



FORUM REVIEW ARTICLE

Base Excision Repair Facilitates a Functional Relationship Between Guanine Oxidation and Histone Demethylation

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Abstract

Significance: Appropriately controlled epigenetic regulation is critical for the normal development and health of an organism. Misregulation of epigenetic control *via* deoxyribonucleic acid (DNA) methylation or histone methylation has been associated with cancer and chromosomal instability syndromes. **Recent Advances:** The main function of the proteins in the base excision repair (BER) pathway is to repair DNA single-strand breaks and deamination, oxidation, and alkylation-induced DNA base damage that may result from chemotherapy, environmental exposure, or byproducts of cellular metabolism. Recent studies have suggested that one or more BER proteins may also participate in epigenetic regulation to facilitate gene expression modulation *via* alteration of the state of DNA methylation or *via* a reaction coupled to histone modification. BER proteins have also been reported to play an essential role in pluripotent stem cell reprogramming. **Critical Issues:** One emerging function for BER in epigenetic regulation is the repair of base lesions induced by hydrogen peroxide as a byproduct of lysine-specific demethylase 1 (LSD1) enzymatic activity (LSD1/LSD2-coupled BER) for transcriptional regulation. **Future Directions:** To shed light on this novel role of BER, this review focuses on the repair of oxidative lesions in nuclear DNA that are induced during LSD1-mediated histone demethylation. Further, we highlight current studies suggesting a role for BER proteins in transcriptional regulation of gene expression *via* BER-coupled active DNA demethylation in mammalian cells. Such efforts to address the role of BER proteins in epigenetic regulation could broaden cancer therapeutic strategies to include epigenetic modifiers combined with BER inhibitors. *Antioxid. Redox Signal.* 18, 2429–2443.

Introduction

THE PROTEINS OF THE base excision repair (BER) pathway participate in the repair of dozens of base modifications that result from oxidative deoxyribonucleic acid (DNA) damage (88). Such damage can arise from numerous exogenous and endogenous sources, resulting in a multitude of detrimental cellular effects, including mutations, genome rearrangements, altered gene expression, and the onset of cell death or senescence (9,49,50). As depicted in Figure 1 and extensively reviewed elsewhere (88), many proteins are involved in the BER pathway to remove the base lesions and complete repair. Briefly, there are 11 human DNA glycosylases that remove the induced base lesions and thereby function to initiate BER. Once the base lesions are removed,

Innovation

Proteins of the base excision repair (BER) pathway help maintain the stability of the genome by repairing deoxyribonucleic acid (DNA) base damage and DNA single-strand breaks that may arise from oxidative stress and cellular metabolism. Recent studies have suggested that one or more BER proteins may also play a role in epigenetic regulation of gene expression. To shed light on this novel role of BER, this review focuses on the repair of oxidative lesions in nuclear DNA that are induced during histone demethylation. Further, we highlight current studies suggesting a role for BER proteins in DNA demethylation.

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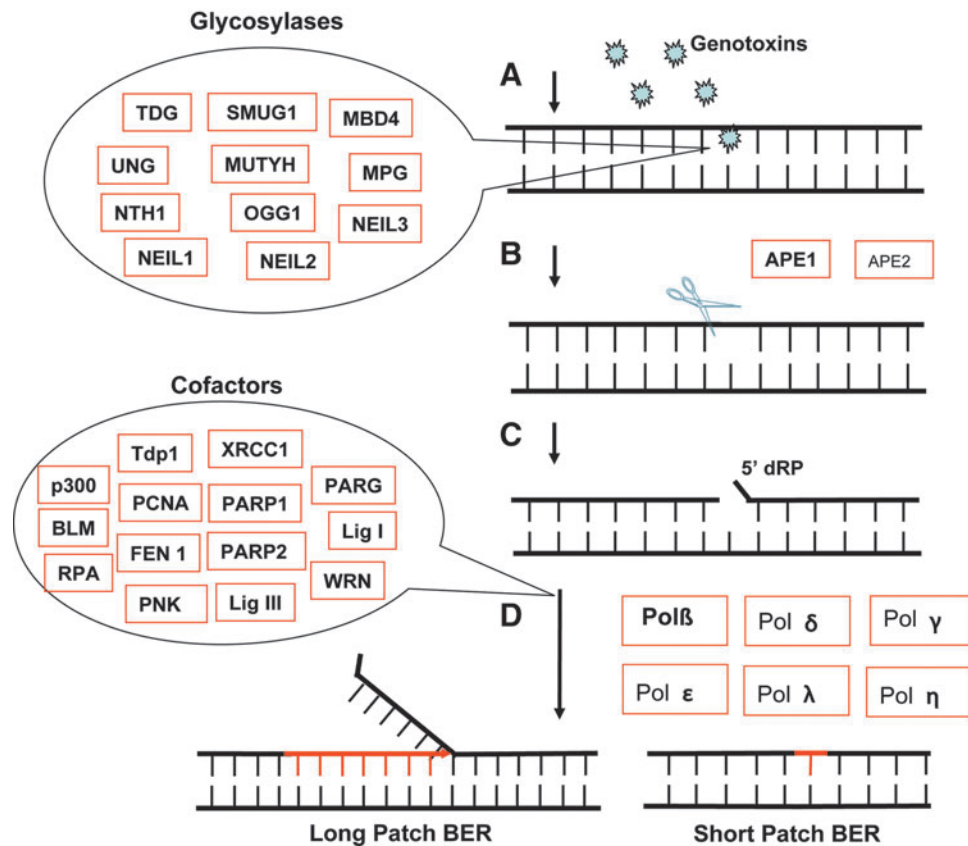


FIG. 1. General model for base excision repair (BER). The BER pathway includes three essential steps: lesion recognition/strand scission, gap tailoring, and deoxyribonucleic acid (DNA) synthesis/ligation. **(A)** When DNA is damaged by genotoxins, BER is initiated with one of 11 human DNA glycosylases to remove base lesions and form the apurinic/aprimidinic (abasic or AP) site or hydrolyze the DNA strand (depending on the glycosylase). **(B)** The AP endonucleases APE1 or APE2 are recruited to the AP site to cleave the DNA backbone and form a single-strand break. **(C,D)** The BER cofactor proteins are recruited to the lesion site working with DNA polymerase β (Pol β) (or other DNA polymerases) to complete the repair. Alternate DNA polymerases have also been suggested to participate in BER, either in a short-patch repair subpathway or an alternate long-patch repair subpathway. The enzymes depicted with smaller fonts suggest that they play a minor role in BER. To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/ars

the product, an apurinic/aprimidinic (abasic or AP) site, is a substrate for an endonuclease specific for AP sites, the AP endonucleases APE1 or APE2 (although the majority activity results from APE1) (3). There is general consensus that the resulting DNA single-strand break after APE1 (or APE2) cleavage forms a nucleation site for scaffold proteins such as poly(adenosine diphosphate [ADP]-ribose) polymerase-1 (PARP1) and XRCC1, followed by recruitment of the proteins needed to complete repair. DNA polymerase β (Pol β) is considered the major end-trimming and DNA polymerase in BER (bolded, Fig. 1D). Alternate DNA polymerases have also been suggested to participate in BER, either in a short-patch repair subpathway or an alternate long-patch repair subpathway, as depicted in Figure 1. As mentioned above, BER has been considered to have a biological role limited to nuclear (and mitochondrial) genome repair in response to endogenous and exogenous genotoxins. However, recently, it has been suggested that these proteins may also have been co-opted to facilitate and enhance gene regulation, as will be discussed herein.

The genome of more than 350 species has been sequenced (34), yet the gap between our understanding of the genotype and phenotype is still considerable. Through a series of spatiotemporal developmental steps, stem and progenitor cells of

a multicellular organism are differentiated into different cell types with unique gene expression profiles to perform their specific functions (11,16,46,68). It is now quite clear that among the many biological and genetic alterations that define cellular function, the pattern of DNA and histone modification plays a main role to define the cellular phenotypes (54). The balance between the DNA methylation/demethylation and histone modification status can impact the structure of chromatin, define the on/off switch for many genes, and eventually change the physiologic outcome. For example, the fate of a honeybee (*Apis mellifera*) as either a worker or a queen is determined by its DNA methylation pattern (82). Approximately 3%–4% of genomic cytosine in a typical mammalian cell is methylated to 5-methylcytosine (5mC) as an epigenetic mark (24). These marks generally occur at the dinucleotide CpG of the promoter region of a gene. Methylation of cytosine to 5mC at the CpG sites is an important step in epigenetic transcriptional regulation related to transcriptional repression, X-chromosome inactivation, imprinting, and suppression of parasitic sequences (13,60,83). Abnormal DNA methylation patterns at the CpG sites are often observed in disease states such as global genome hypomethylation and tumor suppressor gene hypermethylation in cancer (28,31).

In addition, because the DNA is wrapped around histone proteins packed into the chromatin structure, post-translational modification of histone proteins is an important method for controlling DNA access and transcriptional regulation (14,41). The tails of H3 and H4 histones can be covalently modified on several residues by methylation, acetylation, phosphorylation, ubiquitylation, SUMOylation, citrullination, and ADP-ribosylation (19,41,64). Based on the status of these histone modifications, the expression of the modified gene might be upregulated or silenced.

Recent studies have demonstrated that BER may be involved or coupled with both active DNA demethylation (BCADD) and lysine-specific demethylase 1 (LSD1)-mediated histone demethylation (LC-BER) (40,76). In BCADD, three families of enzymes are involved: the Ten–eleven translocation (Tet) protein family, activation-induced deaminase (AID)/apolipoprotein B mRNA-editing enzyme complex (APOBEC), and proteins from the BER pathway. The TET proteins are responsible for the hydroxylation of 5mC to yield 5-hydroxy-methyl-cytosine (5hmC), which can be further oxidized to 5-formylcytosine (5fC) and 5-carboxylcytosine (5caC). AID/APOBEC proteins can deaminate 5mC (or 5hmC) to form thymine or 5-hydroxymethyluracil (5hmU). In the latter case, the resulting modified base would be mispaired with guanine. Once these modified bases are formed, the BER pathway is initiated by TDG to remove base modifications such as 5fC, 5caC, or 5hmU (22).

With regard to histone modification, BER is suggested to be coupled to LSD1-mediated histone demethylation to repair base lesions induced by hydrogen peroxide (H_2O_2) as a by-product of the LSD1 (or LSD2) enzymatic activity (LC-BER).

LSD1 is the first protein demethylase discovered that converts histone H3K4me2 to H3K4me1 or H3K4me0 through a flavin adenine dinucleotide (FAD)-dependent oxidative reaction. In the amine oxidase-mediated demethylation reaction, the co-factor FAD is reduced to $FADH_2$ and then reoxidized to FAD by oxygen with the generation of H_2O_2 (Fig. 2). The DNA damaged by H_2O_2 from LSD1-mediated demethylation triggers the recruitment of the BER machinery to the promoter and regulatory response sites. It is suggested that it is the repair of the LSD1-induced DNA damage that facilitates and enhances transcription initiation (78). Therefore, it is likely that the BER machinery is not only critical for preventing reactive oxygen species (ROS)-induced genome instability, but it is also possible that BER is an essential component of ROS-mediated transcriptional regulation. In this review, the two main epigenetic modifications, DNA demethylation and histone demethylation, are discussed in detail with an emphasis on the roles of BER in transcriptional regulation.

Gene Expression Regulation by Histone Acetylation and Methylation

The basic unit of chromatin consists of 146 base pairs (bp) of DNA wrapped around a histone octamer, which is composed of two copies of each of the four core histones: H2A, H2B, H3, and H4. Thus, post-translational modifications of histones alter the interactions between DNA and histones and modulate DNA access by transcription factors or other regulatory proteins. These alterations result in chromatin structure condensation or relaxation, which leads to the regulation of the expression of a targeted gene (6). The two most common

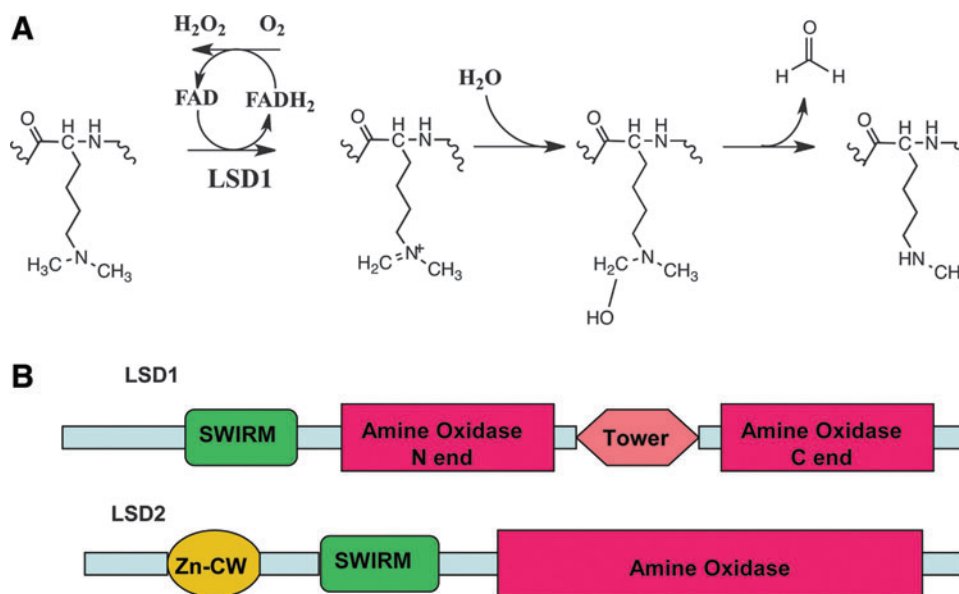


FIG. 2. Comparative analysis of the mammalian flavin-dependent histone demethylases lysine-specific demethylase 1 (LSD1) and LSD2. (A) The reaction of LSD1/LSD2 demethylation: LSD1/LSD2 demethylates H3K4me2 *via* an amine oxidation reaction. Flavin adenine dinucleotide (FAD) is needed as a cofactor. The reaction generates hydrogen peroxide (H_2O_2), which results in local DNA base oxidation. The formation of an imine intermediate is necessary. The imine intermediate further hydrolyzes to an unstable carbinolamine, which spontaneously degrades to H3K4Me1 with the release of formaldehyde. **(B)** The domain differences between LSD1 and LSD2: The first 150 amino acids of LSD1 are predicted to be disordered. On the contrary, LSD2 has a CW-type zinc-finger domain in its N-terminus. Additionally, LSD2 does not have a Tower domain. The Tower domain of LSD1 is responsible for the binding of LSD1 to CoRest. To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/ars

histone modifications are acetylation and methylation. Histone acetylation and deacetylation are highly regulated dynamic processes typically catalyzed by enzymes with a histone acetyltransferase (HAT) or histone deacetylase (HDAC) activity. Acetylation of the (normally) positively charged lysine residue results in an uncharged lysine residue, causing a decreased interaction between the histone and DNA that is generally associated with active transcription. Histone acetylation and deacetylation are usually coregulated with histone methylation and demethylation to facilitate regulation of gene expression. Unlike histone acetylation, methylation of histones does not influence the net charge of the newly modified lysine residues, and hence has no effect on the DNA–histone interaction. However, the histone methylation status is important for the interaction between chromatin and regulatory proteins and therefore can impact transcription. The presence of epsilon-N-methyl-lysine in calf thymus histones was first demonstrated in 1964, and it was suggested that the methyl group might come from methionine (73). In the same year, Allfrey and colleagues reported the possible role of histone methylation and acetylation in regulating RNA synthesis (1). Decades of work on histone modification and regulation of gene expression have developed into a histone-code hypothesis that links the function of histone modifications in chromatin with transcriptional activation or suppression states of related genes (55,87,95). Histone methylation is usually thought to occur at the N-terminal domains of H3 and H4. Histone lysine methyl transferases (HKMTs) can mainly be classified into two families: Su(var)3–9/Enhancer of zeste/Trithorax (SET) domain-containing proteins and the DOT1-like proteins without a SET domain (41). Proteins from both families can transfer a methyl group from S-adenosylmethionine to the epsilon-amino-group of lysine, resulting in the formation of S-adenosylhomocysteine and methyl-lysine. Most HKMTs belong to the SET family and use the SET domain as their catalytic core and almost exclusively act near the N-termini of the histone proteins. On the other hand, DOT1/DOT1L proteins target the lysine tail region of the histone, and DOT1 is the only enzyme known to methylate a lysine residue in the globular core of the histone (103).

Histone Demethylation and Oxidation of DNA

For a long time, histone methylation was considered a permanent and irreversible histone modification and therefore used as an epigenetic mark because of the high thermodynamic stability of the N–CH₃ bond (93). The first histone demethylase, LSD1, discovered in 2004 by Shi and coworkers, brought a completely new field to light that suggested a dynamically controlled balance between HKMTs and demethylases in response to the extracellular or intracellular signals needed for transcriptional regulation (81). LSD1 is often upregulated in various tumors and hence is considered a promising oncogenic target. In line with this thought, the inhibition of LSD1 activity has been reported to reduce cancer cell proliferation or block tumor metastasis (52). LSD1 also plays an important role in embryo development, demonstrated by the observation that a null mutation of the mouse isoform of LSD1 (Kdm1a) causes embryonic lethality (100). LSD1 demethylates its histone substrate *via* an FAD-dependent amine oxidase reaction (Fig. 2A). Because demethylation of trimethylated lysine residues requires a pro-

tonated methyl ammonium group for LSD1-catalyzed oxidation, LSD1 is unable to demethylate trimethylated lysines (81). Subsequently, the other histone demethylase family that contains the Jumonji (JmjC) catalytic domain was identified and characterized and shown to demethylate trimethylated lysines (20,62,94,101,104). The JmjC-driven demethylase reaction is compatible with demethylation of mono-, di-, and trimethylated lysines and has a substrate preference for trimethylated lysine demethylation (23,75). Unlike the LSD1 family proteins, JmjC proteins catalyze lysine demethylation of histones through an oxidative reaction that requires Fe(II) and α -ketoglutarate (α -KG) as cofactors. This demethylation mechanism will not result in DNA damage and therefore does not invoke the BER system. As such, these demethylases are not the focus of this review. The details on the JmjC family of demethylases have been reviewed elsewhere (80).

Based on the proposal that amine oxidases might remove the methyl group from histones *via* an oxidation reaction (7) and the highly homologous sequences between LSD1 and amine oxidases, Shi *et al.*⁸¹ demonstrated that LSD1 catalyzes the removal of the methyl group from both H3K4me₂ and H3K4me₁, but not H3K4me₃ *in vitro via* an FAD-dependent oxidation reaction (Fig. 2A). Forneris *et al.* then demonstrated that molecular oxygen was utilized as the electron acceptor. After exposure of the reduced LSD1, which was generated from the demethylation of H3K4Me₂ under anaerobic conditions, to air for a few minutes, the flavin cofactor of LSD1 was reoxidized by oxygen. The reoxidized LSD1 has the same absorption spectrum as the native enzyme (35). In LSD1-catalyzed histone demethylation, the amino-group of the methylated lysine is oxidized, presumably to generate an imine intermediate that will spontaneously hydrolyze to produce formaldehyde and the corresponding amine residue. Substrate oxidation leads to the two-electron reduction of the cofactor FAD, which is reoxidized by molecular oxygen to produce H₂O₂, which was initially detected with a peroxidase-coupled assay. Because of the formation of the imine intermediate, LSD1-mediated demethylation is critically dependent on the protonation of the nitrogen. Thus, this enzyme can only catalyze demethylation of mono- and dimethylated lysines. In 2009, Karytinov and colleagues discovered a second FAD-dependent H3 lysine demethylase, LSD2. LSD2 was demonstrated to be specific for demethylation of H3K4me₁ and H3K4me₂ *via* a mechanism similar to LSD1 (58). Both proteins have a C-terminal amine oxidase domain hosting the FAD cofactor for catalytic activity that is preceded by a SWIRM domain, a six- α -helical structural module frequently found in chromatin-associated proteins and important to LSD1 protein stability (Fig. 2B). The main difference between LSD1 and LSD2 is in the N-terminus. Unlike the N-terminal sequence of LSD2 that forms a CW-type zinc-finger domain (residues 130–200), the first 150 amino acids of LSD1 are predicted to be disordered (58). Another difference is that LSD2 does not have a Tower domain that in LSD1 is important for CoRest binding, which therefore precludes the possibility of LSD2/CoRest complex formation (58). In addition, the N-terminal CW-type zinc-finger domain found only in LSD2 suggests the possibility of an interaction between LSD2 and nucleosomal DNA (58). The sequence and structural differences between LSD1 and LSD2 may foreshadow functional specificities of the two demethylases. Recently, Huang and colleagues showed that inhibition of LSD1 activity by the

monoamine oxidase inhibitor pargyline or reduction of LSD1 expression by small interfering RNA (siRNA) resulted in increased acetylation of H3K9 (AcH3K9), an epigenetic mark for transcriptionally active chromatin. Further, the reactivation of many tumor suppressor genes resulted in growth inhibition of breast cancer cells. However, the reduction of LSD2 expression by siRNA-mediated knockdown did not change the level of AcH3K9. This result suggests that LSD2 activity may not functionally complement the HDAC activity of LSD1 (52). In 2010, van Essen and colleagues demonstrated that LSD2 was required for the removal of the methyl group from H3K9me2 at the *Mdc* and *Il12b* promoters when stimulus-induced NF- κ B was recruited to the promoter region for activating those genes (97). In addition, Fang *et al.* reported that LSD2 mainly binds to the gene bodies (but not promoters) to demethylate H3K4me2 and may be associated with elongation factors to regulate gene transcription after initiation (29). In both the LSD1- and LSD2-mediated demethylation reaction, the H₂O₂ product generated oxidative damage on the nearby DNA (21). It has been reported that when LSD1-mediated H3K4 demethylation occurred, the percentage of cells labeled with a fluorescein-tagged 8-oxo-7,8-dihydro-2'-deoxyguanosine (8-oxodG)-binding protein increased from 5% at a basal level to 74%, close to the percentage (84%) that resulted from the treatment of H₂O₂ as a positive control (21). Although only the oxidatively modified form of deoxyguanosine (8-oxodG) was reported after LSD1-mediated histone demethylation (4,78), all four deoxynucleosides (deoxyadenosine, deoxyguanosine, thymidine, and deoxycytidine) and the methylated form of deoxycytidine (5-methyl-deoxycytidine) can be oxidatively damaged (88). The most common and intensively studied oxidatively damaged base is 8-oxodG (59). Other major oxidative lesions include 2,6-diamino-4-hydroxy-5-formamidopyrimidine. With the exception of DNA polymerase ι (ι), DNA replication in mammalian cells is not significantly blocked by 8-oxodG damage, and thus the 8-oxodG DNA lesion is minimally cytotoxic and in fact is mostly mutagenic (106). Since 8-oxodG potentially mispairs with A, either DNA replication or DNA repair-mediated DNA synthesis opposite the 8-oxodG lesion yields deoxyadenosine monophosphate (dAMP) insertion (47,65,67,85,107). Eventually, this results in a G \Rightarrow T substitution mutation (85). In general, the 8-oxodG lesion is mainly repaired by the BER pathway, and it has been suggested that the repair (BER) synthesis mediated by Pol- λ (λ) preferentially inserts deoxycytidine triphosphate (dCTP) opposite 8-oxodG (69,70,98,99). Similarly, BER synthesis mediated by Pol β prefers insertion of dCTP (by a factor of 2:1) over insertion of the mutagenic dAMP base (71). If not repaired, the 8-oxodG lesion can be further oxidized to yield several mutagenic base lesions, including guanidinohydantoin and spiroiminodihydantoin (44,74).

Deoxyadenosine can be oxidized into two major products: 8-oxo-7,8-dihydro-2'-deoxyadenosine (8-oxodA) and 4,6-diamino-5-formamidopyrimidine (FapydA) (12). The FapydA lesion was first detected in both normal and cancerous tissues (77) and is the most abundant of the adenine lesions induced by γ -radiation (18). Both lesions are weakly mutagenic (57,92), and tandem 8-oxodA lesions can be induced by hydroxyl radicals. BER is the major pathway to repair such DNA lesions. The major 2'-deoxycytidine-oxidized product is 5-hydroxy-2'-deoxycytidine (OH5dC). Inaccurate replication across the OH5dC lesion incorporated into DNA tends to

generate the C \Rightarrow T transition mutations (30). Thymine can be oxidized to thymine glycol (Tg). The Tg lesion is formed by a hydroxyl radical attack on the double bond of thymine at C5 or C6 or by hydrogen abstraction from the methyl group (56). Tg was originally identified in purified DNA after oxidation with ionizing radiation (36). Although the Tg lesion blocks human replicative DNA polymerases, the lesion only results in a minimal increase in mutations, since several translesion DNA polymerases such as DNA polymerase η , κ , ν , β , and λ readily bypass the lesion (10,33,66,91). Additional oxidized thymine analogs include 5,6-dihydro-thymine and 5-hydroxy-5,6-dihydro-thymine. Further details on the repair of oxidative DNA base lesions have been reviewed previously (88).

BER Pathway and the Repair of Oxidatively Damaged DNA

Both environmental and endogenous factors can induce DNA base damage, requiring repair by multiple DNA repair pathways to maintain genome stability and to prevent cell malignant transformation. The BER pathway is the predominant DNA repair pathway for repairing oxidatively damaged DNA. The BER pathway consists of three functional steps: (i) lesion recognition/strand scission, (ii) gap tailoring, and (iii) DNA synthesis/ligation (3,88). The initial step of recognition and strand scission involves the removal of the oxidatively damaged DNA lesion by one of several DNA glycosylases (Fig. 1) (88). This is followed by hydrolysis of the DNA backbone primarily by APE1, an AP endonuclease. Gap tailoring can be conducted by several enzymes depending on the initial lesion, and finally DNA synthesis allows the insertion of the correct DNA base followed by ligation to seal the DNA backbone (3). There are several classes of DNA glycosylases, monofunctional and bifunctional glycosylases, which differ based on function. Monofunctional glycosylases hydrolyze the *N*-glycosidic bond to remove the damaged base. This class includes UNG, SMUG1, TDG, MBD4, MPG, and MUTYH. Monofunctional DNA glycosylases such as SMUG1 and MYH use an activated water molecule to cleave the *N*-glycosidic bond. After monofunctional glycosylases remove the damaged base, an AP endonuclease (APE1 or APE2) is required to hydrolyze the phosphodiester bond of DNA, creating a single-strand break with a 5'-deoxyribose-phosphate (5'dRP) group that is ultimately gap-tailored by Pol β (86). In contrast, bifunctional glycosylases possess an AP lyase activity in addition to the glycosylase activity. OGG1, NTHL1, and NEIL3 are bifunctional DNA glycosylases that have an associated β -elimination activity, whereas NEIL1 and NEIL2 are bifunctional DNA glycosylases that have an associated β,δ -elimination activity. In human cells, NTHL1 (NTH1), NEIL1, or NEIL2 is usually involved in removing oxidative pyrimidine lesions while OGG1 is primarily responsible for the removal of oxidatively modified purine bases. SMUG1 excises a subset of oxidative base damage, including 5-hydroxyuracil, 5hmU, and 5-formyluracil. MUTYH (MYH) can remove the normal A-base when misincorporated opposite the template 8-oxoguanine (8-oxodG) during DNA replication or repair synthesis. Interestingly, it has been reported that MYH mutations are related to a colorectal adenoma syndrome (MYH-associated polyposis) and high colorectal cancer risk (17). Bifunctional DNA glycosylases (NTH1, NEIL1, NEIL2, and OGG1) use Lys or Pro for direct attack on sugar C1' to

hydrolyze the *N*-glycosidic bond. Based on the initiating lesion and the mechanism of base removal, the BER pathway can be classified into either a long-patch BER subpathway (Fig. 1) or three short-patch subpathways, including a monofunctional DNA glycosylase-initiated subpathway, bifunctional DNA glycosylases with associated β -elimination-initiated subpathway, and bifunctional DNA glycosylases with associated β,δ -elimination-initiated subpathway (88). The monofunctional DNA glycosylase-initiated subpathway uses the classic short-patch BER mechanism starting with hydrolysis of the *N*-glycosidic bond to form an abasic site (3). The gap margins with a 3'-OH and a 5'-dRP group are tailored by the 5'-dRP lyase activity of Pol β , followed by Pol β -mediated gap filling (DNA synthesis). Either DNA ligase I (LigI) or a complex of DNA ligase III (LigIII) and XRCC1 seals the DNA chain (3). Recent evidence indicating that LigIII is not required for nuclear BER may suggest that LigI is the preferred ligase in BER (37,84). Strand-break repair and signaling are mediated by XRCC1 together with PARP1, PARP2, and PARG. XRCC1 acts as a scaffold for protein complex formation (2,3).

The BER subpathway initiated by bifunctional DNA glycosylases associated with β -elimination is the predominant BER mechanism for the removal of oxidatively damaged DNA. A bifunctional DNA glycosylase such as OGG1, NTHL1, or NEIL3 hydrolyzes the DNA backbone, 3' to the incised base, leaving a 3'-unsaturated aldehyde after β -elimination and a 5'-phosphate at the termini of the repair gap. NEIL3 mainly is used for nuclear DNA repair, whereas OGG1 and NTHL1 can repair oxidatively damaged DNA in both the nucleus and the mitochondria. The gap tailoring is performed by the 3'-phosphodiesterase activity of APE1 (3). Next, gap filling (new DNA synthesis) is mediated by Pol β , and the DNA backbone is sealed by the XRCC1/LigIII heterodimer or LigI (3). In some cases, replicative DNA polymerases (δ and ϵ) or low-fidelity DNA polymerases may insert a wrong base opposite many oxidative lesions. To prevent the accumulation and the eventual onset of G \Rightarrow T substitution mutations, failure to repair the 8-oxodG lesion also triggers repair of the A-base opposite the 8-oxodG lesion by the MYH glycosylase (26,85).

Another BER subpathway is initiated by bifunctional DNA glycosylases with an associated β,δ -elimination (NEIL1 and NEIL2). These DNA glycosylases are followed by an APE1-independent repair mechanism. Binding of NEIL2 to the lesion recruits XRCC1 to the DNA (15). It has not yet been established if the same process of XRCC1 recruitment is mediated by NEIL1. NEIL1 or NEIL2 generates a 5'- and 3'-phosphate at the ends of the DNA in the single-base gap, resulting from hydrolysis of the glycosidic bond to release the base, cleavage of the DNA-3' to the abasic site *via* β -elimination, and then cleavage of the DNA-5' to the abasic site *via* δ -elimination, releasing the *trans*-4-hydroxy-2,4-pentadienal (25,102). The phosphatase PNKP is subsequently recruited to the site to remove the 3'-phosphate in the gap and complete the gap-tailoring step. The repair is finalized by DNA synthesis with Pol β and ligation with XRCC1/LigIII (25,102).

BER and Epigenetic Regulation *via* Induction and Repair of 8-oxodG

In almost all types of cells, a detectable low basal level of oxidative DNA modifications (*e.g.*, 8-oxodG) is observed,

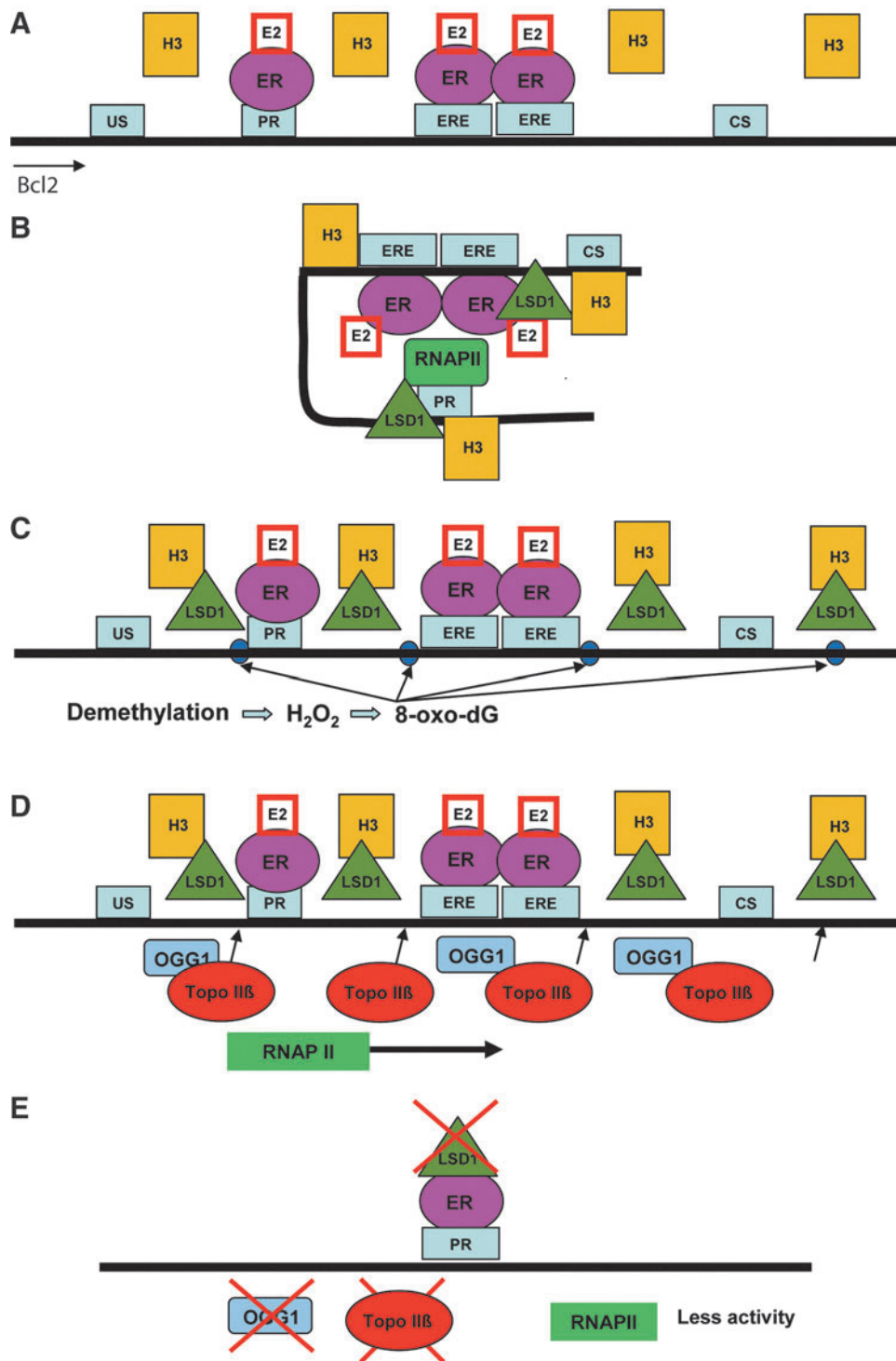
likely due to a steady state between continuous generation of these and related DNA modifications by ROS and simultaneous repair mainly by BER mechanisms (51). The endogenous oxidative nuclear DNA damage is traditionally thought to arise from ROS generated *via* the mitochondrial electron transport chain. This concept was challenged by Hoffmann and colleagues in 2004 (51). The authors reported that a reduction in mitochondrial ROS production by depletion of mitochondrial DNA or an increase in mitochondrial ROS production *via* interfering with the mitochondrial electron transport chain did not change the density of nuclear DNA damage (51). The observation implied there may be additional sources of ROS that contribute to the steady-state level of endogenously induced nuclear DNA base modifications. Moreover, Ziel *et al.* proposed that the oxidative DNA modifications might be utilized as signals for transcriptional regulation (109). Here, the authors observed DNA oxidative modifications enriched at hypoxic-response elements located in the promoter region of the *VEGF* gene. Moreover, compared to the reporter gene with wild-type hypoxic elements, luciferase activity increased with AP-site-modified hypoxic elements. As described above, the AP sites are repair intermediates formed after glycosylase-mediated base lesion removal (Fig. 1). Because AP sites are repaired by the BER pathway, these results suggested that the BER pathway may play a role in transcriptional regulation (109). More recent studies directly demonstrated that the BER pathway may participate in estrogen-induced target gene expression and *Myc*-related transcriptional regulation (see below).

BER and Estrogen-Induced Epigenetic Regulation

The first direct evidence of LC-BER and the involvement of BER in transcriptional regulation was reported by Perillo *et al.* in a study of estrogen-induced target gene expression mediated by LSD1 (78). As shown in Figure 3, the authors used a model system (estrogen receptor [ER]-positive MCF7 cells treated with 17 β -estradiol [E2]), demonstrating that the ER was recruited to the promoter and estrogen-response elements (EREs) and enhancers (Fig. 3A). Binding of activated ER by E2 to both the B-cell CLL/lymphoma 2 (*bcl-2*) promoter and the ERE enhancer regions, which are 1.5-kb apart, formed a chromatin loop between the promoter and the ERE enhancer (Fig. 3B). The chromatin looping lasted for 60 minutes after E2 addition and then disappeared. Assembly of the transcription complex occurred between 30 and 45 minutes after E2 addition. Meanwhile, H3K9me2 demethylation occurred at the promoter and enhancer sites of the *bcl-2* or *pS2* genes. Both chromatin looping and estrogen-induced transcription were prevented when the LSD1 activity was inhibited by the free radical-scavenging drug *N*-acetyl-L-cysteine (NAC) or the LSD1 inhibitor pargyline. The dimethyl lysine at H3K9me2 in both the promoter and enhancer regions is demethylated by LSD1 *via* an FAD-dependent oxidative reaction, yielding the generation of local H₂O₂ that can oxidize the nearby guanine bases to 8-oxo-G (Fig. 3C). Five minutes after E2 treatment, 8-oxodG could be detected by immunofluorescence, and 45 minutes after E2 treatment, the immunofluorescence intensity specific for 8-oxodG was similar to the control experiment after H₂O₂ treatment (78). As expected, deactivation of LSD1 with NAC or pargyline, as well as siRNA knockdown of LSD1, abolished the accumulation of the 8-oxodG lesion.

LSD1-BER-mediated ER-induced expression of Bcl-2 gene

FIG. 3. LSD1/BER-mediated estrogen receptor (ER)-induced expression of the *bcl-2* gene. **(A)** Thirty minutes after E2 stimulation, the E2-ER complex is recruited to the promoter (Pr) and estrogen-response elements (EREs) and enhancer (Enh) sites. **(B)** After E2-ER complex binding to Pr and ERE, a chromatin loop is formed between Pr and Enh. At the same time, LSD1 is recruited to these sites to demethylate H3K9me2. **(C)** H₂O₂, a by-product generated from the reaction of LSD1-mediated H3K9me2 demethylation, oxidizes the nearby guanine bases to 8-oxo-7,8-dihydro-2'-deoxyguanosine (8-oxodG). **(D)** To initiate DNA repair-coupled transcription, OGG1 and Topo IIβ are recruited to the lesion sites (purple dots) to initiate the repair of 8-oxodG. RNA polymerase II assembly starts the transcription of the *bcl-2* gene. **(E)** The results from inhibition experiments indicate that without E2, LSD1 is not recruited to the Pr and Enh sites. The loss of function of OGG1 or Topo IIβ by using targeted siRNA reduces the loading of activated RNAP II on promoters. US, upstream sequence; CS, coding sequence. To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/ars



Next, it was demonstrated that removal of the 8-oxodG lesions from the promoter and enhancer regions of the DNA occurs primarily by OGG1, which specifically recognizes and removes 8-oxodG as a part of the BER pathway (78). Another repair enzyme, topoisomerase IIβ (TopoIIb), which is responsible for recognizing and repairing single-stranded DNA breaks, was present in both sites as well (Fig. 3D). Inhibition of TopoIIb expression with siRNA strongly reduced loading of

the activated RNA Pol II, of which Ser5 was phosphorylated on the promoter. A decrease of loading or assembly of the phospho-RNA polymerase by 30%–40% was achieved by the siRNA-mediated knockdown of OGG1 (Fig. 3E). These results indicated that targeting both TopoIIb and OGG1 to these sites drove transcription initiation.

The proposed mechanism of histone demethylation coupling with BER (LC-BER) for transcriptional regulation is as

follows: LSD1 demethylation results in the accumulation of 8-oxodG lesions *via* the generation of H₂O₂ at the sites of demethylation. The 8-oxodG lesions are repaired by OGG1, ultimately generating DNA single-strand breaks during BER that are recognized by TopoIIb to facilitate chromatin relaxation and formation of the complex process of transcription initiation. As such, H3K9me2 demethylation produced temporally limited and localized H₂O₂ at the promoter and enhancer regions, providing a local signal for the assembly of transcription initiation complex proteins mediated in part by BER proteins such as OGG1 and APE1. Overall, this study demonstrated that LSD1-mediated histone demethylation is coupled with BER to initiate hormone-dependent gene expression (78).

BER and Myc-Induced Epigenetic Regulation

More recently, the same group observed LSD1-BER-mediated transcriptional regulation in Myc-activated transcription of the Myc-target genes *Ncl* and *CAD* (4,5). Myc is one of the most common activators of cell proliferation used by cancer cells to drive disease progression. Because the E-box sequence (CACGTG) for Myc binding was estimated to exist in 15% of the all promoter regions in the human genome (32,42,105), the involvement of BER in the mechanism of Myc-induced transcriptional regulation will have broad significance for cancer therapy research. Amente and colleagues demonstrated that LSD1-BER-coupled epigenetic regulation *via* demethylation of H3K4Me2 by LSD1 at the promoter and E-box sites drives Myc-mediated gene expression (Fig. 4) (4). In this study, the activation of Myc with tamoxifen (OHT) was set as the start point (Fig. 4A). About 30–60 minutes after Myc activation, Myc was recruited mainly on the E-boxes of both the *Ncl* and *CAD* genes and stably accumulated at the E-box until the end point (240 minutes) (Fig. 4B). LSD1 was recruited to the transcription start site (TSS) and the coding region (CR) of both genes shortly after Myc activation (Fig. 4C). The authors suggested that the recruitment of LSD1 to the promoters is an early and transient event, because the existence of LSD1 on both the TSS and the E-box overlapped with H3K4me2 demethylation on those sites only in the period from 30 to 60 minutes. *Ncl* and *Cad* mRNA accumulation was observed 1 hour after Myc induction. Four hours later, not only the methylation status of both sites was back to normal but also the BER proteins were no longer present at the sites. Stable acetylated histone H4 accumulation was detected on the TSS and CR of both the genes with a peak level at 240 minutes after Myc activation. During the demethylation of H3K4me2, the fluorescent signal for detection of 8-oxodG was significantly increased in Myc-activated cells (Fig. 4C), similar to the level of cells exposed to H₂O₂, but not in the Myc-null cells. In separate experiments, addition of siRNA specific for Myc or addition of pargyline (an LSD1 inhibitor) abolished the signal for 8-oxodG detection. It was also demonstrated that two BER enzymes (OGG1 and APE1) were engaged at the TSS and E-boxes of the *Ncl* and *CAD* genes after Myc activation (4). OGG1 appears to be required for the removal of the 8-oxodG lesion, and APE1 was required to complete repair (Fig. 4D). However, no additional BER proteins were evaluated in this initial report. The functional role of BER for transcriptional control was confirmed by the loss of expression of either OGG1 or APE1 *via* targeted siRNA treatment, dramatically

reducing the expression of both the *Ncl* and *CAD* genes after Myc activation (Fig. 4E). The proposed mechanism for Myc-induced transcription is that Myc recruits LSD1 to the target E-box site and initiates histone H3 demethylation, which is then linked to oxidation and DNA repair (LC-BER) to promote the assembly of the transcript initiation complex.

Epigenetic Regulation *via* BCADD

In 1998, Um and colleagues reported that TDG directly interacted with the retinoic acid receptor and the retinoid X receptor (RXR) in a ligand-independent manner (96). Overexpression of TDG induced a fourfold increase of the β -galactosidase reporter gene expression controlled by a retinoic acid-responsive promoter (96). Subsequently, using chicken 5-methylcytosine DNA glycosylase (5-MCDG), a homolog of human TDG, Zhu and colleagues discovered the upregulation of reporter gene expression induced by a similar 5-MCDG-RXR complex that was the result of DNA demethylation of the downstream ecdysone-retinoic acid-responsive enhancer (108). In a nonmammalian model, participation of BER in DNA demethylation is clearly documented. In *Arabidopsis*, the BER proteins Demeter and ROS1 directly remove 5mC *via* their glycosylase activities (39,72). In *Xenopus*, growth arrest and DNA damage-inducible protein 45-alpha (Gadd45a) is the major protein to initiate DNA demethylation, and the interaction of Gadd45a with the nucleotide excision repair protein XPG is suggested (8). In zebrafish embryos, 5mC is converted to thymine by AID, and then the G:T mismatch is removed by the zebrafish thymine glycosylase MBD4, associating Gadd45 with the BER pathway (79). In a mammalian model, the participation of BER in active demethylation has recently been documented using mouse embryos during genome-wide epigenetic reprogramming. In 2010, Hajkova reported the initiation of epigenetic reprogramming at embryonic day 7.25 (E7.25), followed by genome-wide DNA demethylation. Involvement of BER was proposed after an increase in expression of APE1, XRCC1, and the formation of PAR, suggesting the involvement of BER in mouse primordial germ cells, but not the neighboring somatic cells (45). The mechanism of participation of BER proteins in mammalian demethylation was not clear until the discovery of the TET protein family (see Fig. 5) (90). TET protein family members are α -KG and Fe(II)-dependent dioxygenases that include the isoforms TET1, TET2, and TET3. TET1 is mainly expressed in embryonic stem cells (ESCs), whereas TET2 and TET3 are more ubiquitously expressed (90). All TETs can oxidize 5mC to 5hmC (22,48,89,90) (Fig. 5A, B). TET2 mutations were linked to many types of myeloid malignancies and resulted in a significant decrease in the level of 5hmC in bone marrow cells isolated from patients with acute myeloid leukemia as compared to healthy individuals (63). BER was proposed to participate in TET-mediated DNA demethylation by repairing the 5hmU that is formed from 5hmC by the AID/APOBEC family of cytidine deaminases (43) (Fig. 5A). In addition, recent studies in mammalian cells demonstrated two new cytosine modifications, formylcytosine and carboxylcytosine, which are generated by two successive oxidation reactions of 5hmC catalyzed by the TET proteins (22,53) (Fig. 5B). It has recently been reported that the existence of 5fC or 5caC in a DNA template dramatically reduced the RNA Pol II elongation efficiency compared to the DNA templates that

Myc Induced Expression of Ncl Gene

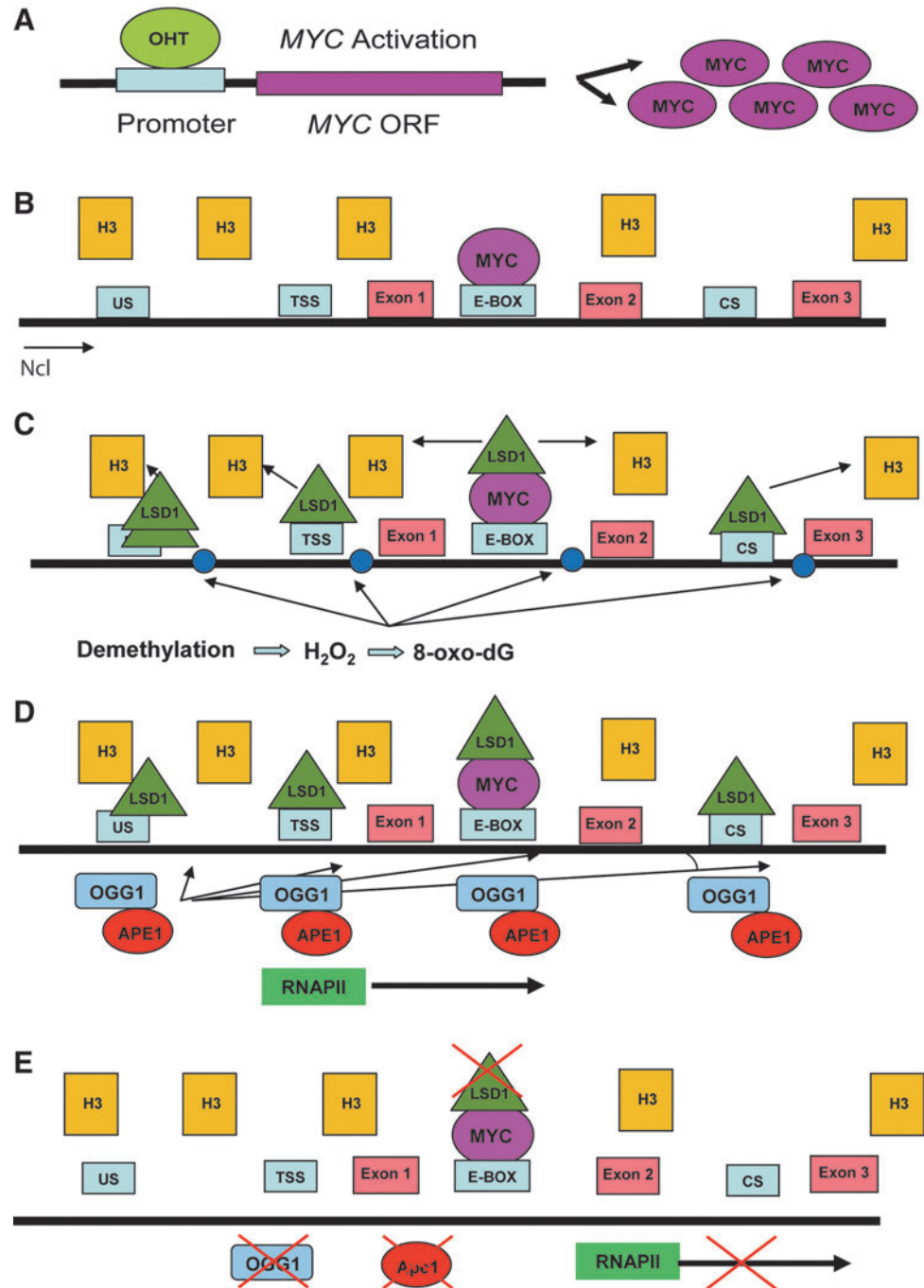


FIG. 4. Myc-induced expression of the *Ncl* gene. (A) The expression of Myc was induced by adding tamoxifen (OHT) to cultured cells. (B) Thirty minutes after Myc activation, Myc is recruited to the E-BOX. (C) After Myc binds to the E-BOX, LSD1 is recruited to US, transcription start site (TSS), E-BOX, and CR sites, and H3K4me2 is then demethylated. LSD1-mediated demethylation of H3K4me2 generates H₂O₂, which results in DNA damage (8-oxodG). (D) OGG1 and APE1 are recruited to the lesion sites to repair DNA damage. This couples with RNAP II to start the transcription of the *Ncl* genes. (E) Knockdown of LSD1, OGG1, or APE1 abolishes the expression of *Ncl* even with the expression of Myc. To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/ars

had unmodified C, 5mC, or 5hmC bases. It was demonstrated that the DNA templates with either 5fC or 5caC resulted in much lower GTP incorporation efficiencies than the others. In addition, 5fC- and 5caC-modified bases also caused a significant level of RNA Pol II complex backtracking, further suggesting that RNA Pol II shifted from an active state to a paused state. In addition, 5fC greatly reduced the fidelity of nucleotide incorporation (61). TDG was shown to be the glycosylase responsible for removal of the carboxylcytosine base that was then followed by complete BER processing to restore the normal cytosine (Fig. 5B). A depletion of TDG leads to accumulation of carboxylcytosine in mouse ESCs (48). Moreover, a loss

of TDG function is lethal to mouse embryos in an early developmental stage associated with epigenetic aberrations affecting the expression of developmental genes (22).

Further, a recent report demonstrated that another BER protein, PARP1, is involved in the regulation of the process required for reprogramming somatic cells into pluripotent stem cells (iPSCs) when using the pluripotency factors *Oct4*, *Sox2*, *Klf4*, and *c-Myc* (OSKM) to mouse embryonic fibroblasts (MEFs) prepared from WT and *Parp1*^{-/-} 13.5-day embryos and tail-tip fibroblasts prepared from WT and *Tet2*^{-/-} mice (27). In this mechanism, PARP1 and Tet2 are recruited to the *Nanog* and *Esrrb* loci to erase the somatic

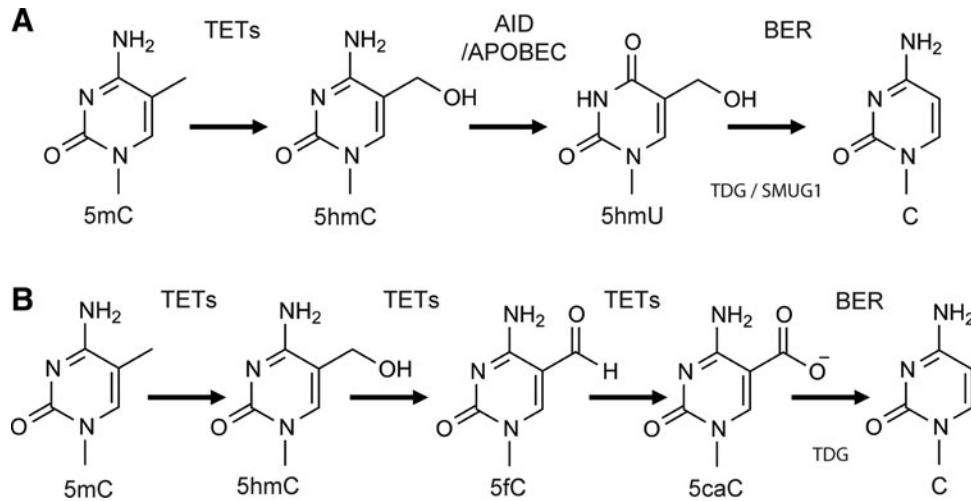


FIG. 5. BER participates in active demethylation. (A) DNA demethylation *via* 5-hydroxymethylcytosine (5hmC)/5-hydroxymethyluracil (5hmU): TET proteins (TET1–3) convert 5mC to 5hmC. Next, an AID/APOBEC enzyme deaminates 5hmC to 5hmU, which is a substrate for TDG or SMUG1. The removal of 5hmU by TDG or SMUG1 results in an abasic (AP) site, inducing recruitment of other BER proteins to complete repair and the conversion of 5hmU to C. **(B)** DNA demethylation *via* 5-formylcytosine (5fC)/5-carboxylcytosine (5caC): TET enzymes iteratively oxidize 5mC to 5hmC and then to 5fC and finally to 5caC. TDG excises the 5caC and forms an AP site. This initiates the BER pathway to convert 5caC to C.

epigenetic signatures of DNA methylation or histone modification and establish alternative epigenetic marks characteristic of ESCs. In a functional screen for epigenetic modification factors that promoted OSKM-mediated somatic cell reprogramming in MEFs, overexpression of PARP1 potentially induced OSKM-MEF reprogramming to generate more iPSCs. Moreover, PARP1 deficiency suppressed the reprogramming of iPSCs. The quantification of total cytosine methylation (5mC plus 5hmC) at the regulatory regions of the pluripotency-related genes *Nanog* or *Esrrb* indicated that PARP1 appeared as a regulator of 5mC. The loss of PARP1 function induced accumulation of 5mC, but not 5hmC, at both the *Nanog* and *Esrrb* loci. However, overexpression of PARP1 did not significantly change the 5mC or 5hmC levels. PARP1 also affected accessibility of the reprogramming factor Oct4 to the regulatory sites of both the *Nanog* and *Esrrb* genes. PARP1 deficiency significantly diminished Oct4 occupancy at both pluripotency loci, as measured by the chromatin immunoprecipitation (ChIP) analysis. Further, PARP1 overexpression robustly promoted exogenous Oct4 binding to the pluripotency loci. PARP1 affected the chromatin state of the *Nanog* and *Esrrb* loci as well. Deficiency of PARP1 diminished the H3K4me2 chromatin mark, which is enriched at the pluripotency loci of iPSCs (both the *Nanog* and *Esrrb* loci) (27). It has also been shown that Tet2 played an important role in iPSC reprogramming. Expression of Tet2 gradually increased along with iPSC reprogramming and kept elevating in iPSCs. In contrast, expression of Tet1 or Tet3 remained at basal levels. iPSC colony formation was abolished when Tet2 expression was knocked down using targeted shRNA. The ChIP analysis indicated that Tet2 was present at the *Nanog* and *Esrrb* pluripotency loci with or without functional PARP1. Tet2 knockdown suppressed the induction of 5hmC and abolished the H3K4Me2 chromatin mark at the pluripotency loci of both the *Nanog* and *Esrrb* genes (27). Overall, these data clearly support a role for PARP1 in the induction of PSCs and suggest that additional BER proteins may have a supportive role. Given

the potential for PARP inhibitors to induce the death of cancer cells defective in homologous recombination DNA repair, the participation of PARP1 in epigenetic regulation may provide support for the use of PARP inhibitors combined with epigenetic inhibitors in cancer. For example, it has been reported that the HDAC inhibitor MS275 potentiated the cytotoxic effect of the PARP inhibitors KU-0058948 and PJ34 in PARP inhibitor-sensitive leukemic cells. However, the DNA methyltransferase inhibitor 5'-aza-2'-deoxycytidine failed to increase the cytotoxicity of PARP inhibitors against primary myeloid leukemic cells and myeloid leukemic cell lines (38). More investigations are clearly needed to fully understand the mechanism of HDAC inhibitor-mediated potentiation of PARP inhibitors with the ultimate goal of improving therapy options using combinations of epigenetic regulators and BER inhibitors.

Summary

Gene expression in eukaryotic cells is regulated at multiple levels of transcriptional control to allow response to growth factors, cellular stress, DNA damage, or development. The primary control comes from genetic information encoded in the DNA sequence, which defines protein sequences, signal sequences, and noncoding regulatory elements for transcription factors, enhancers, or silencers. A secondary, but critical, level of control of gene expression is at the epigenetic level *via* DNA methylation and histone modification. The BER pathway has recently been demonstrated to be necessary for both DNA methylation- and histone modification-mediated epigenetic regulation separate from its main function in maintaining genome stability. Impaired BER can also have significant effects on the cellular DNA methylation status. For example, knockout of TDG resulted in mass DNA methylation changes in many gene promoter regions in mouse embryos and resulted in early embryonic lethality (22). The discovery of the function of the TET family proteins suggests

that the dynamic regulation of DNA methylation by active demethylation requires the BER pathway as the final effector. Two possible TET-BER-regulated active demethylations were proposed. Cortellino *et al.* proposed that 5hmC may first be deaminated by the AID/APOBEC family of cytidine deaminases to generate 5hmU. BER is then engaged to repair the 5hmU:G mismatch, because both TDG and SMUG1 were demonstrated to effectively repair 5hmU in the 5hmU:G mispair in dsDNA, yielding the correct C:G base pair (22). He and colleagues proposed another active DNA demethylation pathway in which the 5mC lesion is iteratively oxidized by TET proteins into 5hmC, 5fC, and 5caC, followed by a conversion back to an unmethylated cytosine *via* TDG-initiated BER (48). Further studies are needed to determine the condition for activating these two pathways. BER also plays an important role in transcriptional regulation at sites of histone demethylation. As we discussed above, defects in BER directly reduced estrogen- or Myc-induced target gene expression. The 8-oxodG lesion, produced from H₂O₂, generated by LSD1 or LSD2 during the process of histone demethylation, serves as the signal to recruit BER enzymes to hydrolyze and relax high GC-content promoter regions, facilitating and enhancing transcription initiation and elongation (5).

Appropriately controlled epigenetic regulation is critical for the normal development and health of an organism. Misregulation of epigenetic control, regarding either DNA methylation or histone methylation, has been associated with cancer, chromosomal instability syndromes, and mental retardation. Recent reports show that BER is indispensable for epigenetic events such as hormone-modulated gene expression and iPSC reprogramming. Such efforts to address the role of BER proteins in epigenetic regulation could broaden cancer therapeutic strategies to include epigenetic modifiers combined with BER inhibitors.

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Author Disclosure Statement

R.W.S. is a scientific consultant for Trevigen, Inc. The remaining authors state that there is no conflict of interest.

References

- Allfrey VG, Faulkner R, and Mirsky AE. Acetylation and methylation of histones and their possible role in the regulation of RNA synthesis. *Proc Natl Acad Sci U S A* 51: 786–794, 1964.
- Almeida KH, and Sobol RW. Increased specificity and efficiency of base excision repair through complex formation. In: *DNA Damage Recognition*, edited by Siede W, Doetsch PW, and Kow YW. New York: Marcel Dekker Inc., 2005, pp. 33–64.
- Almeida KH, and Sobol RW. A unified view of base excision repair: lesion-dependent protein complexes regulated by post-translational modification. *DNA Repair* 6: 695–711, 2007.
- Amente S, Bertoni A, Morano A, Lania L, Avvedimento EV, and Majello B. LSD1-mediated demethylation of histone H3

- lysine 4 triggers Myc-induced transcription. *Oncogene* 29: 3691–3702, 2010.
- Amente S, Lania L, Avvedimento EV, and Majello B. DNA oxidation drives Myc mediated transcription. *Cell Cycle* 9: 3002–3004, 2010.
- Bannister AJ, and Kouzarides T. Reversing histone methylation. *Nature* 436: 1103–1106, 2005.
- Bannister AJ, Schneider R, and Kouzarides T. Histone methylation: dynamic or static? *Cell* 109: 801–806, 2002.
- Barreto G, Schafer A, Marhold J, Stach D, Swaminathan SK, Handa V, Doderlein G, Maltry N, Wu W, Lyko F, and Niehrs C. Gadd45a promotes epigenetic gene activation by repair-mediated DNA demethylation. *Nature* 445: 671–675, 2007.
- Baute J, and Depicker A. Base excision repair and its role in maintaining genome stability. *Crit Rev Biochem Mol Biol* 43: 239–276, 2008.
- Belousova EA, Maga G, Fan Y, Kubareva EA, Romanova EA, Lebedeva NA, Oretskaya TS, and Lavrik OI. DNA polymerases beta and lambda bypass thymine glycol in gapped DNA structures. *Biochemistry* 49: 4695–4704, 2010.
- Bhattacharya B, Puri S, and Puri RK. A review of gene expression profiling of human embryonic stem cell lines and their differentiated progeny. *Curr Stem Cell Res Ther* 4: 98–106, 2009.
- Bjelland S, and Seeberg E. Mutagenicity, toxicity and repair of DNA base damage induced by oxidation. *Mutat Res* 531: 37–80, 2003.
- Boyes J, and Bird A. Repression of genes by DNA methylation depends on CpG density and promoter strength: evidence for involvement of a methyl-CpG binding protein. *EMBO J* 11: 327–333, 1992.
- Butler JS, Koutelou E, Schibler AC, and Dent SY. Histone-modifying enzymes: regulators of developmental decisions and drivers of human disease. *Epigenomics* 4: 163–177, 2012.
- Campalans A, Marsin S, Nakabeppu Y, O'Connor TR, Boiteux S, and Radicella JP. XRCC1 interactions with multiple DNA glycosylases: a model for its recruitment to base excision repair. *DNA Repair (Amst)* 4: 826–835, 2005.
- Cedar H, and Bergman Y. Epigenetics of haematopoietic cell development. *Nat Rev Immunol* 11: 478–488, 2011.
- Chen H, Xu L, Qi Q, Yao Y, Zhu M, and Wang Y. A haplotype variation affecting the mitochondrial transportation of hMYH protein could be a risk factor for colorectal cancer in Chinese. *BMC Cancer* 8: 269, 2008.
- Chetsanga CJ, and Grigorian C. A dose-response study on opening of imidazole ring of adenine in DNA by ionizing radiation. *Int J Radiat Biol Relat Stud Phys Chem Med* 44: 321–331, 1983.
- Chi P, Allis CD, and Wang GG. Covalent histone modifications—miswritten, misinterpreted and mis-erased in human cancers. *Nat Rev Cancer* 10: 457–469, 2010.
- Cloos PA, Christensen J, Agger K, Maiolica A, Rappsilber J, Antal T, Hansen KH, and Helin K. The putative oncogene GASC1 demethylates tri- and dimethylated lysine 9 on histone H3. *Nature* 442: 307–311, 2006.
- Cooke MS, Loft S, Olinski R, Evans MD, Bialkowski K, Wagner JR, Dedon PC, Moller P, Greenberg MM, and Cadet J. Recommendations for standardized description of and nomenclature concerning oxidatively damaged nucleobases in DNA. *Chem Res Toxicol* 23: 705–707, 2010.
- Cortellino S, Xu J, Sannai M, Moore R, Caretti E, Cigliano A, Le Coz M, Devarajan K, Wessels A, Soprano D, Abramowitz LK, Bartolomei MS, Rambow F, Bassi MR, Bruno T,

- Fanciulli M, Renner C, Klein-Szanto AJ, Matsumoto Y, Kobi D, Davidson I, Alberti C, Larue L, and Bellacosa A. Thymine DNA glycosylase is essential for active DNA demethylation by linked deamination-base excision repair. *Cell* 146: 67–79, 2011.
23. Couture JF, Collazo E, Ortiz-Tello PA, Brunzelle JS, and Trievel RC. Specificity and mechanism of JMJD2A, a trimethyllysine-specific histone demethylase. *Nat Struct Mol Biol* 14: 689–695, 2007.
 24. Dahl C, Gronbaek K, and Guldborg P. Advances in DNA methylation: 5-hydroxymethylcytosine revisited. *Clin Chim Acta; International Journal Of Clinical Chemistry* 412: 831–836, 2011.
 25. Das A, Wiederhold L, Leppard JB, Kedar P, Prasad R, Wang H, Boldogh I, Karimi-Busheri F, Weinfeld M, Tomkinson AE, Wilson SH, Mitra S, and Hazra TK. NEIL2-initiated, APE-independent repair of oxidized bases in DNA: evidence for a repair complex in human cells. *DNA Repair (Amst)* 5: 1439–1448, 2006.
 26. David SS, O'Shea VL, and Kundu S. Base-excision repair of oxidative DNA damage. *Nature* 447: 941–950, 2007.
 27. Doege CA, Inoue K, Yamashita T, Rhee DB, Travis S, Fujita R, Guarnieri P, Bhagat G, Vanti WB, Shih A, Levine RL, Nik S, Chen EL, and Abeliovich A. Early-stage epigenetic modification during somatic cell reprogramming by Parp1 and Tet2. *Nature* 488: 652–655, 2012.
 28. Eads CA, Lord RV, Wickramasinghe K, Long TI, Kurumboor SK, Bernstein L, Peters JH, DeMeester SR, DeMeester TR, Skinner KA, and Laird PW. Epigenetic patterns in the progression of esophageal adenocarcinoma. *Cancer Res* 61: 3410–3418, 2001.
 29. Fang R, Barbera AJ, Xu Y, Rutenberg M, Leonor T, Bi Q, Lan F, Mei P, Yuan GC, Lian C, Peng J, Cheng D, Sui G, Kaiser UB, Shi Y, and Shi YG. Human LSD2/KDM1b/AOF1 regulates gene transcription by modulating intragenic H3K4me2 methylation. *Mol Cells* 39: 222–233, 2010.
 30. Feig DI, Sowers LC, and Loeb LA. Reverse chemical mutagenesis: identification of the mutagenic lesions resulting from reactive oxygen species-mediated damage to DNA. *Proc Natl Acad Sci U S A* 91: 6609–6613, 1994.
 31. Feinberg AP, Ohlsson R, and Henikoff S. The epigenetic progenitor origin of human cancer. *Nat Rev Genet* 7: 21–33, 2006.
 32. Fernandez PC, Frank SR, Wang L, Schroeder M, Liu S, Greene J, Cocito A, and Amati B. Genomic targets of the human c-Myc protein. *Genes Dev* 17: 1115–1129, 2003.
 33. Fischhaber PL, Gerlach VL, Feaver WJ, Hatahet Z, Wallace SS, and Friedberg EC. Human DNA polymerase kappa bypasses and extends beyond thymine glycols during translesion synthesis *in vitro*, preferentially incorporating correct nucleotides. *J Biol Chem* 277: 37604–37611, 2002.
 34. Flicek P, Amode MR, Barrell D, Beal K, Brent S, Carvalho-Silva D, Clapham P, Coates G, Fairley S, Fitzgerald S, Gil L, Gordon L, Hendrix M, Hourlier T, Johnson N, Kahari AK, Keefe D, Keenan S, Kinsella R, Komorowska M, Koscielny G, Kulesha E, Larsson P, Longden I, McLaren W, Muffato M, Overduin B, Pignatelli M, Pritchard B, Riat HS, Ritchie GR, Ruffier M, Schuster M, Sobral D, Tang YA, Taylor K, Trevanion S, Vandrovicova J, White S, Wilson M, Wilder SP, Aken BL, Birney E, Cunningham F, Dunham I, Durbin R, Fernandez-Suarez XM, Harrow J, Herrero J, Hubbard TJ, Parker A, Proctor G, Spudich G, Vogel J, Yates A, Zadissa A, and Searle SM. Ensembl 2012. *Nucleic Acids Res* 40: D84–90, 2012.
 35. Forneris F, Binda C, Vanoni MA, Mattevi A, and Battaglioli E. Histone demethylation catalysed by LSD1 is a flavin-dependent oxidative process. *FEBS Lett* 579: 2203–2207, 2005.
 36. Frenkel K, Goldstein MS, Duker NJ, and Teebor GW. Identification of the cis-thymine glycol moiety in oxidized deoxyribonucleic acid. *Biochemistry* 20: 750–754, 1981.
 37. Gao Y, Katyal S, Lee Y, Zhao J, Rehg JE, Russell HR, and McKinnon PJ. DNA ligase III is critical for mtDNA integrity but not Xrcc1-mediated nuclear DNA repair. *Nature* 471: 240–244, 2011.
 38. Gaymes TJ, Shall S, MacPherson LJ, Twine NA, Lea NC, Farzaneh F, and Mufti GJ. Inhibitors of poly ADP-ribose polymerase (PARP) induce apoptosis of myeloid leukemic cells: potential for therapy of myeloid leukemia and myelodysplastic syndromes. *Haematologica* 94: 638–646, 2009.
 39. Gehring M, Huh JH, Hsieh TF, Penterman J, Choi Y, Harada JJ, Goldberg RB, and Fischer RL. DEMETER DNA glycosylase establishes MEDEA polycomb gene self-imprinting by allele-specific demethylation. *Cell* 124: 495–506, 2006.
 40. Gehring M, Reik W, and Henikoff S. DNA demethylation by DNA repair. *Trends Genet* 25: 82–90, 2009.
 41. Greer EL, and Shi Y. Histone methylation: a dynamic mark in health, disease and inheritance. *Nat Rev Genet* 13: 343–357, 2012.
 42. Guccione E, Martinato F, Finocchiaro G, Luzi L, Tizzoni L, Dall'Olio V, Zardo G, Nervi C, Bernard L, and Amati B. Myc-binding-site recognition in the human genome is determined by chromatin context. *Nat Cell Biol* 8: 764–770, 2006.
 43. Guo JU, Su Y, Zhong C, Ming GL, and Song H. Emerging roles of TET proteins and 5-hydroxymethylcytosines in active DNA demethylation and beyond. *Cell Cycle* 10: 2662–2668, 2011.
 44. Hailer MK, Slade PG, Martin BD, Rosenquist TA, and Sugden KD. Recognition of the oxidized lesions spiroiminodihydroantoin and guanidinohydroantoin in DNA by the mammalian base excision repair glycosylases NEIL1 and NEIL2. *DNA Repair (Amst)* 4: 41–50, 2005.
 45. Hajkova P, Jeffries SJ, Lee C, Miller N, Jackson SP, and Surani MA. Genome-wide reprogramming in the mouse germ line entails the base excision repair pathway. *Science* 329: 78–82, 2010.
 46. Hammoud SS, Cairns BR, and Carrell DT. Analysis of gene-specific and genome-wide sperm DNA methylation. *Methods Mol Biol* 927: 451–458, 2013.
 47. Hanes JW, Thal DM, and Johnson KA. Incorporation and replication of 8-oxo-deoxyguanosine by the human mitochondrial DNA polymerase. *J Biol Chem* 281: 36241–36248, 2006.
 48. He YF, Li BZ, Li Z, Liu P, Wang Y, Tang Q, Ding J, Jia Y, Chen Z, Li L, Sun Y, Li X, Dai Q, Song CX, Zhang K, He C, and Xu GL. Tet-mediated formation of 5-carboxylcytosine and its excision by TDG in mammalian DNA. *Science* 333: 1303–1307, 2011.
 49. Hegde ML, Hegde PM, Rao KS, and Mitra S. Oxidative genome damage and its repair in neurodegenerative diseases: function of transition metals as a double-edged sword. *J Alzheimer's Dis: JAD* 24 Suppl 2: 183–198, 2011.
 50. Hoeijmakers JH. Genome maintenance mechanisms for preventing cancer. *Nature* 411: 366–374, 2001.
 51. Hoffmann S, Spitzkovsky D, Radicella JP, Epe B, and Wiesner RJ. Reactive oxygen species derived from the mi-

- tochondrial respiratory chain are not responsible for the basal levels of oxidative base modifications observed in nuclear DNA of mammalian cells. *Free Radic Biol Med* 36: 765–773, 2004.
52. Huang Y, Vasilatos SN, Boric L, Shaw PG, and Davidson NE. Inhibitors of histone demethylation and histone deacetylation cooperate in regulating gene expression and inhibiting growth in human breast cancer cells. *Breast Cancer Res Treat* 131: 777–789, 2012.
 53. Ito S, Shen L, Dai Q, Wu SC, Collins LB, Swenberg JA, He C, and Zhang Y. Tet proteins can convert 5-methylcytosine to 5-formylcytosine and 5-carboxylcytosine. *Science* 333: 1300–1303, 2011.
 54. Jaenisch R, and Bird A. Epigenetic regulation of gene expression: how the genome integrates intrinsic and environmental signals. *Nat Genet* 33 Suppl: 245–254, 2003.
 55. Jenuwein T, and Allis CD. Translating the histone code. *Science* 293: 1074–1080, 2001.
 56. Jovanovic SV, and Simic MG. Mechanism of OH radical reaction with thymine and uracil derivatives. *J Am Chem Soc* 108: 5968–5972, 1986.
 57. Kalam MA, Haraguchi K, Chandani S, Loechler EL, Moriya M, Greenberg MM, and Basu AK. Genetic effects of oxidative DNA damages: comparative mutagenesis of the imidazole ring-opened formamidopyrimidines (Fapy lesions) and 8-oxo-purines in simian kidney cells. *Nucleic Acids Res* 34: 2305–2315, 2006.
 58. Karytinis A, Forneris F, Profumo A, Ciossani G, Battaglioli E, Binda C, and Mattevi A. A novel mammalian flavin-dependent histone demethylase. *J Biol Chem* 284: 17775–17782, 2009.
 59. Kasai H, and Nishimura S. Hydroxylation of deoxyguanosine at the C-8 position by ascorbic acid and other reducing agents. *Nucleic Acids Res* 12: 2137–2145, 1984.
 60. Kass SU, Landsberger N, and Wolffe AP. DNA methylation directs a time-dependent repression of transcription initiation. *Curr Biol* 7: 157–165, 1997.
 61. Kellinger MW, Song CX, Chong J, Lu XY, He C, and Wang D. 5-Formylcytosine and 5-carboxylcytosine reduce the rate and substrate specificity of RNA polymerase II transcription. *Nat Struct Mol Biol* 19: 831–833, 2012.
 62. Klose RJ, Kallin EM, and Zhang Y. JmjC-domain-containing proteins and histone demethylation. *Nat Rev Genet* 7: 715–727, 2006.
 63. Ko M, Huang Y, Jankowska AM, Pape UJ, Tahiliani M, Bandukwala HS, An J, Lamperti ED, Koh KP, Ganetzky R, Liu XS, Aravind L, Agarwal S, Maciejewski JP, and Rao A. Impaired hydroxylation of 5-methylcytosine in myeloid cancers with mutant TET2. *Nature* 468: 839–843, 2010.
 64. Kooistra SM, and Helin K. Molecular mechanisms and potential functions of histone demethylases. *Nat Rev Mol Cell Biol* 13: 297–311, 2012.
 65. Krahn JM, Beard WA, Miller H, Grollman AP, and Wilson SH. Structure of DNA polymerase beta with the mutagenic DNA lesion 8-oxodeoxyguanine reveals structural insights into its coding potential. *Structure* 11: 121–127, 2003.
 66. Kusumoto R, Masutani C, Iwai S, and Hanaoka F. Translesion synthesis by human DNA polymerase eta across thymine glycol lesions. *Biochemistry* 41: 6090–6099, 2002.
 67. Lee DH, and Pfeifer GP. Translesion synthesis of 7,8-dihydro-8-oxo-2'-deoxyguanosine by DNA polymerase eta *in vivo*. *Mutat Res* 641: 19–26, 2008.
 68. Liang Y, Russell I, Walworth C, and Chen C. Gene expression in stem cells. *Crit Rev Eukaryot Gene Expr* 19: 289–300, 2009.
 69. Markkanen E, Hubscher U, and van Loon B. Regulation of oxidative DNA damage repair: the adenine:8-oxo-guanine problem. *Cell Cycle* 11: 1070–1075, 2012.
 70. Markkanen E, van Loon B, Ferrari E, Parsons JL, Dianov GL, and Hubscher U. Regulation of oxidative DNA damage repair by DNA polymerase lambda and MutYH by cross-talk of phosphorylation and ubiquitination. *Proc Natl Acad Sci U S A* 109: 437–442, 2012.
 71. Miller H, Prasad R, Wilson SH, Johnson F, and Grollman AP. 8-oxodGTP incorporation by DNA polymerase beta is modified by active-site residue Asn279. *Biochemistry* 39: 1029–1033, 2000.
 72. Morales-Ruiz T, Ortega-Galisteo AP, Ponferrada-Marin MI, Martinez-Macias MI, Ariza RR, and Roldan-Arjona T. DEMETER and REPRESSOR OF SILENCING 1 encode 5-methylcytosine DNA glycosylases. *Proc Natl Acad Sci U S A* 103: 6853–6858, 2006.
 73. Murray K. The occurrence of epsilon-N-methyl lysine in histones. *Biochemistry* 3: 10–15, 1964.
 74. Neeley WL, and Essigmann JM. Mechanisms of formation, genotoxicity, and mutation of guanine oxidation products. *Chem Res Toxicol* 19: 491–505, 2006.
 75. Ng SS, Kavanagh KL, McDonough MA, Butler D, Pilka ES, Lienard BM, Bray JE, Savitsky P, Gileadi O, von Delft F, Rose NR, Offer J, Scheinost JC, Borowski T, Sundstrom M, Schofield CJ, and Oppermann U. Crystal structures of histone demethylase JMJD2A reveal basis for substrate specificity. *Nature* 448: 87–91, 2007.
 76. Niehrs C. Active DNA demethylation and DNA repair. *Differentiation; Research In Biological Diversity* 77: 1–11, 2009.
 77. Olinski R, Zastawny T, Budzbon J, Skokowski J, Zegarski W, and Dizdaroglu M. DNA base modifications in chromatin of human cancerous tissues. *FEBS Lett* 309: 193–198, 1992.
 78. Perillo B, Ombra MN, Bertoni A, Cuzzo C, Sacchetti S, Sasso A, Chiariotti L, Malorni A, Abbondanza C, and Avvedimento EV. DNA oxidation as triggered by H3K9me2 demethylation drives estrogen-induced gene expression. *Science* 319: 202–206, 2008.
 79. Rai K, Huggins IJ, James SR, Karpf AR, Jones DA, and Cairns BR. DNA demethylation in zebrafish involves the coupling of a deaminase, a glycosylase, and gadd45. *Cell* 135: 1201–1212, 2008.
 80. Seward DJ, Cubberley G, Kim S, Schonewald M, Zhang L, Triplet B, and Bentley DL. Demethylation of trimethylated histone H3 Lys4 *in vivo* by JARID1 JmjC proteins. *Nat Struct Mol Biol* 14: 240–242, 2007.
 81. Shi Y, Lan F, Matson C, Mulligan P, Whetstine JR, Cole PA, and Casero RA. Histone demethylation mediated by the nuclear amine oxidase homolog LSD1. *Cell* 119: 941–953, 2004.
 82. Shi YY, Huang ZY, Zeng ZJ, Wang ZL, Wu XB, and Yan WY. Diet and cell size both affect queen-worker differentiation through DNA methylation in honey bees (*Apis mellifera*, Apidae). *PLoS ONE* 6: e18808, 2011.
 83. Siegfried Z, and Cedar H. DNA methylation: a molecular lock. *Curr Biol* 7: R305–307, 1997.
 84. Simsek D, Furda A, Gao Y, Artus J, Brunet E, Hadjantonakis AK, Van Houten B, Shuman S, McKinnon PJ, and Jasin M. Crucial role for DNA ligase III in mitochondria but not in Xrcc1-dependent repair. *Nature* 471: 245–248, 2011.
 85. Sobol RW. For MutY, it's all about the OG. *Chem Biol* 19: 313–314, 2012.
 86. Sobol RW, Prasad R, Evenski A, Baker A, Yang XP, Horton JK, and Wilson SH. The lyase activity of the DNA repair

- protein β -polymerase protects from DNA-damage-induced cytotoxicity. *Nature* 405: 807–810, 2000.
87. Strahl BD, and Allis CD. The language of covalent histone modifications. *Nature* 403: 41–45, 2000.
 88. Svilar D, Goellner EM, Almeida KH, and Sobol RW. Base excision repair and lesion-dependent sub-pathways for repair of oxidative DNA damage. *Antioxid Redox Signal* 14: 2491–2507, 2011.
 89. Szwagierczak A, Bultmann S, Schmidt CS, Spada F, and Leonhardt H. Sensitive enzymatic quantification of 5-hydroxymethylcytosine in genomic DNA. *Nucleic Acids Res* 38: e181, 2010.
 90. Tahiliani M, Koh KP, Shen Y, Pastor WA, Bandukwala H, Brudno Y, Agarwal S, Iyer LM, Liu DR, Aravind L, and Rao A. Conversion of 5-methylcytosine to 5-hydroxymethylcytosine in mammalian DNA by MLL partner TET1. *Science* 324: 930–935, 2009.
 91. Takata K, Shimizu T, Iwai S, and Wood RD. Human DNA polymerase η (POLN) is a low fidelity enzyme capable of error-free bypass of 5S-thymine glycol. *J Biol Chem* 281: 23445–23455, 2006.
 92. Tan X, Grollman AP, and Shibutani S. Comparison of the mutagenic properties of 8-oxo-7,8-dihydro-2'-deoxyadenosine and 8-oxo-7,8-dihydro-2'-deoxyguanosine DNA lesions in mammalian cells. *Carcinogenesis* 20: 2287–2292, 1999.
 93. Tsukada Y. Hydroxylation mediates chromatin demethylation. *J Biochem* 151: 229–246, 2012.
 94. Tsukada Y, and Zhang Y. Purification of histone demethylases from HeLa cells. *Methods* 40: 318–326, 2006.
 95. Turner BM. Histone acetylation and an epigenetic code. *Bioessays* 22: 836–845, 2000.
 96. Um S, Harbers M, Benecke A, Pierrat B, Losson R, and Chambon P. Retinoic acid receptors interact physically and functionally with the T:G mismatch-specific thymine-DNA glycosylase. *J Biol Chem* 273: 20728–20736, 1998.
 97. van Essen D, Zhu Y, and Saccani S. A feed-forward circuit controlling inducible NF-kappaB target gene activation by promoter histone demethylation. *Mol Cells* 39: 750–760, 2010.
 98. van Loon B, and Hubscher U. An 8-oxo-guanine repair pathway coordinated by MUTYH glycosylase and DNA polymerase lambda. *Proc Natl Acad Sci U S A* 106: 18201–18206, 2009.
 99. van Loon B, Markkanen E, and Hubscher U. Oxygen as a friend and enemy: how to combat the mutational potential of 8-oxo-guanine. *DNA Repair (Amst)*, 2010.
 100. Wang J, Scully K, Zhu X, Cai L, Zhang J, Prefontaine GG, Kronen A, Ohgi KA, Zhu P, Garcia-Bassets I, Liu F, Taylor H, Lozach J, Jayes FL, Korach KS, Glass CK, Fu XD, and Rosenfeld MG. Opposing LSD1 complexes function in developmental gene activation and repression programmes. *Nature* 446: 882–887, 2007.
 101. Whetstine JR, Nottke A, Lan F, Huarte M, Smolnikov S, Chen Z, Spooner E, Li E, Zhang G, Colaiacovo M, and Shi Y. Reversal of histone lysine trimethylation by the JMJD2 family of histone demethylases. *Cell* 125: 467–481, 2006.
 102. Wiederhold L, Leppard JB, Kedar P, Karimi-Busheri F, Rasouli-Nia A, Weinfeld M, Tomkinson AE, Izumi T, Prasad R, Wilson SH, Mitra S, and Hazra TK. AP endonuclease-independent DNA base excision repair in human cells. *Mol Cells* 15: 209–220, 2004.
 103. Wood A, and Shilatifard A. Posttranslational modifications of histones by methylation. *Adv Protein Chem* 67: 201–222, 2004.
 104. Yamane K, Toumazou C, Tsukada Y, Erdjument-Bromage H, Tempst P, Wong J, and Zhang Y. JHDM2A, a JmjC-containing H3K9 demethylase, facilitates transcription activation by androgen receptor. *Cell* 125: 483–495, 2006.
 105. Zeller KI, Zhao X, Lee CW, Chiu KP, Yao F, Yustein JT, Ooi HS, Orlov YL, Shahab A, Yong HC, Fu Y, Weng Z, Kuznetsov VA, Sung WK, Ruan Y, Dang CV, and Wei CL. Global mapping of c-Myc binding sites and target gene networks in human B cells. *Proc Natl Acad Sci U S A* 103: 17834–17839, 2006.
 106. Zhang Y, Yuan F, Wu X, Taylor JS, and Wang Z. Response of human DNA polymerase ϵ to DNA lesions. *Nucleic Acids Res* 29: 928–935, 2001.
 107. Zhang Y, Yuan F, Wu X, Wang M, Rechkoblit O, Taylor JS, Geacintov NE, and Wang Z. Error-free and error-prone lesion bypass by human DNA polymerase κ *in vitro*. *Nucleic Acids Res* 28: 4138–4146, 2000.
 108. Zhu B, Benjamin D, Zheng Y, Anglikler H, Thiry S, Siegmann M, and Jost JP. Overexpression of 5-methylcytosine DNA glycosylase in human embryonic kidney cells ECR293 demethylates the promoter of a hormone-regulated reporter gene. *Proc Natl Acad Sci U S A* 98: 5031–5036, 2001.
 109. Ziel KA, Grishko V, Campbell CC, Breit JF, Wilson GL, and Gillespie MN. Oxidants in signal transduction: impact on DNA integrity and gene expression. *FASEB J* 19: 387–394, 2005.

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Abbreviations Used

α -KG	= α -ketoglutarate
5caC	= 5-carboxylcytosine
5'dRP	= 5'deoxyribose-phosphate
5fC	= 5-formylcytosine
5hmC	= 5-hydroxy-methyl-cytosine
5hmU	= 5-hydroxymethyluracil
5mC	= 5-methylcytosine
5-MCDG	= 5-methylcytosine DNA glycosylase
8-oxodA	= 8-oxo-7,8-dihydro-2'-deoxyadenosine
8-oxodG	= 8-oxo-7,8-dihydro-2'- deoxyguanosine
A	= adenine
AcH3K9	= acetylation of histone H3 lysine9
ADP	= adenosine diphosphate
AID	= activation-induced deaminase
AP	= apurinic/aprimidinic
APE1	= apurinic/aprimidinic endonucleases 1
APE2	= apurinic/aprimidinic endonucleases 2
APOBEC	= apolipoprotein B mRNA-editing enzyme complex

Abbreviations Used (Cont.)

BCADD = BER-coupled active DNA demethylation
 bcl-2 = B-cell CLL/lymphoma 2
 BER = base-excision repair
 bp = base pairs
 C = cytosine
 CAD = carbamoyl-phosphate synthetase 2, aspartate transcarbamylase, and dihydroorotase
 ChIP = chromatin immunoprecipitation
 CoRest = chromatin-modifying corepressor
 CpG = deoxy-cytidylate-phosphate-deoxy-guanylate
 CR = coding region
 CW-type = cysteine and tryptophan-type zinc-finger motif
 dAMP = deoxyadenosine monophosphate
 dCTP = deoxycytidine triphosphate
 Demeter = transcriptional activator DEMETER
 DNA = deoxyribonucleic acid
 DOT1 = disruptor of telomeric silencing 1
 DOT1L = disruptor of telomere silencing 1-like
 E-box = CACGTG
 E2 = 17 β -estradiol
 Enh = enhancers
 ER = estrogen receptor
 EREs = estrogen-response elements
 ESCs = embryonic stem cells
 FAD = flavin adenine dinucleotide
 FADH = flavin adenine dinucleotide, reduced
 FapydA = 4,6-diamino-5-formamidopyrimidine
 G = Guanine
 Gadd45a = DNA damage-inducible protein 45-alpha
 H2A = histone H2A
 H2B = histone H2B
 H₂O₂ = hydrogen peroxide
 H3 = histone H3
 H3K4me0 = unmethylated histone H3 lysine 4
 H3K4me1 = monomethylated histone H3 lysine 4
 H3K4me2 = dimethylated histone H3 lysine 4
 H3K4me3 = trimethylated histone H3 lysine 4
 H3K9me2 = dimethylated histone H3 lysine 9
 H4 = histone H4
 HAT = histone acetyltransferase
 HDAC = histone deacetylase
 HKMTs = histone lysine methyl transferases
 Il12b = interleukin 12B (natural killer cell stimulatory factor 2)
 iPSCs = inducible pluripotent stem cells
 JmjC = Jumonji
 Kdm1a = lysine-specific demethylase 1
 Klf4 = Kruppel-like factor 4
 LC-BER = LSD1/LSD2-coupled BER
 LigI = DNA ligase I
 LigIII = DNA ligase III
 LSD1 = lysine-specific demethylase 1
 LSD2 = lysine-specific demethylase 2
 Lys = lysine

MBD4 = methyl-CpG-binding domain protein 4
 Mdc = ADAM metallopeptidase domain 11
 MEFs = mouse embryonic fibroblasts
 MPG = methyl purine-DNA glycosylase
 MUTYH = mutY homolog
 Myc = V-myc myelocytomatosis viral oncogene homolog (avian)
 NAC = *N*-acetyl-L-cysteine
 Ncl = nucleolin
 NEIL1 = Nei endonuclease VIII-like 1
 NEIL2 = Nei endonuclease VIII-like 2
 NEIL3 = Nei endonuclease VIII-like 3
 NF- κ B = nuclear factor kappa light-chain enhancer of activated B cells
 NTHL1 = endonuclease III-like protein 1
 OGG1 = 8-oxoguanine glycosylase
 OH5dC = 5-hydroxy-2'-deoxycytidine
 OHT = tamoxifen
 OSKM = Oct4, Sox2, Klf4, and c-Myc
 PAR = poly(ADP)ribose
 PARG = poly (ADP-ribose) glycohydrolase
 PARP1 = poly (ADP-ribose) polymerase 1
 PARP2 = poly (ADP-ribose) polymerase 2
 PNKP = bifunctional polynucleotide phosphatase/kinase
 Pol = polymerase
 Pol β = DNA polymerase β
 Pr = promoter
 Pro = proline
 RNA Pol II = RNA polymerase II
 ROS = reactive oxygen species
 ROS1 = repressor of silencing1
 RXR = retinoid X receptor
 Ser = serine
 SET = Su(var)3-9/Enhancer of zeste/Trithorax
 siRNA = small interfering RNA
 SMUG1 = single-strand selective monofunctional uracil DNA glycosylase
 Sox2 = sex determining region Y-box 2
 SUMO = small ubiquitin-related modifier
 SWIRM = Swi3p, Rsc8p, and Moira
 T = thymine
 TDG = thymine-DNA glycosylase
 Tet = Ten-eleven translocation
 Tet1 = Ten-eleven translocation 1
 Tet2 = Ten-eleven translocation 2
 Tet3 = Ten-eleven translocation 3
 Tg = thymine glycol
 TopoIIb = topoisomerase II β
 TSS = transcription start site
 UNG = uracil-DNA glycosylase
 US = upstream sequence
 VEGF = vascular endothelial growth factor A
 XPG = xeroderma pigmentosum, complementation group G
 XRCC1 = X-ray repair cross-complementing protein 1