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Review

Understanding APE1 cellular functions by the structural preference of exonuclease activities

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ABSTRACT

Mammalian apurinic/apyrimidinic (AP) endonuclease 1 (APE1) has versatile enzymatic functions, including redox, endonuclease, and exonuclease activities. APE1 is thus broadly associated with pathways in DNA repair, cancer cell growth, and drug resistance. Unlike its AP site-specific endonuclease activity in Base excision repair (BER), the 3′-5′ exonucleolytic cleavage of APE1 using the same active site exhibits complex substrate selection patterns, which are key to the biological functions. This work aims to integrate molecular structural information and biocatalytic properties to deduce the substrate recognition mechanism of APE1 as an exonuclease and make connection to its diverse functionalities in the cell. In particular, an induced space-filling model emerges in which a bridge-like structure is formed by Arg177 and Met270 (RM bridge) upon substrate binding, causing the active site to adopt a long and narrow product pocket for hosting the leaving group of an AP site or the 3′-end nucleotide. Rather than distinguishing bases as other exonucleases, the hydrophobicity and steric hindrance due to the APE1 product pocket provides selectivity for substrate structures, such as matched or mismatched blunt-ended dsDNA, recessed dsDNA, gapped dsDNA, and nicked dsDNA with 3′-end overhang shorter than 2 nucleotides. These dsDNAs are similar to the native substrates in BER proofreading, BER for trinucleotide repeats (TNR), Nucleotide incision repair (NIR), DNA single-strand breaks (SSB), SSB with damaged bases, and apoptosis. Integration of *in vivo* studies, *in vitro* biochemical assays, and structural analysis is thus essential for linking the APE1 exonuclease activity to the specific roles in cellular functions.

Contents

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1. Introduction

Apurinic/apyrimidinic endonuclease 1 (APE1) is a multifunctional enzyme that plays numerous biological roles by providing redox and nuclease activities[1]. These two functions have been shown to arrive from the N-terminal redox domain and the C-terminal nuclease domain of APE1. Most of the apo-APE1 and APE1-DNA complex structures, though, do not include the 1–36 N-terminal amino acids, and studying the redox activity thus faces the difficulty of lacking structural information. The vital cellular events that involve APE1 includes DNA repair pathways, transcription activation, oxidative stress response, inflammatory responses, and cell proliferation. Controlling such important pathways for cell survival, APE1 is inevitably positioned at core in a wide range of human etiologies, including neurodegeneration, cancer, cardiovascular and other diseases. In these pathologies, APE1 was found to have alterations in expression, subcellular localization, post-transcriptional modification, and/or enzymatic activities[1–4]. Therefore, many recent studies point APE1 as a therapeutic target for cancer and several human diseases[2–10].

Besides the redox function, a key property of APE1 is the ability of acting on nucleic acid substrates as an endonuclease as well as an exonuclease[11,12]. For the 3′-5′ exonucleolytic cleavage of the latter, the activity was found to be two to four orders lower than that of the endonucleolytic digestion[12,13]. Substrate preference of the APE1 endonuclease has been shown to specifically target an Apurinic/apyrimidinic site (abasic site, AP site) during Base excision repair (BER) for fixing the DNA damage due to oxidation, deamination, and alkylation[14] (Table 1). BER is initiated by distinct DNA glycosylases of different base specificities that recognize and remove the damaged bases, leaving an AP site in the substrates. Subsequently, APE1 incises the phosphodiester backbone 5′ upstream of the AP site[15] and the substrate is then further processed by the short-patch repair or the long-patch repair machinery of BER[16]. In establishing the mechanistic role of APE1 as a key endonuclease in BER, protein structural data provide pivotal information[17,18]. Much less attention, though, has been received for the 3′-5′ exonuclease activity of APE1, although it is also identified in many pathways of DNA processing. The lack of structural information leaves many issues unresolved for the exonuclease mode of actions. In particular, the base specificity and structural preference of the APE1 exonucleolytic cleavage behave quite differently from its endonuclease counterpart. An important premise is that the diverse involvement of APE1 in different DNA repair pathways can be understood based on the specific properties of these two nuclease modes.

By in-depth analysis of the atomic structures and cellular functions of APE1, we aim to reveal the connection between its structural and functional behaviors. In different repair pathways, the DNA intermediates encountered tend to have specific structural features. Our focus is thus placed on the preference of APE1 in targeting a variety of structural dsDNAs, since they are key signatures of the DNA-processing pathways in the cell. As discussed later, substrate specificity can indeed offer useful means for linking APE1 with its working partners in DNA repair. Regarding the mechanism by which the APE1 active site is able to perform both the endonucleolytic and exonucleolytic cleavage, structural information can be combined to uncover the critical role played by the conformational variability due to protein dynamics. Therefore, our integrated analysis of APE1 also provides a vivid example for this important property in facilitating cellular functions.

Table 1
The damaged DNA lesions repaired associated with APE1 nuclease activity.

Endonuclease activity		Exonuclease activity	
BER	NIR	Proofreading	Damage bases in SSB
DNA damage from oxidation, deamination and alkylation [14].	1. αdA, DHT, DHU, 5ohU and αT[69]2. 5OH-Hyd and 5OH-5Me-Hyd[71]3. 5ohC and αdC[72]	1. ddT, AZT, D4T[12] 2. L-OddC, dFdC, araC, ddC, L-ddC, L-SddC, L-Fd4c[49]	1. 8-oxoG [50]2. cdA, cdG [36].

*Abbreviation: αdA (alpha-2′-deoxyadenosine), DHT (5,6-Dihydro thymidine), DHU (5,6-dihydro-2′-deoxyuridine), 5ohU (5-Hydroxyuracil), αT (alpha-Thymidine), 5OH-Hyd (5-hydroxyhydantoin), 5OH-5Me-Hyd (5-methyl-5-hydroxyhydantoin), 5-hydroxy-2′-deoxycytidine (5ohC), α-anomeric 2′-deoxycytidine (αdC), ddT (2′,3′-dideoxythymidine), AZT(3′-azido-3′-deoxythymidine), D4T(2,3-didehydro-2, 3-dideoxythymidine), L-OddC (b-L-Dioxolane-cytidine), dFdC (b-D-2′, 2′-difluorodeoxycytidine), araC (b-D-arabinofuranosylcytosine), ddC (b-D-2′,3′-dideoxycytidine), L-ddC (b-L-2′,3′-dideoxycytidine), L-SddC (b-L-2′, 3′-dideoxy-3′-thiocytidine), L-Fd4c (b-L-2′,3′-dideoxy-2′,3′-didehydro-5-fluorocytidine), 8-oxoG (8-oxoguanine), cdA (8,5′-cyclo-2′-deoxyadenosine), cdG (8,5′-cyclo-2′-

2. The versatile active center of APE1 mediates both endonuclease and exonuclease activities

The structure and active site of the APE1 nuclease domain were first identified for understanding the endonuceolytic cleavage that demonstrates high specificity to an AP site[18], and are characterized as in the exoIII structural family named after the *Escherichia coli* (*E. coli*) exonuclease III (exoIII, Xth gene). In addition to acting as an exonuclease, *E. coli* exo III, too, can incise the 5′-upstream phosphodiester backbone next to an AP site[19]. Interestingly, another *E. coli* enzyme, endonuclease IV (endoIV, Nfo gene) [20,21], although having a very different sequence and overall structure, also equips the dual functionalities of an exonuclease and an endonuclease as exoIII and APE1 do[22]. In *E.* coli, exoIII and endoIV participate in similar DNA repair pathways[23]. Mammalian APE1[17,24,25], APE2[26,27], *Drosophila melanogaster* Rrp1 [28], *Caenorhabditis elegans* (*C. elegans*) Exo-3[29], and *S. cerevisiae* Apn2[30] have been identified to be the homologs of exoIII. On the other hand, *C. elegans* APN-1[31] and *S. cerevisiae* Apn1[32] were discovered to be endoIV homologs. However, in species that are evolutionarily higher than *C. elegans*, no endoIV homologs have been reported yet.

In *E. coli*, exoIII is the major AP-site specific endonuclease in DNA repair, but the main player becomes the endoIV homolog APN1 in yeast and endoIV in *Mycobacterium tuberculosis*[33,34]. A mystery is thus the respective roles of exoIII and endoIV types of nucleases in the cell. A potential origin for their overlapping functions is one serving as a backup system for the other. However, the corresponding levels of endonuclease and exonuclease activities [20] and the base preference of these two nuclease types are somewhat different[35]. For example, only exoIII and its homologs can process cdA (8,5′-cyclo-2′-deoxyadenosine)[36], an analog of damaged nucleotides in DNA repair.

The APE1 nuclease domain has ~ 30% sequence identity with *E. coli* exoIII[15,37]. With a similar folding topology, the highly conserved regions of APE1 and exoIII include the residues of catalysis [11,19,24,37,38]. In addition to acting as an endonuclease, both enzyme have also been identified to equip the functionality of a 3′-5′ exonuclease that prefers to digest double-stranded DNA (dsDNA) substrates[11,38]. Mutagenesis and *in vitro* nuclease activity assays[24,39–43] showed that the endonucleolytic and exonucleolytic cleavage of APE1 utilize the same catalytic residues and active center, including Asp70, Glu96, Tyr171, Asn174, Asp210, Asn212, and His309, as those in exoIII[17,24,25].

Since the common group of residues are utilized to hydrolyze phosphodiester bonds in both the endo- and exonucleolytic cleavage, excising dsDNA with matched or mismatched base pairing in the terminus is also expected to proceed by the same active site in APE1 (Fig. 1). The structural data obtained for understanding the enzyme activities in trimming matched or mismatched terminus, though, reveals conformational variation, and has led to the proposition of alternative cleavage sites for APE1 when processing different terminal structures[25]. At core of this issue is how does the single active site selectively accommodate the various substrates that would be encountered in exonucleolytic cleavage. As addressed in the following, detailed comparison over the structures of APE1-substrate and APE1-product complexes shows that the intrinsic organization of the active site residues can vary to discriminate the different structural features for endonuclease as well as exonuclease activities, and for the latter, matched or mismatch base pairing in the dsDNA terminus[24].

In the APE1 structures of endonuclease binding, the active site surface rich in positively charged residues engulfs the AP site containing strand and kinks the dsDNA structure. The AP site is hence in a flipped-out conformation into the product pocket[17,18] (Fig. 1A and 1B). In the product pocket of the APE1 active site, two hydrophobic residues, Phe266 and Trp280, appear to provide the necessary steric hindrance for not allowing normal bases to fit in[25].

The AP site wrapping hydrophobic residues in the product pocket are not highly conserved and their sequence variation is shown to associate with the exonuclease activity. Having a fewer number of hydrophobic residues in the product pocket, such as in *E. coli* exoIII and mammalian APE2 for example, greater preference toward exonuclease substrates is observed[25,26,44]. Unlike the endonucleolytc cleavage, the 3′-end nucleotide in the product pocket adopts a non-base flipping configuration when APE1 acts as an exonuclease[25] (Fig. 1A and 1C). The base pairing of a mismatched terminus being more flexible structurally is thought to facilitate the 3′-end nucleotide to enter the APE1 product pocket [25]. In addition to the product-pocket hydrophobic residues, there are several other amino acids identified to play key roles in the substrate selection of APE1. In particular, Tyr269 in the intercalating loop of human APE1 is required for bending DNA[45], and His167 of two *Neisseria meningitidis* endonucleases was shown to be the structural determinant of their AP site specificity[46].

Integration of the insights emerge from different structures, a general model can potentially be deduced to comprehend the complex substrate selection mechanism of APE1. In this regard, the induced space-filling model[24] recognizes that substrate binding induces particular active site reorganization involving Arg177 and Met270 to form a bridge-like structure, i.e., the RM bridge.

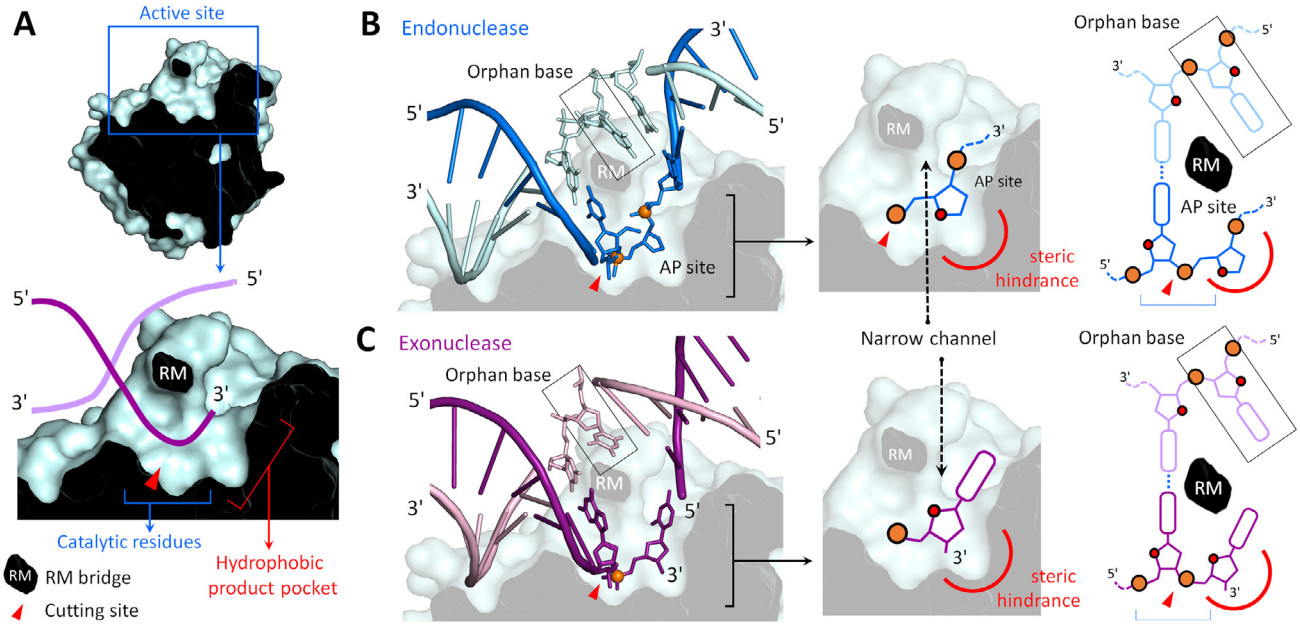

Fig. 1. The substrate selection-related induced space-filling model of APE1 nuclease activity. A. RM bridge is formed by substrate binding and used to unwind the dsDNA to make the 3′-end of the scissile strand into the narrow product for processing. The scissile and non-scissile strands of substrate dsDNA are displayed in light and dark purple. B-C. The close view of the active sites of APE1-substrate complex in endo- or exonuclease manners. The orphan bases are highlight by black boxes, and orange balls display the phosphates in DNA. The narrow product pocket only can be filled by an AP site with downstream phosphate or a nucleotide in endo- and exonuclease manners, respectively. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

The induced space-filling model of the RM bridge provides a unified view for the substrate selection of APE1 in executing both the endo- and exonuclease activities[24]. Formation of the RM bridge partially fills up the active-site space and shapes it into a long and narrow channel that serves as the product pocket (Fig. 1A). After space-filling by the RM bridge, the product pocket can fit the AP site when incising an AP site containing dsDNA and the 3′-end of the single-nucleotide leaving group when excising a damaged dsDNA[17,24,25]. Mutations of both Arg177 and Met270 (mouse R176A/M269A APE1 mutant) are found to disrupt the substrate selection of APE1 based on the profiles of APE1-dsDNA complexes in gel-based experiments[24]. Interestingly, the R176A/M269A APE1 appears to have slightly increased endo- and exonuclease activities[18,24,25], likely due to an elevated rate of product release in absence of the RM bridge[18,47]. In addition to the active-site space-filling mechanism for restricting substrates to those with an allowable product size, the RM bridge also involves in facilitating protein-DNA interactions[18,25,48] and coupling to the orphan base for unwinding the matched base pair when APE1 excises a dsDNA substrate[24]. In the following, the enzyme activities and structures of APE1 as an exonuclease are discussed to delineate the space-filling model.

3. The exonuclease activity of APE1

In the endonucleolytic cleavage, only AP site in the middle of a dsDNA substrate can fit in the narrow product pocket of APE1 composed mostly of hydrophobic residues, including Ala230, Phe266, Trp280 and Leu282[17]. In the product pocket, only a couple of hydrogen bonds are found between the polar sidechains of Asn174 and Asp210 with the ribose oxygen atoms. On the other hand, the exonucleolytic cleavage of APE1 has very different base and structural specificities such that the enzyme can participate in numerous DNA processing pathways. As described below, the selection mechanism of the exonuclease activity can be understood in terms of the induced space-filling model[24].

3.1. APE1 exonuclease activity lacks base preference

As a 3′-5′ exonuclease, APE1 binds and digests dsDNA substrates from the 3′-end to the 5′-end. In such terminal binding, the 3′-OH of the last nucleotide is exposed in absence of the downstream phosphate. Therefore, the nitrogenous base of the 3′-end nucleotide can fit in the product pocket without creating spatial hindrance (Fig. 1). With the product pocket composed mainly of hydrophobic residues, binding the 3′-end is expected to be non-selective. Indeed, in the X-ray structure of a APE1-substrate complex in the exonuclease binding (PDB accession code: 5WN5), no hydrogen bond is found between the nitrogenous base of the 3′-end nucleotide and APE1; only Asn212 interacts to the 3′-OH of the 3′-end nucleotide[25]. Using molecular mechanical modeling based on the X-ray structure also indicates that the product pocket of APE1 can accommodate not only the normal nitrogenous bases in dsDNA but also various damaged DNA bases without coursing steric hindrance[24]. These structural information thus suggest that the exonuclease activity of APE1 would not exhibit clear base preference, and this proposition is consistent with the analysis by using an *in vitro* assay. In testing the exonucleolytic cleavage against a variety of nucleotide analogues as the 3′-end substrate, including 2′,3′-dideoxythymidine (ddT), 3′-azido-3′-deoxythymidine (AZT), 2,3-didehydro-2, 3-dideoxythymidine (D4T), b-L-dioxolane-cytidine (L-OddC), b-D-2′, 2′-difluorodeoxycytidine (dFdC), b-D-arabinofuranosylcytosine (araC), b-D-2′,3′-dideoxycytidine (ddC), b-L-2′,3′-dideoxycytidine (L-ddC), b-L-2′,3′-dideoxy-3′-thiocytidine (L-SddC), b-L-2′,3′-dideoxy-2′,3′-didehydro-5-fluorocytidine (L-Fd4c), 8,5′-cyclo-2′-deoxyadenosine (cdA), and 8,5′-cyclo-2′-deoxyguanosine (cdG)[12,36,49,50], APE1 demonstrates similar activities (Table 1). These results also imply that the APE1 exonuclease can remove damaged nucleotides in various DNA repair pathways.

3.2. APE1 exhibits preference toward substrate structures

As a key player in DNA repair, APE1 has higher activity in processing double-stranded DNAs than that in digesting single-stranded substrates[24,51,52]. Putting together the available X-ray structures of APE1-dsDNA complexes, a consistent interaction zone can be identified to reveal the substrate structural features recognized by APE1[24] and define the nuclease-flanked region on the dsDNA substrate (Fig. 2). Over the consistent interaction zone, APE1 couples the scissile strand through ribose-phosphate backbone near the cleavage site and the non-scissile strand by the ribose-phosphate backbone of three consecutive nucleotides locating two bases downstream of the orphan base that makes specific hydrogen bonds with APE1. The structural information provided by the consistent interaction zone illustrates that APE1 binding involves both stands for the apparent preference toward dsDNA over ssDNA[24].

Further, APE1 demonstrates the abilities of recognizing more subtle features on the substrate structures. As an endonuclease, APE1 specifically works on the AP site (Fig. 2A). When acting as an exonuclease, on the other hand, APE1 shows a rather complicated substrate recognition patterns. For gapped, recessed, and blunt-ended dsDNA, APE1 prefers to bind the scissile strand terminal. Other structural features recognized by APE1 include terminal base pairing being matched or mismatched and the 3′- or 5′-overhang length. APE1 prefers to process dsDNA substrates with a 3′-overhang shorter than 2 nucleotides long (<2 nt) or with a longer 5′-overhang (>5 nt). Longer 3′-overhang may cause steric hindrance in the product pocket. As discussed in the following, these substrate recognition patterns can be understood in terms of the induced space-filling model (Fig. 2B).

Regarding matched or mismatched terminal base pairing, APE1 works on the latter more effectively[12,24,37,53]. Such difference in activity toward a mismatched or matched terminus, though, is less pronounced when processing recessed dsDNAs with a longer 5′-overhang (>5 nt) and gapped dsDNAs[24], reflecting subtlety of the underlying molecular processes. Furthermore, indirect evidence suggests that APE1 may exhibit different binding modes depending on whether the dsDNA substrate has a matched or mismatched terminal base pair. In mixing APE1 with gapped dsDNAs with matched terminal base pairing in a gel shift assay, two APE1-dsDNA complex bands can be observed. For the same substrate but with mismatched terminal base pairing, only one band of APE1-dsDNA complex shows up. Such distinct behaviors suggest that the same binding site can have alternative modes to bind different terminal structures[24].

For nicked dsDNA substrates with matched terminal base pairing, the processing by APE1 prefers the downstream nucleotides having 5′-OH rather than that with 5′-phosphoryl group on ribose since the hydroxyl group is sufficiently small to not to cause steric hindrance that a 5′-phosphoryl group would encounter with the base of the leaving group. The narrow product pocket due to RM bridge space-filling[24] provides the underlying molecular picture for this preference. For nicked dsDNAs with a mismatched terminus, on the other hand, even having a 5′-phosphoryl on the downstream nucleotide would not cause hindrance[25]. As demonstrated in the APE1-dsDNA complex structure (PDB entry: 5WN5), the downstream region of the mismatched nicked dsDNA is bent and shifts about 10° for this distinction between matched and mismatched terminal base pairing to occur (Fig. 2B). This

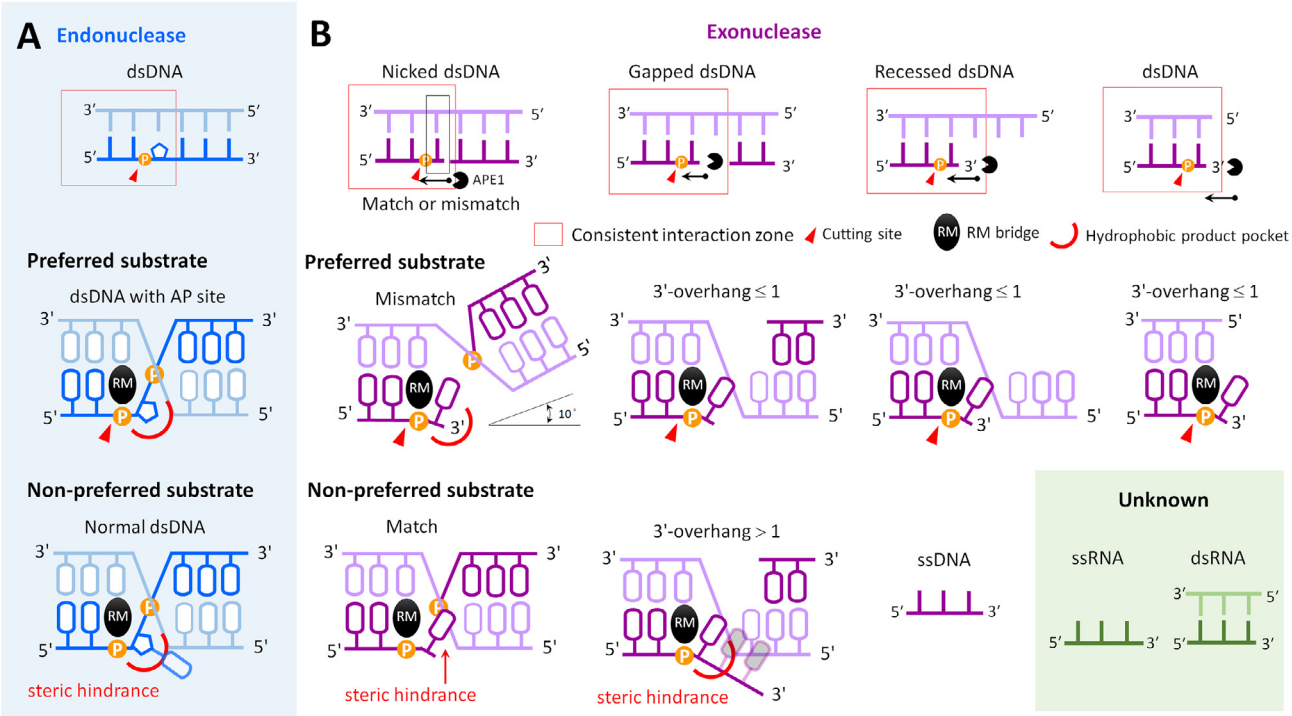

Fig. 2. Structural preference of mAPE1 on various substrates in endo- and exonuclease manners. A-B. The schematic presentation of the various DNA structures (top panel) and the possible DNA structures after APE1 binding. The structure of preferred and non-preferred substrates is shown in the lower panel. The black box highlights the 3′-terminal base pair for APE1 processing, matched or mismatched base pair. The matched or mismatched terminus in recessed or blunt-ended dsDNA are substrates of APE1, but matched substrates show slightly lower activity for APE1 processing. The recessed dsDNA with 3′-overhang longer than 2 nt is a non-preferred substrate of APE1.

structure also provides evident information that shows different binding modes depending on a matched or a mismatched terminus. Structural information is thus of critical importance for elucidating the molecular origin of nuclease functionalities. Recent studies indicate that APE1 is also an RNase and involved in RNA processing[42,54–56], but the underlying mechanism is elusive due to the lack of APE1-RNA complex structures.

4. Cellular functions involving the APE1 exonuclease activity

In vitro biochemical and structural analysis of APE1 established its ability in trimming the dsDNA terminal with matched or 1 nt mismatched base pairing[24,25]. Substrates with such an end are broadly encountered in various DNA repair pathways. Combining the substrate preference of APE1 with the behaviors observed in *in vivo* and mutagenesis studies can thus reveal the specific roles of the APE1 exonuclease in DNA repair pathways and apoptosis. In certain pathways, both the endonucleolytic and exonucleolytic cleavage of APE1 are required, particularly the BER proofreading, BER in trinucleotide repeats, and Nucleotide incision repair (NIR).

4.1. Proofreading in base excision repair

DNA glycosylases and APE1 in BER are responsible for removing the damaged base and nicking the resulting AP site. The lyase function of DNA polymerase β (pol β) then removes the 5′ deoxyribose phosphate (dRP) group of the substrate. Next, pol β can fill the gap by its polymerase activity, but it does not contain a 3′-5′ exonuclease domain and lacks the proofreading function. In this regard, APE1 can serve to proofread and remove any mismatched DNA end in the newly synthesized nicked dsDNA to increase the fidelity of pol β [11,12,25] (Fig. 3A). The co-working mechanism of pol β and APE1 has been established by monitoring protein–protein interactions [57–59] and *in vitro* proofreading assays[12]. In addition the mismatched terminus in a nicked dsDNA, APE1 can exonucleolytically remove the anti-HIV nucleoside analogous AZT and D4T, suggesting its therapeutic impact on the regulation anti-viral treatment[12].

4.2. Base excision repair in trinucleotide repeats

DNA replication, repair, recombination and gene transcription in trinucleotide repeats (TNRs) may lead to TNR expansion, resulting in formation of non-B form DNA structures, including hairpins, triplex, and stick DNAs[60–63]. Therefore, TNRs are hotspots for genome instability and related to over 40 neurodegenerative diseases in human, such as Spinobulbar Muscular Atrophy (SBMA), Huntington Disease (HD) and Spinocerebellar Ataxias (SCAs)[60]. Interestingly, BER of the oxidized DNA base at the hairpin loop of TNRs can lead to the hairpin removal and prevent the expansion of TNRs[63,64]. In this process, DNA glycosylase and APE1 are responsible for removing the damaged base and incising the 5′-side of the AP site to convert the hairpin into a double-flap structure (Fig. 3B). The 3′-5′ exonuclease activity of APE1 or the Mus81/Eme1 complex serves to shorten the upstream 3′-flap region[64–66]. With the induced space-filling of the RM bridge to shape the product pocket, APE1 prefers to process dsDNA substrates with a short 3′-overhang[24]. Therefore, the major nuclease for shortening the 3′-flap is likely the Mus81/Eme1 complex or other nucleases. Subsequently, the APE1 3′-5′ exonuclease activity for processing the matched dsDNA terminal can increase the gap size of the paired dsDNA for the next step of DNA filling by the polymerase β and thereby stimulate the ligation for hairpin removal[64,65].

4.3. Nucleotide incision repair

NIR is a backup/overlapped DNA repair system to BER for repairing oxidatively damaged nucleotides, the major type of endogenous DNA damage associated with human degenerative

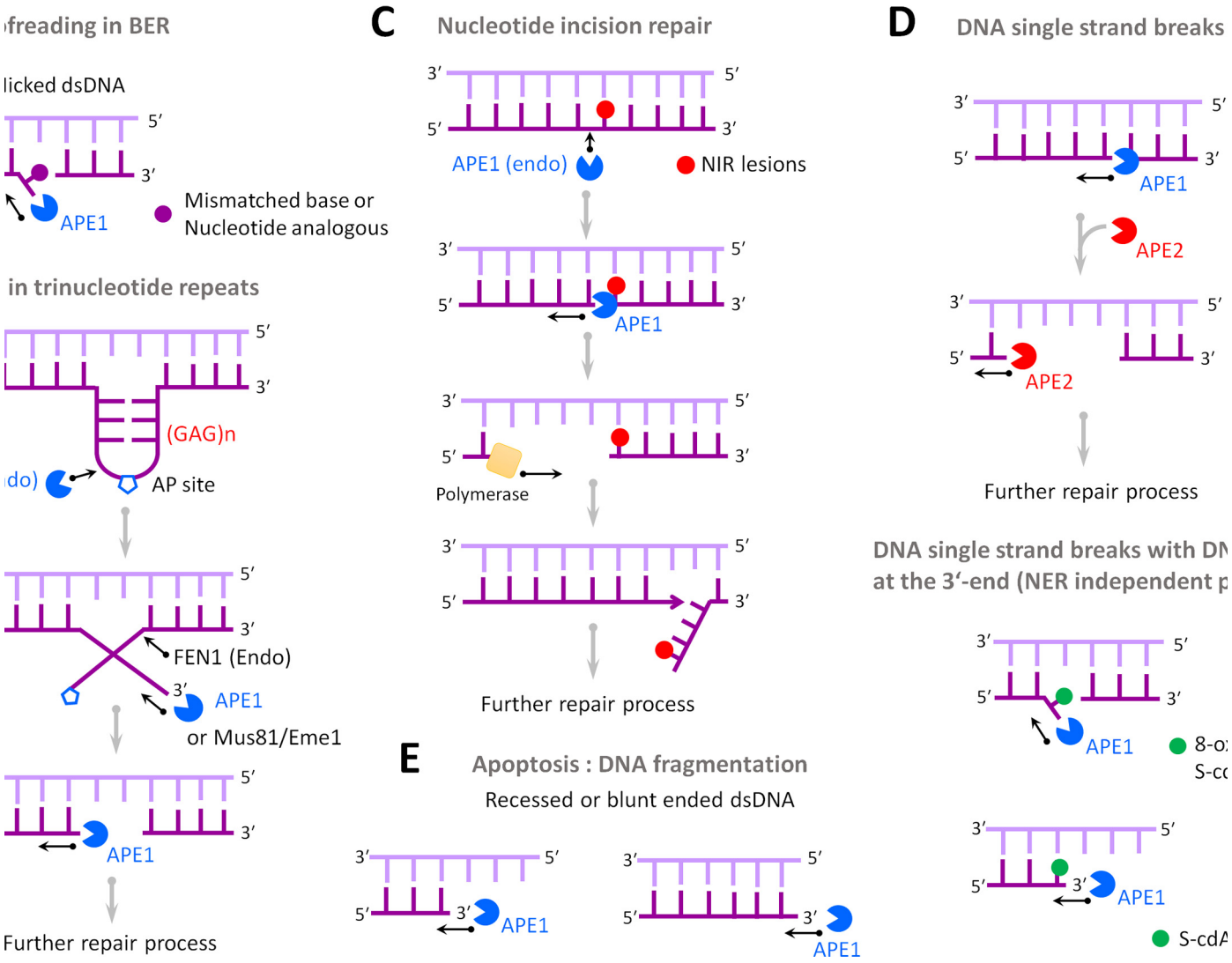

Fig. 3. The DNA processing related cellular functions associated with the exonuclease activity of APE1. A-E. The working mechanisms of various DNA processing related cellular functions associated with APE1 displayed by the cartoon, including Proofreading in BER (A), BER in TNRs (B), NIR (C), SSB, and SSB with damaged bases (D) and Apoptosis (E).

disorders like cancer, cardiovascular, and brain dysfunction[67,68]. The oxidative lesions repaired by NIR are listed in Table 1[69–72]. Unlike BER, NIR is a DNA glycosylase-independent pathway and requires both the endonuclease and exonuclease activities of APE1[67]. APE1 can sense the DNA base with oxidative damage and makes a nick at the 5′-end of the lesion to generate 3′-hydroxyl and 5′-phosphate termini[69–71]. The 3′-5′ exonuclease activity of APE1 then works on the nicked dsDNA to increase the gap size and prepare the substrate for the next repair step by DNA polymerase, FEN1/PCNA, and ligase (Fig. 3C)[72,73]. The 3′-5′ exonuclease activity of APE1 may also serve to prevent the formation of lethal double-strand breaks when repairing the bi-stranded clustered DNA damage by NIR[72,73].

4.4. DNA single-strand breaks (SSB) and SSB with damaged bases

SSB is the most abundant form of DNA damage in the cell; an unrepaired SSB leads to genome instability and is associated with cancer and neurodegenerative diseases. The occurrence of SSB also plays an essential role in triggering the ATR-Chk1-mediated DNA damage response (DDR) pathway for oxidative stress, which requires APE1 and its homologous APE2 [74–76]. APE1 can sense an SSB site and initiate SSB repair by performing the 3′-5′ exonucleolytic cleavage of the SSB terminus in dsDNA with a matched and nicked structure[76]. APE1 is also responsible for recruiting and activating repair proteins such as APE2 and PCNA for participation in the DDR pathway[76,77]. With the gap size of the nicked dsDNA increased by APE1, it then becomes suitable for further end resection by APE2. Afterwards, PCNA and other repair factors would complete the repair progress[27,76,78] (Fig. 3D).

In the DNA damages due to radiation induced ionization or misincorporation of 8-oxoguanine (8-oxoG) by the polymerase, the SSB would contain an 8-oxoG in the 3′-end (3′-8-oxoG). While DNA glycosylase-associated BER repairs the dsDNA with a 8-oxoG base in the middle, 3′-8-oxoG in SSB inhibits the extension mediated by the polymerase, ligation by ligase, and is thus resistant to this BER[50]. In this regard, APE1 provides the major exonuclease activity for removing the 3′-8-oxoG in SSB[32,50] (Fig. 3D) and plays a central role in repairing this type of SSB in both yeast and human[50].

Certain helix-distorting nucleotides such as cdA, cdG and L-OddC (BCH-4556, Troxacitabine, and Troxatyl) (Table 1), are products from oxidative stress and radiation or medicine induced ionization[79]. These abnormal deoxynucleotides in dsDNA can

distort the double-helix structure, block replication and transcription, and cause genome instability and development of diseases [36,79]. Although repairing the dsDNA substrates containing such nucleotides involves APE1, it does not follow the nucleotide excision repair (NER) pathway[36,49]. The actual route for the involvement of APE1 is such repair awaits further studies to establish. Among these structural distorting nucleotides, L-OddC can be used in anticancer therapy since its incorporation into DNA would terminate chain elongation, stop replication, and thereby prevent the proliferation of abnormal cancer cell[80]. L-OddC is currently under phase III clinical trial for treating leukemia[13]. Exonucleolytic removal of such therapeutic analogs of deoxynucleotide by APE1[13,49] is associated with drug resistance, highlighting the importance of the 3′-5′ exonuclease activity in anti-cancer therapy.

4.5. Apoptosis

Various truncated sequences of APE1 have been identified to associate with apoptosis[81,82], including N-terminal truncated Δ31 APE1 (lacking amino acids 1–31)[81] and AN34 APE1 (lacking amino acids 1–35)[82]. The exonuclease activity of AN34 APE1 was shown to be able to process matched blunt-ended dsDNA and recessed dsDNA and may participate in chromatin fragmentation during apoptosis [82] (Fig. 3E). Regarding Δ31 APE1 found in Granzyme A (GzmA)-mediated cell death, the *in vivo* exonuclease activity has not been measured, but *in vitro* and structural analysis showed similar activity and structure as those of the wild-type APE1[24]. These results suggest that Δ31 APE1 potentially plays certain roles in GzmA-mediated cell death by its exonuclease activity. Further investigation, though, is still needed to develop a comprehensive working mechanism by which APE1 participates in apoptosis, including the involvement in upstream apoptotic signaling pathways such as ATR/ATM-mediated DDR[76,77].

5. Potential working partners of APE1 in DNA processing

Another property of the exonucleolytic cleavage of APE1 is the specific limitation due to the induced space-filling of product pocket that does not allow the substrates containing a 3′-overhang longer than 2 nt to fit in. Therefore, it is likely that APE1 works together with other protein partners such as TREX1 in substrate digestion. TREX1 is also a 3′-5′ exonuclease and can complement the insufficiency of APE1 in processing certain dsDNA substrates. In fact, TREX1 provides most of the exonuclease activity in mammalian cells and equips the specificity against duplex DNAs with 3′-overhang[83,84]. Furthermore, TREX1 is in the DEDDh exonuclease superfamily and specifically recognizes the 3′-OH in DNA for executing its exonuclease activity[85,86]. With such base preference, TREX1 does not digest the APE1 substrates, such as the dsDNAs with 8-oxoG or S-cdA [36,84,87,88]. On the other hand, the lack of base preference in APE1 enables it to process the DNA substrates without the 3′-OH. Therefore, APE1 and TREX1 working together can enhance the efficiency of processing the wide variety of duplex DNAs in repair pathways. Moreover, both exonucleases are components of the endoplasmic reticulum (ER)-bound SET complex[81,89]. Even the biological functions of the SET complex in normal cells are yet to be characterized, a plausible assumption would be that APE1 and TREX1 work together. Both enzymes also overlap in various cellular functions, such as apoptosis[82,90], proofreading for DNA pol β[12,57–59,91–93], working with poly (ADP-ribose) polymerases 1 (PARP-1)[94,95], and DNA repair [84,96]. These observations strongly suggest that TREX1 and APE1 work together in cellular responses to DNA damage. It is certainly valuable to further test this hypothesis.

In addition to TREX1, APE1 also likely works with another member of the SET complex, high-mobility group protein 2 (HMGB2) [97]. HMGB2 stimulates the activity of several nucleases such as TREX1[84], DFF40/CAD[98], and RAG[99,100] by bending the dsDNA substrates. Along the same line, the HMGB2 homolog HMGB1 enhances the activities of APE1 and FEN1 in substrate incision[101,102]. This observation thus hints that APE1 and HMGB2 work collaboratively in cellular processing of DNA substrates. A potentially important future direction is thus elucidation of the co-working networks of APE1, TREX1, and HMGB2.

Other working partners of APE1 include APE2 and PCNA in the oxidative stress-induced ATR-Chk1-mediated DDR pathway, which directly interact with APE1 as shown *in vitro* pull-down assays [76,77]. APE1 and APE2 have different catalytic properties, equip non-overlapping functions, and work on different steps in SSB repair [75–77,103]. The main differences in the nuclease activities of APE1 and APE2 are that APE1 is a more active endonuclease, and its 3′-5′ exonuclease can process the nicked dsDNA or gapped dsDNA with small gap size[24]. Therefore, APE1 is better suited for sensing SSB and initiating the DDR pathway. The active site residues of these two nucleases are highly conserved and other aspects are thus responsible for rendering the different exonuclease activities. The key features of APE1 are the RM bridge mediating induced space-filling mechanism and the product pocket containing more hydrophobic residues (Ala213, Phe265 and Trp279 in mouse APE1) for substrate selection. Instead, the product pocket of *Xenopus laevis* APE2 has Ser187, Cys256 and Thr270 [24,103]. In addition, the extra PIP box and Zf-GRF motif in APE2 may also play a role [75–77]. Further studies are thus needed to establish the structural determinants of different exonuclease activities.

6. Discussion

The substrate selection mechanism of APE1 can be understood in terms of the induced space-filling model. Importance of the specific preference toward dsDNA structural features is reflected in the biological roles of APE1 in cellular pathways such as DNA repair and apoptosis. Despite this understanding, several questions regarding the APE1 nuclease activity call for further studies. First, APE1 has been shown to recognize the oxidative bases in NIR (Table 1) and can make a nick at the 5′ side of the damaged base. However, the endonucleolytic cleavage of APE1 only allows an AP site without base to fit in the narrow product pocket. How does an oxidatively damaged base in NIR enter the APE1 product pocket is thus a key issue for revealing the detailed function of APE1 in NIR. The NIR substrates containing oxidized bases may have distorted dsDNA structures for APE1 to bind and cleave, and testing this hypothesis requires additional APE1-DNA complex structures. Secondly, a general trend of APE1 is processing dsDNAs with a mismatched terminus more effectively than handling those with the ends of matched base pairing [12,24,37,53]. For recessed dsDNAs with a longer 5′-overhang, however, this preference becomes less pronounced [24]. This behavior indicates that the 5′-overhang length is an important structural feature for APE1 in processing recessed dsDNA exonucleolytically. Structural analysis indicates that the region in APE1 for binding the 5′-overhang of a recessed dsDNA is different from that in binding a nicked or gapped dsDNA at the non-scissile strand[24]. To further unravel the mechanism by which the 5′-overhang of a recessed dsDNA affects the activity of APE1 exonuclease calls for resolving the structures of APE1-recessed dsDNA complexes. Thirdly, Establishing the molecular determinants of substrate preferences, such as the differences between APE1 and APE2, calls for structural information combined with nuclease activity assays to systematically probe various DNA

substrates on both enzymes. A thorough mutagenesis study at the RM bridge is of particular interest to analyze the space-filling mechanism at the product pocket. It would also be valuable to obtain the crystal structures of full-length APE2 and APE2-DNA complexes. Fourthly, APE1 was also identified to participate in RNA processing, but how does the nuclease recognize and digest RNA molecules and what are the differences comparing to the handling of DNA substrates are open questions [42,54–56]. Measurements of bioactivity and binding assays with different RNA substrates would be of value for identifying the natural RNA substrates of APE1. This knowledge can then be combined with biological analysis such as the *in vivo* knock-down phenotypes for understanding the RNA processing functions of APE1. Finally, as a member in the ER-bound SET complex, the actual functional roles of APE1 are yet to be identified. Establishing the working partners of APE1 that potentially include TREX1, NM23H1 [104], or HMGB2 also awaits further studies. Development of *in vitro* nuclease activity assays with these proteins alone or together and protein–protein interaction assays is required for resolving this issue. Recently, TREX1 and HMGB2 are demonstrated to work together in digesting endogenous DNA in the cytosol for immune regulation and autoimmunity prevention [84,105]. Is APE1 also playing a role in the immune regulation associated with TREX1 is also a plausible question to address. In this regard, experiments with the APE1 single or APE1/TREX1 double knockdown coupled with cytosolic DNA measurements are particularly recommended.

CRediT authorship contribution statement

Tung-Chang Liu: Formal analysis, Investigation. **Kai-Wei Guo:** Investigation. **Jhih-Wei Chu:** Writing - review & editing. **Yu-Yuan Hsiao:** Visualization, Writing - original draft, Project administration, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

[1] Tell G, Quadrifoglio F, Tiribelli C, Kelley MR. The many functions of APE1/Ref-1: not only a DNA repair enzyme. Antioxid Redox Signal 2009;11(3):601–19.
[2] Thakur S, Sarkar B, Cholia RP, Gautam N, Dhiman M, Mantha AK. APE1/Ref-1 as an emerging therapeutic target for various human diseases: phytochemical modulation of its functions. Exp Mol Med 2014; 46:e106.
[3] Shah F, Logsdon D, Messmann RA, Fehrenbacher JC, Fishel ML, Kelley MR. Exploiting the Ref-1-APE1 node in cancer signaling and other diseases: from bench to clinic. NPJ Precis Oncol 2017;1(1). https://doi.org/10.1038/s41698-017-0023-0.
[4] Laev SS, Salakhutdinov NF, Lavrik OI. Inhibitors of nuclease and redox activity of apurinic/apyrimidinic endonuclease 1/redox effector factor 1 (APE1/Ref-1). Bioorg Med Chem 2017;25(9):2531–44.
[5] Simeonov A, Kulkarni A, Dorjsuren D, Jadhav A, Shen M, McNeill DR, et al. Identification and characterization of inhibitors of human apurinic/apyrimidinic endonuclease APE1. PLoS ONE 2009;4(6):e5740. https://doi.org/10.1371/journal.pone.0005740.
[6] Dorjsuren D, Kim D, Vyjayanti VN, Maloney DJ, Jadhav A, Wilson DM, et al. Diverse small molecule inhibitors of human apurinic/apyrimidinic endonuclease APE1 identified from a screen of a large public collection. PLoS ONE 2012;7(10):e47974. https://doi.org/10.1371/journal.pone.004797410.1371/journal.pone.0047974.s00110.1371/journal.pone.0047974.s002.
[7] Luo M, Kelley MR. Inhibition of the human apurinic/apyrimidinic endonuclease (APE1) repair activity and sensitization of breast cancer cells to DNA alkylating agents with lucanthone. Anticancer Res 2004;24:2127–34.
[8] Ströbel T, Madlener S, Tuna S, Vose S, Lagerweij T, Wurdinger T, et al. Ape1 guides DNA repair pathway choice that is associated with drug tolerance in glioblastoma. Sci Rep 2017;7(1). https://doi.org/10.1038/s41598-017-10013-w.
[9] Fishel ML, Kelley MR. The DNA base excision repair protein Ape1/Ref-1 as a therapeutic and chemopreventive target. Mol Aspects Med 2007;28(3-4):375–95.
[10] Whitaker AM, Stark WJ, Flynn TS, Freudenthal BD. Molecular and structural characterization of disease-associated APE1 polymorphisms. DNA Repair (Amst). 2020;91-92:102867. https://doi.org/10.1016/j.dnarep.2020.102867.
[11] Dyrkheeva NS, Khodyreva SN, Lavrik OI. Multifunctional human apurinic/apyrimidinic endonuclease 1: Role of additional functions. Mol Biol 2007;41(3):402–16.
[12] Chou K-M, Cheng Y-C. An exonucleolytic activity of human apurinic/apyrimidinic endonuclease on 3' mispaired DNA. Nature 2002;415(6872):655–9.
[13] Chou K-M, Cheng Y-C. The exonuclease activity of human apurinic/apyrimidinic endonuclease (APE1). Biochemical properties and inhibition by the natural dinucleotide Gp4G. J Biol Chem 2003;278(20):18289–96.
[14] Krokan HE, Bjoras M. Base excision repair. Cold Spring Harb Perspect Biol. 2013;5:a012583.
[15] Dyrkheeva NS, Lebedeva NA, Lavrik OI. AP Endonuclease 1 as a Key Enzyme in Repair of Apurinic/Apyrimidinic Sites. Biochemistry (Mosc). 2016;81(9):951–67.
[16] Sung J-S, Demple B. Roles of base excision repair subpathways in correcting oxidized abasic sites in DNA. FEBS J 2006;273(8):1620–9.
[17] Freudenthal BD, Beard WA, Cuneo MJ, Dyrkheeva NS, Wilson SH. Capturing snapshots of APE1 processing DNA damage. Nat Struct Mol Biol 2015;22(11):924–31.
[18] Mol CD, Izumi T, Mitra S, Tainer JA. DNA-bound structures and mutants reveal abasic DNA binding by APE1 and DNA repair coordination [corrected]. Nature 2000;403:451–6.
[19] Mol CD, Kuo C-F, Thayer MM, Cunningham RP, Tainer JA. Structure and function of the multifunctional DNA-repair enzyme exonuclease III. Nature 1995;374(6520):381–6.
[20] Kerins SM, Collins R, McCarthy TV. Characterization of an endonuclease IV 3'-5' exonuclease activity. J Biol Chem 2003;278(5):3048–54.
[21] Hosfield DJ, Guan Y, Haas BJ, Cunningham RP, Tainer JA. Structure of the DNA repair enzyme endonuclease IV and its DNA complex: double-nucleotide flipping at abasic sites and three-metal-ion catalysis. Cell 1999;98(3):397–408.
[22] Tsutakawa SE, Shin DS, Mol CD, Izumi T, Arvai AS, Mantha AK, et al. Conserved structural chemistry for incision activity in structurally non-homologous apurinic/apyrimidinic endonuclease APE1 and endonuclease IV DNA repair enzymes. J Biol Chem 2013;288(12):8445–55.
[23] Souza LL, Eduardo IR, Padula M, Leitao AC. Endonuclease IV and exonuclease III are involved in the repair and mutagenesis of DNA lesions induced by UVB in Escherichia coli. Mutagenesis. 2006;21:125-30.
[24] Liu T-C, Lin C-T, Chang K-C, Guo K-W, Wang S, Chu J-W, et al. APE1 distinguishes DNA substrates in exonucleolytic cleavage by induced space-filling. Nat Commun 2021;12(1). https://doi.org/10.1038/s41467-020-20853-2.
[25] Whitaker AM, Flynn TS, Freudenthal BD. Molecular snapshots of APE1 proofreading mismatches and removing DNA damage. Nat Commun 2018;9:399.
[26] Burkovics P, Szukacsov V, Unk I, Haracska L. Human Ape2 protein has a 3'-5' exonuclease activity that acts preferentially on mismatched base pairs. Nucleic Acids Res 2006;34(9):2508–15.
[27] Lin Y, Bai L, Cupello S, Hossain MA, Deem B, McLeod M, et al. APE2 promotes DNA damage response pathway from a single-strand break. Nucleic Acids Res 2018;46:2479-94.
[28] Nugent M, Huang SM, Sander M. Characterization of the apurinic endonuclease activity of Drosophila Rrp1. Biochemistry 1993;32(42):11445–52.
[29] Shatilla A, Ishchenko AA, Saparbaev M, Ramotar D. Characterization of Caenorhabditis elegans exonuclease-3 and evidence that a Mg2+-dependent variant exhibits a distinct mode of action on damaged DNA. Biochemistry 2005;44(38):12835–48.

[30] Unk I, Haracska L, Prakash S, Prakash L. 3'-phosphodiesterase and 3'–>5' exonuclease activities of yeast Apn2 protein and requirement of these activities for repair of oxidative DNA damage. Mol Cell Biol 2001;21:1656–61.
[31] Yang X, Fan J, Ishchenko AA, Patel D, Saparbaev MK, Ramotar D. Functional characterization of the Caenorhabditis elegans DNA repair enzyme APN-1. DNA Repair (Amst). 2012;11(10):811–22.
[32] Ishchenko AA, Yang X, Ramotar D, Saparbaev M. The 3'->5' exonuclease of Apn1 provides an alternative pathway to repair 7,8-dihydro-8-oxodeoxyguanosine in Saccharomyces cerevisiae. Mol Cell Biol 2005;25:6380–90.
[33] Mol CD, Hosfield DJ, Tainer JA. Abasic site recognition by two apurinic/apyrimidinic endonuclease families in DNA base excision repair: the 3' ends justify the means. Mutat Res 2000;460(3-4):211–29.
[34] Puri RV, Singh N, Gupta RK, Tyagi AK, Parish T. Endonuclease IV Is the major apurinic/apyrimidinic endonuclease in Mycobacterium tuberculosis and is important for protection against oxidative damage. PLoS ONE 2013;8(8):e71535.
[35] Hang B, Chenna A, Sagi J, Singer B. Differential cleavage of oligonucleotides containing the benzene-derived adduct, 1, N6-benzetheno-dA, by the major human AP endonuclease HAP1 and Escherichia coli exonuclease III and endonuclease IV. Carcinogenesis 1998;19:1339–43.
[36] Mazouzi A, Vigouroux A, Aikeshev B, Brooks PJ, Saparbaev MK, Morera S, et al. Insight into mechanisms of 3'-5' exonuclease activity and removal of bulky 8,5'-cyclopurine adducts by apurinic/apyrimidinic endonucleases. Proc Natl Acad Sci U S A. 2013;110(33):E3071–80.
[37] Wong D, DeMott MS, Demple B. Modulation of the 3'–>5'-exonuclease activity of human apurinic endonuclease (Ape1) by its 5'-incised Abasic DNA product. J Biol Chem 2003;278:36242–9.
[38] Shida T, Noda M, Sekiguchi J. Cleavage of single- and double-stranded DNAs containing an abasic residue by Escherichia coli exonuclease III (AP endonuclease VI). Nucleic Acids Res 1996;24(22):4572–6.
[39] Mundle ST, Fattal MH, Melo LF, Coriolan JD, O'Regan NE, Strauss PR. Novel role of tyrosine in catalysis by human AP endonuclease 1. DNA Repair (Amst). 2004;3(11):1447–55.
[40] He H, Chen Q, Georgiadis MM. High-resolution crystal structures reveal plasticity in the metal binding site of apurinic/apyrimidinic endonuclease I. Biochemistry 2014;53(41):6520–9.
[41] McNeill DR, Wilson DM. A dominant-negative form of the major human abasic endonuclease enhances cellular sensitivity to laboratory and clinical DNA-damaging agents. Mol Cancer Res 2007;5(1):61–70.
[42] Kim W-C, Berquist BR, Chohan M, Uy C, Wilson DM, Lee CH. Characterization of the endoribonuclease active site of human apurinic/apyrimidinic endonuclease 1. J Mol Biol 2011;411(5):960–71.
[43] Wilson DM. Properties of and Substrate Determinants for the Exonuclease Activity of Human Apurinic Endonuclease Ape1. J Mol Biol 2003;330(5):1027–37.
[44] Hadi MZ, Ginalski K, Nguyen LH, Wilson DM. Determinants in nuclease specificity of Ape1 and Ape2, human homologues of Escherichia coli exonuclease III. J Mol Biol 2002;316(3):853–66.
[45] Hoitsma NM, Whitaker AM, Beckwitt EC, Jang S, Agarwal PK, Van Houten B, et al. AP-endonuclease 1 sculpts DNA through an anchoring tyrosine residue on the DNA intercalating loop. Nucleic Acids Res 2020;48:7345–55.
[46] Carpenter EP, Corbett A, Thomson H, Adacha J, Jensen K, Bergeron J, et al. AP endonuclease paralogues with distinct activities in DNA repair and bacterial pathogenesis. EMBO J 2007;26(5):1363–72.
[47] Izumi T, Schein CH, Oezguen N, Feng Y, Braun W. Effects of backbone contacts 3' to the abasic site on the cleavage and the product binding by human apurinic/apyrimidinic endonuclease (APE1). Biochemistry 2004;43(3):684–9.
[48] Peddi SR, Chattopadhyay R, Naidu CV, Izumi T. The human apurinic/apyrimidinic endonuclease-1 suppresses activation of poly(adp-ribose) polymerase-1 induced by DNA single strand breaks. Toxicology 2006;224(1-2):44–55.
[49] Chou K-M, Kukhanova M, Cheng Y-C. A novel action of human apurinic/apyrimidinic endonuclease: excision of L-configuration deoxyribonucleoside analogs from the 3' termini of DNA. J Biol Chem 2000;275(40):31009–15.
[50] Parsons JL, Dianova II, Dianov GL. APE1-dependent repair of DNA single-strand breaks containing 3'-end 8-oxoguanine. Nucleic Acids Res 2005;33:2204–9.
[51] Yao M, Kow YW. Cleavage of insertion/deletion mismatches, flap and pseudo-Y DNA structures by deoxyinosine 3'-endonuclease from Escherichia coli. The Journal of biological chemistry. 1996;271(48):30672–6.
[52] Marenstein DR, Wilson III DM, Teebor GW. Human AP endonuclease (APE1) demonstrates endonucleolytic activity against AP sites in single-stranded DNA. DNA Repair (Amst). 2004;3(5):527–33.
[53] Dyrkheeva NS, Lomzov AA, Pyshnyi DV, Khodyreva SN, Lavrik OI. Efficiency of exonucleolytic action of apurinic/apyrimidinic endonuclease 1 towards matched and mismatched dNMP at the 3' terminus of different oligomeric DNA structures correlates with thermal stability of DNA duplexes. Biochim Biophys Acta, Mol Cell Biol Lipids 2006;1764(4):699–706.
[54] Chohan M, Mackedenski S, Li W-M, Lee CH. Human apurinic/apyrimidinic endonuclease 1 (APE1) has 3' RNA phosphatase and 3' exoribonuclease activities. J Mol Biol 2015;427(2):298–311.
[55] Antoniali G, Serra F, Lirussi L, Tanaka M, D'Ambrosio C, Zhang S, et al. Mammalian APE1 controls miRNA processing and its interactome is linked to cancer RNA metabolism. Nat Commun 2017;8(1). https://doi.org/10.1038/s41467-017-00842-8.
[56] Kim WC, King D, Lee CH. RNA-cleaving properties of human apurinic/apyrimidinic endonuclease 1 (APE1). Int J Biochem Mol Biol 2010;1:12–25.
[57] Bennett RA, Wilson 3rd DM, Wong D, Demple B. Interaction of human apurinic endonuclease and DNA polymerase beta in the base excision repair pathway. Proc Natl Acad Sci U S A 1997;94:7166–9.
[58] Moor NA, Vasil'eva IA, Anarbaev RO, Antson AA, Lavrik OI. Quantitative characterization of protein-protein complexes involved in base excision DNA repair. Nucleic Acids Res 2015;43(12):6009–22.
[59] Quinones JL, Thapar U, Yu K, Fang Q, Sobol RW, Demple B. Enzyme mechanism-based, oxidative DNA-protein cross-links formed with DNA polymerase beta in vivo. Proc Natl Acad Sci U S A 2015;112:8602–7.
[60] Almeida B, Fernandes S, Abreu IA, Macedo-Ribeiro S. Trinucleotide repeats: a structural perspective. Front Neurol 2013;4:76.
[61] Liu G, Leffak M. Instability of (CTG)n*(CAG)n trinucleotide repeats and DNA synthesis. Cell Biosci 2012;2(1):7. https://doi.org/10.1186/2045-3701-2-7.
[62] Liu G, Chen X, Bissler JJ, Sinden RR, Leffak M. Replication-dependent instability at (CTG) x (CAG) repeat hairpins in human cells. Nat Chem Biol 2010;6:652–9.
[63] Liu Y, Wilson SH. DNA base excision repair: a mechanism of trinucleotide repeat expansion. Trends Biochem Sci 2012;37(4):162–72.
[64] Beaver JM, Lai Y, Xu M, Casin AH, Laverde EE, Liu Y. AP endonuclease 1 prevents trinucleotide repeat expansion via a novel mechanism during base excision repair. Nucleic Acids Res 2015;43(12):5948–60.
[65] Lai Y, Beaver JM, Laverde E, Liu Y. Trinucleotide repeat instability via DNA base excision repair. DNA Repair (Amst). 2020;93:102912. https://doi.org/10.1016/j.dnarep.2020.102912.
[66] Xu M, Lai Y, Torner J, Zhang Y, Zhang Z, Liu Y. Base excision repair of oxidative DNA damage coupled with removal of a CAG repeat hairpin attenuates trinucleotide repeat expansion. Nucleic Acids Res. 2014;42:3675-91.
[67] Couvé-Privat S, Ishchenko AA, Laval J, Saparbaev M. Nucleotide Incision Repair: An Alternative and Ubiquitous Pathway to Handle Oxidative DNA Damage; 2007:54-66.
[68] Ishchenko AA, Deprez E, Maksimenko A, Brochon J-C, Tauc P, Saparbaev MK. Uncoupling of the base excision and nucleotide incision repair pathways reveals their respective biological roles. Proc Natl Acad Sci U S A. 2006;103(8):2564–9.
[69] Gros L, Ishchenko AA, Ide H, Elder RH, Saparbaev MK. The major human AP endonuclease (Ape1) is involved in the nucleotide incision repair pathway. Nucleic Acids Res 2004;32:73–81.
[70] Gelin A, Redrejo-Rodríguez M, Laval J, Fedorova OS, Saparbaev M, Ishchenko AA, et al. Genetic and biochemical characterization of human AP endonuclease 1 mutants deficient in nucleotide incision repair activity. PLoS ONE 2010;5(8):e12241.
[71] Redrejo-Rodríguez M, Saint-Pierre C, Couve S, Mazouzi A, Ishchenko AA, Gasparutto D, et al. New insights in the removal of the hydantoins, oxidation product of pyrimidines, via the base excision and nucleotide incision repair pathways. PLoS ONE 2011;6(7):e21039.
[72] Daviet S, Couvé-Privat S, Gros L, Shinozuka K, Ide H, Saparbaev M, et al. Major oxidative products of cytosine are substrates for the nucleotide incision repair pathway. DNA Repair (Amst). 2007;6(1):8–18.
[73] Golan G, Ishchenko AA, Khassenov B, Shoham G, Saparbaev MK. Coupling of the nucleotide incision and 3'–>5' exonuclease activities in Escherichia coli endonuclease IV: Structural and genetic evidences. Mutat Res 2010;685:70–9.
[74] Willis J, Patel Y, Lentz BL, Yan S. APE2 is required for ATR-Chk1 checkpoint activation in response to oxidative stress. Proc Natl Acad Sci U S A. 2013;110(26):10592–7.
[75] Wallace BD, Berman Z, Mueller GA, Lin Y, Chang T, Andres SN, et al. APE2 Zf-GRF facilitates 3'-5' resection of DNA damage following oxidative stress. Proc Natl Acad Sci U S A 2017;114(2):304–9.
[76] Lin Y, Raj J, Li J, Ha A, Hossain MA, Richardson C, et al. APE1 senses DNA single-strand breaks for repair and signaling. Nucleic Acids Res 2020;48:1925-40.
[77] Lin Y, McMahon A, Driscoll G, Bullock S, Zhao J, Yan S. Function and molecular mechanisms of APE2 in genome and epigenome integrity. Mutat Res 2021;787:108347. https://doi.org/10.1016/j.mrrev.2020.108347.
[78] Hossain Md, Lin Y, Yan S. Single-Strand Break End Resection in Genome Integrity: Mechanism and Regulation by APE2. Int J Mol Sci 2018;19(8):2389. https://doi.org/10.3390/ijms19082389.
[79] Jaruga P, Dizdaroglu M. 8,5'-Cyclopurine-2'-deoxynucleosides in DNA: mechanisms of formation, measurement, repair and biological effects. DNA Repair (Amst). 2008;7(9):1413–25.
[80] Grove KL, Guo X, Liu SH, Gao Z, Chu CK, Cheng YC. Anticancer activity of beta-L-dioxolane-cytidine, a novel nucleoside analogue with the unnatural L configuration. Cancer Res 1995;55:3008–11.
[81] Fan Z, Beresford PJ, Zhang D, Xu Z, Novina CD, Yoshida A, et al. Cleaving the oxidative repair protein Ape1 enhances cell death mediated by granzyme A. Nat Immunol 2003;4(2):145–53.
[82] Yoshida A, Urasaki Y, Waltham M, Bergman A-C, Pourquier P, Rothwell DG, et al. Human apurinic/apyrimidinic endonuclease (Ape1) and its N-terminal truncated form (AN34) are involved in DNA fragmentation during apoptosis. J Biol Chem 2003;278(39):37768–76.
[83] Perrino FW, Miller H, Ealey KA. Identification of a 3'–>5'-exonuclease that removes cytosine arabinoside monophosphate from 3' termini of DNA. J Biol Chem 1994;269:16357–63.

[84] Huang KW, Liu TC, Liang RY, Chu LY, Cheng HL, Chu JW, et al. Structural basis for overhang excision and terminal unwinding of DNA duplexes by TREX1. PLoS biology. 2018;16:e2005653.
[85] Hsiao Y-Y, Yang C-C, Lin CL, Lin JLJ, Duh Y, Yuan HS. Structural basis for RNA trimming by RNase T in stable RNA 3'-end maturation. Nat Chem Biol 2011;7 (4):236–43.
[86] Hsiao YY, Duh Y, Chen YP, Wang YT, Yuan HS. How an exonuclease decides where to stop in trimming of nucleic acids: crystal structures of RNase T-product complexes. Nucl Acids Res 2012;40:8144-54.
[87] Kuraoka I, Robins P, Masutani C, Hanaoka F, Gasparutto D, Cadet J, et al. Oxygen free radical damage to DNA. Translesion synthesis by human DNA polymerase eta and resistance to exonuclease action at cyclopurine deoxynucleoside residues. J Biol Chem 2001;276(52):49283–8.
[88] Gehrke N, Mertens C, Zillinger T, Wenzel J, Bald T, Zahn S, et al. Oxidative damage of DNA confers resistance to cytosolic nuclease TREX1 degradation and potentiates STING-dependent immune sensing. Immunity 2013;39 (3):482–95.
[89] Chowdhury D, Beresford PJ, Zhu P, Zhang D, Sung J-S, Demple B, et al. The exonuclease TREX1 is in the SET complex and acts in concert with NM23-H1 to degrade DNA during granzyme A-mediated cell death. Mol Cell 2006;23 (1):133–42.
[90] Wang C-J, Lam W, Bussom S, Chang H-M, Cheng Y-C. TREX1 acts in degrading damaged DNA from drug-treated tumor cells. DNA Repair 2009;8 (10):1179–89.
[91] Chagovetz AM, Sweasy JB, Preston BD. Increased activity and fidelity of DNA polymerase beta on single-nucleotide gapped DNA. J Biol Chem 1997;272:27501–4.
[92] Hoss M, Robins P, Naven TJ, Pappin DJ, Sgouros J, Lindahl T. A human DNA editing enzyme homologous to the Escherichia coli DnaQ/MutD protein. EMBO J 1999;18:3868–75.
[93] Dyrkheeva NS, Khodyreva SN, Sukhanova MV, Safronov IV, Dezhurov SV, Lavrik OI. 3′–5′ exonuclease activity of human apurinic/apyrimidinic endonuclease 1 towards DNAs containing dNMP and their modified analogs at the 3′ end of single strand DNA break. Biochemistry (Moscow). 2006;71 (2):200–10.
[94] Miyazaki T, Kim Y-S, Yoon J, Wang H, Suzuki T, Morse HC. The 3'-5' DNA exonuclease TREX1 directly interacts with poly(ADP-ribose) polymerase-1 (PARP1) during the DNA damage response. J Biol Chem 2014;289 (47):32548–58.
[95] Cistulli C, Lavrik OI, Prasad R, Hou E, Wilson SH. AP endonuclease and poly (ADP-ribose) polymerase-1 interact with the same base excision repair intermediate. DNA Repair (Amst). 2004;3(6):581–91.
[96] Christmann M, Tomicic MT, Aasland D, Berdelle N, Kaina B. Three prime exonuclease I (TREX1) is Fos/AP-1 regulated by genotoxic stress and protects against ultraviolet light and benzo(a)pyrene-induced DNA damage. Nucleic Acids Res. 2010;38:6418-32.
[97] Fan Z, Beresford PJ, Zhang D, Lieberman J. HMG2 interacts with the nucleosome assembly protein SET and is a target of the cytotoxic T-lymphocyte protease granzyme A. Mol Cell Biol 2002;22(8):2810–20.
[98] Kalinowska-Herok M, Widłak P. High mobility group proteins stimulate DNA cleavage by apoptotic endonuclease DFF40/CAD due to HMG-box interactions with DNA. Acta Biochim Pol 2008;55(1):21–6.
[99] Kriatchko AN, Bergeron S, Swanson PC. HMG-box domain stimulation of RAG1/2 cleavage activity is metal ion dependent. BMC Mol Biol 2008;9(1):32. https://doi.org/10.1186/1471-2199-9-32.
[100] Swanson PC. Fine structure and activity of discrete RAG-HMG complexes on V (D)J recombination signals. Mol Cell Biol 2002;22(5):1340–51.
[101] Liu Y, Prasad R, Wilson SH. HMGB1: roles in base excision repair and related function. Biochim Biophys Acta, Mol Cell Biol Lipids 2010;1799(1-2):119–30.
[102] Prasad R, Liu Y, Deterding LJ, Poltoratsky VP, Kedar PS, Horton JK, et al. HMGB1 is a cofactor in mammalian base excision repair. Mol Cell 2007;27 (5):829–41.
[103] Álvarez-Quilón A, Wojtaszek JL, Mathieu M-C, Patel T, Appel CD, Hustedt N, et al. Endogenous DNA 3' Blocks Are Vulnerabilities for BRCA1 and BRCA2 Deficiency and Are Reversed by the APE2 Nuclease. Mol Cell 2020;78 (6):1152–1165.e8.
[104] Zhang Z-M, Yang X-Q, Wang D, Wang Ge, Yang Z-Z, Qing Yi, et al. Nm23-H1 protein binds to APE1 at AP sites and stimulates AP endonuclease activity following ionizing radiation of the human lung cancer A549 cells. Cell Biochem Biophys 2011;61(3):561–72.
[105] Yan N. Immune Diseases Associated with TREX1 and STING Dysfunction. J Interfer Cytok Res Off J Int Soc Interfer Cytok Res 2017;37(5):198–206.