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Mechanisms of Cryptochrome-Mediated Photoresponses in Plants

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blue light, cryptochrome, CRY1, CRY2, photoreceptor, photomorphogenesis, transcription, proteolysis, protein–protein interactions, *Arabidopsis*

Abstract

Cryptochromes are blue-light receptors that mediate photoresponses in plants. The genomes of most land plants encode two clades of cryptochromes, CRY1 and CRY2, which mediate distinct and overlapping photoresponses within the same species and between different plant species. Photoresponsive protein–protein interaction is the primary mode of signal transduction of cryptochromes. Cryptochromes exist as physiologically inactive monomers in the dark; the absorption of photons leads to conformational change and cryptochrome homooligomerization, which alters the affinity of cryptochromes interacting with cryptochrome-interacting proteins to form various cryptochrome complexes. These cryptochrome complexes, collectively referred to as the cryptochrome complexome, regulate transcription or stability of photoresponsive proteins to modulate plant growth and development. The activity of cryptochromes is regulated by photooligomerization; dark monomerization; cryptochrome regulatory proteins; and cryptochrome phosphorylation, ubiquitination, and degradation. Most of the more than 30 presently known cryptochrome-interacting proteins are either regulated by other photoreceptors or physically interacting

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with the protein complexes of other photoreceptors. Some cryptochrome-interacting proteins are also hormonal signaling or regulatory proteins. These two mechanisms enable cryptochromes to integrate blue-light signals with other internal and external signals to optimize plant growth and development.

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1. INTRODUCTION

Cryptochromes are found in all major evolutionary lineages, from archaea to bacteria, algae to terrestrial plants, and sponges to humans, and they were among the first photoreceptors to evolve in plants (15, 42, 135). Cryptochromes were first discovered in *Arabidopsis* (2). Researchers found that the *long hypocotyl 4* (*HY4*) gene responsible for the blue-light inhibition of the hypocotyl elongation of *Arabidopsis* seedlings encodes a protein homologous to DNA photolyases (2). Photolyases are photoresponsive DNA-repairing enzymes that repair the cyclobutane pyrimidine dimer or 6-4 pyrimidine-pyrimidone photoproducts of ultraviolet (UV)-damaged DNA (134). The HY4 protein, later referred to as cryptochrome 1 or CRY1 (82), contains flavin adenine dinucleotide (FAD), which is the primary chromophore of photolyases and cryptochromes, but generally lacks the DNA-repairing enzymatic activity of the photolyase (83, 101). Soon after the discovery of the first cryptochrome in *Arabidopsis*, cryptochromes were also found in other lineages, such as insects and mammals, acting as photoreceptors, transcriptional regulators, or integral parts of the circadian oscillator (31, 53, 108, 147, 157). Cryptochromes are defined by their common two-domain structure: the highly conserved FAD-binding photolyase homologous region (PHR) domain that is approximately 500 residues in length and the divergent CRY C-terminal extension (CCE) domain of various lengths that often contains intrinsically disordered regions (121, 155). The CCE domains of cryptochromes from moss, fern, and angiosperm all contain an evolutionarily

Cryptochrome:
blue-light receptor
that mediates
photoresponses in
plants

HY4: long hypocotyl 4

FAD: flavin adenine
dinucleotide

PHR: photolyase
homologous region

CCE: cryptochrome
C-terminal extension
(also known as CCT,
CTT, etc.)

conserved DQXVP-acidic-STAES (DAS) signature, and researchers hypothesized that the ancestral plant cryptochromes arose by fusion of an ancestral photolyase sequence to a DAS-containing sequence, which may or may not have been lost during evolution (84). Although the PHR domain and CCE domain of cryptochromes were previously thought to act as the light-sensing domain and effector domain, respectively, most presently known cryptochrome-interacting proteins, except constitutive photomorphogenic 1 (COP1), physically interact with the PHR domain of cryptochromes, suggesting that both the PHR and CCE of cryptochromes may act as effect domains.

Based on sequence analyses, cryptochromes are grouped into three major classes: plant (and plant-like), animal (and animal-like), and CRY-DASH (cryptochrome-*Drosophila*, *Arabidopsis*, *Synechocystis*, human) that are widely found in microbes and eukaryotic organelles (35, 71, 105, 110). The ancestral cyclobutane pyrimidine dimer-repairing photolyase might have duplicated at least eight times before the divergence of eubacteria and eukaryotes, and cryptochromes in different lineages, such as those of plants and animals, are thought to have evolved independently (15, 63). Different organisms have different numbers of cryptochromes, ranging from two in *Arabidopsis* and humans to six or seven in soybean and zebrafish (187). Almost all higher plants studied have two phylogenetically distinguishable clades of cryptochromes, CRY1 and CRY2, corresponding to the two *Arabidopsis* cryptochromes originally discovered (84). Most plant cryptochromes, including those in Chlorophyta (green algae), Bryophyta (mosses), and angiosperms, act as sensory photoreceptors, whereas animal cryptochromes are either photoresponsive photoreceptors (type I) or nonphotoresponsive transcription coregulators (type II) (14, 136). It appears that regardless of their photoresponsiveness, most cryptochromes in different organisms act as the regulators of gene expression, especially transcription (151, 162). Plant cryptochromes are nucleocytoplasmic proteins. For example, *Arabidopsis* CRY1 locates and functions in both the nucleus and cytoplasm (172), whereas CRY2 seems to be an exclusively nuclear protein that completes its post-translational life cycle in the nucleus (182). Although cryptochromes were previously defined as photolyase-like proteins without DNA-repairing activity, this earlier definition is modifiable in light of the more recent findings that the CRY-DASH and algal cryptochromes have both DNA-repairing and transcription-regulatory activities (21, 140, 150). The crystal structure of the PHR domain of *Arabidopsis* CRY1 was the first to be solved, and it exhibits a striking similarity to the structure of *Escherichia coli* DNA photolyase despite their evolutionary distance (11). However, the crystal structure of the full-length plant cryptochrome has not been reported because of the technical difficulty of crystallizing a protein with the large, intrinsically disordered CCE domain. The crystal structures of the full-length *Drosophila* and mouse cryptochromes, which have relatively small CCE domains, and their complexes, including the respective partner proteins, have been determined (24, 138, 174, 190). These studies demonstrate the pivotal roles of the FAD-binding pocket and the physical interactions between the PHR and CCE domains in the functions of animal cryptochromes. There seems to be a general consensus that light-dependent changes in the interaction between the PHR and CCE domains of cryptochromes may at least partially explain the photoresponsive conformational changes of plant cryptochromes.

In the last 25 years, the function, photochemistry, and molecular mechanisms of cryptochromes in different evolutionary lineages have been extensively investigated, and the results of these studies have been reviewed periodically. For example, plant cryptochrome has been previously reviewed twice in this journal (18, 84). In this article, we focus on the recent progress of mechanistic studies of cryptochromes in plants, especially the model plant *Arabidopsis*, but we do not recount all of the discoveries of plant cryptochromes. Readers are encouraged to read the previous reviews and their references for a more comprehensive understanding of plant cryptochromes.

DAS: DQXVP-acidic-STAES

COP1: constitutive photomorphogenic 1

DASH: *Drosophila*, *Arabidopsis*, *Synechocystis*, human

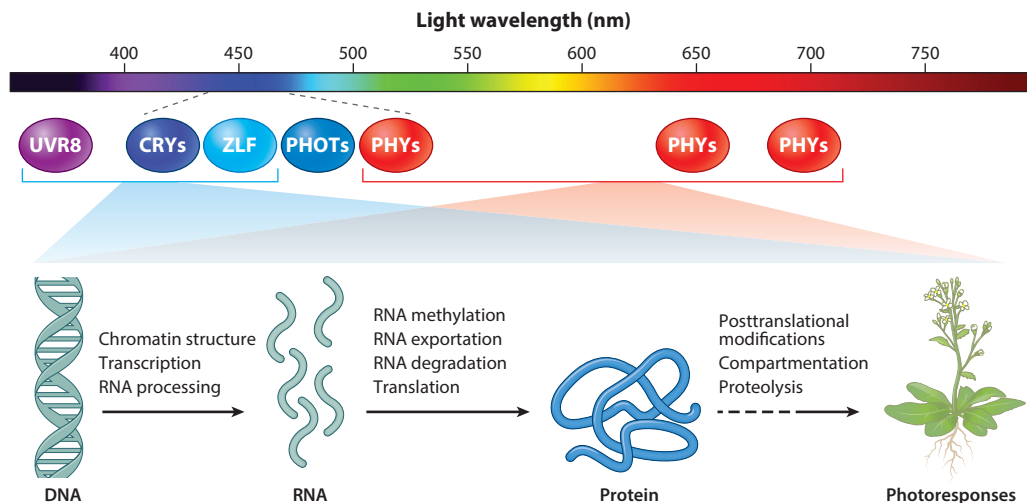


Figure 1

Photoreceptors mediate light regulation of gene expression to modulate plant growth and development. The diagram depicts the general action mechanisms of most plant photoreceptors, except phototropins (PHOTs), which act by modulating gene expression to alter plant growth and development. The ultraviolet (UV)-B receptor UVR8, blue/UV-A light receptors cryptochromes (CRYs), PHOTs, the LOV-domain/F-box proteins ZTL/FKF1/LKP2 represented by ZLF, and red/far-red (FR) light receptors phytochromes (PHYs) have the respective absorption spectra that are indicated by their positions under the light wavelength (nm). Known or potential regulatory mechanisms of gene expression are indicated. PHOTs are found primarily in plasma membrane such that regulation of gene expression is not depicted as the main mechanism of PHOTs. PHYs, especially phyA, are known to absorb blue light in addition to red/FR light, and to regulate blue-light responses.

2. CRYPTOCHROME-MEDIATED PHOTORESPONSES

2.1. Cryptochrome-Mediated Photoresponses in Higher Plants

Plant cryptochromes regulate many aspects of the plant life cycle, by mediating blue-light regulation of various steps of the gene expression process (**Figure 1**). Almost all presently known functions of cryptochromes were originally discovered by phenotypical and physiological analyses of induced mutations or natural variations impairing the *cryptochrome* genes. Cryptochrome-mediated photoresponses in terrestrial plants include photoresponsive genome transcription (102, 161), entrainment of the circadian clock (146), inhibition of the germination of dormant seeds (7), inhibition of hypocotyl elongation (2), stimulation of cotyledon expansion (86), coordination of temperature sensing (10), modulation of gravitropic responses (73, 118), promotion of root greening (154), stimulation of stomata opening and development (64, 102), regulation of shade avoidance (123), control of programmed cell death (25), enhancement of biotic (173) and abiotic stress responses (102), promotion of floral initiation (30, 40), regulation of fruit development (29), and suppression of leaf senescence (106). Cryptochromes may also be involved in the regulation of phototropism (5, 73) and magnetoreception (3, 43, 126). The above list of cryptochrome-mediated photoresponses is hardly complete: Additional cryptochrome-mediated photoresponses, especially those involved in crosstalk between light and other signaling processes, or new functions in plant species other than *Arabidopsis* are likely to be discovered. Among various cryptochrome-mediated photoresponses in land plants, the blue-light inhibition of hypocotyl elongation is the most widely used readout for the studies of cryptochromes. Although cryptochromes can independently regulate photomorphogenesis in the absence of phytochromes (54, 148), cryptochrome-mediated photoresponses are often redundantly, coordinately, or antagonistically coregulated by

other photoreceptors, including phytochromes (109, 128, 130, 176); phototropins (19); the LOV-domain/F-box flavoproteins including ZEITLUPE (ZTL); the flavin-binding, kelch repeat, F-box 1 (FKF1); LOV KELCH PROTEIN 2 (LKP2) (59); and UV-B resistance 8 (UVR8) (45, 61). This is at least partially because cryptochromes mediate blue-light responses by physically interacting with cryptochrome-interacting signaling proteins. Many cryptochrome-signaling proteins can also interact with photoreceptors other than cryptochromes, which may compete with or enhance the signaling process of the respective photoreceptors, and cause change within similar genomic or proteomic expression networks, to modulate similar growth and developmental changes in response to different or similar wavelengths of light.

2.2. Cryptochrome-Mediated Photoresponses in Green Algae

Cryptochromes have also been extensively studied in green algae, which appear to have a more complex set of cryptochromes than higher plants (32, 69, 71). In addition to canonical plant cryptochromes and CRY-DASH, green algae also have plant-like, animal-like, and cryptochrome/photolyase family (CPF) proteins. The algal cryptochromes seem different from those of the higher plants in at least two aspects. First, algal cryptochromes are often bifunctional, acting as both DNA photolyases and transcription regulators. For example, CPF1 of the marine diatom *Phaeodactylum tricornutum* and green alga *Ostreococcus tauri* has been shown to exhibit both 6-4 DNA photolyase activity and transcription regulatory activity (21, 26, 46). When algal CPF1 proteins are expressed in heterologous mammalian cells (Chinese hamster ovary cells), they can bind to the mammalian CLOCK/BMAL1 heterodimer and suppress the activity of CLOCK/BMAL1-regulated expression of the E-box-containing reporter promoter (21, 46). Second, algal cryptochromes may mediate photoresponses to a broader spectrum of light than the cryptochromes of the higher plants mediate. For example, *Chlamydomonas reinhardtii* possesses a plant cryptochrome (pCRY, previously referred to as CPH1) and an animal-like cryptochrome (aCRY), and both aCRY and pCRY respond to red light in addition to blue light (71). pCRY, which is 104 kDa and one of the largest cryptochromes, undergoes proteolysis via the 26S proteasome system in response to either blue light or red light (129), whereas aCRY, which is approximately 70 kDa, regulates the light-dependent expression of genes in response to blue and red light (8, 36). The *Chlamydomonas* pCRY regulates the circadian clock and life cycle progression; the *pcry* mutant exhibits many phenotypic defects, including period lengthening or arrhythmicity, phase shift abnormality in response to blue light, defects in the stimulation of mating by light or inhibition of mating by darkness, and light-dependent germination (111). *Chlamydomonas* aCRY is a bifunctional and broad-spectral photoreceptor that possesses both 6-4 DNA photolyase and photoreceptor activities (36), and it mediates photoresponses to blue, yellow, and red light (but not far-red light) (8, 36). Based on the analyses of absorption spectra of purified aCRY, changes of gene expression in the *acry* mutant, and the crystal structure of the PHR domain of aCRY, researchers proposed that different redox states of the FAD chromophore of aCRY may explain the broad-spectra photoresponses of aCRY (8, 36). According to the current view, the fully oxidized FAD of aCRY absorbs blue light to convert to the neutral radical state that absorbs blue, yellow, and red light to regulate gene expression (8, 36). Because the cryptochrome-signaling protein has not been reported in algae, exactly how algal cryptochromes regulate gene expression remains unclear.

3. CRYPTOCHROME PHOTOACTIVATION AND INACTIVATION

3.1. Cryptochrome Photoreduction and Photoactivation

Photoactivation of a photoreceptor can be broadly defined as photoresponsive changes that activate the photoreceptor, such as submolecular changes of the energy, orbital, or electronic state of

chemical bonds of the chromophore and its interacting amino acids, and subsequent molecular or intermolecular changes, such as conformational changes that affect intermolecular interactions of the photoreceptor. Photoactivation of plant cryptochromes begins with photoexcitation or photon absorption and photoreduction of the FAD chromophore (83, 101, 141), which results in conformational changes, such as disengagement of the PHR and CCE domains (112, 121, 179, 184) and formation of the cryptochrome homooligomers that interact with cryptochrome-interacting proteins to alter gene expression and plant development (91, 131, 137, 161). Partly because the fully oxidized FAD absorbs blue light more effectively than any other redox forms of FAD, the FAD photoreduction has been hypothesized to explain how cryptochromes are photoactivated. The non-redox photolyase-like cyclic electron shuttle mechanism has been proposed as an alternative photochemistry for cryptochrome photoactivation (87). However, this hypothesis has not been experimentally tested nor does it readily explain the long lifetime of the active cryptochromes required for their functions in plant cells (47, 112).

The FAD photoreduction hypothesis argues that the photon-excited FAD is reduced by receiving electrons transferred through three evolutionarily conserved tryptophan residues known as the Trp triad (1, 87). Recently, the fourth residue has been reported to contribute to cryptochrome photoreduction (9, 85, 113, 114, 117), and an Asp residue near the isoalloxazine ring of FAD has been proposed as the proton donor (13, 70). Despite abundant *in vitro* studies of the cryptochrome photoreduction phenomenon (1), exactly how FAD photoreduction is involved in cryptochrome photoactivation remains controversial (1, 87). At least two questions about cryptochrome photoreduction contribute to the controversy of cryptochrome photochemistry. First, why do mutations of the Trp-triad residues of cryptochromes that abolish cryptochrome photoreduction *in vitro* fail to abolish their physiological activities *in vivo*? Most Trp-triad mutants of cryptochromes studied, including *Arabidopsis* CRY1 (38) and CRY2 (79, 89), fail to undergo photoreduction *in vitro* but remain physiologically active *in vivo* (87, 89). Recently, researchers reported that some Trp mutants impaired photoresponses of the *Drosophila* cryptochrome (dCRY) *in vivo* (85), supporting the Trp triad-dependent photoreduction hypothesis for the *Drosophila* cryptochrome. However, the *Arabidopsis cry2* mutations altered in two or all three Trp residues of the Trp triad have recently been shown to retain the physiological activities *in vivo*, imposing further challenges to the Trp triad-dependent FAD photoreduction hypothesis (89). Researchers also noticed that free flavins and flavoenzymes, which have no reported light-dependent functions *in vivo*, commonly undergo photoreduction *in vitro* (104, 124), making it difficult to establish the causal relationship for the correlations between the cryptochrome photoreduction observed *in vitro* and the photoactivation mechanism of the respective cryptochrome *in vivo*. Therefore, new and innovative approaches may be needed to solve this conundrum.

Another aspect of the FAD photoreduction hypothesis is how to maintain FAD in its oxidized state in the dark. Because the oxidized FAD is the predominant redox form that absorbs blue light, it is expected to be the ground state of cryptochromes. However, the midpoint redox potentials of *Arabidopsis* CRY1 have been estimated by two independent studies to be approximately -143 mV to -153 mV for FAD/FADH \cdot and -161 mV to -181 mV for FADH \cdot /FADH $_2$ (6, 83). The redox potential of *Arabidopsis* cytoplasm is measured to be approximately -320 mV (107, 139), which is markedly lower than -143 mV to -153 mV, the potential of the FAD/FADH \cdot couple of CRY1. Under this reduced cellular condition, little CRY1 is expected to spontaneously oxidize to its fully oxidized ground state (87). Researchers have reported recently that the *Drosophila* dCRY has a redox potential of -316 mV for FAD/FAD \cdot^- (85), which is strikingly lower than 125 mV reported previously (122). This new estimation of the midpoint redox potential of dCRY seems to explain how dCRY may be oxidized in the insect cells (85). But it remains to be investigated whether the midpoint redox potential of plant cryptochromes might be lower than what is currently known

or whether the subcellular microenvironment of the nucleus that most cryptochromes reside in might provide a redox potential that is more oxidized than the currently known redox potentials of plant cryptochromes. For example, cryptochrome-interacting proteins may create a protein complex to provide a microenvironment so oxidized that it may force cryptochromes to maintain oxidized states despite the reductive cytoplasmic environment.

3.2. Cryptochrome Photooligomerization and Photoactivation

Regardless of the exact photochemical mechanism of cryptochromes, it must lead to a change in protein conformation in order to activate cryptochromes. Light-dependent conformational changes of cryptochromes were first demonstrated by a partial proteolysis experiment, in which light exposure resulted in a five- to tenfold increase in the proteolysis rate of the *Arabidopsis* CRY1 protein expressed and purified from insect cells (121). Although light-induced conformational change has not been directly observed in a plant cryptochrome due to the lack of a crystal structure, it has been hypothesized that the conformational change may lead to or be associated with a light-induced disengagement of the PHR and CCE domains (112, 121, 179, 184). This domain disengagement model has been demonstrated for the full-length *Drosophila* dCRY photoreceptor (190). In dCRY, the small C-terminal helix of dCRY docks to the FAD pocket in the groove analogous to the one that binds DNA in the DNA photolyases. The dCRY crystal exposed to X-rays photoreduces FAD to its anionic semiquinone state (FAD^{•−}) to facilitate restructuring of the tail helix of dCRY (190). The light-induced domain disengagement model would predict that overexpression of a truncated cryptochrome fragment devoid of the photon-absorbing PHR domain would interfere with the function of endogenous cryptochromes. Indeed, it has been shown that transgenic expression of the β -glucuronidase (GUS)-CCT1 or GUS-CCT2 fusion proteins, which are GUS fused to the C-terminal CCT (i.e., CCE) domains of *Arabidopsis* CRY1 or CRY2, resulted in a constitutive photomorphogenic phenotype similar to that of the *constitutive photomorphogenic 1* (*cop1*) mutant (28, 179). Later studies showed that the constitutive photomorphogenic phenotypes resulting from overexpression of the CCE domain of cryptochromes are caused by physical interaction between the CCE domain of cryptochromes and the WD40 domain of COP1 (52, 74, 75, 125).

Researchers predict that domain disengagement of cryptochromes may lead to changes of intermolecular interactions, resulting in homooligomerization (including homodimer, homotetramer, etc.) of cryptochromes and their interaction with other proteins (131, 137, 161). A number of experimental observations are consistent with this prediction. Blue-light-dependent cryptochrome homooligomerization was first implicated by the finding that the CRY2-RFP (red fluorescence protein) and CRY2-GFP (green fluorescence protein) fusion proteins, as well as the endogenous CRY2 protein, rapidly form nuclear speckles (also known as nuclear bodies or photobodies) in *Arabidopsis* cells exposed to blue light (103, 183). Moreover, *Arabidopsis* CRY2 protein expressed in heterologous mammalian cells forms morphologically similar photobodies in response to blue light, demonstrating that photoexcited CRY2 is capable of homooligomerizing into photobodies in the absence of other proteins (12, 119). At least three earlier studies showed that cryptochrome homooligomerization is required for the functions of *Arabidopsis* CRY1 and CRY2 (131, 137, 184). The first study showed that *Arabidopsis* CRY1 forms homooligomers via its PHR domain [also called Cryptochrome N Terminus (CNT)], that the CNT1 fragment can interact with the endogenous CRY1 to cause dominant-negative inhibition of the activity of CRY1 in transgenic plants, and that the CNT1 fragments of CRY1 mutated in A462V, G347R, or S66N lost their abilities to interact with CRY1 or to inhibit the endogenous CRY1 (137). The second study showed that the chemically induced oligomerization of the C-terminal domain fragments of CRY2 could

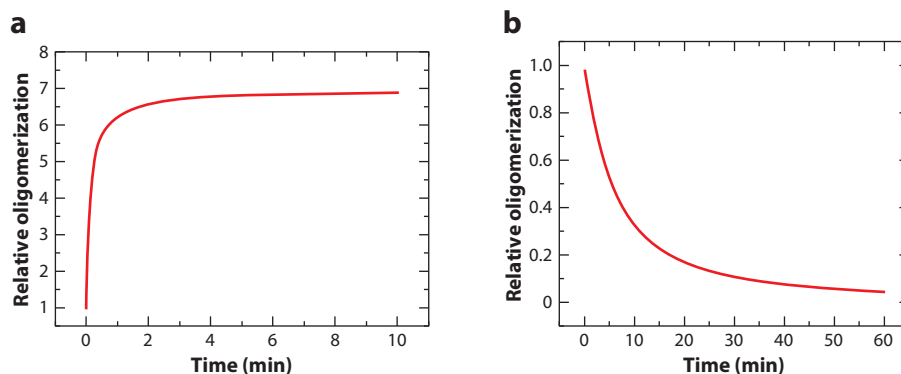


Figure 2

CRY2 photooligomerization and dark-reversion. The Flag- and Myc-tagged *Arabidopsis* CRY2 recombinant proteins were coexpressed in human embryonic kidney (HEK293) cells. (a) HEK293 cells were irradiated with $100 \mu\text{mol m}^{-2} \text{s}^{-1}$ blue light for the indicated time at 21°C ; the kinetics of CRY2 homooligomerization was measured by co-immunoprecipitation at the indicated time after illumination. (b) The kinetics of CRY2 monomerization or dark-reversion was measured by co-immunoprecipitation at the indicated time after light-treated HEK293 cells were transferred to darkness. Results of this experiment indicate that photooligomerization of CRY2 is much faster ($\sim 30\times$) than its dark-reversion under the experimental conditions used.

elicit changes in the expression of the cryptochrome-target genes in the absence of light (131). The third study showed that disengagement of the PHR domain and CCE domain and homooligomerization of *Arabidopsis* CRY2 may expose a small region spanning the two domains (NC80, residues 486–565) to elicit physiological functions of CRY2 (184). Together, these studies suggest that cryptochrome homooligomerization is necessary for the functions of plant cryptochromes. Although the light response of cryptochrome oligomerization was not detected in these three earlier studies, it was later shown that homooligomerization and heterooligomerization of *Arabidopsis* cryptochromes were blue-light-dependent photoreactions (92, 161), which are collectively referred to as cryptochrome photooligomerization (92). In these experiments, homooligomerization or heterooligomerization of recombinant *Arabidopsis* CRY1 or CRY2 proteins fused to different epitope tags was coexpressed in human embryonic kidney 293 (HEK293) cells or *Arabidopsis* plants, and their interactions were analyzed by co-immunoprecipitation (92, 161). Because HEK293 cells contain no other plant proteins to interfere with the co-immunoprecipitation assay by indirect protein-protein interaction, they can be conveniently used to study the light responses and kinetics of cryptochrome photooligomerization. For example, *Arabidopsis* CRY2 expressed in HEK293 cells took about 1 min to reach saturation of homooligomerization when the cells were exposed to $100 \mu\text{mol m}^{-2} \text{s}^{-1}$ blue light (Figure 2a). This method has also been used to demonstrate that *Arabidopsis* CRY1 and CRY2 can form heterooligomers and that photoresponsive cryptochromes from other organisms, such as rice, soybean, liverwort, *Drosophila*, monarch butterfly, and zebrafish, could all undergo photooligomerization (92). These observations are consistent with a hypothesis that photooligomerization may be an evolutionarily conserved photoactivation mechanism of cryptochrome photoreceptors from not only plants but also other evolutionary lineages.

HEK293: human embryonic kidney 293

3.3. Cryptochrome Inactivation and the CRY-BIC Complexes

The inactivation of photoreceptors is usually necessary to maintain the sustained photosensitivity and to avoid the excessive photosensitivity of cells. Because photoreduction of the FAD

chromophore is likely the first step of cryptochrome photoactivation, FAD oxidation in the absence of light might play a role in the inactivation of cryptochrome oligomers. A direct test of this possibility may help to resolve the controversy of cryptochrome photoreduction. However, the physiologically active cryptochrome homooligomers are expected to be at the higher energy state so they undergo spontaneous reversion to monomers in darkness by thermal relaxation. The rate of cryptochrome monomerization in darkness is much slower than that of the cryptochrome photo-oligomerization reaction in light. For example, in the heterologous HEK293 cells, the rate of CRY2 monomerization is at least 30 times slower than that of the photo-oligomerization reaction (**Figure 2b**). The rates of light-induced oligomerization and dark-dependent monomerization of *Arabidopsis* CRY2 appear faster in plant cells than those shown in heterologous HEK293 system (92). This observation suggests that the cellular environment and/or cryptochrome-interacting proteins may affect the equilibrium of cryptochrome photo-oligomerization and monomerization to govern the cryptochrome activity in plants under different light conditions.

BIC: blue-light inhibitor of cryptochrome

In plants, the blue-light-dependent cryptochrome oligomerization can also be suppressed or inactivated by interaction with two closely related cryptochrome inhibitory proteins, known as blue-light inhibitor of cryptochrome 1 (BIC1) and BIC2 (161). These two cryptochrome inhibitors were initially identified in a gain-of-function genetic screen (161). It was found that over-expression of BIC1 or BIC2 in transgenic plants suppressed all known photobiochemical and photophysiological activities of CRY1 and CRY2, including blue-light inhibition of hypocotyl elongation, blue-light-responsive gene expression, photoperiodic promotion of flowering, blue-light-dependent interaction of cryptochromes with cryptochrome-signaling proteins, blue-light-induced formation of photobodies of CRY2, blue-light-dependent phosphorylation of both CRY1 and CRY2, and blue-light-dependent polyubiquitination and degradation of CRY2, whereas the loss-of-function *bic1bic2* double mutants are hypersensitive to blue light (161). When BIC proteins and CRY2 are co-expressed in mammalian or plant cells, they physically interact to inhibit CRY2 oligomerization under blue light. However, these experiments did not distinguish whether BIC proteins might bind to the photoexcited cryptochrome monomer or to the photoactivated cryptochrome oligomer. In other words, whether BIC proteins inhibit cryptochrome photoactivation or promote cryptochrome inactivation remains to be elucidated. Photoactivated cryptochromes trigger a negative feedback circuitry, because expression of the messenger RNAs (mRNAs) of the *BIC* genes increases more than 200–1,200-fold in etiolated seedlings exposed to 100 $\mu\text{mol m}^{-2} \text{s}^{-1}$ blue light for an hour (165). Interestingly, although the function of BIC proteins is blue-light specific, mRNA expression of *BIC* genes is induced by not only blue light but also red light, and the photoinduction of BIC expression requires cryptochromes, phytochromes, COP1, and HY5 (165). This result suggests that photoactivation of cryptochromes triggers a negative-feedback reaction that increases the supply of BIC proteins to prevent excessive levels of active cryptochromes in the cells, and this process requires the actions of both cryptochromes and phytochromes.

In addition to cryptochrome monomerization, light-dependent proteolysis may serve the purpose of actively removing the activated cryptochromes. For example, depending on the plant species, CRY1, CRY2, and other types of cryptochromes have all been shown to undergo light-dependent proteolysis (17, 48, 58, 86, 129, 188). For example, *Arabidopsis* CRY2 undergoes rapid blue-light-specific ubiquitination and 26S proteasome-dependent degradation in the nucleus (86, 182, 192). *Arabidopsis* CRY2 has a half-life of longer than 24 h in etiolated seedlings, in contrast to a half-life of 25 min in etiolated seedlings exposed to 16 $\mu\text{mol m}^{-2} \text{s}^{-1}$ blue light (182). The ubiquitination and degradation of *Arabidopsis* CRY2 are dependent on CRY2 oligomerization and phosphorylation, which suggests that degradation is a mechanism to remove active CRY2 (86, 161, 183). Because both the protein kinases and E3 ubiquitin ligases are required for CRY2

activity and degradation (93, 143, 160, 167), the cryptochrome photoactivation and inactivation processes are expected to be closely coupled in time and space. A model of the photoactivation and inactivation of plant cryptochromes, based on the results of the studies described above, is depicted in **Figure 3**. According to this model, plant cryptochromes exist as inactive monomers in the absence of light, presumably containing the oxidized FAD; photoexcited FAD undergoes photoreduction or other types of photochemical changes to promote conformational changes and oligomerization of the cryptochrome proteins. Cryptochrome homooligomers actively interact with cryptochrome-signaling proteins to alter gene expression and photomorphogenesis, and photoactivated cryptochrome homooligomers are rapidly inactivated by FAD oxidation, monomerization, and possibly BIC interaction, and then are removed by proteolysis. The cryptochrome-mediated blue-light stimulation of the transcription of the *BIC* genes constitutes a negative feedback loop to maintain the homeostasis of the active and inactive cryptochrome pools in plant cells (**Figure 3**; **Table 1**).

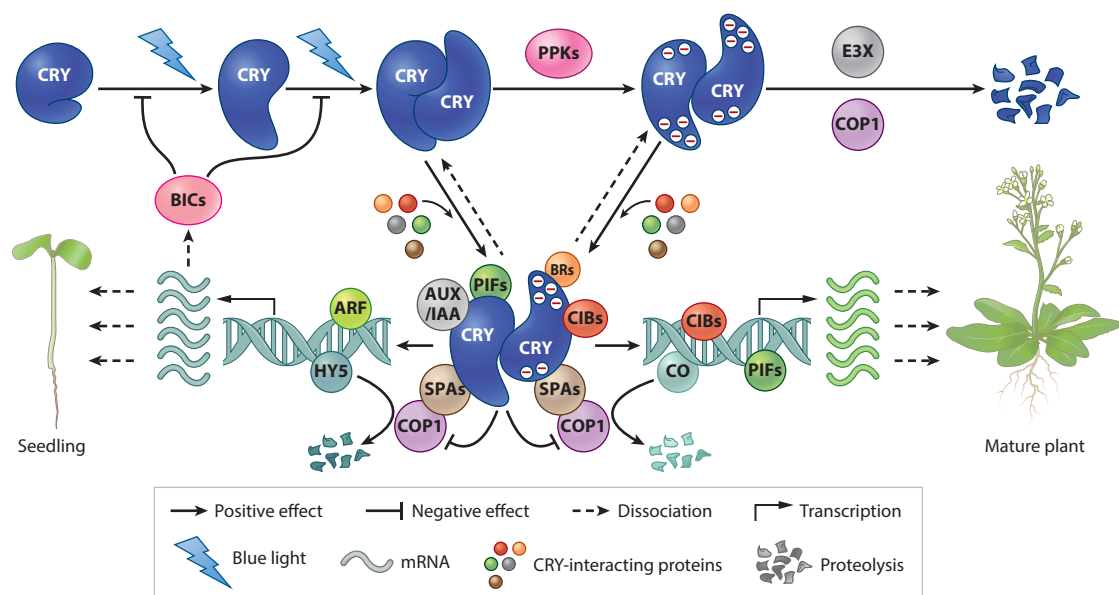


Figure 3

CRY photoactivation, signaling, and inactivation mechanisms. Cryptochromes exist as inactive monomers in darkness. Photoexcited cryptochromes undergo homooligomerization to become biochemically and physiologically active. The cryptochrome homooligomers interact with cryptochrome-interacting proteins. The presently known cryptochrome-interacting proteins, or components of the CRY complexome, include transcription regulators CIB proteins, PIF proteins, AUX/IAA proteins, the cryptochrome regulators BICs and PPKs, the E3 ubiquitin ligase complex COP1/SPA, and the BRs (including BZR1, BES1, BIN2, and BIM1). The CRY complexome mediates blue-light regulation of transcription or protein stability. For example, the CRY-COP1-SPA interaction positively regulates the abundance of the HY5 protein, which promotes transcription of the *BIC* genes. The BIC proteins interact with photoexcited cryptochromes to inhibit cryptochrome homooligomerization and activity. The PPK protein kinases catalyze blue-light-dependent phosphorylation of cryptochromes to positively regulate not only cryptochrome activity but also cryptochrome polyubiquitination. The COP1/SPA proteins and another E3 ubiquitin ligase (E3X) catalyze the polyubiquitination and degradation of phosphorylated cryptochromes. Abbreviations: AUX/IAA, auxin/indole-3-acetic acid; ARFs, auxin response factors; BIC, blue-light inhibitor of cryptochromes; BR, brassinosteroid regulator; CIB, cryptochrome-interacting basic helix-loop-helix; CO, CONSTANS; COP1, CONSTITUTIVE PHOTOMORPHOGENESIS 1; CRY, cryptochrome; E3X, unknown E3 ubiquitin ligases; HY5, LONG HYPOCOTYL 5; PIF, phytochrome-interacting factor; PPK, photoregulatory protein kinase; SPA, suppressor of phytochrome A.

Table 1 Components of the *Arabidopsis* cryptochrome (CRY) complexes

Accession	Abbreviation	Full name	CRY interaction(s)	CRY effects	Activity	Major function(s)	Reference(s)
AT4G34460	AGB1	ARABIDOPSIS G-PROTEIN BETA SUBUNIT 1	CRY1 (blue +), CRY2 (blue +)	–	Trimeric G protein	Auxin signaling, HY5-interacting	80
At3G52740	BIC1	Blue-light inhibitor of cryptochromes	CRY2 (blue +)	+	Cryptochrome inhibitor	Blue light signaling, hypocotyl growth, flowering time	161, 165
AT3G44450	BIC2	Blue-light inhibitor of cryptochromes	CRY2 (blue +)	+	Cryptochrome inhibitor	Blue light signaling, growth promoter, flowering inhibitor	161, 165
AT4G18710	BIN2	BRASSINOSTEROID-INSENSITIVE2	CRY1 (blue+)	+	Protein kinase	BR signaling, blue light signaling	44
AT5G08130	BIM1	BES1-INTERACTING MYC-LIKE1	CRY1 (blue +), CRY2 (blue +)	+	Transcription regulator	BR signaling, blue light signaling	164
AT1G75080	BZR1	BRASSINAZOLE-RESISTANT 1	CRY1 (blue +), CRY2 (blue +)	–	Transcription factor	BR signaling, blue light signaling	44, 164
AT1G19350	BZR2/BES1	BRASSINAZOLE-RESISTANT 2/BR1-EMS SUPPRESSOR1	CRY1 (blue +), CRY2 (blue +)	–	Transcription factor	BR signaling, blue light signaling	44, 164
AT4G34530	CIB1	CRYPTOCROME-INTERACTING BASIC HELIX-LOOP-HELIX 1	CRY2 (blue +)	+	Transcription factor	Blue light signaling, flowering promotion	91, 95, 106
AT5G48560	CIB2	CRYPTOCROME-INTERACTING BASIC HELIX-LOOP-HELIX 2	CRY2	+	Transcription factor	Blue light signaling, flowering promotion	95
AT3G07340	CIB3	CRYPTOCROME-INTERACTING BASIC HELIX-LOOP-HELIX 3	CRY2 (in vitro)	+	Transcription factor	Blue light signaling	95
AT1G10120	CIB4	CRYPTOCROME-INTERACTING BASIC HELIX-LOOP-HELIX 4	CRY2 (in vitro)	+	Transcription factor	Blue light signaling, flowering promotion	95
AT1G26260	CIB5	CRYPTOCROME-INTERACTING BASIC HELIX-LOOP-HELIX 5	CRY2 (blue +)	+	Transcription factor	Blue light signaling, flowering promotion	95
AT2G32950	COP1	CONSTITUTIVE PHOTOMORPHOGENIC 1	CRY1, CRY2	–	E3 ubiquitin ligase	Light signaling, photomorphogenesis	159, 178
AT4G08920	CRY1	CRYPTOCROME 1	CRY1 (blue +), CRY2 (blue +)	+	Blue light receptor	Blue light signaling	92, 137
AT1G04400	CRY2	CRYPTOCROME 2	CRY1 (blue +), CRY2 (blue +)	+	Blue light receptor	Blue light signaling	161, 137
AT2G18300	HBH1	HOMOLOG OF BEE2 INTERACTING WITH IBH 1	CRY1 (blue +), CRY2	–	Transcription factor	BR signaling	163

(Continued)

Table 1 (Continued)

Accession	Abbreviation	Full name	CRY interaction(s)	CRY effects	Activity	Major function(s)	Reference(s)
AT3G23050	IAA7	INDOLE-3-ACETIC ACID 7	CRY1 (blue +), CRY2 (blue +)	+	Transcription regulator	Auxin signaling	175
AT1G04550	IAA12	INDOLE-3-ACETIC ACID 12	CRY1 (blue +)	+	Transcription regulator	Auxin signaling	175
AT1G04250	IAA17	INDOLE-3-ACETIC ACID 17	CRY1 (blue +), CRY2 (blue +)	+	Transcription regulator	Auxin signaling	175
AT1G09570	phyA	PHYTOCHROME A	CRY1	+	Red/far-red light receptor	Red light signaling, blue light signaling	4
AT2G18790	phyB	PHYTOCHROME B	CRY2	+	Red/far-red light receptor	Red light signaling, blue light signaling	103
AT3G13670	PPK1	Photoregulatory protein kinase 1	CRY2 (blue +)	+/-	Protein kinase	CRY regulator, PIF3 regulator	93, 115
AT5G18190	PPK2	Photoregulatory protein kinase 2	CRY2 (blue +)	+/-	Protein kinase	CRY regulator, PIF3 regulator	93, 115
AT3G03940	PPK3	Photoregulatory protein kinase 3	CRY2 (blue +)	+/-	Protein kinase	CRY regulator, PIF3 regulator	93, 115
AT2G25760	PPK4	Photoregulatory protein kinase 4	CRY2 (blue +)	+/-	Protein kinase	CRY regulator, PIF3 regulator	93, 115
AT2G43010	PIF4	PHYTOCHROME-INTERACTING FACTOR 4	CRY1 (blue +), CRY2	-	Transcription factor	Phytochrome signaling	98, 123
AT3G59060	PIF5	PHYTOCHROME-INTERACTING FACTOR 5	CRY1, CRY2	-	Transcription factor	Phytochrome signaling	123
AT2G46340	SPA1	SUPPRESSOR OF PHA-105 1	CRY1 (blue +), CRY2 (blue +)	-	Positive regulator of COP1	Light signaling, photomorphogenesis	81, 88, 191
AT4G11110	SPA2	SPA1-RELATED 2	CRY1 (blue +)	-	Positive regulator of COP1	Light signaling, photomorphogenesis	81
AT3G15354	SPA3	SPA1-RELATED 3	CRY1 (blue +)	-	Positive regulator of COP1	Light signaling, photomorphogenesis	81
AT1G53090	SPA4	SPA1-RELATED 4	CRY1 (blue +)	-	Positive regulator of COP1	Light signaling, photomorphogenesis	81
AT5G57360	ZTL	ZEITLUPE	CRY1	+	Blue light receptor, E3 ubiquitin ligase	Blue light signaling, circadian clock	60, 90

4. THE CRYPTOCHROME COMPLEXOME AND CRYPTOCHROME SIGNAL TRANSDUCTION

More than 30 cryptochrome-interacting proteins have been reported (**Table 1**). Our understanding of the molecular mechanisms of cryptochrome signal transduction has been advanced primarily by the identification and investigation of these cryptochrome-interacting proteins. Blue-light-responsive protein–protein interactions appear to be the primary mechanisms underlying cryptochrome signal transduction, whereby photoactivated cryptochrome oligomers interact with cryptochrome-interacting proteins to regulate gene expression and photoresponses (**Figure 3**; **Table 1**). Plant cryptochromes have been reported to physically interact with COP1 (159, 178), cryptochrome-interacting basic helix-loop-helix 1–5 (CIB1–CIB5) (91), suppressors of phytochrome A 1–4 (SPA1–SPA4) (81, 88, 191), BIC1 and BIC2 (161), phytochrome interacting factors 1–7 (PIF1–PIF7), photoregulatory protein kinases 1–4 (PPK1–PPK4) (93, 115), auxin/indole-3-acetic acid (AUX/IAA) (175), AGB1 (a G-protein β subunit) (80), phytochromes (phyA and phyB) (4, 103), and several brassinosteroid signaling proteins (44, 164). Various cryptochrome complexes composed of cryptochromes and individual cryptochrome-interacting proteins are collectively referred to as the cryptochrome complexome. It is conceivable that in plant cells, the cryptochrome complexome exists in dynamic homeostasis by the reversible formation and disintegration of cryptochrome monomers, cryptochrome homooligomers, and cryptochrome heterooligomers composed of different cryptochrome-interacting proteins. One remarkable feature of the cryptochrome complexome is that many cryptochrome-interacting proteins are either regulated by other photoreceptors or physically interacting with other photoreceptors or their signaling proteins (**Table 1**). COP1/SPA, PIF, PPK, and AGB1 proteins are known to interact with the red/far-red light receptor phytochromes or their signaling proteins. BIC proteins are the only presently known cryptochrome-interacting proteins that exhibit blue-light-specific activity, but their expression is regulated not only by blue light but also by red light (165). This feature of the cryptochrome complexome seems to provide a mechanistic explanation for the long-standing notion of photoreceptor coactions in plants and our naive expectation that plants grown in nature must integrate light signals of different wavelengths into similar genomic, proteomic, or metabolic changes to affect similar cellular activities, such as cell elongation, chloroplast development, metabolism, and cell fate determination.

CIB: cryptochrome-interacting basic helix-loop-helix

SPA: suppressor of phytochrome A and related proteins

AUX/IAA: auxin/indole-3-acetic acid

PIF: phytochrome-interacting factor

PPK: photoregulatory protein kinase

4.1. The CRY-COP1-SPA Complexes

COP1 is the first cryptochrome-signaling protein identified in plants (159, 178). The loss-of-function *cop1* mutant exhibits constitutive photomorphogenic phenotypes, leading to the hypothesis that COP1 is a central repressor of plant photomorphogenesis (28). Indeed, this hypothesis has been supported by studies of many photoreceptors, including cryptochromes. COP1 contains a RING finger motif, a coiled-coil domain, and a WD40-repeat domain (28, 56). COP1 acts by complexing with its related SPA proteins (SPA1 to SPA4), which contain a coiled-coil domain, WD40 repeats, and a kinase-like domain instead of the RING domain of COP1 (50, 51). Like cryptochromes, COP1 is evolutionarily conserved in major evolutionary lineages, including humans, but SPA proteins are specific to the green lineage (42). The COP1-SPA complex is best known for its function as substrate receptors of the cullin 4-based E3 ubiquitin ligase, CUL4^{COP1/SPAs}, which facilitates ubiquitination and degradation of different light-signaling proteins, although they also perform other functions in plant development by interacting with various substrate proteins (50, 56). A conserved VP motif (VPE/D) has been identified in COP1 substrate proteins (52), and it overlaps with the DAS motif of cryptochromes (84). Structural analysis has recently shown that the VP/DAS motif of *Arabidopsis* CRY2 directly interacts with COP1 (74). Transgenic overexpression

of either the oligomerized CCE domain or the DAS-containing 80-residue fragment (NC80) of cryptochromes causes a constitutive photomorphogenic phenotype resembling that of the loss-of-function *cop1* mutant or the *spa* quadruple mutant (28, 179, 184). These genetic studies support the hypothesis that the VP/DAS motif of the CCE domain of cryptochromes interacts with COP1 to exert cryptochrome functions. Because COP1 interacts with many proteins that contain the VP motif (125), the cryptochrome-mediated blue-light signal is expected to integrate into the COP1 multiple signaling hub to regulate plant development.

Although CRY1 interacts with COP1 in a light-independent manner in heterologous systems (159, 178), the light-dependent formation of the CRY1-COP1 complex was detected in plant cells, which is explained by COP1-SPA interactions (132, 142, 189) and light-dependent CRY-SPA interaction in vivo (81, 88, 191). *Arabidopsis* CRY1 and CRY2 interact with SPA proteins in a blue-light-dependent manner. The CRY-COP1-SPA interaction and its suppression of COP1/SPA activity can at least partially explain the blue-light-dependent stabilization of transcription factors, such as LONG HYPOCOTYL 5 (HY5) and CONSTANS (CO) (81, 88, 167, 191), which regulate hypocotyl growth and floral initiation, respectively (156, 159, 178). It is interesting that structurally similar CRY1 and CRY2 interact with SPA1 in different ways. The N-terminal PHR domain of CRY2 interacts with the N-terminal kinase-like domain of SPA1 in response to blue light, which enhances the CRY2-COP1 interaction (191). Exactly how the enhanced CRY2-COP1-SPA interaction inhibits the activity of the CUL4^{COP1-SPAs} E3 ubiquitin ligase remains unclear. In contrast, the C-terminal CCE domain of CRY1 interacts with the C-terminal WD40 domain of SPA1 in a blue-light-dependent manner, which results in suppression of the SPA1-COP1 interaction and CUL4^{COP1-SPAs} ligase activity (81, 88). Therefore, CRY1 apparently acts as a light-dependent competitive inhibitor of the COP1-SPA interaction and CUL4^{COP1-SPAs} activity. Regardless of the complex details of the CRY-COP1-SPA tripartite interaction, it seems clear now that cryptochromes can directly interact with COP1 to form the CRY-COP1 complex, probably in a light-independent manner, whereas cryptochromes also interact with SPA1 but in a light-dependent manner. The CRY-SPA interaction conveys the light signal to alter the CRY-SPA-COP1 tripartite complex, suppressing CUL4^{COP1-SPAs} E3 ligase activity to regulate the stability of other light-signaling proteins.

4.2. The CRY-CIB Complexes

The first blue-light-specific cryptochrome-interacting protein identified in plants was a basic helix-loop-helix (bHLH) transcription factor known as cryptochrome-interacting bHLH 1 (CIB1) (91). Because of its relatively high specificity, affinity, and robust photoresponsiveness, the blue-light-dependent CRY2-CIB1 interaction has been widely utilized as an optogenetic tool for biomedical research in mammalian models (66, 185). CIB1 belongs to the BEE/CIB subfamily (family 18) of bHLH transcription factors (37, 91). The BEE/CIB subfamily contains 17 members (152), including genes encoding BR enhanced expression 1–3 (BEE1–BEE3), CESTA, and ILI1 binding bHLH (IBH1), which regulate brassinosteroid signaling (37, 127, 186); BIGPETALp, which interacts with ARF8 to regulate petal growth (158); and activator of cell elongation (ACE) proteins, which also regulate cell elongation (57). Different family members of the BEE/CIB subfamily of bHLH proteins seem to regulate both overlapping and unique aspects of plant development. CRY2 mediates blue-light stimulation of the CIB1 activation of transcription of the *FLOWERING LOCUST T (FT)* gene, which encodes the central regulator of floral initiation, or florigen, that migrates from leaves to the apical meristem to promote floral meristem development (23, 65, 68, 91, 171, 181). Three other CIB1-like bHLH proteins, CIB2, CIB4, and CIB5, have also been shown to interact with photoactivated CRY2 and bind to the E-box (CANNTG) elements of the promoter of *FT* (91, 95). CRY2 does not seem to affect the affinity of CIB proteins to DNA

or chromatin (91). However, it has recently been reported that CIB proteins directly interact with CO to promote floral initiation (96), implying that CRY2 might affect CIB–CO interaction and *FT* transcription. Recently, more CIB1-related proteins, including BEE2, CIL1, and HBI1, have been shown to interact with photoactivated cryptochromes (163). And at least one of them, HBI1, has been shown to mediate CRY1-dependent blue-light inhibition of hypocotyl elongation (163). Interestingly, in contrast to the lack of effect of CRY2–CIB1 interaction on the affinity of the DNA-binding activity of CIB1, the CRY1–HBI1 interaction inhibits the DNA-binding activity of HBI1 (163). CIB and BIC proteins seem to be the only two groups of cryptochrome-interacting proteins that so far have not been reported to interact with signaling proteins of other photoreceptors. However, the mRNA or protein expression of CIBs and BICs is photoregulated by other photoreceptors. It was found that CIB proteins (CIB1, CIB4, CIB5) are degraded in the dark or red light by the 26S proteasome, whereas blue light suppresses degradation of all of them. Surprisingly, neither cryptochromes nor the dark-active CUL4^{COP1-SPAs} E3 ubiquitin ligase is involved in the control of CIB degradation (90). It is intriguing that photoreceptors and E3 ligase other than the CIB-interacting photoreceptors cryptochromes or the cryptochrome-interacting CUL4^{COP1-SPAs} E3 ubiquitin ligase have emerged in evolution to regulate CIB degradation. Nevertheless, researchers have found that the LOV-domain photoreceptors ZTL and LKP2 mediate blue-light inhibition of CIB degradation (90). The E3 ubiquitin ligase responsible for CIB degradation has not been identified. Cryptochromes are known to interact with ZTL (60) that is a substrate receptor of the SCF^{ZTL} E3 ubiquitin ligases (41). However, exactly how cryptochrome- and ZTL-type photoreceptors interact with each other or with CIB proteins to coordinate blue-light suppression of CIB degradation remains unclear.

Blue-light-dependent CRY2–CIB interactions are evolutionarily conserved, yet the physiological function of the CRY–CIB interaction may be diverse in different plant species. For example, photoactivated CRY2 interacts with CIB1 to promote CIB1 activation of flowering in *Arabidopsis*, whereas CRY2 interacts with CIB1 to suppress CIB1 promotion of leaf senescence in soybean (106). Exactly how the CRY2–CIB1 complex mediates the blue-light-responsive alteration of transcription remains unclear. Although few effects of CRY2 or light were observed for the CIB1–DNA or CIB1–chromatin interaction in an earlier study (91), a more recent study argued that CRY2 may enhance the DNA-binding affinity of CIB1 or that CRY2 itself may be a DNA-binding transcription activator (180). In this study, CRY2 and/or CIB1 was coexpressed in mammalian HEK293 cells; the transcription activation activity of CIB1 was analyzed by reporter genes, and the CIB1 DNA-binding affinity was analyzed by the affinity precipitation of a biotin-labeled DNA fragment mixed with cell lysates. In these assays, photoactivated CRY2 appears to enhance the affinity of CIB proteins to its target DNA (the E-box-containing second intron of the *FT* gene) and the CIB1 transcription activation activity for the reporter gene. However, if CRY2 itself exhibits both DNA-binding and transcription activation activity in the HEK293 system, the above results may be alternatively interpreted by the activity of CRY2. The possibility that CRY2 itself may act as a DNA-binding transcriptional activator seems particularly appealing because the presumed ancestors of cryptochromes, DNA photolyases, are DNA-binding proteins, and animal cryptochromes are transcription repressors, but additional work is needed to further test this possibility.

4.3. The CRY-PIF Complexes

In addition to CIB proteins, plant cryptochromes interact with another group of bHLH transcription factors, PIF (phytochrome interacting factor) proteins, which are phytochrome-interacting and G-box-binding transcription factors that belong to a phylogenetic clade remotely related to

the CIB proteins (77, 120, 152). The *pifq* quadruple mutants (*pif1pif3pif4pif5*) exhibit a constitutive photomorphogenic phenotype, including growth arrest of hypocotyls (78, 145). Although PIF proteins are best known for their roles in the phytochrome-mediated photomorphogenesis responses, such as red-light inhibition of hypocotyl elongation, these positive regulators of growth appear to act as systems integrators that integrate different signals, including hormones, sugar, circadian timing, and temperature (76, 120). In addition to red light, the *pif4pif5* and *pifq* mutants exhibit a short hypocotyl phenotype in blue light, implying that PIF proteins have a role in blue-light inhibition of growth (72, 97). Photoactivated CRY1 and CRY2 can physically interact with PIF4 and PIF5 via the PHR domain of cryptochromes and the N-terminal domain of PIF proteins in a region distinct from the phytochrome-binding motif (98, 123). It was shown that under shade conditions, canopies decrease not only the red-to-far-red ratio but also blue-light intensity and that both phyB and cryptochromes mediate shade-avoidance responses in low blue light (123). phyB binds PIF3 to promote PIF3 phosphorylation, ubiquitination, and degradation (115, 116); the low blue light in shade conditions may suppress phyB-dependent PIF degradation to promote growth. Moreover, it has been reported that cryptochromes suppress degradation of PIF1 in low blue light (16). The decreased blue-light intensity caused by canopy may decrease cryptochrome oligomerization to weaken the CRY–PIF interaction, resulting in increased activity or accumulation of PIF proteins to promote hypocotyl elongation. Cryptochromes have also been previously shown to play important roles in temperature responses of *Arabidopsis* (10, 34, 39). A recent study showed that CRY1 interacts with PIF4 and PIF5 in a blue-light-dependent manner to affect the function of CRY1 in hypocotyl growth at high ambient temperatures (98). Similar to the CRY2–CIB1 interaction, the CRY1–PIF4 interaction does not affect the DNA- or chromatin-binding activity of PIF4, but it suppresses the transcription activation activity of PIF4, affecting transcription of the auxin biosynthesis or signaling genes, and hypocotyl elongation of seedlings grown at high ambient temperatures (98).

4.4. The CRY-PPK Complexes

Arabidopsis CRY1 and CRY2 undergo blue-light-dependent phosphorylation in plant cells (143, 144). Phosphorylation of CRY2 not only enhances its activity but also facilitates its ubiquitination and degradation by CUL4^{COP1/SPA5} and other E3 ubiquitin ligases (94, 143, 167, 182). Label-free quantitative mass spectrometry analyses of *Arabidopsis* CRY2 proteins purified from plants identified at least two dozen phosphorylated residues of CRY2, including 18 serine and 6 threonine residues (93, 160). The level of phosphorylation in almost all those phosphorylated residues increased in response to blue light (160). Surprisingly, both the S-to-A and the phosphomimetic S-to-D mutants of CRY2 exhibited the loss of phosphorylation and partial loss of function in physiological activities. Given that an aspartate (−1 charge per residue) carries relatively fewer negative charges than phosphorylated serine (−1.5 to −2 charges per residue) at pH 7.2 estimated for the *Arabidopsis* nuclear compartment, this observation suggests that photoactivation of cryptochromes may change charge distribution to facilitate domain disengagement (184). Although cryptochromes have been shown to undergo autophosphorylation *in vitro*, their phosphorylation *in vivo* is primarily catalyzed by protein kinases. Four closely related CRY2-associated protein kinases, referred to as photoregulatory protein kinases (PPK1 to PPK4), have been shown to catalyze blue-light-induced phosphorylation of CRY2 *in vivo* or *ex vivo* (93, 115). PPKs are plant-specific protein kinases evolutionarily derived from the ubiquitous casein kinase I, which were also called MUT9-like kinases (166). All four PPKs preferentially interact with photoexcited but unphosphorylated cryptochromes, and they phosphorylate cryptochromes in a partially redundant manner. The blue-light-dependent phosphorylation of CRY2 appears normal in the monogenic

ppk mutants but is largely absent in the *ppk1ppk2ppk3* and *ppk1ppk2ppk4* triple mutants and the artificial microRNA lines (*amiR^{4k}*) with reduced expression of all 4 *PPK* genes. Unphosphorylated CRY2 proteins are neither ubiquitinated nor degraded in the *ppk* triple mutants, confirming the previous prediction that CRY2 phosphorylation is required for its subsequent ubiquitination and degradation (143, 160, 161, 182). These results support a model arguing that photoexcited cryptochromes are phosphorylated by four structurally related and functionally redundant PPK kinases; phosphorylation of cryptochromes causes charge-dependent conformational changes to enhance the physiological activity of both CRY1 and CRY2 as well as the polyubiquitination and degradation of CRY2 (Figure 3). PPKs have other substrates in addition to cryptochromes, including the phytochrome-signaling protein PIF3 (115), histone H2A (149), histone H3 (166), and probably circadian clock proteins (55), suggesting that PPKs may act as a hub of multiple signaling interactions.

ARF: auxin response factor

TIR1/AFB: transport inhibitor response 1/auxin signaling F-box

4.5. The CRY-AUX/IAA and CRY-AGB1 Complexes

Plant hormones are major internal regulatory molecules of plant growth and development, and photoresponses inevitably interact with hormonal regulation networks in plants (27). Cryptochromes mediate genome-wide gene expression changes in response to blue light, including changes in mRNA expression of biosynthesis and signaling genes of various phytohormones, such as auxin, gibberellin, and brassinosteroid (33, 67, 99, 100, 118). Regulation of auxin-responsive gene expression has been proposed to explain the cryptochrome-mediated blue-light inhibition of cell elongation (170). Recent studies found that the photoactivated cryptochromes can physically interact with the AUX/IAA proteins to accomplish this. AUX/IAA proteins are a family of small (25 to 35 kDa), auxin-inducible transcription repressors that bind to auxin response factors (ARFs) to suppress ARF activity and the transcription of auxin-responsive genes (133, 168). The *Arabidopsis* genome encodes 29 AUX/IAA proteins, which, together with 23 ARF proteins, can regulate the diverse auxin responses in plants. The SCF^{TIR/AFB} E3 ubiquitin ligase complex acts as an auxin receptor that binds to auxin to facilitate interaction between the substrate receptor transport inhibitor response 1/auxin signaling F-box (TIR1/AFB) proteins of the SCF^{TIR/AFB} E3 ligase and its substrates, AUX/IAA proteins, leading to the ubiquitination and degradation of the AUX/IAA proteins, release of the inhibition of ARF proteins, and promotion of auxin-responsive gene expression and cell elongation. This general auxin-signaling mechanism allows plants to control growth by regulating the homeostasis of the AUX/IAA and ARF proteins, the TIR/AFB-AUX/IAA interaction, or the AUX/IAA-ARF interaction, in response to different environmental factors, such as light. For example, auxin induces the TIR1-AUX/IAA interaction in the dark to promote cell elongation in etiolated seedlings or plants under shade, whereas light inhibits the auxin-induced TIR1-AUX/IAA interaction and AUX/IAA degradation. In plants exposed to blue light, the PHR domains of photoactivated cryptochromes interact with AUX/IAA proteins, including IAA7, IAA12, and IAA17 (175). The interaction between cryptochromes and those IAA proteins suppresses the TIR1-IAA interaction and IAA degradation (175). Therefore, cryptochromes may act as blue-light-dependent competitive inhibitors of auxin signal transduction and hypocotyl elongation (175). Phytochromes also physically interact with the AUX/IAA proteins in response to red or far-red light (22, 175, 177). And similar to the CRY-AUX/IAA interaction in blue light, the phytochrome-AUX/IAA interaction causes suppression of the TIR1-AUX/IAA interaction, AUX/IAA degradation, and cell elongation under red light or shade conditions.

In addition to interacting with AUX/IAA proteins, *Arabidopsis* cryptochromes also interact with the G-protein β subunit AGB1 (80). The photoactivated CRY1 interacts with AGB1 to disrupt the

BR: brassinosteroid regulator

BES1: BRI1-EMS-SUPPRESSOR 1

BZR1:
BRASSINAZOLE-RESISTANT 1

BIM1: BES1-INTERACTING MYC-LIKE1

BIN2:
BR-INSENSITIVE 2

AGB1–HY5 interaction, which positively regulates the DNA-binding activity of HY5 to promote photomorphogenesis. Although heterotrimeric G proteins are usually plasma membrane proteins that interact with G protein–coupled receptors in response to light, odors, hormones, and growth factors in animals (169), it is not uncommon that in animal cells the subunits of G proteins are imported to the nucleus to regulate the activity of transcription factors (49). Moreover, both HY5 and G proteins are known to participate in the auxin responses in plants (20, 62, 153), which is consistent with the proposition that CRY1–AGB1 regulation may also be associated with the light–auxin crosstalk.

4.6. The CRY-BR (Brassinosteroid Regulator) Complexes

In addition to auxin, plant cryptochromes also integrate blue-light signals with brassinosteroid signals by interacting with brassinosteroid-signaling proteins or brassinosteroid regulators (BRs). It has been reported recently that photoactivated *Arabidopsis* cryptochromes interact with several brassinosteroid-signaling proteins (collectively called brassinosteroid regulators here), including BRI1-EMS-SUPPRESSOR 1 (BES1) and BRASSINAZOLE-RESISTANT 1 (BZR1), two related master transcription factors that positively regulate brassinosteroid signaling; BES1-INTERACTING MYC-LIKE1 (BIM1), a bHLH transcription factor interacting with BES1; and BR-INSENSITIVE 2 (BIN2), a GSK3-like protein kinase catalyzing phosphorylation of BZR1 and BES1 to prevent their nuclear importation and function (44, 164). Plant cryptochromes may interact with these BRs to suppress brassinosteroid signaling by multiple mechanisms. For example, CRY1 interacts with BIN2 to enhance BIN2-dependent phosphorylation and cytoplasmic retention of BZR1, whereas CRY1 preferentially interacts with the dephosphorylated forms of BES1 and BZR1 to inhibit their DNA-binding activity and brassinosteroid-responsive transcription (44, 164). Because these brassinosteroid regulators interact with each other, cryptochromes may form a dynamic complex or complexes composed of these proteins to directly modulate brassinosteroid-responsive transcriptional regulons in response to both the internal brassinosteroid signal and the external light signal.

5. FUTURE CHALLENGES

We anticipate that new components of the cryptochrome complexome, in addition to those reported so far, will be discovered to further our knowledge of plant cryptochromes. However, there are at least three major challenges to a mechanistic understanding of cryptochrome-mediated photoresponses in plants: What are the exact conformational and structural changes of the photoactivated cryptochrome monomer and oligomers, how do cryptochromes mediate photoresponses in different cells, and what are the mechanisms of cryptochrome regulation of gene expression other than their general roles regulating transcription and protein stability? First, it may be fair to say that cryptochromes are among the structurally least understood plant photoreceptors at present. This holdup is partially due to the technical difficulty of obtaining suitable protein crystals of a full-length plant cryptochrome. The domain disengagement hypothesis predicts relatively large photoresponsive conformational changes of plant cryptochromes, whereas the crystal-packing effect of protein crystals and lack of stringent dark control due to inevitable X-ray illumination in diffraction crystallography may impose additional challenges to observing the exact photoresponsive conformational changes of cryptochromes in crystallography. Recent advancements in cryo-electron microscopy technology could potentially overcome some of those technical hurdles. Second, plant cryptochromes appear to express ubiquitously, but light is unlikely to have the same effects on different cells and organs of a plant. Therefore, how to distinguish the specific

functions of cryptochromes in the specific photoresponses of individual cells is another challenge. Recent advances in single-cell RNA and protein analysis technologies would likely bring new insights about the novel and cell-specific functions of plant cryptochromes. Finally, most mechanistic studies of plant cryptochromes are presently limited to the cryptochrome-mediated regulation of the rate of mRNA transcription and ubiquitination-dependent proteolysis. It remains unclear whether or how cryptochromes affect other steps within the gene expression process or the specific mechanisms of individual steps. For example, it remains unknown whether cryptochromes regulate the initiation, elongation, or termination of transcription, or how cryptochromes alter cotranscriptional or post-transcriptional mRNA modifications, such as RNA methylation, to affect mRNA degradation, nuclear exportation, or translation. Again, newly available methodologies for the analyses of transcriptomes, epitranscriptomes, translatomes, proteomes, or individual cryptochrome-targeting genes and proteins will shed more light on the mechanisms underlying cryptochrome-mediated photoresponses in plants.

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103. Discovery of the cryptochrome photobody.

109. Demonstration of the redundant functions of phyA, CRY1, and CRY2 in flowering.

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