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# DNA Repair by Reversal of DNA Damage

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Endogenous and exogenous factors constantly challenge cellular DNA, generating cytotoxic and/or mutagenic DNA adducts. As a result, organisms have evolved different mechanisms to defend against the deleterious effects of DNA damage. Among these diverse repair pathways, direct DNA-repair systems provide cells with simple yet efficient solutions to reverse covalent DNA adducts. In this review, we focus on recent advances in the field of direct DNA repair, namely, photolyase-, alkyltransferase-, and dioxygenase-mediated repair processes. We present specific examples to describe new findings of known enzymes and appealing discoveries of new proteins. At the end of this article, we also briefly discuss the influence of direct DNA repair on other fields of biology and its implication on the discovery of new biology.

Endogenous and environmental agents continuously threaten the genomic integrity of all living organisms. Replication of damaged DNA can lead to mutations that are tumorigenic, whereas DNA lesions that block replication or transcription can result in senescence and cell death. Therefore, cellular DNA must be promptly repaired. Well-known mechanisms include base-excision repair, nucleotide excision repair, mismatch repair, homologous recombination, and nonhomologous end joining. In addition, nature has also evolved several mechanisms in which the damage is directly reversed most often by a single repair protein without the incision of DNA backbone. Although such "direct repair" processes mediate the reversal of a relatively small set of DNA lesions, the simplicity and essentially error-free property of the direct reversal processes make them particularly attractive for a cell. Three major mechanisms of direct DNA repair have been identified to date: (i) photolyases reverse UV light-induced photolesions; (ii) $O^6$-alkylguanine-DNA alkyltransferases (AGTs) reverse a set of *O*-alkylated DNA damage; and (iii) the AlkB family dioxygenases reverse *N*-alkylated base adducts (Fig. 1). This concise article intends to update knowledge since the publication of the second edition of *DNA Repair and Mutagenesis* (ASM) (Friedberg et al. 2006).

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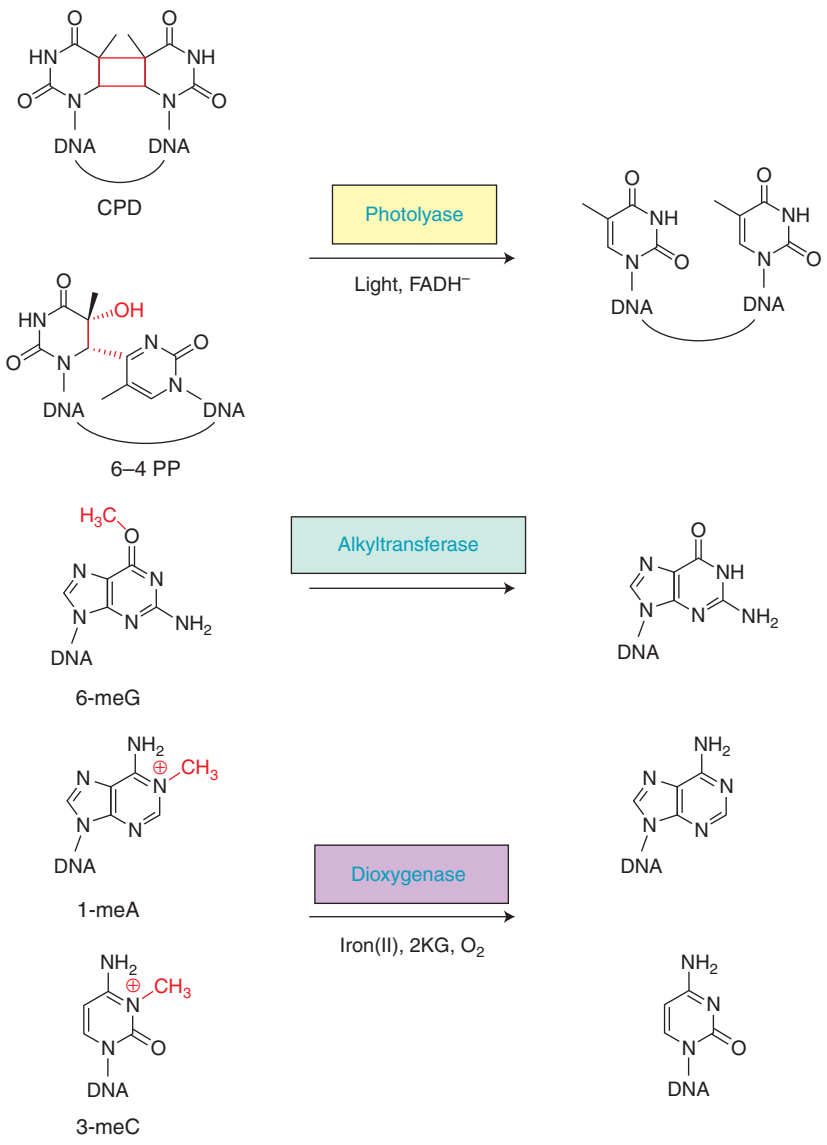


Figure 1. Direct DNA-repair pathways: representative substrates, repair proteins and cofactors, and corresponding repair products.

## PHOTOINDUCED REVERSAL OF UV RADIATION-CAUSED DNA DAMAGE BY PHOTOLYASES

UV radiation produces mainly two types of lesions in DNA: the cyclobutane pyrimidine dimers (CPDs) and the pyrimidine pyrimidones (6–4) photoproducts (6–4 PPs). Photolyases are specific to either CPD or 6–4 PP lesion (thus CPD photolyases and [6–4] photolyases, respectively) and use blue and near-UV light to reverse the UV-light-induced DNA damage. All photolyases bind the essential cofactor flavin adenine dinucleotide (FAD), and only the fully reduced $FADH^-$ is catalytically competent. Proposed mechanisms of photolyases involve an initial electron-transfer step from $FADH^-$ to the UV-induced lesions, a dimer-splitting process,

and a final electron-transfer step from the pyrimidine monomer radical back to $FADH^{\cdot}$, thus regenerating $FADH^{-}$ (Fig. 2) (Muller and Carell 2009; Brettel and Byrdin 2010).

## ADVANCES IN REACTION MECHANISMS OF CPD PHOTOLYASES

The advancing studies of the CPD repair mechanism that are based on direct measurements of Class I CPD photolyases have seen further development with ultrafast spectroscopy. For instance, using an *Escherichia coli* CPD photolyase, Zhong, Sancar, and colleagues observed electron transfer from the excited flavin cofactor to the CPD dimer in 170 ps and back electron transfer from the repaired thymines in 560 ps (Kao et al. 2005); further study by the investigators revealed that the CPD splits in two sequential steps within 90 ps (Liu et al. 2011). In another example by Brettel, Byrdin, and colleagues, splitting rates of the intradimer bond and electron return process of *Aspergillus nidulans* photolyase were estimated to be 0.2 and 1.5 ns, respectively (Thiagarajan et al. 2011). In both examples, the technology advance significantly enhanced our understanding of the spatiotemporal picture of CPD repair.

Class II photolyases have different amino acid sequences compared to those from Class I enzymes and are expected to differ in the pathway of electron transfer and the DNA-repair active site (Fig. 2A,C). The first crystal structure of a Class II photolyase (from *Methanosarcina mazei*), alone and in complex with CPD lesion-containing duplex DNA, revealed a larger lesion-

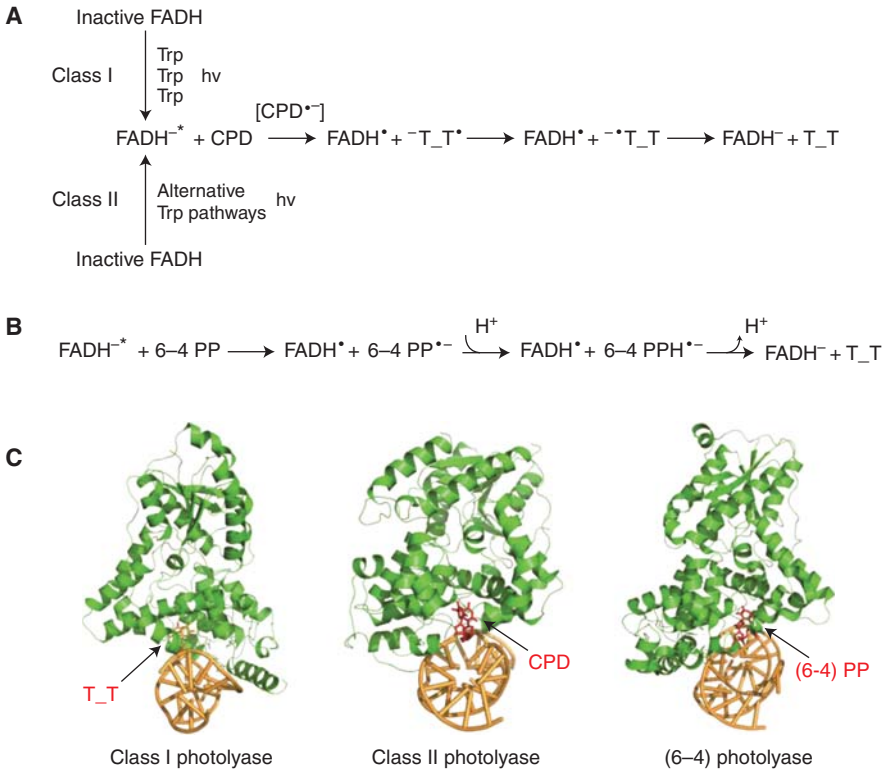


**Figure 2.** Mechanisms and structures of representative photolyases from different classes. (*A*) Mechanisms of photorepair for CPD photolyases. The generation of fully reduced, active $FADH^{-*}$ species is different for Class I and Class II CPD photolyases. (*B*) Repair mechanism of (6–4) photolyases. (*C*) Representative complex structures of photolyases bound to double-stranded DNA (dsDNA) (PDB accession code: 1TEZ, 2XRZ, and 3CVU).

binding site and an unusual (different from that of Class I) tryptophane dyad as the electron-transfer pathway to FAD (Kiontke et al. 2011). In addition, a distinct electron-transfer pathway (three Trp residues in this case) from the Class I pathway was also observed in *Oriza sativa* photolyase (Hitomi et al. 2012). Nevertheless, spectroscopic results for *Arabidopsis thaliana* and *O. sativa* photolyases showed photoreduction kinetics resembling those of Class I enzymes (Okafuji et al. 2010), indicating functional substitution between these different electron-transfer pathways.

## ADVANCES IN THE STRUCTURAL AND FUNCTIONAL RELATIONSHIP OF (6–4) PHOTOLYASE

The reversal of 6–4 PP is thought to be more complex than that of CPD because 6–4 photolyase must catalyze not only covalent bond cleavage between two pyrmidine bases but also undergo a hydroxyl group transfer from the 5′ to the 3′ pyrimidine base. For this reason, an oxetane intermediate was assumed. However, Schlichting, Carell, and colleagues proposed a modified repair mechanism that lacked the strained oxetane intermediate (Maul et al. 2008). They crystallized *Drosophila melanogaster* (6–4) photolyase containing a 6–4 PP and initiated in situ photo repair (Fig. 2C). In the resulting structures, the positions of two conserved histidine residues essential for catalysis (His365 and His369) do not support a mechanism involving an oxetane structure. Instead, a mechanism that involves a water molecule formed in situ, which then attacks the acylimine, was postulated (Maul et al. 2008). More recent structural evidence from *A. thaliana* (6–4) photolyase revealed a narrower and deeper cavity for binding of 6–4 PP, also suggesting a different reaction mechanism compared to that of CPD photolyase (Fig. 2B) (Hitomi et al. 2009).

Zhong et al. used femtosecond spectroscopy to study the dynamics and mechanism of (6−4) photolyase from *A. thaliana*. The investigators observed a key cyclic proton transfer step between an active-site histidine residue and the substrate, which occurs in 425 ps and leads to 6–4 PP repair in tens of nanoseconds (Li et al. 2010). In addition, time-resolved experiments of *Xenopus laevis* (6−4) photolyase uncovered a drastic diffusion change, which was assigned to the rapid dissociation (time constant of ∼50 μs) of the protein from the repaired DNA product (Kondoh et al. 2011). Later on, 6–4 PP repair mediated by the same enzyme was investigated using Fourier transform infrared (FTIR) spectroscopy (Zhang et al. 2011). Differences in FTIR spectra revealed structural changes in the protein and DNA from binding to catalysis. Together, these data depict a general picture of how light is harvested and used to efficiently reverse 6–4 PP by (6−4) photolyase.

## CRYPTOCHROME-DASH MEMBERS SHOW DNA-REPAIR ACTIVITY

The cryptochrome-DASH (CRY-DASH) subfamily is relatively closer to the animal cryptochrome subfamily, rather than the plant cryptochrome subfamily and the CPD photolyase subfamilies (Hitomi et al. 2000; Brudler et al. 2003). Therefore, CRY-DASH proteins were considered at first to be novel cryptochromes, rather than DNA-repair photolyases. However, weak CPD photorepair activities were detected for the Synechocystis protein and two vertebrate CRY-DASHs (Hitomi et al. 2000; Daiyasu et al. 2004). Crystal structure of the Synechocystis CRY-DASH further revealed high similarity to the structures of Class I CPD photolyases (Brudler et al. 2003). In 2008, progress from the Sancar laboratory provided an explanation of the observed weak DNA-repair activity. In actuality, CRY-DASH members robustly bind to and repair CPD in the context of single-stranded DNA (ssDNA), but not in double-stranded DNA (dsDNA) (Selby and Sancar 2006). Crystal structures of Arabidopsis cryptochrome 3 further indicate that the protein most likely lacks an efficient flipping mechanism to access CPD lesions within duplex DNA (Pokorny et al. 2008), and that the CPD-binding cavity in Arabidopsis cryptochrome 3 is less hydrophobic and thus less energetically favorable compared to that of Class I CPD photolyases (Huang et al. 2006). Together, this data reveals that CRY-DASH

proteins are indeed light-driven DNA-repair enzymes, only less competent in base flipping and CPD binding.

## DIRECT REVERSAL OF ALKYLATION DAMAGE BY ALKYLTRANSFERASES

Alkylating agents react with the hetereoatoms of DNA bases to generate a variety of cytotoxic and mutagenic covalent adducts ranging from simple methyl groups to bulky alkyl additions, thereby posing substantial threats to human health (Drablos et al. 2004; Shrivastav et al. 2010). On the other hand, certain alkylating agents are commonly used as chemotherapeutic drugs in cancer patients, with the goal of killing cancer cells. Such double-edged properties of alkylating agents thus impart great biological significance to the studies of the cellular pathways that determine the biological outcome of alkylating agents (Fu et al. 2012). In addition to the base-excision repair pathway that corrects many *N*-alkylated lesions, two direct DNA-repair pathways exist to combat the deleterious effects of alkylating agents: AGTs reverse $O^6$-alkylated guanines and AlkB family dioxygenases reverse mainly *N*-alkylated lesions that block Watson–Crick pairings. Recent progress with regard to these two direct repair pathways is detailed below.

## SUICIDAL REVERSAL OF ALKYLATION DAMAGE BY $O^6$-ALKYLGUANINE-DNA ALKYLTRANSFERASES

$O^6$-methyl guanine (6-meG) is mutagenic and carcinogenic as a result of its ability to cause G:C to A:T transitions during DNA replication. The first 6-meG alkyltransferse, identified in *E. coli* (named C-Ada), is the carboxy-terminal domain of a multifunctional repair protein (Demple et al. 1985; Sedgwick et al. 1988). The amino-terminal domain of *E. coli* Ada protein (N-Ada) mediates a direct removal of the methyl group from $S_p$-methylphosphotriester DNA backbone damage (Sedgwick et al. 1988; Myers et al. 1993; He et al. 2005). It uses a zinc-mediated thiol alkylation mechanism and signals the activation of the *ada* operon, which includes *ada*, *alkA*, *alkB*, and *aidB* (Teo et al. 1986; Lindahl et al. 1988; Sakumi and Sekiguchi 1989). Both Ada and AlkB are direct repair proteins. AlkA is a glycosylase conserved from bacterium to human (Wyatt et al. 1999; Hollis et al. 2000). The exact functional role of AidB is still unknown (Rohankhedkar et al. 2006; Bowles et al. 2008; Rippa et al. 2011).

The human homolog of C-Ada (hAGT) contains two domains, a zinc-bound amino-terminal domain and a carboxy-terminal domain that harbors the methyl recipient Cys145. The *S*-alkylated AGT cannot be restored and undergoes ubiquitin-mediated degradation (Srivenugopal et al. 1996). A recent discovery showed that the AGT homolog in *Saccharomyces cerevisiae* (Mgt1) is cotargeted for degradation by both the Ubr1/Rad6-dependent N-end rule pathway and the Ufd4/Ubc4-dependent ubiquitin-fusion degradation pathway, through a degron near its amino terminus (Hwang et al. 2009). Because Mgt1 and mammalian AGTs share high sequence homology, the next obvious question to be addressed is whether such a mechanism also applies to mammalian AGTs.

AGTs can recognize diverse $O^6$-modified alkyl adducts. For instance, hAGT repairs not only 6-meG, but also many other bigger adducts including ethyl, 2-chloroethyl, and other aliphatic groups, and benzyl and pyridyloxobutyl adducts as well (Tubbs et al. 2007; Pegg 2011). Even $O^6$-G-alkyl-$O^6$-G interstrand cross-links could be repaired (Fang et al. 2008). As a result, hundreds of hAGT pseudosubstrates have been synthesized as inhibitors of hAGT, which are used in combination with therapeutic alkylating agents to improve the efficacy of cancer chemotherapy (Tubbs et al. 2007). One example is the clinical trial of $O^6$-benzyl guanine (6-bzG) and temozolomide. Some responses were observed from patients with refractory central nervous system (CNS) tumors (Hammond et al. 2004; Warren et al. 2005; Quinn et al. 2009a,b). Another example is the combined use of $O^6$-(4-bromothenyl) guanine (or PaTrin-2) and temozolomide in the treatment of refractory acute leukemia. Preclinical in vitro studies have shown that PaTrin-2 increases the inhibitory activity of temozolomide against human acute

leukemia cells (Turriziani et al. 2006). Despite these initial positive feedbacks, it is clear that inhibitors with greater potency and higher tumor specificity are still needed (Zang et al. 2005; Guza et al. 2006; Pegg 2011).

Several structures of hAGT/DNA complex are available for detailed examinations of substrate binding and nucleotide flipping. Tainer and colleagues reported the first structures of hAGT in complex with dsDNA, which revealed that hAGT uses a helix-turn-helix motif to mediate an unusual minor groove DNA binding (Fig. 3A,B) (Daniels et al. 2004). Our group also solved the structure of hAGT bound to a duplex DNA containing $N^4$-*p*-xylylenediamine cytosine. The protein binds at two different sites: the modified cytosine and a partially flipped overhanging thymidine at the sticky ended DNA junction (Fig. 3A) (Duguid et al. 2005). The two different hAGT/DNA interactions suggest that hAGT may search for weakened and/or distorted base pairs to locate the lesion. Results from computational approaches have suggested a two-step base-flipping mechanism of hAGT, in which the existence of an extra-helical intermediate was postulated (Hu et al. 2008). It has also been revealed that while searching along a duplex DNA, hAGT shows 5′ to 3′ preference and binds DNA cooperatively (Daniels et al. 2004; Rasimas et al. 2007; Adams et al. 2009). Last, single-molecule spatial tracking measurements of C-Ada, the *E. coli* equivalence of hAGT, showed that sliding, with essentially no hopping, is the mechanism of C-Ada motion along stretched DNA (Lin et al. 2009).

## REPAIR OF ALKYLATION DAMAGE BY ALKYLTRANSFERASE-LIKE PROTEINS (ATLs)

In silico analysis of genomic sequences suggests the presence of a family of AGT homologs (ATLs) with sequence similarity to the AGT

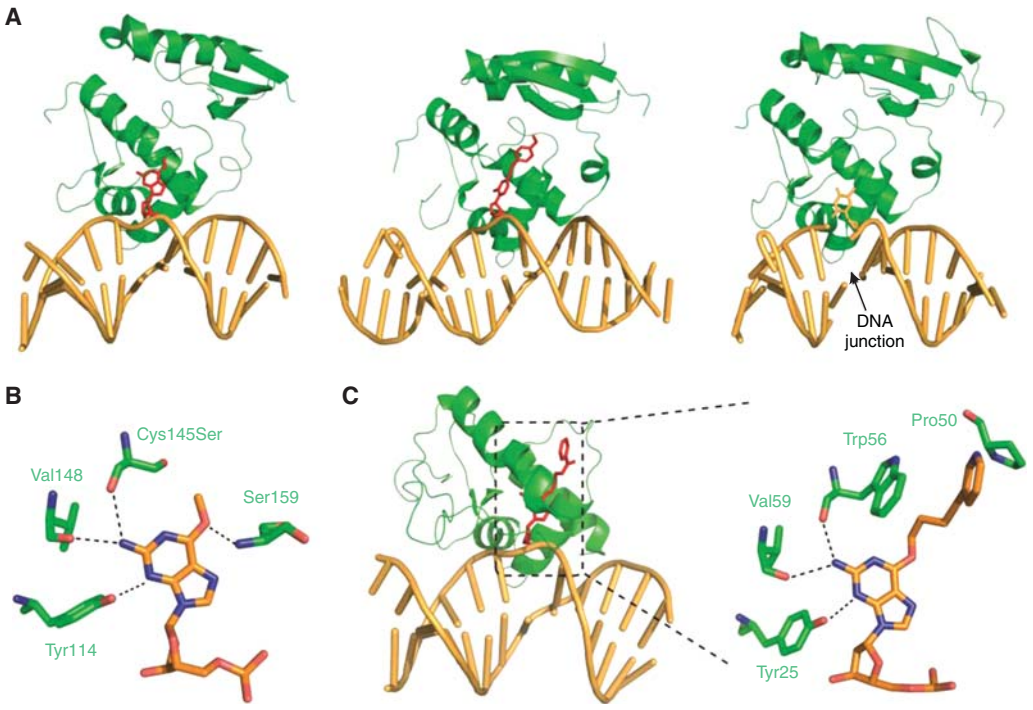


**Figure 3.** Overall structures and damage-binding pockets of hAGT and *Schizosaccharomyces pombe* Atl1. (*A*) Structures of hAGT/DNA bound to a 6-meG (1T38), an $N^4$-*p*-xylylenediamine cytosine, and a partially flipped thymine (1YFH). (*B*) Active site of hAGT C145S with a bound 6-meG. (*C*) Overall and binding-site structure of Atl1 (3GYH).

catalytic domain, with the exception that the active cysteine residue is replaced by tryptophan, alanine, or other residues, all of which are not capable of nucleophilic attack (Margison et al. 2003, 2007). ATLs tightly bind ss/dsDNA that contain $O^6$-alkyl guanine, but they display no alkyltransferase, demethylase, glycosylase, or endonuclease activity (Pearson et al. 2005, 2006; Chen et al. 2008; Morita et al. 2008). In fact, preincubation of alkylated oligonucleotides inhibits the repair activity of hAGT (Pearson et al. 2005, 2006). Yet it was clear that ATL must be playing a role in alkylation damage protection in vivo, because *S. pombe* and *Thermus thermophilus* were rendered more sensitive to alkylating reagents on inactivation of their *ATL* genes (Pearson et al. 2006; Morita et al. 2008). Through a series of genetic and biochemical experiments, ATL was discovered to be linked to the nucleotide excision repair (NER) pathway (Mazon et al. 2009; Tubbs et al. 2009). In a proposed model, ATLs first recognize the often bulky $O^6$-alkyl lesion, then induce extensive DNA bending and switch from an open conformation to a closed state that is not seen in AGTs (Tubbs and Tainer 2010). Large DNA distortions are known to initiate NER recognition, and ATL in the closed state is also believed to facilitate recruitment of NER proteins. Crystal structures of ATLs from *Vibrio parahaemolyticus* and *S. pombe* are also in good agreement with the working model (Fig. 3C) (Tubbs et al. 2009; Aramini et al. 2010).

## OXIDATIVE REVERSAL OF ALKYLATION DAMAGE BY AlkB FAMILY DIOXYGENASES

Oxidative DNA repair mediated by the AlkB family demethylases is one of the most exciting and rapidly growing areas in the field of DNA repair during the past decade since the initial ground-breaking discovery made independently by two groups in 2002 (Falnes et al. 2002; Trewick et al. 2002). Later on, AlkB was shown to repair RNA lesions as well (Aas et al. 2003; Ougland et al. 2004). So far, nine human homologs of *E. coli* AlkB have been identified (termed ALKBH1-8, plus FTO), representing a major class of DNA/RNA demethylases in human cells (Kurowski et al. 2003; Gerken et al. 2007; Sanchez-Pulido and Andrade-Navarro 2007). In the event of oxidative demethylation, the AlkB family proteins use an iron(II) site to activate the dioxygen molecule for oxidation of the aberrant alkyl groups. The hydroxylated alkyl groups, which are attached to the $N^1$ position of adenine or $N^3$ position of cytosine, then undergo facile C–N bond cleavage to yield the unmodified base and formaldehyde (Drablos et al. 2004; Sedgwick 2004; Falnes et al. 2007; Yi et al. 2009). Such an oxidation mechanism is shared by a variety of enzymes within the nonheme iron-containing protein family, which has also inspired the discovery of the JmjC-domain-containing histone demethylases that mediate epigenetic histone demethylation (Tsukada et al. 2006).

### *E. coli* AlkB: UNIQUE BASE-FLIPPING, DIVERSE SUBSTRATES, AND OXIDATIVE DEMETHYLATION

Like photolyases and alkyltransferases, AlkB and related dioxygenases also need to flip the damaged base out of duplex DNA for repair reactions. AlkB has been shown to repair alkylation lesions in both ssDNA and dsDNA, with a preference toward single-stranded substrates. Structures of AlkB bound to a trimer d(T-1-meA-T) DNA provided the first glance of the folding and substrate binding of AlkB (Fig. 4A) (Yu et al. 2006); yet the characterization of the AlkB/dsDNA complex structure had been hampered by AlkB's weak and nonsequence-specific binding property (Dinglay et al. 2000; Mishina et al. 2004a). Utilizing a disulfide cross-linking technique pioneered by Verdine et al. to capture transient protein/DNA interactions (Huang et al. 1998; Verdine and Norman 2003), we stabilized the complex formed between AlkB and dsDNA (Fig. 4B) (Mishina and He 2003; Mishina et al. 2004b). We first engineered the cross-link into the active site of AlkB and determined the structure of the resulting complex (Yang et al. 2008). With the information revealed from the "active-site cross-linked" structure, a distal cross-link was designed to obtain a complex with an intact

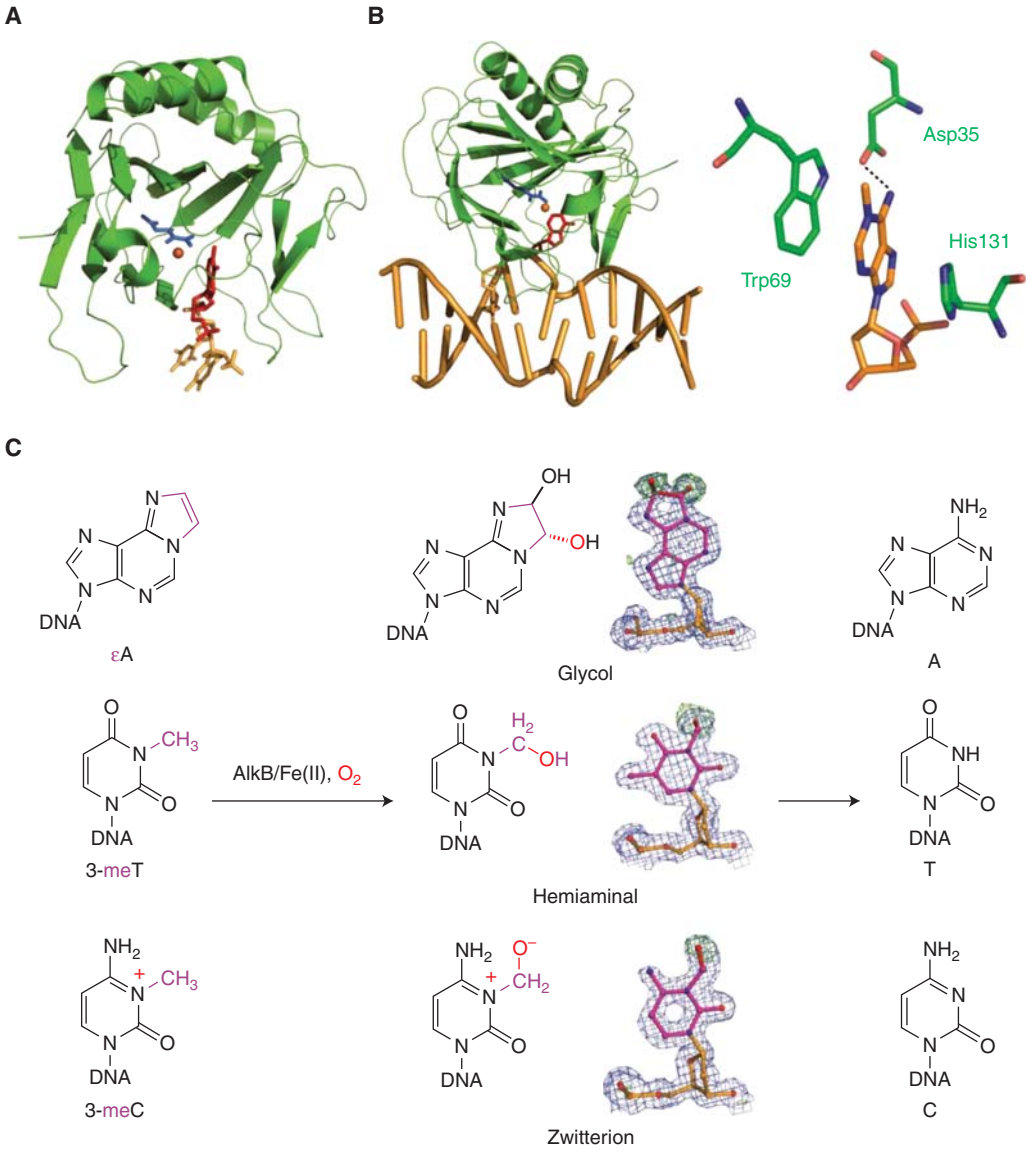


Figure 4. Structure and mechanism of AlkB-mediated oxidative demethylation. (*A*) Structure of AlkB/d(T-1meA-T) (2FD8). (*B*) Structure of overall AlkB/DNA complex and the active site (3BIE). (*C*) Direct observation of oxidative-demethylation intermediates in crystallo.

enzymatic pocket. Both of these structures show an unprecedented base-flipping mechanism. AlkB squeezes together the two bases flanking the flipped-out one to access the damaged base (Fig. 4B). In contrast to DNA distortion in the lesion-containing strand, AlkB has few interactions with the complementary strand, which merely accommodates DNA distortion of the lesion-containing strand through spontaneous conformational rearrangements. Thus, the complementary strand acts as a noncompetitive inhibitor, which explains the observed preference of AlkB toward ssDNA (Yang et al. 2008).

AlkB recognizes diverse substrates: $N^1$-methyl adenine (1-meA) and $N^3$-methyl cytosine (3-meC) are the best substrates (Falnes et al. 2002; Trewick et al. 2002); $N^1$-methyl guanine and $N^3$-methyl thymine (3-meT) are repaired at lower rates (Delaney and Essigmann 2004; Falnes 2004; Koivisto et al. 2004). Furthermore, exocyclic DNA adducts such as 1,$N^6$-ethenoadenine (εA), 1,$N^6$-ethano adenine, and 3,$N^4$-ethenocytosine are also substrates of AlkB (Delaney et al. 2005; Mishina et al. 2005; Frick et al. 2007). Mechanistically, such promiscuous substrate recognition has been extensively investigated with AlkB crystal structures containing different substrates (Yu et al. 2006; Yang et al. 2008; Yu and Hunt 2009; Holland and Hollis 2010; Yi et al. 2010). A "pinch" sequence (Thr51 to Tyr55) and a flexible loop (His72 to Tyr76) anchor the phosphate backbone; His131 and Trp69 stack against the base plane; Tyr78 and a "substrate recognition" loop (Lys134 to Glu136) form specific hydrogen bonds with the flipped bases (Yu and Hunt 2009; Yi et al. 2010). Overall, AlkB strictly controls the final location of substrates in the binding pocket so that the aberrant alkyl group is positioned precisely for efficient oxidative dealkylation.

Knowing how AlkB recognizes and accommodates different lesions, the next question concerns the mechanism of the oxidative repair. It was proposed that oxidative demethylation proceeds through hydroxylation at the aberrant methyl group followed by heterocleavage of the C–N bond; yet direct evidence supporting the process is lacking. To address this question, we took an in crystallo oxidation approach and captured different reaction intermediates (a zwitterionic structure, a hemiaminal, and a glycol) in the demethylation processes of 3-meC, 3-meT, and εA, respectively (Fig. 4C) (Yi et al. 2010). Essigmann and coworkers have also observed an epoxide intermediate in AlkB-mediated εA repair using mass spectrometry (Delaney et al. 2005). These observations provide direct support of the oxidative-demethylation mechanism. Together with solution observations (Bleijlevens et al. 2008, 2012), a more complete picture of cofactor binding, substrate flipping and processing, and product release is now emerging.

## ALKBH1: AP LYASE OR DEMETHYLASE?

Among the nine homolog proteins, ALKBH1 displays the strongest homology with AlkB, although early studies failed to detect any demethylation activities (Duncan et al. 2002; Aas et al. 2003). A later study showed that, as a mitochondrial protein, ALKBH1is able to demethylate 3-meC in vitro (Westbye et al. 2008). Yet this is just the beginning of the enigma of ALKBH1. Two groups then independently showed that both human ALKBH1 and the *S. pombe* homolog Abh1 show AP-lyase activity (Muller et al. 2010; Korvald et al. 2012). The lyase activity of human ALKBH1 and Abh1 is not dependent on iron(II) or 2KG and is not affected by mutation of the putative metal-binding residues; furthermore, Abh1 failed to show demethylase activity against methylated DNA or etheno adducts, nor was the yeast *abh1*$^-$ mutant sensitive toward alkylating agents. But if the in vitro AP-lyase activity has any biological significance, it remains elusive. What does seem clear, however, is that ALKBH1 plays a role in placental trophoblast lineage differentiation and participates in transcriptional regulation (Pan et al. 2008; Nordstrand et al. 2010).

## ALKBH2: A BONA FIDE HOUSEKEEPING DNA-REPAIR ENZYME

ALKBH2 demethylates both 1-meA and 3-meC in vitro (Duncan et al. 2002; Aas et al. 2003); mouse experiments have also established mAlkbh2 as the primary repair enzyme guarding the mammalian genome against these two lesions (Ringvoll et al. 2006). Moreover, ALKBH2 was shown to be the principle enzyme for εA repair in vivo as well (Duncan et al. 2002). Very recent data suggests that ALKBH2 protects against lethality and mutation in primary mouse embryonic fibroblasts (Nay et al. 2012), and that ALKBH2 may be involved in the molecular mechanism of gastric cancer through the inhibition of the proliferating gastric cancer cells (Gao et al. 2011). ALKBH2 ortholog in *A. thaliana* has also been shown to protect Arabidopsis against methylation DNA damage (Meza et al. 2012). Thus, unlike ALKBH1, ALKBH2 has

been convincingly established as a bona fide DNA-repair enzyme that protects the genome against alkylation damage.

Crystallographic evidence has provided insights into the lesion-recognition mode and damage-searching mechanism of ALKBH2 (Fig. 5A). Using a disulfide cross-linking technique, we have succeeded in the structural characterization of ALKBH2 bound to duplex DNA containing different base lesions (Yang et al. 2008; Yi et al. 2012). ALKBH2 uses several active-site residues to recognize 1-meA and uses an aromatic finger residue Phe102 to facilitate base flipping (Yang et al. 2008). In addition, Phe102 can also probe the stability of base pairs when ALKBH2 is interrogating DNA for damage; the unique oxidation chemistry of ALKBH2 then ensures that only a cognate substrate will be modified (Yi et al. 2012). It remains to be seen if ALKBH2 could signal or recruit additional repair factors when it encounters noncognate damage (Gilljam et al. 2009).

## ALKBH3: A DNA-REPAIR ENZYME THAT TARGETS 3-meC WITH ssDNA PREFERENCE

ALKBH3 demethylates both 1-meA and 3-meC in vitro, with a preference toward single-stranded nucleic acids (Duncan et al. 2002; Aas et al. 2003). Inside cells, ALKBH3 has significant roles in both prostate cancers and lung cancers (Liu et al. 2007; Tasaki et al. 2011), and it contributes to cell survival and invasion through discoidin receptor 1 (Shimada et al. 2008). In fact, studies have suggested ALKBH3 as a promising target molecule for developing therapeutic agents to treat castrate-resistant prostate cancer (Koike et al. 2012). To address the relevance of ALKBH3 in mammalian cells, Shi and colleagues revealed that ALKBH3 physically associates with ASCC3, a 3′ to 5′ DNA helicase (Dango et al. 2011). Such an association is critical to the ALKBH3-mediated repair process because ASCC3's DNA-unwinding activity presents the 3-meC substrate in ssDNA context, allowing ALKBH3 to access 3-meC within the double-stranded region of DNA. Thus, for tumors showing ALKBH3 and ASCC3 overexpression, therapeutic approaches that can couple 3-meC cytotoxicity to the disturbance of the activities, or association of these two proteins, may have therapeutic effects against these tumors.

Slupphaug, Tainer, and colleagues solved the crystal structure of the catalytic core of ALKBH3 and identified a flexible hairpin involved in base-flipping and ss/dsDNA discrimination (Fig. 5B) (Sundheim et al. 2006). Sequence alignments of ALKBH2 and ALKBH3 reveal a very hydrophobic Val101-Phe102-Gly103 motif for ALKBH2 and a heavily charged Arg122-Glu123-Asp124 sequence for ALKBH3 at the nucleotide-flipping region (Yi et al. 2009). Swapping the two sequences surprisingly switches the ss/dsDNA preference of the two proteins (Chen et al. 2010; Monsen et al. 2010). Yet a complex structure of ALKBH3/DNA would still be highly desirable to address the exact protein/DNA interactions in ALKBH3.

## ALKBH5: AN OXYGENASE TARGETED BY HYPOXIA-INDUCING FACTOR 1α

Like other members of the ALKB family, ALKBH5 possesses the metal- and 2KG-binding motif. Pollard et al. showed that ALKBH5 is largely localized to the nucleus and is a 2KG oxygenase; however, the decarboxylation of 2KG by ALKBH5 is not stimulated by known AlkB substrates, hinting that ALKBH5 does not act on 1-meA-type DNA damage (Thalhammer et al. 2011). What makes ALKBH5 unique among the *ALKBH* genes is probably that it is a direct transcriptional target of hypoxia-inducible factor-1 and is induced by hypoxia in a range of cell types. Yet the biological function of ALKBH5 is currently unknown.

## ALKBH8: A tRNA MODIFICATION ENZYME RATHER THAN A DNA-REPAIR PROTEIN

ALKBH8 is a multidomain protein that possesses an amino-terminal RNA recognition domain, the AlkB domain, and a carboxy-terminal methyltransferase domain. The methyltransferase domain has sequence homology with the *S. cerevisiae* tRNA methyltransferase Trm9, which catalyzes the methyl esterification of U34 of

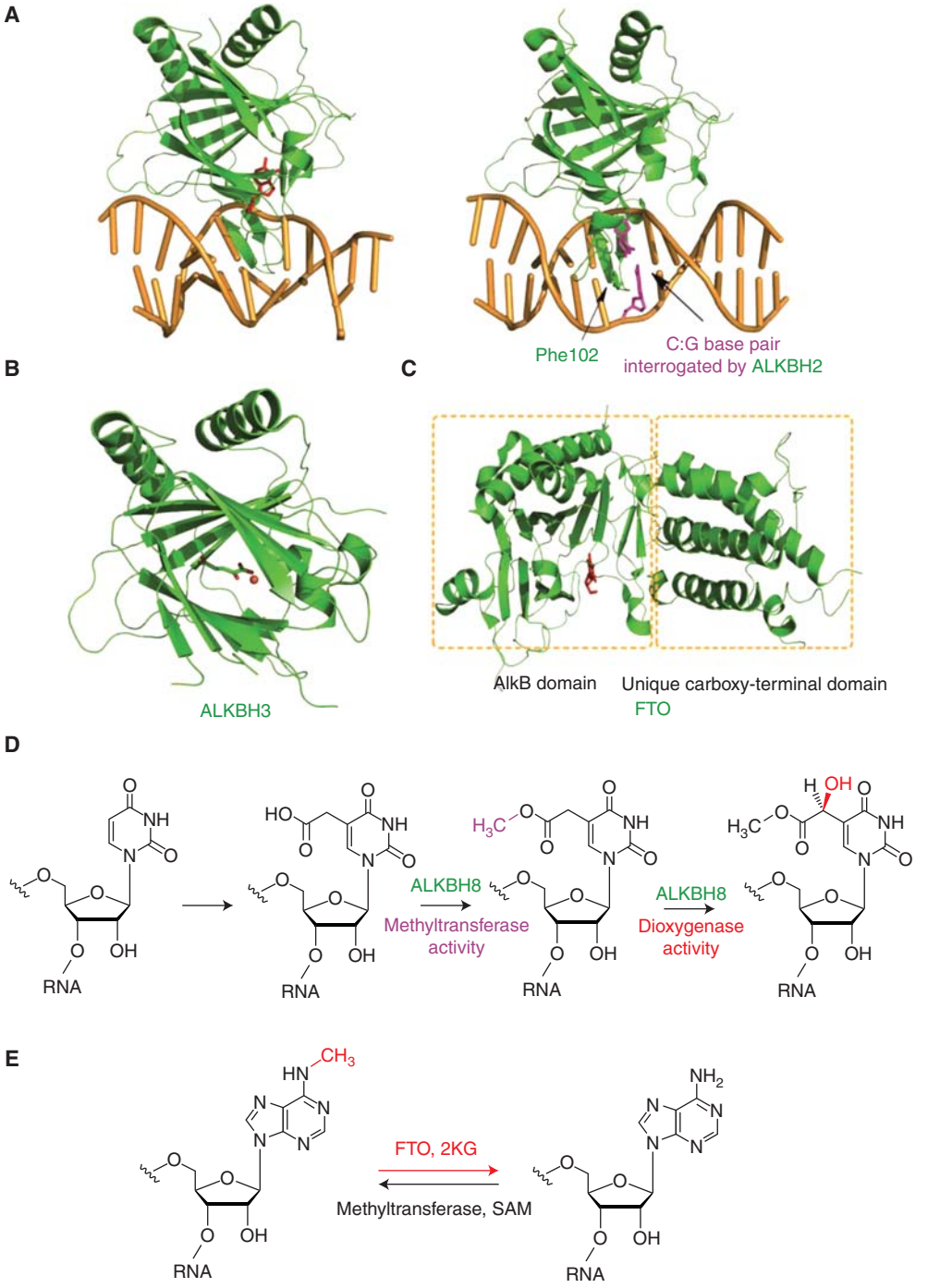


**Figure 5.** Structures and functions of AlkB homologs. (*A*) Structures of ALKBH2 with a bound 1-meA (3BTY) and a central C:G base pair (3RZG), which the protein is interrogating. (*B*) Structure of ALKBH3(2IUW). (*C*) Structure of FTO (3LFM). (*D*) ALKBH8 is a transfer RNA (tRNA) modification enzyme. (*E*) Demethylation of 6-meA by FTO enables reversible RNA modification.

tRNA$^{Arg}$ and tRNA$^{Glu}$, forming 5-methoxycarbonylmethyluridine (mcm$^5$U) and 5-methoxycarbonylmethyl-2-thiouridine, respectively. Two reports independently showed that ALKBH8 is a tRNA methyltransferase required for the biogenesis of mcm$^5$U (Fu et al. 2010a; Songe-Moller et al. 2010). Soon after, we and others showed that the AlkB domain of ALKBH8 catalyzes the hydroxylation of mcm$^5$U into (S)-5-methoxycarbonylhydroxymethyluridine, thereby firmly establishing ALKBH8 as a tRNA hypermodification enzyme (Fig. 5D) (Fu et al. 2010b; van den Born et al. 2011). The recently solved structure of ALKBH8, comprising the RNA recognition and AlkB domains, shows disordered loops flanking the active site in the AlkB domain, providing insights into the evolutionary diversification of AlkB domains (Pastore et al. 2012). Interestingly, ALKBH8 was shown to contribute to the progression of human bladder cancer; and silencing of ALKBH8 significantly suppressed invasion, angiogenesis, and growth of bladder cancers in vivo (Shimada et al. 2009). How the tRNA modification activity can attribute to this observed phenotype remains to be established.

## FTO: LINKING OBESITY TO RNA DEMETHYLAION

A genome-wide association study found the *FTO* (fat-mass and obesity-associated) gene to be associated with body mass index (BMI) (Frayling et al. 2007). In this study, the authors found that a common variant in the *FTO* gene predisposes a person to diabetes through an effect on BMI. Adults who are homozygous for the risk allele weighed about 3 kg more compared to those without a risk allele. Two independent studies in close succession reported other intron 1 *FTO* single-nucleotide polymorphisms (SNPs) and extended the association to other obesity-related traits (Dina et al. 2007; Scuteri et al. 2007). Numerous subsequent studies also confirmed the association among 22 distinct populations, which have been summarized elsewhere (Tung and Yeo 2011).

In the mouse, *FTO* null individuals (*FTO*$^{-/-}$) displayed decreased fat and lean body mass, increased metabolic rate, and elevated food intake, which shows that *FTO* is involved in energy homeostasis through control of energy expenditure (Fischer et al. 2009; Gao et al. 2010). Consistent with this, FTO was found to be highly expressed in the brain, particularly in the hypothalamus. Furthermore, nutritional status directly regulates the expression of FTO in a bidirectional manner. Food intake can also be bidirectionally influenced through manipulation of the FTO level in the arcuate nucleus of hypothalamus (Gerken et al. 2007; Tung et al. 2010). In humans, a catalytic incompetent mutation Arg316Gln renders the affected individuals a polymalformation syndrome (Boissel et al. 2009). Other loss-of-function mutations of *FTO* have also been identified, which appear to influence BMI as well (Meyre et al. 2010).

Shortly after *FTO* was reported, bioinformatics analysis revealed that *FTO* encodes an iron(II)- and 2KG-dependent dioxygenase, which is closely related to *E. coli* AlkB (Gerken et al. 2007; Sanchez-Pulido and Andrade-Navarro 2007). Crystal structure of FTO did show an AlkB-like domain and a unique carboxy-terminal domain (Fig. 5C) (Han et al. 2010). Biochemically, FTO was first shown to demethylate 3-meT in ssDNA and later 3-meU in ssRNA in vitro (Gerken et al. 2007; Jia et al. 2008). However, such demethylation activity is exceedingly low compared to other AlkB-family proteins. We discovered in 2011 that FTO shows efficient demethylation activity toward the abundant $N^6$-methyl adenine (6-meA) residues in RNA, both in vitro and in vivo (Fig. 5E) (Jia et al. 2011). In addition, FTO partially colocalizes with nuclear speckles, which supports the notion that 6-meA in nuclear RNA is a physiological substrate. Future experiments demonstrating the specific messenger RNA (mRNA) targets of FTO is expected to link the demethylation activity to the observed phenotype of FTO.

## CONCLUDING REMARKS AND OUTLOOK

The last decade has witnessed exciting advances and rapid growth in the field of direct DNA repair. Such rapid expansion of knowledge has not only enabled us to understand DNA-repair

pathways in general and for therapeutic purposes (Rabik et al. 2006; Tubbs et al. 2007), but has also accelerated new discoveries in biology (Wu and Zhang 2011). For instance, the oxidative-demethylation mechanism of the AlkB family dioxygenases has inspired the discovery of histone lysine demethylases (Tsukada et al. 2006). The recently identified TET family proteins also share oxidative hydroxylation chemistry with AlkB. These proteins consecutively convert 5-methylcytosine, a hallmark of DNA epigenetics, to 5-hydroxymethylcytosine, 5-formylcytosine, and 5-carboxylcytosine (Tahiliani et al. 2009; Ito et al. 2010, 2011; He et al. 2011). Interestingly, the enzyme that recognizes and excises the oxidation end products of 5-formylcytosine and 5-carboxylcytosine is a DNA-repair glycosylase, thymine DNA glycosylase or TDG, which is a DNA mismatch repair protein (He et al. 2011; Maiti and Drohat 2011; Zhang et al. 2012). These advances are among the most exciting breakthroughs in the field of DNA epigenetics. In addition, the discovery that RNA methylation is reversed by FTO also brought up the notion of reversible RNA modification in biological regulation (Fig. 5E) (He 2010). Nevertheless, many puzzling questions remain. For example, what are enzymatic activities of ALKBH1, 4, 5, 6, and 7 and what are their biological roles? How is the demethylation activity of FTO related to its phenotypic observations? Hopefully, continued investigations will shine light on such questions that are related to or go beyond the field of DNA repair in the future.

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