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Direct DNA damage reversal: elegant solutions for nasty problems

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Abstract. The genomic integrity of all living organisms is constantly jeopardized by physical [e.g. ultraviolet (UV) light, ionizing radiation] and chemical (e. g. environmental pollutants, endogenously produced reactive metabolites) agents that damage the DNA. To overcome the deleterious effects of DNA lesions, nature evolved a number of complex multi-protein repair processes with broad, partially overlapping substrate specificity. In marked contrast, cells may use very simple repair systems, referred to as direct DNA damage reversal, that rely on a single protein, remove lesions in a basically error-free manner, show high substrate specificity, and do not involve incision of the sugar-phosphate backbone or base excision. This concise review deals with two types of direct DNA damage reversal: (i) the repair of alkylating damage by alkyltransferases and dioxygenases, and (ii) the repair of UV-induced damage by spore photoproduct lyases and photolyases. (Part of a Multi-author Review)

Keywords. DNA repair, photolyase, methyltransferease, dioxygenase, spore photoproduct lyase, UV damage, alkylation damage.

Introduction

The integrity of the genome of every organism is continuously threatened by environmental agents [e.g. ultraviolet (UV) light, ionizing radiation, chemicals] and endogenously produced cellular metabolites (i. e. reactive oxygen species and other radicals) that damage DNA. Replication of damaged DNA can cause mutations, which ultimately can lead to cancer, while DNA lesions that obstruct replication and transcription can lead to cellular senescence or apoptosis, which are believed to contribute to diseases of aging [1–4]. In contrast to other biomolecules, DNA cannot be replaced, only repaired. Therefore, cells have acquired a variety of DNA repair mechanisms (i. e. nucleotide excision repair, base excision repair, cross-link repair, homologous recombination and non-homologous end joining) with broad (often overlapping) substrate specificities to counteract the harmful effects of DNA injuries (for review see [1, 3]). In addition to these complex, often error-prone, genome maintenance systems (e. g. nucleotide excision repair, involving over 30 proteins), nature has evolved single enzyme mechanisms that can repair lesions without incision of the DNA sugar-phosphate backbone or base excision. The relative simplicity of these repair mechanisms, referred to as direct damage reversal, predicts essentially error-free repair, with, however, a very narrow substrate range as a trade-off. This concise review deals with the two main types of direct DNA damage reversal systems: (i) repair of alkylating damage by alkyltransferases and dioxygenases, and (ii) repair UV light-induced photolesions by spore photoproduct lyases and photolyases.

Removal of alkylation damage

Alkylation damage

Alkylation damage could be the result of environmental exposure of DNA to compounds like N-

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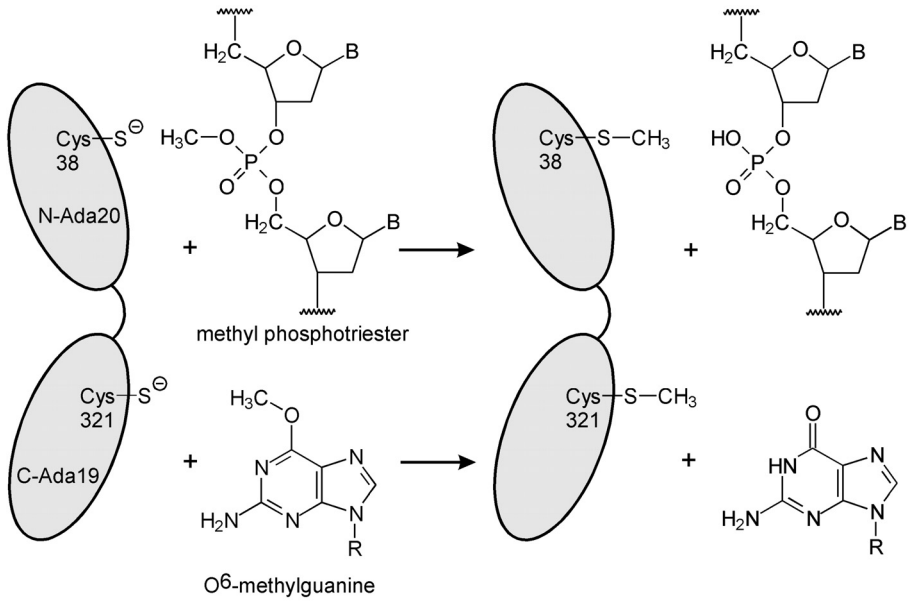

Figure 1. Reaction mechanism for *E. coli* Ada alkyltransferase. Both repair functions are depicted: demethylation of methylphosphotriesters in DNA by the N-terminal part and repair of O^6-meG by the C-terminal part. The methyl groups are transferred to a Cys residue in an irreversible suicide reaction.

methyl-N′-nitro-N-nitroso-guanidine (MNNG), N-methyl-N-nitrosurea (MNU), or methyl methanesulfonate (MMS), which cause a variety of O-alkylated and N-alkylated adducts. Apart from these powerful exogenous alkylating agents, alkylation damage may arise from endogenous sources like S-adenosylmethionine (SAM), a metabolite involved in many biochemical processes (e. g. the immune system, cell membrane maintenance, neurotransmitter degradation) and known to weakly methylate DNA. Biologically important lesions formed in double-stranded DNA (dsDNA) are O^6-methylguanine (O^6-meG), 7-methylguanine (7-meG), and 3-methyladenine (3-meA). In addition, phosphodiester groups in DNA might become methylated. Lesions like O^6-meG are mutagenic and carcinogenic as misincorporation during replication gives rise to G:C → A:T transitions. Although alkylated bases can be removed by multistep excision repair processes, several lesions are also subject to effective and highly specific direct repair mechanisms.

Alkyltransferases

In *Escherichia coli*, the *ada* gene encodes the 39-kD multifunctional repair protein O^6-alkylguanine-DNA alkyltransferase (O^6-Agt), composed of an N-terminal and a C-terminal part separated by a hinge region, and each containing a reactive center. While the 19-kD C-terminal part (C-Ada19) repairs O^6-meG or O^4-methylthymine, the 20-kD N-terminal part (N-Ada20) demethylates methyl phosphotriester lesions in the DNA. In both cases the methyl group is transferred in an irreversible reaction to a cysteine residue, Cys38 in N-Ada20 and Cys321 in C-Ada19 [5, 6] (Fig. 1). The Ada protein is strictly speaking not an enzyme, as it participates in an irreversible suicide reaction and therefore should be considered as a reactant rather than a catalyst.

Further adding to the multifunctionality of the Ada protein, methylation of Cys38 of N-Ada20 converts it into a transcriptional activator that specifically binds to the promoters of the *ada-alkB* operon and the *alkA* and *aidB* genes [7, 8]. The *alkA* gene encodes a 3-meA-DNA glycosylase (involved in base excision repair) and *alkB* a 1-meA/3-meC-DNA dioxygenase (see below). *E. coli* has a second O^6-meG-DNA methyltransferase encoded by the constitutively expressed *ogt* gene. Thus, low levels of methylation damage can be handled by the Ogt protein while in an adaptive response the relatively innocuous methyl phosphotriester lesions serve as a sensor for high methylation levels, invoking a large increase in Ada activity in the cell [9]

In humans, direct damage reversal of O^6-meG lesions requires O^6-meG-DNA methyltransferase (MGMT) also referred to as alkylguanine transferase (AGT). This homolog of C-Ada19 has Cys145, present in a conserved Pro-Cys-His-Arg active site motif, as a methyl recipient [10]. The crystal structure of an MGMT-substrate complex (Fig. 2A) shows that the O^6-meG lesion is flipped out of the DNA duplex with the side-chain of an arginine residue replacing it in the base stack [11]. Methylated MGMT cannot be reused and disappears rapidly from cells by ubiquitin-mediated degradation [12]. The dealkylation activity in human cells might interfere with effective action of alkylating agents used in chemotherapeutic treatment of cancer. The MGMT activity, however, can be

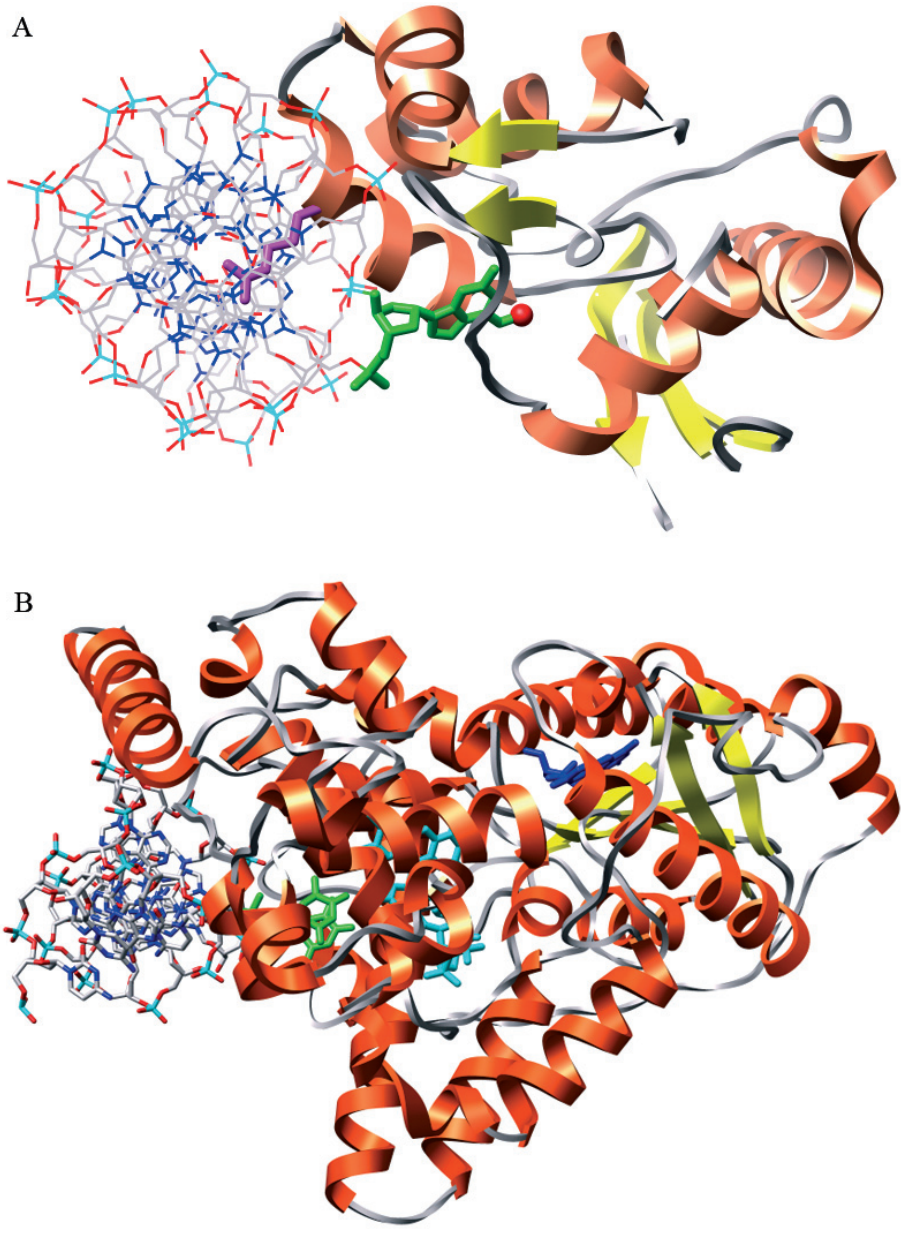

Figure 2. Base flipping in direct repair. (*A*) crystal structure of human MGMT-substrate complex (pdb: 1t38). The MGMT protein is inactive due to a Cys145Ser mutation but still capable of substrate binding. The DNA substrate has a flipped out O^6-meG lesion (green, methyl group in red). Also shown is the side-chain of the Lys128 residue (purple) intruding the DNA duplex to replace O^6-meG. (*B*) Crystal structure of an *Anacystis nidulans* CPD photolyase-substrate postrepair complex (pdb: 1tez). The photolyase chromophores FAD (cyan) and 8-HDF (blue) are shown. The thymine dimer (green) is flipped out of the DNA substrate: its cyclobutane ring is actually broken, probably by the synchrotron radiation used for structure determination. (Figures were prepared using Chimera; α-helices are in orange, sheets in yellow-green and DNA substrates in CPK colors).

reduced by inhibitors like O^6-benzylguanine and combinations of alkylating drugs and MGMT inhibitors are currently tested in clinical trials [10, 13].

Several polymorphisms (mutations) are known for the MGMT gene in the human population, some affecting protein activity or expression level, but associations with cancer risk are not consistent [14]. Some tumor-derived cell lines are very sensitive to alkylating agents. These mex^-/mer^- cells are deficient in O^6-meG repair due to a very low level level of MGMT activity [15]. This repression of MGMT could be linked to extensive CpG methylation in the promoter and transcribed region [16, 17]. Treatment with 5-azacytidine, which reverses CpG methylation, results in upregulation of MGMT activity [18]. The methylation status of the MGMT promoter could be a predictor for the success of tumor treatment with alkylating agents [19, 20]

The role of alkyltransferases in carcinogenesis has been investigated in various transgenic mouse models. Mice expressing the *E. coli ada* gene are protected from hepatocarcinogenic events induced by low doses of alkylating agents [21]. Transgenic mice carrying the human MGMT gene behind a bovine cytokine promoter, which expresses selectively in the epidermis, exhibited a significantly lower tumor incidence in a two-stage topical tumor induction protocol compared to nontransgenic animals (Fig. 3) [22]. Likewise, overexpression of human MGMT in the thymus protects against MNU-induced lymphomas [23],

while brain/liver-specific overexpression of the human gene in a genetic background that predisposes to spontaneous hepatocellular carcinoma reduces tumor prevalence [24].

In contrast, MGMT-deficient mice have been shown to be hypersensitive to alkylating agents like N-methyl-N-nitrosourea and chemotherapeutic drugs as evident from lower LD_{50} values, reduced bone marrow cellularity, and impaired reproductivity of hematopoetic stem cells [25, 26]. Taken together, these experiments show that MGMT protects against carcinogenic events.

Oxidative dealkylation (dioxygenases)

The *E. coli* AlkB protein and its human homologs ABH2 and ABH3 preferentially repair 1-methyladenine and 3-methylcytosine lesions by oxidative demethylation. AlkB has a broader substrate range compared to ABH2/3 as it also repairs e.g. 1-methylguanine and 1-ethyladenine [27]. A polymeric substrate structure is not required as a trinucleotide dTp1-meApT and even 5′-phosphorylated 1-meAMP are demethylated [28]. AlkB and ABH3 prefer single-stranded DNA (ssDNA) or RNA substrates while ABH2 preferentially acts on dsDNA [29].

The subcellular localization in HeLa cells indicates that ABH2 and ABH3 are nuclear enzymes [30], and it has been suggested that ABH2 repairs DNA close to replication forks while ABH3 is involved in repair of nuclear ssDNA and RNA associated with transcriptionally active genes. ABH2 and ABH3 knockout mice as well as double knockout mice are viable and do not show an obvious phenotype. ABH2, but not ABH3, deficient mice accumulate 1-methyl-adenine lesions in the absence of exogenous methylating agents. ABH2-deficient embryonic fibroblasts could not remove MMS-induced lesions [31].

The AlkB and ABH2/3 proteins, belonging to the superfamily of 2-oxoglutarate/iron-dependent dioxygenases [32], contain a Fe^{2+} cofactor and use 2-oxoglutarate and molecular oxygen as co-substrate. The methyl-group of e.g. 1-meA is hydroxylated (Fig. 4) with concurrent formation of succinate and CO_2, followed by the spontaneous release of formaldehyde.

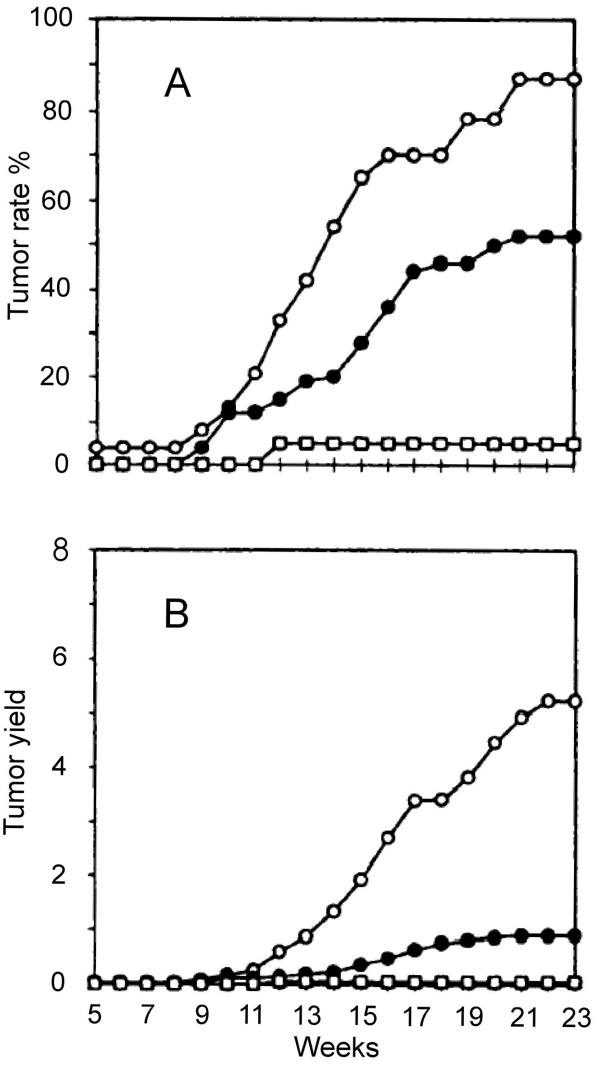

Figure 3. Effect of MGMT expression on tumor response in mice. Transgenic (closed circles) and nontransgenic (open circles) mice were initiated with a single subthreshold dose of MNU (N-nitroso-N-methylurea) followed by treatment with TPA twice a week. Transgenic animals carried the human MGMT gene expressed from the epidermis-specific Ck promoter. Control mice were treated with TPA only (open squares). Tumor response is shown as tumor rate (panel *a*, no. papilloma-bearing mice/no. survivors) and tumor yield (panel *b*, no. tumors/no. survivors). Adapted with permission from [22].

Several crystal structures are available which sustain this reaction mechanism. In the ABH3 structure [33],

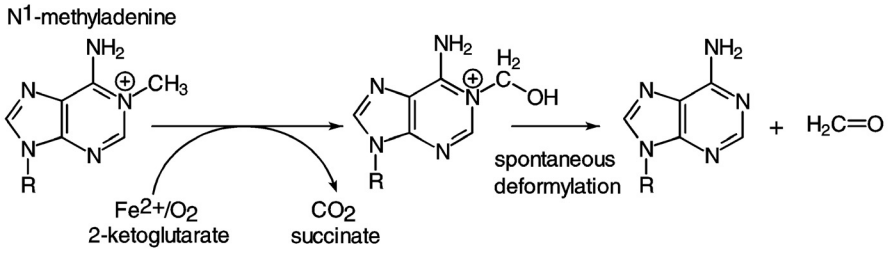

Figure 4. Reaction mechanism for oxidative dealkylation by *E. coli* alkB or human ABH2/ABH3 dioxygenases. The methyl group is hydroxylated using 2-ketoglutarate and O_2 as co-substrates and is then removed as formaldehyde.

a Fe^{2+} ion is coordinated to a conserved His-X-Asp-X_n-His motif and 2-oxoglutarate. Moreover, a flexible hairpin was identified which is probably involved in substrate binding. The AlkB-substrate structure [34] shows, in addition to the flexible lid, a putative tunnel for O_2 diffusion to the active site. The AlkB-substrate structure [35] also shows a base-flipping mechanism with the lesion flipped out by squeezing together the bases flanking the lesion. In the ABH2-substrate complex structure an additional flexible loop is present to bind the DNA strand opposite to the lesion [35].

Reversal of photoproducts

UV-induced photoproducts in DNA

UV radiation induces mainly two types of lesions in DNA, both originating from neighboring pyrimidine bases: cyclobutane pyrimidine dimers (CPDs) and pyrimidine(6–4′)pyrimidones [(6–4)photoproducts or (6–4)PPs]. (6–4)PPs, and to a lesser extent CPDs, distort the DNA double helix [36, 37] and both interfere with ongoing transcription and replication. In eukaryotes, the yield and distribution of DNA lesions may be influenced by a higher-order DNA structure as CPD lesions are found predominantly in nucleosomal DNA [38] while (6–4)PPs are primarily found in linker DNA [39].

Interestingly, CPDs are not observed in UV-irradiated bacterial spores [40]. Due to dehydration, spore DNA is forced in an A-like conformation and small acid soluble proteins (SASPs), expressed during spore formation, are bound to the DNA [41]. The very special conditions within the spore suppress the induction of CPDs, and instead promote the formation of a spore photoproduct (SP) which was identified as 5-thyminyl-5,6-dihydrothymine [42]. Moreover, spores contain large amounts of dipicolinic acid [43], which acts as a photosensitizer, improving the yield of spore photoproduct [44].

Despite the fact that most organisms have acquired the nucleotide excision repair system for removal of helix-distorting lesions and other bulky DNA adducts, highly specific direct damage reversal mechanisms exist for the three types of photoproducts.

Spore photoproduct lyase

SPs are specifically repaired by SP lyase in a light-independent process [45]. Spore formation involves the generation of a forespore within the bacterial cell. During this process, SP lyase is synthesized and packed ready for use in the forespore. As the dormant spore is unable to repair its DNA, UV-induced DNA damage will accumulate. Notwithstanding this deficit, spores are highly UV-resistant, as early in spore germination the DNA-bound SASPs are degraded by a specific protease, allowing efficient reversal of SPs into two thymines by SP lyase or, to a lesser extent, removal by nucleotide excision repair.

SP lyase is a 41-kD protein, encoded by the *spl* gene. The SP lyase amino acid sequence of several *Bacillus* and *Clostridium* species is known [46]. All SP lyases contain a conserved Cys-X_3-Cys-X_2-Cys motif characteristic for the radical SAM (S-adenosylmethionine) protein family (recent review [47]), where it is involved in the binding of a [4Fe-4S] cluster. SP lyase was indeed shown to contain such a Fe-S cluster [48]. His_6-tagged SP lyase can be overexpressed in *Bacillus subtilis* spores or *E. coli* cells and purified under anaerobic conditions [49].

SP lyase specifically binds to SP, but not to CPD, lesions in UV-irradiated DNA [50]. Although SP lyase usually repairs SPs in dsDNA, assays with synthetic substrates showed that the enzyme also acts on single-stranded oligonucleotides. Even the minimal substrates SP-TpT and SP-TT (lacking the phosphodiester bond) are converted [51]. The repair reaction is initiated by the ligation of SAM to the [4Fe-4S] cluster of SP lyase (Fig. 5). Electron transfer from the [4Fe-4S] cluster to SAM generates a free 5′-deoxyadenosyl radical, while the methionine part remains bound to the cluster [52]. The 5′-dAdo· radical abstracts the C6-H atom from the SP [53]. Next, the C-C bond between the thymines undergoes scission, followed by the transfer of a H atom from 5′-dAdoH back to the thymine monomer radical. Finally, SAM is formed and released. Although irreversible splitting of SAM has been reported, it most likely acts as a catalytic cofactor for the reversible generation of the reactive 5′-dAdo· radical rather than as (co)substrate [49].

Photolyases

Photolyases are 50-55-kD single-chain flavoproteins that lesion-specifically bind to UV-induced CPDs or (6–4)PPs in the DNA (reviewed in [54, 55]). When these photolyase + UV-DNA complexes are illuminated with visible (blue) or near-UV light, photon energy is used to repair DNA damage. In nature this is very effective: lesions induced by the UV-component of sunlight are repaired by visible light from the same source.

Absorbtion of visible light requires the presence of a chromophoric group. In fact, photolyases contain two, functionally different, chromophoric cofactors. The first chromophore, FAD, acts as the photochemical reaction center. Various FAD redox states have been found in purified photolyases: oxidized FAD, the half-reduced neutral semiquinone radical $FADH^{\cdot}$, and the deprotonated fully reduced form $FADH^-$. Only the

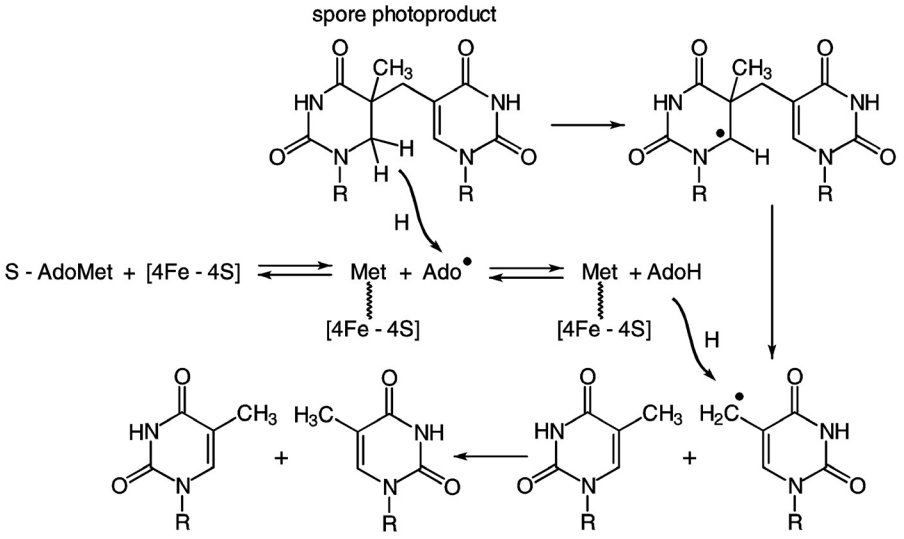

Figure 5. Reaction mechanism of *B. subtilis* spore photoproduct (SP) lyase. A 5′-deoxyadenosyl radical (Ado·), generated by electron transfer from the Fe-S cluster, abstracts an H atom from the SP, which induces scission of the bond between the thymines. The reaction is completed by H-atom transfer from AdoH to the thymine radical.

fully reduced form is biologically active in the repair of UV lesions [56]. It is possible, however, to convert the inactive forms of FAD into $FADH^-$ by photoreduction where an electron is transferred from an exogenous photoreductor through a chain of three tryptophans to FAD [57]. The second chromophore, which is either reduced folate (5,10-methenyl-tetrahydrofolate, MTHF) or 8-hydroxy-5-deazaflavin (8-HDF), acts as an auxiliary light-harvesting antenna. Although the antenna chromophore binding site is specific for either MTHF or 8-HDF, recently a 'promiscuous' binding site was found in *Thermus thermophilus* photolyase which could bind FMN, riboflavin, or 8-HDF [58]. The presence of a second chromophore is not an absolute requirement for activity, as apophotolyases lacking this chromophore are still able to repair UV lesions. Its presence, however, improves the efficiency of photolyase considerably.

Photolyases are specific for either CPD or (6–4)PP lesions. Yet the reaction mechanisms are largely the same: shuttling an electron to the lesion in order to destabilize it. The driving force for this reaction is the energy acquired by the chromophores by absorption of a photon, yielding excited fully reduced $FADH^-$ (Fig. 6). For (6–4)PP photolyases a light-independent step precedes electron transfer. Binding of the (6–4)PP lesion to photolyase induces a rearrangement and an oxetane-ring is formed [59] with the help of two His residues in photolyase [60]. Once a lesion radical is formed, the bond(s) between the pyrimidine rings are broken and finally an electron is transferred back to the catalytic co-factor.

Several crystal structures are available e. g. for *E. coli* [61] and *Anacystis nidulans* [62] photolyase. Structures are very similar, containing an α/β domain with five parallel β-strands and several α-helices and a helical domain containing α-helices only. A similar structure was obtained for the core region of cryptochrome (see below). A photolyase-substrate complex structure [58] clearly shows the dimer lesion completely flipped out of the DNA (Fig. 2B). For a detailed description of reaction mechanism and structures see a recent review [55].

Recently the crystal structure of a *Drosophila* (6–4)PP photolyase-substrate (and product) complex became available [63]. Based on these structures a reaction mechanism has been proposed without an oxetane intermediate.

The photolyase/cryptochrome protein family

At present a vast number of photolyase and photolyase-like genes are known, their gene products sharing a well-conserved core domain of about 500 amino acids to which the two chromophores are bound. Phylogenetic analysis of the amino acid sequence of the core domain of all members of the photolyase family indicates a clear relationship [64]. Yet they are functionally very different, as the phylogenetic tree (Fig. 7) not only comprises genuine photolyases involved in DNA repair but also photolyase-like cryptochromes, which differ from photolyases in having a unique C-terminal extension of varying length. Despite their sequence and structural homology to photolyases, cryptochromes are not capable of repairing UV-induced DNA damage. These findings suggest that multiple gene duplications of an ancestral gene, probably encoding a primordial CPD photolyase, led to functionally diverged proteins [65]. Because of the high UV irradiance due to an anoxic atmosphere, such a photolyase would be essential for survival in early stages of evolution.

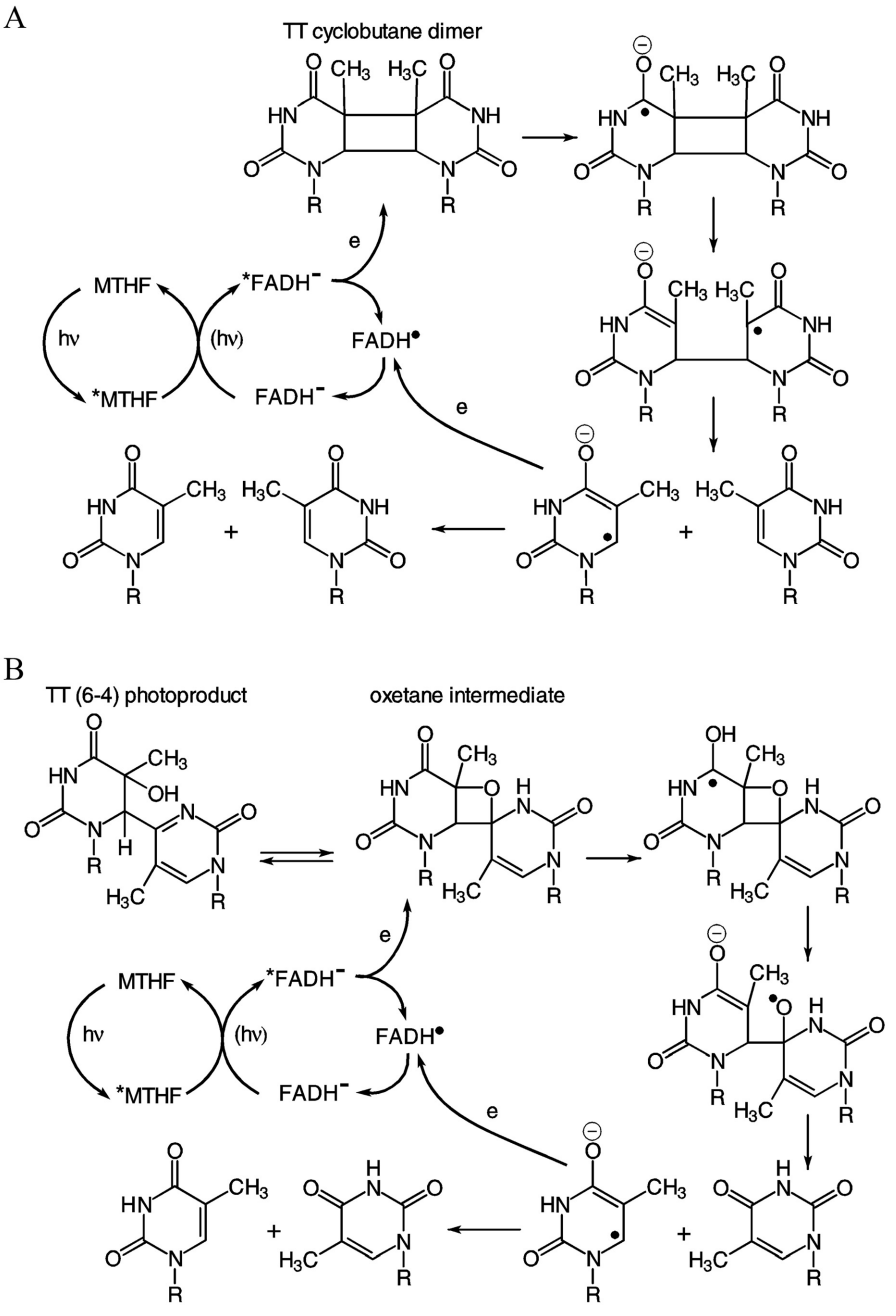

Figure 6. Reaction mechanism of photolyases. In CPD photolyase (*A*) upon absorption of a photon by the MTHF or 8-HDF antenna chromophore energy is transferred to the catalytic co-factor $FADH^-$. Alternatively excited $*FADH^-$ is obtained by direct absorption of a photon. Then an electron is donated from $*FADH^-$ to the dimer lesion which induces breakage of the cyclobutane bonds. Finally, an electron is transferred back from the thymine monomer radical to $FADH^{\cdot}$, returning photolyase to the initial state. A similar mechanism has been proposed for (6–4)PP photolyase (*B*), but previous to electron transfer an oxetane ring (azetidine ring in case of TC(6–4) photoproduct) is formed in an enzyme-mediated reaction.

Based on amino acid sequences, two phylogenetically somewhat distantly related groups can be distinguished (Fig. 7), each containing organisms from all three primary kingdoms of life. The first group comprises a single main cluster with class II CPD photolyases, mainly but not exclusively, from animals and plants. Placental mammals, in contrast to marsupials, do not possess photolyase genes. Among other members of this cluster are several viral photolyases [66]. The other group is quite heterogeneous. Separate clusters can be distinguished containing class I CPD-photolyases, (6–4)PP photolyases, and animal cryptochromes, plant cryptochromes and cryptochromes-DASH.

Cryptochromes were first identified in plants as blue light photoreceptor proteins involved in light-regulated hypocotyl elongation, induction of flowering and photoentrainment of circadian expression of genes (reviewed in [67]). Animal cryptochromes share a variable C-terminal extension in addition to the photolyase-like core and have a function in the circadian clock. This biological clock generates daily rhythms in metabolism, physiology and behavior, and as the intrinsic periodicity of this internal timekeeper

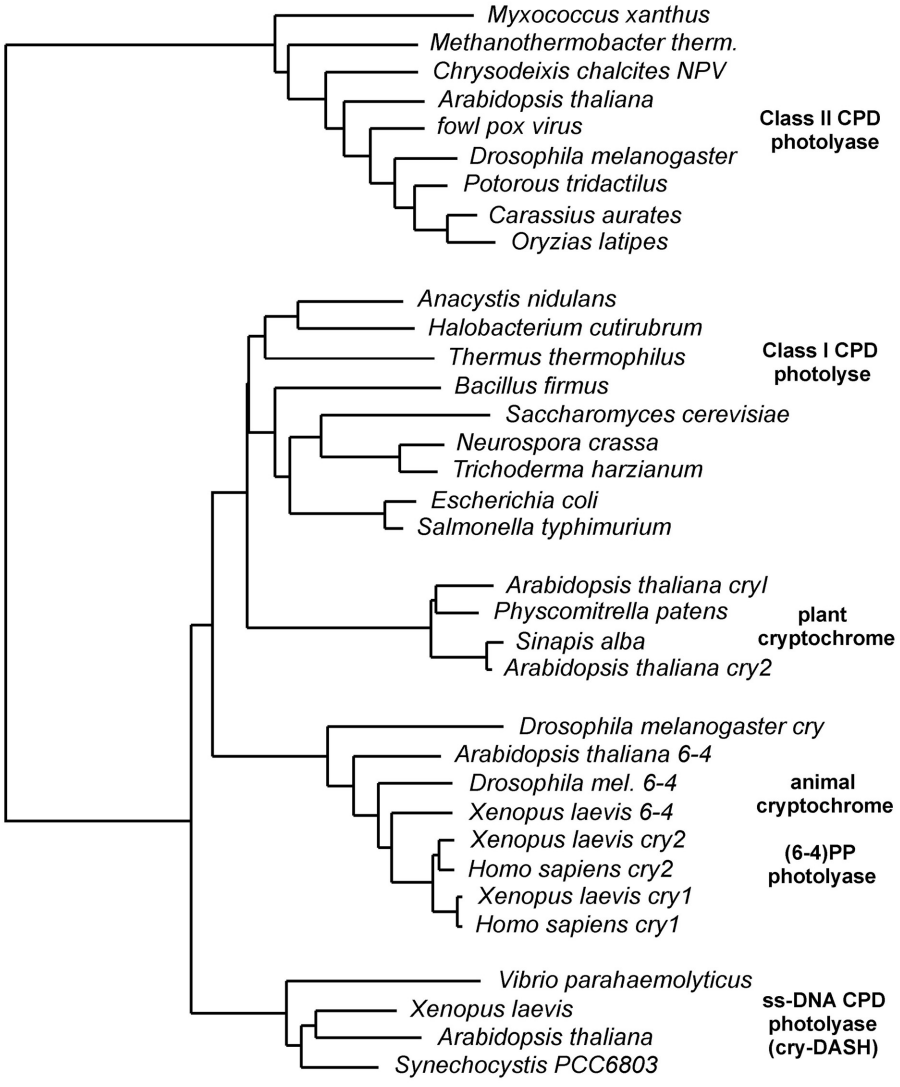

Figure 7. Unrooted phylogenetic tree of the photolyase/cryptochrome family. Amino acid sequences were aligned with ClustalX and a tree was obtained for the core region with the neighbor-joining method (PHYLIP package vs. 3.67 by J. Felsenstein) using the Jones-Taylor-Thornton matrix.

is not exactly 24 h, the clock needs to be reset every day by light. As in plants, *Drosophila* cryptochrome protein acts as a photoreceptor, synchronizing this biological clock to the day-night cycle. In peripheral tissues, but not in the brain, the fly CRY protein is also involved in rhythm generation [68]. Mammals contain two cryptochrome genes (*Cry1* and *Cry2*), inactivation of which, surprisingly, accelerated (*Cry1*) or slowed down (*Cry2*) behavioral rhythms of the mouse [69]. Interestingly, knocking out both genes resulted in a complete loss of rhythmicity, showing that the CRY proteins are indispensable core components of the molecular oscillator underlying circadian rhythmicity [69, 70]. Structure-function analysis of the unique tails of mammalian [71], *Xenopus* [72], *Drosophila* [73], and *Arabidopsis* [74] cryptochromes revealed that the acquirement of different species-specific C-terminal extensions separated cryptochromes from photolyases. Moreover, it caused diversity within the cryptochrome protein family, making cryptochromes act as photoreceptor protein (as in *Arabidopsis*), as combined photoreceptor and core oscillator protein (as in *Drosophila* and zebrafish), or a pure core oscillator protein (as in *Xenopus* and mammals). Intriguingly, the *Drosophila* CRY protein was recently shown to be required for sensing magnetic fields, thus adding a third (clock-independent) function to this class of flavoproteins [75].

The function of cryptochromes-DASH was uncertain as they show aspecific DNA-binding and weak photolyase activity [76, 77] while FAD and MTHF chromophores are present [78, 79]. Eventually they were shown to be photolyases with a clear preference for CPDs in single-stranded nucleic acids [80].

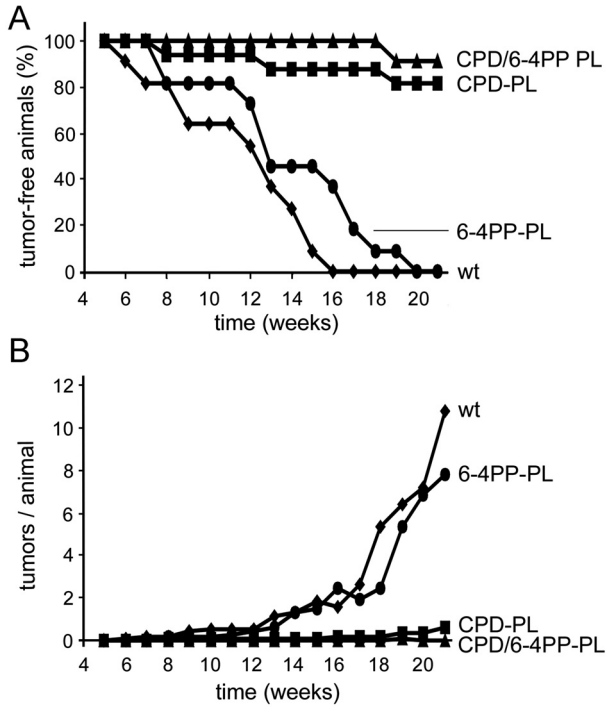

Figure 8. The effect of photoreactivation of CPDs and (6–4)PPs on skin carcinoma occurrence. CPD-photolyase (squares), (6–4)PP-photolyase (circles), and CPD-photolyase/(6–4)PP-photolyase (triangles) transgenic mice, as well as their wild-type littermates (diamonds), received daily treatments of UVB light (500 J/m^2/day), followed by exposure to photoreactivating light for 3 h. (*a*) The fraction of tumor-free mice in time after the first UV treatment. (*b*) The average number of squamous-cell carcinomas per mouse in time after the first UV treatment. Reprinted with permission from [100].

Photolyase in mammals

Except for marsupials, data on the occurrence of photoreactivation in mammals have been conflicting. However, as the only two photolyase-like genes in the human and mouse genome encode cryptochromes (see previous section) [69], the absence of photoreactivating activity in placental mammals seems now generally accepted. Presumably, placental mammals have gone through a nocturnal phase during evolution and consequently lost this important genome protection mechanism against sunlight.

Like most other eukaryotes, mammals remove photolesions from the DNA by the versatile and evolutionary highly conserved nucleotide excision repair (NER) pathway. NER is a complex, multistep process involving the concerted action of 30 or so proteins to sequentially execute damage recognition, chromatin remodeling, incision of the damaged DNA strand on both sides of the lesion, excision of the 27–29mer oligonucleotide containing the damage, and gap-filling DNA synthesis followed by strand ligation. Two NER sub-pathways exist: global genome NER repairs helix-distorting lesions in the entire genome, while transcription-coupled NER specifically repairs transcription-blocking lesions in the transcribed strand of active genes ([1, 3] and a review in this issue by S. Tornaletti). Inborn defects in NER, as in xeroderma pigmentosum, can give rise to striking UV sensitivity and a strong cancer predisposition of the exposed skin [81].

Before the introduction of transgenic technology, mammalian studies on photoreactivation were restricted to photolyase containing non-placental mammals [82, 83]. *Monodelphis domestica* has an efficient photoreactivation pathway which leads to an approximately 80 % reduction in pyrimidine dimers after skin UVB irradiation and to the prevention of UVB-induced local and systemic immunosupression and epidermal Langerhans cell depletion [84]. To date, *M. domestica* and other marsupials are still used as mammalian models for photoreactivation studies since their UV responses have proven to be similar to those of placental mammals [85, 86].

Taking advantage of the absence of photolyases in placental mammals, photolyases have been widely expressed in heterologous mammalian cell systems. This approach led to a better understanding of the specific effects and cellular responses provoked by CPDs and (6–4)PPs. In addition, the structure of CPD and (6–4)PP lesions, their DNA distorting properties, and the interactions between photolysase and DNA has widened the scope for the biological impact of this unique repair enzyme system [87].

After a microinjected microbial photolyase was shown to rapidly eliminate CPD lesions in UV-exposed human fibroblasts [88], in the following years several research groups generated mammalian cells transiently or stably expressing photolyases. Experiments with CPD-photolyase transgenes in normal (NER-proficient) human cell lines revealed that CPDs are an instigator of UV-mediated apoptosis [89, 90]. The introduction of CPD or (6–4)PP photolyases into mouse cells carrying transgenic mutation reporter genes demonstrated that photoreactivation of CPDs, rather than (6–4)PPs, substantially lowered the mutation rate, implying that this lesion type is primarily responsible for the UVB-induced mutations in mammalian cells [91]. Likewise, expression of CPD photolyase in cells from patients with xeroderma pigmentosum complementation group A (XP-A), a cancer-prone NER-deficient syndrome with increased UV sensitivy, demonstrated that the removal of CPD lesions increased UV resistance and reduced the mutation frequency. Since UV survival was increased, but was not the same as in wild-type cells, these results suggested that CPDs, together with other DNA lesions [probably including (6–4)PPs] can lead to

cell killing and mutations [92]. Although these results partially elucidate the role of CPDs in UV-induced cell responses, it should be noted that while NER is efficient at removing (6–4)PPs and other types of helix-distorting damage, CPDs are a poor substrate. Accordingly, except for transcription-coupled NER of the template strand of active genes, CPDs are removed at substantially reduced rates by global genome NER in human cells and virtually not at all in rodent cells [93, 94].

Indeed, by expressing either (6–4)PP or CPD photolyases in XP-A and other NER-deficient cells, it was later shown that removal of the less-abundant (6–4)PPs decreased the apoptotic response to the same extent as or even better than removal of CPDs [95–97]. These studies therefore suggest that (6–4)PP lesions are more potent on the induction of apoptosis than CPD lesions, which tend to induce cell cycle arrest. These results corroborate the hypothesis that in NER-deficient cells both lesions have equally important roles on outcome after UV radiation but that on DNA repair-proficient cells only CPDs are responsible for the induction of apoptosis, probably due to the rapid repair of (6–4)PP by NER [98].

Recently, we generated *Potorous tridactylus* CPD-photolyase and *Arabidopsis thaliana* (6–4)PP-photolyase transgenic mice to assess the relative contribution of CPDs and (6–4)PPs to the detrimental effects of UV light on the skin [99, 100]. Ubiquitous expression of CPD photolyase was shown to protect the UV-exposed mouse skin from hyperplasia, apoptosis, and sunburn in a light-dependent manner [99]. Moreover, expression of CPD photolyase in keratinocytes only was sufficient to block apoptosis in keratinocytes and (non-photolyase-expressing) dermal fibroblasts, which points to intercellular anti-apoptotic signaling. Importantly (as shown in Fig. 8), photoreactivation of CPD lesions provided superior protection against UV-induced carcinogenesis, whereas direct repair of (6–4)PPs provided marginal protection [100]. In line with this finding CPDs formed the main trigger for mutagenic events and formation of mutant *p53* patches, composed of preneoplastic epidermal cells [100]. Likewise, preferential elimination of CPDs (but not of (6–4)PPs) from basal keratinocytes dramatically reduced UV skin cancer induction [101]. Apart from mutation induction, immunesurveillance plays a pivotal role in carcinogenesis [102]. Ubiquitous rather than keratinocyte-specific removal of CPDs abolishes UV-induced immunosuppression, while photoreactivation of (6–4)PPs does not have any effect. These findings suggest that CPD lesions in cells other than keratinocytes are a prerequisite for UV-induced immune suppression. In conclusion, and in line with *in vitro* studies, in repair-proficient placental mammals, CPDs are responsible for the negative effects of UV light. To reveal the true relative potential of CPDs and (6–4)PPs in these processes, the same set of experiments should be performed on photolyase transgenic mice, crossed in an NER-deficient *Xpa* background.

Photoreactivation of CPD lesions is not limited to transgenic animals. Topical application of photolyase-containing liposomes on the UV-exposed skin of volunteers, in combination with exposure to photoreactivating light, was shown not only to reduce the amount of CPDs by 40–45 % but also to prevent UVB-induced immunosupression [103]. Altogether, these results indicate that placental mammalian cells still have the necessary conditions to allow the enzymatic activity of photolyases. Further investigation is still required, but in the near future we might think about the broad use of these enzymes, together with UV sunscreens, to prevent malignant UV sunlight effects.

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