The action mechanisms of plant cryptochromes

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Cryptochromes (CRY) are blue-light receptors that mediate various light responses in plants. The photoexcited CRY molecules undergo several biophysical and biochemical changes, including electron transfer, phosphorylation and ubiquitination, resulting in conformational changes to propagate light signals. Two modes of CRY signal transduction have recently been discovered: the cryptochrome-interacting basic-helix-loop-helix 1 (CIB)-dependent CRY2 regulation of transcription; and the SUPPRESSOR OF PHYA1/CONSTITUTIVELY PHOTOMORPHOGENIC1 (SPA1/COP1)-dependent cryptochrome regulation of proteolysis. Both CRY signaling pathways rely on blue light-dependent interactions between the CRY photoreceptor and its signaling proteins to modulate gene expression changes in response to blue light, leading to altered developmental programs in plants.

Cryptochromes

CRYs are photosensory receptors that regulate growth and development in plants and the circadian clock in both plants and animals [1,2]. Plant CRYs are best studied in *Arabidopsis* (*Arabidopsis thaliana*). The *Arabidopsis* genome encodes three CRY genes, *CRY1*, *CRY2* and *CRY3*. CRY1 and CRY2 act primarily in the nucleus [3,4], whereas CRY3 probably functions in chloroplasts and mitochondria [5]. Plants depend on CRYs and other photoreceptors to sense environmental cues, such as irradiance, day–night transition, photoperiods, and light quality for optimal growth and development. It is well known that *Arabidopsis* CRY1 and CRY2 mediate primarily blue-light regulation of de-etiolation and photoperiodic control of flowering, respectively [6,7]. In addition, these two photoreceptors regulate other aspects of plant growth and development, including entrainment of the circadian clock [8–10], guard cell development and stomatal opening [11,12], root growth [13–15], plant height [16–18], fruit and ovule size [19], tropic growth [20–23], apical dominance [16,17], apical meristem activity [24], programmed cell death [25], the high-light stress response [26,27], osmotic stress response [28], shade avoidance [29] and responses to bacterial and viral pathogens [30,31]. *Arabidopsis* CRY3 belongs to the CRY-DASH clade of the photolyase/CRY superfamily, and it is known to act as a single-stranded DNA-repairing enzyme [5,32–34]. However, CRY-DASH of some organisms have been reported to have both DNA-repairing enzyme activity and photosensory activity [32,35,36]. *Arabidopsis* CRY3 can also act as a dual-function photoreceptor in mitochondria and chloroplasts.

Studies of CRYs have been extensively reviewed during the past decade [1,2,37–46]. In this review, we focus on recent progress in the study of photoexcitation and signal transduction mechanisms of *Arabidopsis* CRYs.

Photoexcitation of cryptochromes

The photoexcitation mechanism of CRYs is not fully understood, although it has been proposed to involve light-dependent electron transport [46]. The CRY apoprotein contains two domains: the N-terminal photolyase-homologous region (PHR) domain of approximately 500 residues, and the C-terminal cryptochrome C-terminal extension (CCE) domain of various lengths and sequences (Figure 1b). PHR is the chromophore-binding domain of CRYs that bind non-covalently to the chromophore flavin adenine dinucleotide (FAD) and possibly the second chromophore, 5,10-methenyltetrahydrofolate (MTHF) [47–50]. FAD is a two-electron carrier that can exist in one of the three different redox states or five different protonated forms: oxidized (FAD), semi-reduced (anion radical $FAD^{\bullet-}$ or neutral radical $FADH^{\bullet}$), and fully reduced flavin ($FADH^{-}$ or $FADH_2$) (Figure 1a). Among the different redox forms, only the oxidized flavin and anion radical semiquinone flavin ($FAD^{\bullet-}$) absorb significant amounts of blue light (approximately 400–500 nm). It has been proposed that the oxidized flavin is the ground-state chromophore of *Arabidopsis* CRYs, because it absorbs blue light most effectively. *Arabidopsis* CRY1 expressed and purified from insect cells contained oxidized FAD, which shows an absorption spectrum that peaks at UV-A and blue-light regions [47,48]. Upon illumination, the purified CRY1 can be reduced to neutral radical semiquinone $FADH^{\bullet}$ that characteristically absorbs green light, and then to fully reduced $FADH_2$ (or $FADH^{-}$) that absorbs little visible wavelengths of light [47,51]. Similarly, purified *Arabidopsis* CRY2 also contains oxidized FAD that can be photoreduced *in vitro* [49]. It was found that addition of green light to blue light partially suppressed blue-light inhibition of hypocotyl elongation and blue-light stimulation of anthocyanin accumulation in *Arabidopsis* seedlings [49,51,52]. The antagonistic effect of adding green light to blue light was also reported for the blue light-induced CRY2 degradation and blue-light promotion of flowering in short day (SD) photoperiods [49,51]. Based on these results, a photoreduction cycle was proposed as the photoexcitation mechanism of *Arabidopsis* CRYs [49,51]. According to this hypothesis, CRYs in the dark

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 1360-1385/$ – see front matter © 2011 Published by Elsevier Ltd. doi:10.1016/j.tplants.2011.09.002 Trends in Plant Science, December 2011, Vol. 16, No. 12

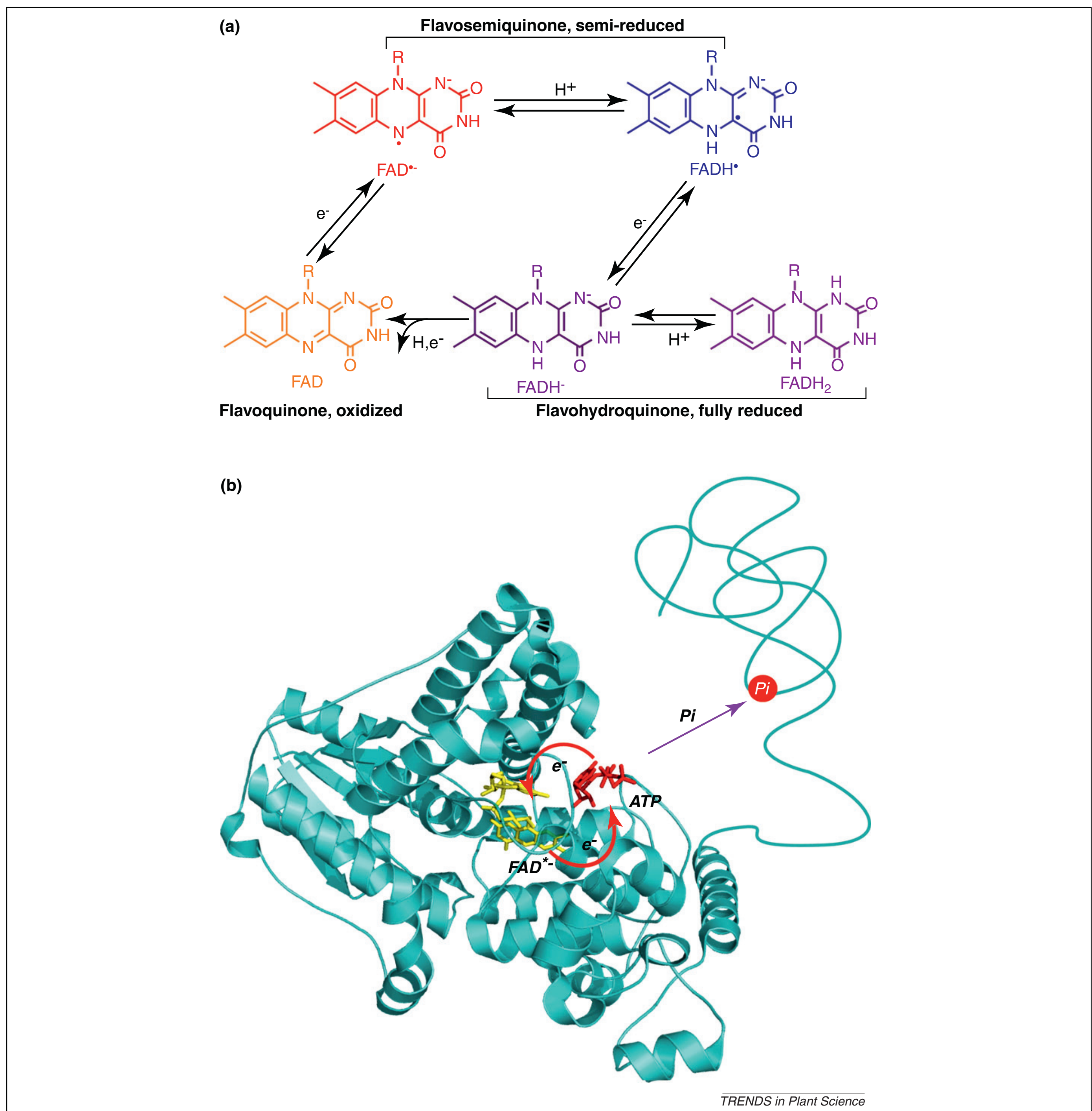

Figure 1. Photoexcitation of cryptochromes. **(a)** Five possible redox forms of flavins. The two different forms of semiquinone radicals: anion radical (e.g. $FAD^{\bullet-}$) and neutral radical (e.g. $FADH^{\bullet}$), and two forms of reduced flavins: protonated hydroquinone (e.g. $FADH_2$) and anionic hydroquinone (e.g. $FADH^{-}$) are shown. R: side groups of flavins. **(b)** The photolyase-like cyclic electron shuttle model of cryptochrome (CRY) photoexcitation. In this model, the resting state of a CRY contains the anion radical semiquinone ($FAD^{\bullet-}$). Upon photon absorption, the excited $FAD^{\bullet-}$ transfers an electron to ATP, triggering phosphotransfer and autophosphorylation of the CRY. The electron is subsequently transferred back to flavin to complete the cycle. The putative locations of the phosphorous group (red circle) and the electron transfer path (red arrows) are indicated. Abbreviation: FAD, flavin adenine dinucleotide.

(ground state) contain oxidized FAD; FAD is reduced to semi-reduced $FADH^{\bullet}$ upon blue-light absorption, which may be further reduced to $FADH_2$ (or $FADH^{-}$); the photoreduction of oxidized FAD to the semi-reduced $FADH^{\bullet}$ triggers a conformational change of the CRYs and the subsequent signal transduction; the reduced flavin is oxidized to complete the photocycle [49,51,53].

Although the photoreduction hypothesis is consistent with results of several experimental observations, whether this model explains the CRY photoexcitation *in vivo* is currently under debate [46,54–57]. An alternative hypothesis argues that the photoexcitation mechanism of CRYs might be similar to that of photolyase and might not involve a *bona fide* redox reaction (Figure 1b) [46]. Instead,

a circular electron shuttle may occur without a net gain or loss of electrons; such a circular electron shuttle may be sufficient to trigger conformational changes of CRYs and subsequent signal transduction [46]. In this regard, it is particularly interesting to compare the structures of photolyase and CRY. *Arabidopsis* CRY1 does not bind pyrimidine dimers or repair DNA; instead, it binds ATP in the FAD-access cavity at the site equivalent to the pyrimidine dimer-binding site of a photolyase [58]. Importantly, the ribose moiety of ATP penetrates deeply into the FAD-access cavity; the nucleotide moiety of ATP has a water-mediated contact with FAD; the phosphates are located near the surface of the PHR domain that probably interacts with the CCE domain [58]. Given that CRY1 catalyzes autophosphorylation [59–61], it is conceivable that photoexcitation may result in an electron transport from $FAD^{\bullet-}$ to ATP (analogous to the pyrimidine dimer) to somehow facilitate phosphotransfer from ATP to a residue of the CCE domain of CRY. The phosphotransfer from ATP to the CCE domain of CRY might contribute to a light-dependent disassociation of the CCE domain from the PHR domain (analogous to disassociation of the repaired DNA), and the backflow of the electron from ADP (analogous to the repaired pyrimidines) to FAD (Figure 1b). This alternative hypothesis appears consistent with several observations [46,54–57], but it remains speculative and needs to be tested directly. Regardless of the photoexcitation mechanism, there seems a present consensus that the photoexcited CRY adapts an open conformation to undergo further biochemical changes that affects its interactions with other proteins, resulting in alterations of gene expression and developmental programs in plants.

Photobiochemistry of cryptochromes

Similar to many photoreceptors studied to date, a photoexcited CRY changes its phosphorylation status. *Arabidopsis* CRY1 and CRY2 undergo phosphorylation in etiolated seedlings exposed to blue light, and the CRY phosphorylation is required for its photoactivation [59,60,62,63]. The CCE domains of *Arabidopsis* CRY1 and CRY2 are approximately 180 and 110 residues in length, respectively, and are thought to at as an effector domain [41–44]. The CCE domain appears intrinsically unstructured, but it may change conformation upon photoexcitation, presumably by light-induced folding [63–68]. It has been proposed that blue light-dependent phosphorylation of CRYs causes electrostatic repelling of the CCE domain from the surface of the negatively charged PHR domain of CRY [63–68], resulting in separation of the two domains to trigger or alter the interaction between CRYs and their signaling partners [68,69]. Because multiple serine residues of a CRY are phosphorylated in response to light, one or more protein kinases are probably involved in CRY phosphorylation in addition to autophosphorylation [59,62]. However, the protein kinase(s) responsible for the complete phosphorylation of *Arabidopsis* CRY has not been identified, although multiple protein kinases, including an AMP-activated protein kinase (AMPK), a casein kinase I (CKIε), a glycogen synthase kinase (GSK-3β) and a mitogen-activated protein kinase (MAPK), have been found to phosphorylate mammalian CRYs [70–74].

Blue light-dependent phosphorylation of *Arabidopsis* CRY appears to trigger ubiquitination and subsequent degradation of the photoreceptor, in addition to the conformational changes [4,69,75]. Similar to phytochromes, for which phyA but not other phytochromes, undergoes rapid degradation in red light, only CRY2 but not CRY1 is rapidly degraded in blue light [75,76]. This observation suggests that different CRYs use different mechanisms for desensitization. As expected, the blue light-dependent CRY2 degradation requires the flavin chromophore, because the $CRY2^{D387A}$ mutant protein that fails to bind FAD no longer undergoes blue light-dependent degradation [77]. Both CRY2 phosphorylation and degradation take place in the nucleus, suggesting that the photoexcited CRY2 is sequentially modified by the kinases and E3 ubiquitin ligases in the nucleus [69]. Results of a domain swap experiment indicate that both the PHR domain and the CCE domain are required for the blue light-dependent degradation of CRY2 [75]. Indeed, the fusion protein GUS–CCT2, which contains only the CCE domain of CRY2 without the PHR domain, is constitutively phosphorylated, but no longer degraded regardless of blue-light treatment [4,62,69]. CRYs in other organisms also undergo ubiquitination-dependent degradation, and the E3 ubiquitin ligases responsible for degradation of mouse (*Mus musculus*) and *Drosophila* (*Drosophila melanogaster*) CRYs have been identified [78–82]. The multifunctional E3 ubiquitin ligase CONSTITUTIVE PHOTOMORPHOGENIC 1 (COP1) seems to be associated with CRY2 degradation in *Arabidopsis*, because CRY2 degradation is partially impaired in *cop1* weak mutant alleles (*cop1-4* and *cop1-6*) [62,66,67]. However, CRY2 still undergoes blue light-dependent degradation in the *cop1* null allele (*cop1-5*) [62] (B. Liu and C. Lin, unpublished data), suggesting a possible involvement of additional E3 ubiquitin ligases in blue light-dependent CRY2 degradation. Consistent with this possibility, the SPA proteins that are important for COP1 activity show no direct involvement in blue light-dependent CRY2 degradation, because CRY2 appears to degrade normally in the *spa1spa2spa3spa4* quadruple mutant (B. Liu, and C. Lin, unpublished data). Further investigations are needed to elucidate the molecular mechanism responsible for the blue light-dependent ubiquitination of CRY2.

Signal transduction of cryptochromes

It seems clear that modulation of nuclear gene expression is the major consequence of blue light-dependent CRY signal transduction, whereby photoexcited CRYs change conformation to interact with CRY-signaling proteins, triggering changes in gene expression and developmental programs in plants (Figure 2). Depending on the conditions of tests, approximately 5–25% of genes in the *Arabidopsis* genome change their expression in response to blue light; most of the changes are mediated by CRY1 and CRY2 [21,83–85]. The expression of many CRY-regulated genes is also regulated by other signaling pathways, such as phytochromes and phytohormones, suggesting that the CRY-dependent photomorphogenesis is intimately integrated with the general regulatory networks that control plant development. CRYs mediate blue-light control of gene expression via at least two mechanisms: light-dependent modulation of transcription

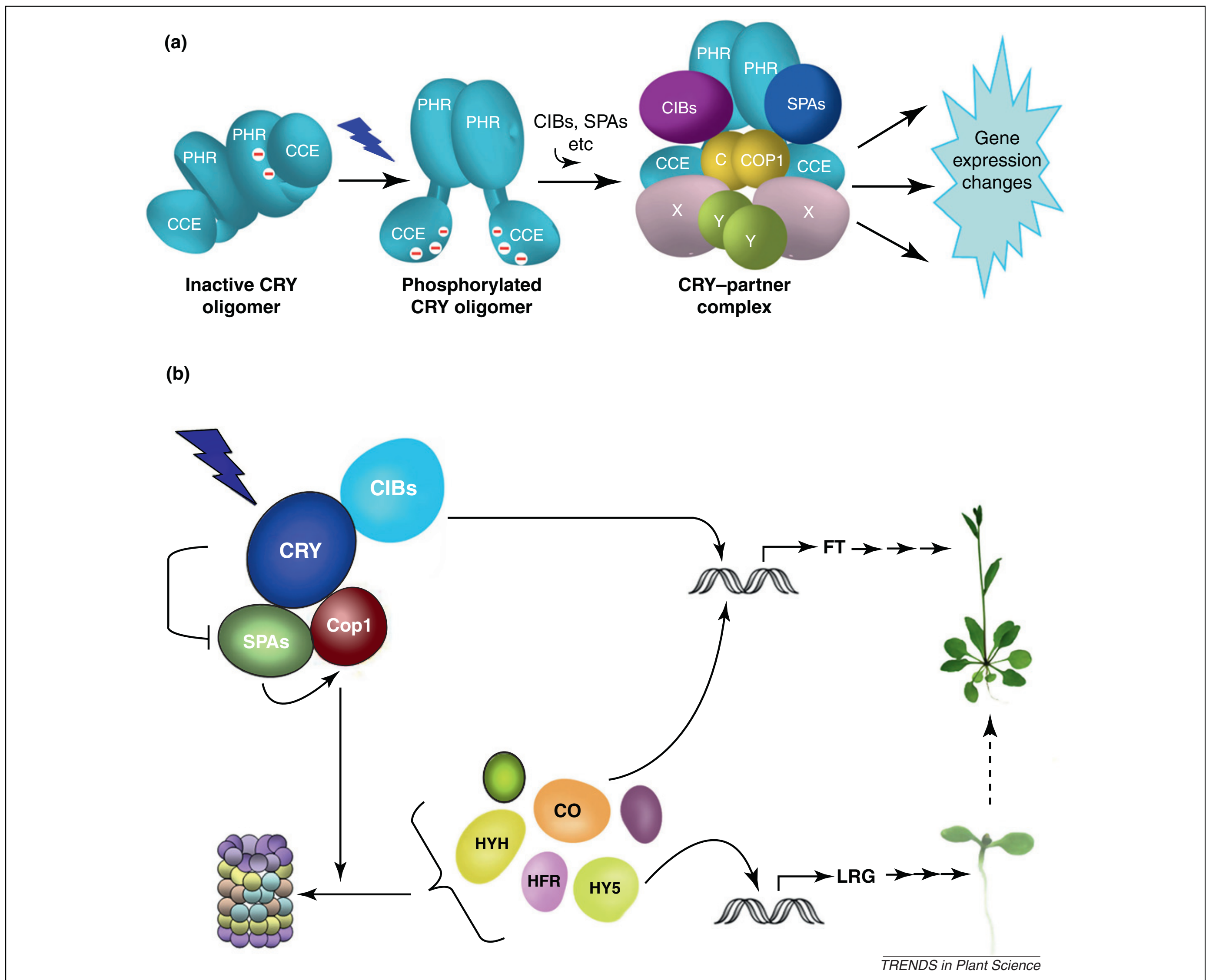

Figure 2. Signal transduction of cryptochromes. **(a)** Photoexcited cryptochrome (CRY) changes conformation to initiate signal transduction by interacting with signaling proteins. This model depicts CRY homodimerization via the PHR domains, light-dependent phosphorylation (negative charges shown), changes of protein conformation by the disengagement of the PHR and CCE domains, and interaction with partner proteins, including CIBs, SPAs, COP1 and other yet to be identified CRY-interacting proteins (X and Y). **(b)** Two mechanisms of CRY signal transduction: regulation of transcription via light-dependent interaction of CRYs with transcription factors CIB1 and its relatives (CIBs), and post-translational regulation of proteolysis via light-dependent interaction of CRYs with SPA1 and its relatives (SPAs). The CRY-interacting CIBs activate *FT* transcription to promote floral initiation. CRYs interact with SPA proteins to suppress the SPA activation of COP1 activity that is required for the degradation of HY5, HYH, CO and other transcription regulators, resulting in changes of transcription of light-regulated genes (LRG) and photomorphogenesis. Abbreviations: CCE, cryptochrome C-terminal extension; CIB1, CRY-interacting basic-helix-loop-helix 1; CO, CONSTANS; COP1, CONSTITUTIVELY PHOTOMORPHOGENIC1; FT, FLOWERING LOCUS T; HY5, LONG HYPOCOTYL5; HYH, HY5 HOMOLOGUE; PHR, photolyase-homologous region; SPA1, SUPPRESSOR OF PHYA 1.

[e.g. the CRY–CIBs) pathway] and light-dependent suppression of proteolysis (the CRY–SPA1/COP1 pathway). Both mechanisms are involved with blue light-dependent protein–protein interactions of CRYs and the signaling proteins.

The CRY2–CIBs pathway

CRY-interacting basic-helix-loop-helix 1) is the first blue light-dependent CRY2-interacting protein identified in plants [77]. *Arabidopsis* CRY2 undergoes blue light-specific interaction with the basic-helix-loop-helix (bHLH) transcription factor CIB1, which was isolated in a blue light-differentiated yeast-two-hybrid screen [77]. CIB1 positively regulates floral initiation in a CRY2-dependent manner and it interacts with the chromatin of the promoter DNA of the *FLOWERING LOCUS T* (*FT*) gene, which encodes a mobile transcriptional regulator that migrates from leaves to the apical meristem to activate transcription of floral meristem identity genes [86]. *Arabidopsis* CIB1 binds to the G-box (CACGTG) DNA sequence with the highest affinity *in vitro*. However, the transcriptional regulatory activity of CIB1 seems to be indiscriminatory toward G and E boxes (CANNTG) in a transient *in vivo* assay, and the *FT* promoter contains E box but not G box sequence [77]. These observations argue for a significant difference of the CIB1 DNA-binding activity *in vitro* and *in vivo*. One possible interpretation of this predicament would be that CIB1 heterodimerizes with other bHLH proteins to alter their preference or affinity to different DNA sequences *in vivo*. Consistent with this hypothesis, it was found that at least

three CIB1-related bHLH proteins, referred to as CIB3, CIB4 and CIB5, can heterodimerize with CIB1. The heterodimers of different CIB proteins, but not the homodimers of individual CIB proteins, bind to the E box of the *FT* promoter *in vitro* (H. Liu, and C. Lin, unpublished data). More importantly, although monogenic mutations of individual *CIB* genes show no apparent phenotypic alterations, the *cib1cib3cib5cib4RNAi* quasi-quadruple mutant exhibits a marked delay of floral initiation [77] (H. Liu, and C. Lin, unpublished data). These results suggest that multiple CIB proteins act redundantly in the CRY2–CIB signal transduction pathway to mediate photoperiodic promotion of floral initiation.

Several questions remain to be investigated about the mechanism and regulation of the CRY2–CIB signaling pathway. For example, it remains unclear whether the blue light-dependent interaction of CIB1 with CRY2 affects the affinity of CIB1 to the E box DNA sequence or the *FT* promoter *in vivo* or exactly how the DNA binding or transcriptional regulatory activity of CIB1 is affected by blue light, CRY2 and other CIB proteins. In addition, CIB1 and other CIB proteins are degraded by the ubiquitin-26S proteasome pathway in plants grown under all light conditions except blue light (H. Liu, and C. Lin, unpublished data). It would be interesting to identify the E3 ubiquitin ligases responsible for the degradation of CIB1 and related proteins, as well as the photoreceptors mediating blue light-specific stabilization of these CRY2-signaling proteins. Furthermore, CIB1 and its related CIB proteins do not seem to play major roles in the de-etiolation responses, because no abnormal de-etiolation phenotype has been observed in the monogenic mutants or the *cib1-cib3cib5cib4RNAi* quasi-quadruple mutant [77] (H. Liu and C. Lin, unpublished results). Given that CRY1 is the major CRY mediating blue-light regulation of de-etiolation, it would be interesting to investigate whether there are blue light-specific CRY1-interacting transcription factors that function in de-etiolation or other blue-light responses.

The CRY–SPA1/COP1 pathway

In addition to the direct regulation of transcription by interacting with transcription factors, CRYs also indirectly modulate gene expression via post-transcriptional mechanisms by interacting with the SPA1/COP1 complex [87–89]. It is well known that CRYs mediate blue-light suppression of the E3 ubiquitin ligase COP1 and COP1-dependent proteolysis to affect gene expression [83,90]. For example, CRY1 mediates blue-light suppression of the COP1-dependent degradation of the bZIP transcription factors LONG HYPOCOTYL5 (HY5), HY5 HOMOLOGUE (HYH), and the bHLH transcription factor Long Hypocotyl in Far-Red 1 (HFR1), which regulate transcription of genes required for the de-etiolation response [90–94]. Many of the target genes of CRY1 and HY5 encode signaling proteins functioning in the phytohormones, such as auxin, brassinosteroid (BR) and gibberellic acid (GA), enzymes catalyzing syntheses and degradation of cell wall components, and photosynthetic and other metabolic enzymes. Altered abundance of those signaling proteins and metabolic enzymes can at least partially explain the CRY1-mediated morphological changes of young seedlings in response to blue light. Similarly, CRY2 mediates blue-light suppression of the COP1-dependent protein degradation of a major transcriptional regulator of floral initiation, *CONSTANS* (*CO*). The CO protein is a critical positive regulator of flowering in LD (long day), which promotes flowering initiation by activating transcription of the florigen gene *FT* [95]. It has been shown recently that COP1 physically interacts with CO *in vivo*, and that COP1 facilitates ubiquitination of CO *in vitro* [96,97]. CRYs are required for the accumulation of the CO protein in blue light [98], whereas COP1 promotes CO degradation in the absence of blue light [96,97]. These observations argue that CRYs mediate blue light-dependent suppression of the COP1 activity to facilitate CO accumulation and floral initiation in response to photoperiodic signals.

It took over a decade to solve the puzzle of how CRYs mediate blue light-dependent suppression of COP1 activity. The first hint of the involvement of COP1 in the CRY signal transduction came from a study showing that overexpression of the GUS-fusion proteins of the CCE domain of CRY1 and CRY2 (referred to as GUS–CCT1 and GUS–CCT2, respectively) resulted in constitutive photomorphogenic phenotype resembling that of the *cop1* mutants [65]. It was subsequently discovered that COP1 physically interacts with CRY1 and CRY2, albeit in a light-independent manner [66,67]. These recent studies argue strongly that the CRY/COP1 complex is involved in CRY signal transduction. However, there are some interesting questions remaining. For example, it is not clear why CRY dimerization is required for the activity of the CCE domain of CRYs (e.g. GUS-–CCT1 and GUS–CCT2) but not for their interaction with COP1. CRY1 and CRY2 form homodimers *in vivo* via the PHR domain, and dimerization is required for the dominant positive (or *cop*) activity of the CCT and/or CCE domains expressed *in vivo* [68,99,100]. However, the CCT and/or CCE domains alone interacted with COP1 in yeast cells or *in vitro*, but they showed no physiological activity in plants [65,67,68,99]. More importantly, COP1 interacts with CRY1 or CRY2 in a light-independent manner [66,67,77], leaving the question of how CRYs mediate blue-light suppression of the COP1 activity open at the time of those studies. Two mechanisms have been proposed to answer this question: CRYs may alter the activity or nuclear and/or cytoplasmic distribution of COP1 with some unknown biochemical mechanisms [101]; alternatively, CRY might undergo a light-dependent interaction with COP1-interacting proteins to affect the COP1 activity [102–106].

Direct tests of the second mechanism have been reported in three recent studies [87–89]. These showed that *Arabidopsis* CRY1 and CRY2 undergo blue light-dependent interaction with the COP1-interacting protein SPA1 [102–106]. It was found that *Arabidopsis* CRY1 and CRY2 interact with SPA1 in response to blue light but not red light; and that SPA1 acts genetically downstream of CRY1 and CRY2 to mediate blue light suppression of the COP1-dependent degradation of HY5 and CO, respectively. Because SPA1 is known to interact physically with COP1 in a light-dependent manner and it is a positive regulator of COP1 [103,105,107,108], the blue

light-dependent CRY–SPA1 interaction appears to at least partially solve the puzzle of how CRYs, which showed no light-dependent interaction with COP1 in the previous studies, mediate light-dependent suppression of COP1. Unexpectedly, the structurally similar CRY1 and CRY2 interact with SPA1 in different ways. The C-terminal CCE domain of CRY1 interacts with the C-terminal CC-WD domain of SPA1, whereas the N-terminal PHR domain of CRY2 interacts with the N-terminal kinase-like domain of SPA1. The different modes of protein–protein interaction of CRY1–SPA1 and CRY2–SPA1 might explain the different modes of action for the two CRYs. It was shown that CRY1–SPA1 interaction suppressed SPA1–COP1 interaction in yeast (*Saccharomyces cerevisiae*) and in plant cells [88,89], arguing that CRY1 may act as a competitive inhibitor of COP1. The finding that CRY1 acts as a light-dependent competitive inhibitor of SPA1–COP1 interaction also provides, at least partially, a molecular explanation of previous observations that SPA1–COP1 interaction is suppressed by light [103]. In contrast to CRY1, CRY2–SPA1 interaction does not seem to affect SPA1-COP1 interaction. Instead, the blue light-dependent CRY2–SPA1 interaction appears to enhance the CRY2–COP1 interaction in yeast cells and formation of the CRY2/COP1 complex in plants [87]. This observation reveals the photobiological aspect of the effect of CRY2 on COP1, although exactly how an augmented CRY2/COP1 complex formation inhibits COP1 activity remains unclear at present. The blue light enhancement of CRY2–COP1 interaction was observed only in a yeast three-hybrid assay and in a co-immunoprecipitation analysis using 'sensitized' *Arabidopsis* transgenic lines that overexpress SPA1 [87], which explains why this phenomenon was not observed previously.

Arabidopsis has three SPA1-related proteins, SPA2, SPA3 and SPA4, which function in a partially redundant manner [108,109]. Among the SPA quartet proteins, SPA4 showed a strong blue light-dependent interaction with CRY1 and CRY2 [87–89]. However, whether SPA4 or other SPA proteins play the same or different role as SPA1 in the CRY signal transduction is not clear. It also remains to be elucidated whether the blue light-dependent interaction of CRYs and the SPA1/COP1 complex might provide a molecular mechanism for the functional interaction, or co-action, of phytochromes and CRYs. SPA1 was originally identified as a phyA signaling protein that plays important roles in the functions of phytochromes and red and/or far-red light suppression of COP1 activity [103,107,110,111]. It has been shown that CRYs mediate blue light regulation of the mRNA expression of the *SPA* genes, suggesting an indirect mechanism for the CRY regulation of phytochrome function [85]. Given that phytochromes might also physically interact with the SPA1/COP1 complex [107], the newly discovered physical association of the SPA1/COP1 complex with CRYs argues for a more direct role of the complex in the co-action of phytochromes and CRYs. How light modulates the cellular homeostasis of the phytochrome–SPAs/COP1 complex and the CRY–SPAs/COP1 complex to affect photoreceptor signal transduction and photomorphogenesis of plants in nature remains to be further investigated.

Concluding remarks and future outlook

Significant progress has been made over the past few years in understanding the mechanism of action of plant CRYs. The blue light-dependent physical interaction of CRYs with the transcriptional or post-transcriptional regulators of gene expression has emerged as the primary mechanism of CRY signal transduction in plants. However, many aspects of the photochemistry, signal transduction and regulatory mechanisms of CRYs still remain to be elucidated. For example, in addition to CIBs, SPAs and COP1, are there additional CRY-interacting proteins directly involved in the early CRY signaling process regulating transcription? What are the protein kinases, phosphatases and E3 ubiquitin ligases required for light regulation of CRYs? How do CRYs regulate hormone metabolic and signaling genes in different cells to modulate development? Continuous investigations of these questions directly related to CRY photobiology and other questions concerning the system-wide interplay of light and hormonal signal transduction pathways are required to understand fully the action mechanisms of plant CRYs.

Acknowledgments

Work in the authors' laboratories is supported, in part, by the National Institute of Health (GM56265 to CL), UCLA faculty research grants, Sol Leshin BGU-UCLA Academic Cooperation programs, and National Transgenic Crop Initiative (to ICS-CAAS).

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