Review

Interplay between DNA Polymerases and DNA Ligases: Influence on Substrate Channeling and the Fidelity of DNA Ligation

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Abstract

DNA ligases are a highly conserved group of nucleic acid enzymes that play an essential role in DNA repair, replication, and recombination. This review focuses on functional interaction between DNA polymerases and DNA ligases in the repair of single- and double-strand DNA breaks, and discusses the notion that the substrate channeling during DNA polymerase-mediated nucleotide insertion coupled to DNA ligation could be a mechanism to minimize the release of potentially mutagenic repair intermediates. Evidence suggesting that DNA ligases are essential for cell viability includes the fact that defects or insufficiency in DNA ligase are casually linked to genome instability. In the future, it may be possible to develop small molecule inhibitors of mammalian DNA ligases and/or their functional protein partners that potentiate the effects of chemotherapeutic compounds and improve cancer treatment outcomes.

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Introduction

DNA ligases are ubiquitous cellular proteins that belong to the large family of nucleotidyl transferases. These enzymes catalyze phosphodiester bond formation to seal the nick consisting of 5′-phosphate (P) and 3′-hydroxyl (OH) termini on ends of the broken DNA strand [1,2]. There are three DNA ligase genes, *Lig I*, *III*, and *IV*, that encode mammalian DNA ligases I, III, and IV, respectively [3]. *Lig III* encodes two polypeptides, DNA ligase IIIα and β [4]. All three mammalian DNA ligases as well as prototypical bacterial DNA ligases contain a highly conserved C-terminal catalytic core consisting of the oligonucleotide binding domain (OBD) and the adenylation domain (AdD) or nucleotidyl transferase domain [5] (Fig. 1). The AdD domain contains the adenosine monophosphate (AMP) binding pocket and the active-site lysine residue that becomes adenylated, while the OBD includes only catalytic residues [6,7]. Mammalian DNA ligases also harbor the DNA binding domain (DBD). This domain stimulates catalytic activity, most likely through its ability to stabilize DNA conformation in which 5′-P and 3′-OH DNA termini at the nick are well positioned to interact with the active-site lysine in the DNA ligase catalytic core [8–10]. In mammalian DNA ligases, the DBD stabilizes the DNA and interacts with both the minor groove of the nicked DNA as well as the OBD to make a ring-shaped protein structure that encircles the DNA during nick sealing (Fig. 2), as shown in the crystal structure of the catalytic core of human DNA ligase I in complex with adenylated DNA [11–13]. This conformational flexibility allows DNA ligase to open and close around the DNA, thereby optimizing the position of the nick in the enzyme active site. In addition to the highly conserved catalytic core, mammalian DNA ligases contain amino (N)- and carboxyl (C)-terminal non-catalytic residues (Fig. 1), which promote protein–protein interactions and subcellular localization [1,5,10]

DNA Ligase Structure and Reaction Mechanism

All three mammalian DNA ligases utilize adenosine triphosphate (ATP) as a high-energy cofactor during

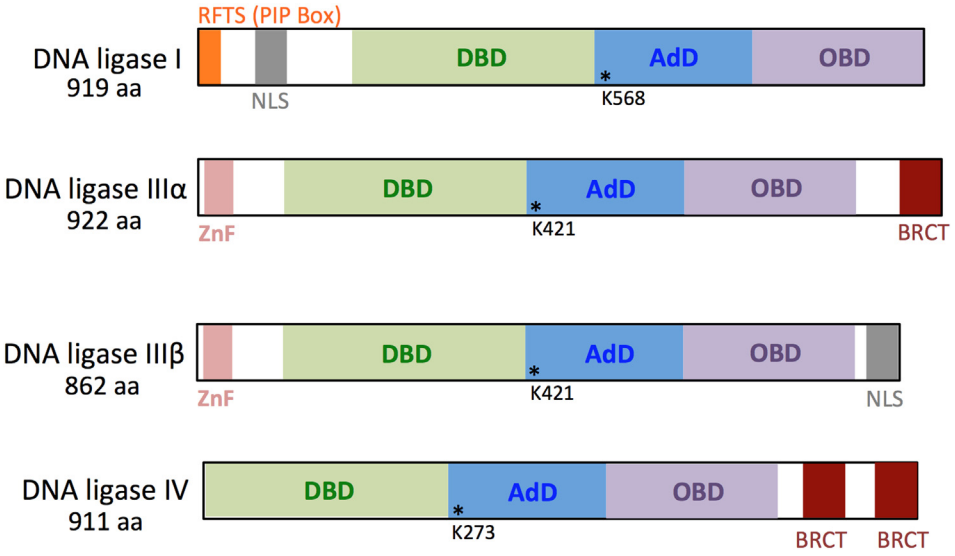

Fig. 1. Structural domain organization of nuclear human DNA ligases. DNA ligases contain the N-terminal DBD (green) and the C-terminal catalytic core consisting of the AdD (blue) and the OBD (purple). DNA ligase III isoforms α and β include an N-terminal zinc finger (ZnF; pink). DNA ligases IIIα and IV have a breast and ovarian cancer susceptibility protein 1 (BRCT) C-terminal domain (red). While DNA ligases I and IIIβ have a nuclear localization signal (NLS; gray), the N-terminal region of DNA ligase I also contains a replication factory targeting sequence (RFTS; orange) also known as a PCNA-interacting peptide (PIP) box. The active-site lysine residues that become adenylated during the first step of ligation reaction in the AdD domain that contains an AMP binding pocket are shown as asterisks.

catalysis [5,10,14,15]. The universally conserved pathway of DNA ligation reaction involves three chemical sequential steps (Fig. 2). In the first step, enzyme adenylation and formation of DNA ligase-adenylate intermediate occur. This step requires that the active-site lysine residue in the AdD attacks and forms a covalent bond with the α-phosphate of ATP [16–18]. During the second step, DNA ligase binds to the nicked DNA, and the AMP is transferred from the adenylated DNA ligase to the 5′-P end of the nicked DNA, through the OBD, generating a 5′-adenylated (5′-AMP) DNA intermediate [19]. In the third and final step, DNA ligase catalyzes phosphodiester bond formation between adjacent 3′-OH and 5′-P DNA termini. In this reaction, the 3′-OH terminus of the nick attacks the 5′-P terminus downstream of the nick, and formation of the phosphodiester bond is coupled to release of AMP [14,20]. Pre-steady-state kinetic studies revealed that all three steps of DNA ligation require at least one divalent metal ion. Release of high-energy pyrophosphate coupled to adenylation of the active-site lysine during the first step of ligation makes the overall reaction irreversible [19,21]. While the three chemical steps occur at similar rates, and the efficiency of ligation is high at saturating concentrations of ATP and Mg^{2+} [22,23]. Also, DNA ligases show different propensity for the joining of DNA ends in response to the salt concentration in the reaction mixture during the ligation of single- and double-strand breaks (DSB) [24].

Fidelity of DNA Ligases and Ligation Failure

The joining of the 5′- and 3′-ends in the phosphodiester backbone of nicked DNA is critical for maintaining genome stability [25]. Single-strand break and DSB in DNA can be generated as a consequence of normal DNA transactions during cellular mechanisms [26]. These strand breaks can also occur as a consequence of DNA lesion removal by DNA repair pathway or be caused directly by a DNA damage-inducing agent, such as oxidative stress-inducing chemicals or ionizing radiation [27,28]. DNA ligases may fail in the presence of damaged or modified DNA ends, resulting in the formation of a 5′-adenylated DNA intermediate [29,30]. This abortive ligation product with the 5′-AMP group is itself very serious damage at the nick and could become a persistent DNA strand break if left unrepaired or left until DNA end processing proteins come to repair them [31,32]. As persistent DNA breaks are expected to be toxic, to block transcription and to be converted into DSBs upon DNA replication, the formation and repair of the ligation failure intermediates are expected to be critical in cellular viability and genome stability.

The multidomain architecture of DNA ligases enables them to sense the nick, which directs the enzyme toward appropriate DNA substrates for efficient ligation [1,5,6,10]. All three mammalian DNA

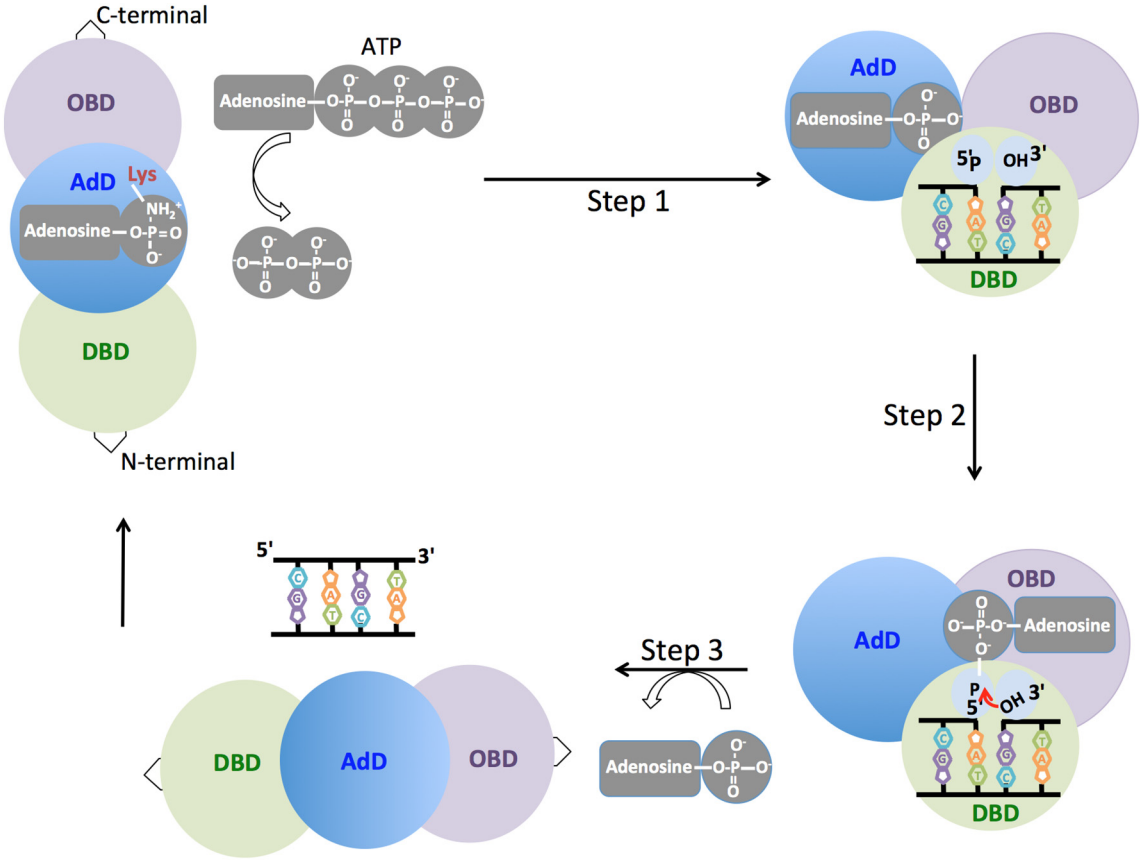

Fig. 2. Three chemical sequential steps of DNA ligation reaction. In the first step, the active-site lysine residue in the AdD (blue) of the DNA ligase interacts with ATP and the enzyme becomes adenylated. In the second step, the adenylated ligase binds to and encircles the nicked DNA through the DBD (green) and OBD (purple). During this step, the AMP is then transferred to the 5′-P end of the nicked DNA. In the third step, the non-adenylated DNA ligase utilizes the 3′-hydroxyl (3′-OH) terminus of the nick as a nucleophile to attack the 5′-adenylated DNA. This results in formation of the phosphodiester bond coupled to release of AMP.

ligases have different ligation fidelity depending on the structural differences in their three-domain core structures. For example, the zinc finger domain of DNA ligase IIIα is not required for catalytic activity but gives the enzyme a broader substrate range in the joining of blunt-ended duplex DNA than that of DNA ligase I, which is also significantly less efficient in the ligation of RNA-containing DNA substrates [24,33,34]. Biochemical studies have shown that heavy metals can also inhibit DNA ligases. For example, all three steps of the DNA ligation reaction are significantly inhibited by zinc (Zn) and cadmium (Cd), while the antitumor agent 9-β-D-arabinofuranosyl-2-fluoroade, known as an analog of adenosine and deoxyadenosine, specifically inhibits the formation of the DNA ligase–AMP complex during the first step of the ligation reaction [35,36]. Moreover, it has been reported that human DNA ligase I can also be inhibited by several DNA binding drugs, such as distamycin, ethidium bromide, and actinomycin [37].

DNA Ligation at the End of Single-Strand Break and DSB Repair

In mammalian cells, the base excision repair (BER) pathway is the major DNA repair mechanism in the cellular defense against single-strand breaks generated by reactive oxygen species (ROS), alkylating agents, and ionizing radiation [38–41]. There are two distinct BER subpathways: short-patch BER (SP-BER) and long-patch BER (LP-BER) [42,43]. These BER subpathways are differentiated by their repair patch sizes and the enzymes involved (Fig. 3). In SP-BER, the repair pathway is initiated by a DNA glycosylase that removes the damaged base (i.e., 8-oxo-7,8-dihydroguanine or 8-oxoG) through hydrolysis of the *N*-glycosidic bond linking the base to the sugar of the phosphodiester backbone [44]. This hydrolysis results in an abasic or apurinic/apyrimidinic (AP) site in double-stranded DNA. In the widely

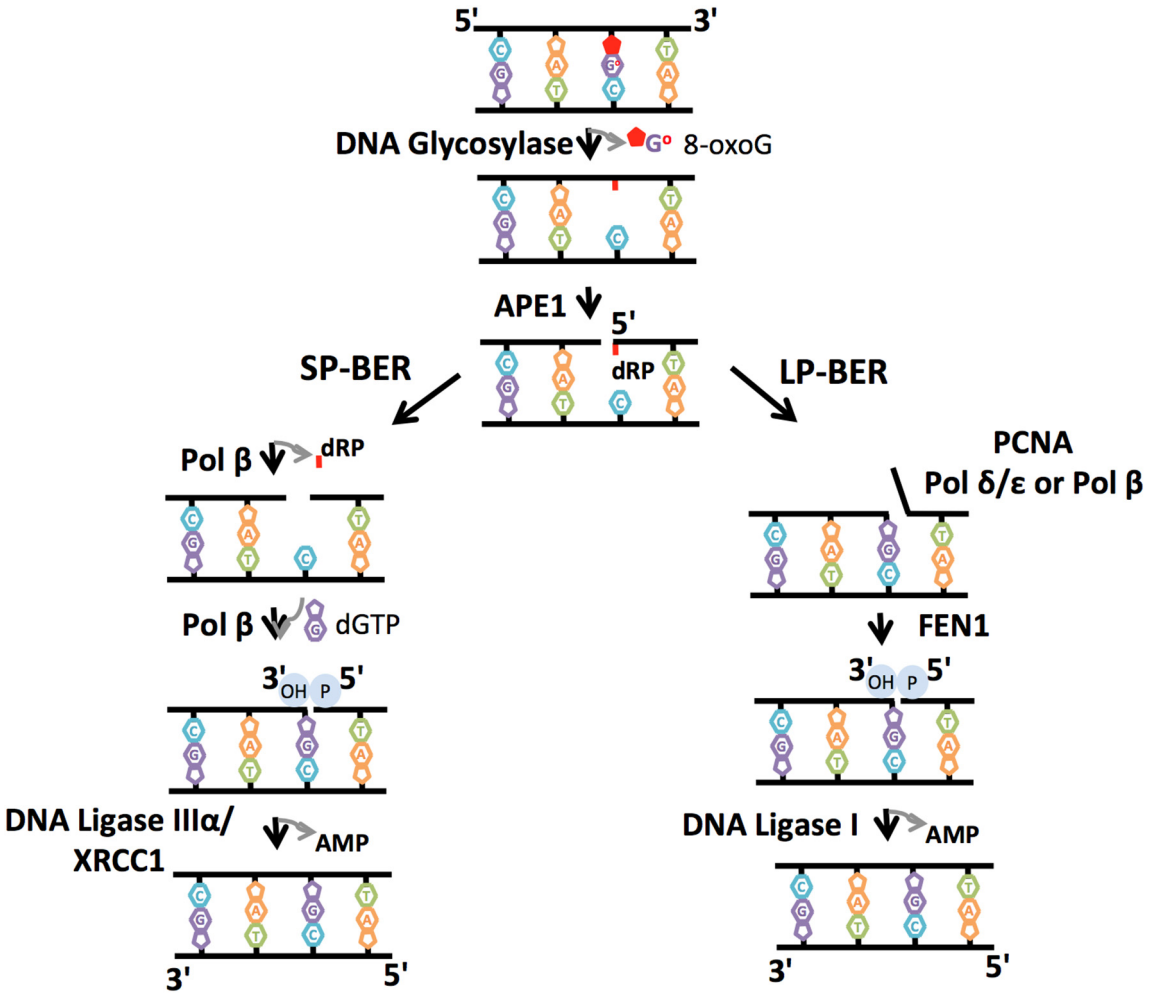

Fig. 3. Substrate channeling of DNA repair intermediates during single-strand break repair. The scheme represents the BER pathway that involves several sequential enzymatic steps and hand-off between BER proteins during short-patch BER (SP-BER) and long-patch BER (LP-BER) subpathways. A single DNA base damage (i.e., 8-oxoG) is initially removed by a DNA glycosylase, leaving an abasic site that is then processed by AP endonuclease 1 (APE1), leaving 3′-OH and 5′-dRP groups. In the SP-BER subpathway, DNA polymerase (pol) β removes the 5′-dRP group and fills the gap by inserting a single nucleotide (i.e., dGTP). In the LP-BER subpathway, if the dRP group is modified, PCNA-dependent DNA synthesis of 2-13 nucleotides by pol δ/ε or pol β and their removal by flap endonuclease 1 (FEN1) occur. DNA ligase IIIα/XRCC1 complex and DNA ligase I joins 3′-OH and 5′-P DNA termini to complete the SP-BER and LP-BER subpathways, respectively.

accepted model of SP-BER, AP endonuclease 1 (APE1) cleaves the phosphodiester backbone at the AP site, leaving 3′-OH and 5′-deoxyribose phosphate (dRP) groups at the termini in a single-nucleotide gap [45]. DNA polymerase (pol) β removes the 5′-dRP group and fills the gap by inserting a single nucleotide [46,47]. However, in the presence of a modified dRP moiety or a reduced AP site, LP-BER becomes the major BER pathway [48]. This subpathway involves strand displacement DNA synthesis by proliferating cell nuclear antigen (PCNA) and pol δ/pol ε, followed by the removal of flap-like structures of 2–10 nucleotides by flap endonuclease 1 (FEN1). The studies also suggest that pol β plays roles in the nucleotide insertion and extension steps during LP-BER [49]. Finally, DNA ligases finalize both BER subpathways at the end. DNA ligase IIIα/x-ray repair cross-complementing protein 1 (XRCC1) complex and DNA ligase I join 3′-OH and 5′-P groups to seal the nicked DNA intermediate to complete the SP-BER and LP-BER subpathways, respectively (Fig. 3). On the other hand, as shown in cell extracts immunodepleted of either DNA ligase I or IIIα, DNA ligase III can complement DNA ligase I during LP-BER, and DNA ligase I is able to efficiently substitute for DNA ligase IIIα/XRCC1 complex during SP-BER [50].

DSBs are the most dangerous type of DNA damage because the integrity of both strands of the DNA duplex is lost [51]. DSBs can be generated either by environmental toxicants, ionizing radiation, and ROS

or during cellular mechanisms such as DNA replication or meiosis [52]. DSBs are repaired through two pathways known as homology-based error-free homologous recombination and error-prone nonhomologous end-joining (NHEJ), which have different impacts on the fidelity and efficiency of DSB repair [53]. As a predominant DSB repair mechanism, classic or canonical NHEJ (cNHEJ) requires the recruitment of the DSB repair protein complex. This complex of core NHEJ factors includes the Ku 70 and 80 heterodimers, DNA protein kinase catalytic subunit (DNAPKcs), x-ray cross-complementing 4 (XRCC4), DNA ligase IV, XRCC-like factor (XLF), and Artemis [54,55]. In contrast to DNA ligases I and DNA ligase IIIα/XRCC1, which play roles in several DNA repair pathways as well as DNA replication, DNA ligase IV functions only in the repair of DSBs during NHEJ, which is mediated by its interaction with XRCC4 [56]. However, it has been shown that alternative end-joining activities including either DNA ligase I or DNA ligase IIIα/XRCC1 can substitute for DNA ligase IV in cells deficient in core cNHEJ factors, and all three DNA ligases may compete for the final ligation step during chromosome translocation [57].

Substrate Channeling during Single-Strand Break and DSB Repair

Based on the product-complex recognition model supported by biochemical and structural studies, the BER pathway proceeds in an orderly fashion through several enzymatic steps and protein–protein interactions (Fig. 3). The steps of the repair pathway involve substrate channeling mechanism [58,59]. The importance of this mechanism is the coordination of the substrate-product hand-off between BER enzymes that coordinate with one another to receive the DNA substrate and efficiently pass the resulting repair product along to the next enzyme [60,61]. This hand-

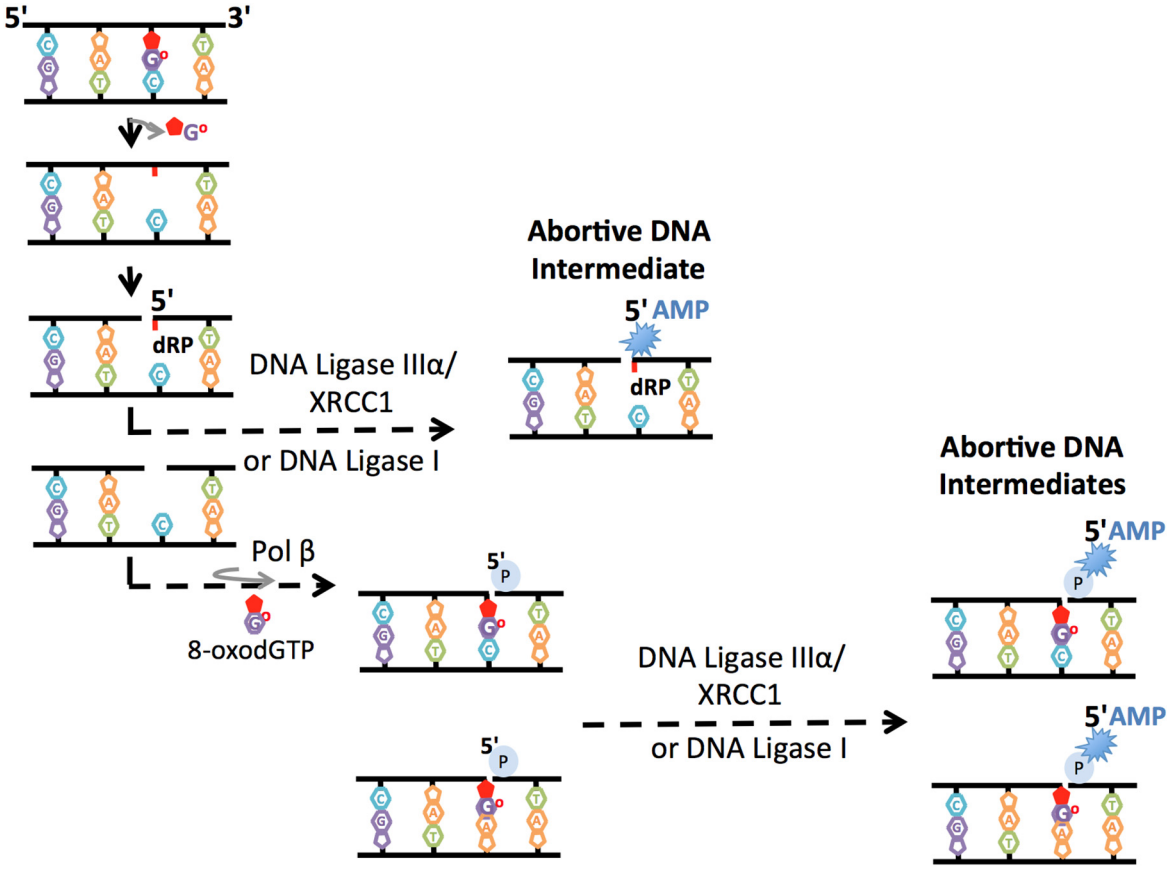

Fig. 4. Functional interaction between DNA polymerase β and DNA ligases during BER. The scheme represents the two possible enzymatic steps in which abortive DNA repair intermediates can be formed as a result of the lack of substrate channeling from DNA polymerase (pol) β to DNA ligase I or DNA ligase IIIα/XRCC1 complex. DNA ligase reaction may abort, and the BER intermediate could become 5′-adenylated, yielding the abortive DNA intermediate with the 5′-AMP-dRP group. Alternatively, pol β oxidized nucleotide (i.e., 8-oxodGTP) insertion opposite the template base A or C may lead to DNA ligase failure and formation of an abortive ligation product with a 3′-inserted oxidized base and a 5′-adenylate group (5′-AMP) on the repair intermediate.

off mechanism prevents the accumulation of toxic DNA strand break intermediates that could trigger cell cycle arrest, necrotic cell death, or apoptosis as well as harmful nuclease activities or recombination events [62]. The reconstitution of human BER with purified repair proteins, from the initial removal of a damaged base by a DNA glycosylase to the final ligation step by a DNA ligase, in a highly regulated manner has been shown in many studies [63–66]. This part of the review focuses on the substrate channeling from pol β to DNA ligase (DNA ligase I or DNA ligase IIIα/XRCC1 complex) during BER pathway (Fig. 4). It has been shown through affinity chromatography developed by crosslinking with an antibody against pol β that the BER proteins pol β and DNA ligase I are coimmunoprecipitated from bovine testis nuclear extracts [67]. Both BER proteins are crucial components of a multiprotein complex that repairs single-base DNA lesions *in vitro*. In another study using purified repair proteins, it was shown that the completely ligated product was observed when pol β and DNA ligase I were preincubated with a single-nucleotide gap including a DNA substrate with a 5′-dRP group. However, the repair intermediate after the pretreatment of the enzymes with trap DNA containing the 5′-P group resulted only in the formation of a ligated trap itself [64]. This finding indicates that the DNA is channeled from pol β to DNA ligase I only when these BER enzymes are prebound to the initial single-nucleotide BER intermediate. In addition, the channeling of substrates was shown using the pol β affinity-capture fractions in the cell extracts isolated from pol β-null mouse embryonic fibroblasts (MEFs) [60].

The assembly of multiprotein complexes and the formation of protein–protein interactions on DNA ends also play a crucial role in NHEJ during DSB repair (Fig. 5). The DNA ends at most DSBs usually

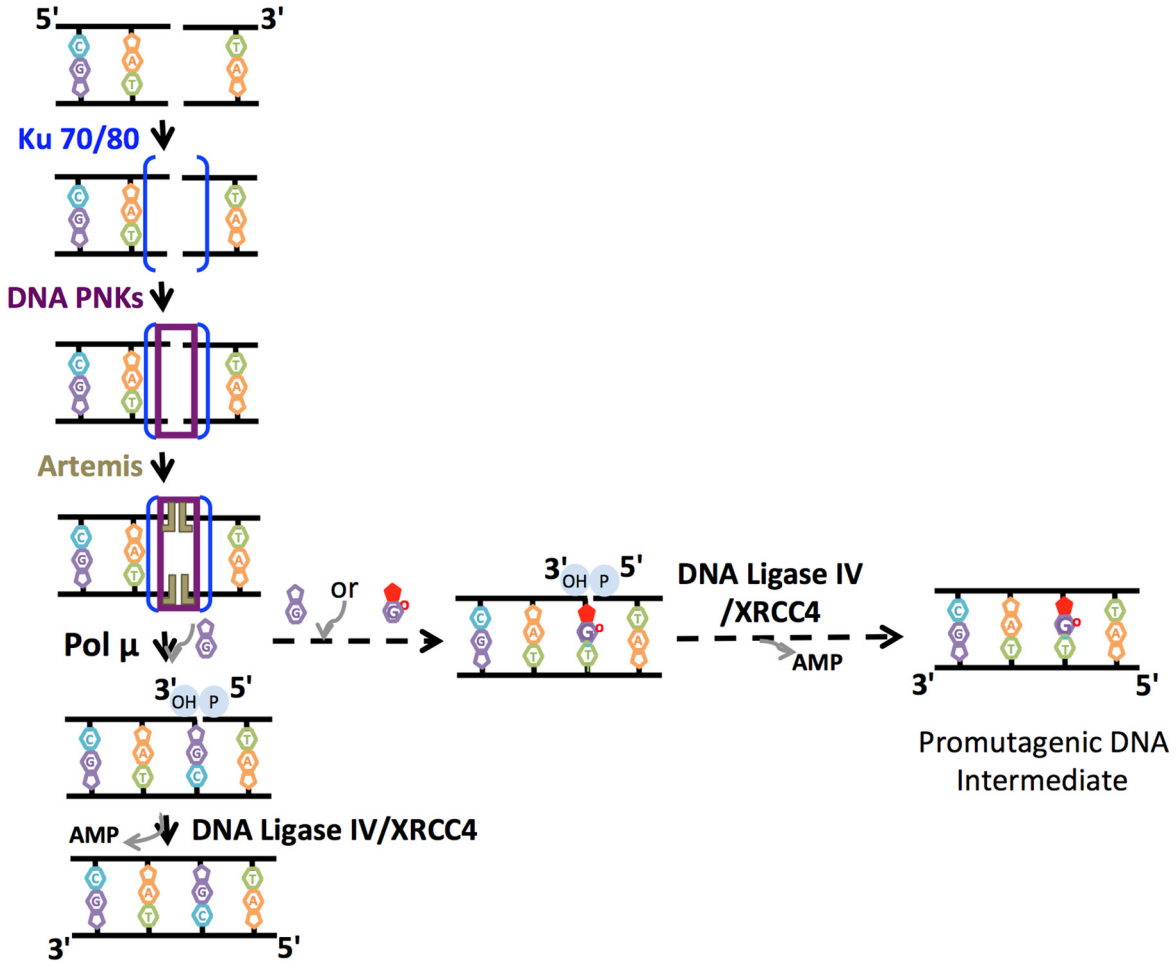

Fig. 5. Substrate channeling of DNA repair intermediates and functional interaction between DNA polymerase μ and DNA ligase IV during DSB repair. The scheme represents the recruitment of multi-protein complex and hand-off between DSB repair proteins during non-homologous end-joining pathway. DNA polymerase μ-mediated dG:T mismatch (dGTP or 8-oxodGTP insertion opposite template base T) insertion may lead to efficient DNA ligation and formation of promutagenic DNA repair intermediate.

require processing prior to the last DNA ligation step catalyzed by DNA ligase IV/XRCC4 complex [68]. This DNA end processing includes DNA synthesis by DNA polymerase (pol) μ, pol λ, or terminal deoxynucleotidyl transferase to fill the gaps generated during DNA end alignment [69,70]. However, the involvement of pol β in the repair of DSBs has also shown [71]. In a recently reported study, the hand-off of ribonucleotide-containing repair intermediates from pol μ-mediated nucleotide insertion to subsequent DNA ligation through "sequential coupled strand break repair reactions" during cellular NHEJ have been suggested in an *in vivo* model system [72].

Effect of DNA Polymerase Nucleotide Insertion on the Fidelity of DNA Ligation

The channeling of the pol β product to the next DNA ligation step enables the recognition of the DNA repair intermediate and its hand-off from pol β to DNA ligase during the repair pathway [59,60,64,73]. I previously demonstrated that BER DNA ligases, namely, DNA ligase I or the DNA ligase IIIα/XRCC1 complex, cannot ligate an abasic site in the absence of 5′-dRP group removal [74,75]. This inability results in uncoordinated DNA ligase activity that could lead to the "5′-adenylate block" of the dRP-containing BER intermediate (i.e., 5′-adenylated-dRP or 5′-AMP-dRP) (Fig. 4). The ligation of this intermediate by BER DNA ligases is highly sensitive to ATP and Mg^{2+} concentrations in the ligation reaction [74]. In another study, using a coupled repair assay that enables the simultaneous observation of DNA polymerase-mediated nucleotide insertion coupled to DNA ligation, I demonstrated that pol β 8-oxodGTP insertion opposite the template base A or C leads to ligase failure and interrupts the BER pathway due to a lack of substrate channeling from pol β to DNA ligase [76,77]. This interruption results in the formation of an abortive ligation product with a 3′-inserted oxidized base and a 5′-adenylate group on the repair intermediate (Fig. 4). In this study, I also reported that the extent of ligation failure is significantly sensitive to both the type of oxidized base inserted by pol β and the nature of the template base on the repair intermediate to be transferred to DNA ligase. Moreover, I demonstrated that BER DNA ligases respond to changes in the active-site residues that make unique contacts in the pol β precatalytic ternary substrate complex by comparing the base pairing of an incoming nucleotide 8-oxodGTP base with A *versus* C, which showed different ligation efficiencies. Overall, the findings indicate that the functional interaction between pol β and BER DNA ligases (DNA ligase I or DNA ligase IIIα/XRCC1 complex) could determine the fate of the repair at the last step of the repair pathway. The loss of this interaction could lead to the formation of abortive DNA intermediates that could become persistent DNA breaks, leading to genome instability, deleterious mutagenesis, and cytotoxicity [73].

All three human DNA ligases are very selective for the chemical characteristics of base pairs and show different abilities to join nicks, including 3′-mismatches [12,13]. It has been known that DNA polymerases with no proofreading capacity, such as pol β, can incorporate mismatch nucleotides during DNA repair [78]. Accordingly, the fidelity of human DNA ligases in terms of the ligation efficiency of joining 5′-P and 3′-mismatch-containing ends at the nick has been investigated. For example, studies using purified human DNA ligases showed that DNA ligase III distinguishes correctly paired bases from mismatches at the 3′-terminus of the nick more efficiently than DNA ligase I [79,80]. In another study, it was reported that both human DNA ligases ligate 3′-dA more efficiently than the 3′-dC terminus paired to 8-oxodG on a template position [81]. However, more biochemical studies are required to further understand the channeling of the repair intermediate after pol β-mediated mismatch insertion coupled to DNA ligation during the BER pathway.

Furthermore, I investigated the NHEJ factors, including the DNA ligase IV/XRCC4 complex and pol μ, in a coupled repair assay and demonstrated that pol μ-mediated dG:T mismatch (dGTP or 8-oxodGTP insertion opposite template base T) insertion facilitates the channeling of the DSB repair intermediate to the next ligation step during NHEJ [82] (Fig. 5). However, the DNA ligase IV/XRCC4 complex is itself not able to join the 3′-end with the Watson–Crick wobble base mismatch [83]. Similarly, a recently published study has reported DNA ligation facilitated by pol μ ribonucleotide incorporation in MEF cells expressing neither pol μ nor terminal deoxynucleotidyl transferase [72]. The observed differences between pol β-BER DNA ligases and pol μ-DSB repair ligase could be due to either the unique features of pol μ or DNA ligase IV structure. For example, unlike other X-family DNA polymerases such as pol β and λ, pol μ undergoes no large-scale active-site conformational changes upon nucleotide binding where the catalytic intermediates with a transient third metal ion were observed [84,85]. Similarly, the crystal structure of the catalytic core of human DNA ligase IV revealed dynamic and extensive interdomain interactions with the nicked DNA in which the active site undergoes a transition between open and closed conformations for efficient DNA nick sealing [86,87]. For example, it has been shown that DNA ligase IV undergoes conformational remodeling and end-alignment configuration for the ligation of diverse DNA end structures on chromosomal breaks, suggesting DNA ligase IV-mediated sensing of damaged and mismatched ends during cellular NHEJ [70]. On the other hand, additional x-ray crystallography studies are required to

capture the DNA ligase/polymerase binary protein complex in action with a DNA repair intermediate containing noncompatible end structures.

Protein–Protein Interactions of DNA Ligases and Their Impact on the Fidelity of DNA Ligation

ATP-dependent DNA ligases from bacteria to humans all share a conserved catalytic core structure that is responsible for nick sealing [88]. However, human DNA ligases have unrelated flanking N- and/or C-terminal regions that play roles in their specific protein–protein interactions with their partner proteins and thereby direct them to participate in different cellular functions [1,2,5,6,10].

Human DNA ligase I is required for the sealing of Okazaki fragments during discontinuous DNA replication and finalizes the last step of LP-BER [89]. The protein contains N-terminal region that has a nuclear localization signal and is responsible for interaction with other proteins, such as DNA-sliding clamps, PCNA, and pol β [90]. The interaction between PCNA and DNA ligase I occurs between the interdomain connector loop of PCNA and the PIP box of DNA ligase I (Fig. 6a). The inactivation of the PCNA-binding site on the ligase completely abolishes the localization of DNA ligase I at the sites of DNA replication [91]. Moreover, PCNA improves the binding of DNA ligase I to nicked single-stranded DNA and stimulates ligation activity *in vitro* [92]. Biochemical studies have reported that the interaction between PCNA and DNA ligase I plays a key role in the coordination of the DNA synthesis and ligation steps during LP-BER. However, this interaction is not required to repair an abasic site through SP-BER [93,94]. PCNA cannot enhance ligation on a circular DNA substrate without the addition of replication factor C, a clamp loader responsible for loading PCNA onto DNA in an ATP-dependent reaction, and the replication factor C itself significantly inhibits the catalytic activity of DNA ligase I [95]. Thermodynamics and domain mapping studies also showed that the N-terminal 8-kDa domain of pol β interacts with the N-terminal noncatalytic part of DNA ligase I (Fig. 6a). This interaction is significantly abolished when the lysine residues (K35, K62, and K72) that are responsible for pol β dRP lyase activity (i.e., 5′-dRP group removal) are mutated to alanine residues, and the C-terminal part of the protein with DNA synthesis activity has no effect on this interaction

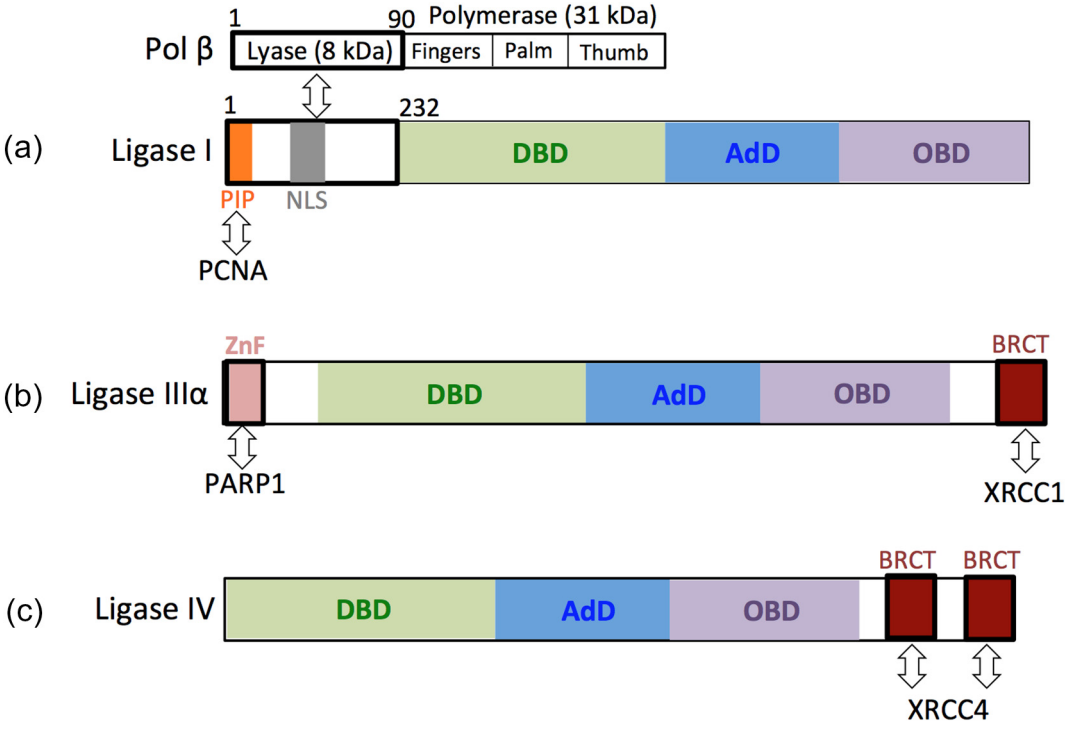

Fig. 6. Protein partners of nuclear human DNA ligases. (a) The N-terminal domain of DNA ligase I, including the first 232 amino acid residues of the protein, interacts with the N-terminal part of DNA polymerase β harboring an 8-kDa lyase domain that is responsible for 5′-dRP removal. The PIP box of DNA ligase I mediates its interaction with PCNA. (b) The ovarian cancer susceptibility protein 1 (BRCT) and zinc-binding (ZnF) domains of DNA ligase IIIα mediates protein–protein interactions with XRCC1 and PARP1, respectively. (c) DNA ligase IV forms a functional complex with x-ray repair cross-complementing protein 4 (XRCC4) through its two BRCA1 C-terminal (BRCT) domains.

[67,96]. Overall, it seems that the distinct interplay between DNA ligase and its partner protein when bound to a repair intermediate with an incompatible end could affect the activity of DNA ligases. Additional biochemical studies are needed to understand the functional importance of the physical interaction between pol β and DNA ligase I and the effect of this interaction on the substrate channeling of repair intermediates during the LP-BER subpathway.

The DNA ligase that plays a role in the SP-BER subpathway, DNA ligase IIIα, contains a C-terminal region with a breast and ovarian cancer susceptibility protein 1 (BRCT) motif that is involved in the interaction with its nuclear partner XRCC1 [97] (Fig. 6b). As a scaffold protein, XRCC1 has no catalytic activity but interacts with protein partners that are involved in all enzymatic steps of SP-BER, such as APE1 and pol β [98]. On the other hand, no stimulatory role of XRCC1 on nick sealing by DNA ligase IIIα has been reported [99,100]. Mammalian DNA ligase IIIα also has an N-terminal zinc-binding domain, termed a zinc finger, that is not required for catalytic activity; however, it serves as a nick sensor and bridges the two ends of DNA strand breaks during NHEJ [101–103]. As an important binding partner of XRCC1 and DNA ligase IIIα, PARP1 (poly(ADP-ribose) polymerase 1) mediates the recruitment of both proteins to DNA damage sites *in vivo*, relying heavily on NAD-dependent-poly (ADP-ribose) synthesis [104]. The N-terminal region of DNA ligase IIIα adjacent to its zinc finger is responsible for its interaction with PARP1 (Fig. 6b). Biochemical *in vitro* studies showed increased DNA end joining activity of DNA ligase IIIα in the presence of PARP1, and this stimulation is highly dependent on the presence of NAD^+ when PARP-1 becomes poly(ADP-ribosyl)ated [104]. Time-lapse single-molecule studies using fluorescently labeled DNA ligases *in vivo* revealed that DNA ligase IIIα in complex with XRCC1 accumulates at sites of oxidative DNA damage before DNA ligase I is recruited through its interaction with PCNA [105].

DNA ligase IV interacts with its protein partner XRCC4 through the linker sequence between two BRCT domains of the protein (Fig. 6c). Many physical and functional interactions have been reported between components of the NHEJ core complex [106,107]. DNA ligase IV plays a central role and is involved in a series of species-specific pairwise protein–protein interactions that coordinate the DNA end processing and ligation reactions that complete the repair of DSBs by NHEJ [53–56]. Some of these interactions also affect the ligation of noncompatible DNA ends by DNA ligase IV. For example, a stimulatory role of XRCC4 on the ligation of double-strand DNA ends with fully incompatible short 3′-overhang configurations that present no potential for base pairing has been reported [108]. Moreover, DNA-PKcs interacts with and promotes intermolecular joining by the DNA ligase IV/XRCC4 complex [86].

Oxidative Stress and DNA Ligation: New Cancer Therapeutic Targets

DNA ligases are promising candidates for the development of small-molecule inhibitors for anticancer therapeutic purposes because of their crucial roles in multiple DNA repair pathways, replication, and recombination [109–111]. DNA ligase inhibitors have been developed using a computer-aided rational drug design strategy based on the crystal structure of human DNA ligase I [112]. For this approach, a less conserved DBD of the DNA ligase is targeted over the OBD and AdD domains, including a common active-site lysine and catalytic residues [113]. Studies have shown that the efficacy or specificity of DNA damage-inducing anticancer drugs in chronic myeloid leukemia, breast cancer, and neuroblastoma cells can be increased by using DNA ligase inhibitors [114–119]. For example, the DNA ligase I-specific inhibitor L87–G17 increased cytotoxicity in MCF7 breast cancer cells when combined with DNA-damaging agents such as the DNA alkylating agent methyl methanesulfonate and decreased colon cancer cell survival under ionizing radiation with no observed effect on sensitivity in the cell lines from normal cells [120]. Similarly, enhanced cell growth arrest in HeLa cells was recently shown in response-combined treatment with the DNA alkylating agent temozolomide and the DNA ligase I, III, and IV inhibitor L189. Moreover, biopsies taken from estrogen receptor- and progesterone receptor-positive and tamoxifen- and aromatase-resistant derivatives of MCF7 breast cancer cells exhibited significantly increased sensitivity to a combination of PARP and DNA ligase III inhibitors [121,122].

Many types of cancer cells have increased levels of ROS [123–125]. These highly reactive hydroxyl radicals react with the components of DNA as well as with the nucleotide pool, leading to oxidative DNA damage (i.e., 8-oxoG) and an oxidized nucleotide pool (i.e., 8-oxodGTP) [126–129]. In fact, the nucleotide pool is more susceptible to oxidation than the DNA molecule itself because of the abundance of water, the highly reactive intracellular environment, and the lack of protective mechanisms, such as the histone-like proteins bound to DNA molecules [127,130–132]. MutT Homolog 1 (MTH1) is the nucleotide pool sanitization enzyme that detoxifies the oxidized nucleotide pool and prevents the incorporation of oxidized nucleotides into genomic DNA by repair DNA polymerases such as pol β, suggesting that this protein could be targeted as an anticancer therapeutic approach [133,134]. It seems that small-molecule inhibitors of MTH1 have strong effects on the cell death of various cancer types [135,136]. On the other hand, these reports were followed by other studies reporting that cellularly active MTH1 inhibitors have no anticancer efficacy either in monotherapy or in combination therapies [137–139]. All reports contribute to the

understanding of the complex MTH1 biology, its role in carcinogenesis, and the utility of its inhibition to cure cancer patients.

I previously demonstrated that BER-proficient MEF cells overexpressing the pol β gene show more cytotoxicity and DNA DSBs in response to oxidative stress than BER-deficient MEF cells with pol β gene deletion [76]. This oxidative stress-induced cell killing was increased in MEF cells co-treated with MTH1 inhibitor or following *mth1* gene deletion, suggesting the involvement of the oxidized nucleotide pool and their pol β-mediated insertion during the BER pathway. However, further investigation is required to elucidate the biological consequences of DNA ligase deficiency or ligation inhibition in MEF cells under oxidative stress conditions *in vivo*. Recently, reduced DNA ligation by DNA ligase III/XRCC1 complex and a significant defect in the repair of oxidative DNA damage through SP-BER *in vivo* were reported in motor neurons derived from ALS patient-derived fibroblast cells [140].

Conclusions and Future Prospects

DNA ligation is the last step of almost all DNA repair pathways, and DNA ligases are known as biomarkers of abnormal DNA repair. DNA ligation failure products could become persistent DNA strand breaks that threaten genome stability. DNA polymerase-mediated ligation failure could be a source of mutagenic and toxic repair intermediates. The functional protein partners of DNA ligases can be considered chemotherapeutic targets with the goal of identifying more selective inhibitors for DNA ligases. In this respect, the protein–protein interactions between DNA ligases and DNA polymerases, particularly through the repair of oxidative DNA damage during the BER pathway, could be considered a potent new class of chemotherapeutic targets to improve the activity and/or specificity of the current generation of small-molecule ligase inhibitors. Therefore, biochemical and structural studies are needed to elucidate the structure–activity relationships and identify important residue(s) that play critical roles in the DNA polymerase-DNA ligase protein–protein interaction. The expression levels of DNA ligases in cancer cells represent an area of investigation that could identify cancers that would be ideal targets for ligase inhibition. The efficacy of anticancer drugs is highly influenced by cellular DNA repair capacity. The toxicity of DNA-damaging drugs can be reduced by the activities of several overlapping DNA repair pathways that remove lesions before the onset of DNA replication. Therefore, DNA repair pathways modulate the efficacy of cancer therapy. Cancer cells under increased oxidative stress are likely to be more vulnerable to damage by further ROS insults. As the BER pathway is the major player in the repair of oxidative DNA damage, BER DNA ligases (DNA ligase I and DNA ligase IIIα) and their interaction partner pol β and the development of new therapeutic agents that target their protein–protein interaction hold promise for potentiating the effects of cancer treatments.

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Abbreviations used:
OBD, oligonucleotide binding domain; AdD, adenylation domain; AMP, adenosine monophosphate; DBD, DNA binding domain; ATP, adenosine triphosphate; BER, base excision repair; dRP, deoxyribose phosphate; PCNA, proliferating cell nuclear antigen; DSB, double-strand break; ROS, reactive oxygen species; NHEJ, nonhomologous end-joining; MEF, mouse embryonic fibroblast.

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