of *cop1det/fus* mutants. Now Yang *et al.* [39**] and Wang *et al.* [40**] have independently shown that cry1 and cry2 can bind directly to COP1. It is thereby proposed that cryptochrome signalling involves rapid, blue light triggered inactivation of COP1 through direct physical contact between the two nuclear-localised molecules and that this abrogates the targeted degradation of HY5 leading to its accumulation and transcriptional activation of target genes, such as *CHS* and *RBCS* ([39**,40**]; Figure 4). It is further proposed that longer-term inactivation of COP1 occurs by subsequent depletion of the molecule from the nucleus [76].

Conclusions – a working model

Examination of the existing literature suggests the possibility that the phytochromes and cryptochromes may signal by two separate cellular mechanisms that converge to regulate the abundance of the HY5 transcriptional activator. It is proposed that the phytochromes induce enhanced transcription of the *HY5* gene, whereas the cryptochromes inhibit degradation of the HY5 protein. Either or both activities lead to increased HY5 levels that drive transcription of G-box-containing, photomorphogenically important genes (Figure 4). Although Yang *et al.* [39**] report that COP1 also binds to the C-terminal domain of phyB in yeast two-hybrid assays, the relevance of this interaction to phytochrome signalling remains to be established as, in contrast to the cryptochromes, the isolated carboxy-terminal domains of the phytochromes do not appear to be active *in vivo* [78**,79]. The same reservation applies to the observed *in vitro* binding of the phyA-specific component SPA1 to COP1 [80]. Similarly, although the recent identification of the F-box protein EID1 suggests that a proteasome-related pathway is involved in phyA activity [52], there is currently no evidence that phyA signals through light-regulated proteolysis.

It seems likely that this apparent intersection of the two photoreceptor pathways through relatively direct regulation of a single transcriptional activator represents only one node in a highly complex signalling and transcriptional network. There is already evidence that the phytochromes target multiple transcription-factor genes for direct transcriptional regulation through PIF3 and possibly other factors [23*,33**], and that the cryptochrome–COP1 system may target multiple transcriptional regulators for proteolytic regulation of abundance [81]. In addition, there is the potential for enormous, uncharted complexity centred on the two characterised transcriptional regulators, PIF3 and HY5. Both belong to large families of related factors (about 135 bHLH and about 81 bZIP proteins in *Arabidopsis*; [82,83]), and both recognise the same core G-box motif in sequence-specific fashion in *in vitro* binding assays [33**,35*,68]. These observations raise many possibilities, ranging from competitive DNA binding to

functional redundancy and combinatorial heterodimerisation of factors at the level of light-regulated promoters [35*]. Thus, although considerable recent progress has been made, we still have much to learn about light signalling in plants.

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References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
 - ** of outstanding interest
1. Kendrick RE, Kronenberg GHM: *Photomorphogenesis in Plants*, 2nd edn. Dordrecht, Netherlands: Kluwer Academic Publishers; 1994.
 2. Cashmore AR, Jarillo JA, Wu YJ, Liu D: **Cryptochromes: blue light receptors for plants and animals**. *Science* 1999, **284**:760-765.
 3. Smith H: **Phytochromes and light signal perception by plants – an emerging synthesis**. *Nature* 2000, **407**:585-591.
 4. Briggs WR, Olney MA: **Photoreceptors in plant photomorphogenesis to date. Five phytochromes, two cryptochromes, one phototropin, and one superchrome**. *Plant Physiol* 2001, **125**:85-88.
 5. Briggs WR, Huala E: **Blue-light photoreceptors in higher plants**. *Annu Rev Cell Dev Biol* 1999, **15**:33-62.
 6. Kagawa T, Sakai T, Suetsugu N, Oikawa K, Ishiguro S, Kato T, Tabata S, Okada K, Wada M: **Arabidopsis NPL1: a phototropin homolog controlling the chloroplast high-light avoidance response**. *Science* 2001, **291**:2138-2141.
 7. Jarillo JA, Gabrys H, Capel J, Alonso JM, Ecker JR, Cashmore AR: **Phototropin-related NPL1 controls chloroplast relocation induced by blue light**. *Nature* 2001, **410**:952-954.
 8. Sakai T, Kagawa T, Kasahara M, Swartz TE, Christie JM, Briggs WR, Wada M, Okada K: **Arabidopsis nph1 and npl1: blue light receptors that mediate both phototropism and chloroplast relocation**. *Proc Natl Acad Sci USA* 2001, **98**:6969-6974.
 9. Briggs WR, Beck CF, Cashmore AR, Christie JM, Hughes J, Jarillo JA, Kagawa T, Kanegae H, Liscum E, Nagatani A *et al.*: **The phototropin family of photoreceptors**. *Plant Cell* 2001, **13**:993-997.
 10. Mathews S, Sharrock RA: **Phytochrome gene diversity**. *Plant Cell Environ* 1997, **20**:666-671.
 11. Whitelam GC, Devlin PF: **Roles of different phytochromes in Arabidopsis photomorphogenesis**. *Plant Cell Environ* 1997, **20**:752-758.
 12. Devlin PF, Patel SR, Whitelam GC: **Phytochrome E influences internode elongation and flowering time in Arabidopsis**. *Plant Cell* 1998, **10**:1479-1487.
 13. Quail PH, Boylan MT, Parks BM, Short TW, Xu Y, Wagner D: **Phytochromes: photosensory perception and signal transduction**. *Science* 1995, **268**:675-680.
 14. Guo H, Duong H, Ma N, Lin C: **The Arabidopsis blue light receptor cryptochrome 2 is a nuclear protein regulated by a blue light-dependent post-transcriptional mechanism**. *Plant J* 1999, **19**:279-287.
 15. Kleiner O, Kircher S, Harter K, Batschauer A: **Nuclear localization of the Arabidopsis blue light receptor cryptochrome 2**. *Plant J* 1999, **19**:289-296.
 16. Quail PH: **An emerging molecular map of the phytochromes**. *Plant Cell Environ* 1997a, **20**:657-665.

17. Yeh K-C, Lagarias JC: Eukaryotic phytochromes: light-regulated serine/threonine protein kinases with histidine kinase ancestry. *Proc Natl Acad Sci USA* 1998, **95**:13976-13981.
18. Fankhauser C, Yeh KC, Lagarias JC, Zhang H, Elich TD, Chory J: PKS1, a substrate phosphorylated by phytochrome that modulates light signaling in *Arabidopsis*. *Science* 1999, **284**:1539-1541.
19. Yamaguchi R, Nakamura M, Mochizuki N, Kay SA, Nagatani A: Light-dependent translocation of a phytochrome B-GFP fusion protein to the nucleus in transgenic *Arabidopsis*. *J Cell Biol* 1999, **145**:437-445.
20. Kircher S, Kozma-Bognar L, Kim L, Adam E, Harter K, Schaefer E, Nagy F: Light quality-dependent nuclear import of the plant photoreceptors phytochrome A and B. *Plant Cell* 1999, **11**:1445-1456.
21. Nagy F, Schäfer E: Nuclear and cytosolic events of light-induced, phytochrome-regulated signaling in higher plants. *EMBO J* 2000, **19**:157-163.
22. Nagy F, Schäfer E: Control of nuclear import and phytochromes. *Curr Opin Plant Biol* 2000b, **3**:450-454.
23. Tepperman JM, Zhu T, Chang H-S, Wang X, Quail PH: Multiple transcription-factor genes are early targets of phytochrome A signaling. *Proc Natl Acad Sci USA* 2001, **98**:9437-9442.
This paper provides evidence, based on microarray analysis of phyA-regulated gene expression, that phyA may regulate seedling de-etiolation by direct targeting of light signals to the promoters of a master-set of transcription factor genes through G-box-bound PIF3 (phytochrome-interacting factor 3).
24. Ma L, Li J, Qu L, Chen Z, Zhao H, Deng X-W: Light control of *Arabidopsis* development entails coordinated regulation of genome expression and cellular pathways. *Plant Cell* 2001, **13**:2589-2607.
25. Deng X-W, Quail PH: Signalling in light-controlled development. *Semin Cell Dev Biol* 1999, **10**:121-129.
26. Fankhauser C, Chory J: Light control of plant development. *Annu Rev Cell Dev Biol* 1997, **13**:203-229.
27. Fankhauser C: The phytochromes, a family of red/far-red absorbing photoreceptors. *J Biol Chem* 2001, **276**:11453-11456.
28. Chory J, Wu DY: Weaving the complex web of signal transduction. *Plant Physiol* 2001, **125**:77-80.
29. Hudson ME: The genetics of phytochrome signalling in *Arabidopsis*. *Semin Cell Dev Biol* 2000, **11**:475-483.
30. Neff MM, Fankhauser C, Chory J: Light: an indicator of time and place. *Genes Dev* 2000, **14**:257-271.
31. Ni M, Tepperman JM, Quail PH: PIF3, a phytochrome-interacting factor necessary for normal photoinduced signal transduction, is a novel basic helix-loop-helix protein. *Cell* 1998, **95**:657-667.
32. Ni M, Tepperman JM, Quail PH: Binding of phytochrome B to its nuclear signaling partner PIF3 is reversibly induced by light. *Nature* 1999, **400**:781-784.
33. Martinez-Garcia, JF, Huq, E, Quail, PH: Direct targeting of light signals to a promoter element-bound transcription factor. *Science* 2000, **288**:859-863.
This study identifies the G-box motif as the specific core-binding motif recognised by phytochrome-interacting factor 3 (PIF3) and demonstrates that phyB binds specifically and reversibly to DNA-bound PIF3 upon light-induced conversion to the active form of Pfr (far red light absorbing conformer of phytochrome). It also establishes that a subset of genes (*CCA1* and *LHY*) with G-box motifs in their promoters exhibit reduced induction in PIF3-deficient *Arabidopsis* seedlings in response to continuous red light; thereby, indicating that PIF3 appears to be necessary for phyB-induced expression of these genes.
34. Choi G, Yi H, Lee J, Kwon Y-K, Soh MS, Shin B, Luka Z, Hahn T-R, Song P-S: Phytochrome signalling is mediated through nucleoside diphosphate kinase 2. *Nature* 1999, **401**:610-613.
35. Quail PH: Phytochrome interacting factors. *Semin Cell Dev Biol* 2000, **11**:457-466.
This review provides a somewhat detailed examination of the data for, and implications of, the physical interactions of the phytochromes with the proteins PIF3, PKS1, NDPK2, cry1 and cry2 that had been reported at that time. It also discusses at some length the complexities raised by the apparent overlapping DNA-binding site specificities of the plant basic helix-loop-helix (bHLH) and bZIP families of factors, and the implications of potential heterodimerisations between multiple members of the large bHLH family for phytochrome signalling.
36. Motchoulski A, Liscum E: *Arabidopsis* NPH3: a NPH1 photoreceptor-interacting protein essential for phototropism. *Science* 1999, **286**:961-964.
37. Sakai T, Wada T, Ishiguro S, Okada K: RPT2: a signal transducer of the phototropic response in *Arabidopsis*. *Plant Cell* 2000, **12**:225-236.
38. Sweere U, Eichenberg K, Lohrmann J, Mira-Rodado V, Bäurle I, Kudla J, Nagy F, Schäfer E, Harter K: Interaction of the response regulator ARR4 with the photoreceptor phytochrome B in modulating red light signaling. *Science* 2001, **294**: 1108-1111.
39. Yang H-Q, Tang R-H, Cashmore AR: The signaling mechanism of **•• Arabidopsis** CRY1 involves direct interaction with COP1. *Plant Cell* 2001, **13**:2573-2587.
Evidence is presented that both the carboxy-terminal domain and the full-length molecule of CRY1 binds to COP1 in yeast two-hybrid and *in vitro* co-immunoprecipitation assays. This binding is also detected in plant extracts and appears to be constitutive, as it is unaffected by light. It is concluded that the observed binding might facilitate the light-induced inactivation of COP1 by cry1; thereby, disrupting the negative, proteolytically based regulation of HY5 by COP1.
40. Wang HY, Ma LG, Li JM, Zhao HY, Deng XW: Direct interaction of **•• Arabidopsis** cryptochromes with COP1 in light control development. *Science* 2001, **294**:154-158.
This study provides independent evidence that cry1 interacts physically with COP1 and extends the observation to include cry2. It is concluded similarly by Yang *et al.* (2000) [39••] that light-activated cryptochrome is likely to inactivate the putative E3 ubiquitin ligase activity of COP1; thereby, abrogating the targeted degradation of HY5, which leads in turn to direct control of light-responsive gene expression and photomorphogenic development.
41. Ahmad M, Jarillo JA, Smirnova O, Cashmore AR: The CRY1 blue light photoreceptor of *Arabidopsis* interacts with phytochrome A *in vitro*. *Mol Cell* 1998, **1**:939-948.
42. Mas P, Devlin PF, Panda S, Kay SA: Functional interaction of phytochrome B and cryptochrome 2. *Nature* 2000, **408**:207-211.
43. Liu XL, Covington MF, Fankhauser C, Chory J, Wagner DRY: ELF3 encodes a circadian clock-regulated nuclear protein that functions in an *Arabidopsis* phyB signal transduction pathway. *Plant Cell* 2001, **13**:1293-1304.
44. Jarillo JA, Capel J, Tang R-H, Yang H-Q, Alonso JM, Ecker JR, Cashmore AR: An *Arabidopsis* circadian clock component interacts with both CRY1 and phyB. *Nature* 2001, **410**:487-490.
45. Colon-Carmona A, Chen DL, Yeh KC, Abel S: Aux/IAA proteins are phosphorylated by phytochrome *in vitro*. *Plant Physiol* 2000, **124**:1728-1738.
46. Hudson M, Ringli C, Boylan MT, Quail PH: The *FAR1* locus encodes a novel nuclear protein specific to phytochrome A signaling. *Genes Dev* 1999, **13**:2017-2027.
47. Fairchild CD, Schumaker MA, Quail PH: *HFR1* encodes an atypical bHLH protein that acts in phytochrome A signal transduction. *Genes Dev* 2000, **14**:2377-2391.
48. Soh MS, Kim YM, Han SJ, Song PS: REP1, a basic helix-loop-helix protein, is required for a branch pathway of phytochrome A signaling in *Arabidopsis*. *Plant Cell* 2000, **12**:2061-2073.
49. Spiegelman JI, Mindrinos MN, Fankhauser C, Richards D, Lutes J, Chory J, Oefner PJ: Cloning of the *Arabidopsis* *RSF1* gene by using a mapping strategy based on high-density DNA arrays and denaturing high-performance liquid chromatography. *Plant Cell* 2000, **12**:2485-2498.
50. Ballesteros ML, Bolle C, Lois LM, Moore JM, Vielle-Calzada J-P, Grossniklaus U, Chua N-H: LAF1, a MYB transcription activator for phytochrome A signaling. *Genes Dev* 2001, **15**:2613-2625.
51. Hoecker U, Tepperman JM, Quail PH: SPA1: a WD-repeat protein specific to phytochrome A signal transduction. *Science* 1999, **284**:496-499.
52. Dieterle M, Zhou YC, Schäfer E, Funk M, Kretsch T: EID1, an F-box protein involved in phytochrome A-specific light signaling. *Genes Dev* 2001, **15**:939-944.
53. Huq E, Tepperman JM, Quail PH: GIGANTEA is a nuclear protein involved in phytochrome signaling in *Arabidopsis*. *Proc Natl Acad Sci USA* 2000, **97**:9789-9794.

54. Pepper A, Delaney T, Washburn T, Poole D, Chory J: *DET1*, a negative regulator of light-mediated development and gene expression in *Arabidopsis*, encodes a novel nuclear-localized protein. *Cell* 1994, **78**:109-116.
55. Schwechheimer C, Deng XW: The COP/DET/FUS proteins – regulators of eukaryotic growth and development. *Sem Cell Dev Biol* 2000, **11**:495-503.
56. Schwechheimer C, Deng XW: COP9 signalosome revisited: a novel mediator of protein degradation. *Trends Cell Biol* 2001, **11**:420-426.
57. Hardtke CS, Deng XW: The cell biology of the COP/DET/FUS proteins. Regulating proteolysis in photomorphogenesis and beyond? *Plant Physiol* 2000, **124**:1548-1557.
58. Wei N, Deng X-W: The role of the *COP/DET/FUS* genes in light control of *Arabidopsis* seedling development. *Plant Physiol* 1996, **112**:871-878.
59. Zhu Y, Tepperman JM, Fairchild CD, Quail P: Phytochrome B binds with greater apparent affinity than phytochrome A to the basic helix-loop-helix factor PIF3 in a reaction requiring the PAS domain of PIF3. *Proc Natl Acad Sci USA* 2000, **97**:13419-13424.
60. Wang Z-Y, Kenigsbuch D, Sun L, Harel E, Ong MS, Tobin EM: A Myb-related transcription factor is involved in the phytochrome regulation of an *Arabidopsis* *Lhcb* gene. *Plant Cell* 1997, **9**:491-507.
61. Schaffer R, Ramsay N, Samach A, Corden S, Putterill F, Carré IA, Coupland G: The late elongated hypocotyl mutation of *Arabidopsis* disrupts circadian rhythms and the photoperiodic control of flowering. *Cell* 1998, **93**:1219-1229.
62. Green RM, Tobin EM: Loss of the circadian clock-associated protein 1 in *Arabidopsis* results in altered clock-regulated gene expression. *Proc Natl Acad Sci USA* 1999, **96**:4176-4179.
63. Wang Z-Y, Tobin EM: Constitutive expression of the *CIRCADIAN CLOCK ASSOCIATED 1 (CCA1)* gene disrupts circadian rhythms and suppresses its own expression. *Cell* 1998, **93**:1207-1217.
64. Fankhauser C: Phytochromes as light-modulated protein kinases. *Sem Cell Dev Biol* 2000, **11**:467-473.
65. Millar AJ, McGrath RB, Chua N-H: Phytochrome phototransduction pathways. *Annu Rev Genet* 1994, **28**:325-349.
66. Koornneef M, Rolff E, Spruit C: Genetic control of light-inhibited hypocotyl elongation in *Arabidopsis thaliana* (L.) Heynh. *Z Pflanzenphysiol* 1980, **100**:147-160.
67. Oyama T, Shimura Y, Okada K: The *Arabidopsis* *HY5* gene encodes a bZIP protein that regulates stimulus-induced development of root and hypocotyl. *Genes Dev* 1997, **11**:2983-2995.
68. Chattopadhyay S, Ang L-H, Puente P, Deng X-W, Wei N: *Arabidopsis* bZIP protein HY5 directly interacts with light-responsive promoters in mediating light control of gene expression. *Plant Cell* 1998, **10**:673-683.
69. Alabadi D, Oyama T, Yanovsky MJ, Harmon FG, Mas P, Kay SA: Reciprocal regulation between *TOC1* and *LHY/CCA1* within the *Arabidopsis* circadian clock. *Science* 2001, **293**:880-883.
- This elegant study provides evidence that *CCA1* and *LHY* negatively regulate *TOC1* gene expression through direct, sequence-specific binding to sites in the *TOC1* promoter, and conversely that *TOC1* positively regulates *CCA1* and *LHY* expression. It is concluded that these results define the basic framework for the clock mechanism in *Arabidopsis*.
70. Strayer C, Oyama T, Schultz TF, Raman R, Somers DE, Mas P, Panda S, Kreps JA, Kay SA: Cloning of the *Arabidopsis* clock gene *TOC1*, an autoregulatory response regulator homolog. *Science* 2000, **289**:768-771.
71. Harmer SL, Hogenesch JB, Straume M, Chang H-S, Han B, Zhu T, Wang X, Kreps JA, Kay SA: Orchestrated transcription of key pathways in *Arabidopsis* by the circadian clock. *Science* 2000, **290**:2110-2113.
- This paper provides the first comprehensive microarray analysis of clock-regulated genes in plants and identifies a DNA-sequence element, designated the 'evening element', conserved in the promoters of 31 of these genes, which is required for circadian control of gene expression.
72. Wei N, Deng XW: Making sense of the COP9 signalosome – a regulatory protein complex conserved from *Arabidopsis* to humans. *Trends Genet* 1999, **15**:98-103.
73. Schwechheimer C, Serino G, Callis J, Crosby WL, Lyapina S, Deshaies RJ, Gray WM, Estelle M, Deng X-W: Interactions of the COP9 signalosome with the E3 ubiquitin ligase SCF^{TIR1} in mediating auxin response. *Science* 2001, **292**:1379-1382.
- The authors provide evidence for the interaction of the COP9 signalosome (CNS) with the E3 ubiquitin ligase SCF^{TIR1}, which is consistent with the proposed function of CNS in targeted, proteasome-mediated proteolysis.
74. Deng X-W, Matsui M, Wei N, Wagner D, Chu AM, Feldmann KA, Quail PH: *COP1*, an *Arabidopsis* photomorphogenic regulatory gene, encodes a protein with both a Zn-binding motif and a Gβ homologous domain. *Cell* 1992, **71**:791-801.
75. Ang L-H, Chattopadhyay S, Wei N, Oyama T, Okada K, Batschauer A, Deng X-W: Molecular interaction between COP1 and HY5 defines a regulatory switch for light control of *Arabidopsis* development. *Mol Cell* 1998, **1**:213-222.
76. Osterlund MT, Ang L-H, Deng X-W: The role of COP1 in repression of *Arabidopsis* photomorphogenic development. *Trends Cell Biol* 1999, **9**:113-118.
77. Osterlund MT, Hardtke CS, Wei N, Deng XW: Targeted destabilization of HY5 during light-regulated development of *Arabidopsis*. *Nature* 2000, **405**:462-466.
- This study provides compelling evidence for the involvement of COP1 and the COP9 signalosome complex in maintaining low levels of HY5 in dark-grown seedlings by proteolytic degradation of the HY5 protein. The authors also show that light reverses this suppression of HY5 levels by reducing degradation.
78. Yang HQ, Wu YJ, Tang RH, Liu DM, Liu Y, Cashmore AR: The C termini of *Arabidopsis* cryptochromes mediate a constitutive light response. *Cell* 2000, **103**:815-827.
- This landmark study provides striking evidence that expression of only the carboxyl terminus of Cry1 or Cry2 fused to a GUS reporter induces a constitutive photomorphogenic response in dark-grown *Arabidopsis* seedlings, which is similar to that of *cop1* mutants. The authors make three conclusions: that the carboxy-terminal domains of the cryptochromes carry the signalling information; that this activity is repressed by the amino-terminal domain in darkness; and that light activation of the photoreceptor relieves this repression, permitting signalling to occur.
79. Wagner D, Fairchild CD, Kuhn RM, Quail PH: Chromophore-bearing NH₂-terminal domains of phytochromes A and B determine their photosensory specificity and differential light lability. *Proc Natl Acad Sci USA* 1996, **93**:4011-4015.
80. Hoecker U, Quail PH: The phytochrome A-specific signaling intermediate SPA1 interacts directly with COP1, a constitutive repressor of light signaling in *Arabidopsis*. *J Biol Chem* 2001, **276**:38173-38178.
81. Holm M, Deng XW: Structural organization and interactions of COP1, a light-regulated developmental switch. *Plant Mol Biol* 1999, **41**:151-158.
82. Arabidopsis Genome Initiative: Analysis of the genome sequence of the flowering plant *Arabidopsis thaliana*. *Nature* 2000, **408**:796-815.
83. Riechmann JL, Heard J, Martin G, Reuber L, Jiang CZ, Keddie J, Adam L, Pineda O, Ratcliffe OJ, Samaha RR et al.: *Arabidopsis* transcription factors: genome-wide comparative analysis among eukaryotes. *Science* 2000, **290**:2105-2110.