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Photolyases and cryptochromes: common mechanisms of DNA repair and light-driven signaling?

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DNA photolyases are extremely efficient light-driven DNA repair enzymes that use the energy of a blue-light photon to 'inject' an electron onto UV-damaged DNA, catalyzing the splitting of mutagenic pyrimidine dimers. By contrast, cryptochromes use blue light to trigger signaling cascades in multicellular organisms, fungi and several prokaryotes. Despite these functional differences, both protein families arose from a common ancestor and share many similarities, such as the overall protein fold, the presence of antenna chromophores and the use of flavin adenine dinucleotide (FAD) as the primary reactive group. Several significant advances in the biophysical and structural characterization of photolyases and cryptochromes are now revealing the details of how light-driven redox reactions can be used for such seemingly different purposes.

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Introduction

DNA photolyases are DNA repair enzymes that revert the genome-damaging effects caused by UV light, namely the covalent cross-linking of neighboring pyrimidine bases due to [2 + 2] photocyclization. Unlike other DNA repair enzymes, DNA photolyases use the energy of a photon from the near-UV/visible spectrum of light (λ = 320–500 nm) to cleave these pyrimidine dimers [1•]. Two types of UV lesions are repaired by structurally related subfamilies of DNA photolyases. *Cis-syn* cyclobutane pyrimidine dimers (CPDs) are formed by the photocyclization of the C5–C6 double bonds of two pyrimidine bases, mostly thymine, and make up about three-quarters of all UV lesions. The remainder are primarily 6-4 pyrimidine–pyrimidone lesions (6-4 PP), which are generated by the photoaddition of the C4 carbonyl of a thymine to the C5–C6 double bond of a neighboring pyrimidine, followed by an Amadori rearrangement (Figure 1).

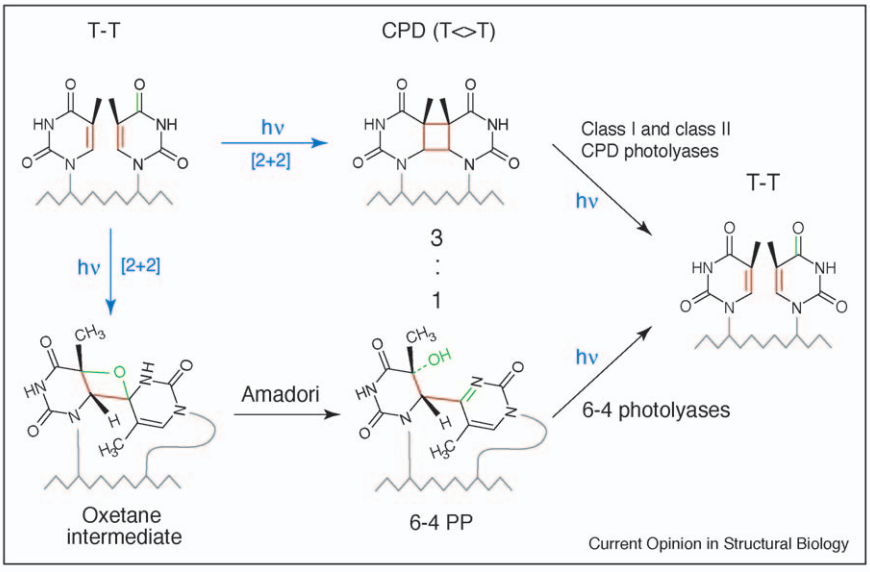
Due to their wide occurrence in eukaryotes, eubacteria and archaea [2], including several viruses (e.g. [3]) and parasites [4], CPD photolyases were presumed to have evolved in the first forms of life, when the UV flux on the surface of the primordial Earth was at least three orders of magnitude higher than nowadays. Unlike the almost ubiquitous CPD photolyases, which can be further subdivided according to sequence similarity into two distinct subfamilies, class I and II, the 6-4 photolyases must have been a later invention during evolution, as they form a uniform subfamily and are mostly restricted to multicellular organisms, such as plants and animals. Interestingly, there are some indirect hints from directed evolution experiments that, even in a primordial 'RNA world', photolyase-like activities might have evolved to protect self-replicating nucleic populations from UV inactivation, although RNA naturally exhibits a significantly better UV resistance than DNA [5•]. *In vitro* selection of single-stranded DNA oligonucleotides with the ability to repair CPD lesions in *cis* and in *trans* yielded a 42-nucleotide DNAzyme that exhibited photolyase activity [6]. This DNAzyme had an activity spectrum resembling that of natural, protein-based photolyases and presumably used a quadruplex structure for light harvesting and electron transfer (ET).

Structures of class I CPD photolyases have been known for a decade [7–9], and a plethora of biochemical and biophysical data have been accumulated (for reviews, see [1•,2]), but only recently has work provided deeper insight into the mechanism of DNA recognition and repair, as well as the potential mechanistic resemblances between photolyases and their cousins among the blue-light receptors, the cryptochromes [10•,11•]. Structurally, photolyases and cryptochromes share a common, conserved architecture of about 420–620 residues comprising an N-terminal α/β domain, responsible for binding a light-harvesting chromophore, and a C-terminal α-helical domain, which contains a photoreducable FAD chromophore and, in the cases of photolyases, the active site for catalysis. This review focuses on how the functional and structural data now give a convergent view of the mechanism and action of photolyases and cryptochromes.

Recognition of CPD lesions by photolyases

Although CPD lesions are genotoxic to cells by causing the arrest of transcriptional and replicational activity, the structural changes that occur upon dimerization of pyrimidine

Figure 1



Formation and repair of DNA UV lesions. Reactive double bonds involved in the [2 + 2] photocyclization are highlighted in red and green. The repair of 6-4 PP lesions by 6-4 photolyases presumably proceeds via an oxetane intermediate that is formed in the active site of the enzyme.

bases are comparatively minor. In B-type DNA, the CPD lesion in its *cis-syn* conformation (T<>T) induces a 22° bend towards the major groove [12], but the bases of the thymine dimer still stack within the duplex DNA and form Watson–Crick-like hydrogen bonds with the complementary adenines (Figure 2a). Accordingly, a prerequisite for the repair of the CPD lesion is the complete flip-out of the thymine dimer into the active site of the CPD photolyase. The X-ray structure of a photolyase–CPD-DNA complex [13••] confirmed such a double flip-out, which is accompanied by local unwinding and an increase in the DNA bend to about 50° (Figure 2a,b). Apart from the thymine dimer, only the phosphoribose backbone of the DNA strand containing the CPD lesion makes specific interactions with the C-terminal catalytic domain of the CPD photolyase, which is highly conserved among class I CPD photolyases [13••,14••]. The structurally defined recognition motif (NpT<>TpNpNp) coincides with previous biochemical footprinting data on the binding of photolyases to DNA; for nucleosome-bound DNA, it also explains the position-dependent modulation of photolyase-mediated DNA repair by steric hindrance [15].

In the photolyase–CPD-DNA complex, the flipped thymine dimer is deeply placed inside the active site, such that its C4 carbonyls hydrogen bond with the N6 amino group of the catalytic $FADH^-$ cofactor [13••]. Its location and intimate interaction with the catalytic flavin corroborates the reported effects of substrate binding on the reduction potential of the flavin cofactor [16,17•] and on the ET reactions within the enzyme [18], and disagrees with models in which the CPD lesion is only peripherally bound along the outer rim of the active site [19•,20•].

Despite concise structural information about the interaction of class I CPD photolyases and CPD-containing DNA [13••,14••], it is not entirely resolved how photolyases recognize their cognate substrate before complex formation and structural rearrangement of the DNA. Unlike many other DNA-binding enzymes, which are able to 'slide' along DNA using a mode of low-dimensional diffusion, DNA photolyases presumably bind to DNA via free three-dimensional diffusion, with association rate constants of $\sim 10^7\ M^{-1}\ s^{-1}$ [1•]. A primary and highly local recognition signal for CPD photolyases is proposed to be the extended BII-type conformation of the phosphoribose backbone of the uncomplexed CPD-DNA on the 3′ side of the T<>T dimer [12,21]. This highly energetic BII conformation differs from the canonical and compact BI conformation, which is a characteristic of B-type DNA, by the switching of the ε/ζ torsion angles from trans/gauche$^-$ to gauche$^-$/trans (Figure 2a). For the P^{+1} phosphate, the X-ray crystallographic and NMR data delineated a crucial salt bridge to a highly conserved arginine sidechain. Conserved interactions are also made with the P^{+2} and P^{+3} phosphates [13••,14••], but the loss of the P^{+1} recognition site causes the largest decrease in activity and specificity for CPD lesions [14••,22]. Interestingly, the crystal structure of the photolyase–DNA complex shows not only two complexes with CPD-DNA, but also two complexes with short stretches of non-specifically bound DNA whose phosphate groups are bound to the P^{+1}, P^{+2} and P^{+3} phosphate-recognition