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Review article

# End-processing nucleases and phosphodiesterases: An elite supporting cast for the non-homologous end joining pathway of DNA double-strand break repair

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ABSTRACT

Nonhomologous end joining (NHEJ) is an error-prone DNA double-strand break repair pathway that is active throughout the cell cycle. A substantial fraction of NHEJ repair events show deletions and, less often, insertions in the repair joints, suggesting an end-processing step comprising the removal of mismatched or damaged nucleotides by nucleases and other phosphodiesterases, as well as subsequent strand extension by polymerases. A wide range of nucleases, including Artemis, Metnase, APLF, Mre11, CtIP, APE1, APE2 and WRN, are biochemically competent to carry out such double-strand break end processing, and have been implicated in NHEJ by at least circumstantial evidence. Several additional DNA end-specific phosphodiesterases, including TDP1, TDP2 and aprataxin are available to resolve various non-nucleotide moieties at DSB ends. This review summarizes the biochemical specificities of these enzymes and the evidence for their participation in the NHEJ pathway.



**Contents**



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## 1. Introduction

DNA double-strand breaks (DSBs) occur spontaneously as a result of endogenous oxidative metabolism, stalled topoisomerase reactions [1], or class-switch/V(D)J recombination [2,3]. They are also induced by exogenous DNA damaging agents such as ionizing radiation [4], radiomimetic drugs [5], and oxidative stress [6]. The repair of these lesions is essential in order to maintain cellular homeostasis and genomic integrity, and a failure to do so causes chromosome loss or rearrangements contributing toward tumorigenesis and cell death. To prevent this, there are two major cellular processes that recognize and repair DNA DSBs: Homologous Recombination Repair (HRR) and Non-Homologous End Joining (NHEJ). HRR is an error-free pathway that is mainly functional during S and $G_2$ phases of the cell cycle and during meiosis, due to the requirement for sister chromatids [7], whereas NHEJ is an error-prone pathway occurring throughout the cell cycle [8]. Microhomology-mediated end joining (MMEJ)/Alternative Nonhomologous end joining (Alt-NHEJ or A-EJ) is a third mechanism involved in DSB repair that is prevalent during late $G_1$/early S phase and makes use of 5–25 bp microhomologous sequences for error-prone end joining [9,10].

NHEJ mainly involves the recognition and repair of DSB ends by direct ligation, and most of the DSBs in the $G_0/G_1$ phase are repaired by NHEJ [11]. NHEJ begins with the binding of Ku heterodimers (Ku70 and Ku80) to DSBs [12,13]. Ku then recruits DNA-PK which forms a stable complex only in the presence of DNA ends. This leads to the interaction of Ku with DNA polymerases μ and λ, the XRCC4-DNA ligase IV complex [14] and the XRCC4 paralogs XLF and PAXX [15]. The binding of this complex is mainly through contacts between Ku and DNA ligase IV, DNA-PKcs and XRCC4 and all these together reinforce a stable association of XRCC4 and DNA ligase IV with DNA-PK at DNA ends [16].

The DNA ends at most naturally occurring DSBs are characterized by various chemical modifications that need to be resolved in order for proper processing of the DNA ends, and this resolution is carried out via the agency of a number of nucleases and other phosphodiesterases. This review will focus on these enzymes and their role in end-processing DNA during DSB repair with special regard to NHEJ. The emphasis will be on mammalian cells, with some reference to other organisms, primarily yeast, in instances where information on mammalian enzymes is limited or conflicting.

## 2. Structural modifications at DNA ends

The sugar-phosphate backbone of DNA is highly prone to oxidation caused by free radical species [17]. DNA DSBs induced by radiation are the result of free radical attack on deoxyribose mainly by the hydroxyl radical, forming carbon-centered free radicals on any of the five deoxyribose carbons and leading to strand cleavage. For example, 3′-phosphoglycoaldehyde (Fig. 1**b**) is one such product that is formed due to the oxidation of the 3′ position of deoxyribose [18,19]. Other free radical induced DNA termini include 3′-phosphoglycolate (PG, Fig. 1a), 3′-formyl phosphate (Fig. 1c), 3′-keto-2′-deoxynucleotide and 5′-aldehyde [20,21]. Most of these are highly unstable and break down spontaneously to give rise to breaks with 3′ and 5′-phosphates. The exception to this rule is the 3′-PG terminus which is stable even under harsh conditions [22].

Free radical-mediated DSBs are also induced by radiomimetic natural products including neocarzinostatin (NCS), bleomycin and calicheamicin [5]. In the case of NCS, the non-protein chromophore of NCS physically binds to DNA first externally and then intercalates between adjacent DNA base pairs followed by a thiol activation to a free radical species which then attacks the deoxyribose moiety of DNA (predominantly at thymidylate residues). The resulting C-5′ radical either reacts with a dioxygen to form a peroxyl radical derivative which ultimately causes a strand break with a 5′-aldehyde (Fig. 1**e**) at the 5′ ends or less frequently forms a drug-deoxyribose covalent adduct after binding to the bound drug [23]. A fraction of these strand breaks are accompanied by simultaneous attack at the C-1′ or C-4′carbons of nucleotides in the opposite strand on a 2-base 3′stagger, yielding bistranded lesions that include some DSBs bearing 3′-PGs on 1-base 3′ overhangs [24]. Also, formyl phosphate-ended DNA can transfer the formyl group to other regions within the DNA and other proteins forming lesions that are difficult to repair [25]. Calicheamicin binds to DNA non-covalently in the minor groove and rearranges to a benzenoid diradical intermediate abstracting hydrogen atoms from deoxyribose in both strands [26]. Calicheamicin-induced DSBs are structurally similar to those formed by NCS, except that they are formed on a 3-base 3′ stagger. Bleomycin-induced DSBs either have either blunt ends or non-complementary single base 5′ extensions [27], with predominantly 3′-PG termini [5,28]. Because they induce a specific subset of the highly diverse DSBs formed by radiation and free radicals, these agents have been useful in determining pathways and enzymes responsible for processing certain types of blocked DSB ends.

DNA DSB ends accompanied by nearby oxidative base damage also need to be processed during DNA repair. These clustered DNA lesions, also referred to as a “locally multiply damaged site”, are induced by closely spaced ionizations along the tracks of secondary electrons discharged by γ-rays [29,30]. Two of the most common and most studied oxidative base lesions are 8-oxoguanine (8-oxoG) and thymine glycol (Tg) (Fig. 1f and g). The 8-oxoG can still base-pair with cytosine, although this interaction is less stable than the canonical G•C base pairing. Earlier studies have shown that 8-oxoG can flip into a *syn* conformation and base-pair with adenine, which renders 8-oxoG a highly mutagenic DNA lesion [31–33]. Tg is the result of oxidation of the 5–6 double bond of thymine, which ultimately distorts the DNA and blocks rejoining when present at the DNA termini [34–37]. In mammalian cells, these lesions are primarily removed by DNA glycosylases and abasic endonucleases [38]. However, when Tg and 8-oxoG occur within the clustered DNA lesions, normal repair by the glycosylases at base excision repair (BER) is often thwarted by adjacent breaks and gaps [39]. As a result, these damaged bases may in some cases require removal by DSB-specific end-trimming mechanisms involving various nucleases discussed in detail below.

Topoisomerases are essential enzymes that relax DNA by forming a covalent DNA-enzyme intermediate between a catalytic tyrosine residue on the enzyme and a broken end of the DNA. Depending on the number of DNA strands broken and religated, these enzymes are classified as; Topoisomerases I (TOP1, TOP1mt) which induce SSBs with 5′-hydroxyl termini and topoisomerase linked to the 3′ terminus (Fig. 1h). TOP1-mediated DSBs result from the collision of replication forks or occasionally, transcription complexes with these SSBs [40].Topoisomerases II (TOP2α and 2β, Spo11) induce DSBs which have 3′-hydroxyl termini and 4-base 5′-overhangs terminating in a tyrosyl-linked topoisomerase [41–43] (Fig. 1i). Normally, these intermediates are almost immediately religated but ligation can be hampered by oxidative base damage or enzyme inactivation. Topoisomerases III (TOP3α and 3β) act on intertwined single strands comprising of DNA (3α) or DNA and RNA (3β). These act by cleaving one strand and form a covalent bond between the catalytic tyrosine and 5′ end of the nucleic acid [44,45].

Ribonucleotide reductases (RNRs) are enzymes that catalyze the conversion of nucleotides to deoxynucleotides that are important precursors for DNA replication and repair. Inhibitors of RNRs such as gemcitabine prevent this process and the mechanism of action