 © 2010 American Society of Plant Biologists

First published on September 23, 2010: e0135. 10.1199/tab.0135

The Cryptochrome Blue Light Receptors

Xuhong Yu, Hongtao Liu, John Klejnot, and Chentao Lin[a,1]

[a]Department of Molecular, Cell and Developmental Biology, University of California, Los Angeles, CA 90095, USA
[1]Address correspondence to clin@mcdb.ucla.edu

Cryptochromes are photolyase-like blue light receptors originally discovered in Arabidopsis but later found in other plants, microbes, and animals. Arabidopsis has two cryptochromes, CRY1 and CRY2, which mediate primarily blue light inhibition of hypocotyl elongation and photoperiodic control of floral initiation, respectively. In addition, cryptochromes also regulate over a dozen other light responses, including circadian rhythms, tropic growth, stomata opening, guard cell development, root development, bacterial and viral pathogen responses, abiotic stress responses, cell cycles, programmed cell death, apical dominance, fruit and ovule development, seed dormancy, and magnetoreception. Cryptochromes have two domains, the N-terminal PHR (Photolyase-Homologous Region) domain that bind the chromophore FAD (flavin adenine dinucleotide), and the CCE (CRY C-terminal Extension) domain that appears intrinsically unstructured but critical to the function and regulation of cryptochromes. Most cryptochromes accumulate in the nucleus, and they undergo blue light-dependent phosphorylation or ubiquitination. It is hypothesized that photons excite electrons of the flavin molecule, resulting in redox reaction or circular electron shuttle and conformational changes of the photoreceptors. The photoexcited cryptochrome are phosphorylated to adopt an open conformation, which interacts with signaling partner proteins to alter gene expression at both transcriptional and posttranslational levels and consequently the metabolic and developmental programs of plants.

INTRODUCTION

Plants possess two types of photoreceptors: photosynthetic pigments that harvest light energy for photosynthesis, and photosensory receptors that mediate non-photosynthetic light responses. Plant photosensory receptors are presently best studied in Arabidopsis. The Arabidopsis genome encodes apoproteins of at least a dozen photoreceptors: five red/far-red light receptors phytochromes (phyA, phyB, phyC, phyD, and phyE), and seven blue light receptors, including two cryptochromes (CRY1 and CRY2), two phototropins (phot1 and phot2), and three LOV/F-box/Kelch-domain proteins (ZTL, FKF, and LKP2). Cryptochromes are photolyase-like flavoproteins that mediate blue-light regulation of gene expression and photomorphogenic responses in Arabidopsis and other organisms (Cashmore, 1997; Lin and Shalitin, 2003; Sancar, 2003). In this chapter, we will discuss the function, structure, photochemistry, and signal transduction mechanisms of Arabidopsis cryptochromes. Readers are encouraged to read other chapters in this book for the related topics, such as photomorphogenesis (Nemhauser and Chory, 2002), phytochromes (Wang and Deng, 2002), phototropins (Liscum, 2002), and the circadian clock (McClung et al., 2002).

The term cryptochrome was first coined three decades ago for the then "cryptic" photoreceptors mediating various UV-A/blue responses in cryptogams, which is an obsolete taxonomic term for plants that do not reproduce by seed, such as algae, fungi, mosses, and ferns (Gressel, 1979). Earlier physiological and photochemical experiments suggested that flavin might be the chromophore of the then unidentified cryptochromes (Briggs and Huala, 1999). In 1980, the Arabidopsis mutant hy4 was identified, which showed elongated hypocotyls when grown in blue light but not in other wavelengths of light or in the dark (Koornneef et al., 1980), suggesting that the gene is involved in blue light sensing . Over a decade later, Ahmad and Cashmore isolated another allele of the hy4 mutant from an Arabidopsis T-DNA insertion population generated by Ken Feldman and cloned the HY4 gene (Ahmad and Cashmore, 1993). The HY4 gene encodes a protein that resembles a DNA photolyase. Because DNA photolyase is a flavoprotein that catalyzes blue/UV-A light-dependent repair of lesions (cyclobutane pyrimidine dimers) in UV-damaged DNA (Sancar, 1990), HY4 was immediately suspected to be the long sought-after cryptochrome. It was subsequently found that the HY4 protein binds to flavin adenine dinucleotide (FAD) and that it lacks DNA-repairing photolyase activity (Lin et al., 1995b; Malhotra et al., 1995). These results, together with the finding that transgenic tobacco seedlings expressing the Arabidopsis *HY4* cDNA were hypersensitive to blue and UV-A light but not to red or far-red light, argued strongly that HY4 is a cryptochrome and so it was renamed cryptochrome 1 or CRY1 (Lin et al., 1995a). The second member of the CRY gene family in *Arabidopsis*, CRY2, was isolated by screening Arabidopsis cDNA libraries with the *CRY1* cDNA probes (Hoffman et al., 1996; Lin et al., 1996b). Studies of the *cry2* mutants demonstrated that CRY2 primarily regulates the photoperiodic promotion of floral initiation (Guo et al., 1998; El-Assal et al., 2001). The third member of the Arabidopsis CRY family, CRY3, is a CRY-DASH protein that can be

detected in chloroplasts and mitochondria (Kleine et al., 2003). CRY-DASH proteins do not have conventional photolyase activity, but they bind DNA or RNA directly. Although some CRY-DASH have been shown to possess cryptochrome activity in regulating transcription or development (Hitomi et al., 2000; Brudler et al., 2003; Worthington et al., 2003; Veluchamy and Rollins, 2008), it was also found that CRY-DASH proteins, including Arabidopsis CRY3, catalyze repair of the cyclobutane pyrimidine dimers of single-stranded DNA *in vitro* (Huang et al., 2006; Selby and Sancar, 2006; Klar et al., 2007; Pokorny et al., 2008). Therefore, Arabidopsis CRY3 and other CRY-DASH proteins may act as single-stranded DNA photolyases or dual-activity photoreceptors that have both photolyase and cryptochrome activities.

Since the discovery of the first cryptochrome in Arabidopsis, this type of photoreceptor has been widely found in organisms ranging from bacteria to human (Cashmore, 2003; Partch and Sancar, 2005b). All cryptochromes share sequence similarity at their N-terminal PHR domains with DNA photolyases, and they act as photoreceptors not only in plants, but also in bacteria, insects, coral, zebrafish, chicken, and mammals (Emery et al., 1998; Stanewsky et al., 1998; Ceriani et al., 1999; Selby et al., 2000; Cermakian et al., 2002; Van Gelder et al., 2003; Tu et al., 2004; Tamai et al., 2007; Hoang et al., 2008b; Zhu et al., 2008b; Hendrischk et al., 2009). Based on phylogenetic analyses, the photolyase/cryptochrome superfamily was divided into five subfamilies: CPD (cyclobutane pyrimidine dimer) photolyase (photolyase without further qualification refers to CPD photolyase), 6-4 photolyase, plant cryptochromes, animal cryptochromes, and CRY-DASH (Partch and Sancar, 2005a). CPD photolyases and 6-4 photolyases repair cyclobutane pyrimidine dimers and pyrimidine (6-4) pyrimidone photoproducts, respectively (Sancar, 2000; Sancar, 2003). Arabidopsis possesses both CPD photolyase and 6-4 DNA photolyase (Ahmad et al., 1997; Nakajima et al., 1998), in addition to CRY1, CRY2, and CRY-DASH (CRY3). Animal cryptochromes are further divided into two classes, based on both phylogenetic analysis of sequences and their light responsiveness. Animal type I cryptochromes, such as *Drosophila* dCRY and monarch butterfly CRY1, act as photoreceptors, whereas animal type II cryptochromes, such as mouse cryptochromes, human cryptochromes, and monarch butterfly CRY2, act as light-independent transcription repressors (Zhu et al., 2005; Yuan et al., 2007). Some insects, such as monarch butterfly and mosquito, have both types of cryptochromes, whereas others, such as Drosophila, has only one (Yuan et al., 2007). Different cryptochromes have been shown to act as the essential components of the circadian clock in mammals (Thresher et al., 1998; van der Horst et al., 1999; Vitaterna et al., 1999), the dual-function photolyase/transcription-regulator in bacteria, fungus, or algae (Hitomi et al., 2000; Bayram et al., 2008; Coesel et al., 2009), and the light-dependent magnetoreceptors in plants, birds, and insects (Ahmad et al., 2007; Liedvogel et al., 2007; Gegear et al., 2008; Liedvogel and Mouritsen, 2009; Gegear et al., 2010). Studies of the last 17 years indicate that cryptochromes are probably the most widely spread photoreceptors in nature that play various biological functions across all three major evolutionary lineages, from bacteria, plants, to animals.

The nomenclature of Arabidopsis cryptochromes was previously suggested in 1998 to follow that of the phytochromes, such that the wild-type genes, mutant genes, apoproteins, and holoproteins of cryptochromes were referred to as *CRY*, *cry*, CRY, and cry, respectively (Quail et al., 1994; Lin et al., 1998). However, because a clear distinction between apocryptochromes and holocryptochromes is often difficult and not used by researchers of cryptochromes in other organisms, the capital and un-italicized symbol, CRY, is now used more frequently (and in this chapter) to describe cryptochrome proteins regardless whether they are apoproteins or holoproteins (Yu et al., 2007b; Yu et al., 2007a).

CRYPTOCHROME FUNCTIONS

The functions of Arabidopsis cryptochromes have been revealed by genetic studies of the loss-of-function mutants of the *CRY1* or *CRY2* genes and gain-of-function transgenic plants overexpressing either the wild type or mutant *CRY1* and *CRY2* genes. Those studies demonstrate that Arabidopsis CRY1 and CRY2 primarily mediate blue-light stimulation of de-etiolation and photoperiodic control of flowering-time, respectively, although they also mediate other light responses in Arabidopsis (Figure 1). The physiological function of CRY3 remains unclear, because a T-DNA insertional *cry3* mutant showed no obvious phenotypic alteration when grown under various visible or UV light conditions (Yu and Lin, unpublished). But given its biochemical activity in repairing ss-DNA, CRY3 is likely involved in protecting organellar genomes in Arabidopsis against UV damage.

Expression and Subcellular Localization of Cryptochromes

Before discussing the physiological functions of cryptochromes, it is useful to first take a look at the expression pattern of the cryptochrome genes, and the cellular location of the CRY1 and CRY2 proteins. Arabidopsis *CRY1* and *CRY2* genes express ubiquitously in all the cell-type and organs examined (Ahmad and Cashmore, 1993; Lin et al., 1996a; Lin et al., 1998; Toth et al., 2001). Analysis of the *CRY* promoter-driven luciferase reporter indicates that *CRY* mRNA expression is not dramatically affected by blue light, but the promoters of both *CRY1* and *CRY2* are under the control of the circadian clock, with peaks in the light phase and troughs in the dark phase (Toth et al., 2001). Ubiquitous expression of *CRY1* and *CRY2* is consistent with the multiple facets of the functions of those two photoreceptors in various light responses associated with different cells and organs of Arabidopsis. The cellular level of CRY1 protein is not significantly affected by light (Lin et al., 1996a), but the level of the CRY2 protein is negatively regulated by blue light (Ahmad et al., 1998a; Lin et al., 1998). The protein expression patterns of CRY1 and CRY2 seem also consistent with the fluence rate-dependence of the function of CRY1 and CRY2 in the control of de-etiolation and the photoperiod-dependence of the function of CRY2 in the control of flowering-time.

Arabidopsis CRY1 and CRY2 are soluble proteins that accumulate in the nucleus (Lin et al., 1996a; Cashmore et al., 1999; Guo et al., 1999; Kleiner et al., 1999; Wu and Spalding, 2007). CRY1 can also be detected in the cytosol in seedlings grown in both dark and light conditions without a drastic change of relative subcellular concentration (Wu and Spalding, 2007). It has been shown that nuclear CRY1 protein is responsible for blue light in-

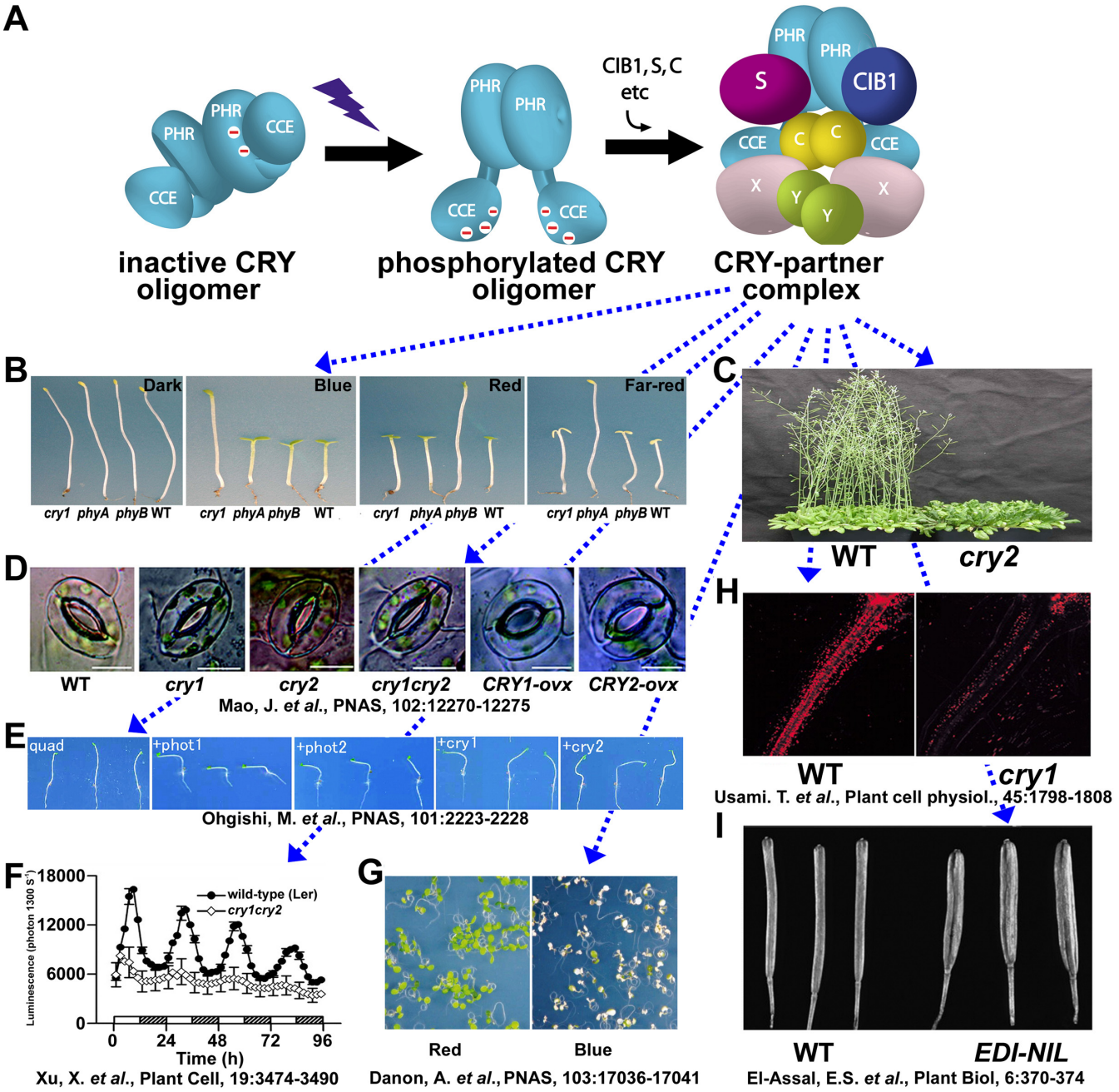

Figure 1. Arabidopsis cryptochromes undergo light-dependent conformational change to interact with multiple signaling proteins and regulate multiple light responses.

(A) A model depicting constitutive homodimerization of cryptochrome via the PHR domain, light-dependent phosphorylation (negative charges shown) and changes of conformation (depicted as the disengagement of the PHR and CCE domains), and interaction with signaling proteins, including CIB1, COP1 (C), SPA's (S), and other unidentified proteins (X and Y). This figure was modified from Figure 4D in Yu et al. (2007a) with permission from the National Academy of Sciences, USA.

(B-I) Different functions of Arabidopsis cryptochromes are shown by comparisons of different genotypes grown under different light conditions. The respective genotypes or light conditions are indicated underneath. **(B)** Arabidopsis seedlings grown in the dark, continuous blue, red, or far-red light, showing blue light-specific long hypocotyl phenotype of the *cry1* mutant. **(C)** Arabidopsis wild-type (WT) and the *cry2* mutant (*cry2*) grown in long-day photoperiod, showing late-flowering phenotype of the *cry2* mutant. **(D-I)** Images taken from the published articles (references are indicated underneath) with permission show cryptochromes mediate blue light stimulation of stomata opening **(D)**, random bending of hypocotyls **(E)**, the circadian oscillation of cytoplasmic calcium concentration **(F)**, programmed cell death (of the *flu* mutant) **(G)**, plastid development **(H)**, and fruit (silique) elongation **(I)**.

hibition of hypocotyl elongation, whereas the cytosolically localized CRY1 mediates blue light stimulation of cotyledon expansion and root elongation (Wu and Spalding, 2007). Remarkably, the nuclear-localized CRY1 also mediates blue light-induced membrane depolarization that occurs within a few seconds upon light exposure (Wu and Spalding, 2007). In contrast, CRY2 appears to complete its post-translational life cycle in the nucleus (Yu et al., 2007b). The nuclear-localized CRY2 mediates floral induction and hypocotyl inhibition. The blue light-dependent CRY2 phosphorylation and degradation also appear to occur only in the nucleus (Yu et al., 2007b). It remains unclear whether there are subtle subcellular redistributions of CRY1 or CRY2 in response to blue light.

Readers of the "Arabidopsis Book" may have noticed the cover of The Arabidopsis Book that shows GFP-"painted" chromosomes of a mitotic cell (http://www.bioone.org/doi/book/10.1199/tab.book) (Figure 2C). The observed GFP "painting" results from association of a fusion protein of GFP and a truncated CRY2 C-terminal region with the chromosomes. This observation was made in a study to investigate subcellular localization of GFP fusion proteins (Cutler et al., 2000). In this study, transgenic Arabidopsis plants expressing random GFP-cDNA fusion constructs were examined for the subcellular localization of individual fusion proteins. One transgenic line that expressed the fusion protein of GFP to the C-terminus region of CRY2 was identified, and it was found to bind to condensed chromosomes in a mitotic cell, whereas the same fusion protein accumulated in the nucleoplasm in non-dividing cells (Cutler et al., 2000). The observation was not due to an artifact of the truncated CRY2 protein, because we found that the full-length CRY2 fused to GFP in either orientation were associated with the condensed chromosomes in mitotic cells (Figure 2A-B). The CRY2-GFP and GFP-CRY2 fusion proteins accumulate in the nucleoplasm in the non-dividing cells, whereas little GFP signal is detected in the cytosol (Figure 2A, and data not shown). Importantly, both CRY2 fusion proteins are physically associated with the condensed chromosomes in mitotic cells (Figure 2A, arrows, and Figure 2B). It has been shown that the gene-rich regions of chromatin were transiently decondensed prior to bolting during flowering and that the cry2 mutant was impaired in this chromatin decondensation response (Tessadori et al., 2007). Given the role of cryptochromes in the blue light regulation of transcription and the recent report of the light-dependent regulation of chromatin remodeling (Charron et al., 2009), one couldn't help but wonder whether CRY2 is associated with chromatin during interphase to affect chromatin remodeling in response to light, whether there is a functional connection between the reported role of CRY2 in chromatin decondensation in interphase and the observed physical association of CRY2 with the condensed chromosome in mitosis, and whether the association of cryptochromes and chromatin may be a general phenomenon in other organisms.

De-etiolation of Seedlings

De-etiolation is the major developmental switch of young seedlings emerging from the darkness under soil and becoming exposed to light. De-etiolation is characterized by several morphological changes, including hypocotyl growth arrest, cotyledon expansion, and chloroplast development. Light inhibits hypocotyl elongation, but stimulates cotyledon expansion and conversion of etioplasts to chloroplasts (Nemhauser and Chory, 2002). It is clear now that phytochromes and cryptochromes both mediate blue light stimulation of de-etiolation in Arabidopsis (Figure 1B), and their functions in de-etiolation are dependent on both transcriptional and post-transcriptional regulation of gene expression (Chen et al., 2004; Jiao et al., 2007). Based on genetic analyses of Arabidopsis mutations impaired in the de-etiolation responses, many genes, such as *COP1, SPA1*, *HY5/HYH, HFR1, PP7, SUB1, SHB1, BIT1, OBP3, HRB1*, and ATAB2 have been found to participate in cryptochrome regulation of de-etiolation (Ang and Deng, 1994; Guo et al., 2001; Yadav et al., 2002; Duek and Fankhauser, 2003; Moller et al., 2003; Kang et al., 2005; Ward et al., 2005; Barneche et al., 2006; Kang and Ni, 2006; Hong et al., 2008). However, how those genes, except *COP1, SPA1*, and *HY5/HYH*, are involved in the cryptochrome function remains largely unknown.

Blue light inhibition of hypocotyl elongation. Light inhibition of hypocotyl elongation is probably the most widely used phenotypic read-out in the study of de-etiolation (Koornneef et al., 1980). As mentioned before, the first cryptochrome was identified in a study of the *cry1* (*hy4*) mutant impaired in blue light inhibition of hypocotyl elongation (Koornneef et al., 1980; Ahmad and Cashmore, 1993; Ahmad et al., 1995) (Figure 1B). Overexpression of CRY1 confers hypersensitive blue-light inhibition of hypocotyl elongation, resulting in shorter hypocotyls and dwarf seedlings grown in continuous blue light (Lin et al., 1995a; Lin et al., 1996a). CRY2 also plays a role, albeit a relatively minor one compared to that of CRY1, in mediating blue-light inhibition of hypocotyl elongation. It seems that the function of CRY2 in de-etiolation is limited to low intensities of blue light (<10 $\mu molem^{-2}s^{-1}$), probably due to a faster turnover of the CRY2 protein under higher fluence rates of blue light (Lin et al., 1998). The *cry1cry2* double mutant exhibited a more pronounced long-hypocotyl phenotype when grown in continuous blue light than the *cry1* or *cry2* monogenic mutant, suggesting a partially redundant function of the two cryptochromes in this response (Mockler et al., 1999). The action spectrum of CRY in mediating light inhibition of hypocotyl elongation has been analyzed by comparisons of wild type, the *cry1cry2* double mutant, and a CRY1-overexpressing transgenic line (Ahmad et al., 2002). The action spectrum of CRY1 mediating hypocotyl inhibition exhibited a broad peak between the UV-A and blue light regions (~390-480nm).

The cellular mechanism underlying blue light- and CRY-dependent growth inhibition has also been extensively studied (Spalding, 2000). It was proposed that cryptochromes activate anion channels, leading to plasma membrane depolarization and inhibition of cell elongation (Spalding and Cosgrove, 1988; Cho and Spalding, 1996; Parks et al., 1998; Spalding, 2000). It was found that nuclear-localized CRY1 is responsible for both hypocotyl inhibition and membrane depolarization (Wu and Spalding, 2007). The nuclear CRY1-dependent membrane depolarization occurs within seconds after illumination, suggesting a mechanism faster than that normally associated with the regulation of transcription or even protein degradation. Furthermore, it was found that the *cry1*, *cry2*, *phyA*, and *phot1* mutants all showed similar defects in the blue light-induced plasma membrane depolarization response (Parks et al., 1998; Parks and Spalding, 1999; Folta

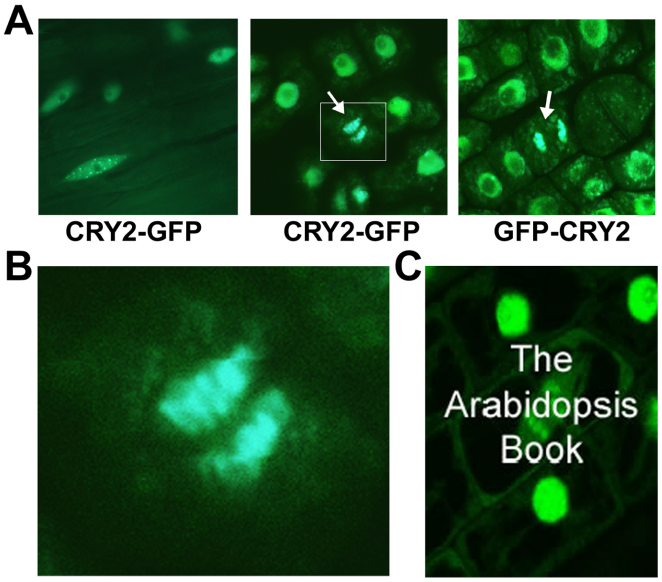

Figure 2. CRY2 is found in nucleoplasm during interphase but it is associated with chromosomes during mitosis.

(A) Fluorescence images of CRY2-GFP (left and center) or GFP-CRY2 (right) fusion proteins taken from mesophyll cells (left) or from shoot apex cells (center and right) of the respective transgenic plants grown in white light. Arrows indicate mitotic cells and CRY2-associated chromosomes. **(B)** An enlarged image of the boxed area of panel A (center) showing association of CRY2-GFP with condensed chromosomes. **(C)** The cover of "The Arabidopsis Book", showing the fusion protein of GFP and the C-terminal region of CRY2 (CCE2) in nuclearplasm (the surrounding cells) or chromosomes (the central cell).

and Spalding, 2001), although the *cry1*, *cry2* and *phyA*, or *phot1* mutants showed severely impaired, modestly impaired, or normal blue-light inhibition of hypocotyl elongation, respectively. A high-resolution growth kinetics analysis detected a growth defect in the *phot1* mutant. However, this defect was manifested only in the *cry1* but not in the *phot1* mutant with respect to the long-hypocotyl phenotype. The growth defect of the *phot1* mutant detected by the growth kinetics assay was restricted in the rapid phase, whereas the defect of the *cry* mutants was limited to the later phase (Folta and Spalding, 2001). Although it was proposed that phototropins might be responsible for initiating the blue light-dependent growth inhibition, whereas cryptochromes are required for the maintenance of the growth inhibition (Spalding, 2000; Parks et al., 2001; Folta and Kaufman, 2003), how might this model explain the different morphological phenotypes between the *cry* and *phot* mutant seedlings remains unclear.

As discussed later in this chapter, the cryptochrome-mediated suppression of COP1-dependent degradation of transcription regulators, such as bZIP transcription factors HY5/HYH and bHLH transcription factor HFR1, is a major mechanism underlying light inhibition of hypocotyl elongation (Osterlund et al., 2000; Duek et al., 2004; Yang et al., 2005; Jiao et al., 2007; Lee et al., 2007). Those transcription factors are usually degraded in seedlings grown in the dark. In response to light, cryptochromes (and phytochromes) suppress the COP1 activity, resulting in accumulation of those transcription factors and altered transcription activities. Altered expression of genes that encode metabolic enzymes or signaling proteins of phytohormones, such as auxin, BR, GA, etc, and other metabolic enzymes, such as those catalyzing syntheses and degradation of cell wall components, can at least partially explain the morphological changes underlying light inhibition of hypocotyl elongation.

Blue light stimulation of cotyledon expansion. In contrast to the inhibitory effect of cryptochromes on cell elongation in hypocotyls, cryptochromes mediate blue-light stimulation of cell expansion in cotyledons (Feldman, 1984; Blum et al., 1994; Casal and Mazzella, 1998; Lin et al., 1998; Neff and Chory, 1998). Both CRY1 and CRY2 mediate blue-light stimulation of cotyledon expansion. It remains puzzling that blue-light stimulation of cotyledon expansion seems mediated by CRY1 located in the cytosol (Wu and Spalding, 2007) but by CRY2 located in the nucleus (Wu and Spalding, 2007; Yu et al., 2007b). As in the case of hypocotyl inhibition, CRY1 stimulates cotyledon expansion in both high and low irradiance, whereas the role of CRY2 is limited to low irradiance (Blum et al., 1994; Lin et al., 1998). Transgenic seedlings overexpressing either CRY1 or CRY2 show larger cotyledons than those of wild type seedlings grown under blue light, suggesting that the cellular concentration of cryptochromes is rate-limiting for cotyledon expansion. The facts that cryptochromes mediate blue-light inhibition of hypocotyl elongation but blue-light stimulation of cotyledon expansion suggest that different signaling mechanisms may be involved in the cryptochrome regulation of cell elongation/expansion in different organs or cell types. Alternatively, the same cryptochrome signaling mechanism may trigger opposite cellular responses in different cells in hypocotyls and cotyledons.

Blue light stimulation of chloroplast development. Blue light-induced change of gene expression in both nuclear and plastid genomes is probably the major mechanism underlying development of plastids during de-etiolation (Kasemir, 1979; Chen et al., 2004; Jiao et al., 2007). Cryptochromes appear to mediate blue light induction of changes in gene expression via both transcriptional and post-transcriptional mechanisms. Cryptochromes mediate blue-light induction of nuclear genes that encode plastid proteins required for not only photosynthesis and other functions of plastids, such as *CAB*, *rbcS,* and *CHS* (Fuglevand et al., 1996; Ma et al., 2001), but also components of the plastid transcriptional apparatus (Ma et al., 2001; Thum et al., 2001; Tsunoyama et al., 2002; Mochizuki et al., 2004). For example, the blue-light-induced transcription of the plastid-encoded *psbA* and *psbD* genes that encode PSII reaction center protein D1 and D2, respectively, are controlled by one of the six nuclear-encoded sigma factors, SIG5. And it has been shown that CRY1 is the major photoreceptor mediating blue-light induction of the expression of the nucleus-encoded *SIG5* gene (Thum et al., 2001; Tsunoyama et al., 2002; Mochizuki et al., 2004; Nagashima et al., 2004). Therefore, cryptochrome regulation of the supply of nuclear-encoded proteins, which are required for the plastid transcription machinery controlled by the plastid-encoded RNA polymerase, may play critical roles in the chloroplast development during de-etiolation. Moreover, cryptochromes also mediate light suppression of COP1-dependent degradation of nuclear-encoded transcription regulators that control the expression of the nuclear genome (Jiao et al., 2007).

Photoperiodic Control of Flowering-time

Among various developmental processes regulated by cryptochromes, the molecular mechanism underlying the control of flowering-time is probably the most extensively studied and best understood (Searle and Coupland, 2004; Alvarez-Buylla et al., 2010). Arabidopsis is a facultative long-day (LD) plant that flowers earlier in LD than in short-day (SD). It is well known that phytochromes mediate photoperiodic control of flowering-time in plants, but the function of cryptochrome in photoperiodic response was unrevealed unexpectedly (Figure 1C). Based on an observation that transgenic seedlings overexpressing CRY2 had hypocotyl lengths intermediate between those of the wild type and CRY1-overexpressing plants, CRY2 was hypothesized to play a minor role in de-etiolation and a genetic screen was designed to isolate the *cry2* mutant. In this screen, fast-neutron mutagenized populations were first screened for seedlings with hypocotyls of intermediate lengths between the wild type and the *cry1* mutant; the putative mutants were further screened using the immunoblot assay probed with the anti-CRY2 antibody (Guo et al., 1998; Lin et al., 1998). Two *cry2* mutant alleles were isolated from this screen. Surprisingly, both *cry2* mutant alleles flowered significantly later than the wild type in LD but not in SD, indicating that CRY2 plays a role in the control of photoperiodic flowering (Guo et al., 1998). Consistent with this finding, the *cry2* mutant was found to be allelic to *fha*, a previously isolated late-flowering mutation that was classified, together with *co, ft,* and *gi*, to the photoperiodic pathway controlling flowering-time (Koornneef et al., 1991; Guo et al., 1998; Koornneef et al., 1998a).

The function of CRY2 in the photoperiodic control of floral initiation was further demonstrated by a study of a natural occurring *CRY2* allele, *EDI* (El-Assal et al., 2001; El-Assal et al., 2003). *EDI* is a major quantitative trait locus (QTL) responsible for the photoperiod-insensitive early flowering phenotype of the Arabidopsis accession Cvi collected from the tropical Cape Verde Islands near the Equator (Alonso-Blanco et al., 1998). A QTL mapping located *EDI* to the *CRY2* gene, and it was found that the day-neutral early flowering phenotype of the *EDI* locus was caused by a V367M substitution in the CRY2 protein (El-Assal et al., 2001). In the laboratory accessions of Arabidopsis, such as Col and L*er*, the cellular level of CRY2 protein exhibits a robust and blue light-dependent diurnal rhythm in SD but not in LD, suggesting that the control of CRY2 protein level may be a mechanism for the perception and/or interpretation of photoperiodic signals in Arabidopsis (El-Assal et al., 2001; Mockler et al., 2003). The $CRY2^{V367M}$ protein is more stable and its abundance is less sensitive to photoperiod in plants grown in white light, although it appears to retain a normal degradation pattern in etiolated seedlings exposed to blue light. The increased stability and the lack of photoperiodic regulation of $CRY2^{V367M}$ appears to explain the day-neutral early flowering phenotype of the Cvi accession.

EDI is the only naturally-occurring *CRY2* allele reported so far that affects flowering-time in Arabidopsis. A linkage disequilibrium mapping of 95 Arabidopsis accessions suggests that two haplotypes (A^S and B) associated with the *CRY2* gene may be responsible for the early-flowering phenotype and that one of the haplotypes (A^S) is characterized by a Q127S replacement of CRY2 (Olsen et al., 2004). However, a direct molecular genetic test of the haplotype A^S or $CRY2^{Q127S}$ in early flowering or altered photoperiodic sensitivity in Arabidopsis accessions has yet to be reported. On the other hand, contrary to a general expectation or that reported in other plants (Thomas and Vince-Prue, 1997; Zhang et al., 2008), a study of 150 Arabidopsis accessions found no clear latitudinal cline in flowering time when grown under LD or SD conditions without vernalization (Lempe et al., 2005). Therefore, it remains unclear whether cryptochromes have a broader contribution to the latitudinal distribution of Arabidopsis in nature.

CRY1 may also affect flowering time in Arabidopsis. Under white light, the *cry1* mutant showed a late-flowering phenotype in some experiments but not others, which may be a result of subtle differences in wavelengths of light or temperatures used in different experiments (Goto et al., 1991; Mozley and Thomas, 1995; Bagnall et al., 1996; Bruggemann et al., 1996; Blazquez et al., 2003; Mockler et al., 2003). The *cry1cry2* double mutant flowers later than the wild type when grown in continuous blue light, suggesting that CRY1 acts redundantly with CRY2 to promote flowering (Mockler et al., 1999; Liu et al., 2008b). The *cry2* mutation suppresses the early-flowering phenotype of the *phyB* mutant, suggesting that CRY2 antagonizes phyB-mediated red light suppression of floral initiation (Guo et al., 1998; Mockler et al., 1999). However, it is not clear whether CRY1 also acts to antagonize phyB action. Several different mechanisms may be involved in the cryptochrome promotion of floral initiation, including suppression of CO degradation (Valverde et al., 2004), activation of transcription activators (Liu et al., 2008a), and modulation of chromatin remodeling (Charron et al., 2009; Kumar and Wigge, 2010).

Earlier genetic analyses of Arabidopsis flowering-time mutants have placed CRY2 upstream from a number of flowering-time genes, including *CO*, *FT*, and *GI* (Koornneef et al., 1998b). The molecular nature of the genetic interactions of various photoreceptors and their downstream flowering-time genes has been at least partially solved (Suarez-Lopez et al., 2001; Yanovsky and Kay, 2002; Valverde et al., 2004). The results of those studies demonstrated that phytochromes and cryptochromes regulate transcriptional and post-translational control of the expression of CO, which is the major regulator responsible for the photoperiod-dependent transcription of the *FT* gene. For example, CRY1 and CRY2 mediates blue-light stabilization of the CO protein, whereas phyB mediates red-light destabilization of the CO protein (Valverde et al., 2004). It has been shown that the RING-Finger E3 ubiquitin ligase COP1 and its partner the WD-repeat protein SPA1 are directly involved in the CRY2 stabilization of CO (Laubinger et al., 2006; Jang et al., 2008; Liu et al., 2008b). The function of CRY2 promoting floral initiation appears to be limited to the vascular bundles (Endo et al., 2007), whereby the florigen protein FT was transcribed, translated, and transmitted to the apical meristem to activate transcription of the floral meristem identity genes (An et al., 2004; Corbesier et al., 2007; Jaeger and Wigge, 2007; Mathieu et al., 2007; Tamaki et al., 2007). Therefore, CRY2 regulation of CO protein stability and *FT* transcription represents another example of cell type-specific action of cryptochromes in Arabidopsis.

In addition to the regulation of CO protein stability, cryptochromes may also affect flowering time via additional mechanisms or signaling pathways. For example, the activity of CRY1 and CRY2 in the light entrainment of the circadian clock may affect clock-dependent transcription of the *CO* and other flowering-time genes (Yu et al., 2008), and CRY2 may interact with phyB to modulate phyB-dependent regulation of transcription (Mas et

al., 2000). CRY1 and CRY2 have also been shown to mediate blue light stimulation of the expression of microRNA Mi172, in a CO-independent manner, to regulate flowering-time (Jung et al., 2007). Moreover, it has been recently shown that CRY2 physically interacts with the bHLH transcription factor CIB1 in a blue light-dependent manner to directly affect transcription of the *FT* gene (Liu et al., 2008a). Although the CIB1-dependent regulation of *FT* transcription appears mechanistically independent from COP1/SPA1-dependent degradation of the CO protein, the CIB1 activity in promoting floral initiation is genetically dependent on the function of not only *CRY2* and *FT*, but also *CO* (Liu et al., 2008a) (Liu, unpublished results). Given that CIB1 is a bHLH protein that directly interacts with the genomic DNA of the *FT* gene, the CO-dependent CIB1 activation of *FT* transcription is intriguing, but the underlying molecular nature remains to be investigated.

Light Entrainment of the Circadian Clock

The circadian clock is the molecular oscillator that drives various overt circadian rhythms in most organisms, allowing the organisms to anticipate and adapt to the environmental changes dominated by the daily 24-hour light/dark cycle as well as the annual photoperiod cycle on earth (Devlin and Kay, 2001; Harmer, 2009). Light is the most dominant environmental cue that enables the circadian clock and physiological activities of an organism to be synchronized to the daily cycles of environmental changes (Devlin and Kay, 2001; Cashmore, 2003; Sancar, 2003). Cryptochrome as a major photoreceptor or intrinsic component of the circadian clock was demonstrated in 1998 in Arabidopsis, Drosophila, and mouse (Emery et al., 1998; Somers et al., 1998; Stanewsky et al., 1998; Thresher et al., 1998). An analysis of the clock-dependent transcription using the luciferase reporter showed that the *cry* mutants are impaired in the circadian clock in Arabidopsis (Somers et al., 1998). The *cry1* mutant has a lengthened period of the circadian rhythms in both high and low fluence rates (photons per unit area and time) of blue lights, whereas the *cry2* mutant has a slightly shortened period in low fluence rates of blue light. The *cry1cry2* double mutant exhibited a longer period under all fluence rates of blue light tested, suggesting that Arabidopsis CRY1 and CRY2 act redundantly to entrain the circadian clock. In contrast to the mouse *cry1cry2* double mutant that is arrhythmic, the Arabidopsis *cry1cry2* double mutant retains rhythmicity, indicating that Arabidopsis cryptochromes are not intrinsic components of the circadian oscillator (van der Horst et al., 1999; Vitaterna et al., 1999; Devlin and Kay, 2000; Yanovsky et al., 2000). The role of Arabidopsis cryptochromes mediating blue light regulation of the circadian clock has also been shown by other phenotypic read-out, such as cytosolic free Ca^{2+} oscillations and leaf movement (Yanovsky et al., 2001; Xu et al., 2007) (Figure 1F). More importantly, the cryptochrome-mediated blue light entrainment of the circadian clock has been demonstrated directly by a blue light-induced phase-shift assay (Yanovsky et al., 2001). It was shown that blue light-induced phase advancement is impaired in the *cry1* mutant and abolished in the *cry1cry2phyA* triple mutant (Yanovsky et al., 2001). This experiment demonstrates unambiguously that the three photoreceptors, CRY1, CRY2 and phyA, act redundantly to mediate blue-light entrainment of the circadian clock in Arabidopsis.

It remains unclear exactly how cryptochromes mediate light entrainment of the circadian clock in Arabidopsis. It was recently reported that COP1 interacts with ELF3 to mediate day-length signaling from CRY2 (Yu et al., 2008). Because the function of ELF3 and GI are closely associated with light regulation of the circadian clock (Hicks et al., 1996; Park et al., 1999; Huq et al., 2000; Sawa et al., 2007), it is conceivable that cryptochromes may suppress the activity of COP1 to affect ELF3 and GI and light regulation of the clockwork. But the detailed mechanism of the entrainment of clock is not known. In this regard, it is interesting that physical interactions between several photoreceptor pairs, including CRY1-phyA, CRY2-phyB, CRY1-ZTL, and phyB-ZTL, have been reported (Ahmad et al., 1998c; Mas et al., 2000; Jarillo et al., 2001). The physical interaction of multiple photoreceptors may have an adaptive advantage in conferring complex responses of the clockwork to the changing light environment. However, exactly how those photoreceptor interactions may affect light responses and what types of biochemical consequence of such interaction may bring about to impact the operation of the circadian oscillator in the cell remain to be investigated.

Light Stimulation of Guard Cell Development and Stomata Opening

Light affects stomata opening and guard cell development. Stomata open in the daytime and close in the evening, allowing gas exchange for photosynthesis and transpiration in the daytime, and reservation of water in the evening (Zeiger and Hepler, 1977). Phototropins are the major photoreceptor regulating stomata opening, which probably mediate blue light-dependent hyperpolarization of membrane potential of guard cells, resulting in the influx of water and the opening of the stomata pore (Kinoshita et al., 2001; Schroeder et al., 2001; Briggs and Christie, 2002). A recent observation that the *cry1cry2* double mutant displayed an increased drought tolerance has led to a finding that cryptochromes also contribute to the blue-light stimulation of stomata opening (Mao et al., 2005) (Figure 1D). It was found that the *cry1cry2* mutant and CRY1-overexpressing plants exhibit a reduced or increased stomata opening in response to blue light, respectively. The residual blue-light response promoting stomata opening in the *phot1phot2* double mutant was eliminated in the *cry1cry2phot1phot2* quadruple mutant, whereas overexpression of CRY1 in the *phot1phot2* mutant background can restore stomata opening in response to blue light, suggesting that cryptochromes function additively with phototropins in mediating blue light-induced stomatal opening (Mao et al., 2005).

In addition to stimulating stomata opening, light also stimulates stomata development. Light stimulation of guard cell development is a complex process that includes initiation of asymmetric cell divisions, proliferation of precursor cells, and differentiation of stomatal guard cells that form stomata. It has been shown that blue, red, and far-red light all stimulate guard cell development (Bergmann and Sack, 2007; Kang et al., 2009). *cry1* mutant plants are impaired in blue-light stimulation of stomata development (Kang et al., 2009). Genetic analyses indicate that cryptochrome-mediated blue-light stimulation of stomata development is independent from the known stomata developmental control network associated with the leucine-rich repeat (LRR) receptor-like kinases,

a MAPK cascade, and a number of bHLH transcription factors (Bergmann and Sack, 2007; Kang et al., 2009). This is consistent with the observations that phototropin-mediated blue-light stimulation of stomata opening is involved with mechanisms initiated from the plasma membrane and that cryptochromes regulate development by modulating gene expression (Ma et al., 2001; Stoelzle et al., 2003; Inada et al., 2004; Ueno et al., 2005). Similar to the activity of cryptochromes in mediating de-etiolation responses, the function of cryptochromes in both stomata opening and stomata development seems also dependent on COP1 (Mao et al., 2005; Kang et al., 2009). The exact molecular relationships between the cryptochromes/COP1-dependent and phototropins-dependent pathways triggering stomata opening or the cryptochromes/COP1-dependent and MAPK-dependent pathways regulating guard cell development are not clear at present.

Other Possible Functions

In addition to the light responses discussed above, cryptochromes may mediate light regulation of other aspects of plant growth and development. For example, cryptochromes in Arabidopsis and other plants have been shown to regulate plant height (Weller et al., 2001; Giliberto et al., 2005; Platten et al., 2005), fruit and ovule development (El-Assal et al., 2004), seed dormancy (Goggin et al., 2008), apical dominance (Weller et al., 2001; Giliberto et al., 2005), the high-light stress response (Kleine et al., 2007), osmotic stress response (Xu et al., 2009), cell cycle program of stem cells (Lopez-Juez et al., 2008), responses to bacterial and viral pathogens (Jeong et al., 2010; Wu and Yang, 2010), as well as magnetoreception, tropic growth, and programmed cell death (Figure 1). Unlike de-etiolation and flowering-time phenotype that are unambiguous and easy to observe, some "minor" phenotypes detected in the loss-of-function cryptochrome mutants are sometimes subtle, ambiguous, controversial, or undetectable unless being in a specific genetic background. Some of those interesting ones are discussed in the following.

Light regulation of root development. Although photomorphogenic changes are most apparent in the aerial tissues, light also regulates many aspects of root development such as root extension, lateral root production, plastid development, and tropic growth (Feldman, 1984). It is not completely dark underneath the soil where roots grow. For example, as much as 5 $\mu molem^{-2}s^{-1}$ of light was detected 5mm under soil (Smith, 1982). Contrary to a conventional wisdom that light should inhibit root elongation (to prevent roots from growing out of the soil), it was reported that blue light stimulated root elongation and that CRY1 mediated this response (Canamero et al., 2006). In contrast, CRY2 appears to antagonize the function of CRY1 in regulating the root cell elongation. The shoot was suggested to be the site of blue-light perception that regulates primary root growth (Canamero et al., 2006), although significant cryptochrome mRNA or protein expression has been detected in roots (Lin et al., 1996a; Toth et al., 2001). The cellular mechanism underlying CRY function in roots is further complicated by the observation that cytoplasmic and nuclear CRY1 may promote or suppress root elongation, respectively (Wu and Spalding, 2007). In addition to cell elongation, blue light also stimulates chloroplast development in roots, and this blue light effect is mediated primarily by CRY1 (Usami et al., 2004) (Figure 1H).

Magnetoreception. Migrating animals such as birds and insects are thought to detect the Earth's magnetic field and use it as a source of directional information to guide migration. The animal magnetoreception is often dependent on light, and it was proposed that the magnetic information is transmitted to the nervous system through the light-induced product of magnetically sensitive radical-pair reaction in photoreceptors (Ritz et al., 2000; Liedvogel and Mouritsen, 2009). It was recently shown that cryptochromes mediate light-dependent magnetoreception in Drosophila, but this function of cryptochrome is dependent on a novel photochemistry unrelated to the Trp-triad-dependent photoreduction of FAD (Gegear et al., 2008; Yoshii et al., 2009; Gegear et al., 2010).

Possible responses to magnetic field, or magnetoreception, has also been studied in plants (Galland and Pazur, 2005). It was reported that magnetic field enhances CRY1-mediated blue light inhibition of hypocotyl elongation, anthocyanin accumulation, and blue light-dependent CRY2 degradation in Arabidopsis (Ahmad et al., 2007; Solov'yov et al., 2007). FAD photoreduction via the Trp-triad-dependent electron transport was proposed to be involved in the light-dependent magnetic-sensing of cryptochromes (Ahmad et al., 2007; Solov'yov et al., 2007). However, it was also reported recently that the blue light-dependent hypocotyl inhibition, anthocyanin accumulation, and mRNA expression of several genes in Arabidopsis showed no statistically significant changes in response to various strengths of magnetic field (Harris et al., 2009). More studies are apparently needed to clarify this controversy.

Programmed cell death (PCD). Molecular mechanisms underlying PCD are thought to contain similar components in animals and plants. However, light-dependent PCD initiated by reactive oxygen species (ROS) resulting from impaired photosynthesis electron transport under stress conditions is unique to plants. It was found that the *cry1* mutation suppresses blue light- and ROS-dependent PCD in Arabidopsis, and this discovery was made possible only in the genetic background of the *flu* mutant (Danon et al., 2006). The *flu* mutant was isolated in a study to investigate tetrapyrrole metabolism for the biosynthesis of compounds such as chlorophyll and phytochromobilin that are the chromophores of the photosynthetic pigments and phytochromes, respectively (Meskauskiene et al., 2001). In contrast to the wild type plants, the *flu* mutant accumulates more protochlorophyllide (Pchlide) in light-grown seedlings transferred to the dark. When the Pchlide-accumulating *flu* mutant plants were re-exposed to light, photosensitized Pchlide molecules transfer light energy to ground state (triplet) oxygen to elevate it to the excited and less stable (singlet) state. As an important source of ROS, singlet oxygen triggers PCD and causes death of the *flu* mutant plants treated with the light-dark-light condition (op den Camp et al., 2003). This PCD phenotype of the *flu* mutant is dependent on blue light but not red light (Figure 1G), so the role of CRY1 was examined. It turned out that the *cry1* mutant rescued the PCD phenotype of the *flu* mutant (in the *flucry1* double mutant), indicating that CRY1 mediates blue light-dependent CPD in Arabidopsis (Danon et al., 2006). A comparison of the expression profiles of the *flu*, *cry1*, and *flucry1* mutants indicate that only 34 of the 588 genes up-regulated in the *flu* mutant were suppressed in the *flucry1* double mutant, and the expression of all those 34 genes was previously known to be responsive to abiotic or biotic

stresses (Danon et al., 2006). Therefore, CRY1 may mediate blue light induction of the expression of those stress-responsive genes to trigger PCD in Arabidopsis.

Phototropism. Similar to the contradictory observations concerning the functions of CRY1 in the control of flowering-time and magnetoreception, the function of CRY1 in phototropism is also complex and sometimes contentious. It was reported that the *cry1cry2* double mutant lacked blue light-dependent phototropism, whereas CRY1 overexpression enhanced phototropism (Ahmad et al., 1998b). However, the major blue light receptor responsible for phototropism was later discovered to be phototropins rather than cryptochromes (Liscum and Briggs, 1995; Christie et al., 1998). A detailed analysis of the *cry1cry2* and *phyAphyB* mutants shows that although both phytochrome and cryptochrome mutants exhibit somewhat reduced amplitude in the first positive phototropism, neither altered other important parameters of phototropism, such as threshold, peak, and saturation fluence, and therefore, neither were considered photoreceptors mediating phototropism *per se* (Lasceve et al., 1999). A more recent report further confirmed that the *cry1cry2* double mutant possesses a near normal phototropism in response to blue light, although the response is impaired in the *cry1cry2phyA* triple mutant (Tsuchida-Mayama et al., 2010). It appears clear now that phototropins are the blue light receptors mediating phototropism and most other blue light-dependent movement responses, such as stomata opening and chloroplast movement. Cryptochromes and phytochromes may act together to modulate the functions of phototropins in many if not all of the phototropin-mediated blue light responses. For example, cryptochromes and phytochromes may act to enhance the phototropin action at lower fluence but antagonized the function of phototropin at high fluence (Whippo and Hangarter, 2003). It was reported recently that CRY1 and CRY2 act redundantly with phyA to positively regulate the RPT2 protein accumulation, phototropin-dependent auxin gradient formation necessary for the phototropic growth, but negatively regulate GA-dependent suppression of phototropism (Tsuchida-Mayama et al., 2010).

Gravitropism. It has been known for some time that light affects gravitropism in plants (Hangarter, 1997). For example, phytochromes mediate red light suppression of gravitropic growth of hypocotyls in Arabidopsis (Liscum and Hangarter, 1993; Robson and Smith, 1996). However, the effect of blue light on gravitropism is usually masked by the blue light-dependent phototropism and it is unraveled only in the background of the *phot1phot2* double mutant (Ohgishi et al., 2004) (Figure 1E). In this elegant experiment, transgenic expression of individual cryptochromes or phototropins was prepared in the *cry1cry2phot1phot2* quadruple mutant to restore the function of one photoreceptor at a time in the absence of the other three. In the absence of blue light-dependent phototropism, the transgenic seedlings expressing *CRY1* or CRY2 (in the background of the *cry1cry2phot1phot2* quadruple mutant) showed random hypocotyl bending (phenotypically similar to agravitropic growth) when grown under blue light (Ohgishi et al., 2004). Importantly, the same plants showed no agravitropic phenotype when grown in the dark. This experiment demonstrates that, analogous to the phytochrome-dependent red light suppression of gravitropism, CRY1 and CRY2 mediate blue-light suppression of gravitropism or blue light activation of random hypocotyl bending (Ohgishi et al., 2004). Light-dependent suppression of gravitropism is likely a consequence of photoreceptor-dependent regulation of the expression of genes involved in hormone metabolism, transport, or signaling. For example, cryptochromes mediate blue-light inhibition of the expression of the *PGP19/ABSB9* gene. *PGP19/ABSB9* encodes an ABC-type auxin transporter required for appropriate auxin transport and gravitropic growth. The *pgp19* mutant shows an agravitropic growth in the absence of phototropism and the phenotype of *pgp19* is epistatic to *cry1cry2* (Nagashima et al., 2008). It appears that the cryptochrome-mediated blue light suppression of *PGP19/ABSB9* expression explains partially the blue light suppression of gravitropic growth (Nagashima et al., 2008). Consistent with a role of *PGP19/ABSB9* in the CRY1 signaling, it has been recently reported that *ABSB9* can partially rescue the long hypocotyl phenotype of the *cry1* mutant, and that transgenic seedlings overexpressing *ABSB9* are hypersensitive to light inhibition of cell elongation (Wu et al., 2010). The CRY-dependent gravitropic response may also involve the COP1 pathway, because similar to the *cop1* mutant that is known to be constitutively photomorphogenic and agravitropic (Hou et al., 1993; Cao et al., 2000; Yang et al., 2000; Yu et al., 2009), the transgenic plants overexpressing GUS-CCT2 or CRY2-GFP also showed both constitutive photomorphogenic and agravitropic phenotypes (Hou et al., 1993; Cao et al., 2000; Yang et al., 2000; Yu et al., 2009) (Yu and Lin, unpublished).

Blue light-independent CRY actions? Besides its blue light-dependent functions, CRY may possess blue light-independent activities in the presence of longer wavelengths of light, such as red light and far-red light. For example, the *cry2* mutant seedlings showed a longer period length of the circadian clock in red light, and a FR-dependent defect in hypocotyl elongation (Mas et al., 2000). The gain-of-function *Cvi CRY2* allele ($CRY2^{V367M}$) exhibits an enhanced cotyledon unfolding in response to a FR pulse, whereas the *cry1cry2* mutant (in Col or Ler backgrounds) shows a reduced cotyledon unfolding under the same condition (El-Assal et al., 2001; Botto et al., 2003). It has also been reported that the mRNA and protein level of certain genes showed alteration in the *cry1cry2* mutant seedlings exposed to red light, in comparison to the wild type plants (Yang et al., 2008). It may not be surprising that most, if not all, of those effects found in the cryptochrome mutants are relatively minor. It is generally accepted that cryptochromes do not absorb long wavelength of light, but cryptochromes containing certain redox or excitation states of FAD (e.g. neutral radical or excited triplet state) that may absorb some photons with the wavelengths beyond 600nm (Song et al., 2006; Henbest et al., 2008; Ozturk et al., 2008). Therefore, a potential effect of long-wavelength light on the cryptochrome structure or function in some minor ways may not be excluded. Alternatively, given that cryptochromes may interact with phytochromes in the absence of blue light (Ahmad et al., 1998c; Mas et al., 2000), the observed phenotypic alterations of the *cry* mutants in the absence of blue light would be interpreted as a result of interactions between the two types of photoreceptors.

CRYPTOCHROME STRUCTURE

Cryptochromes are composed of two major domains, the N-terminal PHR (for Photolyase-Homologous Region) domain of about 500 residues, and the C-terminal extension CCE (for Cryp-

tochrome C-terminal Extension) domain of various lengths. The CCE of Arabidopsis CRY1 and CRY2 are approximately 180 and 110 residues in length, respectively. The PHR domain is required for chromophore-binding and homo-dimerization of Arabidopsis CRY1 and CRY2 (Sang et al., 2005; Yu et al., 2007a; Rosenfeldt et al., 2008), whereas CCE is an effector domain of cryptochrome (Yang et al., 2000; Wang et al., 2001; Yang et al., 2001; Yu et al., 2007a).

The X-ray crystallographic structures of several photolyases, CRY-DASH/CRY3, and the PHR domain of Arabidopsis CRY1 have been solved (Park et al., 1995; Brudler et al., 2003; Brautigam et al., 2004; Huang et al., 2006; Klar et al., 2007; Pokorny et al., 2008; Hitomi et al., 2009) (Figure 3). The three-dimensional structure of the PHR domain of Arabidopsis CRY1 is very similar to that of photolyases, suggesting a photolyase-like photochemistry in the cryptochrome photoexcitation (Figure 3). No crystallographic structure is currently available for a full-length cryptochrome. The difficulty in the crystallization of the full-length cryptochrome may be due to the intrinsically unstructured nature of the CCE domain of cryptochromes.

The Photolyase Homologous Region (PHR)

The PHR domain is the chromophore-binding domain of cryptochromes that shares strong sequence similarity to DNA photolyases. The amino acid sequence of the PHR domain of Arabidopsis CRY1 or CRY2 is 30% or 29% identical to the sequence of *E. coli* photolyase, respectively (Lin and Shalitin, 2003). Based on amino acid sequence comparisons among PHR domains of different plant cryptochromes, it appears that the gene duplication event leading to the divergence of CRY1 and CRY2 might have occurred no earlier than the split between spermatophytes (seed plants) and pteridophyte (seedless vascular plants), but no later than the emergence of angiosperms. The same type of cryptochromes from different angiosperm species share higher sequence similarity than different types of cryptochromes from the same species. For example, the PHR domain of monocot rice CRY1a is ~75% identical to that of dicot Arabidopsis CRY1, whereas the PHR domain of Arabidopsis CRY1 is only ~59% identical to that of Arabidopsis CRY2 (Lin and Shalitin, 2003; Matsumoto et al., 2003; Hirose et al., 2006; Zhang et al., 2006). However, such corresponding relationships appear to be lacking in cryptochromes of more distantly related plant species. For example, the PHR domains of fern (*Adiantum capillus-veneris*) AdiCRY1 share similar sequence identities to the PHR domains of both Arabidopsis CRY1 (69%) and CRY2 (64%) (Kanegae and Wada, 1998; Lin and Shalitin, 2003). Not surprisingly, the FAD-binding pocket of cryptochrome is the most conserved region within the PHR domain (Figure 4A). For example, the sequence of about 27 residues located within the 4Å vicinity of FAD is 81% identical between CRY1 and CRY2. Many residues associated with FAD-binding are conserved in cryptochromes from Arabidopsis to human (Figure 4A). In comparison, the sequences of photolyases involved in folate- or DNA-binding are less conserved in cryptochromes (Lin et al., 1995b; Ozgur and Sancar, 2003; Brautigam et al., 2004; Berndt et al., 2007).

The PHR domains of Arabidopsis CRY1 and CRY2 bind noncovalently to FAD (Lin et al., 1995b; Malhotra et al., 1995; Banerjee et al., 2007; Bouly et al., 2007). The PHR domain of Arabidopsis CRY1 and CRY3 expressed and purified from *E. coli* also contains the second chromophore, 5,10-methenyltetrahydrofolate (MTHF) (Malhotra et al., 1995; Selby and Sancar, 2006; Song et al., 2006). However, unlike CRY3 that binds MTHF stoichiometrically (Huang et al., 2006; Song et al., 2006; Klar et al., 2007), the full-length CRY1 and CRY2 expressed and purified from insect cells bind either no or only trace amount of MTHF, which prevents a more detailed study of this possible chromophore (Lin et

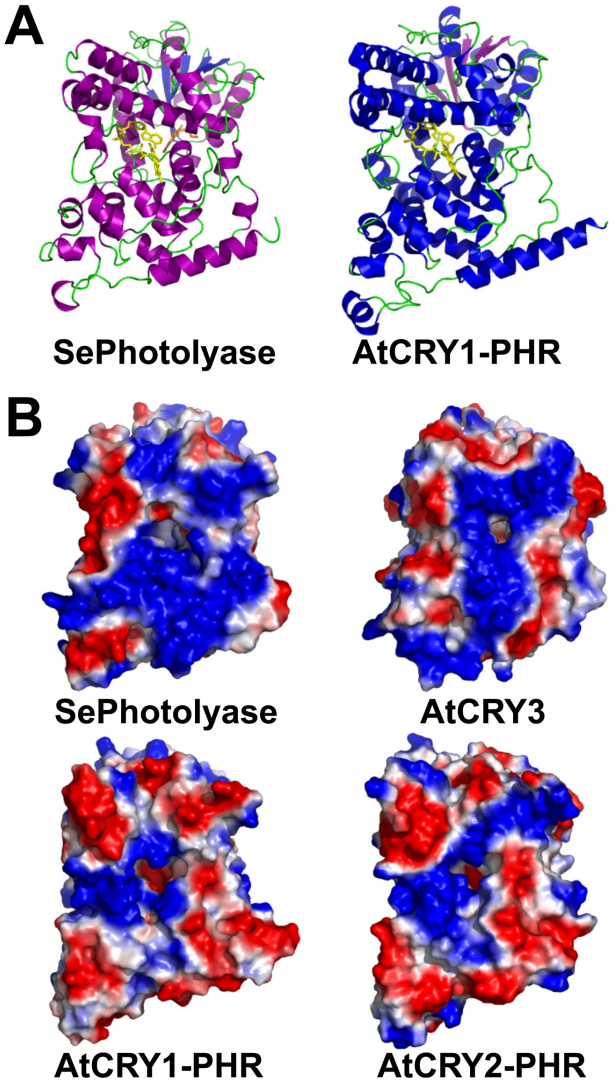

Figure 3. Structure and electrostatic surfaces of photolyase and cryptochromes

(A) Secondary structure of *Synechococcus elongatus* photolyase (PDB ID 1QNF) and the PHR domain of Arabidopsis CRY1 (PDB ID 1U3C). (B) Electrostatic surface potentials of *Se*Photolyase, Arabidopsis CRY-DASH/CRY3 (PDB ID 2IJG), Arabidopsis CRY1-PHR domain, and Arabidopsis CRY2 (predicted structure by homology modeling). Images were generated using Pymol (http://www.pymol.org).

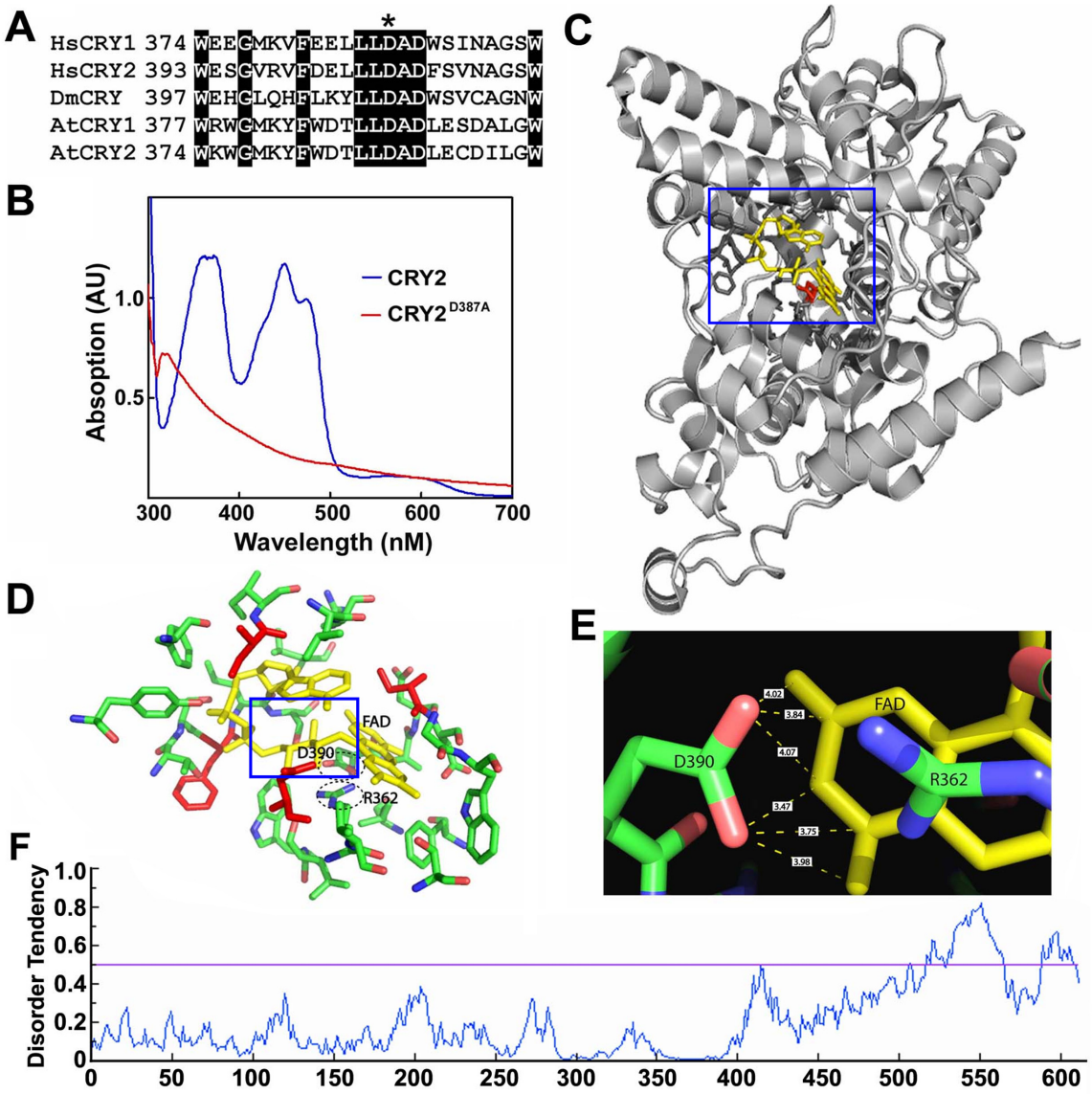

Figure 4. Structure features of Arabidopsis CRY1

(A) Sequence alignment of a conserved region of the FAD-binding pocket of human (HsCRY1 and HsCRY2), Drosophila (DmCRY), and Arabidopsis (AtCRY1 and AtCRY2) cryptochromes. Asterisk indicates D387 of CRY2 and equivalent residues in other cryptochromes. **(B)** Absorption spectrum of Arabidopsis CRY2 and CRY2^{D387A} mutant proteins, showing the lack of blue light absorption of the CRY2^{D387A} mutant protein (panels A and B are from Liu et al., 2008a). **(C)** The secondary structure of the PHR domain of Arabidopsis CRY1 (PDB ID 1U3C), showing the relative position of FAD (yellow) and the residue D390 (red) of CRY1 that is equivalent to D387 of CRY2. **(D)** Atomic structure of the region surrounding FAD and D390 of CRY1. **(E)** Distances (Å) between indicated atoms of D390 and FAD. **(F)** The local disorder tendency of the CCE domain of CRY2 based on an estimated-amino-acid-pairwise-energy-content analysis by IUPred (http://iupred.enzim.hu/), indicating intrinsically unstructured nature of the CCE domain of CRY2.

al., 1995b; Banerjee et al., 2007; Bouly et al., 2007). The lack of a clear MTHF-binding pocket in the crystallographic structure of CRY1-PHR domain is also not helpful to confirm the existence of the second chromophore in Arabidopsis CRY1 and CRY2 (Brautigam et al., 2004). A recent study analyzed the action spectrum of light-dependent degradation of CRY2 in Arabidopsis seedlings (Hoang et al., 2008a). It showed the peak at ~380nm of light, which falls in the absorption spectra of not only flavin but also folate. Moreover, it was reported that illumination of the CRY1-expressing insect cells with 380 nm light stimulated photoreduction of CRY1 expressed in the insect cells and this effect of UV-A light was suppressed by the folate synthesis inhibitor 2,4-diamino-6-hydroxyamino pyrimidine (DAHP) (Hoang et al., 2008a). However, an unequivocal proof of the existence and the molecular identity of the putative second chromophore may have to wait until a study of cryptochrome holoprotein purified from plants can be done.

The structure of the Arabidopsis CRY1-PHR domain has the following features (Brautigam, et al., 2004). (1), similar to photolyase and CRY-DASH, the CRY1-PHR is composed of two sub-domains: an N-terminal α/β sub-domain (residue 13-139) connected via a loop to the C-terminal α helical sub-domain (residues 217-495). The α/β domain has five-stranded parallel β sheet flanked by four α-helices and a 3_{10} helix (a less common helix with 3-residue per turn instead of 3.6-residue per turn in α helix), resembling a dinucleotide-binding domain. The C-terminal helical domain forms a FAD-access cavity (a flat face with a hole in the middle), whereby non-covalently bound FAD is buried. Similar to that of photolyase, FAD binds to CRY1-PHR in an unusual U-shaped conformation with the isoalloxazine and adenine rings in close proximity, which may facilitate intramolecular energy or electron transfer. (2), three tryptophan residues presumably involved in the intramolecular electron transfer from the protein surface to FAD are well conserved in CRY1-PHR. These three tryptophan residues have been hypothesized to conduct intramolecular electron transfer associated with photoexcitation of CRY1. (3), there is a possible disulfide bond formed between Cys-190 of the connector loop and Cys-80 of the α/β domain. This disulfide bond may potentially be involved in a light-dependent redox reaction and conformational changes of CRY1 upon photoexcitation. (4), the PHR domain of Arabidopsis CRY1 binds ATP. The adenine and ribose moieties of ATP penetrate into the FAD-access cavity, whereas the phosphates of ATP are found near the surface of the PHR domain where the CCE domain may interact (Brautigam, et al., 2004). This configuration is important for the blue light-dependent phosphorylation of cryptochromes as discussed later. The close proximity (4-5 Å) between atoms of FAD and ATP in CRY1-PHR would allow electron transfer from photoexcitated flavin to ATP, which may facilitate transfer of the surface-exposed phosphates from ATP to serine residues of the same or homodimerized cryptochromes. (5), the surface of CRY1-PHR is predominantly negatively-charged. This is in striking contrast to photolyases and CRY-DASH, which have a positively charged groove on their surface running through the FAD-access cavity (Figure 3). This difference of the electrostatic surface potential between CRY1 and photolyase may explain the different enzymatic activities between the two proteins and why CRY1 does not specifically bind and repair UV-damaged DNA. Moreover, the negative electrostatic potential of the surface of CRY1-PHR also argues that a phosphorylated and negatively charged CCE domain may be electrostatically repelled from the PHR domain to cause conformational changes upon photoexcitation (Yu et al., 2007a).

The CRY C-terminal Extension (CCE)

The carboxyl-terminal extension of Arabidopsis cryptochromes was previously called CCT (for Cryptochrome Carboxyl Terminus) (Yang et al., 2000). However, the same abbreviation CCT (for CO, CO-like, and TOC1) has also been used for a 45-residue motif common to CO, CO-like, and TOC1 proteins that are important to light-signaling and clock function (Putterill et al., 1995; Strayer et al., 2000)(Pfam motif accession PF06203). Therefore, we refer the cryptochrome c-terminal extension as CCE in this chapter to avoid confusion.

Plant and animal cryptochromes possess CCE's with clearly distinct sequences that do not seems to be evolutionarily related to other protein domains (Kanai et al., 1997; Cashmore et al., 1999; Lin and Shalitin, 2003; Sancar, 2003). It has been hypothesized that the photolyase/cryptochrome superfamily went through several rounds of gene duplication during evolution (Kanai et al., 1997; Todo, 1999). It is conceivable that duplicated copies in different evolutionary lineages might fuse to different sequences to become CCE domains of the progenitor cryptochromes in different lineages. Although the CCE domains of plant cryptochromes share no sequence similarity with the CCE domains of animal cryptochromes, plant cryptochromes from different species do share a common sequence motif DAS (DQXVP –Acidic-STAESSS) in their CCE's (Lin and Shalitin, 2003). Cryptochromes from liverwort, moss, and fern all possess various versions of the DAS motif (Lin and Shalitin, 2003). However, Chlamydomonas (*Chlamydomonas reinhardtii*), the single-cellular green alga presumably derived from the last common ancestor of the plant and animal lineages, has a plant-type cryptochrome (CPH1) (Lariguet and Dunand, 2005). CPH1 is 49% identical in the PHR domain to that of Arabidopsis CRY1, but it contains no DAS motif in the CCE domain (Small et al., 1995; Reisdorph and Small, 2004; Immeln et al., 2007). Some cryptochromes found in other algae also posses no DAS domain, and they appear more closely related to animal cryptochromes than to plant cryptochromes (Coesel et al., 2009; Heijde et al., 2010). These algae cryptochromes interact with CLOCK:BMAL heterodimers to suppress transcription, and some of them may act as dual DNA-repairing enzyme and transcription suppressors.

The CCE domain is important for the functions of cryptochromes in plants and animals, because mutations in this domain often impair the function of the mutant cryptochromes (Ahmad and Cashmore, 1993; Ahmad et al., 1995; Bruggemann et al., 1996; Rosato et al., 2001; Zhu et al., 2003; Busza et al., 2004; Chaves et al., 2006). The presence of a relatively conserved DAS motif in plant cryptochromes from moss, fern, to angiosperm suggests that cryptochromes probably came to existence before early evolution of land plants some 360-480 million years ago (Kenrick and Crane, 1997). Computational analyses of secondary structures of CCEs from Arabidopsis and human cryptochromes predict that this domain is intrinsically unstructured (Partch et al., 2005)(Figure 4F). The unstructured nature of the CCE domain of human CRY2 and Arabidopsis CRY1 has been confirmed, at least *in vitro*, by the circular dichroism and NMR analyses (Partch et al., 2005). Over one third of eukaryotic proteins contain intrinsically unstructured regions that may undergo induced folding or unfolding *in vivo* (Gsponer et al., 2008). The unstructured regions are found more frequently in signaling proteins. It was hypothesized that the unstructured regions of signaling proteins are important for protein-protein interactions, because they confer structural plasticity for interactions with multiple partners, more favorable energetic cost for high specificity/low affinity binding of the partner proteins, and accessible posttranslational modification sites often recognized by not only signaling partners but also regulatory proteins (Dyson and Wright, 2005; Gsponer et al., 2008). It is conceivable that the CCE domains of cryptochromes act as effector modules by undergoing light-induced folding or unfolding to alter their interaction with the PHR domain and to change the overall conformation of the photoreceptors.

Light-induced Conformational Changes

Light-induced conformational changes are instrumental for the signal transduction and regulation of all photoreceptors, which allow alteration of the photoreceptor with signaling partners or modifying enzymes in response to light. No significant conformational change was reported for the crystal structure of the PHR domain of Arabidopsis CRY1 (Brautigam et al., 2004). However, a disassociation between the PHR and CCE domains of a cryptochrome may represent an expected conformational change of cryptochromes as argued above, but such a change cannot be detected in the PHR domain alone (Yu et al., 2007a; Yu et al., 2009) (Figure 1A). A possible conformational change has also been examined using partial proteolysis assay *in vitro* (Partch et al., 2005). It was found that the trypsin sensitivity of the PHR domain of CRY1 was not altered by light. In contrast, the CCE domain of CRY1 increased its susceptibility to trypsin by 5-10 fold in response to light. The light-dependent conformation rearrangement was estimated to occur primarily in a small region of ~45 residues between amino acid 610 to 655 of CRY1, where the highly conserved STAESSS motif resides. The increased trypsin susceptibility suggests that specific regions of the CCE domain become disordered upon photoexcitation. Given a prediction that partially disordered structures favor certain types of protein-protein interactions, one might expect a light-induced protein-protein interaction to occur more likely in the CCE domain of a photoexcited cryptochrome. However, experimental evidence supporting this expectation is presently lacking. The protein-interaction sites of cryptochromes have been mapped for only three CRY-interacting proteins, phyA, COP1 and CIB1 (Ahmad et al., 1998c; Wang et al., 2001; Yang et al., 2001; Liu et al., 2008a). Both phyA and COP1 interact with the CCE domain of cryptochromes, but neither interaction appears to be light-dependent (Ahmad et al., 1998c; Wang et al., 2001; Yang et al., 2001). CIB1 is the only Arabidopsis protein that has been reported to interact with a cryptochrome in the blue light-dependent manner (Liu et al., 2008a). Interestingly, CIB1 appears to interact with the PHR domain but not the CCE domain of CRY2 (Liu and Lin, unpublished). In this regard, the CRY2-CIB1 interaction is reminiscent of the CRY-TIM interaction in Drosophila (Busza et al., 2004). Given that the PHR domain and the CCE domain of a cryptochrome physically interact with each other (Partch et al., 2005), photoexcitation at the PHR domain may alter this intramolecular interaction with the CCE domain to cause change of affinity of the PHR domain to a CRY-signaling partner, such as CIB1.

If CCE acts an effector domain, one would expect that overexpression of CCE would cause dominant phenotypic alterations. Indeed, transgenic plants overexpressing the CCE domains of CRY1 and CRY2 fused to the β-glucuronidase reporter enzyme (called GUS-CCT1 or GUS-CCT2) or part of the CCE domain (GUS-NC80) showed dominant positive or constitutive photomorphogenic phenotypes reminiscent to that observed in the *cop1* mutant (Deng et al., 1992; Yang et al., 2000; Yu et al., 2007a). For example, *cop1* and transgenic plants overexpressing GUS-CCT1, GUS-CCT2, or GUS-NC80 developed short hypocotyls, expanded cotyledons, chloroplast development, expression of light-induced genes when grown in the dark, and accelerated flowering in SD photoperiod. The CCE domain of CRY1 was found to physically interact with COP1 at the WD domain of COP1 (Wang et al., 2001; Yang et al., 2001). It was hypothesized that cryptochromes interact with COP1 to suppress its E3 ubiquitin ligase activity, resulting in altered cellular concentration of the COP1 substrates, such as HY5, to change transcription and plant development in response to light. However, given the lack of light dependence of the CRY-COP1 interaction, some yet-to-be-revealed mechanisms must be responsible to bring the light responsiveness for this mechanism as discussed later.

One hypothesis discussed previously argues that photoexcitation of a CRY by blue light induces phosphorylation of the CCE domain, resulting in electrostatic repelling of the CCE domain from the surface of the PHR domain, separation of the two domains, and altered interaction between CRY and its signaling partners (Yu et al., 2007a) (Figure 1A). Consistent with this hypothesis, it was recently shown that transgenic plants expressing CRY2-GFP, but not GFP-CRY2, exhibited constitutive photomorphogenic or the *cop* phenotype (Yu et al., 2009). It is conceivable that fusion of GFP to the C-terminus of CCE somehow "pull" CCE away from the PHR domain in the absence of light, resulting in light-independent disassociation of the PHR and CCE domain of the CRY2-GFP fusion protein. In other words, a separation of PHR-CCE intramolecular interaction of the CRY2-GFP fusion protein resembles the light-induced conformational change of the photoexcited CRY2 protein, allowing the CRY2-GFP fusion protein to be active in the absence of photoexcitation (Yu et al., 2009). One possible consequence of the proposed domain separation may be to expose regions of the CCE or the PHR domain for specific protein-protein interactions. However, this domain separation hypothesis remains to be tested directly.

CRYPTOCHROME PHOTOBIOCHEMISTRY

A photoreceptor that absorbs a photon is "photoexcited", by which an electron of the chromophore molecule is promoted from the ground state orbital to a higher energy orbital. The exact molecular changes resulting from photoexcitation of Arabidopsis cryptochrome is not clear at present. It has been proposed that the flavin chromophore of cryptochromes undergoes photoreduction, by which the oxidized FAD absorbs blue light to shift its redox equilibrium toward the semireduced flavin (neutral semiquinone radical $FADH^{\bullet}$ or anionic semiquinone radical $FAD^{\bullet-}$) (Figure 5). It was further hypothesized that the semireduced flavin is the signaling state, and it can be oxidized to the ground-state FAD in the dark to complete the photocycle (Banerjee et al., 2007; Bouly et al., 2007). However, whether photoreduction or a *bona fide* redox reaction is required for cryptochrome function *in vivo* is currently under debate, and a detailed discussion of this controversy will be presented elsewhere (Liu et al., 2010). Regardless the exact mechanism of photoexcitation, it seems a current consensus that the photoexcited cryptochromes undergo a series of biophysical and biochemical changes, such as structural rearrangement, protein modifications, protein-protein interactions, and/or additional signal transduction events that eventually lead to changes in gene expression, cellular physiology, and growth/developmental programs.

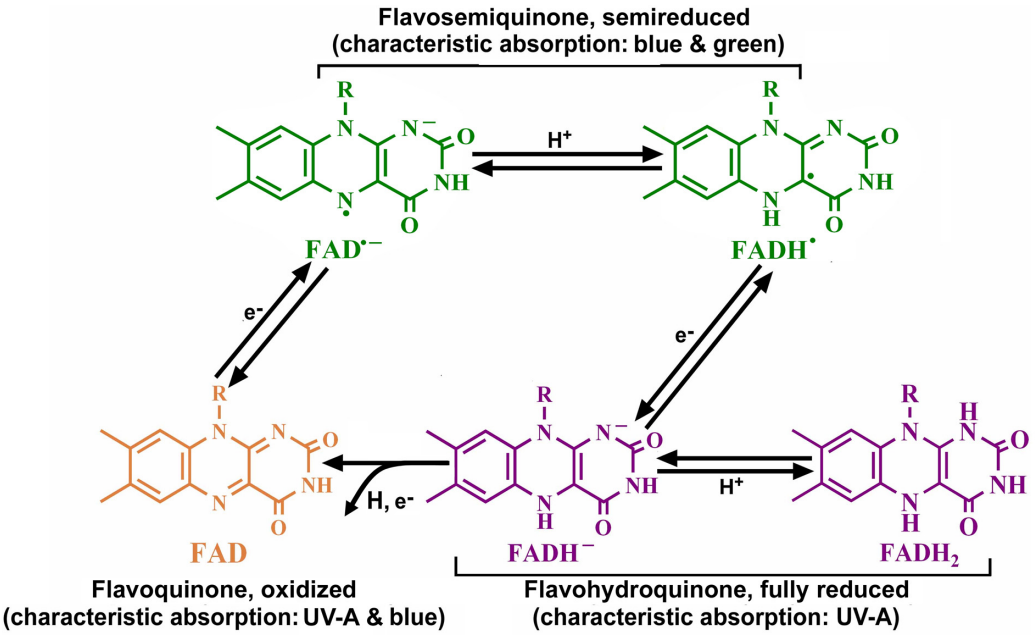

Figure 5. Oxidoreduction of flavins

Five possible redox forms of flavins are shown. R indicates different side groups in different flavins. The two different forms of semiquinone radicals: anion red radical (e.g. $FAD^{\bullet-}$) and neutral blue radical (e.g. $FADH^{\bullet}$), and two forms of reduced flavins: protonated hydroquinone (e.g. $FADH_2$) and anionic hydroquinone (e.g. $FADH^-$) are shown.

Blue Light-dependent Cryptochrome Phosphorylation

Arabidopsis CRY1 and CRY2 undergo phosphorylation in etiolated seedlings exposed to blue light (Shalitin et al., 2002; Shalitin et al., 2003). Cryptochrome phosphorylation is both blue light-specific and fluence rate-dependent. Red light or far-red lights do not trigger cryptochrome phosphorylation (Shalitin et al., 2002; Shalitin et al., 2003). Phosphorylated CRY2 can be detected in seedlings within 5 min after blue light illumination at a fluence rates as low as 5 μmole m^{-2} s^{-1}. The phosphorylated CRY1 or CRY2 protein can be detected as multiple bands in a SDS-PAGE gel, suggesting that cryptochrome phosphorylation occurs on multiple residues (Shalitin et al., 2002; Shalitin et al., 2003). Blue light-dependent phosphorylation of Arabidopsis cryptochromes positively correlates with the function of those photoreceptors in every case examined. For example, in a genetic screen, nine severe mis-sense *cry1* mutations were isolated from an EMS-mutagenized population. These nine *cry1* alleles affect 6 different residues (with some alleles isolated more than once). Those 6 mutant *cry1* proteins showed little blue light-dependent phosphorylation or physiological activity mediating blue light inhibition of hypocotyl elongation (Shalitin et al., 2003). In contrast, the transgenically expressed GUS-CCT2 fusion proteins that caused constitutive photomorphogenic phenotypes were constitutively phosphorylated in the transgenic plants (Yang et al., 2000; Shalitin et al., 2002). In another study of the function and regulation of CRY2 fusion proteins in transgenic plants, it was found that the GFP-CRY2 fusion protein exhibited both blue light-dependent physiological activity and blue light-dependent phosphorylation, whereas the CRY2-GFP fusion protein showed constitutive activity and constitutive phosphorylation (Yu et al., 2009). Blue light-dependent phosphorylation also correlates with the blue light-dependent CRY2 degradation. Partial suppression of CRY2 degradation in two weak alleles of the *cop1* mutant (*cop1-4* and *cop1-6*) correlates with the accumulation of phosphorylated CRY2 (Shalitin et al., 2002). Moreover, it was found that the CRY2-GR fusion protein that was retained in the cytosol (in the absence of dexamethasone) was not phosphorylated, nor physiologically active, and it was not degraded in response to blue light. Conversely, the same CRY2-GR fusion protein transported into the nucleus (in the presence of dexamethasone) was phosphorylated, physiologically active, and degraded in response to blue light in the transgenic plants (Yu et al., 2007b). Given that the protein structure, including chemical modifications such as phosphorylation, determines its biochemical behaviors, the results of those experiments appear to argue for a causal relationship between the blue light-dependent phosphorylation and physiological activity of a cryptochrome. It is not clear exactly how cryptochrome phosphorylation affects its activity. However, the negative charges introduced by phosphorylation on the CCE domain may disrupt the interaction between the PHR and CCE domains to affect the physical interactions between cryptochromes and the signaling partners (Yang et al., 2000; Yu et al., 2007a; Liu et al., 2008a; Burney et al., 2009; Yu et al., 2009). A direct test of this hypothesis will have to wait until the structure of a holocryptochrome is available.

In addition to the *in vivo* studies, cryptochrome phosphorylation has also been studied *in vitro*. It was found that serine residues are the major phosphorylation sites of CRY1 (Bouly et al., 2003). The most surprising finding was that Arabidopsis CRY1, which had no sequence relatedness to protein kinases, showed autophosphorylation activity *in vitro* (Bouly et al., 2003; Shalitin et al., 2003; Ozgur and Sancar, 2006). The *in vitro* autophosphorylation of CRY1 has been reported to be blue light-dependent, blue light-enhanced, or blue light-independent (Bouly et al., 2003; Shalitin et al., 2003; Ozgur and Sancar, 2006). These discrepancies about light dependence of the *in vitro* CRY1 phosphorylation may result from different experimental conditions or experimental artifacts. Nevertheless, it is clear that Arabidopsis CRY1 is capable of autophosphorylation *in vitro*. The Arabidopsis CRY1 bound to ATP has been recently reported to exhibit conformational changes, an increased level of semireduced flavin radicals in response to light, and decreased oxidation of flavin in the absence of light (Burney et al., 2009). These results are consistent with a hypothesis that CRY1 autophosphorylation may also occur *in vivo* and may be functionally important. The autophosphorylation activity has also been found for human and *Chlamydomonas* cryptochromes (Ozgur and Sancar, 2006; Immeln et al., 2007), although human cryptochromes are apparently phosphorylated by an AMP-dependent protein kinase that is a central regulator of metabolisms (Lamia et al., 2009). It is not clear whether CRY1 autophosphorylation is sufficient or additional protein kinases may be involved for its blue light-dependent phosphorylation. No autophosphorylation of CRY2 can be detected in the CRY2 protein expressed and purified from insect cells (Ozgur and Sancar, 2006)(Yu and Lin, unpublished), so it remains unclear whether CRY2 may possess autophosphorylation activity.

Blue Light-dependent CRY2 Degradation

One consequence of blue light-dependent phosphorylation of Arabidopsis CRY2 is its blue light-dependent proteolysis, via the ubuquitin/26S proteasome pathway (Yu et al., 2007b; Yu et al., 2009). It seems a reoccurring theme in the cellular signal transduction that activated signaling proteins may be rapidly degraded to bring the system back to the ground state. However, similar to phytochromes, for which phyA but not other phytochromes undergoes rapid degradation in red light, only CRY2 (but not CRY1) is degraded in blue light (Ahmad et al., 1998a; Lin et al., 1998). As expected, the blue light-dependent CRY2 degradation requires the flavin chromophore, because the $CRY2^{D387A}$ mutant protein that fails to bind FAD no longer undergoes blue light-dependent degradation (Liu et al., 2008a). CRY2 degradation has been reported to be partially attenuated by concurrent exposure to blue light and green light or a short, single pulse of green light following blue light exposure, suggesting that the semiquinone CRY2 is more prone to degradation (Banerjee et al., 2007; Bouly et al., 2007). CRY2 degradation is a high irradiance response that requires continuous blue light illumination (Busza et al., 2004; Yu et al., 2007b). It is interesting to notice that although CRY2 is rapidly degraded to almost completion in etiolated seedlings exposed to blue light for a few hours and that the level of CRY2 did not re-accumulate to the that of etiolated seedlings after the light-treated seedlings were returned to dark for two days, abundant CRY2 protein was detected in seedlings grown in continuous blue light (Yu et al., 2007b). This observation suggests a very different kinetics of the light-dependent CRY2 degradation in cells of etiolated seedlings exposed to blue light and that of seedlings grown under continuous blue light. The underlying mechanism of this phenomenon and its biological implication are not clear. Consistent with the hypothesis that phosphorylation is required for CRY2 ubiquitination and degradation, the constitutively phosphorylated CRY2-GFP fusion protein is more readily observed in the nuclear bodies where degradation presumably takes place (Yu et al., 2009). It seems that both the PHR domain and the CCE domain are required for the blue light-dependent degradation of CRY2 (Ahmad et al., 1998a). Consistent with this notion, another constitutively phosphorylated GUS-CCT2 fusion protein is no longer degraded in response to blue light (Shalitin et al., 2002; Yu et al., 2007b; Yu et al., 2009). The E3 ubiquitin ligase(s) responsible for CRY2 degradation has not been all identified. Although CRY2 degradation is partially suppressed in the *cop1* weak mutant alleles (*cop1-4* and *cop1-6*), CRY2 still undergoes blue light-dependent degradation in the *cop1* null allele (*cop1-5*) (Shalitin et al., 2002)(Liu and Lin, unpublished). This result indicates the involvement of at least another unknown E3 ubiquitin ligase in the blue light-dependent CRY2 degradation.

CRY-INTERACTING PROTEINS AND CRYPTOCHROME SIGNAL TRANSDUCTION

The signal transduction mechanism of Arabidopsis cryptochromes remains not fully understood, although it is generally agreed that cryptochromes interact with signaling proteins to regulate gene expression (Cashmore, 2003; Lin and Shalitin, 2003; Partch and Sancar, 2005b; Jiao et al., 2007) (Figure 1A). According to the results of several mRNA profile analyses by DNA microarrays, approximately 5-25% of genes in the Arabidopsis genome change their expression in response to blue light, most of those blue light-regulated gene expression changes are mediated by CRY1 and CRY2. The expression of many of the CRY-regulated genes are also regulated by other signaling pathways such as phytochromes and phytohormones (Ma et al., 2001; Folta et al., 2003; Ohgishi et al., 2004; Sellaro et al., 2009)(Shalitin and Lin, unpublished), suggesting that the cryptochrome-dependent photomorphogenesis is involved with extensive gene expression regulatory networks. Arabidopsis cryptochromes appear to regulate gene expression by both transcriptional and posttranslational mechanisms (Figure 6).

Although cryptochrome, the role of cryptochromes in the circadian clock, and some common biochemical characteristics of cryptochrome such as cryptochrome phosphorylation and degradation were first discovered in Arabidopsis (Ahmad and Cashmore, 1993; Guo et al., 1998; Lin et al., 1998; Somers et al., 1998; Shalitin et al., 2002), the signaling mechanism of cryptochromes is currently not as well understood in Arabidopsis as that in animal models such as *Drosophila* and mouse (Young and Kay, 2001; Reppert and Weaver, 2002; Partch and Sancar, 2005a). In *Drosophila*, dCRY physically interacts with the PAS-domain proteins TIMELESS (TIM) and PERIOD (PER), whereas in mouse, mCRY1 and mCRY2 physically interact with the bHLH-domain proteins CLOCK and BMAL, as well as with the mammalian TIM

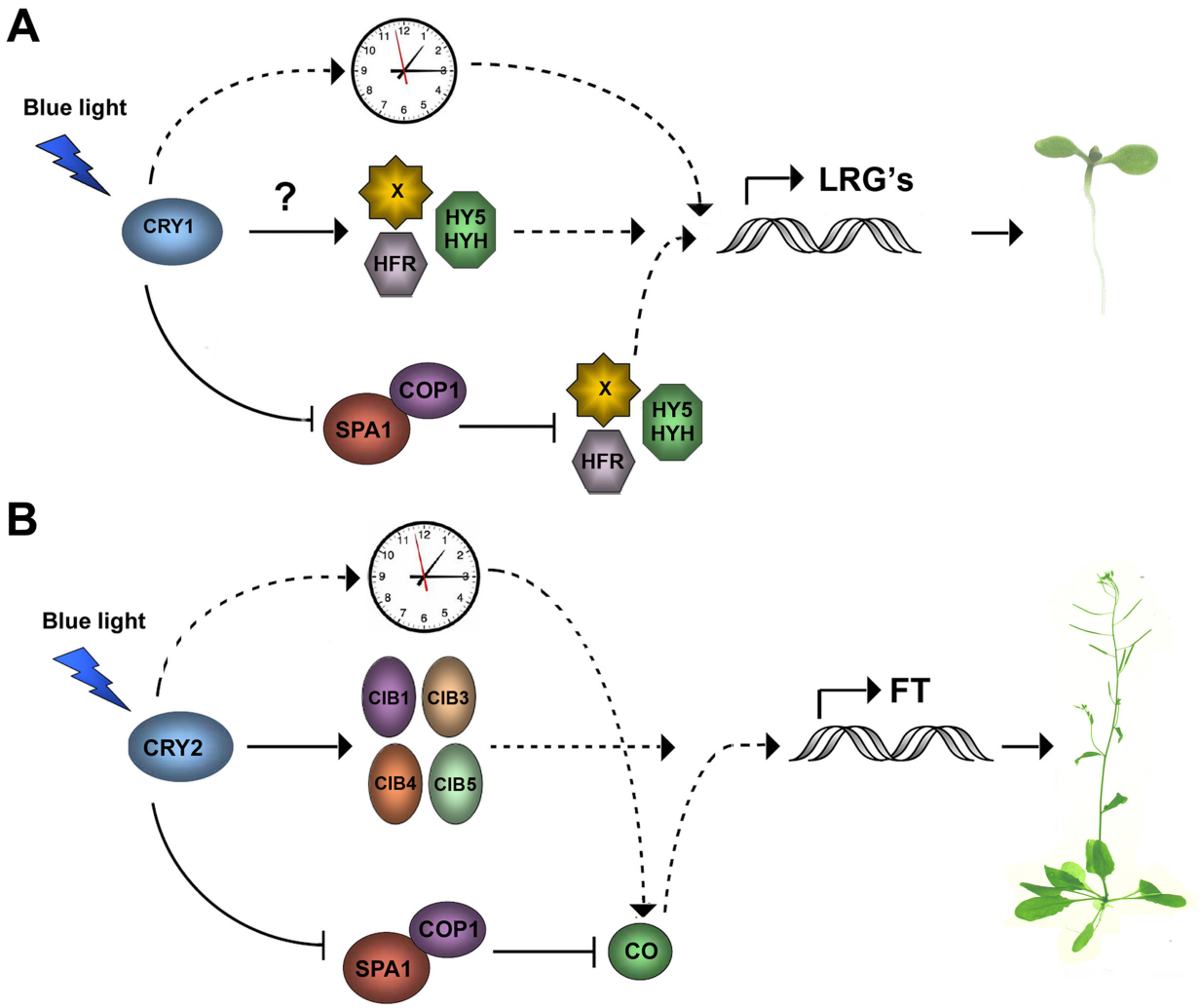

Figure 6. Working models of the signal transduction mechanism of Arabidopsis cryptochromes

Three possible signaling pathways by which CRY1 **(A)** or CRY2 **(B)** may regulate gene expression changes and developments of plants in response to blue light are depicted. **(A)** CRY1 mediates blue light suppression of the COP1-dependent degradation of known (HY5, HYH, HFR1) or unknown (X) transcription regulators to affect expression of light-regulated genes (LRG's). CRY1 may also directly or indirectly interact with unknown (X) transcription regulators. In addition, CRY1 mediates light entrainment of the circadian clock via unknown mechanisms to gate light control of LRG's. **(B)** CRY2 undergoes blue light-dependent interaction with the CIB transcription factors to directly stimulate transcription of the target gene *FT*. In addition, CRY2 also interact with COP1-interacting proteins to suppress COP1-dependent degradation of the CO protein that is a positive regulator of *FT* transcription.

and PER proteins (Ceriani et al., 1999; Griffin et al., 1999; Kume et al., 1999; Rosato et al., 2001). In *Drosophila*, the CLK/CYC complex acts as the transcription activator, whereas the CRY//TIM/PER complex acts as the transcriptional repressor. In mammals, the CLOCK/BMAL1 complex acts as the transcription activator, whereas the CRY/PER complex acts as the transcriptional repressor. Therefore, physical interactions between cryptochromes and transcription regulators directly affect transcription and the activity of the central oscillator complexes in the respective animals. In *Drosophila*, the dCRY-TIM/PER interaction is dependent on blue light (Ceriani et al., 1999; Busza et al., 2004; Peschel et al., 2009), which is consistent with dCRY being a photoreceptor regulating the circadian clock in this animal. In mouse, mCRY-CLOCK/BMAL or mCRY-PER interactions are not dependent on light in transient expression assays using cultured mammalian cells. This observation is also consistent with the light-independent activity of mCRY regulating transcription and the circadian clock in those cells (Griffin et al., 1999; Kume et al., 1999; van der Horst et al., 1999). It remains unclear whether mCRY-CLOCK/BMAL or mCRY-PER interaction is affected by light in the retinal ganglion cells, whereby mCRYs might act as the photoreceptors (Thresher et al., 1998; Selby et al., 2000). In addition to CRY-

interacting proteins directly associated with CRY-dependent transcription regulation, enzymes required for the regulation of cryptochrome activity, such as protein kinases and ubiquitin ligases, have also been identified in *Drosophila* and/or mouse (Eide et al., 2002; Sanada et al., 2004; Harada et al., 2005; Busino et al., 2007; Godinho et al., 2007; Siepka et al., 2007; Lamia et al., 2009; Peschel et al., 2009).

Cryptochromes in different evolutionary lineages may have evolved independently from DNA photolyases (Kanai et al., 1997; Todo, 1999), implying that cryptochromes in plants and animals may have different signaling mechanisms. However, protein-protein interactions are involved in early steps of almost all cellular signal transduction processes, and a light-dependent protein-protein interaction seems a relatively "simple" mechanism that might have occurred multiple times during evolution for cryptochromes. It is intuitive to contemplate that the key to understand the cryptochrome signaling mechanism in Arabidopsis may lie in the identification and characterization of CRY-interacting proteins, especially those that interact with cryptochromes in a blue light-dependent manner or those that change their biochemical activities upon interacting with cryptochromes. In the last decade, several CRY-interacting proteins have been identified and studied in Arabidopsis, contributing to our current understanding of cryptochrome signal transduction mechanisms. This last section attempts to discuss those findings.

Phytochromes

It was previously suggested that phytochromes may be required to amplify the action or signals of cryptochromes (Mohr, 1994), but the results of a recent study of the *phyABCDE* quintuple mutant demonstrate that cryptochromes can function in the absence of phytochromes in Arabidopsis (Strasser et al., 2010). Therefore, phytochromes are most likely involved in the modulation, instead of determination, of the functions of cryptochromes. Phytochrome A (phyA) is the first CRY-interacting protein reported in Arabidopsis (Ahmad et al., 1998c). It was found that phyA interacted with CRY1 in the yeast two-hybrid (Y2H) assay. The CRY1-phyA interaction is independent from blue light. A functional interaction between Arabidopsis phytochromes and cryptochromes has been reported in various light responses such as the control of floral initiation (Ahmad et al., 1998c; Guo et al., 1998), and it was postulated that phyA-cryptochrome interaction might be more relevant to the photoreceptor control of flowering time than to the photoreceptor control of de-etiolation (Ahmad et al., 1998c). It was also reported that phyA phosphorylated CRY1 and CRY2 *in vitro* at the CCE domain of cryptochromes (Ahmad et al., 1998c). The *in vitro* phyA phosphorylation of cryptochromes is dependent on ATP, it occurs in both blue and red light, but it is reversed by FR light (Ahmad et al., 1998c). However, none of the phytochrome mutants tested, including phyA, affected blue light-dependent cryptochrome phosphorylation *in vivo* (Shalitin et al., 2002; Shalitin et al., 2003). Furthermore, because phyA phosphorylates CRY1 in both blue light and red light *in vitro*, whereas the cryptochrome phosphorylation and function *in vivo* are blue light-specific, the reported phyA activity discussed above does not seem to be physiologically relevant to the phosphorylation and function of CRY1 *in vivo*.

phyB is another phytochrome known to both functionally and physically interact with an Arabidopsis cryptochrome. Because the *cry2* mutant flowers later in LD under white light or blue-plus-red light, but not under monochromatic blue or red light, and because the late-flowering phenotype of the *cry2* mutant is epistatic to the early-flowering phenotype of the phyB mutant, the two photoreceptors may antagonize the function of each other to optimize the flowering time in plants (Guo et al., 1998; Mockler et al., 1999). It was found that, somewhat analogous to its flowering phenotype, the *cry2* mutant also showed a more pronounced defect in maintaining the circadian rhythm of transcription under white light than under monochromatic blue or red light, which may be interpreted as co-action of the two photoreceptors (Mas et al., 2000). A direct physical interaction between phyB and CRY2 may partially explain the antagonizing functions of CRY2 and phyB. CRY2 and phyB were detected in the same protein complex in a co-immunoprecipitation experiment; CRY2 was shown to physically interacted with phyB in plant cells by the FRET (fluorescent resonance energy transfer) analyses. Moreover, it was demonstrated that CRY2-RFP and CRY2-GFP fusion proteins "migrate" into "nuclear speckles" (also called nuclear bodies) in response to blue light, and that some (but not all) of the CRY2- RFP nuclear bodies co-localized with the phyB nuclear-bodies (Yamaguchi et al., 1999; Gil et al., 2000; Mas et al., 2000; Yu et al., 2009). These results are consistent with that the photoexcited CRY2 physically interacting with phyB. Several hypotheses may be proposed to explain how the physical interaction between CRY2 and phyB could affect CRY2 function and/or regulation. For example, CRY2-phyB interaction may alter the way CRY2 interacts with other signaling partners or light modification of CRY2. It is also conceivable that phyB may affect CRY2 degradation in the nuclear bodies (Yu et al., 2009), resulting in altered diurnal rhythms of the abundance of CRY2 protein and flowering time in white light. Alternatively, phyB may affect CRY1-COP1 interaction to alter transcription regulation and hypocotyl growth (Yang et al., 2001; Strasser et al., 2010). These hypotheses, however, remain to be further tested.

The LOV-domain F-box Protein ZTL

There are two types of LOV-domain blue light receptors, the blue light-dependent protein kinases phototropins (phot1 and phot2) that control various movement responses in plants (Huala et al., 1997; Christie et al., 1998; Christie et al., 1999; Briggs et al., 2001; Kagawa et al., 2001; Kinoshita et al., 2001), and the F-box/Kelch-repeat proteins ZTL (ZEITLUPE), FKF (FLAVIN-BINDING, KELCH REPEAT, F-BOX 1), and LKP2 (LOV KELCH REPEAT PROTEIN 2), that regulate proteasome-dependent degradation of proteins associated with the circadian clock (Nelson et al., 2000; Somers et al., 2000; Imaizumi et al., 2003; Mas et al., 2003; Imaizumi et al., 2005; Kim et al., 2007; Sawa et al., 2007). Cryptochromes and phototropins mediate distinct blue light responses (although they may affect each other in some minor ways), and there seems no direct association between the signal transductions of these two types of blue light receptors. On the other hand, cryptochromes and the F-box LOV proteins control related physiological responses, such as the circadian clock and flowering time (Guo et al., 1998; Somers et al., 1998; Nelson et al., 2000; Somers et al., 2000; Imaizumi et al., 2003). FKF interacts with

CDF1 (CYCLING DOF FACTOR1) and acts as an E3 ubiquitin ligase to promote CDF1 degradation (Imaizumi et al., 2005). ZTL also interacts with the clock proteins TOC1 (TIMING OF CAB EXPRESSION 1) and PRR5 (PSEUDORESPONSE REGULATOR 5) to promote their degradation (Mas et al., 2003; Fujiwara et al., 2008). FKF1 and ZTL both undergo blue light-dependent interaction with GI (GIGANTEA). But in this case, the GI-FKF1/ZTL interaction results in mutual stabilization of GI and the photoreceptor proteins in a circadian time-dependent manner (Kim et al., 2007; Sawa et al., 2007). Interestingly, it has been reported that ZTL (also called ADO1 or LKP1) physically interacts with CRY1 and phyB in yeast two-hybrid and *in vitro* pull-down assays (Jarillo et al., 2001). It remains unclear what biochemical consequence may result from these interactions. Given the closely related physiological functions of these three types of photoreceptors in the control of plant growth and development, it is an interesting question whether and how such complex photoreceptor interactions may affect the signal transduction of cryptochromes, as well as other photoreceptors.

The E3 Ubiquitin Ligase COP1

COP1 is another E3 ubiquitin ligase extensively involved in photomorphogenic responses and photoreceptor functions (Deng et al., 1989; Deng et al., 1992). Loss-of-function *cop1* mutants showed constitutive photomorphogenic phenotype. The *cop1* mutant seedlings germinated and grown in the dark develop short hypocotyls and expanded cotyledons, increased anthocyanin accumulation, and mis-expression of light-induced genes in the absence of light. It seems clear that cryptochromes mediate light suppression of COP1-dependent degradation of multiple transcriptional regulators to affect gene expression (Figure 6). A possible involvement of COP1 in cryptochrome signal transduction was initially suggested by an experiment in which transgenic plants overexpressing GUS-CCT1 and GUS-CCT2 fusion proteins showed the "*cop*" (constitutive photomorphogenic) phenotype. Namely, transgenic seedlings grown in the dark developed short hypocotyls, expanded cotyledons, accumulation of pigments, and ectopic gene expressions (Yang et al., 2000). The similar phenotypic alterations of the *cop1* loss-of-function mutants and transgenic plants overexpressing GUS-CCT1 or GUS-CCT2 suggest a functional relationship between COP1 and CRY proteins. Indeed, it was reported that the CCT (i.e. CCE) domains of CRY1 and CRY2 physically interact with COP1 in yeast two-hybrid assays, that CRY2 was co-immunoprecipitated with COP1 from Arabidopsis plants, and that GFP-CCT1 co-migrated into nuclear bodies with COP1 in plant cells (Wang et al., 2001). Importantly, over 80% of the gene expression changes profiled by the DNA microarrays are similar in the *cop1* mutant and the transgenic plants overexpressing GUS-CCT1 or GUS-CCT2 (Wang et al., 2001), which is consistent with the hypothetic mode of action suggested by the similar phenotypic alterations of the two genotypes. It has been established that COP1 mediates light-dependent degradation of many photo-signaling proteins, including HY5/HYH (Ang et al., 1998; Hardtke et al., 2000; Osterlund et al., 2000; Saijo et al., 2003), LAF1 (Seo et al., 2003), HFR1 (Duek et al., 2004; Jang et al., 2005), CRY2 (Shalitin et al., 2002), phyA (Seo et al., 2004; Saijo et al., 2008), CO (Laubinger et al., 2006; Jang et al., 2008; Liu et al., 2008b), GI (Yu et al., 2008), etc. and it was hypothesized that photoexcited cryptochromes may interact, directly or indirectly, with COP1 to suppress its E3 ubiquitin ligase activity, resulting in alterations of the cellular level of those COP1 substrates, changes of expression of genes regulated by the substrates of COP1 (often transcription factors), and eventually changes of plant development (Yang et al., 2000; Wang et al., 2001; Yang et al., 2001). This model is straightforward and consistent with numerous genetic and transgenic studies. On the other hand, the action mechanism associated with cryptochromes and COP1 is apparently more complex and there are a number of questions remaining to be answered. For example, it is not clear why dimerization is required for the activity of the CCE domain of cryptochromes (e.g. GUS-CCT1 and GUS-CCT2) but not for their interaction with COP1. CRY1 and CRY2 form homodimers *in vivo* via the PHR domain, and dimerization is required for the dominant positive (or *cop*) activity of the CCT/CCE domains expressed *in vivo* (Sang et al., 2005; Yu et al., 2007a; Rosenfeldt et al., 2008). It is puzzling that the CCT/CCE domains alone showed similar activity as the full-length cryptochromes or GUS-CCT in their interaction with COP1 in yeast cells or *in vitro* (Yang et al., 2000; Wang et al., 2001; Sang et al., 2005; Yu et al., 2007a). More importantly, given that COP1 interacts with CRY1 or CRY2 in a light-independent manner (Wang et al., 2001; Yang et al., 2001; Liu et al., 2008a), it becomes critical to understand how cryptochromes mediate light-dependent suppression of the COP1 activity. At least two mechanisms may confer CRY- and light-dependent regulation of COP1. First, cryptochromes may alter the biochemical activity, or nuclear/cytoplasmic distribution of COP1 in an unknown but light-dependent manner (Von Arnim and Deng, 1994). Alternatively, CRY may undergo a light-dependent interaction with COP1-interacting proteins, such as ELF3 (EARLY FLOWERING 3) or SPA1 (SUPPRESSOR OF PHYA) (Hoecker and Quail, 2001; Saijo et al., 2003; Laubinger et al., 2006; Yu et al., 2008), to affect the COP1 activity. Among those proteins, SPA1, which is the dominant member of the 4-member gene family encoding WD-repeat proteins (Hoecker et al., 1999; Laubinger et al., 2004), is particularly interesting, because SPA1 is known to physically interact with COP1 and to act as a positive regulator of COP1 (Hoecker and Quail, 2001; Saijo et al., 2003; Saijo et al., 2008; Zhu et al., 2008a). We have recently found that CRY1 and CRY2 physically interact with SPA1 in yeast cells and in plant cells in a blue light-dependent manner (Liu, Zuo and Lin, unpublished results). Therefore, one mechanism of cryptochrome signal transduction seems to be that photoexcited cryptochromes interact with SPA1 to somehow suppress COP1-dependent degradation of transcriptional regulators, resulting in genome-wide changes of transcription in response to blue light.

The CIB Transcription Factors

In addition to the posttranslational mechanisms, cryptochromes also regulate transcription directly (Figure 6). A major hypothesis underlying the signaling mechanism of Arabidopsis cryptochromes argues that cryptochromes in the ground state have a "closed" conformation that has relatively lower affinity to the relevant signaling proteins; whereas photoexcited cryptochromes adopt an "open" conformation, allowing high affinity interactions

with signaling proteins to affect gene expression (Yu et al., 2007a) (Figure 1A). The first blue light-specific CRY-interacting protein has been reported recently (Liu et al., 2008a). In this study, a bHLH protein called CIB1 (CRY-interacting bHLH 1) was identified by a blue light-differentiated yeast-two-hybrid screen. In yeast cells, CIB1 interacts with CRY2 in a wavelength-specific, fluence rate-dependent, and FAD-dependent manner (Liu et al., 2008a). In Arabidopsis cells, a significant amount of CIB1 is detected in the CRY2 complex in seedlings exposed to blue light, but little CIB1-CRY2 complex was detected in seedlings exposed to red light. Arabidopsis CIB1 binds to the G-box (CACGTG) with the highest affinity *in vitro*, but it appears to affect transcription associated with both G-box and E-box (CANNTG) *in vivo*. It was shown in a transient Arabidopsis transcription assay that CIB1 acted as a CRY- and blue light-dependent transcription regulator. However, the *in vivo* transcriptional regulation activity of CIB1 seems indiscriminatory toward G- and E-boxes, suggesting a significant difference of the CIB1 DNA-binding activity *in vitro* and its transcription regulatory activity *in vivo*. One possible interpretation of this dilemma would be that CIB1 heterodimerizes with other bHLH proteins to alter their preference or affinity to different DNA sequences *in vivo*. Indeed, at least four CIB1-related proteins (CIB1, CIB3, CIB4, and CIB5) were found to interact with CIB1 and/or CRY2, and some of them could potentially heterodimerize with CIB1 to affect CRY2-regulated gene expression changes (Figure 6). Consistent with this proposition, the monogenic *cib1* mutant shows no phenotypic alterations when grown under laboratory conditions, whereas *cib1cib5* double mutant flower slightly later than the wild type plants. Overexpression of CIB1 results in accelerated flowering in the wild-type background but not in the *cry1cry2* mutant background, demonstrating that the floral promotion activity of CIB1 is dependent on cryptochromes. The CIB1 promoter is active in all organs and cell types examined, including vascular bundle cells where CRY2 regulates *FT* expression and floral initiation (Liu et al., 2008a). Therefore, CIB proteins may be involved in other cryptochrome-mediated light responses, although they do not seem to play a major role in de-etiolation (Liu and Lin, unpublished).

Although the blue light-dependent CIB1-CRY2 interaction argues strongly that a direct interaction between the cryptochrome and transcription factors represents an important mechanism for the cryptochrome signal transduction, several questions remain. First, although the current data suggest that CIB1 interacts with the E box of the *FT* genomic DNA to regulate its transcription, the exact mechanism how CIB1 regulates transcription is unknown. For example, exactly what DNA sequence CIB1 binds to in the *FT* genomic DNA, how the DNA binding or transcriptional regulatory activity of CIB1 is affected by blue light, CRY2, and CIB1-related bHLH proteins remain to be further elucidated. Second, almost all photoreceptor signaling proteins are themselves regulated by light, via various mechanisms such as RNA expression, cellular compartmentation, protein modification, or protein degradation. A blue light-specific reaction for CIB1 other than its interaction with CRY2 has yet to be reported. Third, how the multiple mechanisms associated with cryptochrome regulation of transcription of a target gene may be integrated in plant cells is not clear at present. For example, it is known that CRY2 mediates blue light suppression of the COP1-dependent degradation of CO that activates *FT* transcription (Yanovsky and Kay, 2002; Valverde et al., 2004). In the mean time, CRY2 also interacts with CIB1 to directly activate the *FT* gene (Jang et al., 2008; Liu et al., 2008b). It seems an evolutionary advantage to plants if multiple pathways regulating the same target are physically integrated in the cell. But whether and how different mechanisms associated with the transcriptional and posttranslational regulatory activities of cryptochromes are physically integrated remain to be further investigated.

PERSPECTIVES

Since identification of the first cryptochrome in 1993, significant progress has been made in our understanding of this group of photosensory receptors in plants, including their structure, function, regulation, and the mechanisms of photoexcitation and signal transduction. However, many questions about Arabidopsis cryptochromes still remain to be answered. For example, is the redox reaction *per se* required for the initial photoexcitation of cryptochromes? What is the physiological function of CRY3? How can a holocryptochrome be crystallized to solve its structure and to understand the mechanism of conformational changes of a cryptochrome in response to light? How do CRY1 and CRY2 suppress COP1 activity in response to blue light? What are the protein kinases, phosphotases, and the E3 ubiquitin ligases responsible for blue light-dependent cryptochrome phosphorylation/dephosphorylation and ubiqutination? How do cryptochromes in different cells mediate cell-specific light responses? Continuing investigation of these and other problems will undoubtedly shed more light on this group of light sensors and photomorphogenesis in general.

ACKNOWLEDGEMENT

This work is supported in part by National Institute of Health (GM56265 to CL). The authors thank Dr. Elaine Tobin for critical readings of the manuscript, Hongyun Yang for the result of experiments shown in Figure 2, and Kao Ya-Ting for the preparation of Figure 5.

REFERENCES

Ahmad, M., and Cashmore, A.R. (1993). HY4 gene of *A. thaliana* encodes a protein with characteristics of a blue-light photoreceptor. Nature **366,** 162-166.

Ahmad, M., Lin, C., and Cashmore, A.R. (1995). Mutations throughout an Arabidopsis blue-light photoreceptor impair blue-light-responsive anthocyanin accumulation and inhibition of hypocotyl elongation. Plant J **8,** 653-658.

Ahmad, M., Jarillo, J.A., and Cashmore, A.R. (1998a). Chimeric proteins between cry1 and cry2 arabidopsis blue light photoreceptors indicate overlapping functions and varying protein stability. Plant Cell **10,** 197-208.

Ahmad, M., Jarillo, J.A., Smirnova, O., and Cashmore, A.R. (1998b). Cryptochrome blue-light photoreceptors of Arabidopsis implicated in phototropism. Nature **392,** 720-723.

Ahmad, M., Jarillo, J.A., Smirnova, O., and Cashmore, A.R. (1998c). The CRY1 blue light photoreceptor of Arabidopsis interacts with phytochrome A in vitro. Mol Cell **1,** 939-948.

Ahmad, M., Galland, P., Ritz, T., Wiltschko, R., and Wiltschko, W. (2007).

Magnetic intensity affects cryptochrome-dependent responses in Arabidopsis thaliana. Planta **225,** 615-624.

Ahmad, M., Jarillo, J.A., Klimczak, L.J., Landry, L.G., Peng, T., Last, R.L., and Cashmore, A.R. (1997). An enzyme similar to animal type II photolyases mediates photoreactivation in Arabidopsis. Plant Cell **9,** 199-207.

Ahmad, M., Grancher, N., Heil, M., Black, R.C., Giovani, B., Galland, P., and Lardemer, D. (2002). Action spectrum for cryptochrome-dependent hypocotyl growth inhibition in Arabidopsis. Plant Physiol **129,** 774-785.

Alonso-Blanco, C., El-Assal, S.E., Coupland, G., and Koornneef, M. (1998). Analysis of natural allelic variation at flowering time loci in the Landsberg erecta and Cape Verde Islands ecotypes of Arabidopsis thaliana. Genetics **149,** 749-764.

Alvarez-Buylla, E.R., Benítez, M., Corvera-Poiré, A., Cador, Á.C., de Folter, S., de Buen, A.G., Garay-Arroyo, A., García-Ponce, B., Jaimes-Miranda, F., Pérez-Ruiz, R.V., Piñeyro-Nelson, A., and Sánchez-Corralesa, Y.E. (2010) Flower Development. In: The Arabidopsis Book. The American Society of Plant Biologists, Rockville, MD., doi:10.1199/tab.0127, http://www.aspb.org/publications/arabidopsis/.

An, H., Roussot, C., Suarez-Lopez, P., Corbesier, L., Vincent, C., Pineiro, M., Hepworth, S., Mouradov, A., Justin, S., Turnbull, C., and Coupland, G. (2004). CONSTANS acts in the phloem to regulate a systemic signal that induces photoperiodic flowering of Arabidopsis. Development **131,** 3615-3626.

Ang, L.H., and Deng, X.W. (1994). Regulatory hierarchy of photomorphogenic loci: allele-specific and light-dependent interaction between the HY5 and COP1 loci. Plant Cell **6,** 613-628.

Ang, L.H., Chattopadhyay, S., Wei, N., Oyama, T., Okada, K., Batschauer, A., and Deng, X.W. (1998). Molecular interaction between COP1 and HY5 defines a regulatory switch for light control of Arabidopsis development. Mol Cell **1,** 213-222.

Bagnall, D.J., King, R.W., and Hangarter, R.P. (1996). Blue-light promotion of flowering is absent in hy4 mutants of Arabidopsis. Planta **200,** 278-280.

Banerjee, R., Schleicher, E., Meier, S., Munoz Viana, R., Pokorny, R., Ahmad, M., Bittl, R., and Batschauer, A. (2007). The signaling state of Arabidopsis cryptochrome 2 contains flavin semiquinone. J Biol Chem **282,** 14916-14922.

Barneche, F., Winter, V., Crevecoeur, M., and Rochaix, J.D. (2006). ATAB2 is a novel factor in the signalling pathway of light-controlled synthesis of photosystem proteins. Embo J **25,** 5907-5918.

Bayram, O., Biesemann, C., Krappmann, S., Galland, P., and Braus, G.H. (2008). More Than a Repair Enzyme: Aspergillus nidulans Photolyase-like CryA Is a Regulator of Sexual Development. Mol Biol Cell **19,** 3254-3262.

Bergmann, D.C., and Sack, F.D. (2007). Stomatal development. Annu Rev Plant Biol **58,** 163-181.

Berndt, A., Kottke, T., Breitkreuz, H., Dvorsky, R., Hennig, S., Alexander, M., and Wolf, E. (2007). A novel photoreaction mechanism for the circadian blue light photoreceptor Drosophila cryptochrome. J Biol Chem **282,** 13011-13021.

Blazquez, M.A., Ahn, J.H., and Weigel, D. (2003). A thermosensory pathway controlling flowering time in Arabidopsis thaliana. Nat Genet **33,** 168-171.

Blum, D.E., Neff, M.M., and Van Volkenburgh, E. (1994). Light-stimulated cotyledon expansion in *blu3* and *hy4* mutants of *Arabidopsis thaliana*. Plant Physiol. **105,** 1433-1436.

Botto, J.F., Alonso-Blanco, C., Garzaron, I., Sanchez, R.A., and Casal, J.J. (2003). The cape verde islands allele of cryptochrome 2 enhances cotyledon unfolding in the absence of blue light in Arabidopsis. Plant Physiol **133,** 1547-1556.

Bouly, J.P., Giovani, B., Djamei, A., Mueller, M., Zeugner, A., Dudkin, E.A., Batschauer, A., and Ahmad, M. (2003). Novel ATP-binding and autophosphorylation activity associated with Arabidopsis and human cryptochrome-1. Eur J Biochem **270,** 2921-2928.

Bouly, J.P., Schleicher, E., Dionisio-Sese, M., Vandenbussche, F., Van Der Straeten, D., Bakrim, N., Meier, S., Batschauer, A., Galland, P., Bittl, R., and Ahmad, M. (2007). Cryptochrome blue light photoreceptors are activated through interconversion of flavin redox states. J Biol Chem **282,** 9383-9391.

Brautigam, C.A., Smith, B.S., Ma, Z., Palnitkar, M., Tomchick, D.R., Machius, M., and Deisenhofer, J. (2004). Structure of the photolyase-like domain of cryptochrome 1 from Arabidopsis thaliana. Proc Natl Acad Sci U S A **101,** 12142-12147.

Briggs, W.R., and Huala, E. (1999). Blue-light photoreceptors in higher plants. Ann. Rev. Cell Dev. Biol. **15,** 33-62.

Briggs, W.R., and Christie, J.M. (2002). Phototropins 1 and 2: versatile plant blue-light receptors. Trends Plant Sci **7,** 204-210.

Briggs, W.R., Beck, C.F., Cashmore, A.R., Christie, J.M., Hughes, J., Jarillo, J.A., Kagawa, T., Kanegae, H., Liscum, E., Nagatani, A., Okada, K., Salomon, M., Rudiger, W., Sakai, T., Takano, M., Wada, M., and Watson, J.C. (2001). The phototropin family of photoreceptors. Plant Cell **13,** 993-997.

Brudler, R., Hitomi, K., Daiyasu, H., Toh, H., Kucho, K., Ishiura, M., Kanehisa, M., Roberts, V.A., Todo, T., Tainer, J.A., and Getzoff, E.D. (2003). Identification of a new cryptochrome class. Structure, function, and evolution. Mol Cell **11,** 59-67.

Bruggemann, E., Handwerger, K., C., E., and Storz, G. (1996). Analysis of fast neutron-generated mutants at the *Arabidopsis thaliana* locus. Plant J **10,** 755-760.

Burney, S., Hoang, N., Caruso, M., Dudkin, E.A., Ahmad, M., and Bouly, J.P. (2009). Conformational change induced by ATP binding correlates with enhanced biological function of Arabidopsis cryptochrome. FEBS Lett **583,** 1427-1433.

Busino, L., Bassermann, F., Maiolica, A., Lee, C., Nolan, P.M., Godinho, S.I., Draetta, G.F., and Pagano, M. (2007). SCFFbxl3 controls the oscillation of the circadian clock by directing the degradation of cryptochrome proteins. Science **316,** 900-904.

Busza, A., Emery-Le, M., Rosbash, M., and Emery, P. (2004). Roles of the two Drosophila CRYPTOCHROME structural domains in circadian photoreception. Science **304,** 1503-1506.

Canamero, R.C., Bakrim, N., Bouly, J.P., Garay, A., Dudkin, E.E., Habricot, Y., and Ahmad, M. (2006). Cryptochrome photoreceptors cry1 and cry2 antagonistically regulate primary root elongation in Arabidopsis thaliana. Planta **224,** 995-1003.

Cao, D., Lin, Y., and Cheng, C.L. (2000). Genetic interactions between the chlorate-resistant mutant cr88 and the photomorphogenic mutants cop1 and hy5. Plant Cell **12,** 199-210.

Casal, J.J., and Mazzella, M.A. (1998). Conditional synergism between cryptochrome 1 and phytochrome B is shown by the analysis of phyA, phyB, and hy4 simple, double, and triple mutants in Arabidopsis. Plant Physiol **118,** 19-25.

Cashmore, A.R. (1997). A cryptochrome family of photoreceptors. Plant Cell Environ. **20,** 764-767.

Cashmore, A.R. (2003). Cryptochromes: enabling plants and animals to determine circadian time. Cell **114,** 537-543.

Cashmore, A.R., Jarillo, J.A., Wu, Y.J., and Liu, D. (1999). Cryptochromes: blue light receptors for plants and animals. Science **284,** 760-765.

Ceriani, M.F., Darlington, T.K., Staknis, D., Mas, P., Petti, A.A., Weitz, C.J., and Kay, S.A. (1999). Light-dependent sequestration of TIMELESS by CRYPTOCHROME. Science **285,** 553-556.

Cermakian, N., Pando, M.P., Thompson, C.L., Pinchak, A.B., Selby, C.P., Gutierrez, L., Wells, D.E., Cahill, G.M., Sancar, A., and Sassone-Corsi, P. (2002). Light Induction of a vertebrate clock gene involves signaling through blue-light receptors and MAP kinases. Curr Biol **12,** 844-848.

Charron, J.B., He, H., Elling, A.A., and Deng, X.W. (2009). Dynamic landscapes of four histone modifications during deetiolation in Arabidopsis. Plant Cell **21,** 3732-3748.

Chaves, I., Yagita, K., Barnhoorn, S., Okamura, H., van der Horst, G.T., and Tamanini, F. (2006). Functional evolution of the photolyase/cryptochrome protein family: importance of the C terminus of mammalian CRY1 for circadian core oscillator performance. Mol Cell Biol **26,** 1743-1753.

Chen, M., Chory, J., and Fankhauser, C. (2004). Light signal transduction in higher plants. Annu Rev Genet **38,** 87-117.

Cho, M.H., and Spalding, E.P. (1996). An anion channel on Arabidopsis hypocotyls activated by blue light. Proc. Natl. Acad. Sci. USA **93,** 8134-8138.

Christie, J.M., Salomon, M., Nozue, K., Wada, M., and Briggs, W.R. (1999). LOV (light, oxygen, or voltage) domains of the blue-light photoreceptor phototropin (nph1): binding sites for the chromophore flavin mononucleotide. Proc Natl Acad Sci U S A **96,** 8779-8783.

Christie, J.M., Reymond, P., Powell, G.K., Bernasconi, P., Raibekas, A.A., Liscum, E., and Briggs, W.R. (1998). Arabidopsis NPH1: A flavoprotein with the properties of a photoreceptor for phototropism. Science **282,** 1698-1701.

Coesel, S., Mangogna, M., Ishikawa, T., Heijde, M., Rogato, A., Finazzi, G., Todo, T., Bowler, C., and Falciatore, A. (2009). Diatom PtCPF1 is a new cryptochrome/photolyase family member with DNA repair and transcription regulation activity. EMBO Rep **10,** 655-661.

Corbesier, L., Vincent, C., Jang, S., Fornara, F., Fan, Q., Searle, I., Giakountis, A., Farrona, S., Gissot, L., Turnbull, C., and Coupland, G. (2007). FT protein movement contributes to long-distance signaling in floral induction of Arabidopsis. Science **316,** 1030-1033.

Cutler, S.R., Ehrhardt, D.W., Griffitts, J.S., and Somerville, C.R. (2000). Random GFP::cDNA fusions enable visualization of subcellular structures in cells of Arabidopsis at a high frequency. Proc Natl Acad Sci U S A **97,** 3718-3723.

Danon, A., Coll, N.S., and Apel, K. (2006). Cryptochrome-1-dependent execution of programmed cell death induced by singlet oxygen in Arabidopsis thaliana. Proc Natl Acad Sci U S A **103,** 17036-17041.

Deng, X.-W., Caspar, T., and Quail, P.H. (1989). COP1: a regulatory locus involved in light-controlled development and gene expression in Arabidopsis. Genes Dev. **5,** 1172-1182.

Deng, X.-W., Matsui, M., Wei, N., Wagner, D., Chu, A.M., Feldmann, K.A., and Quail, P.H. (1992). COP1, an Arabidopsis regulatory gene, encodes a protein with both a zinc-binding motif and a Gbeta homologous domain. Cell **71,** 791-801.

Devlin, P.F., and Kay, S.A. (2000). Cryptochromes are required for phytochrome signaling to the circadian clock but not for rhythmicity. Plant Cell **12,** 2499-2510.

Devlin, P.F., and Kay, S.A. (2001). Circadian photoperception. Annu Rev Physiol **63,** 677-694.

Duek, P.D., and Fankhauser, C. (2003). HFR1, a putative bHLH transcription factor, mediates both phytochrome A and cryptochrome signalling. Plant J **34,** 827-836.

Duek, P.D., Elmer, M.V., van Oosten, V.R., and Fankhauser, C. (2004). The degradation of HFR1, a putative bHLH class transcription factor involved in light signaling, is regulated by phosphorylation and requires COP1. Curr Biol **14,** 2296-2301.

Dyson, H.J., and Wright, P.E. (2005). Intrinsically unstructured proteins and their functions. Nat Rev Mol Cell Biol **6,** 197-208.

Eide, E.J., Vielhaber, E.L., Hinz, W.A., and Virshup, D.M. (2002). The circadian regulatory proteins BMAL1 and cryptochromes are substrates of casein kinase Iepsilon. J Biol Chem **277,** 17248-17254.

El-Assal, S.E., Alonso-Blanco, C., Hanhart, C.J., and Koornneef, M. (2004). Pleiotropic effects of the Arabidopsis cryptochrome 2 allelic variation underlie fruit trait-related QTL. Plant Biol (Stuttg) **6,** 370-374.

El-Assal, S.E.-D., Alonso-Blanco, C., Peeters, A.J., Raz, V., and Koornneef, M. (2001). A QTL for flowering time in Arabidopsis reveals a novel allele of CRY2. Nat Genet **29,** 435-440.

El-Assal, S.E.-D., Alonso-Blanco, C., Peeters, A.J., Wagemaker, C., Weller, J.L., and Koornneef, M. (2003). The role of cryptochrome 2 in flowering in Arabidopsis. Plant Physiol **133,** 1504-1516.

Emery, P., So, W.V., Kaneko, M., Hall, J.C., and Rosbash, M. (1998). CRY, a Drosophila clock and light-regulated cryptochrome, is a major contributor to circadian rhythm resetting and photosensitivity. Cell **95,** 669-679.

Endo, M., Mochizuki, N., Suzuki, T., and Nagatani, A. (2007). CRYPTOCHROME2 in vascular bundles regulates flowering in Arabidopsis. Plant Cell **19,** 84-93.

Feldman, L.J. (1984). Regulation of root development. Annu Rev Plant Physiol **35,** 223-242.

Folta, K.M., and Spalding, E.P. (2001). Unexpected roles for cryptochrome 2 and phototropin revealed by high- resolution analysis of blue light-mediated hypocotyl growth inhibition. Plant J **26,** 471-478.

Folta, K.M., and Kaufman, L.S. (2003). Phototropin 1 is required for high-fluence blue-light-mediated mRNA destabilization. Plant Mol Biol **51,** 609-618.

Folta, K.M., Pontin, M.A., Karlin-Neumann, G., Bottini, R., and Spalding, E.P. (2003). Genomic and physiological studies of early cryptochrome 1 action demonstrate roles for auxin and gibberellin in the control of hypocotyl growth by blue light. Plant J **36,** 203-214.

Fuglevand, G., Jackson, J.A., and Jenkins, G.I. (1996). UV-B, UV-A, and blue light signal transduction pathways interact synergistically to regulate chalcone synthase gene expression in Arabidopsis. Plant Cell **8,** 2347-2357.

Fujiwara, S., Wang, L., Han, L., Suh, S.S., Salome, P.A., McClung, C.R., and Somers, D.E. (2008). Post-translational regulation of the Arabidopsis circadian clock through selective proteolysis and phosphorylation of pseudo-response regulator proteins. J Biol Chem **283,** 23073-23083.

Galland, P., and Pazur, A. (2005). Magnetoreception in plants. J Plant Res **118,** 371-389.

Gegear, R.J., Casselman, A., Waddell, S., and Reppert, S.M. (2008). Cryptochrome mediates light-dependent magnetosensitivity in Drosophila. Nature **454,** 1014-1018.

Gegear, R.J., Foley, L.E., Casselman, A., and Reppert, S.M. (2010). Animal cryptochromes mediate magnetoreception by an unconventional photochemical mechanism. Nature **463,** 804-807.

Gil, P., Kircher, S., Adam, E., Bury, E., Kozma-Bognar, L., Schafer, E., and Nagy, F. (2000). Photocontrol of subcellular partitioning of phytochrome-B:GFP fusion protein in tobacco seedlings. Plant J **22,** 135-145.

Giliberto, L., Perrotta, G., Pallara, P., Weller, J.L., Fraser, P.D., Bramley, P.M., Fiore, A., Tavazza, M., and Giuliano, G. (2005). Manipulation of the blue light photoreceptor cryptochrome 2 in tomato affects vegetative development, flowering time, and fruit antioxidant content. Plant Physiol **137,** 199-208.

Godinho, S.I., Maywood, E.S., Shaw, L., Tucci, V., Barnard, A.R., Busino, L., Pagano, M., Kendall, R., Quwailid, M.M., Romero, M.R., O'Neill, J., Chesham, J.E., Brooker, D., Lalanne, Z., Hastings, M.H., and Nolan, P.M. (2007). The after-hours mutant reveals a role for Fbxl3 in determining mammalian circadian period. Science **316,** 897-900.

Goggin, D.E., Steadman, K.J., and Powles, S.B. (2008). Green and blue light photoreceptors are involved in maintenance of dormancy in imbibed annual ryegrass (Lolium rigidum) seeds. New Phytol **180,** 81-89.

Goto, N., Kumagai, T., and Koornneef, M. (1991). Flowering responses to light-breaks in photomorphogenic mutants of *Arabidopsis thaliana*, a long-day plant. Physiol. Plant **83,** 209-215.

Gressel, J. (1979). Blue light photoreception. Photochem. Photobiol. **30,** 749-754.

Griffin, E.A., Jr., Staknis, D., and Weitz, C.J. (1999). Light-independent role of CRY1 and CRY2 in the mammalian circadian clock. Science **286,** 768-771.

Gsponer, J., Futschik, M.E., Teichmann, S.A., and Babu, M.M. (2008). Tight regulation of unstructured proteins: from transcript synthesis to protein degradation. Science **322,** 1365-1368.

Guo, H., Yang, H., Mockler, T.C., and Lin, C. (1998). Regulation of flowering time by Arabidopsis photoreceptors. Science **279,** 1360-1363.

Guo, H., Duong, H., Ma, N., and Lin, C. (1999). The Arabidopsis blue light receptor cryptochrome 2 is a nuclear protein regulated by a blue light-dependent post-transcriptional mechanism. Plant J **19,** 279-287.

Guo, H., Mockler, T.C., Duong, H., and Lin, C. (2001). SUB1, an Arabidopsis Ca2+-Binding protein involved in cryptochrome and phytochrome coaction. Science **291,** 487-490.

Hangarter, R.P. (1997). Gravity, light and plant form. Plant Cell Environ **20,** 796-800.

Harada, Y., Sakai, M., Kurabayashi, N., Hirota, T., and Fukada, Y. (2005). Ser-557-phosphorylated mCRY2 is degraded upon synergistic phosphorylation by glycogen synthase kinase-3 beta. J Biol Chem **280,** 31714-31721.

Hardtke, C.S., Gohda, K., Osterlund, M.T., Oyama, T., Okada, K., and Deng, X.W. (2000). HY5 stability and activity in arabidopsis is regulated by phosphorylation in its COP1 binding domain. Embo J **19,** 4997-5006.

Harmer, S.L. (2009). The circadian system in higher plants. Annu Rev Plant Biol **60,** 357-377.

Harris, S.R., Henbest, K.B., Maeda, K., Pannell, J.R., Timmel, C.R., Hore, P.J., and Okamoto, H. (2009). Effect of magnetic fields on cryptochrome-dependent responses in Arabidopsis thaliana. J R Soc Interface **6,** 1193-1205.

Heijde, M., Zabulon, G., Corellou, F., Ishikawa, T., Brazard, J., Usman, A., Sanchez, F., Plaza, P., Martin, M., Falciatore, A., Todo, T., Bouget, F.Y., and Bowler, C. (2010). Characterization of two members of the cryptochrome/photolyase family from Ostreococcus tauri provides insights into the origin and evolution of cryptochromes. Plant Cell Environ.

Henbest, K.B., Maeda, K., Hore, P.J., Joshi, M., Bacher, A., Bittl, R., Weber, S., Timmel, C.R., and Schleicher, E. (2008). Magnetic-field effect on the photoactivation reaction of Escherichia coli DNA photolyase. Proc Natl Acad Sci U S A **105,** 14395-14399.

Hendrischk, A.K., Fruhwirth, S.W., Moldt, J., Pokorny, R., Metz, S., Kaiser, G., Jager, A., Batschauer, A., and Klug, G. (2009). A cryptochrome-like protein is involved in the regulation of photosynthesis genes in Rhodobacter sphaeroides. Mol Microbiol **74,** 990-1003.

Hicks, K.A., Millar, A.J., Carre, I.A., Somers, D.E., Straume, M., Meeks-Wagner, D.R., and Kay, S.A. (1996). Conditional circadian dysfunction of the Arabidopsis early-flowering 3 mutant. Science **274,** 790-792.

Hirose, F., Shinomura, T., Tanabata, T., Shimada, H., and Takano, M. (2006). Involvement of rice cryptochromes in de-etiolation responses and flowering. Plant Cell Physiol **47,** 915-925.

Hitomi, K., Okamoto, K., Daiyasu, H., Miyashita, H., Iwai, S., Toh, H., Ishiura, M., and Todo, T. (2000). Bacterial cryptochrome and photolyase: characterization of two photolyase-like genes of Synechocystis sp. PCC6803. Nucleic Acids Res **28,** 2353-2362.

Hitomi, K., DiTacchio, L., Arvai, A.S., Yamamoto, J., Kim, S.T., Todo, T., Tainer, J.A., Iwai, S., Panda, S., and Getzoff, E.D. (2009). Functional motifs in the (6-4) photolyase crystal structure make a comparative framework for DNA repair photolyases and clock cryptochromes. Proc Natl Acad Sci U S A **106,** 6962-6967.

Hoang, N., Bouly, J.P., and Ahmad, M. (2008a). Evidence of a light-sensing role for folate in Arabidopsis cryptochrome blue-light receptors. Mol Plant **1,** 68-74.

Hoang, N., Schleicher, E., Kacprzak, S., Bouly, J.P., Picot, M., Wu, W., Berndt, A., Wolf, E., Bittl, R., and Ahmad, M. (2008b). Human and Drosophila cryptochromes are light activated by flavin photoreduction in living cells. PLoS Biol **6,** e160.

Hoecker, U., and Quail, P.H. (2001). The phytochrome A-specific signaling intermediate SPA1 interacts directly with COP1, a constitutive repressor of light signaling in Arabidopsis. J Biol Chem **276,** 38173-38178.

Hoecker, U., Tepperman, J.M., and Quail, P.H. (1999). SPA1, a WD-repeat protein specific to phytochrome A signal transduction. Science **284,** 496-499.

Hoffman, P.D., Batschauer, A., and Hays, J.B. (1996). PHH1, a novel gene from Arabidopsis thaliana that encodes a protein similar to plant blue-light photoreceptors and microbial photolyases. Mol. Gen. Genet. **253,** 259-265.

Hong, S.H., Kim, H.J., Ryu, J.S., Choi, H., Jeong, S., Shin, J., Choi, G., and Nam, H.G. (2008). CRY1 inhibits COP1-mediated degradation of BIT1, a MYB transcription factor, to activate blue light-dependent gene expression in Arabidopsis. Plant J **55,** 361-371.

Hou, Y., von Arnim, A.G., and Deng, X. (1993). A new class of Arabidopsis constitutive photomorphogenic genes involved in regulating cotyledon development. Plant Cell **5,** 329-339.

Huala, E., Oeller, P.W., Liscum, E., Han, I.S., Larsen, E., and Briggs, W.R. (1997). Arabidopsis NPH1: a protein kinase with a putative redox-sensing domain. Science **278,** 2120-2123.

Huang, Y., Baxter, R., Smith, B.S., Partch, C.L., Colbert, C.L., and Deisenhofer, J. (2006). Crystal structure of cryptochrome 3 from Arabidopsis thaliana and its implications for photolyase activity. Proc Natl Acad Sci U S A **103,** 17701-17706.

Huq, E., Tepperman, J.M., and Quail, P.H. (2000). GIGANTEA is a nuclear protein involved in phytochrome signaling in Arabidopsis. Proc Natl Acad Sci U S A **97,** 9789-9794.

Imaizumi, T., Tran, H.G., Swartz, T.E., Briggs, W.R., and Kay, S.A. (2003). FKF1 is essential for photoperiodic-specific light signalling in Arabidopsis. Nature **426,** 302-306.

Imaizumi, T., Schultz, T.F., Harmon, F.G., Ho, L.A., and Kay, S.A. (2005). FKF1 F-box protein mediates cyclic degradation of a repressor of CONSTANS in Arabidopsis. Science **309,** 293-297.

Immeln, D., Schlesinger, R., Heberle, J., and Kottke, T. (2007). Blue light induces radical formation and autophosphorylation in the light-sensitive domain of Chlamydomonas cryptochrome. J Biol Chem **282,** 21720-21728.

Inada, S., Ohgishi, M., Mayama, T., Okada, K., and Sakai, T. (2004). RPT2 is a signal transducer involved in phototropic response and stomatal opening by association with phototropin 1 in Arabidopsis thaliana. Plant Cell **16,** 887-896.

Jaeger, K.E., and Wigge, P.A. (2007). FT protein acts as a long-range signal in Arabidopsis. Curr Biol **17,** 1050-1054.

Jang, I.C., Yang, J.Y., Seo, H.S., and Chua, N.H. (2005). HFR1 is targeted by COP1 E3 ligase for post-translational proteolysis during phytochrome A signaling. Genes Dev **19,** 593-602.

Jang, S., Marchal, V., Panigrahi, K.C., Wenkel, S., Soppe, W., Deng, X.W., Valverde, F., and Coupland, G. (2008). Arabidopsis COP1 shapes the temporal pattern of CO accumulation conferring a photoperiodic flowering response. Embo J **27,** 1277-1288.

Jarillo, J.A., Capel, J., Tang, R.H., Yang, H.Q., Alonso, J.M., Ecker, J.R., and Cashmore, A.R. (2001). An Arabidopsis circadian clock component interacts with both CRY1 and phyB. Nature **410,** 487-490.

Jeong, R.-D., Chandra-Shekara, A.C., Barman, S.R., Navarre, D., Klessig, D.F., Kachroo, A., and Kachroo, P. (2010). CRYPTOCHROME 2 and PHOTOTROPIN 2 regulate resistance protein mediated viral defense by negatively regulating a E3 ubiquitin ligase. Proc Natl Acad Sci U S A **In press**.

Jiao, Y., Lau, O.S., and Deng, X.W. (2007). Light-regulated transcriptional networks in higher plants. Nat Rev Genet **8,** 217-230.

Jung, J.H., Seo, Y.H., Seo, P.J., Reyes, J.L., Yun, J., Chua, N.H., and Park, C.M. (2007). The GIGANTEA-regulated microRNA172 mediates photoperiodic flowering independent of CONSTANS in Arabidopsis. Plant Cell **19,** 2736-2748.

Kagawa, T., Sakai, T., Suetsugu, N., Oikawa, K., Ishiguro, S., Kato, T., Tabata, S., Okada, K., and Wada, M. (2001). Arabidopsis NPL1: a phototropin homolog controlling the chloroplast high-light avoidance response. Science **291,** 2138-2141.

Kanai, S., Kikuno, R., Toh, H., Ryo, H., and Todo, T. (1997). Molecular evolution of the photolyase-blue-light photoreceptor family. J Mol Evol **45,** 535-548.

Kanegae, T., and Wada, M. (1998). Isolation and characterization of homologues of plant blue-light photoreceptor (cryptochrome) genes from the fern *Adiantum capillus- veneris*. Mol Gen Genet **259,** 345-353.

Kang, C.Y., Lian, H.L., Wang, F.F., Huang, J.R., and Yang, H.Q. (2009). Cryptochromes, phytochromes, and COP1 regulate light-controlled stomatal development in Arabidopsis. Plant Cell **21,** 2624-2641.

Kang, X., and Ni, M. (2006). Arabidopsis SHORT HYPOCOTYL UNDER BLUE1 contains SPX and EXS domains and acts in cryptochrome signaling. Plant Cell **18,** 921-934.

Kang, X., Chong, J., and Ni, M. (2005). HYPERSENSITIVE TO RED AND BLUE 1, a ZZ-type zinc finger protein, regulates phytochrome B-mediated red and cryptochrome-mediated blue light responses. Plant Cell **17,** 822-835.

Kasemir, H. (1979). Control of chloroplast formation by light. Cell Biol Int Rep **3,** 197-214.

Kenrick, P., and Crane, R. (1997). The origin and early evolution of plants on land. Nature **389,** 33-39.

Kim, W.Y., Fujiwara, S., Suh, S.S., Kim, J., Kim, Y., Han, L., David, K., Putterill, J., Nam, H.G., and Somers, D.E. (2007). ZEITLUPE is a circadian photoreceptor stabilized by GIGANTEA in blue light. Nature **449,** 356-360.

Kinoshita, T., Michio Doi, M., Suetsugu, N., Kagawa‡, T., Wada, M., and Shimazaki, K.-I. (2001). phot1 and phot2 mediate blue light regulation of stomatal
opening. Nature **414,** 656-660.

Klar, T., Pokorny, R., Moldt, J., Batschauer, A., and Essen, L.O. (2007). Cryptochrome 3 from Arabidopsis thaliana: structural and functional analysis of its complex with a folate light antenna. J Mol Biol **366,** 954-964.

Kleine, T., Lockhart, P., and Batschauer, A. (2003). An Arabidopsis protein closely related to Synechocystis cryptochrome is targeted to organelles. Plant J **35,** 93-103.

Kleine, T., Kindgren, P., Benedict, C., Hendrickson, L., and Strand, A. (2007). Genome-wide gene expression analysis reveals a critical role for CRYPTOCHROME1 in the response of Arabidopsis to high irradiance. Plant Physiol **144,** 1391-1406.

Kleiner, O., Kircher, S., Harter, K., and Batschauer, A. (1999). Nuclear localization of the Arabidopsis blue light receptor cryptochrome 2. Plant J **19,** 289-296.

Koornneef, M., Rolff, E., and Spruit, C.J.P. (1980). Genetic control of light-inhibited hypocotyl elongation in *Arabidopsis thaliana* (L.) Heynh. Z. Pflanzenphysiol. Bd. **100,** 147-160.

Koornneef, M., Hanhart, C.J., and van der Veen, J.H. (1991). A genetic and physiological analysis of late flowering mutants in *Arabidopsis thaliana*. Mol Gen Genet **229,** 57-66.

Koornneef, M., Alonso-Blanco, C., Peeters, A.J.M., and Soppe, W. (1998a). Genetic control of flowering time in Arabidopsis. Annu. Rev. Plant Physiol. Plant Mol. Biol. **49,** 345-370.

Koornneef, M., Alonso-Blanco, C., Blankestijn-de Vries, H., Hanhart, C.J., and Peeters, A.J. (1998b). Genetic interactions among late-flowering mutants of Arabidopsis. Genetics **148,** 885-892.

Kumar, S.V., and Wigge, P.A. (2010). H2A.Z-containing nucleosomes mediate the thermosensory response in Arabidopsis. Cell **140,** 136-147.

Kume, K., Zylka, M.J., Sriram, S., Shearman, L.P., Weaver, D.R., Jin, X., Maywood, E.S., Hastings, M.H., and Reppert, S.M. (1999). mCRY1 and mCRY2 are essential components of the negative limb of the circadian clock feedback loop. Cell **98,** 193-205.

Lamia, K.A., Sachdeva, U.M., DiTacchio, L., Williams, E.C., Alvarez, J.G., Egan, D.F., Vasquez, D.S., Juguilon, H., Panda, S., Shaw, R.J., Thompson, C.B., and Evans, R.M. (2009). AMPK regulates the circadian clock by cryptochrome phosphorylation and degradation. Science **326,** 437-440.

Lariguet, P., and Dunand, C. (2005). Plant photoreceptors: phylogenetic overview. J Mol Evol **61,** 559-569.

Lasceve, G., Leymarie, J., Olney, M.A., Liscum, E., Christie, J.M., Vavasseur, A., and Briggs, W.R. (1999). Arabidopsis contains at least four independent blue-light-activated signal transduction pathways. Plant Physiol **120,** 605-614.

Laubinger, S., Fittinghoff, K., and Hoecker, U. (2004). The SPA quartet: a family of WD-repeat proteins with a central role in suppression of photomorphogenesis in arabidopsis. Plant Cell **16,** 2293-2306.

Laubinger, S., Marchal, V., Le Gourrierec, J., Wenkel, S., Adrian, J., Jang, S., Kulajta, C., Braun, H., Coupland, G., and Hoecker, U. (2006). Arabidopsis SPA proteins regulate photoperiodic flowering and interact with the floral inducer CONSTANS to regulate its stability. Development **133,** 3213-3222.

Lee, J., He, K., Stolc, V., Lee, H., Figueroa, P., Gao, Y., Tongprasit, W., Zhao, H., Lee, I., and Deng, X.W. (2007). Analysis of transcription factor HY5 genomic binding sites revealed its hierarchical role in light regulation of development. Plant Cell **19,** 731-749.

Lempe, J., Balasubramanian, S., Sureshkumar, S., Singh, A., Schmid, M., and Weigel, D. (2005). Diversity of flowering responses in wild Arabidopsis thaliana strains. PLoS Genet **1,** 109-118.

Liedvogel, M., and Mouritsen, H. (2009). Cryptochromes--a potential magnetoreceptor: what do we know and what do we want to know? J R Soc Interface **7 Suppl 2,** S147-162.

Liedvogel, M., Maeda, K., Henbest, K., Schleicher, E., Simon, T., Timmel, C.R., Hore, P.J., and Mouritsen, H. (2007). Chemical magnetoreception: bird cryptochrome 1a is excited by blue light and forms long-lived radical-pairs. PLoS One **2,** e1106.

Lin, C., and Shalitin, D. (2003). Cryptochrome Structure and Signal transduction. Annu. Rev. Plant Biol. **54,** 469-496.

Lin, C., Ahmad, M., and Cashmore, A.R. (1996a). Arabidopsis cryptochrome 1 is a soluble protein mediating blue light- dependent regulation of plant growth and development. Plant J **10,** 893-902.

Lin, C., Ahmad, M., Gordon, D., and Cashmore, A.R. (1995a). Expression of an Arabidopsis cryptochrome gene in transgenic tobacco results in hypersensitivity to blue, UV-A, and green light. Proc Natl Acad Sci U S A **92,** 8423-8427.

Lin, C., Ahmad, M., Chan, J., and Cashmore, A.R. (1996b). CRY2, a second member of the Arabidopsis cryptochrome gene family. Plant

Physiol. **110,** 1047.

Lin, C., Yang, H., Guo, H., Mockler, T., Chen, J., and Cashmore, A.R. (1998). Enhancement of blue-light sensitivity of Arabidopsis seedlings by a blue light receptor cryptochrome 2. Proc Natl Acad Sci U S A **95,** 2686-2690.

Lin, C., Robertson, D.E., Ahmad, M., Raibekas, A.A., Jorns, M.S., Dutton, P.L., and Cashmore, A.R. (1995b). Association of flavin adenine dinucleotide with the Arabidopsis blue light receptor CRY1. Science **269,** 968-970.

Liscum, E. (2002) Phototropism: Mechanisms and Outcomes. In: The Arabidopsis Book. The American Society of Plant Biologists, Rockville, MD., doi: 10.1199/tab.0042, http://www.aspb.org/publications/arabidopsis/.

Liscum, E., and Hangarter, R.P. (1993). Genetic evidence that the red-absorbing form of phytochrome B modulates gravitropism in Arabidopsis thaliana. Plant Physiol **103,** 15-19.

Liscum, E., and Briggs, W. (1995). Mutations in the *NPH1* locus of Arabidopsis disrupt the perception of phototropic stimuli. Plant Cell **7,** 473-485.

Liu, H., Zhong, D., Liu, B., and Lin, C. (2010). In the search for a photocycle of the cryptochrome photoreceptors. Current Opinion in Plant Biology **In press**.

Liu, H., Yu, X., Li, K., Klejnot, J., Yang, H., Lisiero, D., and Lin, C. (2008a). Photoexcited CRY2 interacts with CIB1 to regulate transcription and floral initiation in Arabidopsis. Science **322,** 1535-1539.

Liu, L.J., Zhang, Y.C., Li, Q.H., Sang, Y., Mao, J., Lian, H.L., Wang, L., and Yang, H.Q. (2008b). COP1-Mediated Ubiquitination of CONSTANS Is Implicated in Cryptochrome Regulation of Flowering in Arabidopsis. Plant Cell **20,** 292-306.

Lopez-Juez, E., Dillon, E., Magyar, Z., Khan, S., Hazeldine, S., de Jager, S.M., Murray, J.A., Beemster, G.T., Bogre, L., and Shanahan, H. (2008). Distinct light-initiated gene expression and cell cycle programs in the shoot apex and cotyledons of Arabidopsis. Plant Cell **20,** 947-968.

Ma, L., Li, J., Qu, L., Hager, J., Chen, Z., Zhao, H., and Deng, X.W. (2001). Light control of Arabidopsis development entails coordinated regulation of genome expression and cellular pathways. Plant Cell **13,** 2589-2607.

Malhotra, K., Kim, S.T., Batschauer, A., Dawut, L., and Sancar, A. (1995). Putative blue-light photoreceptors from *Arabidopsis thaliana* and *Sinapis alba* with a high degree of sequence homology to DNA photolyase contain the two photolyase cofactors but lack DNA repair activity. Biochemistry **34,** 6892-6899.

Mao, J., Zhang, Y.C., Sang, Y., Li, Q.H., and Yang, H.Q. (2005). A role for Arabidopsis cryptochromes and COP1 in the regulation of stomatal opening. Proc Natl Acad Sci U S A **102,** 12270-12275.

Mas, P., Devlin, P.F., Panda, S., and Kay, S.A. (2000). Functional interaction of phytochrome B and cryptochrome 2. Nature **408,** 207-211.

Mas, P., Kim, W.Y., Somers, D.E., and Kay, S.A. (2003). Targeted degradation of TOC1 by ZTL modulates circadian function in Arabidopsis thaliana. Nature **426,** 567-570.

Mathieu, J., Warthmann, N., Kuttner, F., and Schmid, M. (2007). Export of FT protein from phloem companion cells is sufficient for floral induction in Arabidopsis. Curr Biol **17,** 1055-1060.

Matsumoto, N., Hirano, T., Iwasaki, T., and Yamamoto, N. (2003). Functional analysis and intracellular localization of rice cryptochromes. Plant Physiol **133,** 1494-1503.

McClung, C.R., Salomé, P.A., and Michael, T.P. (2002) The Arabidopsis Circadian System. In: The Arabidopsis Book. The American Society of Plant Biologists, Rockville, MD., doi: 10.1199/tab.0044, http://www.aspb.org/publications/arabidopsis/.

Meskauskiene, R., Nater, M., Goslings, D., Kessler, F., op den Camp, R., and Apel, K. (2001). FLU: a negative regulator of chlorophyll biosynthesis in Arabidopsis thaliana. Proc Natl Acad Sci U S A **98,** 12826-12831.

Mochizuki, T., Onda, Y., Fujiwara, E., Wada, M., and Toyoshima, Y. (2004). Two independent light signals cooperate in the activation of the plastid psbD blue light-responsive promoter in Arabidopsis. FEBS Lett **571,** 26-30.

Mockler, T., Yang, H., Yu, X., Parikh, D., Cheng, Y.C., Dolan, S., and Lin, C. (2003). Regulation of photoperiodic flowering by Arabidopsis photoreceptors. Proc Natl Acad Sci U S A **100,** 2140-2145.

Mockler, T.C., Guo, H., Yang, H., Duong, H., and Lin, C. (1999). Antagonistic actions of Arabidopsis cryptochromes and phytochrome B in the regulation of floral induction. Development **126,** 2073-2082.

Mohr, H. (1994). Coaction between pigment systems. In Photomorphogenesis in Plants - 2nd Edition, R.E. Kendrick and G.H.M. Kronenberg, eds (Dordrecht: Kluwer Academic Publishers.

Moller, S.G., Kim, Y.S., Kunkel, T., and Chua, N.H. (2003). PP7 Is a positive regulator of blue light signaling in Arabidopsis. Plant Cell **15,** 1111-1119.

Mozley, D., and Thomas, B. (1995). Developmental and photobiological factors affecting photoperiodic induction in *Arabidopsis thaliana* Heynh. Landsberg erecta. J. Exp. Botany **46,** 173-179.

Nagashima, A., Hanaoka, M., Shikanai, T., Fujiwara, M., Kanamaru, K., Takahashi, H., and Tanaka, K. (2004). The multiple-stress responsive plastid sigma factor, SIG5, directs activation of the psbD blue light-responsive promoter (BLRP) in Arabidopsis thaliana. Plant Cell Physiol **45,** 357-368.

Nagashima, A., Suzuki, G., Uehara, Y., Saji, K., Furukawa, T., Koshiba, T., Sekimoto, M., Fujioka, S., Kuroha, T., Kojima, M., Sakakibara, H., Fujisawa, N., Okada, K., and Sakai, T. (2008). Phytochromes and cryptochromes regulate the differential growth of Arabidopsis hypocotyls in both a PGP19-dependent and a PGP19-independent manner. Plant J **53,** 516-529.

Nakajima, S., Sugiyama, M., Iwai, S., Hitomi, K., Otoshi, E., Kim, S.T., Jiang, C.Z., Todo, T., Britt, A.B., and Yamamoto, K. (1998). Cloning and characterization of a gene (UVR3) required for photorepair of 6-4 photoproducts in Arabidopsis thaliana. Nucleic Acids Res **26,** 638-644.

Neff, M.M., and Chory, J. (1998). Genetic interactions between phytochrome A, phytochrome B, and cryptochrome 1 during Arabidopsis development. Plant Physiol **118,** 27-35.

Nelson, D.C., Lasswell, J., Rogg, L.E., Cohen, M.A., and Bartel, B. (2000). FKF1, a clock-controlled gene that regulates the transition to flowering in Arabidopsis. Cell **101,** 331-340.

Nemhauser, J., and Chory, J. (2002) Photomorphogenesis. In: The Arabidopsis Book. The American Society of Plant Biologists, Rockville, MD,, doi: 10.1199/tab.0054, http://www.aspb.org/publications/arabidopsis/.

Ohgishi, M., Saji, K., Okada, K., and Sakai, T. (2004). Functional analysis of each blue light receptor, cry1, cry2, phot1, and phot2, by using combinatorial multiple mutants in Arabidopsis. Proc Natl Acad Sci U S A **101,** 2223-2228.

Olsen, K.M., Halldorsdottir, S.S., Stinchcombe, J.R., Weinig, C., Schmitt, J., and Purugganan, M.D. (2004). Linkage disequilibrium mapping of Arabidopsis CRY2 flowering time alleles. Genetics **167,** 1361-1369.

op den Camp, R.G., Przybyla, D., Ochsenbein, C., Laloi, C., Kim, C., Danon, A., Wagner, D., Hideg, E., Gobel, C., Feussner, I., Nater, M., and Apel, K. (2003). Rapid induction of distinct stress responses after the release of singlet oxygen in Arabidopsis. Plant Cell **15,** 2320-2332.

Osterlund, M.T., Hardtke, C.S., Wei, N., and Deng, X.W. (2000). Targeted destabilization of HY5 during light-regulated development of Arabidopsis. Nature **405,** 462-466.

Ozgur, S., and Sancar, A. (2003). Purification and properties of human

blue-light photoreceptor cryptochrome 2. Biochemistry **42,** 2926-2932.

Ozgur, S., and Sancar, A. (2006). Analysis of autophosphorylating kinase activities of Arabidopsis and human cryptochromes. Biochemistry **45,** 13369-13374.

Ozturk, N., Song, S.H., Selby, C.P., and Sancar, A. (2008). Animal type 1 cryptochromes. Analysis of the redox state of the flavin cofactor by site-directed mutagenesis. J Biol Chem **283,** 3256-3263.

Park, D.H., Somers, D.E., Kim, Y.S., Choy, Y.H., Lim, H.K., Soh, M.S., Kim, H.J., Kay, S.A., and Nam, H.G. (1999). Control of circadian rhythms and photoperiodic flowering by the Arabidopsis GIGANTEA gene. Science **285,** 1579-1582.

Park, H.W., Kim, S.T., Sancar, A., and Deisenhofer, J. (1995). Crystal structure of DNA photolyase from *Escherichia coli*. Science **268,** 1866-1872.

Parks, B.M., and Spalding, E.P. (1999). Sequential and coordinated action of phytochromes A and B during Arabidopsis stem growth revealed by kinetic analysis. Proc Natl Acad Sci U S A **96,** 14142-14146.

Parks, B.M., Cho, M.H., and Spalding, E.P. (1998). Two genetically separable phases of growth inhibition induced by blue light in Arabidopsis seedlings. Plant Physiol **118,** 609-615.

Parks, B.M., Folta, K.M., and Spalding, E.P. (2001). Photocontrol of stem growth. Curr Opin Plant Biol **4,** 436-440.

Partch, C.L., and Sancar, A. (2005a). Photochemistry and photobiology of cryptochrome blue-light photopigments: the search for a photocycle. Photochem Photobiol **81,** 1291-1304.

Partch, C.L., and Sancar, A. (2005b). Cryptochromes and circadian photoreception in animals. Methods Enzymol **393,** 726-745.

Partch, C.L., Clarkson, M.W., Ozgur, S., Lee, A.L., and Sancar, A. (2005). Role of structural plasticity in signal transduction by the cryptochrome blue-light photoreceptor. Biochemistry **44,** 3795-3805.

Peschel, N., Chen, K.F., Szabo, G., and Stanewsky, R. (2009). Light-dependent interactions between the Drosophila circadian clock factors cryptochrome, jetlag, and timeless. Curr Biol **19,** 241-247.

Platten, J.D., Foo, E., Elliott, R.C., Hecht, V., Reid, J.B., and Weller, J.L. (2005). Cryptochrome 1 contributes to blue-light sensing in pea. Plant Physiol **139,** 1472-1482.

Pokorny, R., Klar, T., Hennecke, U., Carell, T., Batschauer, A., and Essen, L.O. (2008). Recognition and repair of UV lesions in loop structures of duplex DNA by DASH-type cryptochrome. Proc Natl Acad Sci U S A **105,** 21023-21027.

Putterill, J., Robson, F., Lee, K., Simon, R., and Coupland, G. (1995). The CONSTANS gene of Arabidopsis promotes flowering and encodes a protein showing similarities to zinc finger transcription factors. Cell **80,** 847-857.

Quail, P.H., Briggs, W., Chory, J., Hangarter, R.P., Harberd, N.P., Kendrick, R.E., Koornneef, M., Parks, B., Sharrock, R.A., Schafer, E., Thompson, W.F., and Whitelam, G.C. (1994). Spotlight on phytochrome nomenclature. Plant Cell **6,** 468-471.

Reisdorph, N.A., and Small, G.D. (2004). The CPH1 gene of Chlamydomonas reinhardtii encodes two forms of cryptochrome whose levels are controlled by light-induced proteolysis. Plant Physiol **134,** 1546-1554.

Reppert, S.M., and Weaver, D.R. (2002). Coordination of circadian timing in mammals. Nature **418,** 935-941.

Ritz, T., Adem, S., and Schulten, K. (2000). A model for photoreceptor-based magnetoreception in birds. Biophys J **78,** 707-718.

Robson, P.R., and Smith, H. (1996). Genetic and transgenic evidence that phytochromes A and B act to modulate the gravitropic orientation of Arabidopsis thaliana hypocotyls. Plant Physiol **110,** 211-216.

Rosato, E., Codd, V., Mazzotta, G., Piccin, A., Zordan, M., Costa, R., and Kyriacou, C.P. (2001). Light-dependent interaction between Drosophila CRY and the clock protein PER mediated by the carboxy terminus of CRY. Curr Biol **11,** 909-917.

Rosenfeldt, G., Viana, R.M., Mootz, H.D., von Arnim, A.G., and Batschauer, A. (2008). Chemically induced and light-independent cryptochrome photoreceptor activation. Mol. Plant **1,** 4-12.

Saijo, Y., Sullivan, J.A., Wang, H., Yang, J., Shen, Y., Rubio, V., Ma, L., Hoecker, U., and Deng, X.W. (2003). The COP1-SPA1 interaction defines a critical step in phytochrome A-mediated regulation of HY5 activity. Genes Dev **17,** 2642-2647.

Saijo, Y., Zhu, D., Li, J., Rubio, V., Zhou, Z., Shen, Y., Hoecker, U., Wang, H., and Deng, X.W. (2008). Arabidopsis COP1/SPA1 complex and FHY1/FHY3 associate with distinct phosphorylated forms of phytochrome A in balancing light signaling. Mol Cell **31,** 607-613.

Sanada, K., Harada, Y., Sakai, M., Todo, T., and Fukada, Y. (2004). Serine phosphorylation of mCRY1 and mCRY2 by mitogen-activated protein kinase. Genes Cells **9,** 697-708.

Sancar, A. (2003). Structure and function of DNA photolyase and cryptochrome blue-light photoreceptors. Chem Rev **103,** 2203-2237.

Sancar, G.B. (1990). DNA photolyases: Physical properties, action mechanisms, and roles in dark repair. Mutation Res earch **236,** 147-160.

Sancar, G.B. (2000). Enzymatic photoreactivation: 50 years and counting. Mutat Res **451,** 25-37.

Sang, Y., Li, Q.H., Rubio, V., Zhang, Y.C., Mao, J., Deng, X.W., and Yang, H.Q. (2005). N-terminal domain-mediated homodimerization is required for photoreceptor activity of Arabidopsis CRYPTOCHROME 1. Plant Cell **17,** 1569-1584.

Sawa, M., Nusinow, D.A., Kay, S.A., and Imaizumi, T. (2007). FKF1 and GIGANTEA complex formation is required for day-length measurement in Arabidopsis. Science **318,** 261-265.

Schroeder, J.I., Allen, G.J., Hugouvieux, V., Kwak, J.M., and Waner, D. (2001). Guard cell signal transduction. Annu Rev Plant Physiol Plant Mol Biol **52,** 627-658.

Searle, I., and Coupland, G. (2004). Induction of flowering by seasonal changes in photoperiod. Embo J **23,** 1217-1222.

Selby, C.P., and Sancar, A. (2006). A cryptochrome/photolyase class of enzymes with single-stranded DNA-specific photolyase activity. Proc Natl Acad Sci U S A **103,** 17696-17700.

Selby, C.P., Thompson, C., Schmitz, T.M., Van Gelder, R.N., and Sancar, A. (2000). Functional redundancy of cryptochromes and classical photoreceptors for nonvisual ocular photoreception in mice. Proc Natl Acad Sci U S A **97,** 14697-14702.

Sellaro, R., Hoecker, U., Yanovsky, M., Chory, J., and Casal, J.J. (2009). Synergism of red and blue light in the control of Arabidopsis gene expression and development. Curr Biol **19,** 1216-1220.

Seo, H.S., Watanabe, E., Tokutomi, S., Nagatani, A., and Chua, N.H. (2004). Photoreceptor ubiquitination by COP1 E3 ligase desensitizes phytochrome A signaling. Genes Dev **18,** 617-622.

Seo, H.S., Yang, J.Y., Ishikawa, M., Bolle, C., Ballesteros, M.L., and Chua, N.H. (2003). LAF1 ubiquitination by COP1 controls photomorphogenesis and is stimulated by SPA1. Nature **423,** 995-999.

Shalitin, D., Yu, X., Maymon, M., Mockler, T., and Lin, C. (2003). Blue light-dependent in vivo and in vitro phosphorylation of Arabidopsis cryptochrome 1. Plant Cell **15,** 2421-2429.

Shalitin, D., Yang, H., Mockler, T.C., Maymon, M., Guo, H., Whitelam, G.C., and Lin, C. (2002). Regulation of Arabidopsis cryptochrome 2 by blue-light-dependent phosphorylation. Nature **417,** 763-767.

Siepka, S.M., Yoo, S.H., Park, J., Song, W., Kumar, V., Hu, Y., Lee, C., and Takahashi, J.S. (2007). Circadian mutant Overtime reveals F-box protein FBXL3 regulation of cryptochrome and period gene expression. Cell **129,** 1011-1023.

Small, G.D., Min, B., and Lefebvre, P.A. (1995). Characterization of a Chlamydomonas reinhardtii gene encoding a protein of the DNA pho-

tolyase/blue light photoreceptor family. Plant Mol Biol **28,** 443-454.

Smith, H. (1982). Light quality, photoperception, and plant strategy. Ann. Rev. Plant Physiol. **33,** 481-518.

Solov'yov, I.A., Chandler, D.E., and Schulten, K. (2007). Magnetic field effects in Arabidopsis thaliana cryptochrome-1. Biophys J **92,** 2711-2726.

Somers, D.E., Devlin, P.F., and Kay, S.A. (1998). Phytochromes and cryptochromes in the entrainment of the Arabidopsis circadian clock. Science **282,** 1488-1490.

Somers, D.E., Schultz, T.F., Milnamow, M., and Kay, S.A. (2000). ZEITLUPE encodes a novel clock-associated PAS protein from Arabidopsis. Cell **101,** 319-329.

Song, S.H., Dick, B., Penzkofer, A., Pokorny, R., Batschauer, A., and Essen, L.O. (2006). Absorption and fluorescence spectroscopic characterization of cryptochrome 3 from Arabidopsis thaliana. J Photochem Photobiol B **85,** 1-16.

Spalding, E., and Cosgrove, D. (1988). Large plasma-membrane depolarization precedes rapid blue-light-induced growth inhibition in cucumber. Planta **178,** 407-410.

Spalding, E.P. (2000). Ion channels and the transduction of light signals. Plant, Cell and Environment **23,** 665-674.

Stanewsky, R., Kaneko, M., Emery, P., Beretta, B., Wager-Smith, K., Kay, S.A., Rosbash, M., and Hall, J.C. (1998). The cryb mutation identifies cryptochrome as a circadian photoreceptor in Drosophila. Cell **95,** 681-692.

Stoelzle, S., Kagawa, T., Wada, M., Hedrich, R., and Dietrich, P. (2003). Blue light activates calcium-permeable channels in Arabidopsis mesophyll cells via the phototropin signaling pathway. Proc Natl Acad Sci U S A **100,** 1456-1461.

Strasser, B., Sanchez-Lamas, M., Yanovsky, M.J., Casal, J.J., and Cerdan, P.D. (2010). Arabidopsis thaliana life without phytochromes. Proc Natl Acad Sci U S A **107,** 4776-4781.

Strayer, C., Oyama, T., Schultz, T.F., Raman, R., Somers, D.E., Mas, P., Panda, S., Kreps, J.A., and Kay, S.A. (2000). Cloning of the Arabidopsis clock gene TOC1, an autoregulatory response regulator homolog. Science **289,** 768-771.

Suarez-Lopez, P., Wheatley, K., Robson, F., Onouchi, H., Valverde, F., and Coupland, G. (2001). CONSTANS mediates between the circadian clock and the control of flowering in Arabidopsis. Nature **410,** 1116-1120.

Tamai, T.K., Young, L.C., and Whitmore, D. (2007). Light signaling to the zebrafish circadian clock by Cryptochrome 1a. Proc Natl Acad Sci U S A **104,** 14712-14717.

Tamaki, S., Matsuo, S., Wong, H.L., Yokoi, S., and Shimamoto, K. (2007). Hd3a protein is a mobile flowering signal in rice. Science **316,** 1033-1036.

Tessadori, F., Schulkes, R.K., van Driel, R., and Fransz, P. (2007). Light-regulated large-scale reorganization of chromatin during the floral transition in Arabidopsis. Plant J **50,** 848-857.

Thomas, B., and Vince-Prue, D. (1997). Photoperiodism in plants. (New York: Academic Press).

Thresher, R.J., Vitaterna, M.H., Miyamoto, Y., Kazantsev, A., Hsu, D.S., Petit, C., Selby, C.P., Dawut, L., Smithies, O., Takahashi, J.S., and Sancar, A. (1998). Role of mouse cryptochrome blue-light photoreceptor in circadian photoresponses. Science **282,** 1490-1494.

Thum, K.E., Kim, M., Christopher, D.A., and Mullet, J.E. (2001). Cryptochrome 1, cryptochrome 2, and phytochrome A co-activate the chloroplast psbD blue light-responsive promoter. Plant Cell **13,** 2747-2760.

Todo, T. (1999). Functional diversity of the DNA photolyase/blue light receptor family. Mutat Res **434,** 89-97.

Toth, R., Kevei, E.E., Hall, A., Millar, A.J., Nagy, F., and Kozma-Bognar, L. (2001). Circadian clock-regulated expression of phytochrome and cryptochrome genes in Arabidopsis. Plant Physiol **127,** 1607-1616.

Tsuchida-Mayama, T., Sakai, T., Hanada, A., Uehara, Y., Asami, T., and Yamaguchi, S. (2010). Role of the phytochrome and cryptochrome signaling pathways in hypocotyl phototropism. Plant J.

Tsunoyama, Y., Morikawa, K., Shiina, T., and Toyoshima, Y. (2002). Blue light specific and differential expression of a plastid sigma factor, Sig5 in Arabidopsis thaliana. FEBS Lett **516,** 225-228.

Tu, D.C., Batten, M.L., Palczewski, K., and Van Gelder, R.N. (2004). Nonvisual photoreception in the chick iris. Science **306,** 129-131.

Ueno, K., Kinoshita, T., Inoue, S., Emi, T., and Shimazaki, K. (2005). Biochemical characterization of plasma membrane H+-ATPase activation in guard cell protoplasts of Arabidopsis thaliana in response to blue light. Plant Cell Physiol **46,** 955-963.

Usami, T., Mochizuki, N., Kondo, M., Nishimura, M., and Nagatani, A. (2004). Cryptochromes and phytochromes synergistically regulate Arabidopsis root greening under blue light. Plant Cell Physiol **45,** 1798-1808.

Valverde, F., Mouradov, A., Soppe, W., Ravenscroft, D., Samach, A., and Coupland, G. (2004). Photoreceptor regulation of CONSTANS protein in photoperiodic flowering. Science **303,** 1003-1006.

van der Horst, G.T., Muijtjens, M., Kobayashi, K., Takano, R., Kanno, S., Takao, M., de Wit, J., Verkerk, A., Eker, A.P., van Leenen, D., Buijs, R., Bootsma, D., Hoeijmakers, J.H., and Yasui, A. (1999). Mammalian Cry1 and Cry2 are essential for maintenance of circadian rhythms. Nature **398,** 627-630.

Van Gelder, R.N., Wee, R., Lee, J.A., and Tu, D.C. (2003). Reduced pupillary light responses in mice lacking cryptochromes. Science **299,** 222.

Veluchamy, S., and Rollins, J.A. (2008). A CRY-DASH-type photolyase/cryptochrome from Sclerotinia sclerotiorum mediates minor UV-A-specific effects on development. Fungal Genet Biol **45,** 1265-1276.

Vitaterna, M.H., Selby, C.P., Todo, T., Niwa, H., Thompson, C., Fruechte, E.M., Hitomi, K., Thresher, R.J., Ishikawa, T., Miyazaki, J., Takahashi, J.S., and Sancar, A. (1999). Differential regulation of mammalian period genes and circadian rhythmicity by cryptochromes 1 and 2. Proc Natl Acad Sci U S A **96,** 12114-12119.

Von Arnim, A.G., and Deng, X.-W. (1994). Light inactivation of Arabidopsis photomorphogenic repressor COP1 involves a cell-specific regulation of its nucleocytoplasmic partitioning. Cell **79,** 1035-1045.

Wang, H., and Deng, X.-W. (2002) Phytochrome Signaling Mechanism. In: The Arabidopsis Book. The American Society of Plant Biologists, Rockville, MD., doi: 10.1199/tab.0074.1, http://www.aspb.org/publications/arabidopsis/.

Wang, H., Ma, L.G., Li, J.M., Zhao, H.Y., and Deng, X.W. (2001). Direct interaction of Arabidopsis cryptochromes with COP1 in light control development. Science **294,** 154-158.

Ward, J.M., Cufr, C.A., Denzel, M.A., and Neff, M.M. (2005). The Dof transcription factor OBP3 modulates phytochrome and cryptochrome signaling in Arabidopsis. Plant Cell **17,** 475-485.

Weller, J.L., Perrotta, G., Schreuder, M.E., Van Tuinen, A., Koornneef, M., Giuliano, G., and Kendrick, R.E. (2001). Genetic dissection of blue-light sensing in tomato using mutants deficient in cryptochrome 1 and phytochromes A, B1 and B2. Plant J **25,** 427-440.

Whippo, C.W., and Hangarter, R.P. (2003). Second positive phototropism results from coordinated co-action of the phototropins and cryptochromes. Plant Physiol **132,** 1499-1507.

Worthington, E.N., Kavakli, I.H., Berrocal-Tito, G., Bondo, B.E., and Sancar, A. (2003). Purification and characterization of three members of the photolyase/cryptochrome family blue-light photoreceptors from Vibrio cholerae. J Biol Chem **278,** 39143-39154.

Wu, G., and Spalding, E.P. (2007). Separate functions for nuclear and cy-

toplasmic cryptochrome 1 during photomorphogenesis of Arabidopsis seedlings. Proc Natl Acad Sci U S A **104,** 18813-18818.

Wu, G., Cameron, J.N., Ljung, K., and Spalding, E.P. (2010). A role for ABCB19-mediated polar auxin transport in seedling photomorphogenesis mediated by cryptochrome 1 and phytochrome B. Plant J **62,** 179-191.

Wu, L., and Yang, H. (2010). CRYPTOCHROME 1 Is Implicated in Promoting R Protein-Mediated Plant Resistance to Pseudomonas syringae in Arabidopsis. Mol Plant **3,** 539-548.

Xu, P., Xiang, Y., Zhu, H., Xu, H., Zhang, Z., Zhang, C., Zhang, L., and Ma, Z. (2009). Wheat cryptochromes: subcellular localization and involvement in photomorphogenesis and osmotic stress responses. Plant Physiol **149,** 760-774.

Xu, X., Hotta, C.T., Dodd, A.N., Love, J., Sharrock, R., Lee, Y.W., Xie, Q., Johnson, C.H., and Webb, A.A. (2007). Distinct light and clock modulation of cytosolic free Ca2+ oscillations and rhythmic CHLOROPHYLL A/B BINDING PROTEIN2 promoter activity in Arabidopsis. Plant Cell **19,** 3474-3490.

Yadav, V., Kundu, S., Chattopadhyay, D., Negi, P., Wei, N., Deng, X.W., and Chattopadhyay, S. (2002). Light regulated modulation of Z-box containing promoters by photoreceptors and downstream regulatory components, COP1 and HY5, in Arabidopsis. Plant J **31,** 741-753.

Yamaguchi, R., Nakamura, M., Mochizuki, N., Kay, S.A., and Nagatani, A. (1999). Light-dependent translocation of a phytochrome B-GFP fusion protein to the nucleus in transgenic Arabidopsis. J Cell Biol **145,** 437-445.

Yang, H.-Q., Wu, Y.-J., Tang, R.-H., Liu, D., Liu, Y., and Cashmore, A.R. (2000). The C termini of Arabidopsis cryptochromes mediate a constitutive light response. Cell **103,** 815–827.

Yang, H.Q., Tang, R.H., and Cashmore, A.R. (2001). The signaling mechanism of Arabidopsis CRY1 involves direct interaction with COP1. Plant Cell **13,** 2573-2587.

Yang, J., Lin, R., Sullivan, J., Hoecker, U., Liu, B., Xu, L., Deng, X.W., and Wang, H. (2005). Light regulates COP1-mediated degradation of HFR1, a transcription factor essential for light signaling in Arabidopsis. Plant Cell **17,** 804-821.

Yang, Y.-J., Zuo, J.-C., Zhao, X.-Y., Lia, X., Klejnot, J., Li, Y., Chen, P.Y., Liang, S.-P., Yu, X.-H., Liu, X.-M., and Lin, C. (2008). Blue-light-independent activity of Arabidopsis cryptochromes in the regulation of steady-state levels of protein and mRNA expression. Molecular Plant **1,** 167–177.

Yanovsky, M.J., and Kay, S.A. (2002). Molecular basis of seasonal time measurement in Arabidopsis. Nature **419,** 308-312.

Yanovsky, M.J., Mazzella, M.A., and Casal, J.J. (2000). A quadruple photoreceptor mutant still keeps track of time. Curr Biol **10,** 1013-1015.

Yanovsky, M.J., Mazzella, M.A., Whitelam, G.C., and Casal, J.J. (2001). Resetting of the circadian clock by phytochromes and cryptochromes in Arabidopsis. J Biol Rhythms **16,** 523-530.

Yoshii, T., Ahmad, M., and Helfrich-Forster, C. (2009). Cryptochrome mediates light-dependent magnetosensitivity of Drosophila's circadian clock. PLoS Biol **7,** e1000086.

Young, M.W., and Kay, S.A. (2001). Time zones: a comparative genetics of circadian clocks. Nat Rev Genet **2,** 702-715.

Yu, J.W., Rubio, V., Lee, N.Y., Bai, S., Lee, S.Y., Kim, S.S., Liu, L., Zhang, Y., Irigoyen, M.L., Sullivan, J.A., Lee, I., Xie, Q., Paek, N.C., and Deng, X.W. (2008). COP1 and ELF3 control circadian function and photoperiodic flowering by regulating GI stability. Mol Cell **32,** 617-630.

Yu, X., Shalitin, D., Liu, X., Maymon, M., Klejnot, J., Yang, H., Lopez, J., Zhao, X., Bendehakkalu, K.T., and Lin, C. (2007a). Derepression of the NC80 motif is critical for the photoactivation of Arabidopsis CRY2. Proc Natl Acad Sci U S A **104,** 7289-7294.

Yu, X., Klejnot, J., Zhao, X., Shalitin, D., Maymon, M., Yang, H., Lee, J., Liu, X., Lopez, J., and Lin, C. (2007b). Arabidopsis cryptochrome 2 completes its posttranslational life cycle in the nucleus. Plant Cell **19,** 3146-3156.

Yu, X., Sayegh, R., Maymon, M., Warpeha, K., Klejnot, J., Yang, H., Huang, J., Lee, J., Kaufman, L., and Lin, C. (2009). Formation of nuclear bodies of Arabidopsis CRY2 in response to blue light is associated with its blue light-dependent degradation. Plant Cell **21,** 118-130.

Yuan, Q., Metterville, D., Briscoe, A.D., and Reppert, S.M. (2007). Insect cryptochromes: gene duplication and loss define diverse ways to construct insect circadian clocks. Mol Biol Evol **24,** 948-955.

Zeiger, E., and Hepler, P. (1977). Light and stomatal function: blue light stimulates swelling of guard cellmprotoplasts. Science **196,** 887-889.

Zhang, Q., Li, H., Li, R., Hu, R., Fan, C., Chen, F., Wang, Z., Liu, X., Fu, Y., and Lin, C. (2008). Association of the circadian rhythmic expression of GmCRY1a with a latitudinal cline in photoperiodic flowering of soybean. Proc Natl Acad Sci U S A **105,** 21028-21033.

Zhang, Y.C., Gong, S.F., Li, Q.H., Sang, Y., and Yang, H.Q. (2006). Functional and signaling mechanism analysis of rice CRYPTOCHROME 1. Plant J **46,** 971-983.

Zhu, D., Maier, A., Lee, J.H., Laubinger, S., Saijo, Y., Wang, H., Qu, L.J., Hoecker, U., and Deng, X.W. (2008a). Biochemical characterization of Arabidopsis complexes containing CONSTITUTIVELY PHOTOMORPHOGENIC1 and SUPPRESSOR OF PHYA proteins in light control of plant development. Plant Cell **20,** 2307-2323.

Zhu, H., Conte, F., and Green, C.B. (2003). Nuclear localization and transcriptional repression are confined to separable domains in the circadian protein CRYPTOCHROME. Curr Biol **13,** 1653-1658.

Zhu, H., Yuan, Q., Briscoe, A.D., Froy, O., Casselman, A., and Reppert, S.M. (2005). The two CRYs of the butterfly. Curr Biol **15,** R953-954.

Zhu, H., Sauman, I., Yuan, Q., Casselman, A., Emery-Le, M., Emery, P., and Reppert, S.M. (2008b). Cryptochromes define a novel circadian clock mechanism in monarch butterflies that may underlie sun compass navigation. PLoS Biol **6,** e4.