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The action mechanisms of plant cryptochromes

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Abstract

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

Cryptochromes

Cryptochromes (CRY) are photosensory receptors that regulate growth and development in plants and the circadian clock in plants and animals [1, 2]. Plant cryptochromes are best studied in *Arabidopsis* (*Arabidopsis thaliana*). The Arabidopsis genome encodes three cryptochrome 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 cryptochromes 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/cryptochrome 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 possess 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.

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

Photoexcitation of cryptochromes

The photoexcitation mechanism of cryptochrome is not fully understood, although it has been proposed to involve light-dependent electron transport [46]. The cryptochrome apoprotein contains two domains, the N-terminal PHR (Photolyase-Homologous Region) domain of about 500 residues, and the C-terminal CCE domain (Cryptochrome C-terminal Extension)) of various lengths and sequences (Figure 1b). PHR is the chromophore-binding domain of cryptochromes that bind non-covalently to the chromophore flavin adenine dinucleotide (FAD) and possibly the second chromophore, 5,10-methenyltetrahydrofolate (MTHF) [47–50]. The chromophore of cryptochromes, flavin adenine dinucleotide, is a two-electron carrier that can exist in one of the three different redox states or five different protonated forms: oxidized (FAD), semireduced (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 ($FAD^{\bullet -}$) absorbs significant amounts of blue light (~400–500nm). It has been proposed that the oxidized flavin may be the ground state chromophore of *Arabidopsis* cryptochromes, 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 green light to blue light was also reported for the blue light-induced CRY2 degradation and blue light promotion of flowering in SD (short day)photoperiods [49, 51]. Based on these results, a photoreduction cycle was proposed as the photoexcitation mechanism of *Arabidopsis* cryptochromes [49, 51]. According to this hypothesis, cryptochromes in the dark (ground state) contain oxidized FAD; FAD is reduced to semireduced $FADH^{\bullet}$ upon blue light absorption, which may be further reduced to $FADH_2$ (or $FADH^{-}$); the photoreduction of oxidized FAD to the semireduced $FADH^{\bullet}$ triggers a conformational change of the cryptochromes 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 cryptochrome photoexcitation *in vivo* is currently under debate [46, 54–57]. An alternative hypothesis argues that the photoexcitation mechanism of cryptochromes might be similar to that of photolyase and 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 cryptochromes and subsequent signal transduction [46]. In this regard, it is particularly interesting to compare the structures of photolyase and cryptochrome. *Arabidopsis* CRY1 does not bind pyrimidine dimer or repair DNA, but 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 likely 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 CCE domain from 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 a number of observations [46, 54–57], but it remains speculative and needs to be tested directly. Regardless of the photoexcitation mechanism, it seems a present consensus that the photoexcited cryptochrome 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

Like many photoreceptors studied to date, a photoexcited cryptochrome changes its phosphorylation status. *Arabidopsis* CRY1 and CRY2 undergo phosphorylation in etiolated seedlings exposed to blue light, and the cryptochrome phosphorylation is required for its photoactivation [59, 60, 62, 63]. The CCE domain of *Arabidopsis* CRY1 and CRY2 is approximately 180 and 110 residues in length, respectively, and is 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 cryptochromes causes electrostatic repelling of the CCE domain from the surface of the negatively charged PHR domain of cryptochrome [63–68], resulting in separation of the two domains to trigger or alter interaction between cryptochromes and their signaling partners [68, 69]. Because multiple serine residues of a cryptochrome are phosphorylated in response to light, one or more protein kinases are likely involved in cryptochrome phosphorylation in addition to autophosphorylation [59, 62]. However, the protein kinase(s) responsible for the complete phosphorylation of *Arabidopsis* cryptochrome 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 cryptochromes [70–74].

Blue light-dependent phosphorylation of *Arabidopsis* cryptochrome 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 cryptochromes can 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 may be 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 that 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]. Cryptochromes in other organisms also undergo ubiquitination-dependent degradation, and the E3 ubiquitin ligases responsible for degradation of mouse (*Mus musculus*) and *Drosophila* (*Drosophila melanogaster*) cryptochromes have been identified [78–82]. The multifunctional E3 ubiquitin ligase COP1 seems associated with CRY2 degradation in *Arabidopsis*, because CRY2 degradation is partially impaired in the *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 the blue light-dependent CRY2 degradation. Consistent with this possibility, the SPA proteins that are important for the COP1 activity show no direct involvement in the 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 cryptochrome signal transduction, whereby photoexcited cryptochromes change conformation to interact with CRY-signaling proteins, triggering changes of gene expression and developmental programs of 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 those blue light-regulated gene expression changes are mediated by CRY1 and CRY2 [21, 83–85]. The expression of many CRY-regulated genes are also regulated by other signaling pathways such as phytochromes and phytohormones, suggesting that the cryptochrome-dependent photomorphogenesis is intimately integrated with the general regulatory networks that control plant development. Cryptochromes mediate blue light control of gene expression via at least two mechanisms: light-dependent modulation of transcription (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 cryptochromes and the signaling proteins.

The CRY2–CIBs pathway

CIB1 (cryptochrome-interacting basic-helix-loop-helix 1) is the first blue light-dependent CRY2-interacting proteins identified in plants [77]. *Arabidopsis* CRY2 undergoes blue light-specific interaction with the 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 *FT* gene, which encodes a mobile transcriptional regulator that migrates from leaves to 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 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. Secondly, 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 *cib1cib3cib5cib4RNAi* quasi-quadruple mutant [77] (H. Liu and C. Lin, unpublished results). Given that CRY1 is the major cryptochrome 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, cryptochromes also indirectly modulate gene expression *via* post-transcriptional mechanisms by interacting with the SPA1/COP1 complex [87–89]. It is well known that cryptochromes 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 HY5 (LONG HYPOCOTYL5), HYH (HY5 HOMOLOGUE), and the bHLH (basic helix-loop-helix) transcription factor HFR1 (Long Hypocotyl in Far-Red 1), 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, BR (brassinosteroid), and GA (gibberellic acid), 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]. Cryptochromes 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 cryptochromes 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 cryptochromes mediate blue light-dependent suppression of COP1 activity. The first hint for the involvement of COP1 in the cryptochrome 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) 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 the cryptochrome signal transduction. However, there are some interesting questions left unsolved. For example, it is not clear why

cryptochrome dimerization is required for the activity of the CCE domain of cryptochromes (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/CCE domains expressed *in vivo* [68, 99, 100]. However, the CCT/CCE domains alone could interact 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 cryptochromes mediate blue light suppression of the COP1 activity open at the time of those studies. Two mechanisms have been proposed to answer this question: cryptochromes may alter the activity or nuclear/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 studies 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 physically interact 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 appear to at least partially solve the puzzle how cryptochromes, 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 cryptochromes. 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 only observed in a yeast three-hybrid assay or in the 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 cryptochrome signal transduction is not very clear. It also remains to be elucidated whether the blue light-dependent interaction of cryptochromes and the SPA1/COP1 complex might provide a molecular mechanism for the functional interaction, or co-action, of phytochromes and cryptochromes. SPA1 was originally identified as a phyA signaling protein that plays important roles in the functions of phytochromes and red/far-red light suppression of COP1 activity [103, 107, 110, 111]. It has been shown that cryptochromes mediate blue light

regulation of the mRNA expression of the *SPA* genes, suggesting an indirect mechanism for the cryptochrome 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 cryptochromes argues for a more direct role of SPA1/COP1 complex in the co-action of phytochromes and cryptochromes. How light modulate the cellular homeostasis of the phytochrome–SPAs/COP1 complex and the cryptochrome–SPAs/COP1 complex to affect photoreceptor signal transduction and photomorphogenesis of plants in nature remain to be further investigated.

Conclusion and future outlook

Significant progress has been made in the last few years in our understanding of the action mechanism of plant cryptochromes. The blue light-dependent physical interaction of cryptochromes with the transcriptional or post-transcriptional regulators of gene expression has emerged as the primary mechanism of cryptochrome signal transduction in plants. However, many aspects of the photochemistry, signal transduction and regulatory mechanisms of cryptochromes 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 requir ed for light regulation of cryptochromes? How do cryptochromes regulate hormone metabolic and signaling genes in different cells to modulate development? Continuous investigations of these questions directly related to cryptochrome photobiology and other questions concerning the system-wide interplay of light and hormonal signal transduction pathways are required to fully understand the action mechanisms of plant cryptochromes.

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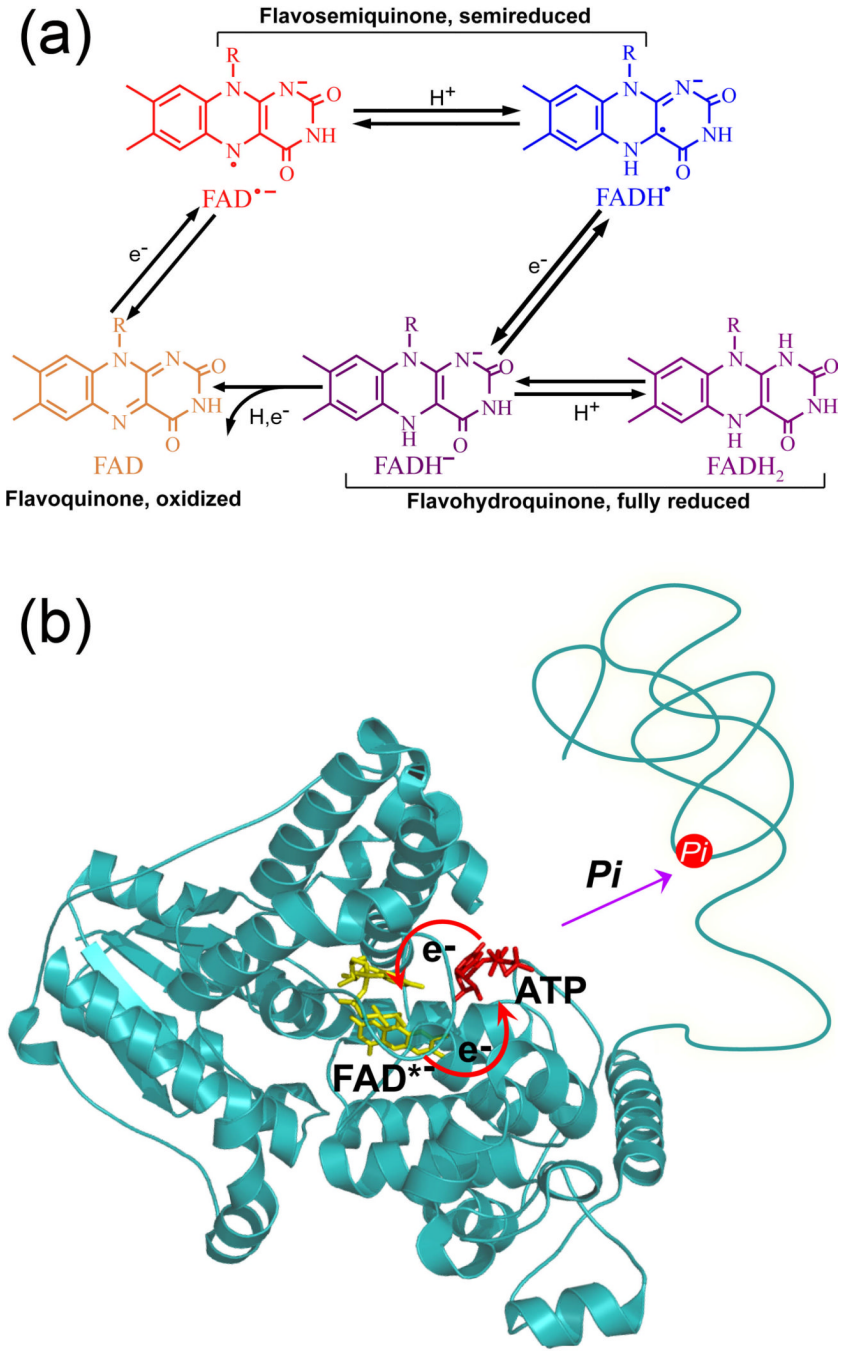

Figure 1.

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 photoexcitation. In this model, the resting state of a cryptochrome 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 cryptochrome. The electron is subsequently

transferred back to flavin to complete the cycle. The putative locations of phosphorous group (red circle) and electron transfer path (red arrows) are indicated.

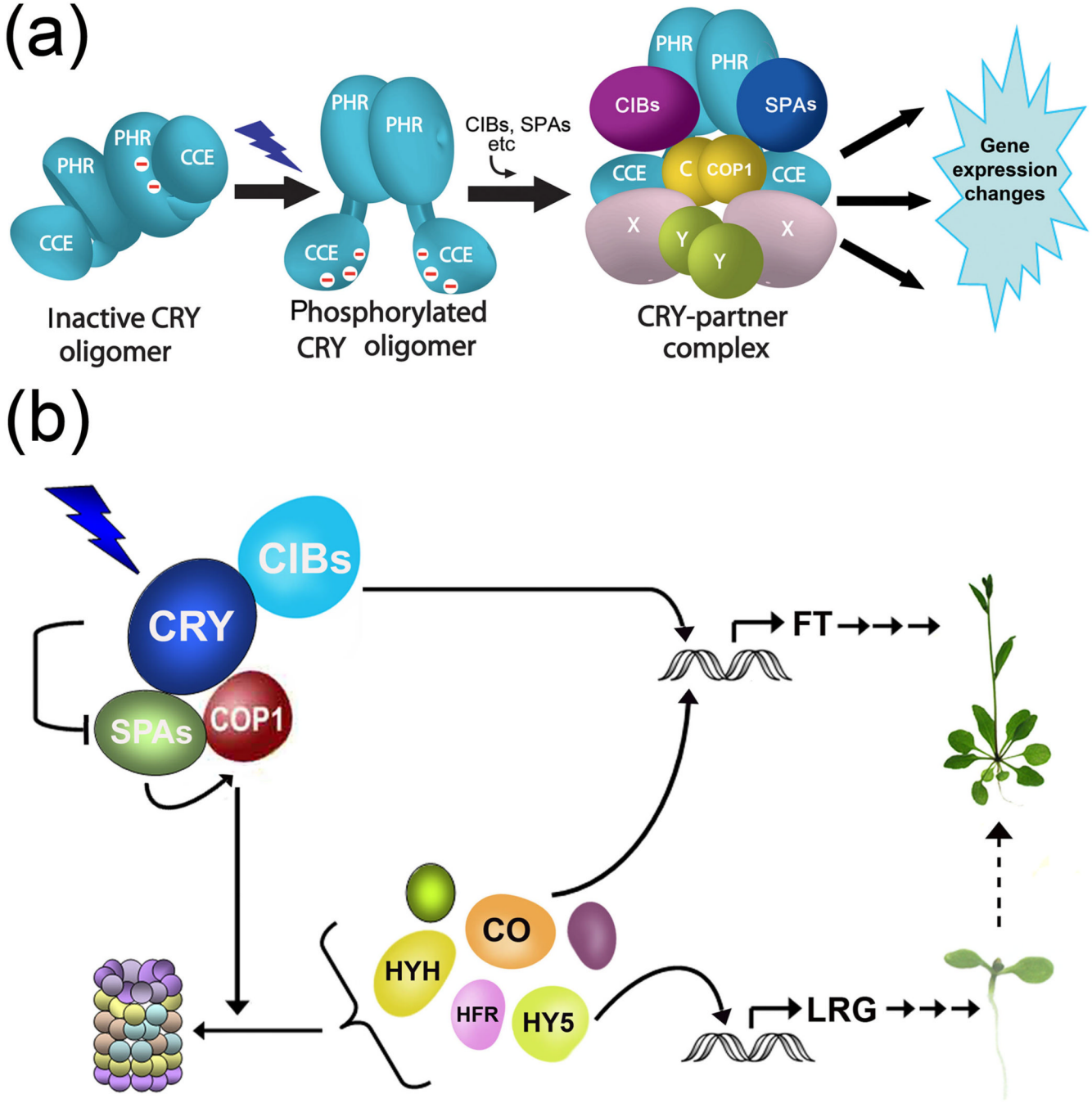

Figure 2.

Signal transduction of cryptochromes.(**a**) Photoexcited cryptochrome change conformation to initiate signal transduction by interacting with signaling proteins. This model depicts cryptochrome 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 cryptochrome signal transduction: regulation of transcription via light-dependent interaction of cryptochromes with transcription factors CIB1 and its relatives (CIBs), and post-translational regulation of proteolysis via light-dependent interaction of cryptochromes with

SPA1 and its relatives (SPAs). The cryptochrome-interacting CIBs activate *FT* transcription to promote floral initiation. Cryptochromes 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.