Nucleic Acid Oxidation in DNA Damage Repair and Epigenetics

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Nucleic acid oxidation in DNA damage repair and epigenetics

Guanqun Zheng, Ye Fu, Chuan He*

Department of Chemistry and Institute for Biophysical Dynamics, the University of Chicago, 929 East 57th Street, Chicago, Illinois, USA

*To whom correspondence should be addressed. Phone: 773-702-5061. Fax: 773-702-0805. E-mail: chuanhe@uchicago.edu

1. Introduction

Methylating agents, extracellular or intracellular, can attack vulnerable sites on DNA, leading to cytotoxic and/or cancerogenic DNA damages. The methylomes of biomolecules are also continuously sculpted by cellular methyltransferases, and demethylating enzymes. Methyltransferases can use *S*-adenosylmethionine (SAM) as a universal electrophilic source of a methyl group to modify DNA, RNA, and proteins.[1-2] While methylation has been well recognized as one of the most important modifications for nucleic acids and proteins, the demethylation process, especially oxidative demethylation is receiving growing attention owing to its cellular regulatory functions.

DNA lesions caused by methylating agents can occur on different positions of bases or backbones, dependent on the type of chemical reactions (S_N1 or S_N2 nucleophilic substitution) as well as the susceptibility of each position. These resulting lesions exhibit different toxicity and mutagenic influence to cells.[3-4] The S_N2 type methylating agents tend to attack the nucleophilic *N*-position of exposed bases on single-stranded DNA (ssDNA), which generates a large portion of N^1-methyladenine (m^1A) and N^3-methylcytosine (m^3C) lesions. Methylation on these two positions are quite toxic because they block the Watson-Crick base paring during DNA replication.[5] The AlkB family of Fe^{II}- and α-ketoglutarate (α-KG)-dependent dioxygenases were identified to repair these DNA lesions though an unprecedented oxidative demethylation mechanism over 10 years ago,[6-7] which provided an example of oxidative demethylation on macromolecules.

Methylation is also widely present in RNA as a modification which has been thought to fine-tune the structure and function of mature RNA.[8] A significant amount of

methylation is present at the nitrogen atoms of the base, such as N^3-methylcytosine on ribosomal RNA (rRNA), N^1-methyladenosine on transfer RNA (tRNA), and N^7-methylguanosine on messenger RNA (mRNA) (http://rna-mdb.cas.albany.edu/RNAmods/). Inspired by the demethylation mechanism involved in DNA repair, we proposed that RNA methylation could be reversible through similar mechanistic avenue to serve potential regulatory roles.[9] The recent discovery of two RNA N^6-methyladenosine (m^6A) demethylases, FTO (Fat mass and obesity-associated) and ALKBH5, has strongly supported the hypothesis that reversible RNA methylation plays regulatory roles.[10-11]

The nucleotide variant 5-methylcytosine (5mC) has been long established as a landmark modification in mammalian genomic DNA in terms of epigenetic regulation. It is recognized as the "fifth base" which represents another layer of heritable information upon the DNA code. DNA methylation occurs primarily at CpG dinucleotides in high eukaryotes, whereas DNA methylation in plants shows little sequence preference.[12] In plants (e.g., *A. Thaliana*), it has been well documented that the Demeter (DME)/repressor of silencing 1 (ROS) family of 5mC glycosylases function to remove 5mC through the base excision repair (BER) pathway.[13] In mammals, although the enzymes that catalyze DNA methylation have been characterized,[14-16] enzymes responsible for demethylation remained elusive for a long time. A recent ground-breaking discovery was the identification of TET (Ten-eleven translocation) proteins that can oxidize 5mC to 5-hydroxymethylcytosine (5hmC), 5-formylcytosine (5fC), then 5-carboxylcytosine (5caC) through stepwise oxidations; 5fC and 5caC can be further converted to cytosine through BER to provide the first biochemically confirmed active demethylation pathway in

mammalian cells.[17-20] The TET protein family includes three members, TET1-3, which all adopt the conserved dioxygenase motif to catalyze the consecutive oxidations in an Fe^{II}- and α-KG-dependent manner, just like AlkB.

This review covers direct oxidative demethylations in DNA repair as well as demethylation of RNA. Oxidation of 5mC on DNA by the TET family of mononuclear non-heme Fe^{II}-dependent dioxygenases will also be discussed. Besides discussing the mechanisms, structures, and functions of these oxygenases, we also highlight the significance of some of these processes in epigenetics.

2. AlkB and its human homologues

2.1 Oxidative dealkylation mediated by AlkB

2.1.1 *E. coli* AlkB

Cellular DNAs are constantly challenged by endogenous and environmental alkylation chemicals, resulting in cytotoxic and mutagenic adducts. Accumulation of these alkylation adducts can lead to senescence, cancer, and even cell death. To ensure the genomic integrity and maintain cellular function, organisms have evolved a variety of housekeeping enzymes to efficiently remove alkylation adducts, including DNA glycosylases in base-excision repair, suicidal O^6-methylguanine-methyltransferases in guanine methylation repair, and AlkB family proteins in direct oxidative repair.[5] Although the *alkB* gene in *E. coli* was identified in a mutant strain with increased sensitivity to the S_N2 type alkylating agent methyl methane sulfonate (MMS) as early as in 1983,[21] this field has come a long way to characterize this protein as a member of Fe^{II}-

and α-KG-dependent dioxygenase, which has now emerged as the largest and extremely important family of non-heme oxidation enzymes.[22]

Since its discovery, early evidence suggested that AlkB is capable of protecting cells from lethal effects by repairing MMS-induced DNA damage under alkylation threatens,[23-25] in a process different from the known DNA repair mechanisms at the time.[26] AlkB protein could be expressed and purified;[27-28] however, it was challenging to biochemically determine the activity of AlkB *in vitro*. Early studies did suggest that AlkB prefers ssDNA and might repair m^1A and m^3C that are most likely DNA damage induced by MMS.[26] This field was driven forward by a bioinformatics study which predicted AlkB as an Fe^{II}- and α-KG-dependent dioxygenase by employing sequence profile analysis.[29] These results inspired subsequently biochemical tests of the catalytic activity of the AlkB protein.

2.1.2 Mechanism

The breakthrough was made by two independent groups in 2002 showing that AlkB directly reverses m^1A and m^3C to unmethylated bases in DNA through an unprecedented oxidative demethylation mechanism in the presence of iron(II), α-KG, and dioxygen.[6-7] To date, the substrates of AlkB has been extended to N^1-methylguanine (m^1G), N^3-methylthymine (m^3T),[30-32] 1,N^6-ethenoadenine (εA),[33-37] 3,N^4-ethenocytosine (εC),[37-40] 1,N^6-ethanoadenine (EA),[41-42] 3,N^4-α-hydroxypropanocytosine,[43] and N^6-methyladenosine (m^6A) (**Figure 1**).[41] DNA lesions of m^1A and m^3C are believed to be the physiologically relevant substrates for AlkB. Reduced toxicity of DNA damaging

agents that induce hydroxyethyl, propyl, and hydroxypropyl adducts has been observed in bacteria owing to the presence of AlkB.[44]

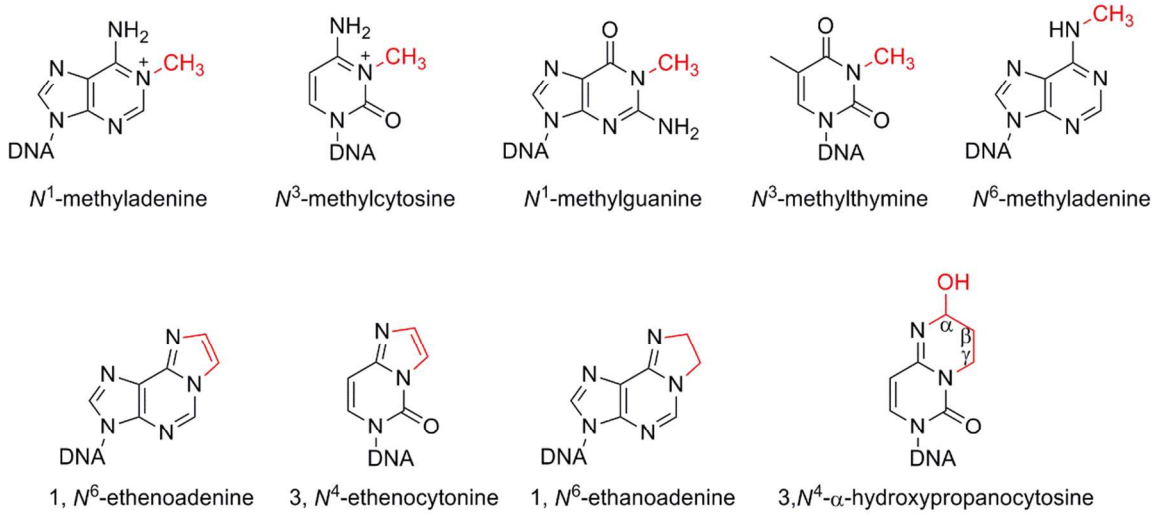

Figure 1. DNA/RNA methylations that can be repaired by AlkB.

AlkB protein uses a mononuclear iron(II) center to donate two electrons for the reduction of dioxygen;[5,45-48] α-KG serve as another cofactor to provide the other two electrons required for the four-electron reduction (**Figure 2**). This catalysis is composed of two phases: activation of dioxygen and oxidation of substrates. In the first phase, AlkB incorporates both α-KG and iron(II) to become catalytically ready.[49] The active site iron(II) then binds and activates the dioxygen molecule which attacks the bound α-KG. Cleavage of the O-O bond then yields a high-valent, high-spin (S = 2), iron(IV)-oxo species,[50] accompanied by the conversion of α-KG to succinate. It is proposed that iron(IV)-oxo species will then undergo conformational reorientation upon the releasing of CO_2 from the active site.[47] The Fe^{IV}=O double bond is reoriented to position *trans* to His187, which draws closer the distance between the Fe^{IV}=O oxygen and the target

methyl carbon.[51] In the second phase, the highly oxidative Fe^{IV}=O species abstracts an H atom from the methyl group of the aberrant adduct to yield a Fe^{III}-OH species and a carbon radical, which is likely the rate-determining step. Rebound of the iron-associated OH to the carbon radical yields a hydroxylated intermediate which dissociates from the active site and subsequently decomposes in water to afford the final demethylated product (**Figure 3**). Replacement of iron(II) with nickel or other metals abolishes this enzymatic activity.[52-53] With exocyclic DNA adducts such as εA and εC,[37,40] the epoxide intermediates were observed using mass spectrometry in the AlkB-mediated oxidation, providing further evidence for the direct reversal mechanism. Recently, the hydroxylated intermediates, glycol (from εA), hemiaminal (from m^3T), and a zwitterionic intermediate (from m^3C), have been captured and characterized in the AlkB-mediated repair during oxidation in crystals,[54] which confirmed the oxidative demethylation mechanism (**Figure 4**).

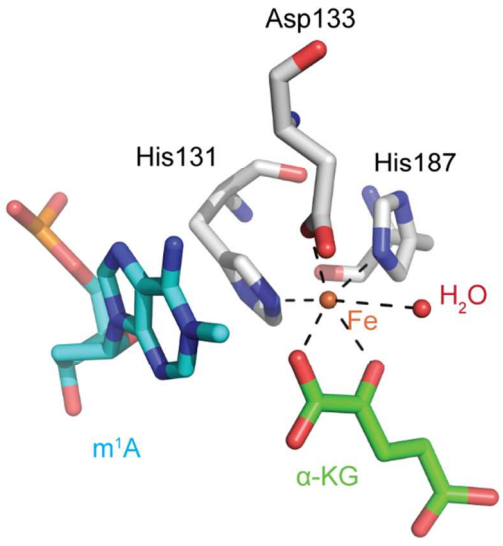

Figure 2. View of the active site stereochemistry with ligand coordination (Protein Data Bank (PDB) code: 2FD8).

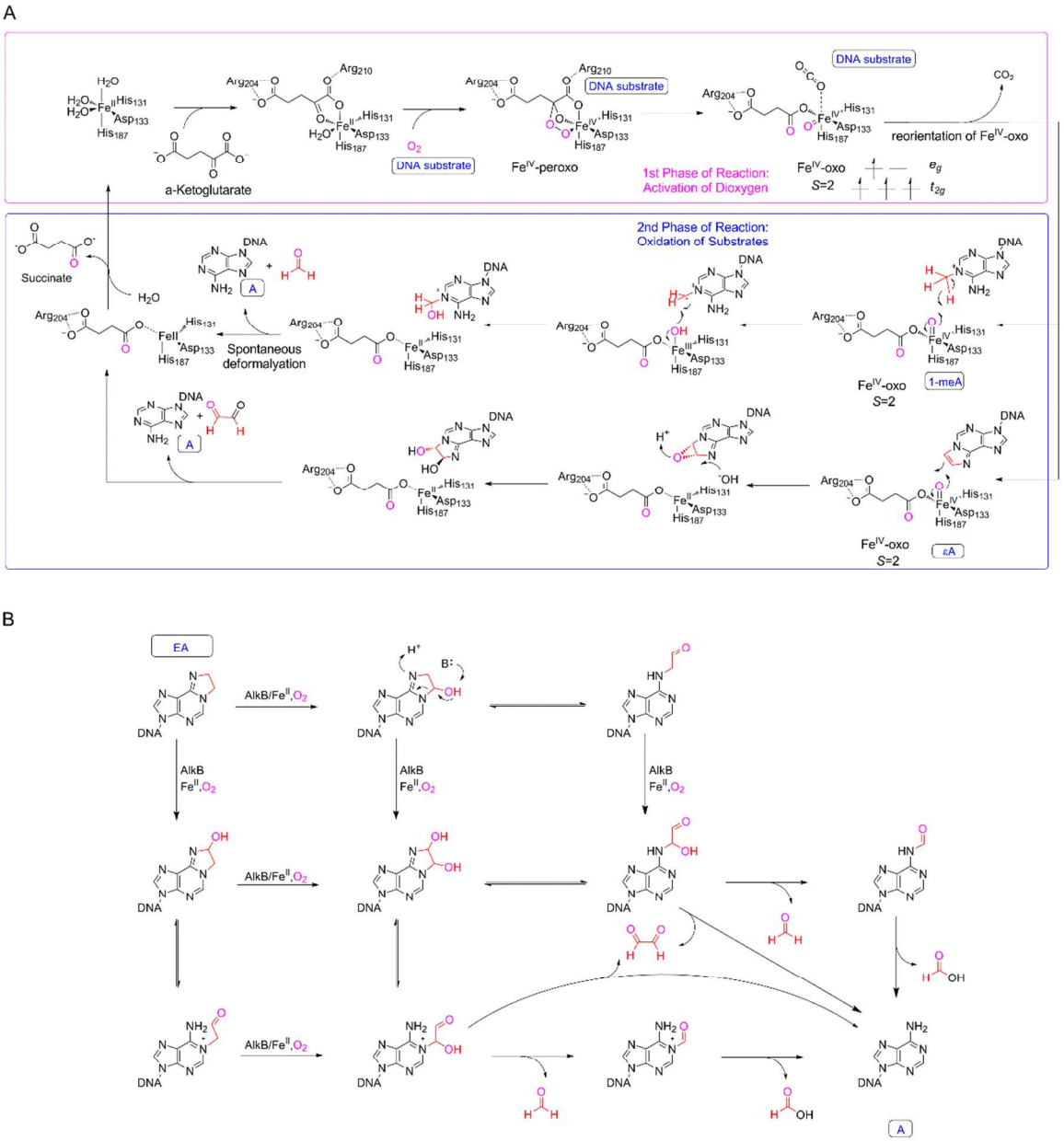

Figure 3. Proposed mechanisms of AlkB-mediated oxidative demethylations towards (A) m^1A, 1,N^6-ethenoadenine (εA), and (B) 1,N^6-ethanoadenine (EA).

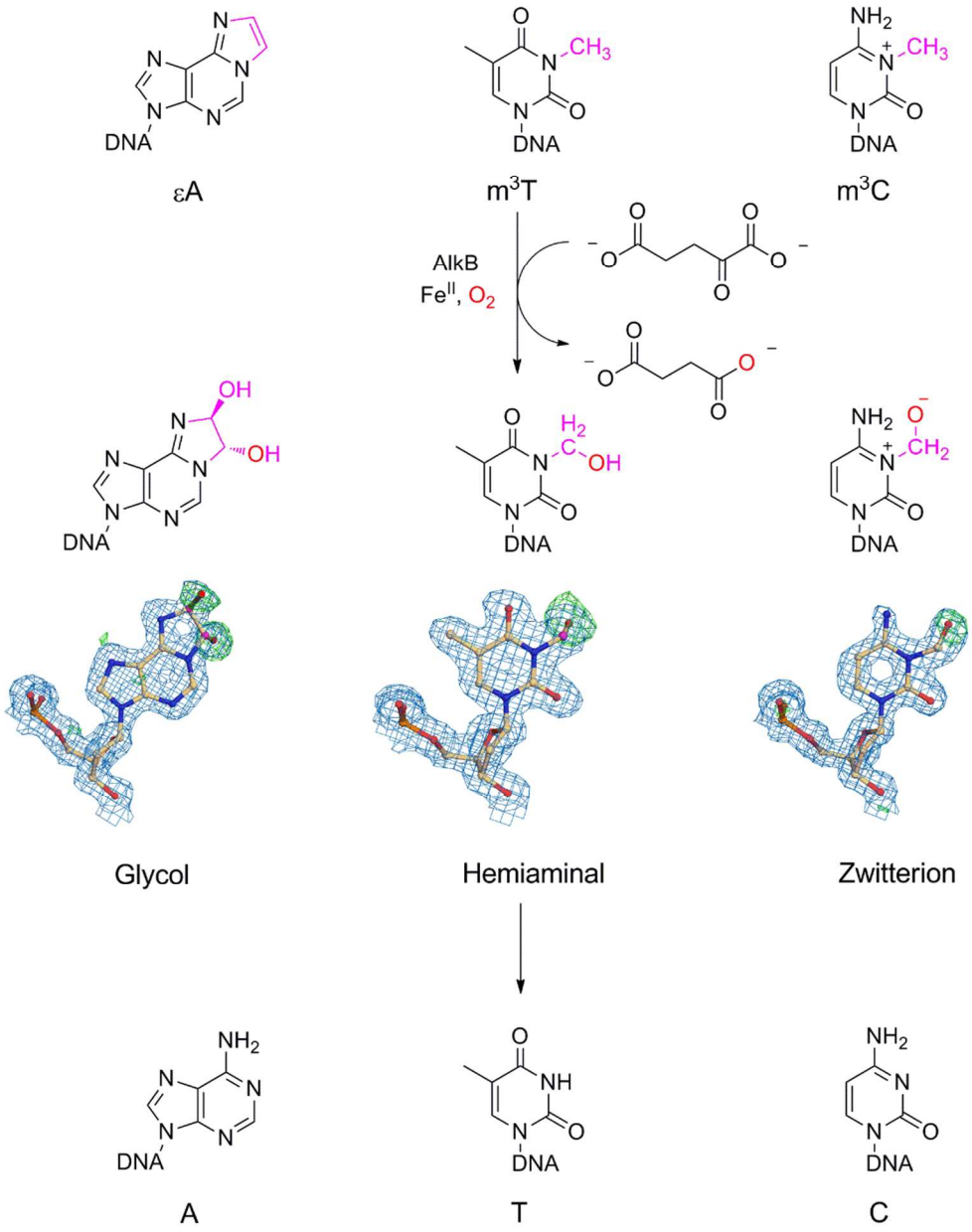

Figure 4. Oxidative demethylation mediated by AlkB. Oxidative repair of εA, m^3T, and m^3C by AlkB with intermediates glycol (3O1U), hemiaminal (3O1P), and a zwitterion (3O1S) captured during oxidation in crystals.

During the first phase of dioxygen activation, high-valent Fe^{IV}-oxo species is generated when AlkB mediates the decarboxylation of the co-substrate α-KG to form

succinic acid and CO_2. The by-products are released through conformational change of AlkB.[55-57] In the absence of substrates, this reaction has a modest α-KG turnover, and the produced Fe^{IV}-oxo species can oxidize the side chain of AlkB, leading to irreversible modifications of the protein.[46] The α-KG turnover could be significantly stimulated by the addition of any methylated DNA, suggesting that the methyl group in the DNA substrates is akin to initiating the oxidation of α-KG.[6-7,58] Analogues of α-KG were found to inhibit AlkB with high specificity.[58] Fluorescence-based assays have been developed to screen inhibitors to target this protein for therapeutic interventions of its human homologues.[59-63]

2.1.3 Substrate site specificity

The promiscuous substrate recognition of AlkB has been investigated through kinetic analyses and crystallographic studies. Positively charged lesions are preferentially repaired by AlkB.[39-40] These positively charged substrates could be favorably positioned in the active site pocket via interaction with the negatively charged side chain carboxylic group of Asp135 in the enzyme.[43] A polynucleotide structure is not essential but a nucleotide 5' phosphate group is required for AlkB to repair the substrate effectively.[44] AlkB creates an electropositive binding groove (Thr51 to Tyr55, Ser129, and Lys127) to anchor the phosphodiester backbone of the substrate. Trp69 and His131 stabilize the base by π-π stacking in the active site pocket. Tyr78, Lys134, Asp135, and Glu136 coordinately recognize the flipped bases through forming specific hydrogen bonds. Compared with other similar dioxygenases, AlkB possesses 90 unique N-terminal

residues to form a flexible substrate-binding lid to dock diverse alkylated nucleotide substrates in optimal catalytic geometry (**Figure 5**).[35,51,54,64-65]

AlkB prefers to repair methylation damages in ssDNA than those in double-stranded DNA (dsDNA).[59,66-68] Methylations at the N^1 site of adenine and N^3 site of cytosine alter the Waston-Crick base paring, thus disrupting DNA double-helices.[69-70] These two modifications could also occur in RNA and are exposed as in DNA. Later, AlkB was indeed shown to repair these adducts in RNA both *in vitro* and *in vivo*.[66,71-72] Repairing these lesions in mRNA and tRNA may serve as part of adaptive response to protect bacteria against chemical methylations.[71,73-75]

An advance in probing the structure and substrate preferences of AlkB was made by adopting a disulfide cross-linking approach to stabilize the labile protein-DNA complex.[76-77] AlkB interacts almost exclusively with the damage-containing strand, and utilizes a base-flipping mechanism to access the damaged base. It squeezes together the two bases flanking the flipped-out base to maintain the base stack. Therefore, it is thermal dynamically less favorable to access and repair the damage in the dsDNA of a rigid duplex structure compared to the same damage in ssDNA. Since AlkB binds to DNAs regardless of their sequences, the complementary strand also serves as a noncompetitive inhibitor for the repair, which is another factor contributing to the preference of AlkB towards ssDNA over dsDNA (**Figure 5**).[65,78]

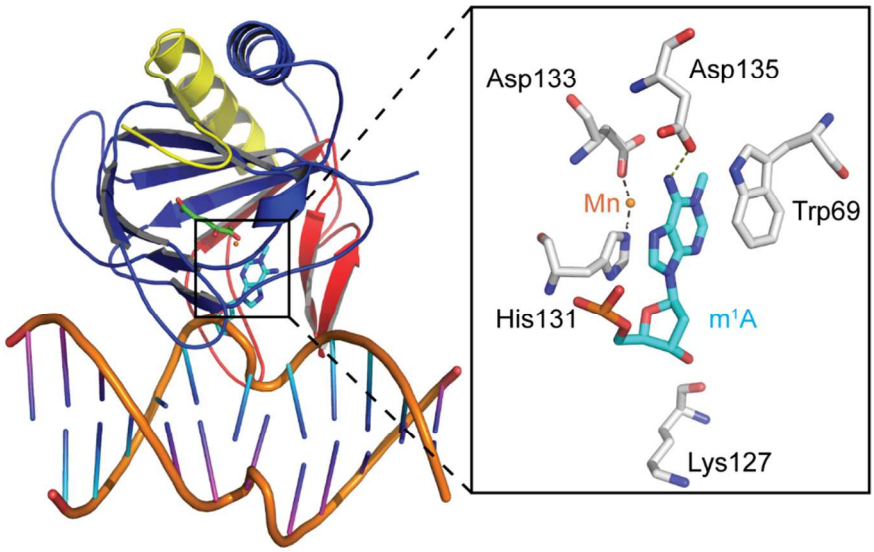

Figure 5. Crystal structure of an AlkB-dsDNA complex (3BIE). The protein is colored according to subdomain organization with the N-terminal extension in yellow (residues 13-44), nucleotide-recognition lid in red (residues 45-90), and catalytic core in blue (residues 91-214). Iron(II) is replaced with manganese(II) (orange) in the structure to quench the catalytic activity. The flipped base m^1A is shown in blue, DNA backbone in beige, bases in the m^1A-containing DNA strand in cyan, and those in the complementary strand in purple.

2.2 AlkB homologues

Bioinformatics and functional analyses reveal that AlkB homologues are widely expressed in many organisms from bacteria to human and carry out diverse biological functions.[29,79-87] The majority of the bacterial AlkB homologues are DNA repair proteins.[84,87] Two AlkB homologues have been found in the genome of fission yeast *S. pombe*.[85,88] One of them, Ofd2, has been characterized to be an Fe^{II}- and α-KG-dependent dioxygenase interacting with histones. In mammals, nine homologues of AlkB have been identified so far, termed ALKBH1-8 and FTO (fat mass and obesity associated protein).[79,81,89-90] All of them possess a double-stranded β-helix (DSBH) catalytic core

that is conserved for this family of dioxygenases,[91] including an $HXDX_nH$ motif for the iron binding, as well as a RXXXXXR motif for the α-KG binding (**Figure 6**).[5,90,92] Among these proteins, ALKBH2 and ALKBH3 are most alike to AlkB. They function as DNA repair proteins to protect the genome integrity of mammalian cells.

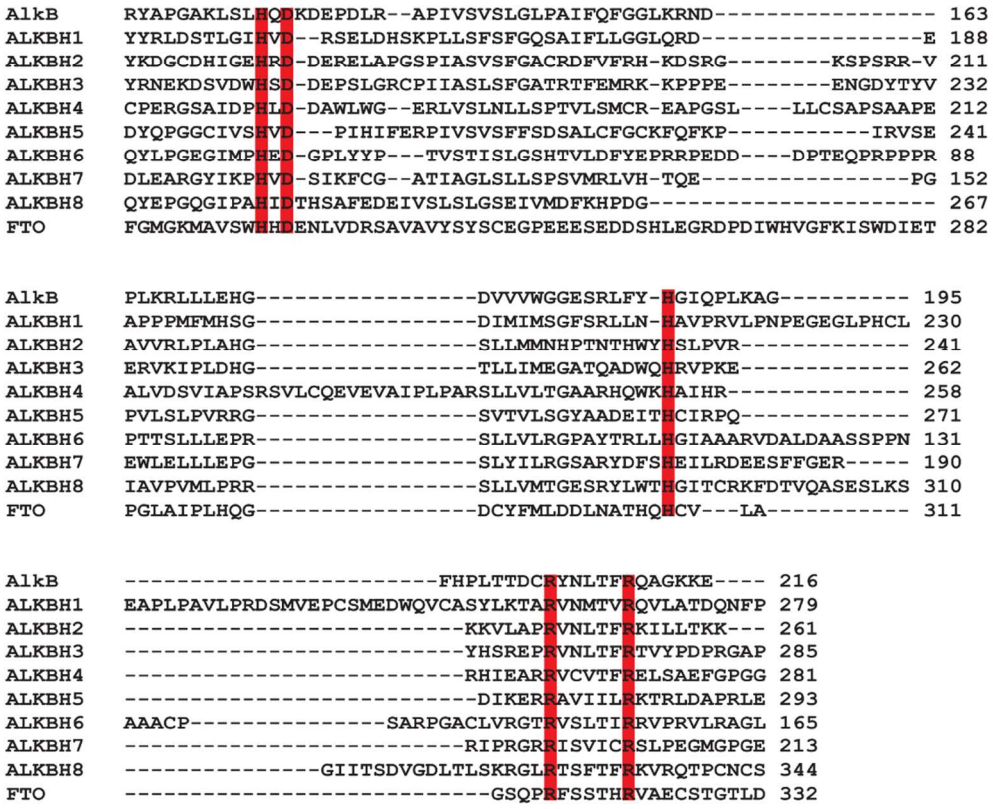

Figure 6. Sequence alignment of human AlkB family proteins with AlkB. Conserved residues highlighted in red are histidines and carboxylates as iron(II)-binding residues, as well as the characteristic RXXXXXR region, which binds cofactor α-KG.

2.2.1 ALKBH2

ALKBH2 has been characterized as a *bona fide* DNA repair enzyme guarding the mammalian genome. It displays robust repair activity against cytotoxic m^1A and m^3C *in vitro* and *in vivo*,[66,93-94] as well as a reduced activity towards m^3T in dsDNA.[30] It also

protects the mammalian genome against εA and εC through direct oxidative dealkylation, in complementary to DNA glycosylase that repair the same lesions through the BER pathway.[95-96] In mouse model, mouse Alkbh2 serves as the primary oxidative demethylase for repairing m^1A and m^3C lesions in DNA,[97] thus protecting mouse genome when primary mouse embryonic fibroblasts are threatened by exogenous methylating agents.[98] In addition, the homologue of ALKBH2 in *A. thaliana* also acts as an important enzyme for protecting *A. thaliana* against methylation DNA damage.[99] These results suggest that homologues of ALKBH2 in other organisms share a similar function of DNA repair.

ALKBH2 reverses DNA damage in the same oxidative dealkylation mechanism by using an active iron center as AlkB (**Figure 2**),[100-101] except that ALKBH2 prefers repairing damage in DNA duplex.[66-67,76] Unlike AlkB, ALKBH2 makes extensively contacts with both strands of duplex DNA as revealed by crystallographic studies.[77] ALKBH2 does not contain a damage-checking site as many glycosylases do.[102] It appears to detect damaged base by probing base-pair stability.[103] Taking the substrate of m^1A as an example, the Hoogsteen base pair with a *syn*-conformation adopted by m^1A, when paired with an opposite T, has much reduced base-pairing stability as compared to the normal Watson-Crick A-T base pair.[104] This reduced stability facilitates the recognition and repair of m^1A by ALKBH2. Compared with AlkB, ALKBH2 possesses a unique short hydrophobic β-hairpin in proximity of the active site which is important for its preference to double-stranded DNA substrates (**Figure 7A**).[105-106] In particular, the aromatic finger residue, Phe102, intercalates into the duplex stack to facilitate the base

flipping. Phe124, His171, and Glu175 coordinate with other protein residues to recognize and flip the damaged base.

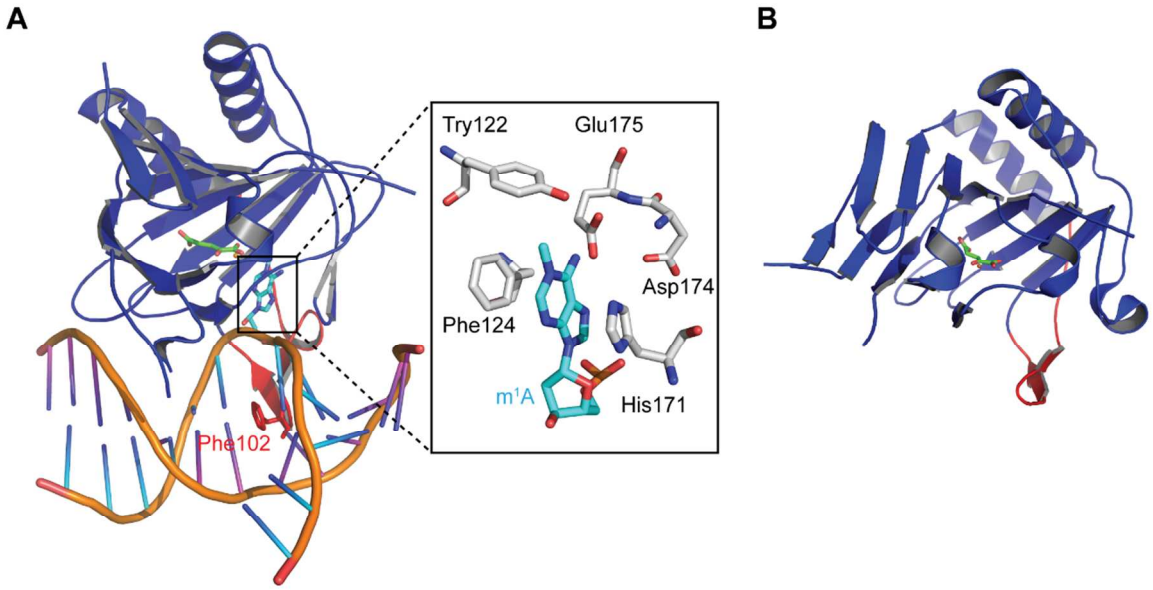

Figure 7. Crystal structure of AlkB human homologues. (A) Crystal structure of an ALKBH2-dsDNA complex (3BUC). (B) Crystal structure of ALKBH3 (2IUW). The protein is colored in blue with the β-hairpin (ALKBH2: residues 89-108; ALKBH3: residues 113-129) in red. Manganese(II) is shown in orange, α-KG in green, DNA backbone in beige, bases in the m^1A-containing DNA strand in cyan, and those in the complementary strand in purple.

The function of ALKBH2 as a DNA repair protein suggests its potential involvement in cancer therapy. It is frequently down regulated in gastric cancer[107] and is also involved in the growth of brain tumor cells,[108] glioblastoma,[109] colon carcinogenesis,[110] and bladder cancer.[111] Knockdown of ALKBH2 increases the sensitivity of cancer therapies, such as photodynamic therapy (PDT) mediated by Photofrin, and chemotherapy with cisplatin.[112-113] Based on these observations, ALKBH2, together with its repair partners,[114] may serve as a biological marker for cancer monitoring as well as a potential target for therapy.

2.2.2 ALKBH3

ALKBH3 is a close homologue of ALKBH2 and was identified at the same time as ALKBH2. ALKBH3 demethylates both m^1A and m^3C *in vitro*, with lowered activity towards m^1T and εA.[30,34,44,66-67,93] Although these two proteins work on similar substrates, ALKBH2 prefers double-stranded substrates while ALKBH3 favors single-stranded nucleic acid substrates.[66-67,76] ALKBH3 can also demethylate m^1A and m^3C in RNA,[66,74,94] suggesting a possibility of repairing RNA lesions.[66,71,115] Recently, ALKBH3 was found to maintain genomic integrity by coordinating with ASCC3 (Activating signal cointegrator 1 complex subunit 3) in a cell-line-specific manner.[116] ASCC3 encodes a 3'-5' DNA helicase that unwinds duplex DNA to generate ssDNA and exposes DNA lesions for the access of ALKBH3-mediated repair. Loss of ALKBH3 or ASCC3 abrogates the cells' tolerance towards DNA damage, which infers their significance upon genome integrity. Interestingly, only one of these two proteins, either ALKBH2 or ALKBH3, functions in a specific cell line to resist alkylation, suggesting a reciprocal nature of these two repair pathways.[116]

Analogous to ALKBH2, ALKBH3 also possesses a flexible hairpin which is proposed to be involved in base flipping and discrimination of single-stranded versus double-stranded substrate (**Figure 7B**).[105,117-118] However, this hairpin is quite hydrophilic with heavily charged amino acids in ALKBH3. Swapping of these two loops leads to the switching of the ssDNA/dsDNA substrate preference of the proteins,[118] which may provide hints for substrate recognition differences. In spite of these findings, a

crystal structure of substrate-bounded ALKBH3 complex is highly desirable to interpret the features required for substrate recognition.

Because ALKBH3 contributes to DNA repair, it not only guards genome integrity in normal cells, but also impacts cancer cell survival and invasion.[119] ALKBH3 is overexpressed in various cancer cells[116] and exhibits a potential role in brain tumor,[108] lung cancer,[120] rectal carcinoma,[121] papillary thyroid cancer,[122] colon carcinogenesis,[110] prostate cancer,[123] pancreatic cancer,[124] and urothelial carcinoma.[125] The understanding of ALKBH3 in the mammalian cells may provide novel therapeutic approaches for the treatment of cancers.

3. RNA demethylases

One of the exciting breakthroughs in recent years is the discovery of RNA demethylation mediated by RNA demethylases FTO and ALKBH5, both are members of the AlkB family proteins.

3.1 FTO

The *FTO* gene was firstly described as one of the six genes deleted in a fused toe (*Ft*) mutant mouse.[126] It was then identified in several genome-wide-association-studies (GWAS) in 2007 to be associated with human fat mass and obesity.[127-129] *Fto* knockout mice showed multiple phenotypes including increased possibility of postnatal lethality, postnatal growth retardation, and reduced fat mass.[130] FTO has the highest expression in brain.[90] It is also suggested to be associated with food intake,[131] development,[132] cancer,[133] and other emerging functions.[134] Mutation of an amino-acid in the conserved active-site abolished the catalytic activity (**Figure 6**), and leads to symptoms such as postnatal growth retardation,

facial dysmorphism, certain brain malformations.[132] FTO was found to be homologous to the AlkB family dioxygenases,[89-90] and possess weak demethylation activity towards m^3T in ssDNA[90] and m^3U in ssRNA.[135] The preference of FTO on ssRNA was supported by its crystal structure, in which an extra loop collapses with the complimentary strand of a potential duplex substrate (**Figure 8**).[136] FTO has an N-terminal AlkB-like domain and a C-terminal domain with novel fold comprised mainly of α-helices.[136] In 2011, our group discovered FTO as the first RNA demethylase that reverses m^6A methylation in mammalian messenger RNA and other nuclear RNAs.[10]

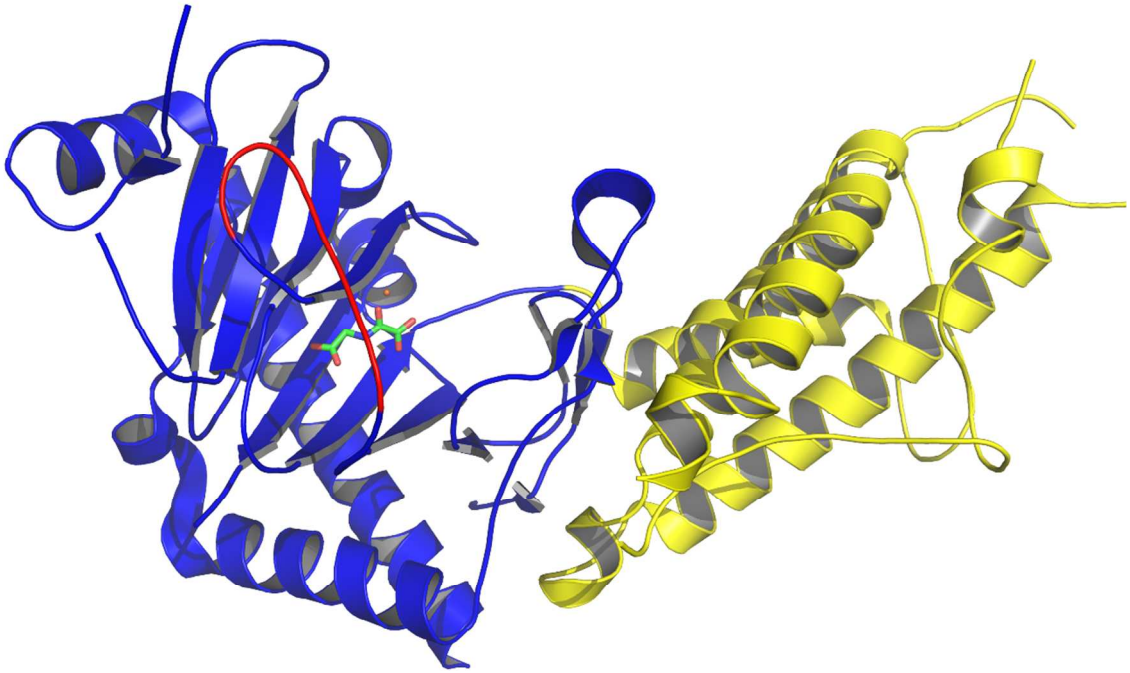

Figure 8. Structure of FTO (3LFM). The N-terminal AlkB-like domain (residues 32-326) and C-terminal domain (residues 327-498) of FTO are colored in blue and yellow, respectively. The extra loop for single-stranded substrate recognition is highlighted in red. Iron(II) is shown in orange. *N*-oxalylglycine (green) was used to substitute α-KG to form a catalytically inert FTO.

m^6A has not been detected in the genomic DNA of higher eukaryotes;[11,137] however, it is the most prevalent internal modification in mRNA for higher eukaryotes at a frequency of about 3-5 sites on average per each mRNA.[8] It is also presented in tRNA, rRNA, small nuclear RNA (snRNA), and long non-coding RNA (lncRNA).[138-140] This methylation on mRNA is installed by mRNA m^6A methyltransferases at a consensus sequence of Pu[G>A]m^6AC[A/C/U].[140-143] Transcriptome-wide profiling of m^6A revealed that this modification is present in all regions of mRNA but are highly enriched around the stop codon region of the mRNA, yet the exact function of this enrichment is currently unclear.[140,142]

FTO forms discrete granules in cell nucleus, and partially co-localizes with nuclear speckles, where mRNA methylation and splicing takes place.[10] This co-localization pattern is enhanced upon transcription inhibition by Actinomycin D, which supports that RNA is a direct substrate of FTO *in vivo*.[10] Recently, through comparing the m^6A-IP (Immunoprecipitation)-enriched mRNA peaks from wild-type and *Fto*$^{-/-}$ mouse brain, several mRNA substrates of Fto, including *Drd3*, *Kcnj6*, and *Grin1*, in Dopaminergic signaling pathway have been identified.[144] The protein levels of these species are lowered with little change of the mRNA level, suggesting a potential effect of m^6A in suppressing mRNA translation.[144]

The demethylation mechanism of FTO was thought to be similar to the AlkB family proteins. However, it was later shown that FTO not only converts m^6A to N^6-hydroxymethyladenosine (hm^6A), but also hm^6A to N^6-formyladenosine (f^6A) in sequential oxidation steps in RNA, albeit with lower efficiency (**Figure 9**).[145] Unlike the oxidation products from demethylation of m^1A and m^3C by AlkB, both hm^6A and f^6A are

relatively stable, and can be detected and characterized *in vitro* and *in vivo*.[145] The increased stability of hm^6A was thought to come from the difference between hydroxymethylation on exocyclic nitrogen in hm^6A and endocyclic nitrogen in other hemiaminal intermediates such as hm^3T. Molecular Dynamics simulation suggests hm^6A fits the active site of FTO very well, similar to m^6A.[145] Both hm^6A and f^6A decompose automatically in water with a half-life of about 3 hrs under physiological conditions, suggesting a possible role of these oxidation products in mRNA *in vivo*, such as modulating the RNA-protein interactions.[145]

As the functions of FTO are mostly dependent on its catalytic activity, small molecular inhibitors of FTO could serve as potential drugs for modulating the function of FTO.[146-147]

3.2 ALKBH5

ALKBH5 is a ubiquitously expressed protein which could be regulated by either protein arginine methyltransferase 7 (PRMT7) upon genotoxic stresses or by hypoxia-inducible factor 1α (HIF-1α) under hypoxia conditions.[148-149] It is mainly localized in nucleus and has been identified as an α-KG-dependent dioxygenase that is able to activate the decarboxylation of α-KG in the presence of iron(II) and ascorbic acid.[148] Photo-crosslinking-based mRNA-bound proteomics profilings have revealed ALKBH5 as a potential mRNA-binding protein.[150-151] Little was known about the biological roles of ALKBH5 until very recently, when this protein has been characterized to be a mammalian RNA demethylase that is capable of removing the methyl group of m^6A from RNA both *in vitro* and *in vivo*.[11]

The recombinant ALKBH5 was shown to efficiently demethylate m^6A-containing nucleic acids, with a preference towards single-stranded substrates. Considering the absence of m^6A in genomic DNA, m^6A on RNA becomes the most likely substrates for ALKBH5 *in vivo*. Indeed, knockdown of ALKBH5 in HeLa cells for 48 hrs results in a ~9% increase of the m^6A level in total mRNA, while overexpression of ALKBH5 for 24 hrs leads to a ~29% decrease of the m^6A level in total mRNA. Therefore, m^6A on mRNA is the primary physiologically-relevant substrate for ALKBH5, although m^6A on other RNA species, such as rRNA and lncRNA, could also serve as potential substrates.[11]

ALKBH5 exhibits a higher demethylation activity towards m^6A-containing consensus sequences over non-consensus sequences.[11] In contrast to FTO, neither hm^6A nor f^6A can be detected during the oxidative demethylation of m^6A by ALKBH5, perhaps due to difference of the protein active sites (**Figure 9**).[152] A structure of ALKBH5 revealing its active site will be valuable to explain its substrate selectivity and catalytic mechanism.

ALKBH5 colocalizes with nuclear speckles which is rich in various mRNA processing factors. ALKBH5 appears to play a broad role in mRNA transport and other RNA metabolism pathways in an m^6A-demethylation-dependent manner. In addition, knockout of the *Alkbh5* gene in mice leads to increased m^6A levels in mRNA isolated from the mouse organs compared with those from wild-type littermates, which provided another strong evidence that m^6A on mRNA is a physiologically-relevant substrate for ALKBH5. *Alkbh5*-deficient mice display impaired male fertility resulting from compromised spermatogenesis, aberrant apoptosis, as well as altered gene expression in the testes[11]. The discoveries of functionally significant ALKBH5 and FTO as RNA

demethylases indicate that the dynamic methylation and demethylation of m^6A on RNA have broad functions in mammals.[11]

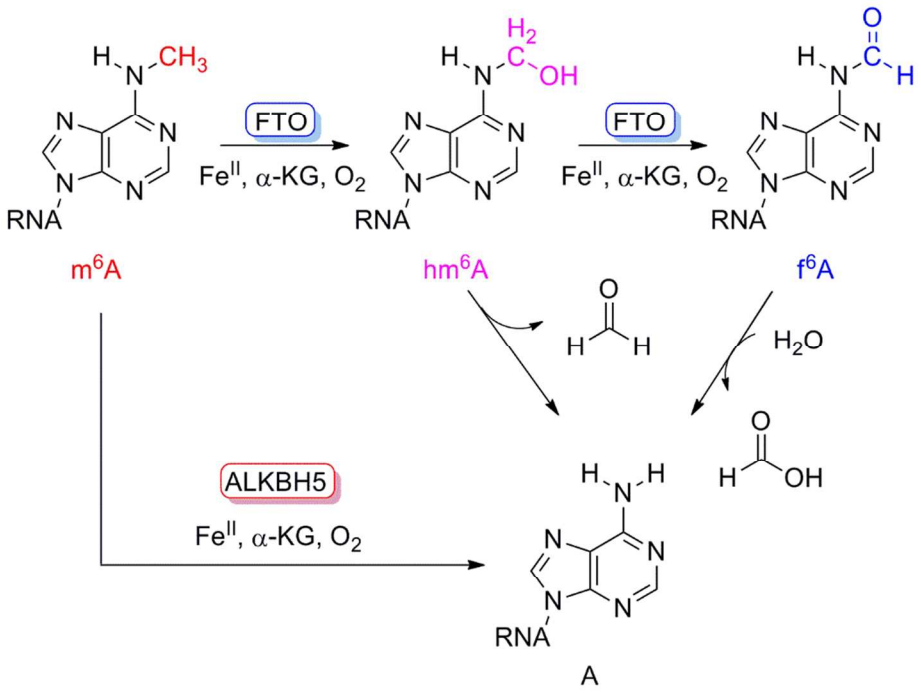

Figure 9. Oxidative demethylation of m^6A in RNA mediated by FTO and ALKBH5 proteins. Note that ALKBH5-mediated oxidation of m^6A should also generate hm^6A as an intermediate, which may decompose in the active site of the protein. In the case of FTO, both hm^6A and f^6A are observed during the oxidation reaction.

4. Other AlkB homologues

In addition to those four AlkB homologues described above, other AlkB homologues have also been shown to catalyze oxidative reactions and play functional roles in biological systems. So far, only ALKBH8 has been conclusively demonstrated as a tRNA hypermodification enzyme. ALKBH6 has no documented function. The

functions of most other homologues are still unclear. Further investigation is required to uncover the enigma of these proteins.

4.1 ALKBH8

ALKBH8 protein is the only tRNA-hypermodification enzyme characterized in the AlkB family. In addition to the AlkB domain, ALKBH8 also possesses an N-terminal RNA-recognition motif (RRM) and a C-terminal Trm9-like methyltransferase domain.[153-156] ALKBH8 has been suggested to contribute to bladder cancer progression by increasing reactive oxygen species production.[157] It is conserved in most multicellular eukaryotes, from plant (*A. thaliana*), worm (*C. elegans*), and insects (*A. mellifera*) to mammals.[156] Knockdown of ALKBH8 produces a fatal defect in cardiac development in *D. melanogaster*,[158] while an internal deletion in the gene encoding ALKBH8 causes embryonic lethality or sterility in animals surviving to adulthood in *C. elegans*.[159] The expression of ALKBH8 protein is also temporally and spatially regulated. It is widely spread in larvae, yet only expressed in a small number of neurons for adults in *C. elegans*.[160]

The crystal structure of the RRM and AlkB domain of ALKBH8 shows that the RRM domain provides a strong but mostly nonspecific binding of RNA, with a basic N-terminal α-helix making critical contributions.[160] The protein loops that interact with the nucleotide substrate are completely disordered, and a disorder-to-order transition is likely responsible for the substrate specificity. The iron(II)-binding site in ALKBH8 is solvent-exposed, which may lead to uncoupled α-KG turnover. However, the basal α-KG oxidation level of ALKBH8 is lower than that of AlkB in the absence of nucleic acid

substrate. This lowered α-KG turnover of ALKBH8 accounts from a catalytically inactive orientation of α-KG and Arg334 in the active site, which require a conformational change upon tRNA binding to be catalytically active.[160]

The Trm9-like methyltransferase domain of ALKBH8 catalyzes the methylation of 5-carboxymethyluridine (cm^5U) to 5-methoxycarbonylmethyluridine (mcm^5U) in tRNAs with UPyN (Py = C/U) as anticodon triplet sequence, while the AlkB domain catalyzes the hydroxylation of mcm^5U to *S*-5-(methoxycarbonylhydroxymethyl)uridine ((*S*)-$mchm^5U$) specifically in $tRNA^{Gly}_{(UCC)}$ (**Figure 10**).[153,155,161] ALKBH8 is the first enzyme found in the AlkB family that mediates hydroxylation instead of demethylation of nucleic acid.[154,162] It uses a similar mechanism as other AlkB family proteins. However, the hydroxylation product is stable because the hydroxyl group is at the 5-α position which is connected to a stable C-C bond instead of a C-N linkage that leads to decomposition in water. The additional hydroxyl group has been suggested to enhance certain codon-codon interactions, and may promote the decoding ability to specific codons.[162] The decoding ability of the total tRNA pool can regulate the translation of individual mRNA depending on the codon bias of the specific mRNA, thus identifying and characterizing genes that are translationally affected by these modifications is an interesting future question to explore.

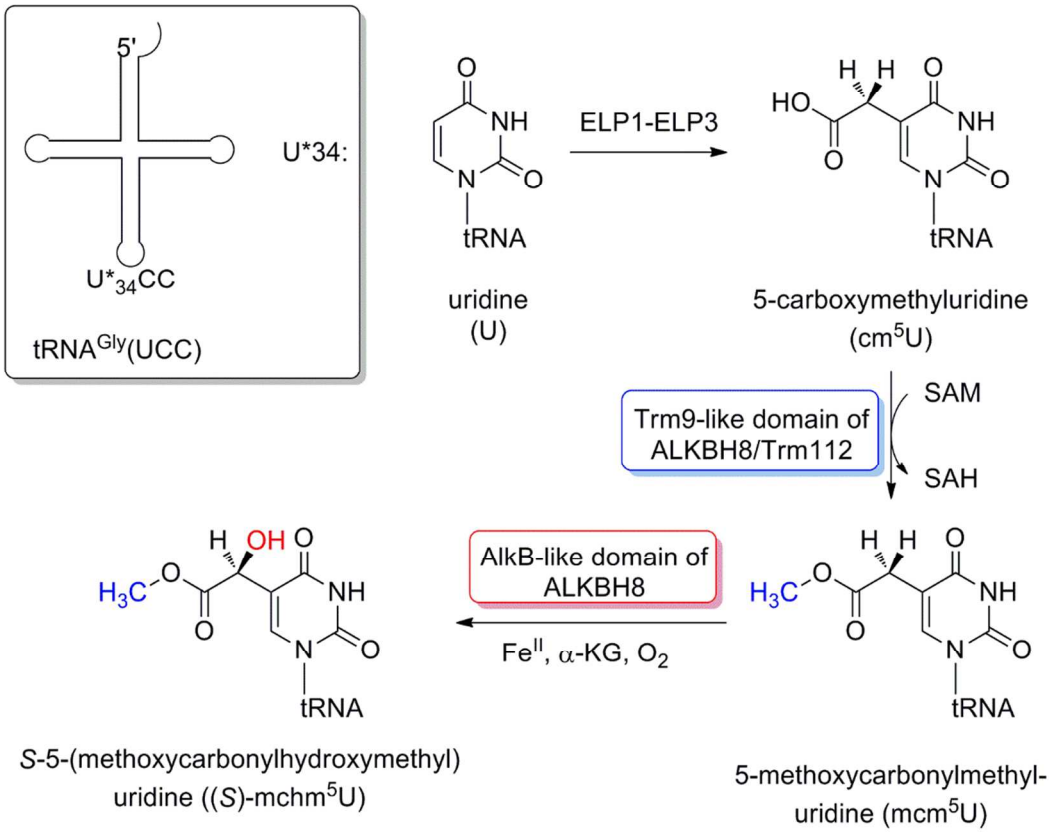

Figure 10. ALKBH8 catalyzes the hypermodification of tRNA wobble uridine. The Trm9-like methyltransferase domain of ALKBH8 catalyzes the methylation of cm^5U to mcm^5U in several tRNAs. And the AlkB-like domain of ALKBH8 catalyzes the hydroxylation of mcm^5U to (*S*)-$mchm^5U$ in $tRNA^{Gly}(UCC)$.

4.2 ALKBH1, 4, and 7

4.2.1 ALKBH1

The first human protein described as a functional AlkB homologue was ALKBH1,[79] which shows the strongest similarity to AlkB,[81] yet potential demethylation activity towards m^1A and m^3C was not detected at first.[66,93] Later, progresses from different groups make this protein a matter of dispute in terms of enzymatic activity as well as biological roles. One line of evidence showed its weak demethylation activity towards m^3C *in vitro*,[163] which was supported by computational modeling,[164] while another line of evidence detected its lyase activity at abasic sites independent of iron(II) or α-KG.[165-166]

Recently, another group demonstrated that ALKBH1 can serve as a histone dioxygenase that acts specifically on histone H2A *in vitro* and *in vivo*.[167] The biological significance of the oxidation functions discovered for ALKBH1 remains unclear. ALKBH1 has been shown to have highest expression level in heart and muscle tissue when analyzed by using Northern blot,[163] yet in spleen when analyzed by using real time PCR and microarray in other studies.[168-169] *Alkbh1*-deficient mice display sex-ratio distortion and impaired differentiation in placental trophoblast lineage and neurons.[167,170-172]

4.2.2 ALKBH4

ALKBH4 has been found to activate the decarboxylation of α-KG, yet it has not been observed to repair nucleic acid substrates so far.[94,173] Yeast two-hybrid screens identified protein partners involved in the interaction with DNA or chromatin, suggesting a function of ALKBH4 in gene regulation.[174] Very recently, ALKBH4 was shown to mediate demethylation of a monomethylated site in actin (K84me1) *in vivo* to regulate actin–myosin interaction and actomyosin-dependent processes such as cytokinesis and cell migration.[175] Such an ALKBH4-mediated regulation of actomyosin dynamics is dependent on the conserved residues of the active site pocket (**Figure 6**), suggesting the involvement of its catalytic activity. Further efforts are needed to biochemically verify this demethylation reaction *in vitro*. While overexpression of ALKBH4 only marginally altered the global gene expression pattern in HEK293 cell line, homozygous *Alkbh4* mutant mice display early embryonic lethality,[174-175] suggesting an essential function of *Alkbh4* during the early developmental process.

4.2.3 ALKBH7

ALKBH7 is a mitochondrial resident protein that does not manifest repair activity towards nucleic acid substrates.[94,176] Human ALKBH7 is required for alkylation and oxidation-induced programmed necrosis by triggering the collapse of mitochondrial membrane potential and large-scale loss of mitochondrial function that lead to energy depletion and cellular demise.[177] Deletion of mouse *Alkbh7* dramatically increased body weight and body fat, indicating its involvement in fatty acid metabolism.[176]

5. TET family dioxygenases

5.1 Active DNA demethylation in mammals

DNA methylation in the form of 5mC is among the best-characterized epigenetic modifications and is essential for genomic imprinting, gene regulation, and development in mammals.[16,178] Methylation patterns are initially established by *de novo* DNA methyltransferases (DNMTs): DNMT3A and DNMT3B,[15,179] then maintained by maintenance methyltransferase: DNMT1, during DNA replication.[180-181] The proper function of DNA methylation requires the dynamic regulation of reciprocal processes. Although the enzymes that catalyze DNA methylation have been well characterized, the demethylation process is complicated and remains elusive.

DNA methylation could be lost at the newly synthesized DNA strand during replication in the absence of DNMT1, termed as passive demethylation. However, such replication-dependent passive demethylation could not explain all of the cellular demthylation events. For example, the male pronucleus has been observed to lose almost all 5mC immediately after fertilization.[182] The genetic materials from the sperm and the

egg have not fused to form one nucleus yet, so that the rapid demethylation at this stage could not result from replication, suggesting the existence of an alternative "active demethylation pathway". Indeed, TET proteins were found to catalyze the sequential oxidation of 5mC to 5hmC, 5fC, and 5caC.[17-20] The resulting derivatives could be removed by thymine DNA glycosylase (TDG) and replaced with unmethylated cytosine through BER.

5.2 TET proteins and mechanism of oxidation

The detection of 5hmC and the studies on thymine hydroxylase have fueled the discovery of the active demethylation by TET proteins. In addition to "the fifth base" of 5mC, 5hmC was found to be highly abundant in the genome of neuron cells and mouse embryonic stem cells (ESC).[17,183] It is known that the methyl group on thymine could be oxidized to its alcohol, aldehyde, and carboxylic acid successively,[184] indicating the possibility of converting 5mC to 5hmC, 5fC, and 5caC through oxidation. While thymine hydroxylase belongs to the family of Fe^{II}- and α-KG-dependent dioxygenases, by using computational analysis, novel homologues of thymine hydroxylases were identified in mammals, including TET family proteins.[185-186]

TET proteins (TET1-3), which were initially identified as a fusion partner of the histone H3K4 methyltransferase MLL (mixed-lineagge leukemia),[187-188] are mammalian homologues of trypanosomal thymine hydroxylase J-binding protein 1 (JBP1) and JBP2.[17] They contain several conserved domains, including a CXXC region that specifically recognizes clustered unmethylated CpG dinucleotides, a cysteine-rich region, and an α-KG dioxygenase DSBH core fold (**Figure 11**).[16,189] The CXXC region binds to

the unmodified cytosine followed by any bases as revealed by biochemical and crystallographic studies, and is thought to target unmethylated C clusters.[190] The other two domains account for the catalytic activity both *in vitro* and *in vivo*.[191] The DSBH fold of TET proteins, featured by all dioxygenases, contains the signature $HXDX_nH$ motif to coordinate iron(II) and a conserved R residue for α-KG binding. A putative Fe^{IV}-oxo species is generated to oxidize the inert C-H bond of 5mC to form 5hmC, 5fC, and 5caC sequentially. Consistent with this mechanism, introducing mutations to the iron-binding sites or adding inhibitors of α-KG dioxygenases to TET proteins would abolish their activity.[17,191-192] The capacity to initiate iterative oxidation towards 5mC, 5hmC, and 5fC suggests a promiscuous substrate-binding site to accommodate all of these substrates. The structure of the catalytic domain has not been reported yet and will be very interesting to dissect the substrate recognition and oxidation mechanism.

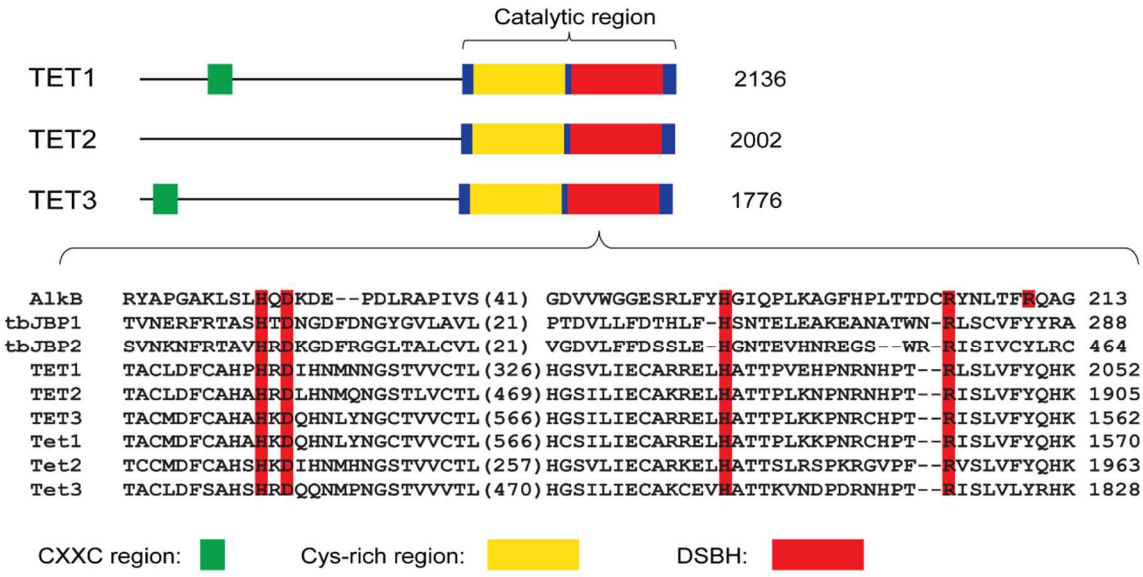

Figure 11. Domain architecture of TET proteins. TET proteins contain a DNA-binding CXXC region in N-terminus and a catalytic core in C-terminus. The catalytic core is composed of a Cys-rich region and a DSBH fold. The number of amino acids for each protein is indicated. Sequence alignment of the catalytic motif is shown. Sequences used in the alignment include AlkB, *Trypanosoma brucei* JBP1 (tbJBP1), tbJBP2, human

TET1-3, and mouse Tet1-3. Conserved iron(II)- and α-KG binding sites are highlighted in red columns.

Although TET1-3 proteins all possess the capacity to oxidize 5mC, their express levels as well as functions vary among cell types and tissues. Mouse Tet1 and Tet2 are highly expressed in ESC cells. They are proposed to regulating pluripotency and lineage differentiation.[189] On the other hand, mTet3 protein is specifically enriched in the paternal pronucleas at the zygotic stage, in agreement with the loss of 5mC and the appearance of 5hmC in the paternal genome,[193-195] suggesting that mTet3 plays a critical role in zygotic epigenetic reprogramming.

5.3 TET-mediated DNA demethylation

In contrast to *N*-demethylation, in which the C-N bond is destabilized because of the hydroxylation, with the oxidized product undergoing hydrolytic deformylation to liberate unmethylated substrate, *C*-demethylation is less likely to occur under ambient conditions due to the stability of C-C bond under physiological conditions. Therefore, all of the oxidative derivatives, 5hmC, 5fC, and 5caC are quite stable under cellular conditions. Although the oxidation of 5mC could function as "demethylation" as the oxidized derivatives would recruit different binding proteins, the complete reversion of methylation requires additional process for the conversion.

In addition to the passive demethylation as cytosine derivatives being diluted during replication, three replication-independent demethylation mechanisms have been proposed following TET-catalyzed 5mC oxidation (**Figure 12**). The first mechanism has been confirmed, in which TDG is recruited to excise 5fC or 5caC to generate an abasic site which is then replaced by cytosine through BER pathway.[18,196-197] In the second

mechanism, 5hmC is proposed to be deaminated to form 5-hydroxyuracil (5-hmU) by AID (activation-induced cytidine deaminase) and APOBEC (apolipoprotein B mRNA editing enzyme, catalytic polypeptide-like) family proteins, then removed by TDG or SMUG1 (single-stranded-selective monofunctional uracil DNA glycosylase 1) and finally repaired by BER.[198] This mechanism is still under debate because purified AID/APOBEC deaminases disfavor modified cytosines *in vitro*.[199] The third mechanism proposes a putative decarboxylase to directly convert 5caC to cytosine, which remains to be revealed.[200]

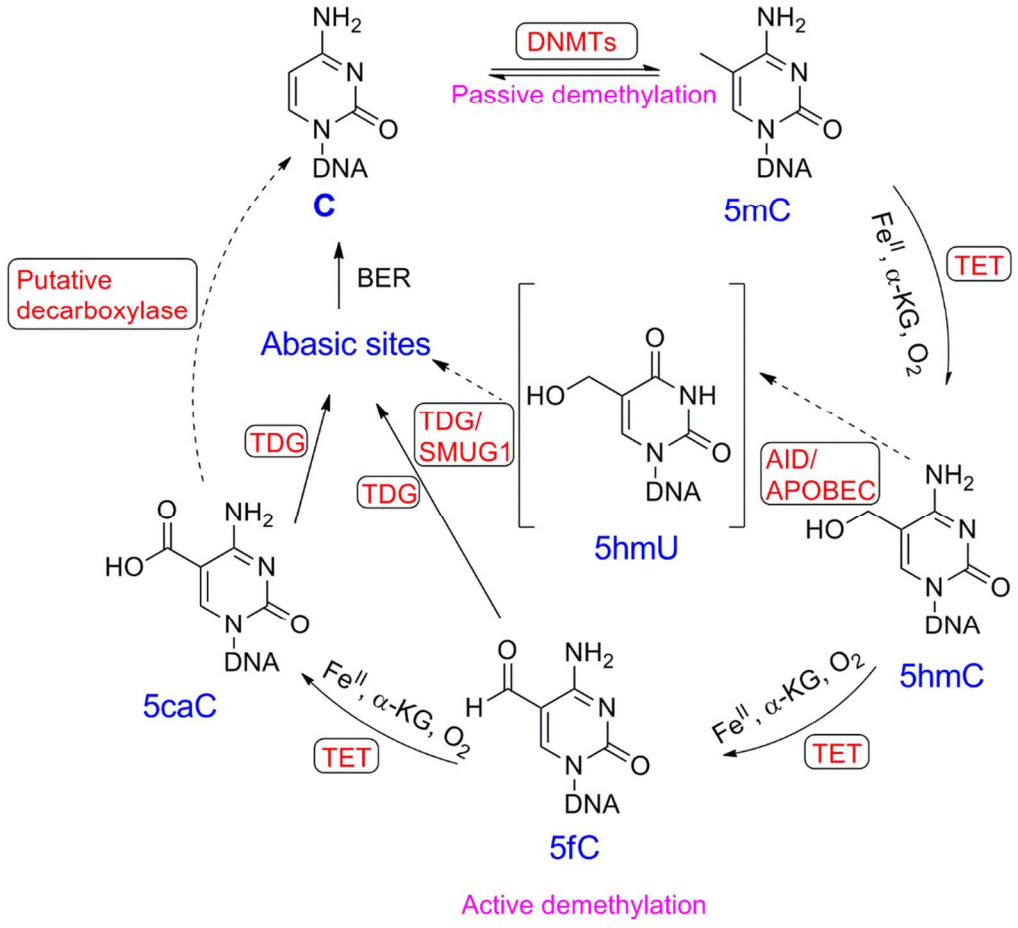

Figure 12. Dynamic regulation of cytosine methylation/demethylation in DNA. DNA 5mC pattern is established and maintained by DNMTs, but can be either passively diluted during replication or actively demethylated through TET-mediated demethylation.

TDG is deeply involved in these demethylation processes. It is a member of the uracil DNA glycosylase (UDG) superfamily that use a base-flipping mechanism to excise damaged base in dsDNA and initiate BER.[201-202] TDG was first shown to remove mismatched pyrimidines from G-U and G-T pairs in dsDNA. Recently, it was identified to recognize TET-oxidized derivatives of 5caC and 5fC, which represents a novel pathway for the TET-mediated active demethylation in mammals.[18,196] In fact, TDG exhibits a slightly higher binding affinity towards G-5fC and G-5caC base pairs than to G-U and G-T mismatches.[196] Crystal structure of the TDG catalytic domain in complex with 5caC-containing dsDNA revealed its preference towards 5caC over other bases.[203] These lines of evidence suggest that instead of DNA repair, DNA demethylation might be the primary function for TDG, supporting its critical roles in transcriptional regulation and mouse embryonic development.[203-204]

5.4 5mC oxidation products 5hmC, 5fC, and 5caC

Although originally identified as TET-mediated oxidation intermediates, 5hmC, 5fC, and 5caC are chemically stable under physiological conditions as discussed above, which enables them to serve as novel epigenetic markers with biological functions. To effectively explore their roles, massive sequencing methods have been developed to profile them genome-wide and with base-resolution.[205]

Increasing evidence suggested that 5hmC could be a unique regulatory marker other than a transient oxidative intermediate.[206-207] 5hmC is not as evenly distributed as 5mC. It is most abundant in ESCs and brain tissues (~1% of total cytosines) with distinct patterns.[19] 5hmC is enriched at distal regulatory elements in ESCs while it is enriched at

the 5mC-depleted gene bodies of neuronal function-related genes.[208-209] Specific 5hmC-binding proteins have been identified, some of which can result in altered chromatin structure and gene expression.[209]

In contrast to 5hmC, 5fC, and 5caC are much less abundant and consistently low among all cells and tissues examined so far,[19] indicating that they are more likely committed as transient demethylation intermediates. In mouse ESCs, the distribution of 5fC and 5caC represents the portion of 5hmC undergoing demethylation, with a preference to distal regulatory elements.[210-211] However, further investigation is required to fully depict these intermediates.

6. Conclusions and perspectives

In this review, we have discussed versatile biological oxidations on nucleic acids mediated by Fe^{II}- and α-KG-dependent dioxygenases, from DNA repair to RNA/DNA demethylation. The discovery of the unique oxidative dealkylation mediated by AlkB in DNA repair opened up this new paradigm. Nine human homologues of AlkB proteins have vividly illustrated how diverse such a mechanism can impact cellular functions and regulations. While some of the homologues have been well studied to uncover the myths of biological pathways, functions of other homologues are still unclear and call for efforts. The discoveries of oxidative demethylation of RNA and DNA methylations have added additional lays of complexity to gene regulation. Identification of specific binding proteins for all of these novel modifications is a future research direction needed to reveal their biological functions. Interest on RNA methylation has been revived owing to the recent identification of RNA m^6A demethylases, yet the RNA m^6A methyltransferase

remains to be fully characterized. High-throughput sequencing methods of base-resolution are particularly urgent to precisely define the RNA methylome. Reversible DNA methylation has attracted and will continue to attract extensive attentions from researchers in broad areas of biology and medicine. The enigma of demethylation events during differentiation and development has yet to be solved. Our knowledge of these oxidation reactions will continue to drive us uncovering biological significance and develop potential therapies taking advantage of some of these proteins.

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8. References

(1) Cantoni, G. L. *J. Am. Chem. Soc.* **1952**, *74*, 2942.
(2) Walport, L. J.; Hopkinson, R. J.; Schofield, C. J. *Curr. Opin. Chem. Biol.* **2012**, *16*, 525.
(3) Drablos, F.; Feyzi, E.; Aas, P. A.; Vaagbo, C. B.; Kavli, B.; Bratlie, M. S.; Pena-Diaz, J.; Otterlei, M.; Slupphaug, G.; Krokan, H. E. *DNA repair* **2004**, *3*, 1389.
(4) Sedgwick, B. *Nat. Rev. Mol. Cell Bio.* **2004**, *5*, 148.
(5) Mishina, Y.; He, C. *J. Inorg. Biochem.* **2006**, *100*, 670.
(6) Trewick, S. C.; Henshaw, T. F.; Hausinger, R. P.; Lindahl, T.; Sedgwick, B. *Nature* **2002**, *419*, 174.
(7) Falnes, P. O.; Johansen, R. F.; Seeberg, E. *Nature* **2002**, *419*, 178.
(8) Grosjean, H. *Fine-tuning of RNA functions by modification and editing* Springer: Berlin ; New York, 2005.
(9) He, C. *Nat. Chem. Biol.* **2010**, *6*, 863.
(10) Jia, G.; Fu, Y.; Zhao, X.; Dai, Q.; Zheng, G.; Yang, Y.; Yi, C.; Lindahl, T.; Pan, T.; Yang, Y. G.; He, C. *Nat. Chem. Biol.* **2011**, *7*, 885.
(11) Zheng, G.; Dahl, J. A.; Niu, Y.; Fedorcsak, P.; Huang, C. M.; Li, C. J.; Vagbo, C. B.; Shi, Y.; Wang, W. L.; Song, S. H.; Lu, Z.; Bosmans, R. P.; Dai, Q.; Hao, Y. J.; Yang, X.; Zhao, W. M.; Tong, W. M.; Wang, X. J.; Bogdan, F.; Furu, K.; Fu, Y.;

Jia, G.; Zhao, X.; Liu, J.; Krokan, H. E.; Klungland, A.; Yang, Y. G.; He, C. *Mol. Cell* **2013**, *49*, 18.
(12) Suzuki, M. M.; Bird, A. *Nat. Rev. Genet.* **2008**, *9*, 465.
(13) Zhu, J. K. *Annu. Rev. Genet.* **2009**, *43*, 143.
(14) Li, E.; Bestor, T. H.; Jaenisch, R. *Cell* **1992**, *69*, 915.
(15) Okano, M.; Bell, D. W.; Haber, D. A.; Li, E. *Cell* **1999**, *99*, 247.
(16) Smith, Z. D.; Meissner, A. *Nat. Rev. Genet.* **2013**, *14*, 204.
(17) Tahiliani, M.; Koh, K. P.; Shen, Y. H.; Pastor, W. A.; Bandukwala, H.; Brudno, Y.; Agarwal, S.; Iyer, L. M.; Liu, D. R.; Aravind, L.; Rao, A. *Science* **2009**, *324*, 930.
(18) He, Y. F.; Li, B. Z.; Li, Z.; Liu, P.; Wang, Y.; Tang, Q. Y.; Ding, J. P.; Jia, Y. Y.; Chen, Z. C.; Li, L.; Sun, Y.; Li, X. X.; Dai, Q.; Song, C. X.; Zhang, K. L.; He, C.; Xu, G. L. *Science* **2011**, *333*, 1303.
(19) Ito, S.; Shen, L.; Dai, Q.; Wu, S. C.; Collins, L. B.; Swenberg, J. A.; He, C.; Zhang, Y. *Science* **2011**, *333*, 1300.
(20) Pfaffeneder, T.; Hackner, B.; Truss, M.; Munzel, M.; Muller, M.; Deiml, C. A.; Hagemeier, C.; Carell, T. *Angew. Chem. Int. Ed.* **2011**, *50*, 7008.
(21) Kataoka, H.; Yamamoto, Y.; Sekiguchi, M. *J. Bacteriol.* **1983**, *153*, 1301.
(22) Schofield, C. J.; Zhang, Z. *Curr. Opin. Struct. Biol.* **1999**, *9*, 722.
(23) Samson, L.; Derfler, B.; Waldstein, E. A. *Proc. Natl. Acad. Sci. U.S.A.* **1986**, *83*, 5607.
(24) Volkert, M. R.; Gately, F. H.; Hajec, L. I. *Mutat. Res.* **1989**, *217*, 109.
(25) Chen, B. J.; Carroll, P.; Samson, L. *J. Bacteriol.* **1994**, *176*, 6255.
(26) Dinglay, S.; Gold, B.; Sedgwick, B. *Mutat. Res.* **1998**, *407*, 109.
(27) Kataoka, H.; Sekiguchi, M. *Mol. genet. genomics* **1985**, *198*, 263.
(28) Kondo, H.; Nakabeppu, Y.; Kataoka, H.; Kuhara, S.; Kawabata, S.; Sekiguchi, M. *J. Biol. Chem.* **1986**, *261*, 15772.
(29) Aravind, L.; Koonin, E. V. *Genome Biol.* **2001**, *2*.
(30) Koivisto, P.; Robins, P.; Lindahl, T.; Sedgwick, B. *J. Biol. Chem.* **2004**, *279*, 40470.
(31) Delaney, J. C.; Essigmann, J. M. *Proc. Natl. Acad. Sci. U.S.A.* **2004**, *101*, 14051.
(32) Sedgwick, B.; Robins, P.; Lindahl, T. *Methods Enzymol.* **2006**, *408*, 108.
(33) Maciejewska, A. M.; Sokolowska, B.; Nowicki, A.; Kusmierek, J. T. *Mutagenesis* **2011**, *26*, 401.
(34) Mishina, Y.; Yang, C. G.; He, C. *J. Am. Chem. Soc.* **2005**, *127*, 14594.
(35) Yu, B.; Hunt, J. F. *Proc. Natl. Acad. Sci. U.S.A.* **2009**, *106*, 14315.
(36) Kang, T. M.; Yuan, J.; Nguyen, A.; Becket, E.; Yang, H.; Miller, J. H. *Antimicrobial agents and chemotherapy* **2012**, *56*, 3216.
(37) Delaney, J. C.; Smeester, L.; Wong, C.; Frick, L. E.; Taghizadeh, K.; Wishnok, J. S.; Drennan, C. L.; Samson, L. D.; Essigmann, J. M. *Nat. Struct. Mol. Biol.* **2005**, *12*, 855.
(38) Kim, M. Y.; Zhou, X.; Delaney, J. C.; Taghizadeh, K.; Dedon, P. C.; Essigmann, J. M.; Wogan, G. N. *Chem. Res. Toxicol.* **2007**, *20*, 1075.
(39) Maciejewska, A. M.; Ruszel, K. P.; Nieminuszczy, J.; Lewicka, J.; Sokolowska, B.; Grzesiuk, E.; Kusmierek, J. T. *Mutat. Res.* **2010**, *684*, 24.

(40) Li, D.; Delaney, J. C.; Page, C. M.; Chen, A. S.; Wong, C.; Drennan, C. L.; Essigmann, J. M. *J. Nucleic Acids* **2010**, *2010*, 369434.
(41) Li, D.; Delaney, J. C.; Page, C. M.; Yang, X.; Chen, A. S.; Wong, C.; Drennan, C. L.; Essigmann, J. M. *J. Am. Chem. Soc.* **2012**, *134*, 8896.
(42) Frick, L. E.; Delaney, J. C.; Wong, C.; Drennan, C. L.; Essigmann, J. M. *Proc. Natl. Acad. Sci. U.S.A.* **2007**, *104*, 755.
(43) Maciejewska, A. M.; Poznanski, J.; Kaczmarska, Z.; Krowisz, B.; Nieminuszczy, J.; Polkowska-Nowakowska, A.; Grzesiuk, E.; Kusmierek, J. T. *J. Biol. Chem.* **2013**, *288*, 432.
(44) Koivisto, P.; Duncan, T.; Lindahl, T.; Sedgwick, B. *J. Biol. Chem.* **2003**, *278*, 44348.
(45) Mishina, Y.; Chen, L. X.; He, C. *J. Am. Chem. Soc.* **2004**, *126*, 16930.
(46) Henshaw, T. F.; Feig, M.; Hausinger, R. P. *J. Inorg. Biochem.* **2004**, *98*, 856.
(47) Liu, H.; Llano, J.; Gauld, J. W. *J. Phys. Chem. B* **2009**, *113*, 4887.
(48) Cisneros, G. A. *Interdiscip. Sci.* **2010**, *2*, 70.
(49) Bleijlevens, B.; Shivarattan, T.; van den Boom, K. S.; de Haan, A.; van der Zwan, G.; Simpson, P. J.; Matthews, S. J. *Biochemistry* **2012**, *51*, 3334.
(50) Que, L., Jr. *Acc. Chem. Res.* **2007**, *40*, 493.
(51) Yu, B.; Edstrom, W. C.; Benach, J.; Hamuro, Y.; Weber, P. C.; Gibney, B. R.; Hunt, J. F. *Nature* **2006**, *439*, 879.
(52) Chen, H.; Costa, M. *Biometals* **2009**, *22*, 191.
(53) Chervona, Y.; Arita, A.; Costa, M. *Metallomics* **2012**, *4*, 619.
(54) Yi, C.; Jia, G.; Hou, G.; Dai, Q.; Zhang, W.; Zheng, G.; Jian, X.; Yang, C. G.; Cui, Q.; He, C. *Nature* **2010**, *468*, 330.
(55) Bleijlevens, B.; Shivarattan, T.; Sedgwick, B.; Rigby, S. E.; Matthews, S. J. *J. Inorg. Biochem.* **2007**, *101*, 1043.
(56) Bleijlevens, B.; Shivarattan, T.; Flashman, E.; Yang, Y.; Simpson, P. J.; Koivisto, P.; Sedgwick, B.; Schofield, C. J.; Matthews, S. J. *EMBO Rep.* **2008**, *9*, 872.
(57) Shivarattan, T.; Chen, H. A.; Simpson, P.; Sedgwick, B.; Matthews, S. *J. Biomol. NMR* **2005**, *33*, 138.
(58) Welford, R. W.; Schlemminger, I.; McNeill, L. A.; Hewitson, K. S.; Schofield, C. J. *J. Biol. Chem.* **2003**, *278*, 10157.
(59) Roy, T. W.; Bhagwat, A. S. *Nucleic Acids Res.* **2007**, *35*, e147.
(60) Karkhanina, A. A.; Mecinovic, J.; Musheev, M. U.; Krylova, S. M.; Petrov, A. P.; Hewitson, K. S.; Flashman, E.; Schofield, C. J.; Krylov, S. N. *Anal. Chem.* **2009**, *81*, 5871.
(61) Krylova, S. M.; Karkhanina, A. A.; Musheev, M. U.; Bagg, E. A. L.; Schofield, C. J.; Krylov, S. N. *Anal. Biochem.* **2011**, *414*, 261.
(62) Woon, E. C. Y.; Demetriades, M.; Bagg, E. A. L.; Aik, W.; Krylova, S. M.; Ma, J. H. Y.; Chan, M. C.; Walport, L. J.; Wegman, D. W.; Dack, K. N.; McDonough, M. A.; Krylov, S. N.; Schofield, C. J. *J. Med. Chem.* **2012**, *55*, 2173.
(63) Krylova, S. M.; Koshkin, V.; Bagg, E.; Schofield, C. J.; Krylov, S. N. *J. Med. Chem.* **2012**, *55*, 3546.
(64) Holland, P. J.; Hollis, T. *PLOS ONE* **2010**, *5*, e8680.
(65) Yi, C.; He, C. *Cold Spring Harb. Perspect. Biol.* **2013**, *5*, a012575.

(66) Aas, P. A.; Otterlei, M.; Falnes, P. O.; Vagbo, C. B.; Skorpen, F.; Akbari, M.; Sundheim, O.; Bjoras, M.; Slupphaug, G.; Seeberg, E.; Krokan, H. E. *Nature* **2003**, *421*, 859.
(67) Falnes, P. O.; Bjoras, M.; Aas, P. A.; Sundheim, O.; Seeberg, E. *Nucleic Acids Res.* **2004**, *32*, 3456.
(68) Dinglay, S.; Trewick, S. C.; Lindahl, T.; Sedgwick, B. *Genes Dev.* **2000**, *14*, 2097.
(69) Yang, H.; Zhan, Y.; Fenn, D.; Chi, L. M.; Lam, S. L. *FEBS Lett.* **2008**, *582*, 1629.
(70) Yang, H.; Lam, S. L. *FEBS Lett.* **2009**, *583*, 1548.
(71) Ougland, R.; Zhang, C. M.; Liiv, A.; Johansen, R. F.; Seeberg, E.; Hou, Y. M.; Remme, J.; Falnes, P. O. *Mol. Cell* **2004**, *16*, 107.
(72) Begley, T. J.; Samson, L. D. *Nature* **2003**, *421*, 795.
(73) Falnes, P. O. *RNA Biol.* **2005**, *2*, 14.
(74) Feyzi, E.; Sundheim, O.; Westbye, M. P.; Aas, P. A.; Vagbo, C. B.; Otterlei, M.; Slupphaug, G.; Krokan, H. E. *Curr. Pharm. Biotechnol.* **2007**, *8*, 326.
(75) Vagbo, C. B.; Svaasand, E. K.; Aas, P. A.; Krokan, H. E. *DNA repair* **2013**, *12*, 188.
(76) Mishina, Y.; Lee, C. H.; He, C. *Nucleic Acids Res.* **2004**, *32*, 1548.
(77) Yang, C. G.; Yi, C. Q.; Duguid, E. M.; Sullivan, C. T.; Jian, X.; Rice, P. A.; He, C. *Nature* **2008**, *452*, 961.
(78) Sundheim, O.; Talstad, V. A.; Vagbo, C. B.; Slupphaug, G.; Krokan, H. E. *DNA repair* **2008**, *7*, 1916.
(79) Wei, Y. F.; Carter, K. C.; Wang, R. P.; Shell, B. K. *Nucleic Acids Res.* **1996**, *24*, 931.
(80) Colombi, D.; Gomes, S. L. *J. Bacteriol.* **1997**, *179*, 3139.
(81) Kurowski, M. A.; Bhagwat, A. S.; Papaj, G.; Bujnicki, J. M. *BMC Genomics* **2003**, *4*, 48.
(82) Bratlie, M. S.; Drablos, F. *BMC Genomics* **2005**, *6*.
(83) van den Born, E.; Omelchenko, M. V.; Bekkelund, A.; Leihne, V.; Koonin, E. V.; Dolja, V. V.; Falnes, P. O. *Nucleic Acids Res.* **2008**, *36*, 5451.
(84) van den Born, E.; Bekkelund, A.; Moen, M. N.; Omelchenko, M. V.; Klungland, A.; Falnes, P. O. *Nucleic Acids Res.* **2009**, *37*, 7124.
(85) Korvald, H.; Moe, A. M. M.; Cederkvist, F. H.; Thiede, B.; Laerdahl, J. K.; Bjoras, M.; Alseth, I. *PLOS ONE* **2011**, *6*.
(86) Mielecki, D.; Zugaj, D. L.; Muszewska, A.; Piwowarski, J.; Chojnacka, A.; Mielecki, M.; Nieminuszczy, J.; Grynberg, M.; Grzesiuk, E. *PLOS ONE* **2012**, *7*.
(87) Simmons, J. M.; Koslowsky, D. J.; Hausinger, R. P. *Exp. Parasitol.* **2012**, *131*, 92.
(88) Korvald, H.; Falnes, P. O.; Laerdahl, J. K.; Bjoras, M.; Alseth, I. *DNA repair* **2012**, *11*, 453.
(89) Sanchez-Pulido, L.; Andrade-Navarro, M. A. *BMC Biochem.* **2007**, *8*, 23.
(90) Gerken, T.; Girard, C. A.; Tung, Y. C. L.; Webby, C. J.; Saudek, V.; Hewitson, K. S.; Yeo, G. S. H.; McDonough, M. A.; Cunliffe, S.; McNeill, L. A.; Galvanovskis, J.; Rorsman, P.; Robins, P.; Prieur, X.; Coll, A. P.; Ma, M.; Jovanovic, Z.; Farooqi, I. S.; Sedgwick, B.; Barroso, I.; Lindahl, T.; Ponting, C. P.; Ashcroft, F. M.; O'Rahilly, S.; Schofield, C. J. *Science* **2007**, *318*, 1469.
(91) Aik, W.; McDonough, M. A.; Thalhammer, A.; Chowdhury, R.; Schofield, C. J. *Curr. Opin. Struct. Biol.* **2012**, *22*, 691.

(92) Sedgwick, B.; Bates, P. A.; Paik, J.; Jacobs, S. C.; Lindahl, T. *DNA repair* **2007**, *6*, 429.
(93) Duncan, T.; Trewick, S. C.; Koivisto, P.; Bates, P. A.; Lindahl, T.; Sedgwick, B. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 16660.
(94) Lee, D. H.; Jin, S. G.; Cai, S.; Chen, Y.; Pfeifer, G. P.; O'Connor, T. R. *J. Biol. Chem.* **2005**, *280*, 39448.
(95) Ringvoll, J.; Moen, M. N.; Nordstrand, L. M.; Meira, L. B.; Pang, B.; Bekkelund, A.; Dedon, P. C.; Bjelland, S.; Samson, L. D.; Falnes, P. O.; Klungland, A. *Cancer Res.* **2008**, *68*, 4142.
(96) Fu, D.; Samson, L. D. *DNA repair* **2012**, *11*, 46.
(97) Ringvoll, J.; Nordstrand, L. M.; Vagbo, C. B.; Talstad, V.; Reite, K.; Aas, P. A.; Lauritzen, K. H.; Liabakk, N. B.; Bjork, A.; Doughty, R. W.; Falnes, P. O.; Krokan, H. E.; Klungland, A. *EMBO J.* **2006**, *25*, 2189.
(98) Nay, S. L.; Lee, D. H.; Bates, S. E.; O'Connor, T. R. *DNA repair* **2012**, *11*, 502.
(99) Meza, T. J.; Moen, M. N.; Vagbo, C. B.; Krokan, H. E.; Klungland, A.; Grini, P. E.; Falnes, P. O. *Nucleic Acids Res.* **2012**, *40*, 6620.
(100) Chen, H.; Giri, N. C.; Zhang, R.; Yamane, K.; Zhang, Y.; Maroney, M.; Costa, M. *J. Biol. Chem.* **2010**, *285*, 7374.
(101) Giri, N. C.; Sun, H.; Chen, H.; Costa, M.; Maroney, M. J. *Biochemistry* **2011**, *50*, 5067.
(102) David, S. S.; O'Shea, V. L.; Kundu, S. *Nature* **2007**, *447*, 941.
(103) Yi, C.; Chen, B.; Qi, B.; Zhang, W.; Jia, G.; Zhang, L.; Li, C. J.; Dinner, A. R.; Yang, C. G.; He, C. *Nat. Struct. Mol. Biol.* **2012**, *19*, 671.
(104) Lu, L.; Yi, C.; Jian, X.; Zheng, G.; He, C. *Nucleic Acids Res.* **2010**, *38*, 4415.
(105) Monsen, V. T.; Sundheim, O.; Aas, P. A.; Westbye, M. P.; Sousa, M. M.; Slupphaug, G.; Krokan, H. E. *Nucleic Acids Res.* **2010**, *38*, 6447.
(106) Pang, X.; Han, K.; Cui, Q. *J. Comput. Chem.* **2013**.
(107) Gao, W.; Li, L. J.; Xu, P.; Fang, J. Y.; Xiao, S. D.; Chen, S. L. *J. Gastroenterol. Hepatol.* **2011**, *26*, 577.
(108) Cetica, V.; Genitori, L.; Giunti, L.; Sanzo, M.; Bernini, G.; Massimino, M.; Sardi, I. *J. Neurooncol.* **2009**, *94*, 195.
(109) Johannessen, T. C. A.; Prestegarden, L.; Grudic, A.; Hegi, M. E.; Tysnes, B. B.; Bjerkvig, R. *Neuro Oncol.* **2013**, *15*, 269.
(110) Calvo, J. A.; Meira, L. B.; Lee, C. Y. I.; Moroski-Erkul, C. A.; Abolhassani, N.; Taghizadeh, K.; Eichinger, L. W.; Muthupalani, S.; Nordstrand, L. M.; Klungland, A.; Samson, L. D. *J. Clin. Invest.* **2012**, *122*, 2680.
(111) Fujii, T.; Shimada, K.; Anai, S.; Fujimoto, K.; Konishi, N. *Cancer Sci.* **2013**, *104*, 321.
(112) Lee, S. Y.; Luk, S. K.; Chuang, C. P.; Yip, S. P.; To, S. S. T.; Yung, Y. M. B. *Br. J. Cancer* **2010**, *103*, 362.
(113) Wu, S. S.; Xu, W.; Liu, S.; Chen, B.; Wang, X. L.; Wang, Y.; Liu, S. F.; Wu, J. Q. *Acta Pharmacol. Sin.* **2011**, *32*, 393.
(114) Gilljam, K. M.; Feyzi, E.; Aas, P. A.; Sousa, M. M. L.; Muller, R.; Vagbo, C. B.; Catterall, T. C.; Liabakk, N. B.; Slupphaug, G.; Drablos, F.; Krokan, H. E.; Otterlei, M. *J. Cell. Biol.* **2009**, *186*, 645.

(115) Sundheim, O.; Vagbo, C. B.; Bjoras, M.; Sousa, M. M.; Talstad, V.; Aas, P. A.; Drablos, F.; Krokan, H. E.; Tainer, J. A.; Slupphaug, G. *EMBO J.* **2006**, *25*, 3389.
(116) Dango, S.; Mosammaparast, N.; Sowa, M. E.; Xiong, L. J.; Wu, F.; Park, K.; Rubin, M.; Gygi, S.; Harper, J. W.; Shi, Y. *Mol. Cell* **2011**, *44*, 373.
(117) Yi, C.; Yang, C. G.; He, C. *Acc. Chem. Res.* **2009**.
(118) Chen, B. E.; Liu, H. C.; Sun, X. X.; Yang, C. G. *Mol. Biosyst.* **2010**, *6*, 2143.
(119) Camps, M.; Eichman, B. F. *Mol. Cell* **2011**, *44*, 343.
(120) Tasaki, M.; Shimada, K.; Kimura, H.; Tsujikawa, K.; Konishi, N. *Br. J. Cancer* **2011**, *104*, 700.
(121) Choi, S. Y.; Jang, J. H.; Kim, K. R. *Clin. Exp. Med.* **2011**, *11*, 219.
(122) Neta, G.; Brenner, A. V.; Sturgis, E. M.; Pfeiffer, R. M.; Hutchinson, A. A.; Aschebrook-Kilfoy, B.; Yeager, M.; Xu, L.; Wheeler, W.; Abend, M.; Ron, E.; Tucker, M. A.; Chanock, S. J.; Sigurdson, A. J. *Carcinogenesis* **2011**, *32*, 1231.
(123) Koike, K.; Ueda, Y.; Hase, H.; Kitae, K.; Fusamae, Y.; Masai, S.; Inagaki, T.; Saigo, Y.; Hirasawa, S.; Nakajima, K.; Ohshio, I.; Makino, Y.; Konishi, N.; Yamamoto, H.; Tsujikawa, K. *Curr. Cancer Drug Targets* **2012**, *12*, 847.
(124) Yamato, I.; Sho, M.; Shimada, K.; Hotta, K.; Ueda, Y.; Yasuda, S.; Shigi, N.; Konishi, N.; Tsujikawa, K.; Nakajima, Y. *Cancer Res.* **2012**, *72*, 4829.
(125) Shimada, K.; Fujii, T.; Tsujikawa, K.; Anai, S.; Fujimoto, K.; Konishi, N. *Clin. Cancer Res.* **2012**, *18*, 5247.
(126) Peters, T.; Ausmeier, K.; Ruther, U. *Mamm. Genome* **1999**, *10*, 983.
(127) Dina, C.; Meyre, D.; Gallina, S.; Durand, E.; Korner, A.; Jacobson, P.; Carlsson, L. M. S.; Kiess, W.; Vatin, V.; Lecoeur, C.; Delplanque, J.; Vaillant, E.; Pattou, F.; Ruiz, J.; Weill, J.; Levy-Marchal, C.; Horber, F.; Potoczna, N.; Hercberg, S.; Le Stunff, C.; Bougneres, P.; Kovacs, P.; Marre, M.; Balkau, B.; Cauchi, S.; Chevre, J. C.; Froguel, P. *Nat. Genet.* **2007**, *39*, 724.
(128) Frayling, T. M.; Timpson, N. J.; Weedon, M. N.; Zeggini, E.; Freathy, R. M.; Lindgren, C. M.; Perry, J. R. B.; Elliott, K. S.; Lango, H.; Rayner, N. W.; Shields, B.; Harries, L. W.; Barrett, J. C.; Ellard, S.; Groves, C. J.; Knight, B.; Patch, A. M.; Ness, A. R.; Ebrahim, S.; Lawlor, D. A.; Ring, S. M.; Ben-Shlomo, Y.; Jarvelin, M. R.; Sovio, U.; Bennett, A. J.; Melzer, D.; Ferrucci, L.; Loos, R. J. F.; Barroso, I.; Wareham, N. J.; Karpe, F.; Owen, K. R.; Cardon, L. R.; Walker, M.; Hitman, G. A.; Palmer, C. N. A.; Doney, A. S. F.; Morris, A. D.; Smith, G. D.; Hattersley, A. T.; McCarthy, M. I.; Control, W. T. C. *Science* **2007**, *316*, 889.
(129) Scott, L. J.; Mohlke, K. L.; Bonnycastle, L. L.; Willer, C. J.; Li, Y.; Duren, W. L.; Erdos, M. R.; Stringham, H. M.; Chines, P. S.; Jackson, A. U.; Prokunina-Olsson, L.; Ding, C. J.; Swift, A. J.; Narisu, N.; Hu, T.; Pruim, R.; Xiao, R.; Li, X. Y.; Conneely, K. N.; Riebow, N. L.; Sprau, A. G.; Tong, M.; White, P. P.; Hetrick, K. N.; Barnhart, M. W.; Bark, C. W.; Goldstein, J. L.; Watkins, L.; Xiang, F.; Saramies, J.; Buchanan, T. A.; Watanabe, R. M.; Valle, T. T.; Kinnunen, L.; Abecasis, G. R.; Pugh, E. W.; Doheny, K. F.; Bergman, R. N.; Tuomilehto, J.; Collins, F. S.; Boehnke, M. *Science* **2007**, *316*, 1341.
(130) Fischer, J.; Koch, L.; Emmerling, C.; Vierkotten, J.; Peters, T.; Bruning, J. C.; Rüther, U. *Nature* **2009**, *458*, 894.

(131) Church, C.; Moir, L.; McMurray, F.; Girard, C.; Banks, G. T.; Teboul, L.; Wells, S.; Bruning, J. C.; Nolan, P. M.; Ashcroft, F. M.; Cox, R. D. *Nat. Genet.* **2010**, *42*, 1086.
(132) Boissel, S.; Reish, O.; Proulx, K.; Kawagoe-Takaki, H.; Sedgwick, B.; Yeo, G. S.; Meyre, D.; Golzio, C.; Molinari, F.; Kadhom, N.; Etchevers, H. C.; Saudek, V.; Farooqi, I. S.; Froguel, P.; Lindahl, T.; O'Rahilly, S.; Munnich, A.; Colleaux, L. *Am. J. Hum. Genet.* **2009**, *85*, 106.
(133) Iles, M. M.; Law, M. H.; Stacey, S. N.; Han, J.; Fang, S.; Pfeiffer, R.; Harland, M.; Macgregor, S.; Taylor, J. C.; Aben, K. K.; Akslen, L. A.; Avril, M. F.; Azizi, E.; Bakker, B.; Benediktsdottir, K. R.; Bergman, W.; Scarra, G. B.; Brown, K. M.; Calista, D.; Chaudru, V.; Fargnoli, M. C.; Cust, A. E.; Demenais, F.; de Waal, A. C.; Debniak, T.; Elder, D. E.; Friedman, E.; Galan, P.; Ghiorzo, P.; Gillanders, E. M.; Goldstein, A. M.; Gruis, N. A.; Hansson, J.; Helsing, P.; Hocevar, M.; Hoiom, V.; Hopper, J. L.; Ingvar, C.; Janssen, M.; Jenkins, M. A.; Kanetsky, P. A.; Kiemeney, L. A.; Lang, J.; Lathrop, G. M.; Leachman, S.; Lee, J. E.; Lubinski, J.; Mackie, R. M.; Mann, G. J.; Martin, N. G.; Mayordomo, J. I.; Molven, A.; Mulder, S.; Nagore, E.; Novakovic, S.; Okamoto, I.; Olafsson, J. H.; Olsson, H.; Pehamberger, H.; Peris, K.; Grasa, M. P.; Planelles, D.; Puig, S.; Puig-Butille, J. A.; Randerson-Moor, J.; Requena, C.; Rivoltini, L.; Rodolfo, M.; Santinami, M.; Sigurgeirsson, B.; Snowden, H.; Song, F.; Sulem, P.; Thorisdottir, K.; Tuominen, R.; Van Belle, P.; van der Stoep, N.; van Rossum, M. M.; Wei, Q.; Wendt, J.; Zelenika, D.; Zhang, M.; Landi, M. T.; Thorleifsson, G.; Bishop, D. T.; Amos, C. I.; Hayward, N. K.; Stefansson, K.; Bishop, J. A.; Barrett, J. H.; Geno, M. E. L. C.; Q, M.; Investigators, A. *Nat. Genet.* **2013**, *45*, 428.
(134) Muller, T. D.; Tschop, M. H.; Hofmann, S. *PloS Genet.* **2013**, *9*.
(135) Jia, G. F.; Yang, C. G.; Yang, S. D.; Jian, X.; Yi, C. Q.; Zhou, Z. Q.; He, C. *FEBS Lett.* **2008**, *582*, 3313.
(136) Han, Z. F.; Niu, T. H.; Chang, J. B.; Lei, X. G.; Zhao, M. Y.; Wang, Q.; Cheng, W.; Wang, J. J.; Feng, Y.; Chai, J. J. *Nature* **2010**, *464*, 1205.
(137) Ratel, D.; Ravanat, J. L.; Berger, F.; Wion, D. *Bioessays* **2006**, *28*, 309.
(138) Saneyoshi, M.; Harada, F.; Nishimura, S. *Biochim. Biophys. Acta* **1969**, *190*, 264.
(139) Iwanami, Y.; Brown, G. *Arch. Biochem. Biophys.* **1968**, *126*, 8
(140) Meyer, K. D.; Saletore, Y.; Zumbo, P.; Elemento, O.; Mason, C. E.; Jaffrey, S. R. *Cell* **2012**, *149*, 1635.
(141) Bodi, Z.; Button, J. D.; Grierson, D.; Fray, R. G. *Nucleic Acids Res.* **2010**, *38*, 5327.
(142) Dominissini, D.; Moshitch-Moshkovitz, S.; Schwartz, S.; Salmon-Divon, M.; Ungar, L.; Osenberg, S.; Cesarkas, K.; Jacob-Hirsch, J.; Amariglio, N.; Kupiec, M.; Sorek, R.; Rechavi, G. *Nature* **2012**, *485*, 201.
(143) Harper, J. E.; Miceli, S. M.; Roberts, R. J.; Manley, J. L. *Nucleic Acids Res.* **1990**, *18*, 5735.
(144) Hess, M. E.; Hess, S.; Meyer, K. D.; Verhagen, L. A.; Koch, L.; Bronneke, H. S.; Dietrich, M. O.; Jordan, S. D.; Saletore, Y.; Elemento, O.; Belgardt, B. F.; Franz, T.; Horvath, T. L.; Ruther, U.; Jaffrey, S. R.; Kloppenburg, P.; Bruning, J. C. *Nat. Neurosci.* **2013**.

(145) Fu, Y.; Jia, G. F.; Pang, X. Q.; Wang, R. N.; Wang, X.; Li, C. J.; Smemo, S.; Dai, Q.; Bailey, K. A.; Nobrega, M. A.; Han, K. L.; Cui, Q.; He, C. *Nat. Commun.* **2013**, *4*.
(146) Chen, B.; Ye, F.; Yu, L.; Jia, G.; Huang, X.; Zhang, X.; Peng, S.; Chen, K.; Wang, M.; Gong, S.; Zhang, R.; Yin, J.; Li, H.; Yang, Y.; Liu, H.; Zhang, J.; Zhang, H.; Zhang, A.; Jiang, H.; Luo, C.; Yang, C. G. *J. Am. Chem. Soc.* **2012**, *134*, 17963.
(147) Aik, W.; Demetriades, M.; Hamdan, M. K.; Bagg, E. A.; Yeoh, K. K.; Lejeune, C.; Zhang, Z.; McDonough, M. A.; Schofield, C. J. *J. Med. Chem.* **2013**, *56*, 3680.
(148) Thalhammer, A.; Bencokova, Z.; Poole, R.; Loenarz, C.; Adam, J.; O'Flaherty, L.; Schodel, J.; Mole, D.; Giaslakiotis, K.; Schofield, C. J.; Hammond, E. M.; Ratcliffe, P. J.; Pollard, P. J. *PLOS ONE* **2011**, *6*, e16210.
(149) Karkhanis, V.; Wang, L.; Tae, S.; Hu, Y. J.; Imbalzano, A. N.; Sif, S. *J. Biol. Chem.* **2012**, *287*, 29801.
(150) Castello, A.; Fischer, B.; Eichelbaum, K.; Horos, R.; Beckmann, B. M.; Strein, C.; Davey, N. E.; Humphreys, D. T.; Preiss, T.; Steinmetz, L. M.; Krijgsveld, J.; Hentze, M. W. *Cell* **2012**, *149*, 1393.
(151) Baltz, A. G.; Munschauer, M.; Schwanhausser, B.; Vasile, A.; Murakawa, Y.; Schueler, M.; Youngs, N.; Penfold-Brown, D.; Drew, K.; Milek, M.; Wyler, E.; Bonneau, R.; Selbach, M.; Dieterich, C.; Landthaler, M. *Mol. Cell* **2012**, *46*, 674.
(152) Zheng, G.; Dahl, J. A.; Niu, Y.; Fu, Y.; Klungland, A.; Yang, Y. G.; He, C. *RNA Biol.* **2013**, *10*.
(153) Fu, D.; Brophy, J. A. N.; Chan, C. T. Y.; Atmore, K. A.; Begley, U.; Paules, R. S.; Dedon, P. C.; Begley, T. J.; Samson, L. D. *Mol. Cell. Biol.* **2010**, *30*, 2449.
(154) Fu, Y.; Dai, Q.; Zhang, W.; Ren, J.; Pan, T.; He, C. *Angew. Chem. Int. Ed.* **2010**, *49*, 8885.
(155) Songe-Moller, L.; van den Born, E.; Leihne, V.; Vagbo, C. B.; Kristoffersen, T.; Krokan, H. E.; Kirpekar, F.; Falnes, P. O.; Klungland, A. *Mol. Cell. Biol.* **2010**, *30*, 1814.
(156) Leihne, V.; Kirpekar, F.; Vagbo, C. B.; van den Born, E.; Krokan, H. E.; Grini, P. E.; Meza, T. J.; Falnes, P. O. *Nucleic Acids Res.* **2011**, *39*, 7688.
(157) Shimada, K.; Nakamura, M.; Anai, S.; De Velasco, M.; Tanaka, M.; Tsujikawa, K.; Ouji, Y.; Konishi, N. *Cancer Res.* **2009**, *69*, 3157.
(158) Kim, Y. O.; Park, S. J.; Balaban, R. S.; Nirenberg, M.; Kim, Y. *Proc. Natl. Acad. Sci. U.S.A.* **2004**, *101*, 159.
(159) Byrne, A. B.; Weirauch, M. T.; Wong, V.; Koeva, M.; Dixon, S. J.; Stuart, J. M.; Roy, P. J. *J. Biol.* **2007**, *6*, 8.
(160) Pastore, C.; Topalidou, I.; Forouhar, F.; Yan, A. C.; Levy, M.; Hunt, J. F. *J. Biol. Chem.* **2012**, *287*, 2130.
(161) Begley, U.; Dyavaiah, M.; Patil, A.; Rooney, J. P.; DiRenzo, D.; Young, C. M.; Conklin, D. S.; Zitomer, R. S.; Begley, T. J. *Mol. Cell* **2007**, *28*, 860.
(162) van den Born, E.; Vågbø, C. B.; Songe-Møller, L.; Leihne, V.; Lien, G. F.; Leszczynska, G.; Malkiewicz, A.; Krokan, H. E.; Kirpekar, F.; Klungland, A.; Falnes, P. Ø. *Nat. Commun.* **2011**, *2*, 172.
(163) Westbye, M. P.; Feyzi, E.; Aas, P. A.; Vagbo, C. B.; Talstad, V. A.; Kavli, B.; Hagen, L.; Sundheim, O.; Akbari, M.; Liabakk, N. B.; Slupphaug, G.; Otterlei, M.; Krokan, H. E. *J. Biol. Chem.* **2008**, *283*, 25046.

(164) He, C. Q.; Ma, L. Y.; Wang, D.; Li, G. R.; Ding, N. Z. *Virology* **2009**, *384*, 51.
(165) Muller, T. A.; Meek, K.; Hausinger, R. P. *DNA repair* **2010**, *9*, 58.
(166) Muller, T. A.; Andrzejak, M. M.; Hausinger, R. P. *Biochem. J.* **2013**.
(167) Ougland, R.; Lando, D.; Jonson, I.; Dahl, J. A.; Moen, M. N.; Nordstrand, L. M.; Rognes, T.; Lee, J. T.; Klungland, A.; Kouzarides, T.; Larsen, E. *Stem Cells* **2012**, *30*, 2672.
(168) Su, A. I.; Cooke, M. P.; Ching, K. A.; Hakak, Y.; Walker, J. R.; Wiltshire, T.; Orth, A. P.; Vega, R. G.; Sapinoso, L. M.; Moqrich, A.; Patapoutian, A.; Hampton, G. M.; Schultz, P. G.; Hogenesch, J. B. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 4465.
(169) Tsujikawa, K.; Koike, K.; Kitae, K.; Shinkawa, A.; Arima, H.; Suzuki, T.; Tsuchiya, M.; Makino, Y.; Furukawa, T.; Konishi, N.; Yamamoto, H. *J. Cell. Mol. Med.* **2007**, *11*, 1105.
(170) Pan, Z. S.; Sikandar, S.; Witherspoon, M.; Dizon, D.; Nguyen, T.; Benirschke, K.; Wiley, C.; Vrana, P.; Lipkin, S. M. *Dev. Dynam.* **2008**, *237*, 316.
(171) Nordstrand, L. M.; Svard, J.; Larsen, E.; Nilsen, A.; Ougland, R.; Furu, K.; Lien, G. F.; Rognes, T.; Namekawa, S. H.; Lee, J. T.; Klungland, A. *PLOS ONE* **2010**, *5*.
(172) Nordstrand, L. M.; Furu, K.; Paulsen, J.; Rognes, T.; Klungland, A. *Nucleic Acids Res.* **2012**, *40*, 10950.
(173) Bjornstad, L. G.; Zoppellaro, G.; Tomter, A. B.; Falnes, P. O.; Andersson, K. K. *Biochem. J.* **2011**, *434*, 391.
(174) Bjornstad, L. G.; Meza, T. J.; Otterlei, M.; Olafsrud, S. M.; Meza-Zepeda, L. A.; Falnes, P. O. *PLOS ONE* **2012**, *7*.
(175) Li, M. M.; Nilsen, A.; Shi, Y.; Fusser, M.; Ding, Y. H.; Fu, Y.; Liu, B.; Niu, Y.; Wu, Y. S.; Huang, C. M.; Olofsson, M.; Jin, K. X.; Lv, Y.; Xu, X. Z.; He, C.; Dong, M. Q.; Rendtlew Danielsen, J. M.; Klungland, A.; Yang, Y. G. *Nat. Commun.* **2013**, *4*, 1832.
(176) Solberg, A.; Robertson, A. B.; Aronsen, J. M.; Rognmo, O.; Sjaastad, I.; Wisloff, U.; Klungland, A. *J. Mol. Cell Biol.* **2013**, *5*, 194.
(177) Fu, D.; Jordan, J. J.; Samson, L. D. *Genes Dev.* **2013**, *27*, 1089.
(178) Jones, P. A. *Nat. Rev. Genet.* **2012**, *13*, 484.
(179) Okano, M.; Xie, S.; Li, E. *Nat. Genet.* **1998**, *19*, 219.
(180) Bestor, T.; Laudano, A.; Mattaliano, R.; Ingram, V. *J. Mol. Biol.* **1988**, *203*, 971.
(181) Hermann, A.; Goyal, R.; Jeltsch, A. *J. Biol. Chem.* **2004**, *279*, 48350.
(182) Mayer, W.; Niveleau, A.; Walter, J.; Fundele, R.; Haaf, T. *Nature* **2000**, *403*, 501.
(183) Kriaucionis, S.; Heintz, N. *Science* **2009**, *324*, 929.
(184) Thornburg, L. D.; Lai, M. T.; Wishnok, J. S.; Stubbe, J. *Biochemistry* **1993**, *32*, 14023.
(185) Iyer, L. M.; Anantharaman, V.; Wolf, M. Y.; Aravind, L. *Int. J. Parasitol.* **2008**, *38*, 1.
(186) Iyer, L. M.; Tahiliani, M.; Rao, A.; Aravind, L. *Cell Cycle* **2009**, *8*, 1698.
(187) Ono, R.; Taki, T.; Taketani, T.; Taniwaki, M.; Kobayashi, H.; Hayashi, Y. *Cancer Res.* **2002**, *62*, 4075.
(188) Lorsbach, R. B.; Moore, J.; Mathew, S.; Raimondi, S. C.; Mukatira, S. T.; Downing, J. R. *Leukemia* **2003**, *17*, 637.

(189) Wu, H.; Zhang, Y. *Genes Dev.* **2011**, *25*, 2436.
(190) Xu, Y.; Xu, C.; Kato, A.; Tempel, W.; Abreu, J. G.; Bian, C.; Hu, Y.; Hu, D.; Zhao, B.; Cerovina, T.; Diao, J.; Wu, F.; He, H. H.; Cui, Q.; Clark, E.; Ma, C.; Barbara, A.; Veenstra, G. J.; Xu, G.; Kaiser, U. B.; Liu, X. S.; Sugrue, S. P.; He, X.; Min, J.; Kato, Y.; Shi, Y. G. *Cell* **2012**, *151*, 1200.
(191) Ito, S.; D'Alessio, A. C.; Taranova, O. V.; Hong, K.; Sowers, L. C.; Zhang, Y. *Nature* **2010**, *466*, 1129.
(192) Xu, W.; Yang, H.; Liu, Y.; Yang, Y.; Wang, P.; Kim, S. H.; Ito, S.; Yang, C.; Wang, P.; Xiao, M. T.; Liu, L. X.; Jiang, W. Q.; Liu, J.; Zhang, J. Y.; Wang, B.; Frye, S.; Zhang, Y.; Xu, Y. H.; Lei, Q. Y.; Guan, K. L.; Zhao, S. M.; Xiong, Y. *Cancer Cell* **2011**, *19*, 17.
(193) Gu, T. P.; Guo, F.; Yang, H.; Wu, H. P.; Xu, G. F.; Liu, W.; Xie, Z. G.; Shi, L.; He, X.; Jin, S. G.; Iqbal, K.; Shi, Y. G.; Deng, Z.; Szabo, P. E.; Pfeifer, G. P.; Li, J.; Xu, G. L. *Nature* **2011**, *477*, 606.
(194) Iqbal, K.; Jin, S. G.; Pfeifer, G. P.; Szabo, P. E. *Proc. Natl. Acad. Sci. U.S.A.* **2011**, *108*, 3642.
(195) Wossidlo, M.; Nakamura, T.; Lepikhov, K.; Marques, C. J.; Zakhartchenko, V.; Boiani, M.; Arand, J.; Nakano, T.; Reik, W.; Walter, J. *Nat. Commun.* **2011**, *2*, 241.
(196) Maiti, A.; Drohat, A. C. *J. Biol. Chem.* **2011**, *286*, 35334.
(197) Zhang, L.; Lu, X. Y.; Lu, J. Y.; Liang, H. H.; Dai, Q.; Xu, G. L.; Luo, C.; Jiang, H. L.; He, C. *Nat. Chem. Biol.* **2012**, *8*, 328.
(198) Guo, J. U.; Su, Y.; Zhong, C.; Ming, G. L.; Song, H. *Cell* **2011**, *145*, 423.
(199) Nabel, C. S.; Jia, H.; Ye, Y.; Shen, L.; Goldschmidt, H. L.; Stivers, J. T.; Zhang, Y.; Kohli, R. M. *Nat. Chem. Biol.* **2012**, *8*, 751.
(200) Schiesser, S.; Hackner, B.; Pfaffeneder, T.; Muller, M.; Hagemeier, C.; Truss, M.; Carell, T. *Angew. Chem. Int. Ed.* **2012**, *51*, 6516.
(201) Lindahl, T. *Nature* **1993**, *362*, 709.
(202) Stivers, J. T.; Jiang, Y. L. *Chem. Rev.* **2003**, *103*, 2729.
(203) Cortazar, D.; Kunz, C.; Selfridge, J.; Lettieri, T.; Saito, Y.; MacDougall, E.; Wirz, A.; Schuermann, D.; Jacobs, A. L.; Siegrist, F.; Steinacher, R.; Jiricny, J.; Bird, A.; Schar, P. *Nature* **2011**, *470*, 419.
(204) Cortellino, S.; Xu, J. F.; Sannai, M.; Moore, R.; Caretti, E.; Cigliano, A.; Le Coz, M.; Devarajan, K.; Wessels, A.; Soprano, D.; Abramowitz, L. K.; Bartolomei, M. S.; Rambow, F.; Bassi, M. R.; Bruno, T.; Fanciulli, M.; Renner, C.; Klein-Szanto, A. J.; Matsumoto, Y.; Kobi, D.; Davidson, I.; Alberti, C.; Larue, L.; Bellacosa, A. *Cell* **2011**, *146*, 67.
(205) Song, C. X.; Yi, C.; He, C. *Nat. Biotechnol.* **2012**, *30*, 1107.
(206) Koh, K. P.; Rao, A. *Curr. Opin. Cell Biol.* **2013**, *25*, 152.
(207) Shen, L.; Zhang, Y. *Curr. Opin. Cell Biol.* **2013**, *25*, 289.
(208) Yu, M.; Hon, G. C.; Szulwach, K. E.; Song, C. X.; Zhang, L.; Kim, A.; Li, X.; Dai, Q.; Shen, Y.; Park, B.; Min, J. H.; Jin, P.; Ren, B.; He, C. *Cell* **2012**, *149*, 1368.
(209) Mellen, M.; Ayata, P.; Dewell, S.; Kriaucionis, S.; Heintz, N. *Cell* **2012**, *151*, 1417.

(210) Song, C. X.; Szulwach, Keith E.; Dai, Q.; Fu, Y.; Mao, S.-Q.; Lin, L.; Street, C.; Li, Y.; Poidevin, M.; Wu, H.; Gao, J.; Liu, P.; Li, L.; Xu, G.-L.; Jin, P.; He, C. *Cell* **2013**, *153*, 678.
(211) Shen, L.; Wu, H.; Diep, D.; Yamaguchi, S.; D'Alessio, A. C.; Fung, H. L.; Zhang, K.; Zhang, Y. *Cell* **2013**, *153*, 692.