Minireview

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DASH-type cryptochromes – solved and open questions

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Abstract: *Drosophila*, *Arabidopsis*, *Synechocystis*, human (DASH)-type cryptochromes (cry-DASHs) form one subclade of the cryptochrome/photolyase family (CPF). CPF members are flavoproteins that act as DNA-repair enzymes (DNA-photolyases), or as ultraviolet(UV)-A/blue light photoreceptors (cryptochromes). In mammals, cryptochromes are essential components of the circadian clock feed-back loop. Cry-DASHs are present in almost all major taxa and were initially considered as photoreceptors. Later studies demonstrated DNA-repair activity that was, however, restricted to UV-lesions in single-stranded DNA. Very recent studies, particularly on microbial organisms, substantiated photoreceptor functions of cry-DASHs suggesting that they could be transitions between photolyases and cryptochromes.

Keywords: cryptochrome; DNA-photolyase; DNA-repair; photoreceptor; protein structure.

Introduction

Cryptochrome/photolyase family (CPF) members are present in all major taxa from archaea to mammals (Mei and Dvornyk 2015) and can be divided into 10 subfamilies based on sequence similarity and function (Öztürk 2017). Simply spoken, CPF members act as light-driven DNA-repair enzymes (DNA-photolyases), or as photoreceptors (cryptochromes) by using the same spectral range (UV-A/blue light) for repair and signaling, respectively. Light absorption in CPF members is caused by the presence of non-covalently bound U-shaped flavin adenine dinucleotide (FAD). In addition, many CPF members, contain a second cofactor that functions as antenna chromophore and transfers excitation energy to FAD (Chaves et al. 2011). Second chromophores identified are 5,10-methenyltetrahydofolate (MTHF), 7,8-didemethyl-8-hydroxy-5-deazariboflavin (8-HDF, F_0), FMN, FAD and, in a more recently discovered subclade that also contains an 4Fe–4S cluster, 6,7-dimethyl-8-ribityl-lumazin (DLZ) (Geisselbrecht et al. 2012). Although second cofactors were not identified for classical animals and plant cryptochromes, MTHF-binding cannot be excluded for the latter based on structural similarities with class III photolyase that accommodates MTHF via two Trp residues (Scheerer et al. 2015) and differ in this aspect from class I photolyase and cry-DASH (see below). The two major ultraviolet (UV) photoproducts in DNA that are formed between adjacent pyrimidines are cyclobutane pyrimidine dimers (CPDs) and (6–4) pyrimidine-pyrimidone photoproducts (6–4PP). Both DNA lesions can be repaired by DNA photolyases that are substrate-specific. Accordingly, these photolyases are named CPD photolyase or (6–4) photolyase, respectively (Sancar 2003). Although the repair mechanisms between the two types of photolyases differ including a proton transfer between a histidine in the catalytic pocket and the 6–4PP (Li et al. 2010; Tan et al. 2015) both require the FAD cofactor for catalysis. In the fully reduced and photoexcited state ($FADH^{-}$*) an electron is injected by photolyase into the DNA-lesion. Furthermore, the lesion dimer has to be flipped out of the DNA-strand into the catalytic pocket to get in close vicinity to $FADH^{-}$*. The resulting dimer radical is unstable and bonds between the pyrimidines, which were formed before by UV-irradiation, are split (Sancar, 2003). Phylogenetic studies suggest that CPF members arose very early in evolution, possibly to repair UV-induced lesions in DNA under conditions where such damage was prevalent owing to the lack of an ozone layer (Gehring and Rosbash 2003). Cry-DASHs have a large distribution ranging from archaea to vertebrates (Mei and Dvornyk 2015).

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Discovery and distribution of DASH-type cryptochromes

The first Cry-DASH was discovered in the cyanobacterium *Synechocystis* sp. PCC6803. Its ORF *sll1629* showed homology to photolyases, and the protein bound FAD. However, *sll1629* did not contribute to the UV-resistance and the recombinant protein was unable to repair a thymine dimer (T<>T) in a double-stranded (ds) DNA probe (Hitomi et al. 2000). The same authors also noted that *sll1629* is closely related to animal cryptochromes and (6–4) photolyases and concluded that it is likely a cryptochrome photoreceptor. A second photolyase-related gene in *Synechocystis* (*slr0854*) is highly similar to class I photolyases and contributed to photoreactivation (Hitomi et al. 2000). Photoreactivation is the process that increases the survival rate of UV-treated cells or phages by visible light given simultaneously or immediately after UV exposure (Dulbecco 1949; Kelner 1949). These conclusions were confirmed by an independent study (Ng and Pakrasi 2001), which also showed that a *slr0854/sll1629* (*phrA/phrB*) double mutant is slightly more sensitive against UV-B than the *phrA* single mutant, suggesting that *phrB* could have some photolyase activity. The name Cry-DASH was invented in 2003 (Brudler et al. 2003) together with the discovery of another photolyase homolog in *Arabidopsis thaliana* (*At*Cry3; AT5G24850.1) that contained FAD and did bind to undamaged dsDNA and single-stranded (ss) DNA, but was unable to complement photoreactivation in photolyase-deficient *E. coli* cells (Kleine et al. 2003). An unrooted phylogenetic tree of 12 CPF members showed that the two novel *Synechocystis* and *Arabidopsis* proteins form a separate subclade that was most closely related to animal type cryptochromes from *Drosophila melanogaster* and *Homo sapiens* leading to the name Cry-DASH. More recently, an alternative and probably more suitable name for Cry-DASH was suggested, namely class 0 PHR (Öztürk 2017). However, for historical reasons, we stay with the name Cry-DASH in this review.

Cry-DASH members have been identified in species ranging from archaea up to vertebrates including birds, but not in the mammalian clades Metatheria, Monotremata and Eutheria (Mei and Dvornyk 2015). Thus, Cry-DASHs are the CPF subclade with the most widespread distribution.

Biological functions of Cry-DASHs

The initial characterization of *Synechocystis* Cry-DASH (Brudler et al. 2003) indicated a regulatory role. Microarray-based comparison of the wild type and Cry-DASH mutant transcriptomes showed some upregulation of at least two transcripts encoding unknown proteins, suggesting that Cry-DASH suppresses the expression of these genes. Considering the accepted role of Cry-DASH as cryptochrome photoreceptors it was a big surprise when the group of Aziz Sancar, who is a 2015 Nobel Prize Laureate in Chemistry, demonstrated for Cry-DASH from *A. thaliana* (*At*Cry3), *Xenopus laevis* (*Xl*Cry) and *Vibrio cholerae* (*Vc*Cry1) highly efficient repair of T<>T in ssDNA but not in dsDNA probes (Selby and Sancar 2006). Accordingly, these authors suggested to reclassify Cry-DASH as ssDNA photolyases but also noted that this does not exclude a second function as photoreceptor as described for canonical class I photolyase from fungi (Bayram et al. 2008; Berrocal-Tito et al. 2007). Indeed, photoreceptor functions of Cry-DASHs are described for microbial organisms. The fungal plant pathogen *Sclerotinia sclerotiorum* (Ascomycota) encodes a Cry-DASH (Cry1) that is upregulated upon UV-A exposure. Deletion of this gene has some but not essential role in sclerotia mass production under UV-A light (Veluchamy and Rollins 2008). The dinoflagellate *Karenia brevis* encodes one Cry-DASH (*Kb*Cry) that is localized in the chloroplast. Under blue light, cells enter earlier into the S phase. Since *Kb*Cry is apparently the only blue light photoreceptor candidate in this organism it was speculated that it could control the cell cycle (Brunelle et al. 2007), but genetic evidence is lacking. Five members of the CPF have been identified in the green alga *Ostreococcus tauri*. Two of them were functionally characterized and belong to the (6–4) photolyases (*Ot*CPF1) and Cry-DASHs (*Ot*CPF2), respectively. The recombinant *E. coli*-expressed *Ot*CPF2 binds FAD and MTHF. Interestingly, *Ot*CPF2, when co-expressed in COS cells together with the mammalian clock components CLOCK and BMAL, suppressed the CLOCK:BMAL heterodimer-mediated activation of an chimeric luciferase reporter gene that included an E-box in its promoter region to which the CLOCK:BMAL heterodimer binds (Heijde et al. 2010). This is reminiscent of the repressor function of Cry in the mammalian cell context and suggests that this *O. tauri* Cry-DASH could play a role as component of the circadian clock. However, the inhibitory effect of *Ot*CPF2 was much weaker than of mouse CRY and *Ot*CPF1. The same authors also discovered binding of about 50 nt long RNA to both *O. tauri* proteins after purification from *E. coli*. Moreover, it was shown that *Ot*CPF2 repairs a CPD-containing dsDNA probe. However, it remains unclear whether this result was specific since a several-hundred-fold excess of enzyme over probe was used in the assay. Likewise, it is not clear whether *Ot*CPF2 contributes to photoreactivation in *O. tauri*. A role of Cry-

DASH as oscillator is also described for *Neurospora crassa*, whereas entrainment of the circadian clock in this organism requires White Collar 1 (Nsa et al. 2015) that together with White Collar two operates as positive element in the oscillator loop (Froehlich et al. 2002).

Fusarium fujikuroi (*Gibberella fujikuroi*, Ascomycota) encodes one Cry-DASH (*Ff*CryD). *E. coli* expressed *Ff*CryD carried MTHF and FAD cofactors and was fully photoreducible to the $FADH^-$ state. As expected, it repaired T<>T in ss probe, but surprisingly bound undamaged and damaged ssDNA and dsDNA probes with similar affinities as well as RNA of not defined sequence. Bound RNA accelerated its photoreduction (Castrillo et al. 2015). The previously made conclusion that the PHR domain of *Ff*CryD binds RNA (Castrillo et al. 2015) needs to be reconsidered based on more recent studies showing that the approximately 230 amino acid long C-terminal extension, which contains three Arg-Gly-Gly (RGG) motifs known from other proteins to be engaged in *e.g.*, RNA interaction (Godin and Varani 2007) is responsible for RNA binding of *Ff*CryD (Javier Pardo Medina, Stephan Kiontke, Alfred Batschauer, Javier Ávalos, unpublished data). The expression of *Ff*CryD is induced by light under control of the white collar one gene *wcoA*. The *Ff*CryD mutant shows several light-dependent alterations compared to wild type, particularly under nitrogen limitation such as formation of macroconidia, increased production of bikaverin and reduced formation of gibberellin. The effects seen by the *Ff*CryD knock-out on secondary metabolism were not caused by alterations in the expression of biosynthetic genes, strongly suggesting that *Ff*CryD regulates these pathways at the posttranscriptional level (Castrillo et al. 2013). Whether RNA-binding activity of *Ff*CryD is directly connected with its function as photoreceptor needs to be clarified. Likewise, it is unclear, which if any biological function RNA-binding has for Cry-DASH from *V. cholerae* (Worthington et al. 2003) and *N. crassa* (Froehlich et al. 2010). Besides the secondary metabolites mentioned above, also photoinduced carotenogenesis is controlled in part by *Ff*CryD in a later phase, whereas WcoA controls this pathway in the early and late phase and much stronger than *Ff*CryD (Castrillo and Avalos 2015).

Like in some fungal species, a clear role of Cry-DASH as photoreceptor has been found for *Synechocystis* PCC 6803. This photosynthetic cyanobacterium suppresses photosystem II (PSII) activity and the amount of the PSII core proteins D1 and D2 during UV-B and high visible light exposure. The Cry-DASH mutant (Δ*sll1629*) is unaffected in the repair of CPD lesions, but strongly reduced in PSII activity and had a lower D1 protein level under UV-B and high light stress (Vass et al. 2014). The same authors also showed that the transcript levels of *psbA3*, encoding D1, are undistinguishable between wild type and the Δ*sll1629* mutant under low light and similarly induced by UV-B. Based on differences in the proteomes of wild type and the Δ*sll1629* mutant it was concluded that the role of *Synechocystis* Cry-DASH as photoreceptor on the recovery of PSII is likely to be indirect via regulating CO_2 transport and fixation or by regulating the level of the pili protein PilA1 (Vass et al. 2014).

A stray CPF member is CryP from the diatom *Phaeodactylum tricornutum* because its sequence matches better with plant cryptochromes, but the spectroscopic properties of the *E. coli* expressed protein is more similar to Cry-DASH (Juhas et al. 2014). As seen for other Cry-DASHs it binds FAD and MTHF and the FAD is present in different redox states after isolation from the cells, which is not seen for plant CRYs. Similar to cry-DASH from *A. thaliana* (*At*Cry3), its FAD cofactor can be fully reduced to $FADH^-$ concomitant with bleaching of MTHF that results from reduction of 5,10-methenylTHF (MTHF) to 5,10-methyleneTHF (Moldt et al. 2009). Analysis of knock-down mutants of *Pt*CryP revealed upregulation of light-harvesting complex proteins Lhcf1-Lhcf11 and downregulation of Lhcx light protection proteins likewise suggesting a photoreceptor role of *Pt*CryP (Juhas et al. 2014).

Cordyceps militaris (Ascomycota) produces secondary metabolites such as carotenoids and cordycepin. Several developmental processes such as primordium differentiation, production of fruiting bodies and pigmentation are light-induced in this fungus. It encodes one Cry-DASH (*Cm*Cry-DASH) that has, like other fungal Cry-DASHs, a C-terminal extension of about 120 amino acids containing RGG motifs of so far unknown function (Wang et al. 2017). The *Cm*Cry-DASH mutant shows several alterations compared to wild type, including increased levels of carotenoids and cordycepin in white light opposite to the white collar 1 (*wc-1*) mutant, lower production of conidia, and lack of light-induction of *wc-1* expression, clearly indicating that Cry-DASH has a photoreceptor function in *C. militaris* (Wang et al. 2017). Although many of the above-mentioned studies support a function of Cry-DASHs as photoreceptors, it was surprising to find that the Cry-DASH of *Phycomyces blakesleeanus* (*Pb*CryA) is able to repair CPD lesions in dsDNA (Tagua et al. 2015). *Phycomyces* does photoreactivate, but encodes only one CPF member, which is *Pb*CryA. Therefore, it was concluded that this Cry-DASH has not only a canonical photolyase function, but it is also the light-driven enzyme for photoreactivation. Although genetic evidence for this statement is lacking due to the inaccessibility of this organism of genetic manipulation, it is tempting to speculate that Cry-DASHs could have

canonical dsDNA repair photolyase activity, which was maintained after the loss of a class I photolyase (present in other fungal clades), and thus representing an early stage in Cry-DASH evolution (Tagua et al. 2015). Our own work on *Ustilago maydis* (Basidiomycota), a biotrophic fungus causing corn smut disease, identified two Cry-DASH genes in this organism named *Um*Cry1 (UMAG_01131) and *Um*Cry2 (UMAG_05917). Recombinant *E. coli* expressed *Um*Cry1 bound only FAD, whereas *Um*Cry2 contained FAD and MTHF. Both enzymes repair T<>T at least in ssDNA probes. It is unlikely that *Um*Cry1 and *Um*Cry2 have canonical photolyase activity because *U. maydis* encodes two other genes, *Um*Phr1 (UMAG_06079) and *Um*Phr2 (UMAG_02144), which have confirmed and non-compromised CPD- or (6–4)-photolyase activity, respectively (Brych et al. 2016).

The first plant Cry-DASH characterized in detail is from *A. thaliana* (Kleine et al. 2003). It was named *At*Cry3 and found to be localized in chloroplasts and mitochondria. In addition to *Vc*Cry1 (Worthington et al. 2003), *At*Cry3 is a rare example for a CPF member for which the cofactor composition, MTHF and FAD, was determined from its natural source (Göbel et al. 2017). Unpublished data based on a PCR assay from our group showed that *At*Cry3 does most likely not contribute to DNA repair in the organelles, consistent with its compromised photolyase activity. However, other sensitive assays for the detection of CPD-repair *in vivo* could be applied to reinvestigate this issue. Surprisingly, some *At*Cry3-GFP was also detected in the nucleus in addition to the before described localization in organelles (Kleine et al. 2003).

Structural insights into Cry-DASHs

First structural information on cry-DASHs was based on the crystal structures of the cyanobacterial cry-DASH from *Synechocystis* sp. PCC6803 (*Syn*Cry-DASH; pdb code 1NP7; Brudler et al. 2003) and the plant ortholog from *A. thaliana* (*At*Cry3; pdb code 2IJG; Huang et al. 2006). *At*Cry3 was established as a model for further in-depth structural and functional studies. Both crystal structures revealed that Cry-DASHs possess a bilobial architecture common to all CPF members, with the N-terminal α/β subdomain and the C-terminal α-helical subdomain. The C-terminal α-helical subdomain contains the non-covalently bound catalytic cofactor FAD in its typical U-shaped conformation (Figure 1A). *Syn*Cry-DASH and *At*Cry3 share a similar overall fold with r.m.s.d. of 0.6 Å for 345 superimposed C_α-atoms. Moreover, the structural similarity with all members of the CPF is reflected in low r.m.s.d. of superimposed C_α-traces (Kiontke et al. 2011). However, in addition to this core fold, which is often denoted as photolyase homology region (PHR) domain, Cry-DASHs can feature both N-terminal and C-terminal extensions (Figure 1B). *At*Cry3 bears an N-terminal extension of about 40 amino acids that mediates import into chloroplasts and mitochondria (Kleine et al. 2003). Other Cry-DASHs e.g., *Fusarium* CryD possess a C-terminal extension. Interestingly, based on bioinformatics prediction methods, this C-terminal extension of potential RNA-protein interaction appears to be largely unstructured.

In addition to the common catalytic cofactor FAD, members of the CPF usually bind an auxiliary cofactor (also called antenna chromophore). Whereas DNA photolyases have been characterized with antenna chromophores of flavin derivatives (FAD, FMN, DLZ, and 8-HDF) and the folate derivative MTHF, the antenna of Cry-DASHs is restricted to MTHF. All flavin antennas are deeply buried in the N-terminal α/β subdomain, which corresponds to the Rossman fold. In contrast, the MTHF cofactor (folate derivative) is bound in a cleft between the N-terminal α/β-subdomain and the α-helical catalytic subdomain (Figure 1A). A single, glutamate residue (*At*Cry3: E149) highly conserved in class I photolyase and cry-DASH is essential for binding of MTHF via hydrogen bonds (Figure 1A). Mutation of this glutamate to alanine (E149A) leads to a complete loss of the antenna chromophore (Zirak et al. 2009). This exclusive role of the glutamate residue in binding of MTHF has also been reported for the structural analogous glutamate E109 in the canonical class I CPD photolyase from *E. coli* (*Ec*CPDI; Henry et al. 2004). The distances and orientations of MTHF in both proteins are similar and well suited for Förster energy transfer to the catalytic cofactor FAD (*At*Cry3: 15.2 Å; *Ec*CPDI: 16.8 Å). Both proteins show a typical red-shift of MTHF's absorbance maximum of approximately 25 nm in comparison to MTHF ($\lambda_{max} \approx 360$ nm) in aqueous solution. This spectral shift of the antenna is caused by protein-MTHF interactions (Song et al., 2006). Noteworthy, the *E. coli* expressed recombinant *Syn*Cry-DASH lacks the antenna chromophore MTHF although the corresponding glutamate (E114) is structurally conserved (Brudler et al., 2003).

For DNA repair activity, the catalytic cofactor FAD in Cry-DASHs (and canonical DNA photolyases) must be converted to its fully reduced state $FADH^-$, a process called photoreduction or photoactivation. *In vitro*, this process can be achieved by blue-light illumination in the presence of an external reducing agent. During photo-reduction a chain of three tryptophan residues (tryptophan triad) mediates the electron transfer from the protein surface to the excited catalytic cofactor FAD (Figure 1C). In *At*Cry3, the

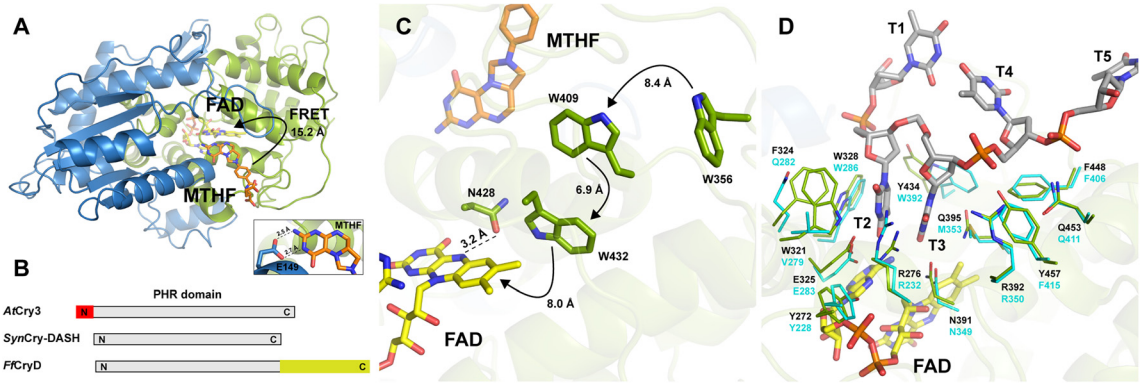

Figure 1: Structural insights into *Drosophila, Arabidopsis, Synechocystis*, human (DASH)-type cryptochromes (Cry-DASHs). (A) Overall structure of *Arabidopsis thaliana* (*At*)Cry3 (pdb code 2J4D). The N-terminal α/β-subdomain is highlighted in blue. The C-terminal α-helical domain (green) harbors the catalytic cofactor flavin adenine dinucleotide (FAD) (yellow). The antenna chromophore 5,10-methenyltetrahydofolate (MTHF) (orange) is bound in a cleft between both subdomains in a distance of 15.2 Å suitable for Förster energy transfer to FAD. The exclusive role of E149 in binding of MTHF is illustrated in the inset. (B) Architecture of several Cry-DASHs. The N-terminal chloroplast/mitochondria import sequence of *At*Cry3 is depicted in red. The unstructured C-terminal extension of *Fusarium fujikuroi*(*Ff*)CryD is shown in olive. (C) Structural overview of the main functional elements in *At*Cry3. The tryptophan triad (W356 → W409 → W432) mediates the electron transfer to the catalytic cofactor FAD during photoreduction. The asparagine residue N428 ensures the accumulation of the catalytic active, fully reduced $FADH^-$ state. (D) Detailed view of the *At*Cry3 cyclobutane pyrimidine dimer (CPD)-binding pocket with the catalytic cofactor FAD (yellow) and the CPD lesion (gray). *At*Cry3 shares a high structural similarity with the canonical class I CPD photolyase from *A. nidulans* (cyan; pdb code 1TEZ).

residues of the tryptophan triad (W356 → W409 → W432) are functionally and structurally conserved compared to class I CPD photolyases, e.g., from *E. coli* (Huang et al. 2006). Mutation of the surface-exposed tryptophan W356 or the tryptophan in proximity of the catalytic cofactor FAD (W432) to redox inactive phenylalanine (W356F; W432F) leads to a loss of photoactivation *in vitro* (Moldt et al. 2009). This functional and structural similarity to class I CPD photolyases also applies to the asparagine residue (N428) in proximity to the *N*5-atom of FAD's isoalloxazine ring (Figure 1C; Klar et al. 2007). It was shown by FTIR spectroscopy that the interaction of asparagine N378 to the *N*5-atom in *Ec*CPDI is crucial for the transition to the catalytically active, fully reduced $FADH^-$ state upon photoactivation (Wijaya et al. 2016). Moreover, mutation of this asparagine residue to an aspartate leads in the class II CPD photolyase from *M. mazei* to an accumulation of the neutral radical FADH state with blue-shifted absorbance maxima during *in vitro* photoreduction (Kiontke et al. 2011). This photocycle arrest along with the blue-shifted maxima represents the signaling state of plant cryptochromes such as *At*Cry1 and *At*Cry2, which possess a structurally conserved aspartate residue (*At*Cry1: D396; *At*Cry2: D393) at this position (Brautigam et al. 2004). The asparagine residue close to the *N*5-atom of FAD's isoalloxazine ring is conserved in all members of the CPF which exhibit DNA repair activity and make a major difference between DNA repair activity and signaling from a structural point of view.

In line with the above-mentioned structural requirements for repair activity was the finding that Cry-DASHs repair CPD-lesions in ssDNA (Selby and Sancar 2006) and partially melted dsDNA (Pokorny et al. 2008), in which UV-induced dimerization of adjacent pyrimidines is more efficient than in duplex DNA (Becker and Wang 1989). Moreover, the crystal structure of *At*Cry3 in complex with an *in situ* repaired CPD lesion (in ssDNA) provided detailed structural insight into the molecular mechanisms of the Cry-DASH subfamily (Pokorny et al. 2008). *At*Cry3 shares a high structural similarity of the CPD-binding pocket compared to the canonical class I CPD photolyase from *A. nidulans* (*An*CPDI; Figure 1D). However, the substitution of the hydrophilic glutamine side chain in *An*CPDI (Q395) with a non-polar, bulky methionine in *At*Cry3 (M353) makes a tremendous structural difference and has been proposed as a unique discriminant between CPD photolyases and Cry-DASHs (Miyazawa et al., 2008). It also was concluded that *At*Cry3 lacks the ability to interfere with the Watson-Crick base pairing at least at one thymine of the CPD lesion in dsDNA hindering base flipping into the active site (Klar et al. 2007; Pokorny et al. 2008).

Surprisingly, the *Phycomyces* CryA, showed all structural and functional characteristics of a Cry-DASH but was capable to repair CPD lesions in dsDNA with a similar catalytic efficiency as in ssDNA and represents an ancient subtype of Cry-DASHs (Tagua et al. 2015). Structural

information on this particular subtype in complex with CPD-containing dsDNA would help to gain molecular insight into their CPD repair mechanism and the evolution of CPF members.

Concluding remarks

Discovered almost 20 years ago, the Cry-DASHs are meanwhile among the best analyzed subclades of the CPF regarding both, structure and function. From an evolutionary point of view Cry-DASHs are a fascinating link between DNA-repair enzymes and photoreceptors. However, there are still a couple of unsolved questions to be addressed. For example, it is unknown how these proteins bind RNA and what biological role this has if any? Can the photoreceptor function of Cry-DASHs particularly in fungi be uncoupled from their compromised DNA-repair activity? Is the repair activity in ssDNA and loop-structured dsDNA of biological relevance and what is the mechanistic and structural reason for this compromised repair activity? And finally, is the recently discovered canonical photolyase function of Cry-DASHs in Mucorales species an evolutionary relic or was this activity regained after the loss of canonical photolyases?

We apologize to those colleagues whose works are not cited in this review due to space limitations.

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