pated. LXRα is an oxysterol receptor that serves as a cholesterol rheostat, regulating the conversion of excess cholesterol to bile acids for removal from the body. The finding that FXR is a bile acid receptor suggests that this orphan also contributes to cholesterol homeostasis. The discovery that PXR and CAR are steroid receptors that modulate the expression of steroid hydroxylases suggests a mechanism for regulating the amounts of steroid hormones. Thus, these orphan receptors not only function as steroid receptors but also regulate key steps in steroid metabolism. A final theme is that orphan receptors represent a tremendous opportunity in terms of understanding and treating human disease. Historically, nuclear receptors have been important drug targets. The discovery that some orphan receptors regulate key metabolic pathways suggests that they will be useful targets for intervention in disease processes. We now know that the inadvertent activation of other orphan receptors can contribute to detrimental side effects of drugs. Thus, knowledge of orphan receptor signaling pathways will be important both for the discovery of new drugs and for minimizing the side effects of these compounds.

The first decade of orphan nuclear receptor research has yielded a large number of new family members and many tantalizing clues as to their biological functions. However, ligands have been identified for only a handful of the orphans. Given the large number of remaining orphan nuclear receptors and the recent advances in combinatorial chemistry, high-throughput screening, and functional genomics, the next decade of reverse endocrinology promises an explosion in our understanding of nuclear hormone signaling pathways.

### References and Notes


1. R. M. Evans, *Science* **240**, 889 (1988); M. Beato, P. Herrlich, G. Schütz, *Cell* **83**, 851 (1995).
2. D. J. Mangelsdorf *et al.*, *Cell* **83**, 835 (1995).
3. B. O'Malley, *Mol. Endocrinol.* **4**, 363 (1990).
4. D. J. Mangelsdorf and R. M. Evans, *Cell* **83**, 841 (1995); P. Kastner, M. Mark, P. Chambon, *ibid.*, p. 859; E. Enmark and J.-A. Gustafsson, *Mol. Endocrinol.* **10**, 1293 (1996); P. J. Willy and D. J. Mangelsdorf, in *Hormones and Signaling*, B. W. O'Malley, Ed. (Academic Press, San Diego, 1998), vol. 1, pp. 307–358; B. Blumberg and R. M. Evans, *Genes Dev.* **12**, 3149 (1998).
5. R. A. Heyman *et al.*, *Cell* **68**, 397 (1992); A. A. Levin *et al.*, *Nature* **355**, 359 (1992).
6. T. Lemberger, B. Desvergne, W. Wahli, *Annu. Rev. Cell Dev. Biol.* **12**, 335 (1996).
7. I. Isseman and S. Green, *Nature* **347**, 645 (1990).
8. B. Staels and J. Auwerx, *Curr. Pharm. Des.* **3**, 1 (1997).
9. S. S.-T. Lee *et al.*, *Mol. Cell. Biol.* **15**, 3012 (1995); J. M. Peters *et al.*, *J. Biol. Chem.* **272**, 27307 (1997); P. Costet *et al.*, *ibid.* **273**, 29577 (1998).
10. B. M. Spiegelman, *Diabetes* **47**, 507 (1998).
11. J. Lehmann *et al.*, *J. Biol. Chem.* **270**, 12953 (1995); T. M. Willson *et al.*, *J. Med. Chem.* **39**, 665 (1996).
12. A. Chawla, E. J. Schwartz, D. D. Dimaculangan, M. A. Lazar, *Endocrinology* **135**, 798 (1994); P. Tontonoz, E. Hu, R. A. Graves, A. I. Budavari, B. M. Spiegelman, *Genes Dev.* **8**, 1224 (1994).
13. P. Tontonoz, E. Hu, B. M. Spiegelman, *Cell* **79**, 1147 (1994); E. Hu, P. Tontonoz, B. M. Spiegelman, *Proc. Natl. Acad. Sci. U.S.A.* **92**, 9856 (1995).
14. M. Göttlicher, E. Widmark, Q. Li, J.-A. Gustafsson, *Proc. Natl. Acad. Sci. USA* **89**, 4653 (1992); G. Krey *et al.*, *J. Steroid Biochem. Mol. Biol.* **47**, 65 (1993).
15. K. Yu *et al.*, *J. Biol. Chem.* **270**, 23975 (1995); B. Forman *et al.*, *Cell* **83**, 803 (1995); B. M. Forman, J. Chen, R. M. Evans, *Proc. Natl. Acad. Sci. U.S.A.* **94**, 4312 (1997); S. A. Kliewer *et al.*, *Cell* **83**, 813 (1995); S. A. Kliewer *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **94**, 4318 (1997); G. Krey *et al.*, *Mol. Endocrinol.* **11**, 779 (1997).
16. L. Nagy, P. Tontonoz, J. G. A. Alvarez, H. Chen, R. M. Evans, *Cell* **93**, 229 (1998).
17. R. T. Nolte *et al.*, *Nature* **395**, 137 (1998); J. Uppenberg *et al.*, *J. Biol. Chem.* **273**, 31108 (1998); E. Xu *et al.*, *Mol. Cell*, in press.
18. P. Tontonoz, L. Nagy, J. G. A. Alvarez, V. A. Thomazy, R. M. Evans, *Cell* **93**, 241 (1998); M. Ricote *et al.*, *Proc. Natl. Acad. U.S.A.* **95**, 7614 (1998).
19. M. Ricote, A. C. Li, T. M. Willson, C. J. Kelly, C. K. Glass, *Nature* **391**, 79 (1998); C. Jiang, A. T. Ting, B. Seed, *ibid.*, p. 82; B. Staels *et al.*, *ibid.* **393**, 790 (1998).
20. S. Altiok, M. Xu, B. M. Spiegelman, *Genes Dev.* **11**, 1987 (1997); P. Tontonoz *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **94**, 237 (1997); E. Mueller *et al.*, *Mol. Cell* **1**, 465 (1998); P. Sarraf *et al.*, *Nature Med.* **4**, 1046 (1998).
21. A.-M. Lefebvre *et al.*, *Nature Med.* **4**, 1053 (1998); E. Saez *et al.*, *ibid.*, p. 1058.
22. R. Apfel *et al.*, *Mol. Cell. Biol.* **14**, 7025 (1994); P. J. Willy *et al.*, *Genes Dev.* **9**, 1033 (1995).
23. J. M. Lehmann *et al.*, *J. Biol. Chem.* **272**, 3137 (1997).
24. B. A. Janowski, P. J. Willy, T. R. Devi, J. R. Falck, D. J. Mangelsdorf, *Nature* **383**, 728 (1996); B. Forman, B. Ruan, J. Chen, G. Schroepfer, R. Evans, *Proc. Natl. Acad. Sci. U.S.A.* **94**, 10588 (1997); B. A. Janowski *et al.*, *ibid.* **96**, 266 (1999).
25. T. A. Spencer, *Acc. Chem. Res.* **27**, 83 (1994).
26. D. Peet *et al.*, *Cell* **93**, 693 (1998).
27. C. Song, J. Kokontis, R. Hiipakka, S. Liao, *Proc. Natl. Acad. U.S.A.* **91**, 10809 (1994); D. Shinar *et al.*, *Gene* **147**, 273 (1994); M. Teboul *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **92**, 2096 (1995).
28. W. Seol, H.-S. Choi, D. Moore, *Mol. Endocrinol.* **9**, 72 (1995).
29. P. Maurel, in *Cytochromes P450: Metabolic and Toxicological Aspects*, C. Ioannides, Ed. (CRC Press, Boca Raton, FL, 1996), pp. 241–270.
30. S. A. Kliewer *et al.*, *Cell* **92**, 73 (1998); J. M. Lehmann *et al.*, *J. Clin. Invest.* **102**, 1016 (1998); G. Bertilsson *et al.*, *Proc. Natl. Acad. U.S.A.* **95**, 12208 (1998).
31. B. Blumberg *et al.*, *Genes Dev.* **12**, 3195 (1998).
32. M. Baes *et al.*, *Mol. Cell. Biol.* **14**, 1544 (1994).
33. B. Forman *et al.*, *Nature* **395**, 612 (1998).
34. P. Honkakoski, I. Zelko, T. Sueyoshi, M. Negishi, *Mol. Cell. Biol.* **18**, 5652 (1998).
35. B. M. Forman *et al.*, *Cell* **81**, 687 (1995).
36. A. M. Zavacki *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **94**, 7909 (1997).
37. D. Parks and J. M. Lehmann, unpublished results; D. D. Moore, personal communication; D. J. Mangelsdorf, personal communication.

38. We thank R. Buckholz, C. Hart, and J. Oberfield for comments on the manuscript.

REVIEW

# Cryptochromes: Blue Light Receptors for Plants and Animals

**Anthony R. Cashmore,* Jose A. Jarillo, Ying-Jie Wu, Dongmei Liu**

Cryptochromes are blue, ultraviolet-A photoreceptors. They were first characterized for *Arabidopsis* and are also found in ferns and algae; they appear to be ubiquitous in the plant kingdom. They are flavoproteins similar in sequence to photolyases, their presumptive evolutionary ancestors. Cryptochromes mediate a variety of light responses, including entrainment of circadian rhythms in *Arabidopsis*, *Drosophila*, and mammals. Sequence comparison indicates that the plant and animal cryptochrome families have distinct evolutionary histories, with the plant cryptochromes being of ancient evolutionary origin and the animal cryptochromes having evolved relatively recently. This process of repeated evolution may have coincided with the origin in animals of a modified circadian clock based on the PERIOD, TIMELESS, CLOCK, and CYCLE proteins.

In an early description of a biological response to blue light, Charles Darwin noted that the heliotropic movement of plants was eliminated if the light was first filtered through a solution of potassium dichromate (*1*). As passage through a dichromate solution reduces the blue content of the radiant light, this experiment demonstrated that plants were selectively sensing the blue region of the spectrum. It is now realized that this ability to sense and respond to blue light (400 to 500 nm) is widespread throughout the biological kingdom. Other examples of such responses include the production of anthocyanins and carotenoids in plants and fungi and the entrainment of behavioral rhythms in flies and mammals. The action spectrum of many responses to blue light is similar to the absorption spectrum of flavins, which prompted Galston to postulate the involvement of a flavoprotein (*2*). However, for several decades the nature of this photoreceptor continued to be hotly debated—some argued in favor of a flavoprotein, and others speculated that the photoreceptor contained a ca-

rotenoid or a retinal chromophore. The elusive nature of this photoreceptor gave rise to the name cryptochrome (*3*).

### Photolyases Mediate Redox Reactions in Response to Light

Photolyases, the presumptive evolutionary precursors for cryptochromes, are flavoproteins that mediate repair of DNA in a light-dependent manner (*4*). Irradiation of organisms with ultraviolet-B (UV-B) light results in DNA damage through the formation of cyclobutane pyrimidine dimers and the pyrimidine (6-4) pyrimidone photoproducts (Fig. 1). In many organisms this damaged DNA can be repaired by photolyases activated by blue/UV-A light. Flavin-adenine dinucleotide (FAD) is the catalytic chromophore for photolyases. The enzyme has a second chromophore, which can be either a pterin (methenyltetrahydrofolate; MTHF) or a deazaflavin (7,8-didemethyl-8-hydroxy-5-deazariboflavin; 8-HDF) (Fig. 1). The second chromophore functions in light harvesting, and the resulting excitation energy is transferred to the catalytic chromophore.

Photolyases bind selectively to pyrimidine dimers present in UV-damaged DNA and mediate DNA repair by transferring an electron from the excited state of the flavin to the pyrimidine dimer, which then isomerizes to yield the original pyrimidine and returns the electron to the flavin (*4*). Although no net change occurs in the oxidation state of the reactants, light-dependent redox reactions are involved.

Two classes of photolyases (types I and II) repair cyclobutane pyrimidine dimers. Another class capable of repairing (6-4) photoproducts was first identified by a gene from *Drosophila* with a sequence divergent from but related to that of the types I and II photolyases (*5*).

### Cryptochrome Photoreceptors in Plants

The power of *Arabidopsis* genetics led to the first isolation of a cryptochrome blue light photoreceptor (*6*). *Arabidopsis* seedlings grown under light have a shorter hypocotyl than seedlings grown in darkness; this response can be mediated by blue, red (600 to 700 nm), or far-red (700 to 750 nm) light (Fig. 2). Certain mutants of *Arabidopsis* (*hy* mutants) have selectively lost the capacity to respond to one or more portions of the spectrum (*7*).

One of these *hy* mutants (the *hy4/cry1* mutant) is selectively deficient in its capacity to respond to blue light. This feature prompted us to speculate that the *cry1* mutant may correspond to a lesion in the structural gene for the blue light photoreceptor. We isolated a T-DNA–tagged allele of *cry1* that encodes a protein with sequence similarity to DNA photolyases (*6*). The protein encoded by the *cry1* gene was shown to be a flavoprotein; however, the protein lacked detectable photolyase activity and contained a distinguishing COOH-terminal extension (*6, 8*). In view of the photobiological, genetic, and molecular properties of this protein, we concluded that the protein was a long-sought blue light receptor and we named it cryptochrome 1 (CRY1).

A second member of the *Arabidopsis* cryptochrome family, CRY2, like CRY1, mediates blue light–induced shortening of the hypocotyl, cotyledon expansion, and anthocyanin production (*9, 10*). Transgenic *Arabidopsis* plants that overexpress either photoreceptor are hypersensitive to blue light—they exhibit enhanced light-induced shortening of the hypocotyl and increased anthocyanin synthesis. Mutations in the *cry2* gene confer a late-flowering phenotype, observed under blue plus red light but not under blue light alone, apparently reflecting a repression of PHYB activity by CRY2 in wild-type plants (*11*). The *cry2* mutant is allelic to *fha*, a late-flowering mutant. The CRY2 protein contrasts with CRY1 in that it is unstable under blue light intensities in excess of 5 μmol $m^{-2}$ $s^{-1}$ (*9, 10*).

Many plant genes exhibit circadian rhythms in their expression, and recent studies indi-

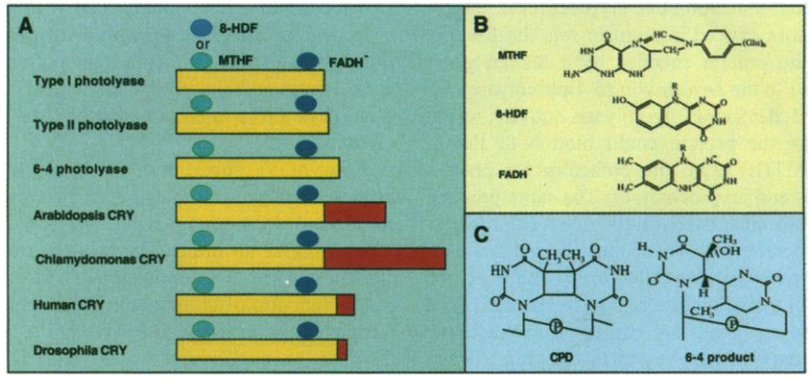


**Fig. 1.** (**A**) Photolyase and cryptochrome structures. For each type of photolyase or cryptochrome, one representative member is shown. Type I photolyase, *Escherichia coli* (472 amino acids); type II photolyase, *Arabidopsis thaliana* (496 amino acids); (6-4)photolyase, *A. thaliana* (537 amino acids); *Arabidopsis* CRY, *A. thaliana* CRY1 (681 amino acids); *Chlamydomonas* CRY, *C. reinhardtii* (867 amino acids); human CRY, *Homo sapiens* CRY1 (586 amino acids); *Drosophila* CRY, *Drosophila melanogaster* CRY (542 amino acids). GenBank accession numbers for the sequences are provided in the legend to Fig. 3. (**B**) Structures of flavin, pterin, and deazaflavin cofactors. Photolyases are characterized by two chromophores: $FADH^-$, present in all photolyases, and a second chromophore, either a pterin (MTHF) or deazaflavin (8-HDF). (**C**) Structures of pyrimidine substrates. Photolyases bind selectively to pyrimidine dimers present in UV-damaged DNA. Two types of products are repaired: cyclobutane pyrimidine dimer (CPD) and the (6-4) pyrimidine dimer, constituting 70% to 80% and 20% to 30% of total UV photoproducts, respectively.

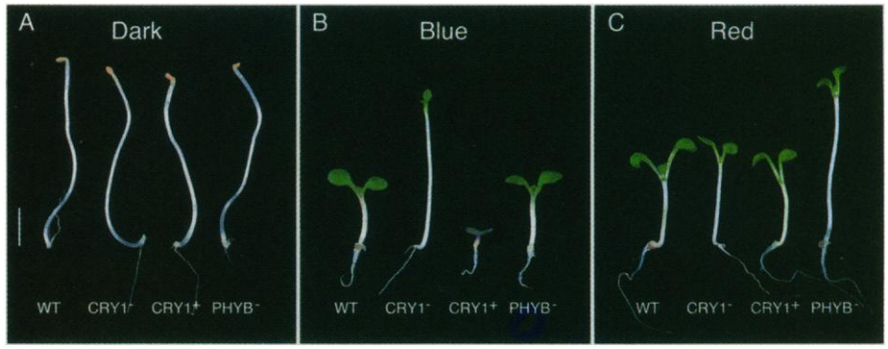


**Fig. 2.** Color-blind mutant *Arabidopsis* seedlings. Six-day-old *Arabidopsis* seedlings are shown after growth under darkness (**A**), blue light (**B**) (25 μmol $m^{-2}$ $s^{-1}$), or red light (**C**) (75 μmol $m^{-2}$ $s^{-1}$). The *cry1* mutant ($CRY1^-$) shows a long hypocotyl under blue light (similar to growth of the wild-type in darkness) but is like wild-type under red light. Conversely, the *phyB* mutant ($PHYB^-$) shows an elongated hypocotyl under red light but not under blue light. The CRY1-overexpressing seedling ($CRY1^+$) is hypersensitive to blue light (but not to red light), exhibiting an unusually short hypocotyl and enhanced anthocyanin production. Scale bar, 2.5 mm.

Plant Science Institute, Department of Biology, University of Pennsylvania, Philadelphia, PA 19104–6018, USA.

*To whom correspondence should be addressed. E-mail: cashmore@upenn.sas.edu

cate that CRY1 mediates photoentrainment of this circadian expression. Under low-intensity blue light (less than 3 μmol $m^{-2}$ $s^{-1}$) the period of gene expression in the *cry1* mutant is increased by about 4 hours, which indicates that CRY1 functions as a blue photoreceptor in rhythm entrainment (*12*). Somewhat surprisingly, the *cry2* mutant, which affects the sensitivity of *Arabidopsis* flowering to photoperiod (*11*), did not affect rhythm entrainment (*12*).

Cryptochrome photoreceptors appear to be present in organisms throughout the plant kingdom; they have been found in the alga *Chlamydomonas reinhardtii* (*13*) as well as in the fern *Adiantum capillus-veneris* (*14*).

## Mammalian Cryptochromes and Circadian Rhythms

The first indication that cryptochrome photoreceptors existed in animals was the finding that the protein encoded by a human gene related to the *Drosophila* (6-4)photolyase (*5*) lacked detectable photolyase activity, even though the protein could bind both flavin and MTHF (*15*), the cofactors for photolyases and cryptochromes. The same properties are characteristic of the *Arabidopsis* cryptochrome proteins; thus the mammalian proteins were also called cryptochromes, although their function remained unclear (*15*). Mouse *cry* genes are expressed in most tissues; the *cry2* gene is expressed at high levels in the central and peripheral retina and *cry1* expression is high in the suprachiasmatic nucleus where it undergoes circadian oscillations (*16*). Thus it was proposed that the mammalian cryptochromes function in the entrainment of behavioral rhythms (*16*). Such entrainment is selectively responsive to light of 500 nm (*17*); furthermore, because mammals deficient in the retinal photoreceptors required for vision are still able to undergo photic entrainment, the photoreceptors mediating these two processes are in some manner distinct (*18*).

Mice lacking the *cry2* gene show reduced levels of light induction of the *mPer1* gene (*19*), a homolog of the *Drosophila per* gene that plays a central role in the circadian clock (*20*). The mutant mice show oscillations in their behavior under constant darkness, with a period about 1 hour longer than wild-type mice, and an increased magnitude of phase-shifting in response to saturating pulses of light. It was concluded that the mouse *cry2* gene plays a role in entrainment of circadian rhythms (*19*).

Some of the properties of CRY2 described in these transgenic studies might be considered more in keeping with cryptochrome functioning as an integral component of the clock instead of in entrainment (*21*). However, the function of cryptochrome as a photoreceptor does not exclude it having a role in the dark, as photolyases, which repair DNA in response to light, in the dark may interact with the excision repair system (*19*). An alternative explanation for the dark phenotype is that these may not be null mutants, and, in a manner similar to certain *Arabidopsis cry1* alleles (*22*), the mouse *cry2* mutation may be conferring a dominant negative phenotype.

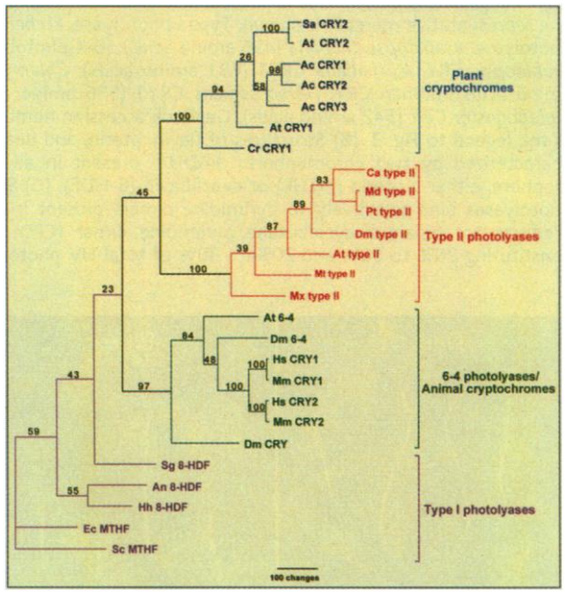


**Fig. 3.** Phylogenetic analysis of the photolyase-cryptochrome family. Sequences analyzed in this study were retrieved from public electronic databases: *Sinapis alba* (Sa) CRY2 (accession number P40115); *A. thaliana* (At) CRY2 (U43397); *A. thaliana* CRY1 (S66907); *Adiantum capillus-veneris* (Ac) CRY1 (AB012629), CRY2 (AB012630), and CRY3 (AB012631); *C. reinhardtii* (Cr) CRY1 (S57795); *Carassius auratus* (Ca) type II (A45098); *Monodelphis domestica* (Md) type II (D31902); *Potorous tridactylis* (Pt) type II (D26020); *D. melanogaster* (Dm) type II (S52047); *A. thaliana* type II (AF053365); *Methanobacterium thermoautotrophicum* (Mt) type II (P12769); *Myxococcus xanthus* (Mx) type II (U44437); *A. thaliana* (6-4)photolyase (At 6-4) (AB003687); *D. melanogaster* (6-4)photolyase (Dm 6-4) (D83701); *H. sapiens* (Hs) CRY1 (D83702); *Mus musculus* (Mm) CRY1 (AB000777); *H. sapiens* CRY2 (AB014558); *M. musculus* CRY2 (AB003433); *D. melanogaster* CRY (AF099734); *Streptomyces griseus* (Sg) 8-HDF (P12768); *Anacystis nidulans* (An) 8-HDF (P05327); *Halobacterium halobium* (Hh) 8-HDF (P20377); *E. coli* (Ec) MTHF (P00914); and *Saccharomyces cerevisiae* (Sc) MTHF (P05066). ClustalW 1.7 (*46, 47*) was used to align proteins; the regions that aligned with amino acids 15 to 488 of *A. thaliana* CRY1 (*6*) were used. Two methods of phylogenetic analysis were employed, parsimony (PAUP 4.0b1) and neighbor joining (PAUP 4.0b1) (*48*). Parsimony analysis was performed with a heuristic search using 500 random addition replicates and 100 bootstrap replicates. Neighbor-joining analysis employed 100 bootstrap replicates. Results from the two analyses were qualitatively similar (even when the length of the input sequences were substantially altered); the results from parsimony analysis are shown.

## Cryptochrome Photoreceptors in *Drosophila*

Proof that cryptochrome proteins are involved in functioning of the circadian clock in animals has come from studies with *Drosophila* (*23–26*). A mutant ($cry^b$) was isolated from a transgenic line of flies harboring a luciferase reporter fused to the *Drosophila per* gene (*23*). In wild-type flies the transgene exhibits cyclical luciferase expression with a period of 24 hours when the flies are subjected to a 12-hour light-dark cycle. In contrast, the $cry^b$ mutant lacks cyclical luciferase expression (*23*). The mutation maps to a position on chromosome III in the vicinity of a deletion encompassing a *Drosophila* cryptochrome gene (*24*). In the mutant $cry^b$ strain, the *cry* gene contains a missense mutation within a codon for a conserved flavin-binding residue. Both *cry* RNA and CRY protein oscillated in a circadian manner, and this oscillation appeared to be regulated at the level of transcription (*24*). In the $cry^b$ mutant, oscillation of *cry* RNA no longer occurs, and CRY protein quantities are greatly reduced.

The $cry^b$ mutation resulted in arrhythmic expression of both PER and timeless (TIM) proteins in the photoreceptor cells—TIM, like PER, represents an integral component of the *Drosophila* circadian clock (*27*). This phenotype reflects a mutation that affects an input into the clock and not a lesion in the clock itself, as demonstrated by continued rhythmic expression of PER and TIM in the $cry^b$ mutant upon entrainment to temperature cycles (*23*).

The $cry^b$ mutant shows relatively normal rhythmic behavior in spite of the arrhythmic expression of PER and TIM. However, when this mutation is combined with the *norpA* mutation—which results in the compound eye being visually unresponsive—the double mutant flies show noticeable deficiencies in behavioral entrainment. Similarly, the double $per^S$; $cry^b$ mutant shows entrainment properties not observed in either single mutant. Furthermore, the $cry^b$ mutant is deficient in its ability to entrain to pulses of light. The ability of the $cry^b$ flies to exhibit circadian oscillations in their behavior suggests that the lateral neurons (the central pacemaker cells) are still functioning effectively. Indeed, for the $cry^b$ mutant, both the dorsal and ventral lateral neurons show some degree of oscillations in expression of both PER and TIM protein expression.

These properties of the $cry^b$ mutant flies

(*23*), the demonstration that photosensitivity is increased in a CRY-overexpressing strain (*24*), and the finding that the fly CRY protein lacks photolyase activity yet apparently binds both flavin and pterin chromophores (*25, 26*) is convincing evidence that the CRY protein is critical for rhythm entrainment in *Drosophila*.

### Repeated Evolution of Cryptochromes

One of the more interesting features of the animal cryptochromes concerns their evolutionary relationship to the plant cryptochromes. At first it might be assumed that these two photoreceptors are encoded by orthologous genes, direct evolutionary descendants of a common photolyase ancestral gene. However, such an assumption appears to be incorrect. Sequence comparison reveals that the mammalian and fly cryptochromes are more closely related to the (6-4)photolyases—including the *Arabidopsis* (6-4)photolyase—than they are to the plant cryptochromes (Fig. 3). It follows that the plant and animal cryptochromes are likely to have arisen from independent evolutionary events. Thus, the cryptochromes represent an example of repeated evolution, a special case of convergent evolution in which a new genetic function arises independently in two different lineages from orthologous (or paralogous) genes (*28*). This phenomenon contrasts with classic convergent evolution, where the ancestral genes are unrelated.

When did these cryptochromes evolve and what happened to the animal counterpart of the plant cryptochromes? The latter are equally divergent from the three different classes of photolyases. This observation, plus the fact that cryptochromes appear to be absent from eubacteria and archaebacteria, prompt us to speculate that the first cryptochromes—the progenitors of the plant cryptochromes—evolved soon after the origin of eukaryotic organisms. In contrast, and given the sequence similarity of the animal cryptochromes and *Arabidopsis* (6-4)photolyase, it appears that the animal cryptochromes evolved soon after the plant–animal divergence.

Given that the function of this animal cryptochrome was likely that of photoentrainment of circadian rhythms, we propose that the origin of the animal cryptochrome coincided with the coevolution of a modified circadian clock based on the PER, TIM, CLOCK, and CYCLE proteins (*20*). This hypothesis suggests that such a clock (the PTCC clock) will not be found in plants. In keeping with this is the finding that MYB-related proteins—distinct from the proteins associated with the animal PTCC clock—are closely associated with the circadian clock in *Arabidopsis* (*29, 30*). In the plant kingdom the original cryptochrome has survived, performing in conjunction with phytochrome a myriad of functions including the entrainment of circadian rhythms. The PAS domain, a distinguishing feature of several mammalian, fly, and fungal clock-related proteins (*20, 31*), is also found in phytochrome (*32*) and a phytochrome-associated protein (*33*)—thus, a common feature of animal clock-associated proteins has also been conserved in plants.

### Distinguishing Features of a Flavin-Based Photoreceptor

Photolyases are photoreceptors, initiating a redox reaction in response to absorption of a photon. This distinguishing feature of photolyases, coupled with the genetic and photobiological data of the *Arabidopsis cry1* mutant, prompted us to conclude that CRY1 was a photoreceptor (*6*). Determining the features required for a flavoprotein to function as a photoreceptor may help define important avenues of future research for the cryptochromes. Note that the redox properties of any flavin will change in response to absorption of a photon, and the rate of electron transfer decreases dramatically with donor-acceptor distance (*34*).

It follows that any flavoprotein could initiate a light-driven redox reaction provided that a redox partner is bound in appropriate juxtaposition and that electron transfer is energetically favored after absorption of light by the flavin. However, this analysis results in the following quandary: why (in an evolutionary sense) have photolyases been "chosen" as the progenitor for the cryptochrome photoreceptors? One might argue that there was an element of chance—it just so happened that photolyase served as the progenitor for cryptochromes. However, this is unlikely as this evolutionary event occurred not once but twice; these independent events gave rise to the plant and animal cryptochromes.

Thus, photolyases have some feature that distinguishes them from other flavoproteins to make them uniquely suitable for functioning as a photoreceptor. The amino acid residues involved in flavin binding, identified from the *Escherichia coli* photolyase crystal structure (*35*), are conserved within the photolyases and cryptochromes (*24, 36*). Possibly there is some feature by which the flavin is bound to the photolyase-cryptochrome apoproteins that facilitates light-induced electron transfer. Indeed the flavin in photolyase is bound in a hairpin-like configuration instead of the extended configuration observed for all other flavoproteins (*35*).

Photolyases are distinguished by not one but two chromophores (*4*). The second chromophore—a pterin or a deazaflavin—functions as a light-harvesting chromophore. The excitation energy resulting from photon absorption is transferred with high efficiency to the flavin, and the latter initiates electron transfer. Because of their high extinction coefficients, these secondary chromophores substantially enhance the overall sensitivity of photolyases to light (*4*) and function as do light-harvesting chlorophylls associated with photosynthetic reaction centers. The possession of this second light-harvesting chromophore distinguishes photolyases from other flavoproteins in their capacity to efficiently respond to photons in the blue/UV-A region of the visible spectrum. This may be the feature that determines the role of photolyases in the evolution of plant and animal cryptochromes.

### Cryptochrome Translocation to the Nucleus

Our emphasis on the light-harvesting and redox properties of photolyase may be misplaced. Both mouse and the human CRY2 proteins localize to the nucleus (*19, 37*). The

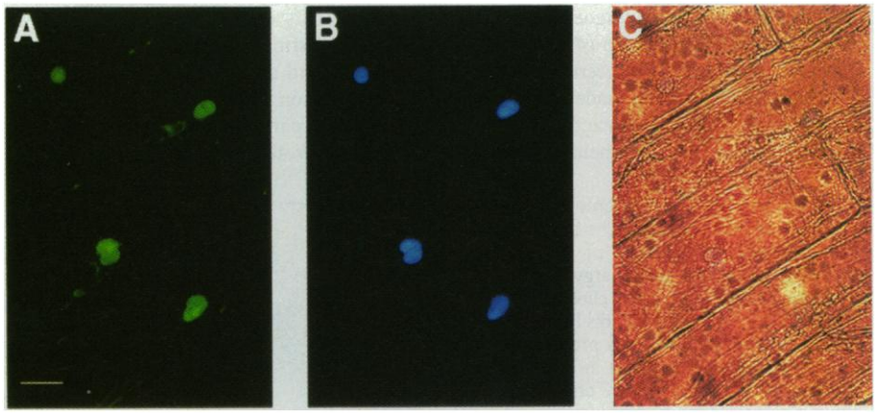


**Fig. 4.** Nuclear localization of *Arabidopsis* CRY1 protein. A gene encoding the *Arabidopsis* CRY1 protein fused to the $NH_2$-terminus of GFP was introduced into onion epidermal cells by biolistic transformation (*49*). Localization of CRY1 GFP was seen to be nuclear (**A**) by comparison with 4′,6-diamidino-2-phenylindole staining (**B**) with fluorescence optics. Cellular structure (**C**) was visualized under bright-field optics. Bar = 5 μm.

mouse CRY1 protein localizes to the mitochondria and, like the human CRY1 protein, binds to DNA (*37*). We have demonstrated that a fusion protein prepared from *Arabidopsis* CRY1 and green fluorescent protein (GFP) localizes to the nucleus on transient expression in onion epidermal cells (Fig. 4). Similarly, for transgenic *Arabidopsis* seedlings a fusion protein of CRY1 and β-glucuronidase is also seen to be nuclear.

If nuclear localization and DNA binding capacity are important features of the cryptochromes, then these properties may be important in the evolutionary history of the cryptochromes. As cryptochrome-mediated signaling in both plants and animals involves transcriptional events, the structural features of photolyases required for DNA binding may be retained in the cryptochromes, their presumptive evolutionary descendants [see (*24*) for a similar proposal].

## Multiplicity of Photoreceptors

In *Arabidopsis* there are two cryptochrome genes and five genes for the phytochrome photoreceptors. The latter, commonly thought of as red far-red light receptors, also function as blue light photoreceptors (*38*). These two classes of photoreceptors overlap in function, with physiological responses such as inhibition of hypocotyl elongation, anthocyanin production, and sensitivity of flowering to photoperiod mediated by both receptors. Similarly, the entrainment of circadian rhythms by blue light is affected by input from both CRY1 as well as phytochrome (*12*). Genetic evidence indicates that phytochrome (*39*), cryptochrome (*40*), and another flavoprotein (NPH1) (*41*) are required for phototropism. The mechanism of action of these proteins and the nature of their interdependence is not well understood. However, it is known that phytochrome has the properties of a serine/threonine protein kinase (*32*), that cryptochrome serves as a substrate for this kinase (*22*), and that NPH1 undergoes blue light-induced autophosphorylation (*41*).

Similar observations concerning multiple photoreceptors have been made in both flies and mammals. The *cry*$^b$ mutant of *Drosophila* exhibits normal cyclical behavior and undergoes entrainment, even when exposed to low light intensities (*23*). However, entrainment is diminished in the double *cry*$^b$*; norpA* mutant. The product of the *norpA* gene (phospholipase C) is downstream of the rhodopsin photoreceptors, and both the single mutants *norpA* and *ninaE* (lacking the major opsin) show deficient entrainment to light-dark cycles. Thus, opsin, as well as cryptochrome, is believed to function in entrainment of the fly's behavioral rhythms (*23*).

Multiple photoreceptors are also involved in rhythm entrainment in mice. Mutant mice lacking CRY2 undergo photoentrainment of their behavioral rhythms, which indicates a role in this process for at least one other photoreceptor (*19*), perhaps CRY1, the other member of the mouse cryptochrome family that is expressed in a cyclical fashion in the suprachiasmatic nucleus (*16*). Whether opsins support rhythm entrainment in mammals, as they appear to in flies, is not so clear. Retinal degeneration caused by the *rd* mutation results in mice that have lost most of their visual sensitivity and opsins yet retain apparently normal sensitivity for photic entrainment of behavioral rhythms (*18*). Further studies in this area are likely to include doubly mutant *cry1 cry2* mice—here a central question is whether these mice retain any capacity to undergo photoentrainment.

## Models for Cryptochrome Function

Cryptochromes, like photolyases, presumably function by mediating a light-dependent redox reaction. In contrast to photolyases, however, pyrimidine dimers are not substrates for this reaction and relatively little is known about the identities of cryptochrome signaling partners. *Arabidopsis* CRY1 binds to and is phosphorylated by phytochrome A in vitro and undergoes phosphorylation in vivo in a red light-dependent manner (*22*). The human CRY2 protein interacts in vitro with a nuclear serine/threonine phosphatase and modulates its activity (*42*). Given the apparently distinct evolutionary histories of the plant and animal cryptochromes, there is little reason to believe that the presumptive redox partners will be the same for these two proteins.

A likely role for the COOH-terminal extensions that distinguish plant cryptochromes from photolyases is to bind a presumptive redox-signaling partner (Fig. 5) that may be activated by a redox reaction—possibly transfer of an electron from $FADH^-$ in a manner similar to the activation of pyrimidine dimers by photolyases. For such a reaction to proceed efficiently, it is necessary for some "useful fraction" of the cryptochrome to be bound to its signaling partner as the flavin excited state is likely to have a half-life on the order of a nanosecond (*43*)—in the absence of electron transfer this excited state will decay by the process of fluorescence.

An alternative way to maximize the efficiency of a signaling process characterized by an intermediate with a short half-life is for the signal to be trapped by an intramolecular process. Such intramolecular processes are used in both phytochrome and rhodopsin signaling. In the former case, the tetrapyrolle chromophore undergoes cis-trans isomerization as a consequence of the absorption of a photon—this isomerization of the chromophore induces a corresponding change in the conformation of the protein (*44*). Similarly, in the case of rhodopsin, the absorption of a photon results in cis-trans isomerization of the associated retinal chromophore that induces a change in conformation of the associated opsin protein (*45*). In view of the potential efficiency of any intramolecular signaling system, it is plausible that the first event in cryptochrome signaling after absorption of a photon may well be an intramolecular redox reaction.

An alternative mode of cryptochrome signaling could involve intermolecular excitation energy transfer to a chromophore associated with a signaling partner. Mechanistically this would work in a manner similar to transfer of energy from the light-harvesting chromophores of photolyases to the flavin. Potential partners in such a reaction are phytochromes in the case of plant cryptochromes and opsins in the case of animal cryptochromes.

## Concluding Thoughts

There have been major recent advances concerning the identity and role of cryptochrome photoreceptors in plants as well as animals. The studies we have reviewed shed light on a fundamental component of the entrainment of animal behavioral rhythms and also provide an example of an interesting evolutionary mechanism. These cryptochrome blue light receptors of plants and animals, both apparently functioning in the photoentrainment of circadian rhythms, are likely the result of repeated evolutionary events. The signaling processes initiated by these receptors remain to be determined. How similar will this process be for the plant and animal crypto-

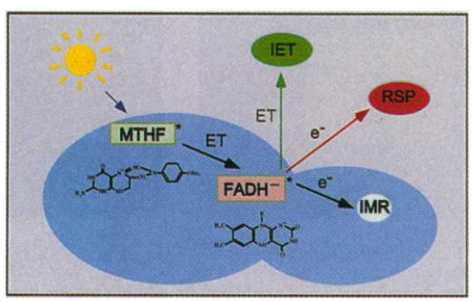


**Fig. 5.** Models for cryptochrome function. Excitation of MTHF (the light-harvesting chromophore) by absorption of a photon; the resulting excitation energy is then transferred to the catalytic chromophore $FADH^-$. In the excited state, this flavin may transfer an electron ($e^-$) to a presumptive redox signaling partner (RSP) in a manner analogous to the reaction of photolyases with pyrimidine dimers. Alternatively, the reaction may involve an intramolecular redox reaction with the electron being transferred to a residue (IMR) within the CRY1 protein. An alternative mode of cryptochrome signaling could involve intermolecular excitation energy transfer (IET) to a chromophore associated with a signaling partner.

chromes and how similar will either be to the light-dependent redox reaction mediated by photolyases? Darwin, surprisingly uninterested in his blue light experiment described above, would surely be excited by these latest findings.

*Note added in proof*: A recent report (*50*) confirmed and extended earlier observations concerning the role of cryptochromes in mammalian circadian rhythms.

**References and Notes**


1. C. Darwin, *The Power of Movement in Plants* (Appleton, New York, 1881).
2. A. W. Galston, *Science* **111**, 619 (1950).
3. J. Gressel, *Photochem. Photobiol.* **30**, 749 (1979).
4. A. Sancar, *Biochemistry* **33**, 2 (1994).
5. T. Todo *et al.*, *Science* **272**, 109 (1996).
6. M. Ahmad and A. R. Cashmore, *Nature* **366**, 162 (1993).
7. M. Koornneef, E. Rolff, C. J. P. Spruit, *Z. Pflanzenphysiol.* **100**, 147 (1980).
8. C. Lin *et al.*, *Science* **269**, 968 (1995).
9. C. Lin *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **95**, 2686 (1998).
10. M. Ahmad *et al.*, *Plant Cell* **10**, 197 (1998).
11. H. Guo *et al.*, *Science* **279**, 1360 (1998).
12. D. E. Somers *et al.*, *ibid.* **282**, 1488 (1998).
13. D. B. Small, B. Min, P. A. Lefebvre, *Plant Mol. Biol.* **28**, 443 (1995).
14. T. Kanegae and M. Wada, *Mol. Gen. Genet.* **259**, 345 (1998).
15. D. S. Hsu *et al.*, *Biochemistry* **35**, 13871 (1996).
16. Y. Miyamoto and A. Sancar, *Proc. Natl. Acad. Sci. U.S.A.* **95**, 6097 (1998).
17. J. S. Takahashi, P. J. DeCoursey, L. Bauman, M. Menaker, *et al.*, *Nature* **308**, 186 (1984).
18. R. G. Foster *et al.*, *J. Comp. Physiol.* **169**, 39 (1991).
19. R. J. Thresher *et al.*, *Science* **282**, 1490 (1998).
20. J. C. Dunlap, *Cell* **96**, 271 (1999).
21. M. Barinaga, *Science* **282**, 1628 (1998).
22. M. Ahmad *et al.*, *Mol. Cell* **1**, 939 (1998).
23. R. Stanewsky *et al.*, *Cell* **95**, 681 (1998).
24. P. Emery *et al.*, *ibid.*, p. 669.
25. C. P. Selby and A. Sancar, *Photochem. Photobiol.* **69**, 105 (1999).
26. S. Okano *et al.*, *ibid.*, p. 108.
27. M. Hunter-Ensor *et al.*, *Cell* **84**, 677 (1996).
28. L. Cseke, N. Dudareva, E. Pichersky, *Mol. Biol. Evol.* **15**, 1491 (1998).
29. R. Schaffer *et al.*, *Cell* **93**, 1219 (1998).
30. Z. Y. Wang and E. M. Tobin, *ibid.*, p. 1207.
31. Z. J. Huang, I. Edery, M. Rosbash, *Nature* **364**, 259 (1993).
32. K.-C. Yeh and J. C. Lagarias, *Proc. Natl. Acad. Sci. U.S.A.* **95**, 13976 (1998).
33. M. Ni, J. M. Tepperman, P. H. Quail, *Cell* **95**, 659 (1998).
34. R. S. Farid, C. C. Moser, P. L. Dutton, *Curr. Opin. Struct. Biol.* **3**, 225 (1993).
35. H.-W. Park, S.-T. Kim, A. Sancar, J. Deisenhofer, *Science* **268**, 1866 (1995).
36. S. Kanai, R. Kikuno, H. Toh, H. Ryo, T. Todo, *J. Mol. Evol.* **45**, 535 (1997).
37. K. Kobayashi *et al.*, *Nucleic Acids Res.* **26**, 5086 (1998).
38. P. H. Quail *et al.*, *Science* **268**, 675 (1995).
39. A. Janoudi, R. Konjevic, G. Whitelam, W. Gordon, K. L. Poff, *Physiol. Plant* **101**, 278 (1997).
40. M. Ahmad, J. A. Jarillo, O. Smirnova, A. R. Cashmore, *Nature* **392**, 720 (1998).
41. J. M. Christie *et al.*, *Science* **282**, 1698 (1998).
42. S. Zhao and A. Sancar, *Photochem. Photobiol.* **66**, 727 (1997).
43. S. T. Kim *et al.*, *Biochemistry* **30**, 11262 (1991).
44. M. Furuya and P.-S. Song, in *Photomorphogenesis in Plants*, R. E. Kendrick and G. H. M. Kronenberg, Eds. (Kluwer, Dordrecht, Netherlands, 1994), pp. 105–140.
45. G. Wald, *Science* **162**, 230 (1968).
46. D. G. Higgins, A. J. Bleasby, R. Fuchs, *Comput. Appl. Biosci.* **8**, 189 (1992).
47. J. D. Thompson, D. G. Higgins, T. J. Gibson, *Nucleic Acids Res.* **11**, 4673 (1994).
48. D. L. Swofford, PAUP*. Phylogenetic Analysis Using Parsimony (* and Other Methods), Version 4 (Sinauer Associates, Sunderland, MA, 1998).
49. M. J. Varagona, R. J. Schmidt, N. V. Raikhel, *Plant Cell* **4**, 1213 (1992).
50. G. T. J. van der Horst *et al.*, *Nature* **398**, 627 (1999).

51. Supported by National Institutes of Health grants GM51956 and GM38409. We thank J. Kissinger for valuable assistance in the phylogenetic analysis. We are also indebted to N. Bonini, M. Bucan, F. Daldal, E. Pichersky, A. Sehgal, and M. Jorns for their reading of the manuscript and for helpful suggestions.

REVIEW

# A Cell's Sense of Direction

**Carole A. Parent and Peter N. Devreotes***

**In eukaryotic cells directional sensing is mediated by heterotrimeric guanine nucleotide–binding protein (G protein)–linked signaling pathways. In *Dictyostelium discoideum* amoebae and mammalian leukocytes, the receptors and G-protein subunits are uniformly distributed around the cell perimeter. Chemoattractants induce the transient appearance of binding sites for several pleckstrin homology domain–containing proteins on the inner face of the membrane. In gradients of attractant these sites are persistently present on the side of the cell facing the higher concentration, even in the absence of a functional actin cytoskeleton or cell movement. Thus, the cell senses direction by spatially regulating the activity of the signal transduction pathway.**

All living cells can sense their environment. The term "directional sensing" refers to the ability of a cell to determine the direction and proximity of an extracellular stimulus. Directional sensing is needed to detect morphogens that control differentiation and attractants that direct cell migration, as in chemotaxis. This fascinating response is critical in immunity, angiogenesis, wound healing, embryogenesis, and neuronal patterning. Chemotaxis is strikingly exhibited during the life cycle of the social amoebae, *D. discoideum* (*1*). During growth, these cells track down and phagocytose bacteria. When starved, they move toward secreted adenosine 3′,5′-monophosphate (cAMP) signals, form aggregates, and differentiate into spore and stalk cells. The fundamental role of chemotaxis in this simple eukaryote provides a powerful system for its genetic analysis. Recent observations in *D. discoideum*, as well as in yeast and mammalian leukocytes, have clarified views of directional sensing. In this review, we focus on the signal transduction events involved in gradient detection. Other important aspects of chemotaxis, such as the regulation of adhesion, motility, and cell shape, have been reviewed and will not be discussed (*2*).

Both leukocytes and amoebae use G protein–linked signaling pathways to detect chemoattractants (Fig. 1). Binding of the attractants to receptors of the seven–transmembrane helix class leads to the dissociation of the G proteins into $\alpha$ and $\beta\gamma$ subunits. It is likely that chemotaxis is mediated through the $\beta\gamma$ subunits. In both leukocytes and amoebae, chemoattractants elicit rapid and transient increases in $Ca^{2+}$ influx, in the intracellular messengers inositol 1,4,5-trisphosphate ($IP_3$), cAMP, and guanosine 3′,5′-monophosphate (cGMP), and in the phosphorylation of myosins I and II. Chemoattractants also induce actin polymerization, most likely through the activation of the Rho family of small guanosine trisphosphatases (GTPases). All these events rapidly subside in the presence of persistent stimulation. This rapid inhibition may allow a migrating cell to "subtract" the ambient concentration of attractant and more accurately sense the direction of a gradient.

Models of chemotaxis should take into account the following behaviors of chemotactic cells (*3*). First, chemotactic cells are extremely sensitive. The accuracy of chemotaxis depends on the relative steepness of the gradient rather than the mean concentration of the attractant, and concentration differences as low as 2% between the front and the back of the cell can direct movement (*4*). Second, cells can regulate polarity. Although they display sensitivity at all points on their perimeter, when amoebae are oriented by

Department of Biological Chemistry, Johns Hopkins University School of Medicine, Baltimore, MD 21205, USA.

*To whom correspondence should be addressed. E-mail: pnd@welchlink.welch.jhu.edu