

Photoreceptors and Associated Signaling II: Cryptochromes

Chentao Lin

University of California, Los Angeles, California, U.S.A.

INTRODUCTION

Blue light affects many aspects of plant growth and development. The blue light responses of plants can be roughly divided into two large categories: photomovement responses and photomorphogenetic responses. Photomovement responses, including phototropic curvature, chloroplast relocation, and stomata opening, are mediated by phototropins. Plant photomorphogenetic responses, including inhibition of hypocotyl elongation, stimulation of cotyledon expansion, regulation of flowering time, entrainment of the circadian clock, and regulation of gene expression, are controlled by both phytochromes (in response to red/far-red light) and cryptochromes (in response to blue/UV-A light).

CRYPTOCHROME GENES AND PROTEINS

The term cryptochrome was coined in the late 1970s as a laboratory nickname for blue/UV-A light receptors that mediate plant blue light responses with the specific action spectra of a peak in the UV-A region (approximately 350–400 nm) and a peak with fine structures in the blue region (approximately 400–500 nm). The compound word is composed of -chrome for “pigment” (from the Greek *chroma* meaning color or pigment) and crypto- as in “cryptic” or “cryptogam.” This term was chosen because the molecular nature of blue light receptors remained hidden (cryptic) at the time in spite of extensive researches, and because the blue light responses are prevalent in cryptogams (plants without true flowers and seeds, such as ferns, mosses, algae, and fungi).^[1]

Cryptochrome now refers to proteins that share sequence similarity to DNA photolyase but lack the photolyase activity. The first cryptochrome gene was isolated from *Arabidopsis thaliana*, which is a small weed widely used in laboratories because of its easy handling and small genome. In 1980, a number of *Arabidopsis* photomorphogenesis mutants were reported, one of which, called *hy4*, showed long hypocotyls when grown in blue light.^[2] The gene corresponding to the *hy4*

mutation was isolated about a decade later, and it was found to encode a protein of 681 amino acids, for which the N-terminal sequence of approximately 500 amino acids was 30% identical to that of *E. coli* DNA photolyase.^[3] DNA photolyases catalyze the blue/UV-A light-dependent cleavage of cyclobutane pyrimidine dimers that is a major type of DNA damage caused by short-wavelength (<300 nm) UV light.^[4] To investigate whether the *HY4* gene indeed encoded a blue light receptor, a full-length recombinant *HY4* gene was expressed in insect cells and the recombinant protein purified.^[5] The recombinant *HY4* gene product is a yellow-colored soluble protein that bound noncovalently to flavin adenine dinucleotide (FAD). Cryptochromes may also contain a pterin as the second chromophore.^[4] The facts that the *HY4* gene product mediates a blue light response, shares sequence similarity to a blue light-dependent enzyme, and contains FAD, which absorbs both blue light and UV-A light, as a prosthetic group, indicated that it is a blue light receptor. The *HY4* gene was renamed *CRY1* (for cryptochrome 1) in 1995.^[5]

Cryptochromes have been found throughout the plant kingdom, including among the angiosperms, ferns, mosses, and algae, and in animals including fishes, frogs, flies, mice, and humans.^[1,4,6] Most plant species studied contain multiple members of the photolyase/cryptochrome gene family. For example, *Arabidopsis* has two cryptochrome genes, *CRY1* and *CRY2*, and two photolyase genes; tomato and barley each have at least 3 cryptochrome genes, *CRY1a*, *CRY1b*, and *CRY2*; ferns and mosses have five and at least two cryptochrome genes, respectively.^[1] The amino acid sequences of tomato *CRY1* (*CRY1a* or *CRY1b*) and *CRY2* are more similar to their *Arabidopsis* counterparts than to each other, indicating that the gene duplication event resulting in *CRY1* and *CRY2* occurred more than 100 million years ago at least, before the divergence of Brassicaceae (e.g., *Arabidopsis*) and Solanaceae (e.g., tomato).

Most plant cryptochromes have two domains, an N-terminal domain called PHR (for photolyase-related) that shares sequence homology with DNA photolyase, and a C-terminal domain called CCT (for cryptochrome C-

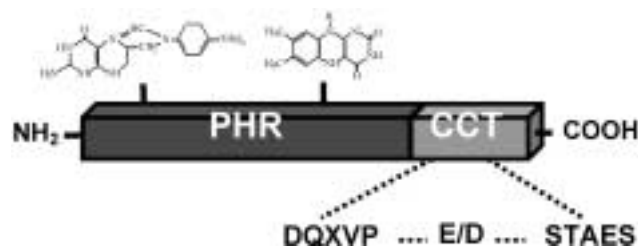


Fig. 1 Diagram depicting the structure of a plant cryptochrome. PHR: photolyase-related domain. CCT: Cryptochrome C-terminal domain. Motifs of the CCT domain are conserved in some plant cryptochromes, and the amino acids of these motifs are shown (X represents any amino acid). (View this art in color at www.dekker.com.)

terminus) that is unrelated to photolyase or any known proteins, although many plant cryptochromes contain conserved motifs in this domain (Fig. 1).^[1] The PHR domain of cryptochrome is evidently the chromophore-binding domain. The CCT domain is also required for the cryptochrome function. The reaction mechanism and 3-D structure of photolyases are well known,^[4] but neither has been extensively studied for cryptochromes. However, given the sequence similarity between cryptochrome and photolyase, at least some aspects of the cryptochrome structure and reaction mechanism may resemble that of a photolyase.

CRYPTOCHROMES MEDIATE VARIOUS PHOTOMORPHOGENETIC RESPONSES

Photomorphogenetic responses mediated by cryptochromes include blue light inhibition of stem elongation (Fig. 2), stimulation of leaf expansion, control of photoperiodic flowering, entrainment of the circadian clock, and regulation of gene expression.^[1,6] The functions of cryptochromes are conserved in different plants. It has been demonstrated that cryptochromes regulate hypocotyl inhibition in *Arabidopsis* and tomato. Different cryptochromes in the same plant can regulate the same light response. For example, in addition to CRY1, *Arabidopsis* CRY2 also contributes to the blue light inhibition of hypocotyl elongation response; and moss (*Physcomitrella patens*) CRY1a and CRY1b are both required for the blue light induction of side-branching on protonema. Different cryptochromes can also regulate distinct light responses. For instance, *Arabidopsis* CRY1 plays a more important role in hypocotyl inhibition, whereas *Arabidopsis* CRY2 is more involved in flowering-time control.

Although phytochromes are known to regulate photoperiodic flowering, it is now clear that cryptochromes also play significant roles in this response. The *Arabidopsis* laboratory strains Columbia (Col) and Landsberg *erecta* (Ler), both collected in the Northern Hemisphere, are nonobligate long-day plants that flower earlier in long days than in short days. The *cry2* mutants isolated from these strains flower later than the wild type in long days but not in short days, so these *cry2* mutants are late-flowering but more or less day-neutral.^[7] Another *Arabidopsis* ecotype, Cvi, collected from the tropical Cape Verde Islands, flowers earlier than many other *Arabidopsis* strains, and Cvi plants flower at about the same time in long days or in short days. The major QTL (quantitative trait locus) responsible for the day-neutral early flowering of the Cvi strain was determined to be the *CRY2* gene.^[8] A valine to methionine substitution (V367M) in the CRY2 protein of the Cvi strain was found to be responsible for its day-neutral early-flowering phenotype. A cryptochrome can act as a day-length sensor by changing its relative abundance in response to photoperiods. The abundance of *Arabidopsis* *cry2* protein shows a day-length-dependent diurnal rhythm. In short-day photoperiods, the level of CRY2 is lower in the day but higher in the night. In long-day photoperiods, such a diurnal rhythm of the CRY2 abundance is significantly diminished.^[1,8]



Fig. 2 *Arabidopsis* cryptochromes mediate blue light inhibition of hypocotyl elongation. *Arabidopsis* wild-type (col4) and *cry1cry2* double-mutant seedlings grown in continuous blue light for 5 days are shown. (View this art in color at www.dekker.com.)





Cryptochrome is a major photoreceptor regulating the circadian clock in both plants and animals. In animals, cryptochromes act redundantly with rhodopsins to regulate the circadian clock.^[4] In plants, the entrainment of the circadian clock is controlled by both phytochromes and cryptochromes.^[9] Although it has been shown that cryptochrome is a photoreceptor for the entrainment of the circadian clock in the fruit fly, whether cryptochromes also provide light entrainment to the circadian clock in mammals such as humans or mice is still being debated. Plant cryptochromes act mainly as photoreceptors rather than as critical components of the circadian clock; in contrast,^[9] mammalian cryptochromes are critical components of the central oscillator.^[4]

CRYPTOCHROME SIGNAL TRANSDUCTION

The initial photoreaction of cryptochrome remains unclear. Because electron transport is involved in the photolyase-catalyzed DNA repairing reaction, a redox reaction has been proposed to be likely involved in the cryptochrome photochemistry.^[6] It is also not clear what the signal transduction mechanism is underlying cryptochrome regulation of photomorphogenetic responses. It has been proposed that cryptochromes may act to change ion homeostasis in the cell, or that cryptochromes, which are often found in the nucleus, may regulate gene expression to alter developmental processes.^[11] And at least two types of biochemical reactions have been demonstrated in the cryptochrome function: protein-protein interactions and a blue light-induced cryptochrome phosphorylation.

Arabidopsis cryptochromes can interact with different proteins, including phytochrome B, COP1 (a putative subunit of E3 ubiquitin ligase complex known to be important for light-regulation of gene expression and hypocotyl inhibition), and ZTL (a PAS-domain-containing protein known to be important for the circadian clock and photoperiodic flowering).^[1] The functional significance of cry-COP1 interaction is demonstrated by the constitutive photomorphogenetic phenotype of *Arabidopsis* transgenic plants overexpressing CCT domain fusion proteins.^[10,11] The cry-phyB interaction may provide an explanation of why cry2 function is dependent on phyB.^[7,12] *Arabidopsis* cryptochromes have also been found to undergo a blue light-dependent phosphorylation, and the blue light-induced phosphorylation of *Arabidopsis* cry2 is important for its function and degradation.^[13] It has been proposed that cryptochromes are unphosphorylated and inactive in dark; blue light induces phosphorylation of cryptochromes by an unknown protein kinase; the phosphorylated cryptochrome becomes active in

triggering photomorphogenetic responses; and phosphorylation-induced cryptochrome degradation is a mechanism to desensitize the photoreceptor.^[13]

CONCLUSION

Cryptochromes are blue/UV-A light receptors regulating various photomorphogenetic responses of plants, but the detailed molecular mechanisms of signal transduction of cryptochromes is not clear at present. Investigation of the interaction among signaling processes of cryptochromes, phytochromes, and phytohormones will also be critical to our understanding of how plants regulate their developmental processes in response to light.

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ARTICLES OF FURTHER INTEREST

- Arabidopsis Thaliana Characteristics and Annotation of a Model Genome*, p. 47
- Circadian Clock in Plants*, p. 278
- Photoperiodism and the Regulation of Flowering*, p. 877
- Photoreceptors and Associated Signaling I: Phytochromes*, p. 881
- Photoreceptors and Associated Signaling III: Phototropins*, p. 889
- Photoreceptors and Associated Signaling IV: UV Receptors*, p. 893
- Phytochrome and Cryptochrome Functions in Crop Plants*, p. 920

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