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Cryptochrome-mediated light responses in plants

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Abstract

Cryptochromes (CRY) are photolyase-like flavoproteins that have been found in all evolutionary lineages. Plant and animal cryptochromes are no longer DNA repairing enzymes but they apparently gained other biochemical functions in evolution. Plant cryptochromes are UV-A/blue light photoreceptors and play a pivotal role in plant growth and development, whereas animal cryptochromes act as either photoreceptors or transcription regulators. The first cryptochrome gene was isolated from *Arabidopsis thaliana*, which regulate stem growth, flowering time, stomatal opening, circadian clock, and other light responses. Cryptochromes are also found in all major crops investigated, with additional functions discovered, such as seed germination, leaf senescence, and stress responses. In this chapter, we will review some aspects of cryptochrome-mediated light responses in plants. Readers are referred to other review articles for photochemistry and signal transduction mechanism of plant cryptochromes [1-3].

I. Introduction

The first cryptochrome gene was cloned from *Arabidopsis thaliana* over two decades ago, in searching for the gene corresponding to *HY4* locus whose loss-of-function alleles (*hy4*) are deficient in response to blue/UV-A light [4-6]. *HY4* encodes a protein with significant homology to DNA photolyases, a class of flavoproteins using UV-A/blue light to repair cyclobutane pyrimidine dimers (CPD) or (6-4) photoproducts in UV-damaged DNA [7, 8]. *HY4* binds flavin adenine dinucleotide (FAD) as photolyases do, but lacks DNA repair activity [9, 10], arguing that *HY4* is a blue light receptor in *Arabidopsis*. The word cryptochrome has been long used for dubbing the mysterious blue light photoreceptor, so the *HY4* has been renamed as Cryptochrome 1 (CRY1) [9]. Another cryptochrome gene in *Arabidopsis*, *CRY2*, was subsequently isolated by screening cDNA libraries with the *CRY1* cDNA probes [11, 12].

After discovery of the *Arabidopsis* cryptochromes, similar cryptochrome genes have also been found in other organisms, including bacteria [13-17], insects [18-20], coral [21], sponges [22], krill [23], zebrafish [24, 25], birds [26, 27], and mammals [28-30]. Archaea genomes contain PHR/CRY-like sequences, but the existence of cryptochromes in this

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evolutionary branch remains unclear [31]. All cryptochromes have a similar PHR (Photolyase-Homologous Region) domain, leading to the hypothesis that cryptochromes are probably the evolutionary descendants of DNA photolyases [32]. Cryptochromes can be simplistically divided into three classes: plant cryptochromes, animal cryptochromes and CRY-DASH [15, 33]. All the plant cryptochromes examined so far are photoreceptors [1]. Animal cryptochromes can be further clustered into two types based on both sequence and photobiochemical properties: type I cryptochromes are light-responsive photoreceptors, including those found in *Drosophila* (dCRY) and other insects (e.g. monarch butterfly CRY1); whereas type II cryptochromes act as light-independent transcription repressors, including those found in mouse (mCRY1 and mCRY2), human (hCRY1 and hCRY2) and monarch butterfly (CRY2) [20, 34]. CRY-DASH is found in bacteria and plants [15], which includes the third member of *Arabidopsis* cryptochromes, CRY3 [35].

Beyond the conserved N-terminal PHR domains (~500 residues), plant and animal cryptochromes are structurally distinguished by the C-terminal CCE (Cryptochrome C-terminal Extensions) domains with varied lengths (ranging from 30 to 250 residues) [36]. In general, plant cryptochromes have a longer CCE (>100 aa) than animal cryptochromes whereas the CCE domain is completely missing in CRY-DASH [15]. The PHR domains of cryptochromes are essential for light absorption through non-covalently binding to the chromophore FAD and possibly the second chromophore, 5,10-methenyltetrahydrofolate (MTHF) [9, 10, 30, 37] (whose presence in cryptochromes is still under debate [38]). The CCE domains lack ordered structure in solution but important for light signal transduction and protein functions such as nuclear localization, protein stability, posttranslational modification, and protein-protein interactions [36, 39-45].

Upon absorption of photons, cryptochromes are believed to be photoexcited by the mechanism involving electron transfer and flavin reduction, but how this is accomplished remains not fully understood at present [1,46]. The flavin chromophore bound in cryptochrome can have different redox states: oxidized (e.g. FAD), semi-reduced semiquinones (e.g. neutral radical FADH[•] or anion radical FAD^{•-}), or fully reduced hydroquinones (e.g. FADH⁻ or FADH₂) [1,47]. In *Arabidopsis* CRYs, oxidized FAD is proposed to be the ground-state chromophore and can convert into its signaling state, the semi-reduced semiquinone (FADH[•] or FAD⁻) after absorbing blue light, then the semi-reduced flavin can be oxidized to ground-state FAD in the dark to complete the photocycle [48, 49]. However, this hypothesis does not seem to explain the photoexcitation mechanism of cryptochromes *in vivo*. A detailed discussion concerning photoexcitation mechanism of cryptochromes can be found in other reviews [1, 46] and research articles [50-53]. Upon photoexcitation, cryptochromes undergo a series of biophysical and biochemical events, such as conformational changes, dimerization, phosphorylation, degradation and protein-protein interactions, to eventually lead to changes in gene expression and physiological responses of plants [54].

In the past two decades, extensive investigations of cryptochromes in diverse plant species have revealed various important roles of cryptochromes in mediating light responses during plant growth and development [32]. It is generally agreed that cryptochromes mediate those light-dependent physiological responses by modulating gene expressions through the

interactions with signal proteins [2, 32, 54]. In *Arabidopsis* genome, approximately 5–25% of genes change their expression in response to blue light and most of these changes are mediated by CRY1 and CRY2 [55–59]. CRYs 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 protein degradation (the CRY-SPA1/COP1 pathway). Both mechanisms are involved with blue light-dependent protein-protein interactions of CRYs and the signaling proteins [2]. Readers can find more details about the signal transduction mechanisms of *Arabidopsis* CRYs in other papers [2, 3].

II Physiological responses mediated by plant cryptochromes

Cryptochromes are widely distributed in plant kingdoms, from algae to angiosperms, but to date, no sequence information of cryptochromes from gymnosperms has been reported [32, 60]. The studies in *Arabidopsis* and other plant species underscored the broad effects of cryptochromes on many physiological and developmental processes, such as photomorphogenesis [4, 56, 61, 62], shade avoidance [63], flowering time control [54, 64, 65], circadian clock regulation [66–69], root growth [70–72], guard cell development and stomata opening [73, 74], fruit and ovule size [75], plant height and apical dominance [76, 77], apical meristem activity [78], programmed cell death [79], the high-irradiance stress response [80, 81], osmotic stress response [82], responses to bacterial and viral pathogens [83, 84], magnetoreception [85, 86], tropic growth [55, 87, 88], seed dormancy [89, 90] and leaf senescence [91]. Although sharing common structural features, different cryptochromes may have independent functions in mediating light responses. For example, in *Arabidopsis*, three cryptochromes have been identified (CRY1, CRY2 and CRY3) [54] and all of them have their major functions: CRY1, present both in nucleus and cytoplasm, primarily mediates blue-light-simulated photomorphogenesis [4, 92]; CRY2 is a nuclear protein and mainly contributes to photoperiodic control of flowering [64, 93]; whereas CRY3 may act both as a DNA photolyase and a photoreceptor in mitochondria or plastids [35, 94, 95], although its physiological functions, other than DNA repair, remain to be discovered. In this chapter, we will discuss some aspects of CRY-mediated light responses in *Arabidopsis* and also briefly review the studies of physiological functions of cryptochromes in other plants.

Blue-light stimulated photomorphogenesis

Photomorphogenesis occurs after seed germination when, upon exposure to light, the seedlings undergo de-etiolation. Cryptochromes participate in many aspects of blue-light stimulated photomorphogenesis (or de-etiolation) (Figure 1), such as hypocotyl elongation arrest, anthocyanin accumulation, cotyledon expansion and chloroplast development. Light inhibits hypocotyl elongation, but promotes the expansion of cotyledons and the conversion of etioplasts to chloroplasts [96–98].

Arabidopsis cry1 (hy4) mutants exhibit elongated hypocotyl under blue light [4, 6], accentuating a main function of CRY1 in mediating photomorphogenesis. Consistently, overexpression of *Arabidopsis CRY1* cDNA in both *Arabidopsis* and tobacco resulted in enhanced light responses in transgenic seedlings [92, 99]. Mutation of CRY2 has no apparent effects on hypocotyl elongation of seedlings grown under high fluence rate of blue

light, however, the *cry1cry2* double mutants have stronger long-hypocotyl phenotypes than either single mutant, suggesting that CRY2 plays a minor role in this response [100]. Interestingly, the long-hypocotyl phenotype of *cry2* becomes much evident under medium-low fluence rate of blue light ($<10 \mu\text{mol m}^{-2} \text{s}^{-1}$), suggesting that the function of CRY2 in early photomorphogenesis of seedlings is largely restricted to medium-low light conditions. Besides hampering hypocotyl elongation, CRY1 and CRY2 also share partially redundant functions in other aspects of light-stimulated de-etiolation. For example, transgenic seedlings overexpressing either CRY1 or CRY2 showed larger cotyledons than those of wild type seedlings grown under blue light [101-103], indicating that both CRY1 and CRY2 mediate blue-light stimulation of cotyledon expansion. It is interesting that different subcellular pools of the CRY1 have different functions. Specifically, the blue-light mediated inhibition of hypocotyl elongation completely depended on the nuclear CRY1; conversely, cotyledon expansion in blue light was promoted by cytoplasmic CRY1 and inhibited by nuclear CRY1; anthocyanin accumulation in response to blue light was strongly stimulated by nuclear CRY1 and, to a lesser extent, by cytoplasmic CRY1 [104]. It remains unclear how CRY1 in different subcellular compartment trigger such different responses. On the other hand, CRY2 is a nuclear protein and mediate all known physiological responses inside the nucleus [102]. Therefore, different CRYs may undertake multiple modes of action to mediate different blue light responses.

Photoperiodic control of flowering-time

Flowering transition is a major physiological change in plant life cycles and light, as one of the most important environmental factors, has profound effects on this process. It has long been known that blue light (wavelength of ~ 400 to 500 nm) and red light (~ 600 to 700 nm) promote and suppress the transition to flowering in *Arabidopsis*, respectively [105, 106], hinting that different photoreceptors may have distinct functions on flowering induction. Apart from light quality, the photoperiod also plays a significant role in flowering time determination. *Arabidopsis* is a facultative long-day (LD) plant that flowers earlier under LD than under short-day (SD). The photoperiodic signal appears to trigger changes of the expression of CONSTANS (CO), a B-box-type zinc-finger protein, which induces transcription of the floral integrator gene, *FLOWERING LOCUS T* (*FT*), in a light-dependent manner [107-109].

In *Arabidopsis*, CRY2 is a major photoreceptor in photoperiodic control of flowering in *Arabidopsis* [64] (Figure 2). Different *cry2* mutant alleles, including the *fha-1* allele that was discovered earlier, all showed similar phenotype: they flowered significantly later than the wild type in LD but showed normal flowering time in SD [64, 110, 111]. The studies on natural variation of flowering time in *Arabidopsis* also identified *EDI* (Early Day length Insensitive) as a natural occurring allele of CRY2 [93, 112]. *EDI* is a major quantitative trait locus (QTL) responsible for most of the difference in flowering response to day-length between two natural accessions, Landsberg *erecta* (*Ler*) and Cape Verde Islands (*Cvi*). *Ler* plants flower much earlier under LD than SD, while *Cvi* flower early under both LD and SD, suggesting that *Cvi* is less sensitive to day-length change than *Ler* [113]. Positional cloning of *EDI* QTL located it to CRY2 locus, molecular characterization of CRY2-*Cvi* allele showed that it is a gain-of-function allele with a single V367M substitution in CRY2

protein [93]. In SD conditions, CRY2 protein stability seems to increase significantly due to this change. The increased stability of CRY2^{V367M} appears explain the day-neutral early flowering phenotype of the Cvi accession [93]. Although DNA microarray data (eFP Browser and Genevestigator) showed that CRY2 is widely expressed in all the organs and tissues examined, the function of CRY2 in flowering-time regulation has a tissue-specificity. Using different tissue-specific promoters to drive CRY2-GFP expression in *cry2* background, it was found that only CRY2-GFP expressed in vascular bundles could accelerate flowering, whereas CRY2-GFP expressed in other tissues failed to rescue the *cry2* mutant phenotype [114]. This observation contrasts with the observation of phyB-GFP, which when expressed in mesophyll cells, but not in vascular bundles, affects the flowering time [115]. Interestingly, both mesophyll cell-expressed phyB and vascular bundle-expressed CRY2 control flowering through modulating *FT* expression in vascular bundles [114, 115], although with the opposite consequences that CRY2 stimulates *FT* expression while phyB represses it. It is likely that blue and red light photoreceptors, CRY2 and phyB, can act in different tissues to antagonistically regulate flowering in response to light. Besides affecting photoperiod-induced flowering, CRY2 also plays a role in ambient temperature-dependent floral initiation. Given the observation that the late-flowering phenotype of *tha-1/cry2* was much more obvious at 16 °C than at 23 °C, it was proposed that *tha-1/cry2* causes an exaggerated response to changes in ambient temperature [116]. The temperature response in *cry2* mutants could be strongly suppressed by *tfl1-1*, a mutant that lost temperature sensitivity [117]. These studies raised an interesting question how a photoreceptor takes part in temperature-dependent signaling transduction.

Currently available experimental results suggest that CRY1 also has a possible effect on flowering, albeit much weaker than CRY2. The flowering phenotype of *cry1* alleles seems not fully reproducible, the late-flowering phenotype could be observed in some experiments but not others [118, 119]. However, the *cry1/cry2* double mutants flower later than *cry2* or *cry1* single mutants under both LD and continuous blue light conditions, which seem to argue a role of CRY1 as a flowering regulator [100]. It has been reported that a gain-of-function allele of *cry1*, *cry1-L407F*, which is originally isolated in a mutant screen for suppression of the late-flowering phenotype of *msi1-tap1* plants [120], strongly accelerates flowering especially under SD [121]. Another gain-of-function mutant of CRY1, *CRY1^{G380R}*, also has the similar effect to *cry1-L407F*. Overexpression of either mutant form of CRY1 in *Arabidopsis* resulted in a photoperiod-independent early-flowering phenotype [122]. In addition, *cry1* mutants flowered much later than wild type plants at 16 °C, but not at 23 °C [116], suggesting that CRY1 may also have a temperature-dependent activity in flowering-time regulation.

So far, two main mechanisms of CRY2-involved flowering promotion have been well suggested: suppression of CO degradation (CRY2-SPA1/COP1 pathway) [123, 124] and activation of transcription activators (CRY2-CIB pathway) [125, 126]. But how either mechanism may be involved in the regulation of flowering-time under different environmental conditions remains unclear.

Cryptochromes and clock: light entrainment and temperature compensation

The Circadian clock is an endogenous timekeeping mechanism that allows plants to anticipate daily environmental changes, such as light and temperature, and optimize the timing of various physiological events [127]. To synchronize the phase of internal and external times, the circadian clock needs to be reset by environmental cues daily, or undergo entrainment. Light is the predominant entraining stimulus to plants and photoreceptors must mediate light entrainment of the circadian clock [128].

In *Arabidopsis*, CRY1 and CRY2 are involved in the light input to the clock [66, 67]. The plant circadian clock can be investigated by different phenotypic read-outs, such as leaf movement, delayed fluorescence and Ca^{2+} oscillations [129-131] or firefly luciferase reporter activity (LUC) [132]. Overexpression of *Arabidopsis* CRY1 showed a shortened period of *CAB2::LUC* expression rhythm in both high-fluence white ($50 \mu\text{mol m}^{-2} \text{s}^{-1}$) and blue ($18 \mu\text{mol m}^{-2} \text{s}^{-1}$) light [66], while deficient in CRY1 resulted in a lengthened period in blue light in two ranges ($>10 \mu\text{mol m}^{-2} \text{s}^{-1}$ or $<3 \mu\text{mol m}^{-2} \text{s}^{-1}$), suggesting that other blue light receptors mediate signaling in the missing region [66]. However, CRY2 seemed unlikely the one that compensated the function of CRY1, because loss of CRY2 only caused a slight period shortening in low-fluence blue light. Nevertheless, the *cry1cry2* double mutant showed an even stronger long-period phenotype in all tested fluence rates of blue light [66], suggesting that CRY1 and CRY2 may act additively and redundantly in mediating light input to the clock and the effect of *cry2* deficiency becomes apparent only in the absence of CRY1. Besides, the long-period phenotype of *cry1cry2* was also observed in measuring Ca^{2+} oscillation and leaf movement [68, 131]. Furthermore, the blue-light induced phase shift of leaf movement rhythm strongly reduced in *cry1* and *cry1cry2* mutants, demonstrating that cryptochromes in *Arabidopsis* mediate blue light entrainment of the circadian clock. Nevertheless, despite the important effect of cryptochromes in light-input signaling to clock, they do not play an oscillator role in plants as they do in mammals [133], because either overexpression of cryptochromes or *cry1cry2* double mutations won't abolish the rhythmicity of circadian clock under examined growth conditions. However, one just can not rule out the possibility that CRYs could act as a component in the circadian oscillator under specific conditions, such as high temperature. In addition, the expression of cryptochromes themselves is regulated by the circadian clock [134], indicating the existence of a regulatory loop that modulates entraining and resetting of the circadian clock.

A recent study uncovered additional roles of cryptochromes in another defining feature of circadian clock, temperature compensation [127]. Relative to wild type plants, *cry1* mutants had no detectable effect on period at 12 °C or 17 °C under blue light but resulted in a long period phenotype at 27 °C, suggesting that CRY1 is involved in temperature compensation at 27 °C under blue light [69]. The *cry2* mutation had no detectable phenotype at different temperatures in blue light. Not surprisingly, the *cry1cry2* double mutant had a long period in all temperatures tested under blue light. More interestingly, the *cry1cry2* double mutant failed to sustain a robust circadian rhythm at 27 °C under blue light, suggesting a conditional involvement of cryptochromes in sustaining circadian rhythmicity [69]. This striking finding is in a parallel with previous work in other systems. For example, in mouse, lacking of both Cry1 and Cry2 proteins causes a complete loss of free-running rhythmicity, indicating that

both proteins are essential for the maintenance of circadian rhythmicity [133]. The *Drosophila* cryptochrome (dCRY) also mediates both light and temperature signaling to the clock by forming complex with the clock proteins PERIOD (PER) and TIMELESS (TIM) in response to light and heat pulse [135]. Arabidopsis CRYs are not known to interact directly with other clock proteins except for the reported interaction of CRY1 with ZTL in yeast system [136], so a question comes up and needs to be answered hereafter, whether and how CRYs interact with clock proteins in a light- or temperature-dependent manner to perform independent functions in clock regulation. However, it also remains unclear whether plant CRYs would act, similar to that found in other systems, as transcription repressors of the circadian oscillator. Neither CRY mRNA or protein levels are dependent on temperature [69], suggesting a novel mechanism underlying this newly discovered function of CRY in the control of the circadian clock.

Light-controlled stomatal opening and development

Stomata regulate gas exchange between plants and atmosphere, optimize the uptake of CO₂ and concomitant loss of water vapor [137]. It has been well known that light can trigger the opening of stomata even with very low irradiance, suggesting that the excited photoreceptors may mediate this response [138]. Blue light is well known for its strong activity in inducing stomata opening than red light [137, 139]. The blue light-induced stomatal opening is primarily mediated by the blue light receptors phototropins (PHOT1 and PHOT2) [140]. Independently of phototropins, cryptochromes may also be required for blue light-stimulated stomatal opening in *Arabidopsis* (Figure 3A). Similar to *phot1phot2*, stomata of the *cry1cry2* double mutants exhibited a reduced blue light response, but this effect of *cry1cry2* was relatively weaker than that of *phot1phot2* [74]. Accordingly, overexpression of CRY1 caused a hypersensitivity to blue light [74]. Unlike *phot1phot2*, for which the stomatal opening response is insensitive to the fluence rate below 5 $\mu\text{mol m}^{-2} \text{s}^{-1}$, the stomata of *cry1cry2* are still able to respond to low fluence rate of blue light ($>1 \mu\text{mol m}^{-2} \text{s}^{-1}$), suggesting that cryptochromes are primarily functioned under high irradiance while phototropins are functioned under a much wider fluence rates of blue light in terms of inducing stomatal opening [74]. Furthermore, the stomata of the *cry1cry2 phot1phot2* quadruple mutants hardly open in response to any fluence rate of blue light tested and this phenotype is apparently stronger than that of either *phot1phot2* or *cry cry2* double mutants, suggesting that cryptochromes work additively with phototropins in mediating blue light-induced stomata opening. This notion is also supported by the observation that overexpression of CRY1 in *phot1phot2* rescued the blue light-insensitivity in this response [74]. Both the cryptochrome and phototropin signaling pathways in controlling stomata opening can be negatively regulated by CONSTITUTIVE PHOTOMORPHOGENIC1 (COP1) [74]. Earlier studies have demonstrated that COP1 interacts directly or indirectly with cryptochromes [124, 141, 142], but the biochemical relationship between COP1 and phototropins remains not very clear. Therefore, it will be interesting to investigate the different mechanisms of how CRY and PHOT modulate COP1 activity to regulate the stomatal opening in response to light at molecular level.

In addition to regulating stomatal opening, cryptochromes have been shown to be involved in blue light-induced stomatal development (Figure 3B). The first evidence supporting this

conclusion is that CRY1-overexpression led to dramatic clustered stomata in cotyledon epidermis under blue light [73]. Then an inhibited stomatal development is observed in *cry1 cry2* double mutants in a blue light-dependent manner [73]. These data indicate that cryptochromes are the primary photoreceptors mediating blue light-induce stomatal development of cotyledon epidermis. Genetic studies further demonstrate that the cryptochrome act antagonistically with the known stomatal development control pathways [73]. As in CRY-mediated de-etiolation, floral induction, and stomata opening responses, COP1 also acts genetically downstream of cryptochromes to regulate stomatal development [73]. But the exact molecular mechanism underlying this developmental response remains to be further studied.

The functions of cryptochrome in other plants

Although most physiological functions of cryptochromes were discovered in *Arabidopsis*, more and more case studies of cryptochrome function have been reported in other plant species, from evolutionarily more primitive plant species such as fungi, moss, and fern, to crops. For example, CryA of the filamentous fungus *Aspergillus nidulans* has been found to act as a negative regulator of sexual development under UV-A light and possesses both near-UV/blue light photosensory and photoreactivated DNA repair activity [143]. In moss *Physcomitrella patens*, two similar cryptochrome genes PpCRY1a and PpCRY1b have been shown to redundantly regulate moss development at many steps under blue light, including induction of side branching in protonema and control of differentiation and growth of gametophores, quite likely through modulating auxin sensitivities [144]. In fern *Adiantum capillus-veneris*, five CRY genes have been isolated, two of them, CRY3 and CRY4 mediate blue light-dependent inhibition of spore germination [145].

Cryptochromes have been studied in several dicots besides *Arabidopsis*, such as *Brassica napus*, *Cardamine nipponica*, tomato, pea and soybean. *Brassica napus* has one copy of CRY1, BnCRY1, although it is a natural allotetraploid. Similar to that found in *Arabidopsis*, decreased or increased expression of BnCRY1 results in decreased or increased blue light sensitivity in the light inhibition of hypocotyl elongation [60]. Overexpression of BnCRY1 also caused enhanced anthocyanin accumulation, increased expansion in cotyledons and leaves, as well as reduced internode elongation of the stem [146]. Furthermore, BnCRY1 overexpression alters the mRNA abundance of genes associated with phytohormones and stress responses, arguing a broader effect of BnCRY1 in plant growth [146].

Two CRY1, one CRY2, and one CRY3 gene have been isolated in tomato [77]. CRY1a, one of the CRY1 genes, controls seedling photomorphogenesis, anthocyanin accumulation and adult plant development, including stem elongation, apical dominance, and the chlorophyll content of leaves and fruits[76]. Overexpression of CRY2 in tomato not only shortens the hypocotyl and internode length under blue light, but also causes several novel phenotypes, such as overproduction of anthocyanins and chlorophyll in leaves and of flavonoids and lycopene in fruits, increased outgrowth of axillary branches and interestingly, an unexpected delay in flowering under both long- and short-day conditions, suggesting that CRY2 is a central player in tomato development[77].

In pea, three cryptochromes genes are present in its genome, including a single CRY1 orthologue and two distinct CRY2 genes [147]. The phenotypic observations of *cry1* mutants reveal that CRY1 participates in de-etiolation of seedlings and stem elongation and leaflet expansion of adult plants [148].

In comparison to other dicot plants excluding Arabidopsis, the soybean cryptochrome may be the best studied with respect to its signaling mechanism. It has been shown that soybean cryptochromes GmCRY1a and GmCRY2a both affect blue light inhibition of cell elongation, but only GmCRY2a undergoes blue light-induced proteolysis via a 26S proteasome-dependent mechanism. In soybean, GmCRY1a seems a more predominant regulator of photoperiodic flowering, the photoperiod-dependent circadian rhythmic expression of the GmCRY1a protein correlates with photoperiodic flowering and latitudinal distribution of different soybean accessions [149]. More recently, a study of GmCRY2a has uncovered a new function of cryptochrome not reported in Arabidopsis, which is to mediate light regulation of leaf senescence. Overexpression of GmCRY2a in transgenic soybean plants delays leaf senescence, whereas knock-down GmCRY2a expression by RNA interference leads to accelerated leaf senescence [91], suggesting that GmCRY2a negatively regulates leaf senescence in soybean. Similar to CRY2-CIB module in Arabidopsis, the photoexcited GmCRY2a physically interacts with soybean bHLH transcription activator CIB1 (Cryptochrome-Interacting bHLH1). The CRY2a-CIB1 interaction inhibits the DNA-binding activity of CIB1 under blue light. It seems that in vivo, formation of the CRY2a-CIB1 complex suppresses CIB1-DNA interaction and mRNA expression of the CIB1 target gene, such as WRKY53b [91]. Because WRKY53b is a positive regulator of leaf senescence in Arabidopsis, it was hypothesized that CRY2a mediates blue light inhibition of leaf senescence by interacting with CIB1 to suppress WRKY53b transcription. However, it remains to be determined whether WRKY53b is a *bona fide* positive regulator for leaf senescence in soybean as it is in Arabidopsis [150]. The CIB1-dependent signaling mechanism of CRY2a in soybean is reminiscent of that in Arabidopsis [125], but some notable differences exist between the two. For example, the CRY2-CIB1 complex in Arabidopsis primarily controls flowering time, whereas the CRY2a-CIB1 complex in soybean mainly regulates leaf senescence; the photoexcited Arabidopsis CRY2 interacts with CIB1 to suppress reporter transcription in transient assays but appears to stimulate the transcriptional activation activity of CIB1 [125], while photoexcited soybean CRY2a interacts with CIB1 to suppress the DNA binding and transcriptional activation activity of CIB1 in planta [91]. These findings supports a hypothesis that the direct physical interaction between CRY and a transcription factor is an evolutionarily conserved CRY-signaling mechanism in plants, and this mechanism is opted in evolution to mediate light regulation of different aspects of plant development in different plant species.

The cryptochrome genes have also been studied in monocot plants. Three cryptochrome genes have been characterized in rice, including OsCRY1a, OsCRY1b and OsCRY2 [151]. OsCRY1s are responsible for blue-light inhibition of coleoptile and leaf elongation during seedling development [151, 152], whereas OsCRY2 is involved in the promotion of flowering [151].

By mining wheat dbESTs, two cryptochrome genes, TaCRY1a and TaCRY2 have been isolated [82]. TaCRY1a exhibits a light-dependent nucleocytoplasmic relocation, whereas TaCRY2 is a nuclear protein that degrades in blue light. A study in transgenic *Arabidopsis* suggests that both TaCRY1a and TaCRY2 may be involved in osmotic stress and abscisic acid (ABA) responses [82]. This effect of TaCRY may also be the case in wheat itself, because the transcription of TaCRY1a and TaCRY2, especially the latter, are apparently regulated by osmotic stress and ABA in wheat roots and germinating embryos [82]. Moreover, the EST analysis suggests that TaCRY2 transcripts in roots increase about ten folds under desiccation treatment; the TaCRY1a ESTs increase seven folds in crown and leaf tissues after a long exposure to low temperature [153]. However, due to their different expression patterns and protein behaviors, TaCRY1a and TaCRY2 likely regulate stress responses through different mechanisms. A similar case also seen in *Arabidopsis*, deficient in CRYs confers enhanced drought-tolerance on plants and overexpression of CRY1 results in greater water-loss [74]. This involvement of *Arabidopsis* CRYs in drought response is largely dependent on their functions on inducing stomata opening in response to blue light [74]. Therefore, it would be intriguing to compare different modes of action of monocot and dicot CRYs in mediating stress response.

Barley also has two CRY1 (CRY1a and CRY1b) and one CRY2 genes [154]. Decreased expression of CRY1a/b in transgenic barley plants lead to reduced blue light inhibition of germination of barley grains, while knockdown of CRY2 mRNA has no such effect, demonstrating that CRY1 is the major photoreceptor in mediating the blue light inhibition of grain germination in barley [90]. Further analysis has found that CRY1 induced expression of the ABA biosynthesis gene 9-cis-epoxycarotenoid dioxygenase (NCED1) and the increase of ABA contents in blue light-illuminated barley grains that exhibit stronger dormancy [90]. This study provides the first direct evidence arguing that cryptochrome may mediate blue light suppression of grain germination in cereal. Interestingly, in *Arabidopsis*, blue light can also repress seed germination, but this light response is mediated by phyB [90, 155].

These functional studies of cryptochromes in crops may have exposed their potentials as useful tools towards crop improvement in terms of agronomical traits such as plant height, flowering time, seed germination, and leaf senescence.

III. Perspectives

The past two decades have witnessed exciting progresses in the studies of plant cryptochromes, especially their physiological functions relevant to crop productivity. Although many aspects of cryptochrome-mediated light responses in plants have been characterized, in most cases we do not fully understand how the cryptochrome modulates plant development at the molecular level. For example, how exactly does plant cryptochrome regulate transcription? How does plant cryptochrome regulate the circadian clock? How does cryptochromes work in concert with other photoreceptors or other signaling pathways to coordinate plant growth? Continuing exploration of these and other questions will undoubtedly shed more light on our understanding of the action mechanisms of plant

cryptochromes and how this type of photoreceptors may be modified to improve agriculture productivity.

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Figure 1. Cryptochrome-mediated light inhibition of hypocotyl elongation

The *Arabidopsis* seedlings of WT (Col) and *cry1cry2* double mutant grown in dark or continuous blue, red, or far-red light conditions, showing blue light-specific long hypocotyl phenotype of the *cry1cry2* double mutant.

Figure 2. Cryptochrome-mediated photoperiodic regulation of floral initiation
WT (Col) and *cry1cry2* double mutants grown under long-day (LD) or short-day (SD) conditions, showing the late-flowering phenotype of *cry1cry2* double mutants under LD.

Figure 3. Cryptochrome-mediated stomatal opening and development in response to lights. (Images are taken from published articles in PNAS. 102: 12270-12275 and Plant Cell. 21: 2624-2641 with the authors' permission)

A. Stomata opening of the WT, *cry1*, *cry2*, *cry1cry2*, *CRY1-ox*, and *CRY2-ox* plants after illuminated with blue light, showing the enhanced light response of stomata opening in *CRY1-ox* plants. (Mao, J. *et al.*, PNAS. 102: 12270-12275)

B. The abaxial cotyledon epidermis of WT and *35Spro-CRY1* seedlings grown in continuous blue, red and far-red light, showing blue light-specific clustered stomata in *35Spro-CRY1*. (Kang, C. *et al.*, Plant Cell. 21: 2624-2641)