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The Photolyase/cryptochrome Family of Proteins as DNA Repair Enzymes and Transcriptional Repressors[†]

Ibrahim Halil Kavakli[1,2,3*], Ibrahim Barış[2], Mehmet Tardu[3], Şeref Gül[1], Haşimcan Öner[1], Sibel Çal, [2]Selma Bulut[1], Darya Yarparvar[1], Çağlar Berkel[2], Pınar Ustaoğlu[2], Cihan Aydın[4]

[1]Department of Chemical and Biological Engineering, [2] Department of Molecular Biology and Genetics, [3] Department of Computational Science and Engineering, Koc University, Rumeli Feneri Yolu, Sariyer, Istanbul, Turkey

[4]Department of Molecular Biology and Genetics, Istanbul Medeniyet University, D-100 Karayolu Yanyol, Uskudar, Istanbul

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*Corresponding author e-mail: hkavakli@ku.edu.tr (Ibrahim Halil Kavakli)

ABSTRACT

Light is a very important environmental factor that governs many cellular responses in organisms. As a consequence, organisms possess different kinds of light-sensing photoreceptors to regulate their physiological variables and adapt to a given habitat. The cryptochrome/photolyase family (CPF) includes photoreceptors that perform different functions in different organisms. Photolyases repair ultraviolet-induced DNA damage by a process known as photoreactivation using photons absorbed from the blue end of the light spectrum. On the

other hand, cryptochromes act as blue-light circadian photoreceptors in plants and *Drosophila* to regulate growth and development. In mammals, cryptochromes have light-independent functions and are very important transcriptional regulators that act at the molecular level as negative transcriptional regulators of the circadian clock. In this review, we highlight current knowledge concerning the structural and functional relationships of CPF members.

INTRODUCTION

Light is one of the main environmental cues that affect organismal physiology and behavior. Depending on its wavelength and intensity, light can be either beneficial or severely harmful to organisms. Consequently, during evolution, organisms have developed diverse light-sensitive proteins, known as photoreceptors, and corresponding signaling pathways to continuously monitor light exposure and adapt their physiological activities to environmental changes (1-6). Various types of photoreceptors, such as rhodopsin, melanopsin, phytochromes, phototropins, and cryptochromes (CRYs), absorb light at different spectral wavelengths and regulate a range of cellular processes.

The cryptochrome/photolyase family (CPF) includes structurally similar proteins that function differently in the cell. Photolyases absorb near UV-visible light in the 300-500 nm range and repair DNA while cryptochromes act as photoreceptors and transcriptional regulators depending the type of organisms (7). Recent advances in genomics and metagenomics have greatly increased the number of identified CPF members (8). Phylogenetic analyses of more than 250 CPF members have revealed several major classes (9, 10). A simplified phylogenetic tree based on these analyses is shown in Fig. 1. Although several classes exist, CPF members can be categorized into two groups based on their functions: (i) photolyases, which absorb blue light

energy to repair ultraviolet (UV) photoproducts in DNA (e.g., class I, II, and III photolyases and [6–4] photolyase); and (ii) cryptochromes (CRYs), which function either in a blue light-dependent or -independent manner to regulate processes ranging from circadian entrainment in insects to growth and development in plants.

Photolyases

DNA absorbs UV light, which results in pyrimidine dimer formation (11). As a result, UV is responsible for the formation of two major types of damage-associated photoproducts: cyclobutane pyrimidine dimers (Pyr<>Pyr) (CPDs) and pyrimidine–pyrimidone (6–4) photoproducts (Pyr [6–4] Pyr) (Fig. 2) (11). From a cellular perspective, failure to correct such damage causes cells to become non-functional and eventually die. In the early 1930s, Hollander et al. performed a series of experiments demonstrating that UV-light exposure leads to the death of bacteria (12). To sustain viability and reproductivity, cellular repair of this damage is therefore essential. Indeed, UV-induced damage in bacteria has been subsequently shown to be reversed by visible light (13) through the reparative activity of photolyases (14). Two different types of photolyases were eventually discovered: CPD and (6–4) photolyases. CPD photolyases repair Pyr<>Pyr dimers while (6-4) photolyases repair Pyr [6–4] Pyr photoproducts (15, 16).

Photolyases, which are present in bacteria, fungi, plants, and marsupial animals, are monomeric proteins with molecular masses ranging from 50 to 61 kDa depending on the species. These proteins use blue light as an energy source to repair DNA damage caused by UV light. Previous research has indicated that two cofactors play a significant role in the enzymatic function of photolyases: flavin adenine dinucleotide (FAD) and methenyltetrahydrofolate (MTHF) (17). Some photolyases possess 8-hydroxy-7,8-didemethyl-5-deaza-riboflavin (8-HDF)

instead of MTHF as the second chromophore (17). Studies on the role of these cofactors have revealed that FAD is not only essential for enzyme catalysis but also for damage recognition, while MTHF or 8-HDF is required to enhance the rate of DNA repair 10- to 100-fold under low light conditions (18-20). After photolyase purification, FAD has been found in different chemical states *in vitro*, namely, fully oxidized (FAD_{ox}), fully reduced ($FADH^-$), and radical (FADH°) forms (21), with $FADH^-$ being the active form during the course of catalysis. The role of $FADH^-$ during catalysis is described below.

Crystal structures of photolyases

Escherichia coli CPD photolyase was the first CPF enzyme whose structure was completely solved by X-ray crystallography among various types of photolyases (22). Analysis of the 3D structures of these photolyases reveals CPD photolyases of *Anacystis nidulans* (23), *Thermus thermophilus* (24), and *Agrobacterium tumefaciens* (25), and (6–4) photolyases of *Arabidopsis thaliana* (26) and *Agrobacterium tumefaciens* (27). Comparison of these crystal structures has revealed valuable information about their structural features and enzyme mechanisms. Although the primary structures of photolyases are not well conserved, the 3D structures are. As can be seen in Fig. 3, root-mean-square deviation of Cα values between different structures exhibit a 2 Å difference, reflecting a remarkable 3D similarity among various types of photolyases. Analysis of these 3D structures reveals two domains connected by a loop (Fig. 3).

The N-terminal α/β domain is a typical dinucleotide-binding domain with five β-sheets and five α-helices. In *E. coli* CPD photolyase, MTHF is observed in the cleft between the two domains, where it interacts with two critical amino acid residues, Cys-292 and Lys-293 (Fig. 3a).

The C-terminal α-helical domain consists of 14 α-helices, with structural analysis indicating a cavity at the center where the FAD is found. Further analysis of *E. coli* CPD photolyase has revealed that FAD is held in a U-shaped conformation by interaction with 14 conserved amino acid residues (Fig. 3a) (28). Analyses of crystal structures have suggested that the DNA phosphodiester backbone binds with the photolyase on the flat surface of the helical domain along a trace of positive electrostatic potential. Consequently, mutations at these positively charged residues reduce the affinity of the enzyme to substrate (29). The T<>T is shown to be 'flipped out' of the DNA helix into the central cavity, thereby coming within van der Waals contact distance of the FAD molecule (22). This central pocket is lined on one side with hydrophobic residues and with polar residues on the other, thus matching the asymmetric polarity of the thymidine dimer. In contrast, the *Arabidopsis thaliana* (6–4) photolyase cavity for binding of substrate is narrower and deeper than that of the CPD photolyase because of the presence of residues within the channel. Within this cavity, His-His-Leu-Ala-Arg-His motif is shown to play important role for catalysis (30).

A single-strand DNA repair enzyme: cryptochrome DASH (CRY-DASH)

A class of the CPF known as CRY-DASHs, found in algae, bacteria, plants, and animals (31), was originally classified as a CRY because no photolyase activity could be detected *in vitro* or *in vivo* by photolyase complementation assays (9, 32-34). VcCRY1, a CRY-DASH from *Vibrio cholerae*, was initially co-purified with RNA, which indicated the affinity of CRY-DASHs towards single-stranded nucleic acids (34). Subsequent studies showed that CRY-DASH proteins have photolyase activity. Because they specifically repair CPD photoproducts in single-stranded

DNA (ssDNA) rather than double-stranded DNA (dsDNA), they are designated as ssDNA photolyases (35).

Photosynthetic organisms living in aquatic environments, specifically algae, possess multiple CPF genes that encode CRY-DASHs (36). Why these organisms possess multiple copies of CRY-DASHs is not clear. A recent study by our research group uncovered seven genes displaying homology to the CPF in *Cyanidioschyzon merolae*, an extremophilic microalga. Characterization of these seven putative CPF members identified three genes encoding CRY-DASHs, accordingly named as CmPHR2, CmPHR5, and CmPHR6 (9). Biochemical characterization of these CRY-DASHs revealed that CmPHR2 and CmPHR5 specifically repair ssDNA, while the other CRY-DASH (CmPHR6) repairs neither (6–4) PP nor CPD damage in ssDNA or dsDNA. Although CmPHR6 might represent a transitional form between ssDNA repair and CRYs, its cellular function has not yet been determined (9). A recent RNA-seq study indicated that 13 % of the total genes in *C. merolae* were regulated by blue light (37). Consistent with their role in global gene regulation, cryptochrome genes (*CmPHR2*, *CmPHR3*, and *CmPHR7*) were differentially regulated, suggesting that they have a potential role in light-dependent transcriptional regulation in *C. merolae* (37).

Our recent work using surface plasmon resonance to measure the affinity of photolyases to DNA during the active repair period has indicated that VcCRY1 exhibits high affinity towards damaged ssDNA rather than dsDNA (38) (Table 1). This observation may explain why CRY-DASH cannot repair dsDNA. As has been previously shown, *Arabidopsis thaliana* CRY-DASH has a structure very similar to CPD photolyase but has lost the ability to flip the CPD lesion out of the duplex (39, 40). This finding explains why CRY-DASH has a reduced affinity towards damaged dsDNA.

Several studies indicating that the photochemistry of CRY-DASHs is very similar to that of DNA photolyases are reviewed in (41-43). Measurement of energy transfer from MTHF to FAD using the femtosecond-resolved fluorescence up-conversion technique gave a calculated $MTHF^*$ lifetime of 845 ps in VcCRY1, which is two times longer than that in *E. coli* photolyase (42). This dissimilarity indicates that MTHF binding regions and local structures are different in these two proteins.

Reaction mechanism of DNA repair in photolyases

The reaction mechanism of CPD repair performed by photolyases has been well characterized (16, 44) (Fig. 4). Photolyase binds to the backbone of the damaged DNA strand via ionic interactions. The dimer is then flipped out into the center cavity of the protein, bringing it into proximity of the flavin. This interaction between the substrate and the enzyme enhances specificity towards photolyase and results in a stable enzyme–substrate complex (45, 46, 21). During the course of catalysis, second photo-antenna folate (or deazaflavin) is excited by a blue-light photon to a high-energy state with a lifetime of 0.5 ns (MTHF) or 2.0 ns (8-HDF). Fluorescence resonance energy transfer occurs from the folate to the flavin ($FADH^-$) with a quantum yield of 0.8 (47). Electron transfer then takes place from the flavin to Pyr<>Pyr, and the cyclobutane ring is broken. Finally, the electron is transferred back to FADH° to regenerate the active form of flavin ($FADH^-$) (48). A study was recently conducted to determine how CPD photolyase achieves such high quantum yield efficiency during DNA repair (49). After mutation of amino acid residues important to the substrate/cofactor interaction and those in the active site, DNA repair efficiency of the mutant enzymes was measured by femtosecond spectroscopy. Rather than depending on the ultrafast photoinduced process, repair efficiency was found to rely

on synergistic relationships among all DNA repair steps (i.e., three electron-transfer and two bond-breaking steps) (49). Although not yet fully characterized, the (6–4) photolyase mechanism is expected to function similarly to that of CPD photolyase. One main proposed difference is that (6–4) photolyase, during the course of catalysis, first thermally converts the photoproduct to an oxetane intermediate, with photo-induced electron transfer then breaking the ring and repairing the bases (50, 51). An alternative scenario based on a major discovery is that a unique proton-coupled electron transfer mechanism is responsible for repair of the (6–4) photoproduct. Results based on femtosecond spectroscopy and site-directed mutagenesis suggest the existence of a one-photon repair mechanism in which initial electron transfer from excited flavin induces transfer of a proton from a histidine on the active site of the enzyme to the (6–4) photoproduct (52, 53).

In the presence of oxygen, flavin exists in the FADH° neutral radical form (54). Enzyme bound to the neutral radical form is inactive until FADH° is reduced to $FADH^-$ by dithionite or by a process of photoreduction (at 300-420nm) (55). Studies using enzyme-bound FADH° have revealed that the photoreduction reaction occurs in two steps (56, 57). First, FADH° absorbs a photon, either directly or from MTHF, thereby generating the flavin radical excited state (FADH°)*. Second, an electron is taken up by (FADH°)* from a Trp residue within the enzyme to yield $FADH^-$ and $TrpH^{+\bullet}$, with further reduction to $FADH^-$ via electron uptake from the solvent. During the course of DNA repair, absorption of light energy by reduced $FADH^-$ leads to conversion to $FADH^-$*, which transfers an electron to Pyr<>Pyr to generate a charge-separated radical pair (FADH°···Pyr<>$Pyr^{\bullet-}$); the cyclobutane ring is broken, and the flavin radical is rebuilt to $FADH^-$ by back electron transfer.

Time-resolved absorption (58) and electron paramagnetic resonance (59) spectroscopy have been applied to identify the amino acid donating an electron to flavin. Further analysis has

demonstrated that Trp306 (W306) is the primary electron donor in the photo-reduction of flavin (58). Biochemical characterization of a photolyase-W306F mutant revealed a CPD binding ability comparable to that of wild-type photolyase but no DNA repair activity in either the presence of blue light or excess dithiothreitol. Photolyase-W306F, however, has been shown to be activated by the reduction of FADH° with dithionite, with the loss of activity in this mutant demonstrated to be due to its inability to be photo-reduced (58). Analysis of the crystal structure of photolyase has revealed that Trp306 is located on the enzyme surface 13 Å away from the flavin (60), too large of a distance for electron transfer to take place from the amino acid to the cofactor. Two electron transfer pathways from Trp306 to FADH° have consequently been proposed (60). One pathway is a Trp306 → Trp359 → Trp382 → FADH° transfer involving three electron hops through the "Trp triad"; the other includes 15 residues (358 to 366) in the α-helix and side chain of Phe366. These proposed electron transfer mechanisms may be components of the photolyase mechanism operating during DNA repair *in vivo*. To test this hypothesis, photolyase-W306F was expressed in *E. coli*, and the mutant enzyme in fact displayed wild-type activity *in vivo* (58, 16). These results clearly indicate that photolyase repairs Pyr<>Pyr by a nonreductive cyclic electron transfer reaction (Fig. 4). To further determine whether FADH° photo-reduction is an integral part of the photolyase photocycle, W306F and W382F mutant *E. coli phr* genes were integrated into the bacterial chromosome of an UNC 523 *E. coli* strain (*phr*$^{-}$*)* (61). The integrated genes were driven by their native promoters and therefore produced the mutant enzymes at a low constitutive level. Using this system, we demonstrated that *E. coli* contains around 16 molecules of photolyase. This amount of either mutant (W306F) or wild-type enzyme repairs at least 400 Pyr<>Pyr dimers at the same rate (Fig.

5). In summary, all these results clearly show that proposed intra-protein electron transfer from W306 to FADH° is not a part of the normal photolyase photocycle *in vivo* (61).

CRYs

CRY was first identified as a blue light photoreceptor in *Arabidopsis thaliana*. Characterization and complementation of a *Hy4 Arabidopsis thaliana* mutant indicated that *Hy4* encodes CRY and is responsible for hypocotyl elongation in a blue light-dependent manner (62). Following this report, CRYs were discovered in many other plant species and found to affect multiple aspects of plant growth and development (36, 63-66). CRYs were subsequently found to act as a photoreceptor in *Drosophila* (67, 68) and as transcriptional regulators regulating the circadian clock at the molecular level in animals (69, 70). Several reviews have been published on the structure–functional relationships of plant and *Drosophila* CRYs (71-73). We briefly discuss the structure and functional relationships of mammalian CRYs.

In mammals, the circadian clock mechanism involves several proteins that participate in positive and negative transcriptional feedback loops (Fig. 6). Proteins involved in the positive feedback loop include BMAL1 (for brain and muscle aryl hydrocarbon receptor nuclear translocator (ARNT)-like protein 1) and CLOCK (circadian locomotor output cycles kaput) proteins containing basic helix-loop-helix domains and PAS domains. The proteins form heterodimers and bind to E-box elements (CACGTG) in Period (*Per*), Cryptochrome (*Cry*), and other clock-controlled genes (74-76). The mammalian PERIOD proteins (PER1 and PER2, collectively designated as PER) and CRY act as negative regulators of transcription driven by the BMAL1:CLOCK heterodimer (77, 78, 69, 70) (Fig. 6). PER and CRY form heterodimers that

interact with casein kinase Iε (CKIε) and then translocate into the nucleus where CRY acts as a negative regulator of BMAL1:CLOCK (79). This basic molecular clock is present in all tissues. Peripheral clocks, however, are synchronized via humoral and neural signals to the master clock originating in the suprachiasmatic nucleus, a region in the hypothalamus of the brain responsible for controlling circadian rhythm.

Although extensive genetic and cell biology data support the canonical clock model at the molecular level, recent studies have suggested that CRY acts differently than in the proposed canonical model (Fig. 6) (80). CRY binds to CLOCK-BMAL1 dimer and inhibits CLOCK-BMAL1-driven transcription on the E-box. On the other hand, it has been shown that CRY together with PER displaces CLOCK-BMAL1 complex from the E-box (81, 82). In addition, no PERs are associated with the CLOCK:BMAL1:CRY DNA complex during the early morning in mouse liver (83). These findings suggest that the ternary complex operates in a poised but repressed state until the appropriate time of day. A recent study has revealed how CRY1 participates in the regulation of circadian timing (84). In that study, CRY1 was demonstrated to compete with co-activators to bind unstructured C-terminal domain of BMAL1 to establish a functional switch between activation and repression of the CLOCK:BMAL1 heterodimer in mouse fibroblasts.

The role of the CRY C-terminus

Alignments of various CRYs and photolyases show that mammal, plant, and insect CRYs have variable extended C-terminal domains that range, depending on the species, from 30 to 300 amino acids (85) (Fig. 7). Studies of *Arabidopsis thaliana* CRY proteins have revealed that C-termini of CRYs mediate blue light signals by interacting with COP proteins (86, 87). In

addition, the *Drosophila* CRY (dCRY) protein transmits the blue light signal by interacting with TIM proteins (88). Subsequent studies provide insight about the role of dCRY C-terminus in phototransduction. They showed that the photolyase homology region of dCRY mediates the response to blue light and that the C-terminal 19 amino acids were required for inhibition of the CRY-TIM interaction in the dark (89, 90). This determines the photosensitivity of the circadian clock by regulating CRY proteasomal degradation (89). Previous studies have suggested that mammalian CRYs together with PER2-CK1ε inhibit BMAL1:CLOCK-driven transcription (77). Both yeast and mammalian two-hybrid systems have been used to indicate that CRY directly interacts with both BMAL1 and PER2 (91, 78) and that the interaction between CRY and CLOCK:BMAL1 occurs through the CRY C-terminus (92, 93). Mammalian CRY C-termini possesses a highly conserved helix that has a periodic spacing of nonpolar residues common to coiled coils (CC), which is thus called the CC helix (92). In addition, this CRY region is important for interaction with different types of proteins such as FBXL3 (94) and PER2 (95). Our group has shown that two amino acid residues of mouse CRY2, Arg-501 and Lys-503, are essential for the interaction with PER2 (95). According to the co-crystal structure of CRY1-PER2, Arg-483 (homolog of Arg-503 of CRY2) is in fact required for the formation of salt bridges with Asp-1167 of PER2 and contributes to the stable interaction between them (96) (Fig. 8). Another crystal structural analysis has revealed that the CC helix of mouse CRY2, including Arg-501 and Lys-503, is masked by the leucine-rich-repeat domain of FBXL3 (94). That study found that this region is indeed important for the PER interaction and that the CC helix of CRYs is the region where both PER2 and FBXL3 bind and compete with each other.

CONCLUSIONS

Although several classes of CPF proteins exist, they perform essentially only two distinct functions. The first functional category is that of photolyases, which reverse UV-induced DNA damage using blue light as an energy source and subsequently help maintain DNA integrity. Photolyases are further subdivided according to the photoproducts repaired, namely, into CPD, (6–4), and ssDNA photolyases. Even though the structure–function relationships of photolyases have been extensively studied, they are still being actively investigated with respect to long- and short-range electron transfer during catalysis. CRYs, the other functional category, act as photoreceptors in various organisms and as transcriptional repressors regulating the mammalian circadian clock. Studies have shown that disruption of clocks in humans and other animals increases the risk of different diseases such as cancer, diabetes, and certain metabolic disorders (97). Considering that CRY is one of the most important core clock components, efforts to find small molecules regulating the activity of CRYs will open new avenues in medicine and enable researchers to introduce new drugs into the market.

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REFERENCES

1. Galvao, V. C. and C. Fankhauser (2015) Sensing the light environment in plants: photoreceptors and early signaling steps. *Curr. Opin. Neurobiol.* **34C,** 46-53.
2. Hegemann, P. (2008) Algal sensory photoreceptors. *Annu. Rev. Plant Biol.* **59,** 167-189.
3. Losi, A. and W. Gartner (2012) The evolution of flavin-binding photoreceptors: an ancient chromophore serving trendy blue-light sensors. *Annu. Rev. Plant Biol.* **63,** 49-72.
4. Montgomery, B. L. (2007) Sensing the light: photoreceptive systems and signal transduction in cyanobacteria. *Mol. Microbiol.* **64,** 16-27.

5. Rockwell, N. C., Y. S. Su and J. C. Lagarias (2006) Phytochrome structure and signaling mechanisms. *Annu. Rev. Plant Biol.* **57,** 837-858.
6. Sancar, A. (2000) Cryptochrome: The second photoactive pigment in the eye and its role in circadian photoreception. *Annu. Rev. Biochem.* **69,** 31-67.
7. Sancar, A., C. Thompson, R. J. Thresher, F. Araujo, J. Mo, S. Ozgur, E. Vagas, L. Dawut and C. P. Selby (2000) Photolyase/cryptochrome family blue-light photoreceptors use light energy to repair DNA or set the circadian clock. *Cold Spring Harb. Symp. Quant. Biol.* **65,** 157-171.
8. Fortunato, A. E., R. Annunziata, M. Jaubert, J. P. Bouly and A. Falciatore (2015) Dealing with light: The widespread and multitasking cryptochrome/photolyase family in photosynthetic organisms. *J. Plant Physiol.* **172,** 42-54.
9. Asimgil, H. and I. H. Kavakli (2012) Purification and characterization of five members of photolyase/cryptochrome family from Cyanidioschyzon merolae. *Plant Sci.* **185-186,** 190-198.
10. Ozturk, N., Y. T. Kao, C. P. Selby, I. H. Kavakli, C. L. Partch, D. P. Zhong and A. Sancar (2008) Purification and characterization of a type III photolyase from Caulobacter crescentus. *Biochemistry* **47,** 10255-10261.
11. Mitchell, D. L. and R. S. Nairn (1989) The biology of the (6-4) photoproduct. *J. Photochem. Photobiol.* **49,** 805-819.
12. Hollaender, A. and W. D. Claus (1936) The bacterial effect of ultraviolet radiation on Eschericihia coli in liquid suspension. *J. Gen. Physiol.* **19,** 753-765.
13. Kelner, A. (1949) Effect of Visible light on the recovery of Streptomyces griseus condia from ultraviolet irradiation injury. *Proc. Natl. Acad. Sci.* **35,** 73-79.
14. Rupert, C. S., S. H. Goodgal and R. M. Herriott (1958) Photoreactivation in vitro of ultraviolet-inactivated Hemophilus influenzae transforming factor. *J. Gen. Physiol.* **41,** 451-471.
15. Menck, C. F. (2002) Shining a light on photolyases. *Nat. Genet.* **32,** 338-339.
16. Sancar, A. (2003) Structure and function of DNA photolyase and cryptochrome blue-light photoreceptors. *Chem. Rev.* **103,** 2203-2237.
17. Sancar, G. B. and A. Sancar (1987) Structure and Function of DNA Photolyases. *Trends Biochem. Sci.* **12,** 259-261.
18. Jorns, M. S., E. T. Baldwin, G. B. Sancar and A. Sancar (1987) Action Mechanism of Escherichia-Coli DNA Photolyase .2. Role of the Chromophores in Catalysis. *J. Biol. Chem.* **262,** 486-491.
19. Sancar, G. B., M. S. Jorns, G. Payne, D. J. Fluke, C. S. Rupert and A. Sancar (1987) Action Mechanism of Escherichia-Coli DNA Photolyase .3. Photolysis of the Enzyme-Substrate Complex and the Absolute Action Spectrum. *J. Biol. Chem.* **262,** 492-498.
20. Sancar, G. B., F. W. Smith, R. Reid, G. Payne, M. Levy and A. Sancar (1987) Action Mechanism of Escherichia-Coli DNA Photolyase .1. Formation of the Enzyme-Substrate Complex. *J. Biol. Chem.* **262,** 478-485.
21. Kim, S. T. and A. Sancar (1991) Effect of base, pentose, and phosphodiester backbone structures on binding and repair of pyrimidine dimers by Escherichia coli DNA photolyase. *Biochemistry* **30,** 8623-8630.
22. Park, H. W., S. T. Kim, A. Sancar and J. Deisenhofer (1995) Crystal-Structure of DNA Photolyase from Escherichia-Coli. *Science* **268,** 1866-1872.

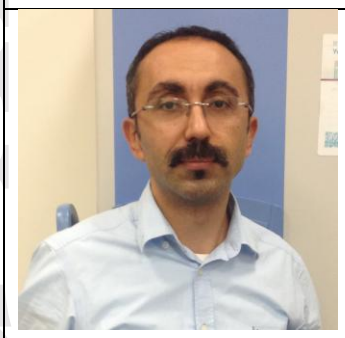
23. Tamada, T., K. Kitadokoro, Y. Higuchi, K. Inaka, A. Yasui, P. E. deRuiter, A. P. M. Eker and K. Miki (1997) Crystal structure of DNA photolyase from Anacystis nidulans. *Nat. Struct. Biol.* **4,** 887-891.
24. Komori, H., R. Masui, S. Kuramitsu, S. Yokoyama, T. Shibata, Y. Inoue and K. Miki (2001) Crystal structure of thermostable DNA photolyase: Pyrimidine-dimer recognition mechanism. *Proc. Natl. Acad. Sci.* **98,** 13560-13565.
25. Scheerer, P., F. Zhang, J. Kalms, D. von Stetten, N. Krauss, I. Oberpichler and T. Lamparter (2015) The Class III Cyclobutane Pyrimidine Dimer Photolyase Structure Reveals a New Antenna Chromophore Binding Site and Alternative Photoreduction Pathways. *J. Biol. Chem.* **290,** 11504-11514.
26. Hitomi, K., L. DiTacchio, A. S. Arvai, J. Yamamoto, S. T. Kim, T. Todo, J. A. Tainer, S. Iwai, S. Panda and E. D. Getzoff (2009) Functional motifs in the (6-4) photolyase crystal structure make a comparative framework for DNA repair photolyases and clock cryptochromes. *Proc. Natl. Acad. Sci.* **106,** 6962-6967.
27. Zhang, F., P. Scheerer, I. Oberpichler, T. Lamparter and N. Krauss (2013) Crystal structure of a prokaryotic (6-4) photolyase with an Fe-S cluster and a 6,7-dimethyl-8-ribityllumazine antenna chromophore. *Proc. Natl. Acad. Sci.* **110,** 7217-7222.
28. Sancar, A. (2008) Structure and function of photolyase and in vivo enzymology: 50th anniversary. *The J. Biol. Chem.* **283,** 32153-32157.
29. Baer, M. E. and G. B. Sancar (1993) The Role of Conserved Amino-Acids in Substrate-Binding and Discrimination by Photolyase. *J. Biol. Chem.* **268,** 16717-16724.
30. Hitomi, K., H. Nakamura, S. T. Kim, T. Mizukoshi, T. Ishikawa, S. Iwai and T. Todo (2001) Role of two histidines in the (6-4) photolyase reaction. *J. Biol. Chem.* **276,** 10103-10109.
31. Brudler, R., K. Hitomi, H. Daiyasu, H. Toh, K. Kucho, M. Ishiura, M. Kanehisa, V. A. Roberts, T. Todo, J. A. Tainer and E. D. Getzoff (2003) Identification of a new cryptochrome class: Structure, function, and evolution. *Mol. Cell.* **11,** 59-67.
32. Hitomi, K., K. Okamoto, H. Daiyasu, H. Miyashita, S. Iwai, H. Toh, M. Ishiura and T. Todo (2000) Bacterial cryptochrome and photolyase: characterization of two photolyase-like genes of Synechocystis sp PCC6803. *Nucleic Acids Res.* **28,** 2353-2362.
33. Kleine, T., P. Lockhart and A. Batschauer (2003) An Arabidopsis protein closely related to Synechocystis cryptochrome is targeted to organelles. *Plant J.* **35,** 93-103.
34. Worthington, E. N., I. H. Kavakli, G. Berrocal-Tito, B. E. Bondo and A. Sancar (2003) Purification and characterization of three members of the photolyase/cryptochrome family blue-light photoreceptors from Vibrio cholerae. *J. Biol. Chem.* **278,** 39143-39154.
35. Selby, C. P. and A. Sancar (2006) A cryptochrome/photolyase class of enzymes with single-stranded DNA-specific photolyase activity. *Proc. Natl. Acad. Sci.* **103,** 17696-17700.
36. Chaves, I., R. Pokorny, M. Byrdin, N. Hoang, T. Ritz, K. Brettel, L. O. Essen, G. T. van der Horst, A. Batschauer and M. Ahmad (2011) The cryptochromes: blue light photoreceptors in plants and animals. *Annu. Rev. Plant Biol.* **62,** 335-364.
37. Tardu, M., U. M. Dikbas, I. Baris and I. H. Kavakli (2016) RNA-seq analysis of the transcriptional response to blue and red light in the extremophilic red alga, Cyanidioschyzon merolae. *Funct. Integr. Genomics.* **16,** 657-669.

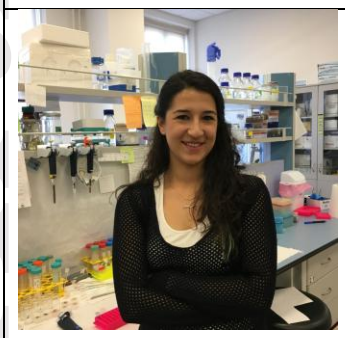
38. Kizilel, R., E. Demir, S. Azizoglu, H. Asimgi, I. H. Kavakli and S. Kizilel (2012) Investigation of Real-Time Photorepair Activity on DNA via Surface Plasmon Resonance. *PloS one* **7,** e44392.
39. Huang, Y. H., R. Baxter, B. S. Smith, C. L. Partch, C. L. Colbert and J. Deisenhofer (2006) Crystal structure of cryptochrome 3 from Arabidopsis thaliana and its implications for photolyase activity. *Proc. Natl. Acad. Sci.* **103,** 17701-17706.
40. Pokorny, R., T. Klar, U. Hennecke, T. Carell, A. Batschauer and L. O. Essen (2008) Recognition and repair of UV lesions in loop structures of duplex DNA by DASH-type cryptochrome. *Proc. Natl. Acad. Sci.* **105,** 21023-21027.
41. Moldt, J., R. Pokorny, C. Orth, U. Linne, Y. Geisselbrecht, M. A. Marahiel, L. O. Essen and A. Batschauer (2009) Photoreduction of the Folate Cofactor in Members of the Photolyase Family. *J. Biol. Chem.* **284,** 21670-21683.
42. Saxena, C., H. Y. Wang, I. H. Kavakli, A. Sancar and D. P. Zhong (2005) Ultrafast dynamics of resonance energy transfer in cryptochrome. *J. Am. Chem. Soc.* **127,** 7984-7985.
43. Song, S. H., B. Dick, A. Penzkofer, R. Pokorny, A. Batschauer and L. O. Essen (2006) Absorption and fluorescence spectroscopic characterization of cryptochrome 3 from Arabidopsis thaliana. *J. Photochem. Photobiol. B* **85,** 1-16.
44. Thompson, C. L. and A. Sancar (2002) Photolyase/cryptochrome blue-light photoreceptors use photon energy to repair DNA and reset the circadian clock. *Oncogene* **21,** 9043-9056.
45. Jordan, S. P., J. L. Alderfer, L. P. Chanderkar and M. S. Jorns (1989) Reaction of Escherichia coli and yeast photolyases with homogeneous short-chain oligonucleotide substrates. *Biochemistry* **28,** 8149-8153.
46. Jorns, M. S., G. B. Sancar and A. Sancar (1985) Identification of Oligothymidylates as New Simple Substrates for Escherichia-Coli DNA Photolyase and Their Use in a Rapid Spectrophotometric Enzyme Assay. *Biochemistry* **24,** 1856-1861.
47. Kim, S. T., P. F. Heelis and A. Sancar (1992) Energy transfer (deazaflavin-->FADH2) and electron transfer (FADH2-->T <> T) kinetics in Anacystis nidulans photolyase. *Biochemistry* **31,** 11244-11248.
48. Sancar, A. (2016) Mechanisms of DNA Repair by Photolyase and Excision Nuclease (Nobel Lecture). *Angew. Chem. Int. Ed. Engl.* **55,** 8502-8527.
49. Tan, C., Z. Y. Liu, J. Li, X. M. Guo, L. J. Wang, A. Sancar and D. P. Zhong (2015) The molecular origin of high DNA-repair efficiency by photolyase. *Nat. Commun.* **6,** 7302.
50. Kim, S. T., K. Malhotra, C. A. Smith, J. S. Taylor and A. Sancar (1994) Characterization of (6-4)-Photoproduct DNA Photolyase. *J. Biol. Chem.* **269,** 8535-8540.
51. Todo, T., H. Takemori, H. Ryo, M. Ihara, T. Matsunaga, O. Nikaido, K. Sato and T. Nomura (1993) A New Photoreactivating Enzyme That Specifically Repairs Ultraviolet Light-Induced (6-4)Photoproducts. *Nature* **361,** 371-374.
52. Li, J., Z. Y. Liu, C. Tan, X. M. Guo, L. J. Wang, A. Sancar and D. P. Zhong (2010) Dynamics and mechanism of repair of ultraviolet-induced (6-4) photoproduct by photolyase. *Nature* **466,** 887-890.
53. Zhong, D. P. (2015) Electron Transfer Mechanisms of DNA Repair by Photolyase. *Annu. Rev. Phys. Chem.* **66,** 691-715.
54. Jorns, M. S., G. B. Sancar and A. Sancar (1984) Identification of a neutral flavin radical and characterization of a second chromophore in Escherichia coli DNA photolyase. *Biochemistry* **23,** 2673-2679.

55. Jorns, M. S., E. T. Baldwin, G. B. Sancar and A. Sancar (1987) Action mechanism of Escherichia coli DNA photolyase. II. Role of the chromophores in catalysis. *The J. Biol. Chem.* **262,** 486-491.
56. Heelis, P. F., G. Payne and A. Sancar (1987) Photochemical properties of Escherichia coli DNA photolyase: selective photodecomposition of the second chromophore. *Biochemistry* **26,** 4634-4640.
57. Sancar, G. B., M. S. Jorns, G. Payne, D. J. Fluke, C. S. Rupert and A. Sancar (1987) Action mechanism of Escherichia coli DNA photolyase. III. Photolysis of the enzyme-substrate complex and the absolute action spectrum. *The J. Biol. Chem.* **262,** 492-498.
58. Li, Y. F., P. F. Heelis and A. Sancar (1991) Active site of DNA photolyase: tryptophan-306 is the intrinsic hydrogen atom donor essential for flavin radical photoreduction and DNA repair in vitro. *Biochemistry* **30,** 6322-6329.
59. Kim, S. T., A. Sancar, C. Essenmacher and G. T. Babcock (1993) Time-resolved EPR studies with DNA photolyase: excited-state FADH0 abstracts an electron from Trp-306 to generate FADH-, the catalytically active form of the cofactor. *Proc. Natl. Acad. Sci.* **90,** 8023-8027.
60. Park, H. W., S. T. Kim, A. Sancar and J. Deisenhofer (1995) Crystal structure of DNA photolyase from Escherichia coli. *Science* **268,** 1866-1872.
61. Kavakli, I. H. and A. Sancar (2004) Analysis of the role of intraprotein electron transfer in photoreactivation by DNA photolyase in vivo. *Biochemistry* **43,** 15103-15110.
62. Ahmad, M. and A. R. Cashmore (1993) Hy4 Gene of a-Thaliana Encodes a Protein with Characteristics of a Blue-Light Photoreceptor. *Nature* **366,** 162-166.
63. Christie, J. M. (2007) Phototropin blue-light receptors. *Annu. Rev. Plant Biol.* **58,** 21-45.
64. Lin, C. and D. Shalitin (2003) Cryptochrome structure and signal transduction. *Annu. Rev. Plant Biol.* **54,** 469-496.
65. Pedmale, U. V., S. S. Huang, M. Zander, B. J. Cole, J. Hetzel, K. Ljung, P. A. Reis, P. Sridevi, K. Nito, J. R. Nery, J. R. Ecker and J. Chory (2016) Cryptochromes Interact Directly with PIFs to Control Plant Growth in Limiting Blue Light. *Cell* **164,** 233-245.
66. Wang, H. (2005) Signaling mechanisms of higher plant photoreceptors: a structure-function perspective. *Curr. Top. Dev. Biol.* **68,** 227-261.
67. Emery, P., W. V. So, M. Kaneko, J. C. Hall and M. Rosbash (1998) CRY, a Drosophila clock and light-regulated cryptochrome, is a major contributor to circadian rhythm resetting and photosensitivity. *Cell* **95,** 669-679.
68. Stanewsky, R., M. Kaneko, P. Emery, B. Beretta, K. Wager-Smith, S. A. Kay, M. Rosbash and J. C. Hall (1998) The cry(b) mutation identifies cryptochrome as a circadian photoreceptor in Drosophila. *Cell* **95,** 681-692.
69. van der Horst, G. T. J., M. Muijtjens, K. Kobayashi, R. Takano, S. Kanno, M. Takao, J. de Wit, A. Verkerk, A. P. M. Eker, D. van Leenen, R. Buijs, D. Bootsma, J. H. J. Hoeijmakers and A. Yasui (1999) Mammalian Cry1 and Cry2 are essential for maintenance of circadian rhythms. *Nature* **398,** 627-630.
70. Vitaterna, M. H., C. P. Selby, T. Todo, H. Niwa, C. Thompson, E. M. Fruechte, K. Hitomi, R. J. Thresher, T. Ishikawa, U. Miyazaki, J. S. Takahashi and A. Sancar (1999) Differential regulation of mammalian period genes and circadian rhythmicity by cryptochromes 1 and 2. *Proc. Natl. Acad. Sci.* **96,** 12114-12119.

71. Cashmore, A. R. (2003) Cryptochromes: enabling plants and animals to determine circadian time. *Cell* **114,** 537-543.
72. Hardin, P. E. (2011) Molecular genetic analysis of circadian timekeeping in Drosophila. *Adv. Genet.* **74,** 141-173.
73. Ito, C. and K. Tomioka (2016) Heterogeneity of the Peripheral Circadian Systems in Drosophila melanogaster: A Review. *Front. Physiol.* **7,** 8.
74. Gekakis, N., D. Staknis, H. B. Nguyen, F. C. Davis, L. D. Wilsbacher, D. P. King, J. S. Takahashi and C. J. Weitz (1998) Role of the CLOCK protein in the mammalian circadian mechanism. *Science* **280,** 1564-1569.
75. Hogenesch, J. B., Y. Z. Gu, S. J. Jain and C. A. Bradfield (1998) The basic-helix-loop-helix-PAS orphan MOP3 forms transcriptionally active complexes with circadian and hypoxia factors. *Proc. Natl. Acad. Sci.* **95,** 5474-5479.
76. King, D. P., Y. L. Zhao, A. M. Sangoram, L. D. Wilsbacher, M. Tanaka, M. P. Antoch, T. D. L. Steeves, M. H. Vitaterna, J. M. Kornhauser, P. L. Lowrey, F. W. Turek and J. S. Takahashi (1997) Positional cloning of the mouse circadian Clock gene. *Cell* **89,** 641-653.
77. Kume, K., M. J. Zylka, S. Sriram, L. P. Shearman, D. R. Weaver, X. W. Jin, E. S. Maywood, M. H. Hastings and S. M. Reppert (1999) mCRY1 and mCRY2 are essential components of the negative limb of the circadian clock feedback loop. *Cell* **98,** 193-205.
78. Shearman, L. P., S. Sriram, D. R. Weaver, E. S. Maywood, I. Chaves, B. H. Zheng, K. Kume, C. C. Lee, G. T. J. van der Horst, M. H. Hastings and S. M. Reppert (2000) Interacting molecular loops in the mammalian circadian clock. *Science* **288,** 1013-1019.
79. Vielhaber, E. L., D. Duricka, K. S. Ullman and D. M. Virshup (2001) Nuclear export of mammalian PERIOD proteins. *J. Biol. Chem.* **276,** 45921-45927.
80. Ye, R., C. P. Selby, N. Ozturk, Y. Annayev and A. Sancar (2011) Biochemical analysis of the canonical model for the mammalian circadian clock. *J. Biol. Chem.* **286,** 25891-25902.
81. Chiou, Y. Y., Y. Yang, N. Rashid, R. Ye, C. P. Selby and A. Sancar (2016) Mammalian Period represses and de-represses transcription by displacing CLOCK-BMAL1 from promoters in a Cryptochrome-dependent manner. *Proc. Natl. Acad. Sci.* **113,** E6072-E6079.
82. Ye, R., C. P. Selby, Y. Y. Chiou, I. Ozkan-Dagliyan, S. Gaddameedhi and A. Sancar (2014) Dual modes of CLOCK:BMAL1 inhibition mediated by Cryptochrome and Period proteins in the mammalian circadian clock. *Gene. Dev.* **28,** 1989-1998.
83. Koike, N., S. H. Yoo, H. C. Huang, V. Kumar, C. Lee, T. K. Kim and J. S. Takahashi (2012) Transcriptional architecture and chromatin landscape of the core circadian clock in mammals. *Science* **338,** 349-354.
84. Xu, H. Y., C. L. Gustafson, P. J. Sammons, S. K. Khan, N. C. Parsley, C. Ramanathan, H. W. Lee, A. C. Liu and C. L. Partch (2015) Cryptochrome 1 regulates the circadian clock through dynamic interactions with the BMAL1 C terminus. *Nat. Struct. Mol. Biol.* **22,** 476-U470.
85. Partch, C. L., M. W. Clarkson, S. Ozgur, A. L. Lee and A. Sancar (2005) Role of structural plasticity in signal transduction by the cryptochrome blue-light photoreceptor. *Biochemistry* **44,** 3795-3805.

86. Wang, H. Y., L. G. Ma, J. M. Li, H. Y. Zhao and X. W. Deng (2001) Direct interaction of Arabidopsis cryptochromes with COP1 in light control development. *Science* **294,** 154-158.
87. Yang, H. Q., Y. J. Wu, R. H. Tang, D. M. Liu, Y. Liu and A. R. Cashmore (2000) The C termini of Arabidopsis cryptochromes mediate a constitutive light response. *Cell* **103,** 815-827.
88. Ceriani, M. F., T. K. Darlington, D. Staknis, P. Mas, A. A. Petti, C. J. Weitz and S. A. Kay (1999) Light-dependent sequestration of TIMELESS by CRYPTOCHROME. *Science* **285,** 553-556.
89. Busza, A., M. Emery-Le, M. Rosbash and P. Emery (2004) Roles of the two Drosophila CRYPTOCHROME structural domains in circadian photoreception. *Science* **304,** 1503-1506.
90. Dissel, S., C. V, R. Fedic, K. J. Garner, R. Costa, C. P. Kyriacou and E. Rosato (2004) A constitutively active cryptochrome in Drosophila melanogaster. *Nat. Neurosci.* **7,** 834-840.
91. Griffin, E. A., D. Staknis and C. J. Weitz (1999) Light-independent role of CRY1 and CRY2 in the mammalian circadian clock. *Science* **286,** 768-771.
92. Chaves, I., K. Yagita, S. Barnhoorn, H. Okamura, G. T. J. van der Horst and F. Tamanini (2006) Functional evolution of the photolyase/cryptochrome protein family: Importance of the C terminus of mammalian CRY1 for circadian core oscillator performance. *Mol. Cell Biol.* **26,** 1743-1753.
93. Langmesser, S., T. Tallone, A. Bordon, S. Rusconi and U. Albrecht (2008) Interaction of circadian clock proteins PER2 and CRY with BMAL1 and CLOCK. *BMC Mol. Biol.* **9,** 41.
94. Xing, W. M., L. Busino, T. R. Hinds, S. T. Marionni, N. H. Saifee, M. F. Bush, M. Pagano and N. Zheng (2013) SCFFBXL3 ubiquitin ligase targets cryptochromes at their cofactor pocket. *Nature* **496,** 64-68.
95. Ozber, N., I. Baris, G. Tatlici, I. Gur, S. Kilinc, E. B. Unal and I. H. Kavakli (2010) Identification of two Amino Acids in the C-terminal Domain of Mouse CRY2 Essential for PER2 Interaction. *BMC Mol. Biol.* **11,** 1.
96. Schmalen, I., S. Reischl, T. Wallach, R. Klemz, A. Grudziecki, J. R. Prabu, C. Benda, A. Kramer and E. Wolf (2014) Interaction of Circadian Clock Proteins CRY1 and PER2 Is Modulated by Zinc Binding and Disulfide Bond Formation. *Cell* **157,** 1203-1215.
97. Roenneberg, T., K. V. Allebrandt, M. Merrow and C. Vetter (2012) Social Jetlag and Obesity. *Curr. Biol.* **22,** 939-943.

	I. Halil Kavakli is a molecular biologist and interested in understanding the structure-function of cryptochromes (CRYs) in unicellular and mammalian organisms, and identification of small molecules that regulate mammalian CRYs activity. He received his PhD from Washington State University. He was a post-doctoral fellow at University of North Carolina at Chapel Hill with Aziz Sancar for 4.5 years in the Department of Biochemistry and Biophysics. He is currently Professor of Chemical and Biological Engineering and Molecular Biology and Genetics at Koc University Istanbul, Turkey.
	Ibrahim Baris is a molecular biologist and interested in understanding the structure-function of mammalian CRYs. He works on CRY SNPs on protein level. He earned his PhD degree from Bogazici University. He is currently senior instructor of Molecular Biology and Genetics at Koc University Istanbul, Turkey.
	Mehmet Tardu is a computational and experimental biologist. He is interested in understanding the role of CRY in unicellular organisms. He earned his PhD degree from Koc University. He is currently a post-doctoral fellow at Koc University with Halil Kavakli in Chemical and Biological Engineering.
	Seref Gul is a computational chemist and interested in identification of small molecules that regulate the activity of mammalian CRYs. He is currently a PhD student at Koc University with Halil Kavakli in Chemical and Biological Engineering.

	Hasimcan Oner is a molecular biologist interested in understanding the bidirectional relationship between immune system and circadian clock. He is currently a PhD student at Koc University with Halil Kavakli in Chemical and Biological Engineering.
	Sibel Cal is a molecular biologist interested in understanding the relationship between metabolism and circadian clock in mammalian organisms. She is currently a PhD student at Koc University with Halil Kavakli in Molecular Biology and Genetics.
	Selma Bulut is a molecular biologist interested in identification of the new core-clock components. She is currently a PhD student at Koc University with Halil Kavakli in Chemical and Biological Engineering.
	Darya Yarparvar has got her bachelor's degree in chemical engineering from Sharif University of Technology. She is now a biochemist interested in identification of small molecules that regulate the activity of mammalian CLOCK. She is currently a PhD candidate at Koc University with Halil Kavakli in Chemical and Biological Engineering.

	Caglar Berkel is a molecular biologist interested in understanding the role of CRY SNPs on biological clock in mammals. He is currently a PhD student at Koc University with Halil Kavakli in Molecular Biology and Genetics.
	Pinar Ustaoglu is a molecular biologist. She is interested in identification of small molecules that regulate the activity of mammalian CRYs. She earned her PhD degree from Birmingham University. She is currently a post-doctoral fellow at Koc University with Halil Kavaklı in Molecular Biology and Genetics.
	Cihan Aydin is a molecular biologist and structural biologist interested in understanding the bidirectional relationship between immune system and circadian clock, and how viral infections affect the biological rhythm. He earned his PhD degree from UMass Medical School in Biochemistry and Molecular Pharmacology. He performed his post-doctoral fellowship in Prof. Halil Kavaklı's lab at Koc University. He is currently an Assistant Professor of Molecular Biology and Genetics in Istanbul Medeniyet University.

Tables

Table 1. Comparison of the binding constants of *V. cholerae* CDP photolyase and CRY-DASH by surface plasmon resonance (38).

Protein	**SPR Kd**	**Biochemical Assay**
VcPHR/UV damaged ssDNA	0.8-1.8 (± 0.3) nM	0.5 nM
VcPHR/Undamaged ssDNA	8.49-22.91 (± 1.4) μM	NA
VcPHR/UV damaged dsDNA	18.53-26.08 (± 0.5) nM	0.79 nM [45] 4.76 nM [28]
VcPHR/Undamaged dsDNA	8.46-41.30 (± 0.6) μM	NA
CRY-DASH/UV damaged ssDNA	29-140 (± 7.5) nM	NA
CRY-DASH/Undamaged ssDNA	106.4-128.6 (± 12.2) μM	NA
CRY-DASH/UV damaged dsDNA	11.6-27.3 (± 15.3) μM	NA
CRY-DASH/Undamaged dsDNA	33.8-80.2 (± 10.7) μM	NA

Figure Captions

Figure 1. Phylogenetic tree of the cryptochrome/photolyase family (CPF). The unrooted phylogenetic tree of CPF members was inferred using the neighbor-joining method. The optimal tree with the sum of branch lengths = 10.04393617 is shown. Bootstrap support percentages based on 1,000 replicates are displayed. Reproduced from reference (10) with permission.

Figure 2. DNA photoproducts that occur as a result of UV irradiation. Two major types of compounds resulting from DNA damage are formed: pyrimidine cyclobutane dimers (CPDs) and pyrimidine−pyrimidone (6−4) photoproducts. Photoproducts between adjacent thymines are shown. Similar photoproducts can also occur between any two adjacent pyrimidines, i.e., T−T, C−C, C−T, and T−C, except that the (6−4) photoproduct does not form at C−T sites. Reproduced from (16) with permission.

Figure 3. Crystal structures of (a) *Escherichia coli* CPD photolyase, (b) *Anacystis nidulans* CPD photolyase, (c) *Thermus thermophilus* CPD photolyase, (d) *Arabidopsis thaliana* (6–4) photolyase, and (e) the superimposition of photolyases. MTHF: methenyltetrahydrofolate, FAD: flavin adenine dinucleotide

Figure 4. Repair mechanism of CPD photolyase. First, folate absorbs a photon from the blue light wavelengths and transfers the energy to flavin. Finally, the excited electron breaks the cyclobutane ring and is transferred back to flavin. Reproduced from (16) with permission.

Figure 5. Photo-reactivation by two Trp triad mutant and wild-type strains. Cells were irradiated with 10 J/m^2 and photo-reactivated for the indicated times. According to the increase in the level of survival after photo-reactivation, the number of repaired dimers was calculated (after correcting for the residual photo-reactivation in the *phr* null mutant) and plotted as a function of photo-reactivation time (the length of time cells were exposed to blue light after UV irradiation). Circle, wild type; triangle, W306F mutant. Reproduced from (61) with permission.

Figure 6. Schematic outline of the molecular mechanism contributing to the generation of circadian rhythms. CLOCK and BMAL1 form a heterodimer which binds to the E-box (CACGTG) in the promoters of *Per* and *Cry* genes as well as those of other clock-controlled genes. The CRY and PER proteins dimerize and, after a time lag, enter the nucleus and inhibit CLOCK:BMAL1-driven transcription of *Per*, *Cry*, and other clock-controlled genes, thus generating an oscillatory pattern of gene expression. A second feedback loop is formed by the action of RORα and REV-ERBα proteins on *Bmal1* transcription; these nuclear receptors activate (ROR) and repress (REV-ERB) transcription through their competitive action on response elements (ROREs) on the *Bmal1* promoter.

Figure 7. Schematic representation of cryptochrome/photolyase family (CPF) proteins. Depending on the type of protein, lengths range from 500 to 700 amino acids. All CPF members contain an N-terminal α/β domain and an α-helical domain associated with FAD. In addition, most CRYs have C-terminal extensions ranging in size from 40 to 250 amino acids. The approximate binding sites of the two cofactors MTHF and FAD are indicated in yellow and blue colors, respectively.

Figure 8. Schematic illustration of the PER2-CRY1 interface between helix α3 of PER2 and coiled coils helix (CC helix) of CRY1.