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CRYPTOCHROME STRUCTURE AND SIGNAL TRANSDUCTION

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■ **Abstract** Cryptochromes are photosensory receptors mediating light regulation of growth and development in plants. Since the isolation of the Arabidopsis *CRY1* gene in 1993, cryptochromes have been found in every multicellular eukaryote examined. Most plant cryptochromes have a chromophore-binding domain that shares similar structure with DNA photolyase, and a carboxyl terminal extension that contains a DQXVP-acidic-STAES (DAS) domain conserved from moss, to fern, to angiosperm. In Arabidopsis, cryptochromes are nuclear proteins that mediate light control of stem elongation, leaf expansion, photoperiodic flowering, and the circadian clock. Cryptochromes may act by interacting with proteins such as phytochromes, COP1, and clock proteins, or/and chromatin and DNA. Recent studies suggest that cryptochromes undergo a blue light–dependent phosphorylation that affects the conformation, intermolecular interactions, physiological activities, and protein abundance of the photoreceptors.

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INTRODUCTION

Cryptochromes are flavoproteins in plants and animals that share structural similarity to DNA photolyase but lack photolyase activity. Most cryptochromes act as blue/UV-A light receptors, although whether mammalian cryptochromes also act

as photoreceptors remains controversial (20, 64, 96). The meaning and definition of cryptochrome have undergone an interesting evolution in the past three decades. Cryptochrome was initially dubbed as a laboratory nickname for plant blue light receptors with action spectra comprising two peaks, one in the UV-A light region (~320–400 nm), and the other with fine structures in the blue light region (~400–500 nm) (35, 98). It was so called because blue light responses appeared prevalent in cryptogams (an old primary division of plants comprising those without true flowers and seeds, including fern, moss, algae, and fungi), and the molecular nature of blue light receptors was frustratingly cryptic at the time (97). It is intriguing that, prior to the isolation of the first cryptochrome gene, DNA photolyase was suspected to be a likely prototype of plant blue light receptors (71). It was also recognized at the time that different blue/UV-A light receptors might be evolutionarily unrelated, and therefore, a single nomenclature of cryptochrome might not be able to cover all the blue light receptors (98). Indeed, after the term cryptochrome was claimed for the photolyase-like blue light receptors (2, 20, 68), the molecular nature of another type of blue light receptors—the LOV domain–containing protein kinases that mediate phototropic responses—was identified (48). It is a bit ironic that although phototropism was one of the most prominent blue light responses at which our earlier hunt for cryptochromes was largely aimed (97), a different name, phototropin, had to be invented for the blue light receptors mediating this response (14, 16). It seems clear now that plants have at least two types of blue light receptors, phototropins that mediate photomovement responses including phototropism, chloroplast relocation, and stomatal opening (15, 63); and cryptochromes that act concurrently with phytochromes to mediate photomorphogenetic responses such as inhibition of stem elongation, stimulation of leaf expansion, control of photoperiodic flowering, entrainment of the circadian clock, and regulation of gene expression (20, 64).

Since isolating the first cryptochrome gene in 1993 (2), studies of cryptochromes have been extensively reviewed and commented on (1, 3, 16, 17, 19, 20, 26, 41, 46, 53, 61, 62, 64, 67, 96, 100, 111, 116). Some of these review articles provide detailed accounts of the general molecular characteristics and possible mode of actions of cryptochromes (20), the interesting history of the study of plant blue light receptors (16), and the diverse functions of these photoreceptors (64). In this article, we focus on some of the recent discoveries of cryptochromes, especially that of the two cryptochromes in Arabidopsis for which most studies of plant cryptochromes have been carried out. We also try to address two basic questions: What are the structural characteristics of cryptochromes, and what is our current understanding of the signal transduction mechanism of plant cryptochromes?

CRYPTOCHROME STRUCTURE

Our current understanding of cryptochrome structure derives mostly from analyses of cryptochrome sequences, studies of cryptochrome mutations, and investigation of recombinant cryptochromes in vitro or in transgenic plants. Sequence analyses

show that most plant cryptochromes have two domains, an N-terminal photolase-related (PHR) domain that shares sequence homology to DNA photolyase, and a C-terminal extension that is unrelated to photolyase. The PHR domain of cryptochrome is the chromophore-binding domain, whereas the C-terminal extension is important for the nuclear/cytosol trafficking and protein-protein interactions. The crystal structure of cryptochromes has not been solved, but the structure of photolyases is known. Given the sequence similarity between cryptochrome and photolyase, it is likely that the structure of at least the PHR domain of a cryptochrome may resemble that of a photolyase.

The Photolyase/Cryptochrome Gene Family

DNA PHOTOLYASES Members of the photolyase/cryptochrome gene family encode either light-dependent DNA repairing enzymes (photolyases) or photosensory receptors (cryptochromes). DNA photolyases are ~55–65-kD flavoproteins widely found in prokaryotes and eukaryotes, and they are thought to be the evolutionary progenitors of cryptochromes. DNA photolyases catalyze blue/UV-A light-dependent repair of DNA damages resulting from exposure to high-energy short-wavelength UV light (95, 109). There are two types of structurally related DNA photolyases: The photolyase or CPD photolyase repairs cyclobutane pyrimidine dimers, and the 6-4 photolyase repairs pyrimidine-pyrimidine 6-4 photoproducts (95, 107). Based on sequence similarity, the CPD photolyases are further divided into two classes, type I photolyase (mostly found in unicellular organisms) and type II photolyase (found in both unicellular and multicellular organisms) (95).

The reaction mechanism of photolyase has been well characterized (95, 96). All the photolyases studied contain two chromophores, a light-harvesting antenna chromophore, being either a folate (methenyltetrahydrofolate) or a deazaflavin, and a catalytic chromophore that is fully reduced flavin adenine dinucleotide ($FADH^-$). Using the antenna chromophore allows photolyases to repair UV-damaged DNA with high photon efficiency (95). A photolyase recognizes and binds to the CPD-containing DNA. The light-harvesting chromophore absorbs blue light and transfers the excited energy in the nonradiative form to the $FADH^-$. The excited $FADH^-$ passes an electron to the CPD substrate, and the resulting negative charge induces splitting of the cyclobutane ring of the CPD pyrimidine dimer. Subsequently, the electron moves back to flavin to regenerate the catalytic active flavin, and the DNA with the two neighboring pyrimidines restored is released from the photolyase (95). The crystal structure has been reported for photolyases from *Escherichia coli* and the cyanobacterium *Synechocyctis* sp PCC6301 (previously called *Anacystis nidulan*) (88, 105). These two photolyases, which share only ~30% amino acid sequence identity and use different light-harvesting chromophores (pterin or deazaflavin), have similar structures that are almost superimposible.

THE STRUCTURE OF PHOTOLYASE/CRYPTOCHROME One prominent feature of a photolyase structure is a positively charged groove running through the enzyme,

which is believed to be the DNA-binding site (88) (Figure 1). There is a cavity in the center of the groove opposing the flavin-binding site, and the CPD of a bound DNA can fit into the hole to accept the electron from the flavin. Despite the lack of the cryptochrome's crystal structure, computational protein modeling showed that the structure of a cryptochrome is similar to that of a photolyase (107). A structural model of the PHR domain of Arabidopsis cry2 is shown in Figure 1 (H. Nakamura, personal communication). Based on this model, the PHR domain of Arabidopsis cry2 is relatively hydrophobic, which appears to be consistent with the observation that Arabidopsis cry2 or PHR domain purified from heterologous expression systems tends to aggregate and precipitate (X. Yu & C. Lin, unpublished data). This hypothetical model shows that the positively charged groove found in photolyase is partially conserved in cry2, although the groove in cry2 appears shorter, wider, and with a relatively lower surface electrostatic potential than that of the photolyase (Figure 1). Such a positively charged groove structure may allow cry2 to bind to DNA. There also seems to be a cavity located in the predicted groove of cry2, which is larger in size compared to the CPD-binding cavity in photolyase (Figure 1). The concentrated positive charge around the relatively larger cavity in cry2 indicates that it is likely an important interface for interaction with other molecules such as DNA or proteins. It has been proposed that a redox reaction(s) is likely associated with photochemistry of cryptochromes (20). The cavity in cry2, like that in a photolyase, is positioned toward the FAD-binding site, which may allow an intermolecular redox reaction to occur. It is not clear how inclusion of the C-terminal extension may affect this structure model.

EVOLUTION OF THE PHOTOLYASE/CRYPTOCHROME GENE FAMILY Most plant species studied have multiple members of the photolyase/cryptochrome gene family. For example, Arabidopsis has at least four members of the photolyase/cryptochrome gene family, including two cryptochromes, one type II photolyase, and one 6-4 photolyase (20). Since the discovery of the first cryptochrome gene in Arabidopsis (2), cryptochromes have also been found in animals including Drosophila (30), fish (58), Xenopus (126), chicken (10), and human (47, 96, 108). An early phylogenetic analysis suggested that ancestral cryptochrome genes may have emerged before the divergence of prokaryotes and eukaryotes (53). Based on the observation that animal cryptochromes are more closely related to 6-4 photolyases, including the Arabidopsis 6-4 photolyase, than to the plant cryptochromes, and the lack of report of a prokaryotic cryptochrome at the time, it was argued that plant and animal cryptochromes might have arisen independently after the divergence of the two lineages in evolution (20). Recent phylogenetic studies and the discovery of a putative cryptochrome from a prokaryote appear to be more consistent with the earlier view (43, 58, 84, 107).

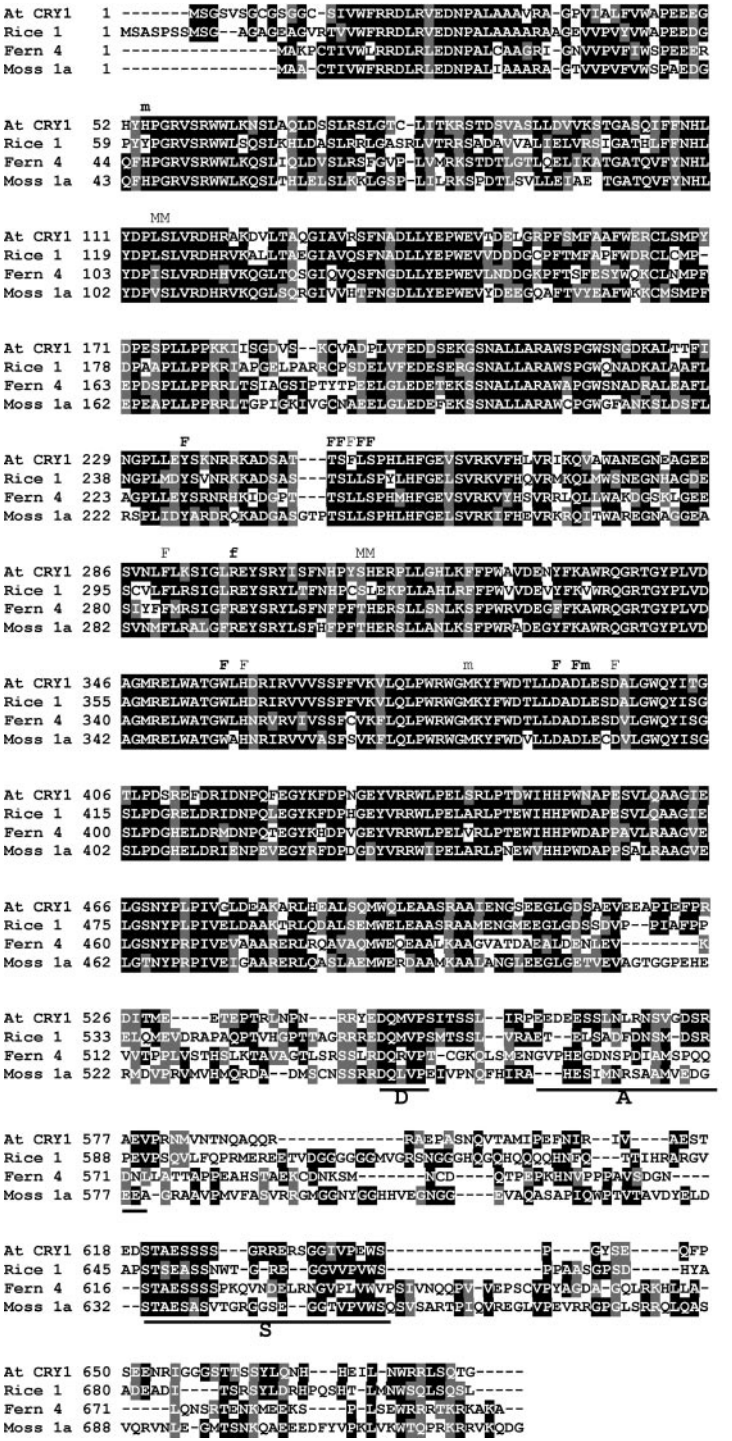
The photosynthetic cyanobacterium *Synechocyctis* sp PCC6803 contains two photolyase-like sequences, slr0854 and sll1629 (also called *phrA* and *phrB*, respectively) (43, 84). Neither gene product contains a C-terminal extension, but both noncovalently bind to partially oxidized FAD. A phylogenetic analysis suggests

slr0854 and sll1629 are more closely related to the type I CPD photolyase or animal cryptochromes (and 6-4 photolyases), respectively. The recombinant slr0854 gene product showed CPD photolyase activity in vitro, and expression of slr0854 rescued the photolyase-deficient *E. coli* mutant in a light-dependent manner. The wild-type cyanobacterial cells showed a white light-dependent resistance to UV-light irradiation, but red light irradiation had little effect. The cyanobacterial mutant disrupted in slr0854 was over a million times more sensitive to UV light than the wild type, confirming that the slr0854 gene product is a photolyase. In contrast, the sll1629 gene product showed no photolyase activity, in vitro or in *E. coli*, and the cyanobacterial mutant disrupted in sll1629 showed little defect in UV sensitivity (43, 84). The double mutant missing both slr0854 and sll1629 genes was only slightly more sensitive to UV than the monogenic slr0854 mutant. Because the sll1629 gene product had little photolyase activity, it may represent the first prokaryotic cryptochrome identified. A study of the genome-wide gene expression changes resulting from the sll1629 mutation suggests that sll1629 may regulate gene expression in *Synechocyctis* (K. Hitomi & E. Getzoff, personal communication).

It has been proposed that at least four gene duplication events may have occurred in evolution to give rise to the present-day photolyases and cryptochromes (107). The first gene duplication produced the ancestral type I CPD photolyase and type II CPD photolyases. The ancestral type I photolyase gene duplicated again to become the present-day type I photolyase and the progenitor of cryptochrome/6-4 photolyase. The progenitor cryptochrome/6-4 photolyase later duplicated a third time. One copy evolved to become the present-day cryptochromes in higher plants, whereas the other copy duplicated again to give rise to the 6-4 photolyases as well as the cryptochromes in the animal lineage.

PLANT CRYPTOCHROMES Cryptochromes have been found throughout the plant kingdom (Figures 2 and 3) (Table 1), including angiosperm, fern, moss, and algae (2, 11, 44, 50, 51, 54, 66, 69, 86, 90, 101, 115). Most plant cryptochromes are 70–80-kD proteins with two recognizable domains, an N-terminal PHR domain that shares sequence homology with photolyases, and a C-terminal extension that has little sequence similarity to any known protein domain (Figure 2) (Table 1). Most residues known to be important for flavin-binding in photolyase are conserved in cryptochromes, whereas residues of photolyases that are critical for the binding of the second chromophore and DNA lesions are less well conserved in cryptochromes (2, 51, 108) (Figure 2).

Arabidopsis has two cryptochrome genes, *CRY1* and *CRY2* (2, 44, 66). Tomato has at least three cryptochrome genes, *CRY1a*, *CRY1b*, and *CRY2* (90, 91). Fern and moss have at least five and at least two cryptochrome genes, respectively (49, 51, 54). The amino acid sequences of tomato CRY1 (CRY1a or CRY1b) and CRY2 are more similar to their Arabidopsis counterparts than to each other (90). As shown in Table 1, the PHR domain of tomato CRY1 is ∼85% identical to the PHR domain of Arabidopsis CRY1, but only ∼63% identical to that of the tomato CRY2. Similarly, the C-terminal extension of tomato CRY1 is ∼50% identical to


At CRY1 1 -------MSGSVSGCGSGGC-SIVWFRRDLRVEDNPALAAAVRA-GPVIALFVWAPEEEG
Rice 1 1 MSASPSSMSG---ACAGEAGVRTVVWFRRDLRVEDNPALAAAARAAGEVVPVYVWAPEEDG
Fern 4 1 ----------------MAKPCTIVWLRRDLRLEDNPALCAAGRI-GNVVPVFIWSPEEER
Moss 1a 1 ----------------MAA-CTIVWFRRDLRLEDNPALIAAARA-GTVVPVFVWSPAEDG
At CRY1 52 HYHPGRVSRWWLKNSLAQLDSSLRSLGTC-LITKRSTDSVASLLDVVKSTGASQIFFNHL
Rice 1 59 EYYPGRVSRWWLSQSLKHLDASLRRLGASRLVTRRSADAVVALIELVRSIGATHLFFNHL
Fern 4 44 QFHPGRVSRWWLKQSLIQLDVSLRSFGVP-LVMRKSTDTLGTLQELIKATGATQVFYNHL
Moss 1a 43 QFHPGRVSRWWLKQSLTHLELSLKKLGSP-LILRKSPDTLSVLLEIAE TGATQVFYNHL
At CRY1 111 YDPLSLVRDHRAKDVLTAQGIAVRSFNADLLYEPWEVTDELGRPFSMFAAFWERCLSMPY
Rice 1 119 YDPLSLVRDHRVKALLTAEGIAVQSFNADLLYEPWEVVDDDGCPFTMFAPFWDRCLCMPF
Fern 4 103 YDPISLVRDHHVKQGLTCSGIQVQSFNGDLLYEPWEVLNDDGKPFTSFESYWQKCLNMPF
Moss 1a 102 YDPVSLVRDHRVKQGLSQRGIVVHTFNGDLLYEPWEVYDEEGQAFTVYEAFWKKCMSMPF
At CRY1 171 DPESPLLPPKKIISGDVS--KCVADPLVFEDDSEKGSNALLARAWSPGWSNGDKALTTFI
Rice 1 178 DPAAPLLPPKRIAPGELPARRCPSDELVFEDESERGSNALLARAWSPGWQNADKALAAFL
Fern 4 163 EPDSPLLPPRRLTSIAGSIPTYTPPELGLEDETEKSSNALLARAWAPGWSNADRALEAFL
Moss 1a 162 EPEAPLLPPRRLTGPIGKIVGCNAEELGLEDEPEKSSNALLARAWCPGWGFANKSLDSFL
At CRY1 229 NGPLLEYSKNRRKADSAT---TSPLSPHLHFGEVSVRKVFHLVRIKQVAWANEGNEAGEE
Rice 1 238 NGPLMDYSVNRRKADSAS---TSLLSPYLHFGELSVRKVFHQVRMKQLMWSNEGNHAGDE
Fern 4 223 AGPLLEYSRNRHKIDGP----TSLLSPHMHFGEVSVRKVYHSVRRLQLLWAKDCSKLGEE
Moss 1a 222 RSPLIDYARDRQKADGASGTPTSLLSPHLHFGELSVRKIFHEVRKRQITWAREGNAGGEA
At CRY1 286 SVNLFLKSIGLREYSRYISFNHPYSHERPLLGHLKFFPWAVDENYFKAWRQGRTGYPLVD
Rice 1 295 SCVLFLRSIGLREYSRYLTFNHPCSLEKPLLAHLRFFPWVVDEVYFRVWRQGRTGYPLVD
Fern 4 280 SIYEFMRSIGFREYSRYLSFNFPFTHERSLLSNLKSFPWRVDEGFFKAWRQGRTGYPLVD
Moss 1a 282 SVNMFLRALGFREYSRYLSFHFPFTHERSLLANLKSFPWRADEGYFKAWRQGRTGYPLVD
At CRY1 346 AGMRELWATGWLHDRIRVVVSSFFVKVLQLPWRWGMKYFWDTLLDADLESDALGWQYITG
Rice 1 355 AGMRELWATGWLHDRIRVVVSSFFVKVLQLPWRWGMKYFWDTLLDADLESDALGWQYISG
Fern 4 340 AGMRELWATGWLHNRVRVIVSSFCVKFLQLPWRWGMKYFWDTLLDADLESDVLGWQYISG
Moss 1a 342 AGMRELWATGWAHNRIRVVVASFSVKFLQLPWRWGMKYFWDVLLDADLECDVLGWQYISG
At CRY1 406 TLPDSREFDRIDNPQFEGYKFDPNGEYVRRWLPELSRLPTDWIHHPWNAPESVLQAAGIE
Rice 1 415 SLPDGRELDRIDNPQLEGYKFDPHGEYVRRWLPELARLPTEWIHHPWDAPESVLQAAGIE
Fern 4 400 SLPDGHELDRMDNPQTEGYKHDPVGEYVRRWLPELVRLPTEWIHHPWDAPPAVLRAAGVE
Moss 1a 402 SLPDGHELDRIENPEVEGYRFDPDGDYVRRWIPELARLPNEWVHHPWDAPPSALRAAGVE
At CRY1 466 LGSNYPLPIVGLDEAKARLHEALSQMWQLEAASRAAIENGSEEGLGDSAEVEEAPIEPPR
Rice 1 475 LGSNYPLPIVELDAAKTRLQDALSEMWELEAASRAAMENGMEEGLGDSSDVP--PIAPPP
Fern 4 460 LGSNYPRPIVEVAAARERLRQAVAQMWEQEAALKAAGVATDAEALDPNLEV--------K
Moss 1a 462 LGTNYPRPIVEIGAARERLQASLAEMWERDAAMKAALANGLEEGLGETVEVAGTGGPEHE
At CRY1 526 DITME----ETEPTRLNPN---RFYEDQMVPSITSSL--IPPEDEDESSLNLRNSVGDSR
Rice 1 533 ELQMEVDRAPAQPTVHGPTTAGRRREDQMVPSMTSSL--VRAET--ELSADFDNSMGDSR
Fern 4 512 VVTPPLVSTHSLKTAVACTLSRSSLRDQRVPT-CGKQLSMENGVPHEGDNSPDIAMSPQQ
Moss 1a 522 RMDVPRVMVHQRDA--DMSCNSSRRDQLVPEIVPNQFHIRA---HESIMNRSAAMVEDG
At CRY1 577 AEVERNVVNTNQAQQR-------------RAEPLSNQVTAMIPEFNHR---IV----AEST
Rice 1 588 PEVESQVLFQPRMEREEVTVDGGGGGGMVGRSNGCGHQCQHQQQCHNFQ---TTIHRARGV
Fern 4 571 DNLLATTAPPEAHSTAEKCDNKSM--------NCD-----QTPEPKHNVPPPAVSDGN----
Moss 1a 577 EEA-GRAAVPMVFASVRFGMGGNYGGHHVEGNGC----EVAQASAPICWPTVTAVDYELD
At CRY1 618 EDSTAESSSS---GRRERSGGIVPEWS----------------P----GYSE------QFP
Rice 1 645 APSTSEASSNWT-GRRE---GGVVPVWS----------------PPAASGPSD-----HYA
Fern 4 616 --STAESSSSPKQVNDELRNGVPLVWVPSIVNQQPV-VEPSCVPYAGDA-GQLRKHLLA-
Moss 1a 632 --STAESASVTGRGGSE--GGTVPVWSQSVSARTPIQVREGLVEEVRRGPCLSRRQLQAS
At CRY1 650 SEENRIGGGSTTSSYLQNH---HEIL-NWRRLSQTG-----
Rice 1 680 ADEADI-----TSRSYLDRHPQSHT-LMNWSQLSQSL-----
Fern 4 671 -----LQNSRTENKMEEKS----P-LSEWRRRTKRKAKA--
Moss 1a 688 VQRVNLE-GMTSNKQAEEDFYVPKLVKWTQPRKRRVKQDG

that of Arabidopsis CRY1, but only ~15% identical to the C-terminal extension of tomato CRY2 (Table 1). In comparison, the PHR domain and the C-terminal extension of Arabidopsis CRY1 and CRY2 share only 58% or 13% amino acid sequence identity, respectively. This observation indicates that the gene duplication event resulting in plant *CRY1* and *CRY2* occurred at least 100 million years ago, before the divergence of *Brassicaceae* (e.g., Arabidopsis) and *Solanaceae* (e.g., tomato) (59, 90). However, it is not clear whether orthologs of both Arabidopsis *CRY1* and *CRY2* exist in other major branches of the plant kingdom. Two putative cryptochrome genes were found from a search of the newly completed rice genome (Figure 3) (Table 1) (34, 124). But both cryptochrome genes found in the rice genome are more similar to Arabidopsis *CRY1* than *CRY2* (Table 1). Although the putative cryptochrome sequences are also found in other monocot species, including maize and barley, most of those sequences are partial sequences derived from ESTs. A more detailed analysis of cereal cryptochromes would likely answer this question.

Due to its low abundance in general, the chromophore composition of cryptochromes has never been studied in holoproteins purified from any host organism. However, studies of recombinant cryptochromes expressed and purified from heterologous expression systems demonstrated that cryptochromes noncovalently bind to flavin ($FADH^-$) and possibly pterin [methenyltetrahydrofolate (MTHF)], and that the PHR domain is the chromophore-binding domain (68, 76). No photolyase activity was found for any plant cryptochromes tested (43, 51, 68, 76).

Different cryptochromes share a much higher sequence similarity in the PHR domain than in the C-terminal extension (51, 69, 90) (Table 1). For example,

Figure 2 An amino acid sequence alignment of four representative plant cryptochromes. The four cryptochromes were aligned using ClustalW. Black-boxed and gray-boxed letters represent identical or similar residues, respectively. The characters F/f and M/m above sequences indicate residues known to interact with FAD or methenyltetrahydrofolate (MTHF), respectively (53). Bolded letters **F**, **f**, **M**, and **m** indicate residues that are conserved between cryptochromes and photolyases; nonbold letters indicate those that are not conserved between cryptochromes and photolyases. Letters F and M indicate the positions in which the corresponding residues of *E. coli* photolyase form direct H bonds with the chromophores; f and m indicate positions in the photolyase that form water-mediated H bonds with the chromophores. The arrowhead indicates junctions between PHR domains and the C-terminal extensions (e.g., residue 494 in At-CRY1) used to calculate sequence similarities in Table 1. Underlines mark the DAS domain in the C-terminal extension. At CRY1: *Arabidopsis thaliana CRY1*, a representative of dycotyledon plants (accession AAB28724); Rice 1: *Oryza sativa CRY1a*, a representative of monocotyledon plants (BAB70686); Fern 4: *Adiantum capsillus-veneris CRY4*, a representative of ferns (BAA88425); Moss 1a: *Physcomitrella patens CRY1a*, a representative mosses (BAA83338).

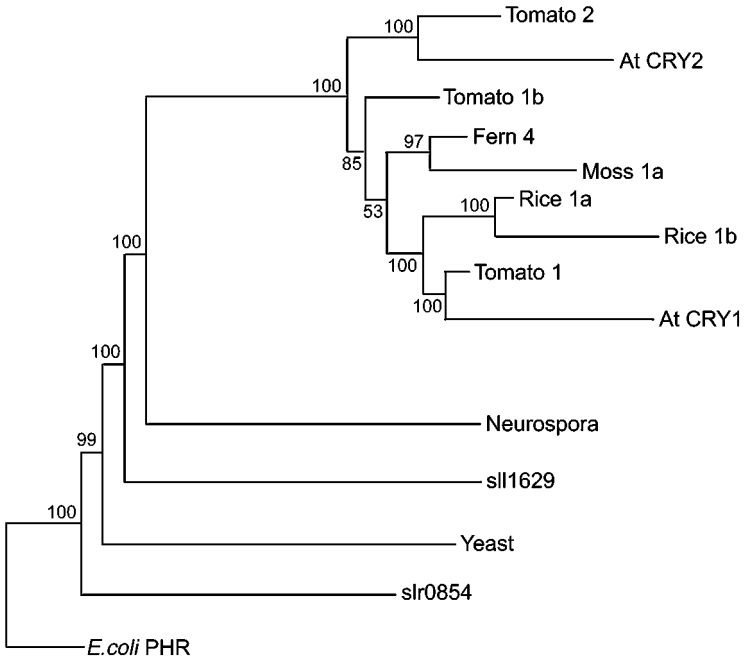


Figure 3 Sequence relatedness of photolyases and plant cryptochromes generated using the neighbor joining analysis software AliBee program (http://www.genebee.msu.su/services/malign_reduced.html). Numbers indicate bootstrap probabilities. Accession numbers are *E. coli* PHR (AAG55031); slr0854 (BAA17790, *Synechocystis* photolyase), Yeast (NP_015031, *Saccharomyces cerevisiae* photolyase); sll1629 (BAA17766, putative *Synechocystis* cryptochrome), Neurospora (S18667, *Neurospora crassa* photolyase); At CRY1 (AAB28724, Arabidopsis *CRY1*); At CRY2 (AAD09837, Arabidopsis *CRY2*); Tomato 1 (AAF72555, tomato *CRY1*); Tomato 2 (AAF72557, tomato *CRY2*); Rice 1a (BAB70686, rice *CRY1a*, also referred to as rice cry1); Rice 1b (BAB70688, rice *CRY1b*, also referred to as rice cry2); Moss 1a (BAA83338, *Physcomitrella patens CRY1a*); Fern 4 (BAA88425, *Adiantum capsillus-veneris CRY4*).

Arabidopsis CRY1 and CRY2 are 59% identical in the PHR domain. However the C-terminal extension of Arabidopsis CRY1 and CRY2 is only ∼13% identical, which is lower than that between the PHR domain of either Arabidopsis cryptochromes compared with the *E. coli* photolyase (∼30% identical) (Table 1). The size of the C-terminal extension of cryptochromes also varies significantly, from ∼380 amino acids long in algae (*Chlamydomonas*), 181 amino acids and 123 amino acids in Arabidopsis CRY1 and CRY2, respectively, to almost no C-terminal extension in the SH-PHR of the white mustard *Sinapis alba* (11, 76) and AcCRY5 of the fern *Adiantum capillus-veneris* (51).

TABLE 1

C-terminal extension

	At CRY1	At CRY2	Tomato 1	Tomato 2	Rice 1a	Rice 1b	Fern 4	Moss 1a
At CRY1		13/16	50/61	18/25	35/44	43/51	19/26	22/33
At CRY2	59/76		13/17	23/30	14/23	6/28	14/18	15/22
Tomato 1	85/92	61/77		15/23	42/50	47/57	23/32	23/32
Tomato 2	60/78	71/83	63/78		18/24	16/23	18/24	15/23
Rice 1a	75/83	59/70	76/84	57/72		64/71	21/31	7/16
Rice 1b	75/86	60/74	76/88	60/78	88/92		16/23	21/31
Fern 4	67/82	64/78	68/83	67/81	65/75	65/79		18/27
Moss 1a	62/79	63/78	64/81	64/80	63/73	63/79	71/84	
E.coli PHR	30/47	29/44	28/39	27/40	28/39	28/39	31/47	31/50

PHR

THE CRYPTOCHROME C-TERMINAL EXTENSIONS AND THE DAS DOMAIN Despite the lack of overall structural similarity in the C-terminal extensions of different cryptochromes, there are three recognizable motifs in this region of cryptochromes in most plants examined (64, 69). These motifs are (*a*) DQXVP near the amino end of the C-terminal extension, (*b*) a region containing a varying number of acidic residues (E or D), and (*c*) STAES followed by another conserved sequence, GGXVP, at the carboxyl end. Because these three motifs and their linear order are well conserved in plant cryptochromes from moss to Arabidopsis, the region of cryptochrome C-terminal extension containing these motifs is referred to as DAS (Figure 2) (64). The presence of the DAS sequence in cryptochromes from moss to angiosperm suggests that the evolutionary history of cryptochromes in plants is likely over 400 million years old, dating back to before the wide spread of vascular plants on the earth (55). It appears that the ancestral plant cryptochrome emerged from a gene fusion of a photolyase sequence to a DAS-containing sequence, which may or may not be lost during evolution. Genetic studies indicate that the DAS domain is important for cellular localization, intermolecular interaction, and physiological functions of a cryptochrome (Figure 4).

The Structure-Function Relationships of Plant Cryptochromes

Researchers have used two approaches to investigate the structure-function relationship of cryptochromes: one to analyze how mutations of individual amino acids may affect cryptochrome function, and the other to investigate how a fusion protein containing a marker enzyme fused to a partial or a full-length

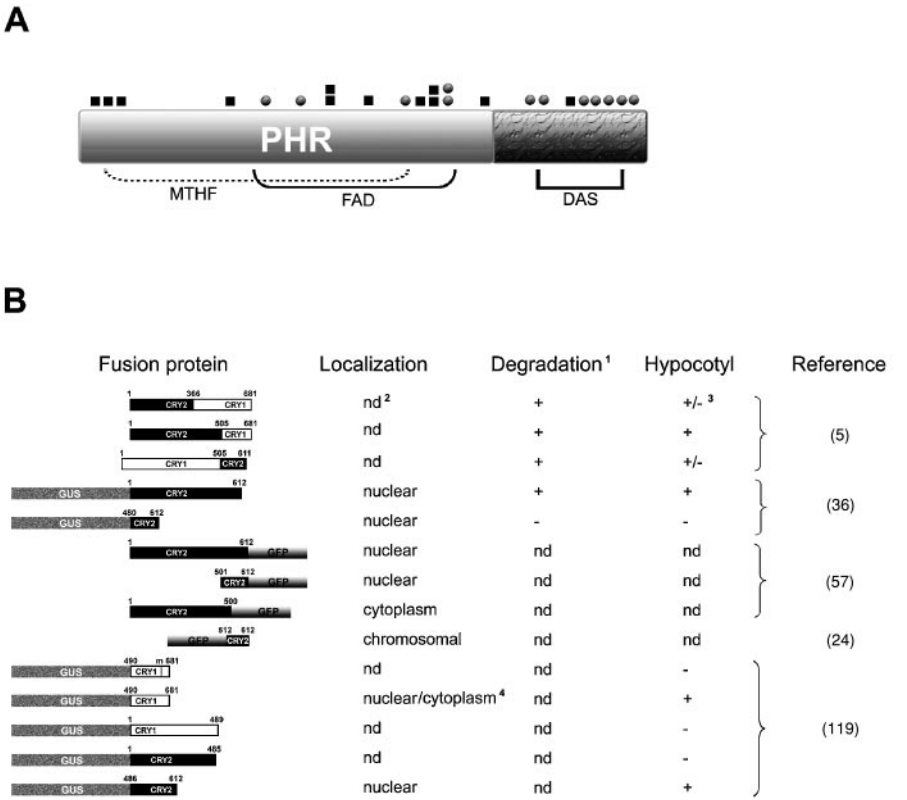


Figure 4 Structure-function relationships of cryptochromes. (*A*) Distribution of mutations found in Arabidopsis *CRY1* and *CRY2* genes. Arabidopsis cryptochromes are roughly divided into the PHR domains—and the C-terminal extensions—as indicated. The approximate FAD- and MTHF-binding regions and the DAS domains are shown by brackets. Circles and squares denote missense and in-frame-deletion mutations found in CRY1 (7) and CRY2 (H. Guo & C. Lin, unpublished data), respectively. (*B*) A summary of experiments showing how recombinant proteins of Arabidopsis CRY1 and CRY2 affect cellular localization, protein degradation, and blue light inhibition of hypocotyl elongation in transgenic plants. "1": degradation detected; "2": nd (not defined); "3": +/− means weak phenotypes detected; "4": CCT1 localizes in the nucleus in dark and in the cytoplasm in light. See references listed for details.

cryptochrome sequence may behave in planta. Figure 4 summarizes the results of some of the studies of Arabidopsis CRY1 and CRY2 with respect to how a cryptochrome's structure may affect its cellular localization, stability, and activity.

When the first approach was used to study Arabidopsis *CRY1* (7) and *CRY2* (H. Guo & C. Lin, unpublished data), missense mutations were found throughout

the coding sequence of both cryptochromes (Figure 4*A*). In addition to the expected mutations found in the PHR chromophore-binding domain, some of the missense mutations were found in the DAS domain of CRY1 and CRY2. These DAS-domain mutations have a similar effect on CRY1 function as that of the mutations in the chromophore-binding PHR domain, indicating the functional importance of the DAS domain. No specific region of the cryptochrome sequence was found to accumulate mutations at a significantly higher frequency. In this regard, cryptochromes seem different from the phytochrome for which a relatively higher frequency of mutations was found in a 160-residue region of the C-terminal domain (93). The lack of an apparent mutation-prone region of cryptochromes may simply be because the mutagenesis of cryptochromes has not been saturating. Alternatively, the overall protein conformation may be more important for the function of a cryptochrome.

NUCLEAR LOCALIZATION Arabidopsis cry1 and cry2 are nuclear proteins (20, 36, 57). It appears that Arabidopsis cry2 is more or less constitutively imported to the nucleus regardless of light treatment. However, Arabidopsis cry1 may be imported to the nucleus in the dark but may be exported or remain in the cytosol in response to light. It was found that the GUS-CCT1 (CRY1 C-terminus) fusion protein was mostly located in the nucleus in root hair cells of dark-grown transgenic plants, but the fusion protein was mostly cytosolic in the light-grown transgenic plants (119). Consistent with the notion that cry1 may be largely cytosolic in light-grown plants, the relative amount of cry1 detected in the nuclear extract obtained from the green tissue of light-grown Arabidopsis was significantly lower than that detected in the total protein extract (36). In contrast, the same nuclear extract was highly enriched for cry2 (36).

Although one may expect that the PHR domain of a cryptochrome would contain the nuclear localization signal (NLS), because DNA photolyase, the presumed ancestor of the PHR domain of cryptochromes, has to move into the nucleus to carry out its DNA-repairing function, the C-terminal extension is sufficient to direct nuclear transportation for both cryptochromes in Arabidopsis (24, 36, 57, 114, 119). A putative bipartite nuclear localization signal was found within the DAS domain of cry2, and fusion proteins of β-glucuronidase (GUS) to the C-terminal extension of cry2 are constitutively nuclear (24, 36, 57). Although no apparent bipartite NLS is found in CRY1, the C-terminal extension has proven sufficient for nuclear/cytoplasmic trafficking of CRY1 (114, 119).

The function of the C-terminal extension in directing subcellular localization of a cryptochrome is conserved in plants from fern to angiosperm. Among the five cryptochromes that have been identified in the fern *Adiantum*, two (CRY3 and CRY4) are apparently nuclear proteins (51). CRY3 is imported to the nucleus in dark or in red light, but it is mostly cytosolic in blue light, whereas CRY4 accumulates constitutively in the nucleus. Studies of the fusion proteins of GUS and the C-terminal extension of CRY3 and CRY4 indicate that, like Arabidopsis cryptochromes, the C-terminal extension also contains

signals regulating nuclear/cytosol trafficking of the fern cryptochromes (51).

CRYPTOCHROME DEGRADATION The Arabidopsis cry2 protein undergoes a rapid blue light–induced degradation (5, 36, 69). In etiolated seedlings exposed to blue light, the level of cry2 decreased to a new steady state in about 15 min after blue light exposure, which is less than 10% that of dark-grown seedlings (99). Both the PHR domain and the C-terminal extension appear to be important for the blue light–induced degradation of cry2 (Figure 4*B*). The GUS-CRY2C (residue 480-612 of cry2) fusion protein was stable in transgenic plants exposed to blue light, suggesting that the C-terminal extension of cry2 by itself is insufficient to mediate its degradation (36). However, using a domain-swap approach, both the C1C2 fusion protein (the PHR domain of CRY1 fused to the C-terminus of CRY2) and the C2C1 fusion protein (the PHR domain of CRY2 fused to the C-terminus of CRY1) were degraded in response to blue light (5) (Figure 4*B*). This result is consistent with a proposition that blue light–induced conformational change of cry2 may play an important role in triggering its degradation, and both the PHR domain and the C-terminal extension are required for appropriate turnover of Arabidopsis cry2.

It is not clear whether ubiquitination is associated with cry2 degradation. However, application of a proteosome inhibitor to Arabidopsis seedlings suppresses blue light–dependent cry2 degradation in vivo, indicating the involvement of this general protein degradation apparatus in the blue light regulation of cry2 turnover (D. Shalitin & C. Lin, unpublished data). It is worth pointing out that Drosophila cryptochrome has been reported to undergo a light-induced and proteosome-dependent degradation and that the light-dependent conformational change appears to be responsible for the degradation of the Drosophila cryptochrome (70).

CHROMATIN INTERACTION A recent report showed that Arabidopsis cry2 may be associated with chromosomes (24). When transgenic Arabidopsis plants expressing random GFP-cDNA fusions were examined for the subcellular localization of individual fusion proteins, one transgenic line that expressed GFP-CRY2C (CRY2 C-terminal extension) was identified. Interestingly, the GFP-CRY2C fusion protein was found to bind to all five chromosome pairs in a mitotic cell (24) (http://deepgreen.stanford.edu/). The cryptochrome-chromatin interaction can also be detected using the chromatin immunoprecipitation (ChIP) method (M. Ong & C. Lin, unpublished data). However, it remains to be determined whether the cryptochrome-chromatin interaction is involved with a direct cryptochrome-DNA interaction and whether cryptochromes bind to chromosomes via protein-protein interactions. It is now clear that cryptochromes can physically interact with other proteins associated with light signal transduction, for which the C-terminal extension of Arabidopsis cryptochromes often acts as the protein-protein interaction domain.

CRYPTOCHROME SIGNAL TRANSDUCTION

A major difficulty in addressing this question has been that, except for cryptochrome mutants, extensive genetic screens in Arabidopsis have not turned up other blue light–specific mutants (7, 9, 18, 73) (H. Guo & C. Lin, unpublished data). This result may be explained if genetic redundancy is associated with most genes involved in cryptochrome signaling. Alternatively, it may reflect a significant convergence between the signal transduction of cryptochromes and phytochromes. Consistent with the latter hypothesis, cryptochromes have been found to interact with phytochromes and other proteins such as COP1 known to be associated with blue light responses as well as phytochrome-mediated photomorphogenetic responses.

Mechanisms of Cryptochrome Signal Transduction

Based largely on genetics and photophysiology studies, it has been established that, in at least Arabidopsis, cryptochromes can mediate blue light inhibition of hypocotyl elongation, blue light modification of gene expression, photoperiodic flowering, and regulation of the circadian clock. Readers are referred to recent review articles and references for more detailed analyses of physiological roles of cryptochromes (16, 20, 40, 61, 62, 64, 82, 89, 96). In the following sections, we briefly discuss possible modes of action of cryptochromes to mediate various light responses and then describe molecular aspects of cryptochrome signal transduction.

DE-ETIOLATION De-etiolation refers to a collection of light-dependent developmental changes in germinating young seedlings, including inhibition of stem elongation, stimulation of leaf expansion, changes in gene expression, and induction of chloroplast development (31, 112). Cryptochromes are involved in mediating many, if not all, of the blue light–dependent de-etiolation responses (20, 64). For example, action spectra studies demonstrated that Arabidopsis cry1 and cry2 are the major photoreceptors mediating blue light inhibition of hypocotyl elongation (4, 123). The function of cryptochrome in mediating de-etiolation responses has also been reported in tomato (86, 115).

There are at least two ways a photoreceptor may trigger a developmental response. A photoreceptor may amplify a light signal via cytosolic second messages that provoke other cellular activities including regulation of gene expression. Alternatively, a nuclear photoreceptor may directly interact with a transcription or posttranscription regulatory apparatus to alter gene expression and developmental patterns. Currently, evidence exists for the involvement of both mechanisms in the cryptochrome-mediated de-etiolation responses.

Based on analyses of blue light effects on plasma membrane depolarization, anion channel activity, and growth inhibition kinetics, it was proposed that cryptochromes activate anion channel activity, resulting in plasma membrane

depolarization, and the inhibition of cell elongation (89, 104). This hypothesis may explain why Arabidopsis cry1, which is the primary blue light receptor mediating blue light inhibition of hypocotyl elongation, is exported to the cytosol in response to light, where it may regulate cytosolic or plasma membrane proteins. It was shown recently that the Arabidopsis *cry1*, *cry2*, and *phot1* mutants were similarly impaired in the blue light–induced membrane depolarization, suggesting that all three photoreceptors play a role in the regulation of blue light activation of anion channels (33). Indeed, all three photoreceptors may regulate leaf expansion via light-dependent control of plasma membrane anion channels, because defects in leaf expansion have been observed in not only cryptochrome mutants but also in phototropin mutants (65, 69, 94). On the other hand, blue light inhibition of hypocotyl elongation appears more complicated. The hypocotyl inhibition response as measured by hypocotyl length for seedlings grown in blue light, is significantly impaired in the *cry1* mutant, slightly affected in the *cry2* mutant, but almost unaffected in the *phot1* mutant (2, 69, 72). However, a high-resolution growth kinetics analysis showed that both *cry1* and *cry2* are similarly impaired in the slow phase (30–120 min after light on) of growth inhibition whereas *phot1* mutant is defective in the rapid phase (<30 min after light on) of growth inhibition (33). It was proposed that *phot1* might be associated with the initiation of blue light inhibition of growth, whereas cryptochromes are required for the maintenance of the growth inhibition (33, 89). Identification of genes encoding the specific anion channels regulated by cryptochromes and/or phototropins would help elucidate the cellular mechanisms underlying cryptochrome-dependent growth response.

In addition to calcium's possible involvement in the phytochrome signal transduction (13, 83), it may also be used as a second message for cryptochrome signal transduction (22, 37, 74). For example, when Arabidopsis cell culture was used to monitor blue/UV-A light–induced chalcone synthase (*CHS*) expression, blue light promoted calcium efflux in the cytosol, and compounds that inhibit voltage-gated calcium channel or Ca^{2+}-ATPase significantly altered blue/UV-A light–induced *CHS* expression (22, 74). An Arabidopsis cell culture system has been used to study how cryptochrome mediate blue/UV-A light–induced *CHS* expression (22, 74). In this system, cryptochomre-mediated *CHS* expression correlates with blue light promotion of calcium efflux in the cytosol. The involvement of calcium homeositasis in cryptochrome-mediated *CHS* expression was indicated by the observation that compounds that inhibit voltage-gated calcium channel or Ca^{2+}-ATPase significantly altered blue/UV-A light–induced *CHS* expression. A possible role of calcium homeostasis in cryptochrome signaling is consistent with a recent study of the Arabidopsis *SUB1* gene, which encodes a calcium-binding protein that acts downstream from cryptochromes in the hypocotyl inhibition response (37). However, a direct demonstration of whether and how cryptochromes act through calcium channels or Ca^{2+}-ATPase to regulate *CHS* gene expression or hypocotyl inhibition depends on the identification of the specific genes encoding those proteins and the corresponding mutations. Moreover, like the anion channel hypothesis discussed above, the role of calcium in cryptochrome function is also

complicated by phototropins. Using Ca^{2+} microelectrodes or transgenic plants expressing the calcium-dependent luminescent protein aequorin targeted to specific cellular compartments, it has been found that blue light stimulates a transient increase of cytosolic calcium (8, 12). However, the blue light–induced elevation of the cytosolic calcium is impaired only in the *phot1* and *phot1/phot2* mutants but not in the *cry1* or *cry2* mutants (8, 12). Since phot1 and phot2 are not the major photoreceptors regulating hypocotyl inhibition or *CHS* expression in response to blue light, the phototropin-dependent change in calcium homeostasis is not likely directly involved in those cryptochrome-dependent responses. It remains to be examined whether there is a more subtle change of calcium homeostasis associated with cryptochrome function.

Recent studies demonstrate that gene expression regulation is a major signaling mechanism underlying cryptochrome action. In Arabidopsis, cry1 and cry2 are known to regulate sets of similar genes in a partially redundant manner. A DNA microarray analysis demonstrated that the expression of about one third of Arabidopsis genes change in response to blue light, and cryptochromes are the major photoreceptors mediating these gene expression alterations (75). More than 71% of blue light–induced gene expressions and more than 40% of blue light–suppressed gene expressions are affected in etiolated *cry1cry2* double mutants exposed to blue light, suggesting the two photoreceptors regulate expression of these genes in response to blue light (75). The rest of the blue light–dependent gene expression change is probably mediated partly or completely by phyA (23, 106). It is unclear which genes regulated by cryptochromes are directly involved in individual reactions of the de-etiolation responses and how cryptochromes regulate gene expression. One possibility is that cryptochromes regulate transcriptional or posttranscriptional processes by interacting with the respective regulatory complexes in the nucleus (62). A direct test of this hypothesis requires identification of transcriptional or posttranscriptional regulators associated with cryptochrome-mediated light responses and a demonstration of how these regulators interact with cryptochromes.

PHOTOPERIODIC FLOWERING AND THE CIRCADIAN CLOCK Cryptochromes also work together with phytochromes to control photoperiodic flowering and the circadian clock (61). Genetic studies of the Arabidopsis *cry2* mutant have demonstrated that cry2 is a major photoreceptor regulating photoperiodic flowering in Arabidopsis (38). Taking together a large number of studies by various laboratories, it was proposed that cryptochromes and phytochromes act in both an antagonistic and redundant manner to regulate floral initiation (61, 80, 81). The molecular mechanism underlying cry2 promotion of floral initiation is not clear, although it may regulate expression of important flowering-time genes such as FT and *CO* (38, 82, 119a). Photoperiodic responses rely on photoreceptors to perceive changes of day length and on the circadian clock to memorize such changes. Investigations of how Arabidopsis cryptochrome and phytochrome mutants affect the circadian clock–controlled transcription demonstrated that phyA, phyB, cry1, and cry2 are all involved in the regulation of the circadian clock in Arabidopsis (102, 121, 125).

However, unlike mammalian cryptochromes, plant cryptochromes may not be the essential part of the central oscillator (27, 120). It remains to be examined how cryptochromes and phytochromes regulate the circadian clock.

Although each of the five phytochromes and two cryptochromes may potentially convey the day-length signal in Arabidopsis, cry2 and phyA are the only two photoreceptors for which a photoperiod-dependent change has been shown (29, 64, 80). Like other photoreceptors in Arabidopsis, the transcription of cry2 and phyA are regulated by the circadian clock (39, 110). But cry2 and phyA are the only ones that are known to be degraded in light, and their protein level in plants grown in photoperiod showed a largely diurnal rhythm. The cellular levels of cry2 and phyA proteins decline in daytime but increase in the evening, a phenomenon likely associated with their light-dependent degradation. More importantly, the diurnal rhythm of cry2 and phyA protein abundance is dependent on photoperiods: The daily rhythmic change of cry2 and phyA abundance is more pronounced in short days than in long days. The functional relevance of the diurnal rhythm of cry2 is clearly demonstrated in a recent study (29). In this elegant study, the early day–length insensitive (*EDI*) quantitative trait loci (QTL) of the Cvi accession (ecotype), collected in the tropical Cape Verde Islands, was mapped and cloned. The *EDI* locus, which is dominant and largely responsible for the photoperiod-insensitive early flowering trait of the Cvi accession, turned out to be the *CRY2* gene. *CRY2-Cvi* encodes the CRY2 protein with a methionine substitution for the valine at position 376 (V367M). Val^{376} was completely conserved among eight different cryptochrome genes compared except for *CRY2-Cvi*. Transgenic plants expressing the mutated *CRY2-Ler* (*CRY2* gene of L*er* accession) with the V367M substitution flowered earlier than L*er*, whereas plants expressing the mutated *CRY2-Cvi* with a M367V substitution flowered later than Cvi. This experiment confirmed that the single V367M substitution in the CRY2-Cvi protein is the cause of the photoperiodic-insensitive early flowering of the Cvi ecotype. Moreover, the V367M substitution of the CRY2 protein resulted in a change in the photoperiod-dependent diurnal cycling of CRY2 expression. The CRY2-Cvi type protein with the V367M substitution had a reduced amplitude of the diurnal rhythm of the cry2 abundance in short day, suggesting that the reduced diurnal rhythm of CRY2-Cvi protein abundance in response to photoperiods is responsible for the reduced day-length insensitivity and early flowering of Cvi plants.

Cryptochrome-Interacting Proteins

Direct protein-protein interaction(s) has been associated with almost every well-characterized signal transduction system. Cryptochrome signal transduction, despite the current lack of full understanding of its mechanism, is no exception. A convincing demonstration of the involvement of protein-protein interaction in cryptochrome signal transduction requires at least two pieces of evidence: identification of the interacting partner(s) and demonstration of the involvement of the interacting proteins in the biological processes mediated by cryptochromes. Both lines of evidence have been reported for Arabidopsis cryptochromes.

CRYPTOCHROME-COP1 INTERACTION It may be expected that if cryptochrome function is dependent on a direct protein-protein interaction, overexpression of the protein-protein interaction domain of a cryptochrome would result in competitive inhibition of the endogenous cryptochrome and dominant phenotypic changes in the transgenic plants. Indeed, transgenic overexpression of fusion proteins of GUS and cryptochrome C-terminal domains confer a dominant positive phenotype (119). Transgenic plants expressing GUS-CCT1 (CRY1 C-terminus) or GUS-CCT2 (CRY2 C-terminus) fusion proteins showed short hypocotyls, opened cotyledons, and increased anthocyanin accumulation when they were grown in dark or in light regardless of wavelength of illumination (119). These phenotypes are reminiscent of those found for the *cop/det/fus* mutants, although *cop/det/fus* mutants are recessive and often lethal, whereas the GUS-CCT expression caused a dominant but not lethal phenotype (21, 25, 119). Mutations in the CRY1 C-terminal domain, including E515K, E531K, and R576K, which had previously been shown to affect cry1 activity (7), eliminated the ability of fusion proteins to confer the *cop/det/fus* phenotype. Interestingly, transgenic plants expressing the GUS-CRY2C fusion protein, which contains seven additional residues at the amino terminus of CRY2C (CRY2 C-terminal domain) than GUS-CCT2 does, did not show the constitutive photomorphogenic phenotype (36). This could be due to the relatively lower level of expression of GUS-CRY2C compared to GUS-CCT2, or because the two different fusion proteins possess significantly different conformations due to the additional seven residues in GUS-CRY2C. Nevertheless, it was proposed that cryptochromes interact with COP1 or other COP/DET proteins in a light-dependent manner to suppress the activity of COP/DET proteins in wild-type plants (119). This hypothesis satisfactorily explains the phenotypes of the *cop1* mutant, the cryptochrome mutants, and the GUS-CCT transgenic plants (114, 119, 118). Several lines of evidence support this hypothesis. Full-length CRY1, CCT1, or GUS-CCT1 fusion proteins interact with COP1 in yeast two-hybrid assays (114, 118). Further evidence of the COP1-CRY1 interaction came from the observation that onion cells coexpressing GFP-CCT1 and COP1 exhibited fluorescent nuclear speckles, which were detected when GFP-COP1 was expressed alone but not when GFP-CCT1 was expressed alone (114). The cry1-COP1 interaction does not seem to be dependent on light. Therefore, protein-protein interaction alone is insufficient to explain cryptochrome-mediated responses. It is also noted that the recruitment of GFP-CCT1 to the nuclear speckles by coexpression of COP1 does not necessarily suggest that cry1 is associated with nuclear speckles because neither this study nor another study discussed later detected GFP-CRY1 in the nuclear speckles (78).

Cry2 may also interact with COP1. It was found that GUS-CCT2 interacted with COP1 in both yeast two-hybrid and coimmunoprecipitation assays (114), although CRY2-COP1 interaction was not detected using a similar yeast two-hybrid assay in another study (118). Transgenic plants expressing GUS-CCT1 or GUS-CCT2 fusion proteins accumulated more of the bZIP transcription factor HY5 in the dark, a finding that is similarly observed in the *cop1* mutant (87). Moreover, genome-wide gene expression profiles of dark-grown GUS-CCT1 and

GUS-CCT2 transgenic seedlings are also similar to that of blue light–grown wild-type seedlings or the dark-grown *cop1* mutant (114).

COP1 is a zinc-finger and WD40-repeat protein that has a light-regulated nucleocytoplasmic partitioning pattern similar to CRY1 in that both are enriched in the nucleus in the dark but are enriched in the cytosol in light (113, 119). COP1 has been proposed to act as a subunit of an E3-ubiquitin ligase complex associated with degradation of the bZIP transcription factor HY5 in the dark (87). These observations are consistent with a transcription regulation model for the cryptochrome-mediated de-etiolation response (114, 119). According to this model, COP1 interacts with HY5 in the dark to facilitate its degradation, ensuring the off status of light-induced gene expression and thus etiolated development. In light, photoactivated cry1 is excluded, together with COP1, from the nucleus, allowing an accumulation of the transcription factor HY5 and transcription activation for genes required for photomorphogenesis. It was further hypothesized that, although cryptochrome-COP1 interaction may be light-independent, absorption of light by the cryptochromes may result in an intramolecular redox reaction and a change of the cryptochrome conformation, which in turn leads to an alteration of the conformation or activity of the COP1 protein and developmental responses (118).

CRYPTOCHROME-PHYTOCHROME INTERACTION Arabidopsis cry2 directly interacts with phyB (78). The cry2-phyB interaction was shown by both yeast two-hybrid assays and coimmunoprecipitation tests. In addition, using fluorescent resonance energy transfer (FRET) microscopy, an intermolecular energy transfer was shown to occur between cry2-RFP and phyB-GFP fusion proteins, indicating that these two photoreceptors interact in vivo (78). Further evidence that cry2-phyB interaction is essential for the function of cry2 came from a finding that CRY2-RFP, but not CRY1-RFP, was colocalized with phyB in the nuclear speckles (78). In light of the recent discovery that phyB could mediate light regulation of transcription via its interaction with the transcription factor PIF3 (77, 85), the direct interaction between phyB and cry2 suggests that alteration of phytochrome-mediated regulation of transcription may be an important mechanism of cryptochrome signal transduction. In addition, cry1 has also been reported to interact, via its C-terminal domain, with phyA in a yeast two-hybrid assay (6). cry1 may also interact with phyB, at least indirectly, because cry1 and phyB can each interact with COP1 (118).

CRYPTOCHROME-ZTL/LKP1/ADO1 INTERACTION COP1 may not be the only protein that can interact with both phytochromes and cryptochromes. Arabidopsis CRY1 and PHYB both interact with ZTL/LKP1/ADO1 in yeast two-hybrid assays and in vitro pull-down tests (52, 56, 103). ZTL/LKP1/ADO1 is a PAS domain–containing protein that also possesses an F-box and six Kelch repeats that were originally identified in a study of the circadian clock in Arabidopsis (103). ZTL/LKP1/ADO1 plays an important role in regulating the

circadian clock and photoperiodic flowering in Arabidopsis. Mutations or overexpression of the *ZTL/LKP1/ADO1* gene caused an alteration of the free-running period of clock-controlled transcription, hypocotyl elongation, leaf movement, and photoperiodic flowering (52, 56, 103). The demonstration of direct interactions between cry1/phyB and ZTL/LKP1/ADO1 indicates that the input pathway from photoreceptors to the central oscillator may be short even though neither photoreceptor appears to be an integral part of the central oscillator.

ABOUT THE NUCLEAR SPECKLES It is intriguing that cry2, phyB, and COP1 not only physically interact with one another, but also associate with nuclear speckles (78, 114). Speckles are poorly defined nuclear substructures that are even less well understood in plant systems. In animal cells, nuclear speckles are most often associated with spliceosomes, although transcription factors, proteins associated with RNA export, and translation factors have also been found in the nuclear speckles (60, 79). Apparently, many intriguing questions remain to be addressed with respect to the function of nuclear speckle in plant cells and their association with photoreceptor functions. For example, what are the basic structure and function of plant nuclear speckles? Do nuclear speckles in plant cells share the similar protein composition and function as their counterparts in animal cells? And if so, what is the function of cryptochromes in nuclear speckles and how may transcription, RNA splicing, or RNA export be associated with cryptochrome regulation of plant growth and development?

Cryptochrome Phosphorylation

Light-dependent protein phosphorylation has been associated with almost every class of photoreceptors, including rhodopsins, phytochromes, and phototropins (16, 32, 45, 122). The first attempt to investigate whether cryptochromes may undergo light-dependent protein phosphorylation was reported in 1998 (6). In this study, a recombinant CRY1 protein was shown to be phosphorylated in vitro by the recombinant oat phyA protein. The in vitro phosphorylation of cry1 by phyA occurs similarly under either red light (30 μmole m^{-2} s^{-1}) or blue light (30 μmole m^{-2} s^{-1}), and to a lesser degree in the dark. The C-terminal domain but not the PHR domain of cry1 was phosphorylated by phyA in vitro. The recombinant C-terminal domain of some *cry1* mutants, including that of *hy4-9*, *hy4-22*, and one containing a deletion in the STAES motif showed decreased phosphorylation. The cry1 phosphorylation was also reported in vivo in transgenic plants overexpressing CRY1 in a red light–dependent and far red light–reversible manner, again suggesting an involvement of phytochromes in the phosphorylation of cry1.

Another attempt to investigate cryptochrome phosphorylation was reported recently (99). In this study, Arabidopsis cry2 underwent a blue light–dependent phosphorylation in vivo using both P^{32}-label and SDS-PAGE migration shift assays. The cry2-phosphorylation is dependent on blue light, because cry2

phosphorylation was not detected in etiolated seedlings, or in seedlings transferred to dark following the blue light treatment. In contrast to the study described previously for cry1 (6), the cry2 phosphorylation was not detected in seedlings exposed to red light. Moreover, normal cry2 phosphorylation was found to occur in every phytochrome mutant tested, including *phyA*, *phyB*, *phyAB*, *phyABD*, *phyBDE*, and *hy1*. A similar study showed that cry1 underwent a blue light–dependent phosphorylation. Similar to the cry2 phosphorylation, the cry1 phosphorylation was not affected by phytochrome mutations (D. Shalitin & C. Lin, unpublished data). Therefore, it appears that phytochromes are not responsible for the blue light–dependent cryptochrome phosphorylation. Recently, a mouse cryptochrome (mCRY1) was shown to undergo a phosphorylation catalyzed by the Casein kinase Iε (28). It would be interesting to test whether CKI-like protein kinases are involved in cryptochrome phosphorylation in Arabidopsis.

The lack of effect of phytochrome mutations on the cryptochrome phosphorylation is somewhat surprising because phytochrome has demonstrated protein kinase activity (32, 117, 122) and phytochromes can physically interact with cryptochromes (6, 78). However, phytochrome involvement in the cryptochrome phosphorylation can not be completely excluded. This is because, first, as far as the red light–induced in vivo cryptochrome phosphorylation is concerned, different experimental conditions employed in different studies, such as genotypes, age of plants, total fluence, etc., may explain the different results observed. Second, it is possible that there is a complete redundancy among the five different phytochromes in Arabidopsis, and any residual phytochrome activity may be sufficient to catalyze cryptochrome phosphorylation. This possibility could be tested when a phytochrome null mutant lacking all five phytochromes becomes available.

A kinetics analysis showed that cry2 phosphorylation is dependent on both the fluence rate of blue light and the time period in which the seedlings were exposed to blue light. The relative level of phosphorylated cry2 increased initially when etiolated seedlings were exposed to blue light, but decreased when the total fluence increased to a certain level. This result suggests that the phosphorylated cry2 may be degraded because it has been shown previously that cry2 is degraded in blue light, especially in high-fluence blue light (5, 36, 69). This hypothesis was supported by the observed correlation between an increase in the relative abundance of phosphorylated cry2 and a decrease in cry2 degradation in the *cop1-6* mutant that was impaired in cry2 degradation (99).

Protein phosphorylation affects not only cry2 regulation but also cry2 function. When the transgenic plants overexpressing GUS-CCT2 fusion protein were analyzed, it was found that, unlike the endogenous cry2 that is phosphorylated in response to blue light, the GUS-CCT2 fusion protein was constitutively phosphorylated (99). Given that the transgenic plants overexpressing GUS-CCT2 fusion protein exhibited a *cop/det/fus*-like constitutive photomorphogenic phenotype (119), it was suggested that the constitutively phosphorylated GUS-CCT2 fusion protein can constitutively trigger a photomorphogenic response. Based on these

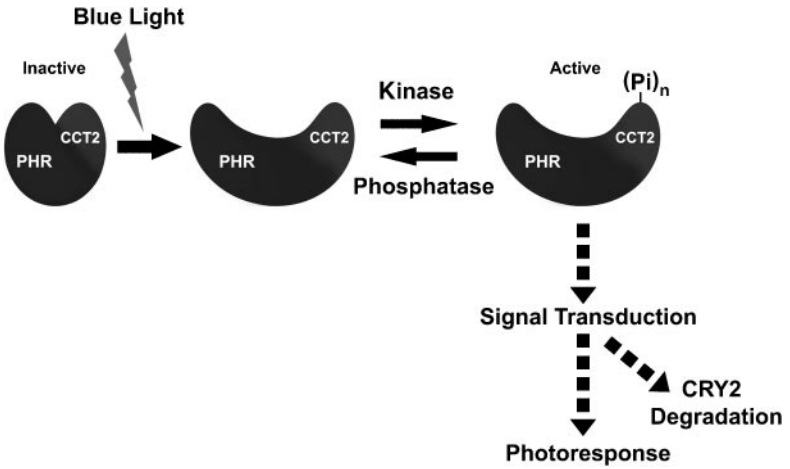


Figure 5 A hypothetical model for the action and regulation of Arabidopsis cry2.

results, a model was proposed to explain how cry2 phosphorylation is related to its function and regulation (Figure 5). According to this model, cry2 remains unphosphorylated, inactive, and stable in dark. Absorption of photons by cry2 changes its conformation, enabling phosphorylation of the photoreceptor by an unknown protein kinase. The phosphorylated cry2 is active, which triggers signal transduction and physiological responses. In the mean time, the phosphorylation of cry2 also marks it for degradation. The light-induced degradation of the active form of cry2 may serve to regulate its activity in the presence of light and to desensitize the photoreceptor in the absence of light.

CONCLUSIONS AND PERSPECTIVES

The past decade has witnessed exciting progresses in the study of plant blue light responses and blue light receptors. Characterization of cryptochromes in Arabidopsis and other plant species have revealed a general picture of the structural characteristics and likely signal transduction mechanisms of plant cryptochromes. At least in Arabidopsis, cryptochromes are nuclear proteins that function by interacting with other proteins to regulate gene expressions. Further investigation of the intermolecular interactions of cryptochromes, examination of how light affects these interactions, and characterization of the proteome composition and functions of nuclear speckles would likely shed more light on the mechanism of cryptochrome signal transduction. It is also expected that studies of cryptochromes, phytochromes, COP/DET/FUS proteins, and the circadian clock will converge to bring about a better understanding of the molecular mechanism of photomorphogenesis.

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LITERATURE CITED

1. Ahmad M. 1999. Seeing the world in red and blue: insight into plant vision and photoreceptors. *Curr. Opin. Plant Biol.* 2:230–35
2. Ahmad M, Cashmore AR. 1993. HY4 gene of *A. thaliana* encodes a protein with characteristics of a blue-light photoreceptor. *Nature* 366:162–66
3. Ahmad M, Cashmore AR. 1996. Seeing blue: the discovery of cryptochrome. *Plant Mol. Biol.* 30:851–61
4. Ahmad M, Grancher N, Heil M, Black RC, Giovani B, et al. 2002. Action spectrum for cryptochrome-dependent hypocotyl growth inhibition in Arabidopsis. *Plant Physiol.* 129:774–85
5. Ahmad M, Jarillo JA, Cashmore AR. 1998. Chimeric proteins between cry1 and cry2 arabidopsis blue light photoreceptors indicate overlapping functions and varying protein stability. *Plant Cell* 10:197–208
6. Ahmad M, Jarillo JA, Smirnova O, Cashmore AR. 1998. The CRY1 blue light photoreceptor of Arabidopsis interacts with phytochrome A *in vitro*. *Mol. Cell.* 1:939–48
7. Ahmad M, Lin C, Cashmore AR. 1995. Mutations throughout an Arabidopsis blue-light photoreceptor impair blue-light-responsive anthocyanin accumulation and inhibition of hypocotyl elongation. *Plant J.* 8:653–58
8. Babourina O, Newman I, Shabala S. 2002. Blue light-induced kinetics of H+ and Ca2+ fluxes in etiolated wild-type and phototropin-mutant Arabidopsis seedlings. *Proc. Natl. Acad. Sci. USA* 99:2433–38
9. Bagnall DJ, King RW, Hangarter RP. 1996. Blue-light promotion of flowering is absent in hy4 mutants of Arabidopsis. *Planta* 200:278–80
10. Bailey MJ, Chong NW, Xiong J, Cassone VM. 2002. Chickens' Cry2: molecular analysis of an avian cryptochrome in retinal and pineal photoreceptors. *FEBS Lett.* 513:169–74
11. Batschauer A. 1993. A plant gene for photolyase: An enzyme catalyzing the repair of UV-light-induced DNA damage. *Plant J.* 4:705–9
12. Baum G, Long JC, Jenkins GI, Trewavas AJ. 1999. Stimulation of the blue light phototropic receptor NPH1 causes a transient increase in cytosolic Ca(2+). *Proc. Natl. Acad. Sci. USA* 96:13554–59
13. Bowler C, Neuhaus G, Yamagata H, Chua N-H. 1994. Cyclic GMP and calcium mediate phytochrome phototransduction. *Cell* 77:73–81
14. Briggs WR, Beck CF, Cashmore AR, Christie JM, Hughes J, et al. 2001. The phototropin family of photoreceptors. *Plant Cell* 13:993–97
15. Briggs WR, Christie JM. 2002.

Phototropins 1 and 2: versatile plant blue-light receptors. *Trends Plant Sci.* 7: 204–10
16. Briggs WR, Huala E. 1999. Blue-light photoreceptors in higher plants. *Annu. Rev. Cell Dev. Biol.* 15:33–62
17. Briggs WR, Olney MA. 2001. Photoreceptors in plant photomorphogenesis to date: five phytochromes, two cryptochromes, one phototropin, and one superchrome. *Plant Physiol.* 125:85–88
18. Bruggemann E, Handwerger K, Essex C, Storz G. 1996. Analysis of fast neutron-generated mutants at the *Arabidopsis thaliana* locus. *Plant J.* 10:755–60
19. Cashmore AR. 1997. A cryptochrome family of photoreceptors. *Plant Cell Environ.* 20:764–67
20. Cashmore AR, Jarillo JA, Wu YJ, Liu D. 1999. Cryptochromes: blue light receptors for plants and animals. *Science* 284:760–65
21. Chory J, Peto C, Feinbaum R, Pratt L, Ausubel F. 1989. *Arabidopsis thaliana* mutant that develops as a light-grown plant in the absence of light. *Cell* 58:991–99
22. Christie JM, Jenkins GI. 1996. Distinct UV-B and UV-A/blue light signal transduction pathways induce chalcone synthase gene expression in Arabidopsis cells. *Plant Cell* 8:1555–67
23. Chun L, Kawakami A, Christopher DA. 2001. Phytochrome a mediates blue light and UV-A-dependent chloroplast gene transcription in green leaves. *Plant Physiol.* 125:1957–66
24. Cutler SR, Ehrhardt DW, Griffitts JS, Somerville CR. 2000. Random GFP:: cDNA fusions enable visualization of subcellular structures in cells of Arabidopsis at a high frequency. *Proc. Natl. Acad. Sci. USA* 97:3718–23
25. Deng X-W, Caspar T, Quail PH. 1989. COP1: a regulatory locus involved in light-controlled development and gene expression in Arabidopsis. *Genes Dev.* 5: 1172–82
26. Devlin PF, Kay SA. 1999. Cryptochromes–bringing the blues to circadian rhythms. *Trends Cell Biol.* 9:295–98
27. Devlin PF, Kay SA. 2000. Cryptochromes are required for phytochrome signaling to the circadian clock but not for rhythmicity. *Plant Cell* 12:2499–510
28. Eide EJ, Vielhaber EL, Hinz WA, Virshup DM. 2002. The circadian regulatory proteins BMAL1 and cryptochromes are substrates of casein kinase Iepsilon. *J. Biol. Chem.* 277:17248–54
29. El-Din El-Assal S, Alonso-Blanco C, Peeters AJ, Raz V, Koornneef M. 2001. A QTL for flowering time in Arabidopsis reveals a novel allele of CRY2. *Nat. Genet.* 29:435–40
30. Emery P, So WV, Kaneko M, Hall JC, Rosbash M. 1998. CRY, a Drosophila clock and light-regulated cryptochrome, is a major contributor to circadian rhythm resetting and photosensitivity. *Cell* 95:669–79
31. Fankhauser C, Chory J. 1997. Light control of plant development. *Annu. Rev. Cell Dev. Biol.* 13:203–29
32. Fankhauser C, Yeh KC, Lagarias JC, Zhang H, Elich TD, et al. 1999. PKS1, a substrate phosphorylated by phytochrome that modulates light signaling in Arabidopsis. *Science* 284:539–41
33. Folta KM, Spalding EP. 2001. Unexpected roles for cryptochrome 2 and phototropin revealed by high-resolution analysis of blue light-mediated hypocotyl growth inhibition. *Plant J.* 26:471–78
34. Goff SA, Ricke D, Lan TH, Presting G, Wang RL, et al. 2002. A draft sequence of the rice genome (Oryza sativa L. ssp. japonica). *Science* 296:92–100
35. Gressel J. 1979. Blue light photoreception. *Photochem. Photobiol.* 30:749–54
36. Guo HW, Duong H, Ma N, Lin CT. 1999. The Arabidopsis blue light receptor cryptochrome 2 is a nuclear protein

regulated by a blue light-dependent post-transcriptional mechanism. *Plant J.* 19 279–87
37. Guo HW, Mockler TC, Duong H, Lin CT. 2001. SUB1, an Arabidopsis Ca2+-binding protein involved in cryptochrome and phytochrome coaction. *Science* 291:487–90
38. Guo HW, Yang WY, Mockler TC, Lin CT. 1998. Regulation of flowering time by Arabidopsis photoreceptors. *Science* 279:1360–63
39. Harmer SL, Hogenesch JB, Straume M, Chang HS, Han B, et al. 2000. Orchestrated transcription of key pathways in arabidopsis by the circadian clock. *Science* 290:2110–13
40. Harmer SL, Panda S, Kay SA. 2001. Molecular bases of circadian rhythms. *Annu. Rev. Cell Dev. Biol.* 17:215–53
41. Hinnemann H. 1995. Some aspects of blue light research during the last decade. *Photochem. Photobiol.* 61:22–31
42. Hitomi K, Nakamura H, Kim ST, Mizukoshi T, Ishikawa T, et al. 2001. Role of two histidines in the (6-4) photolyase reaction. *J. Biol. Chem.* 276:10103–9
43. Hitomi K, Okamoto K, Daiyasu H, Miyashita H, Iwai S, et al. 2000. Bacterial cryptochrome and photolyase: characterization of two photolyase-like genes of Synechocystis sp. PCC6803. *Nucleic Acids Res.* 28:2353–62
44. Hoffman PD, Batschauer A, Hays JB. 1996. PHH1, a novel gene from Arabidopsis thaliana that encodes a protein similar to plant blue-light photoreceptors and microbial photolyases. *Mol. Gen. Genet.* 253:259–65
45. Holmes MG. 1991. *Photoreceptor Evolution and Function.* New York: Academic
46. Horwitz BA. 1994. Properties and transduction chains of UV and blue light photoreceptors. In *Photomorphogenesis in Plants,* ed. RE Kendrick, GHM Kronenberg, pp. 327–50. Boston: Kluwer Acad. 2nd ed.
47. Hsu DS, Zhao X, Zhao S, Kazantsev A, Wang RP, et al. 1996. Putative human blue-light photoreceptors hCRY1 and hCRY2 are flavoproteins. *Biochemistry* 35:13871–77
48. Huala E, Oeller PW, Liscum E, Han IS, Larsen E, et al. 1997. Arabidopsis NPH1: a protein kinase with a putative redox-sensing domain. *Science* 278:2120–23
49. Imaizumi T, Kadota A, Hasebe M, Wada M. 2001. Cryptochrome light signals control development to suppress auxin sensitivity in the moss *Physcomitrella patens*. *Plant Cell* 14:373–86
50. Imaizumi T, Kadota A, Hasebe M, Wada M. 2002. Cryptochrome light signals control development to suppress auxin sensitivity in the moss physcomitrella patens. *Plant Cell* 14:373–86
51. Imaizumi T, Kanegae T, Wada M. 2000. Cryptochrome nucleocytoplasmic distribution and gene expression are regulated by light quality in the fern adiantum capillus-veneris. *Plant Cell* 12:81–96
52. Jarillo JA, Capel J, Tang RH, Yang HQ, Alonso JM, et al. 2001. An Arabidopsis circadian clock component interacts with both CRY1 and phyB. *Nature* 410:487–90
53. Kanai S, Kikuno R, Toh H, Ryo H, Todo T. 1997. Molecular evolution of the photolyase-blue-light photoreceptor family. *J. Mol. Evol.* 45:535–48
54. Kanegae T, Wada M. 1998. Isolation and characterization of homologues of plant blue-light photoreceptor (cryptochrome) genes from the fern Adiantum capillus-veneris. *Mol. Gen. Genet.* 259:345–53
55. Kenrick P, Crane R. 1997. The origin and early evolution of plants on land. *Nature* 389:33–39
56. Kiyosue T, Wada M. 2000. LKP1 (LOV kelch protein 1): a factor involved in the regulation of flowering time in Arabidopsis. *Plant J.* 23:807–15

57. Kleiner O, Kircher S, Harter K, Batschauer A. 1999. Nuclear localization of the Arabidopsis blue light receptor cryptochrome 2. *Plant J.* 19:289–96
58. Kobayashi Y, Ishikawa T, Hirayama J, Daiyasu H, Kanai S, et al. 2000. Molecular analysis of zebrafish photolyase/cryptochrome family: two types of cryptochromes present in zebrafish. *Genes Cells* 5:725–38
59. Ku HM, Vision T, Liu J, Tanksley SD. 2000. Comparing sequenced segments of the tomato and Arabidopsis genomes: large-scale duplication followed by selective gene loss creates a network of synteny. *Proc. Natl. Acad. Sci. USA* 97: 9121–26
60. Lamond AI, Earnshaw WC. 1998. Structure and function in the nucleus. *Science* 280:547–53
61. Lin CT. 2000. Photoreceptors and regulation of flowering time. *Plant Physiol.* 123:39–50
62. Lin CT. 2000. Plant blue-light receptors. *Trends Plant Sci.* 5:337–42
63. Lin CT. 2002. Phototropin blue light receptors and light-induced movement responses in plants. *Science STKE* 2002:E5
64. Lin CT. 2002. Blue light receptors and signal transduction. *Plant Cell* 14(Suppl.):S207–25
65. Lin CT, Ahmad M, Cashmore AR. 1996. Arabidopsis cryptochrome 1 is a soluble protein mediating blue light-dependent regulation of plant growth and development. *Plant J.* 10:893–902
66. Lin CT, Ahmad M, Chan J, Cashmore AR. 1996. CRY2, a second member of the Arabidopsis cryptochrome gene family. *Plant Physiol.* 110:1047
67. Lin CT, Cashmore AR. 1996. Cryptochrome and plant photomorphogenesis. In *Regulation of Plant Growth and Development by Light*, ed. WR Briggs, RL Heath, EM Tobin, pp. 30–39. Rockville, MD: Am. Soc. Plant Physiol.
68. Lin CT, Robertson DE, Ahmad M, Raibekas AA, Jorns MS, et al. 1995. Association of flavin adenine dinucleotide with the Arabidopsis blue light receptor CRY1. *Science* 269:968–70
69. Lin CT, Yang HY, Guo HW, Mockler T, Chen J, Cashmore AR. 1998. Enhancement of blue-light sensitivity of Arabidopsis seedlings by a blue light receptor cryptochrome 2. *Proc. Natl. Acad. Sci. USA* 95:2686–90
70. Lin FJ, Song W, Meyer-Bernstein E, Naidoo N, Sehgal A. 2001. Photic signaling by cryptochrome in the Drosophila circadian system. *Mol. Cell. Biol.* 21:7287–94
71. Lipson ED, Horwitz BA. 1991. Photosensory reception and transduction. In *Sensory Receptors and Signal Transduction*, ed. JL Spudich, BH Satir. New York: Wiley-Liss. 64 pp.
72. Liscum E, Briggs W. 1995. Mutations in the *NPH1* locus of Arabidopsis disrupt the perception of phototropic stimuli. *Plant Cell* 7:473–85
73. Liscum E, Hangarter R. 1991. *Arabidopsis* mutants lacking blue light-dependent inhibition of hypocotyl elongation. *Plant Cell* 3:685–94
74. Long JC, Jenkins GI. 1998. Involvement of plasma membrane redox activity and calcium homeostasis in the UV-B and UV-A/blue light induction of gene expression in Arabidopsis. *Plant Cell* 10:2077–86
75. Ma LG, Li JM, Qu LJ, Hager J, Chen ZL, et al. 2001. Light control of Arabidopsis development entails coordinated regulation of genome expression and cellular pathways. *Plant Cell* 13:2589–607
76. Malhotra K, Kim ST, Batschauer A, Dawut L, 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* 34:6892–99
77. Martinez-Garcia JF, Huq E, Quail PH. 2000. Direct targeting of light signals to

a promoter element-bound transcription factor. *Science* 288:859–63
78. Mas P, Devlin PF, Panda S, Kay SA. 2000. Functional interaction of phytochrome B and cryptochrome 2. *Nature* 408:207–11
79. Mintz PJ, Spector DL. 2000. Compartmentalization of RNA processing factors within nuclear speckles. *J. Struct. Biol.* 129:241–51
80. Mockler T, Yang H, Yu X, Guo H, Parikh D, et al. 2003. Regulation of photoperiodic flowering by Arabidopsis photoreceptors. *Proc. Natl. Acad. Sci. USA* 100:2140–45
81. Mockler TC, Guo HW, Yang HY, Duong H, Lin CT. 1999. Antagonistic actions of Arabidopsis cryptochromes and phytochrome B in the regulation of floral induction. *Development* 126:2073–82
82. Mouradov A, Cremer F, Coupland G. 2002. Control of flowering time: interacting pathways as a basis for diversity. *Plant Cell* 14 (Suppl.):S111–30
83. Neuhaus G, Bowler C, Kern R, Chau N. 1993. Calcium/calmodulin-dependent and -independent phytochrome signal transduction pathways. *Cell* 73:937–52
84. Ng WO, Pakrasi HB. 2001. DNA photolyase homologs are the major UV resistance factors in the cyanobacterium Synechocystis sp. PCC 6803. *Mol. Gen. Genet.* 264:924–30
85. Ni M, Tepperman JM, Quail PH. 1998. PIF3, a phytochrome-interacting factor necessary for normal photoinduced signal transduction, is a novel basic helix-loop-helix protein. *Cell* 95:657–67
86. Ninu L, Ahmad M, Miarelli C, Cashmore AR, Giuliano G. 1999. Cryptochrome 1 controls tomato development in response to blue light. *Plant J.* 18:551–56
87. Osterlund MT, Hardtke CS, Wei N, Deng XW. 2000. Targeted destabilization of HY5 during light-regulated development of Arabidopsis. *Nature* 405:462–66
88. Park HW, Kim ST, Sancar A, Deisenhofer J. 1995. Crystal structure of DNA photolyase from *Escherichia coli*. *Science* 268:1866–72
89. Parks BM, Folta KM, Spalding EP. 2001. Photocontrol of stem growth. *Curr. Opin. Plant Biol.* 4:436–40
90. Perrotta G, Ninu L, Flamma F, Weller JL, Kendrick RE, et al. 2000. Tomato contains homologues of Arabidopsis cryptochromes 1 and 2. *Plant Mol. Biol.* 42:765–73
91. Perrotta G, Yahoubyan G, Nebuloso E, Renzi L, Giuliano G. 2001. Tomato and barley contains duplicated copies of cryptochrome 1. *Plant Cell Environ.* 24:991–97
92. Delete in proof
93. Quail PH, Boylan MT, Parks BM, Short TW, Xu Y, et al. 1995. Phytochromes: Photosensory perception and signal transduction. *Science* 268:675–80
94. Sakamoto K, Briggs WR. 2002. Cellular and subcellular localization of phototropin 1. *Plant Cell* 14:1723–35
95. Sancar A. 1994. Structure and function of DNA photolyase. *Biochemistry* 33:2–9
96. Sancar A. 2000. Cryptochrome: the second photoactive pigment in the eye and its role in circadian photoreception. *Annu. Rev. Biochem.* 69:31–67
97. Senger H. 1980. *The Blue Light Syndrome.* Berlin: Springer-Verlag
98. Senger H. 1984. Cryptochrome, some terminological thoughts. *Blue Light Effects in Biological Systems,* p. 72. Berlin: Springer-Verlag
99. Shalitin D, Yang HY, Mockler TC, Maymon M, Guo HW, et al. 2002. Regulation of Arabidopsis cryptochrome 2 by blue-light-dependent phosphorylation. *Nature* 417:763–67
100. Short TW, Briggs WR. 1994. The transduction of blue light signals in higher plants. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 45:143–71
101. Small DG, Min B, Lefebvre PA. 1995. Characterization of a *Chlamydomonas*

reinhardtii gene encoding a protein of the DNA photolyase/blue light photoreceptor family. *Plant Mol. Biol.* 28:443–54
102. Somers DE, Devlin PF, Kay SA. 1998. Phytochromes and cryptochromes in the entrainment of the Arabidopsis circadian clock. *Science* 282:1488–90
103. Somers DE, Schultz TF, Milnamow M, Kay SA. 2000. ZEITLUPE encodes a novel clock-associated PAS protein from Arabidopsis. *Cell* 101:319–29
104. Spalding EP. 2000. Ion channels and the transduction of light signals. *Plant Cell Environ.* 23:665–74
105. Tamada T, Kitadokoro K, Higuchi Y, Inaka K, Yasui A, et al. 1997. Crystal structure of DNA photolyase from Anacystis nidulans. *Nat. Struct. Biol.* 4:887–91
106. Thum KE, Kim M, Christopher DA, Mullet JE. 2001. Cryptochrome 1, cryptochrome 2, and phytochrome a co-activate the chloroplast psbD blue light-responsive promoter. *Plant Cell* 13:2747–60
107. Todo T. 1999. Functional diversity of the DNA photolyase/blue light receptor family. *Mutat. Res.* 434:89–97
108. Todo T, Ryo H, Yamamoto K, Toh H, Inui T, et al. 1996. Similarity among the Drosophila (6-4) photolyase, a human photolyase homolog, and the DNA photolyase-blue-light photoreceptor family. *Science* 272:109–12
109. Todo T, Takemori H, Ryo H, Ihara M, Matsunaga T, et al. 1993. A new photoreactivating enzyme that specifically repairs ultraviolet light-induced (6-4) photoproducts. *Nature* 361:371–74
110. Toth R, Kevei EE, Hall A, Millar AJ, Nagy F, et al. 2001. Circadian clock-regulated expression of phytochrome and cryptochrome genes in Arabidopsis. *Plant Physiol.* 127:1607–16
111. Van Gelder RN. 2002. Tales from the crypt(ochromes). *J. Biol. Rhythms* 17: 110–20
112. von Arnim A, Deng X-W. 1996. Light control of seedling development. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 47: 215–43
113. von Arnim AG, Deng X-W. 1994. Light inactivation of Arabidopsis photomorphogenic repressor COP1 involves a cell-specific regulation of its nucleocytoplasmic partitioning. *Cell* 79:1035–45
114. Wang H, Ma LG, Li JM, Zhao HY, Deng XW. 2001. Direct interaction of Arabidopsis cryptochromes with COP1 in light control development. *Science* 294:154–58
115. Weller JL, Perrotta G, Schreuder ME, Van Tuinen A, Koornneef M, et al. 2001. Genetic dissection of blue-light sensing in tomato using mutants deficient in cryptochrome 1 and phytochromes A, B1 and B2. *Plant J.* 25:427–40
116. Whitelam G. 1995. A green light for cryptochrome research. *Curr. Biol.* 5:1351–53
117. Wong YS, Cheng HC, Walsh DA, Lagarias JC. 1986. Phosphorylation of Avena phytochrome *in vitro* as a probe of light-induced conformational changes. *J. Biol. Chem.* 261:12089–97
118. Yang HQ, Tang RH, Cashmore AR. 2001. The signaling mechanism of Arabidopsis CRY1 involves direct interaction with COP1. *Plant Cell* 13:2573–87
119. Yang H-Q, Wu Y-J, Tang R-H, Liu D, Liu Y, et al. 2000. The C termini of Arabidopsis Cryptochromes mediate a constitutive light response. *Cell* 103:815–27
119a. Yanovsky MJ, Kay SA. 2002. Molecular basis of seasonal time measurement in Arabidopsis. *Nature* 419:308–12
120. Yanovsky MJ, Mazzella MA, Casal JJ. 2000. A quadruple photoreceptor mutant still keeps track of time. *Curr. Biol.* 10:1013–15
121. Yanovsky MJ, Mazzella MA, Whitelam GC, Casal JJ. 2001. Resetting of the circadian clock by phytochromes and cryptochromes in Arabidopsis. *J. Biol. Rhythms* 16:523–30
122. Yeh KC, Lagarias JC. 1998. Eukaryotic

phytochromes: light-regulated serine/threonine protein kinases with histidine kinase ancestry. *Proc. Natl. Acad. Sci. USA* 95:13976–81

123. Young JC, Liscum E, Hangarter RP. 1992. Spectral-dependence of light-inhibited hypocotyl elongation in photomorphogenic mutants of *Arabidopsis*: Evidence for a UV-A photosensor. *Planta* 188:106–14
124. Yu J, Hu SN, Wang J, Wong GKS, Li SG, et al. 2002. A draft sequence of the rice genome (Oryza sativa L. ssp. indica). *Science* 296:79–92
125. Zhong HH, Resnick AS, Straume M, McClung CR. 1997. Effects of synergistic signaling by phytochrome A and cryptochrome 1 on circadian clock-regulated catalase expression. *Plant Cell* 9:947–55
126. Zhu H, Green CB. 2001. A putative flavin electron transport pathway is differentially utilized in Xenopus CRY1 and CRY2. *Curr. Biol.* 11:1945–49

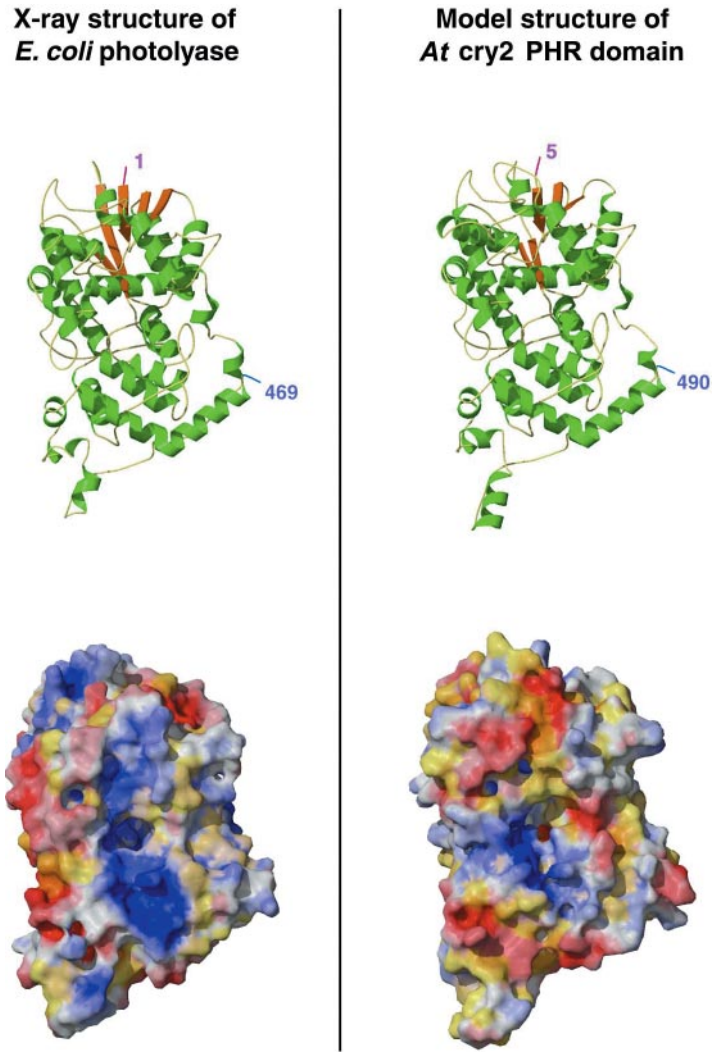

Figure 1 Structure of *E. coli* photolyase and a model structure of Arabidopsis cry2. (*A*) Structure of *E. coli* photolyase is shown in two forms, a ribbon diagram (*top*) and a solvent accessible surface and electrostatic surface potential diagram (*bottom*). (Atomic coordinates were from the Protein Data Bank, Accession number 1DNP). (*B*) A hypothetical structure of the Arabidopsis cry2 PHR domain (residue 5–490) modeled based on the crystal structure of *E. coli* photolyase. The amino acid sequence alignment and tertiary model construction was carried out by Nakamura using the method described previously (42). Colors indicate the surface electrostatic potential being above 0.1 Volt (blue), neutral (white), or below −0.1 Volt (red), or the solvent accessible surface that is hydrophobic (yellow).