Understanding APE1 cellular functions by the structural preference of exonuclease activities

Tung-Chang Liu, Kai-Wei Guo, Jhih-Wei Chu, Yu-Yuan Hsiao

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.

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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 specifically targeted 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 3′-terminal amino acids, and studying the redox activity thus 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.

Table 1

| BER Endonuclease activity | Exonuclease activity | Proofreading | Damage bases in SSB |
|---------------------------|----------------------|--------------|---------------------|
| DNA damage from oxidation, deamination and alkylation [14]. | 1. αdA, DHT, DHU, 5OHU and αT [69,2]. | 1. dDT, AZT, D4T [12]. | 1. 8-oxoG |
| | 5OH-Hy and 5OH-Hyd [71]. | 2. L-OddC, dOddC, araC, dOddC, L-SddC, L-Fd4c [49]. | 50.2. |
| | 5Me-Hyd [3]. | cdA, cdG | 36. |

Abbreviation: αdA (alpha-2′-deoxyadenosine), DHT (5,6-Dihydro thymidine), DHU (5,6-dihydro-2′-deoxyuridine), 5OHU (5-Hydroxyuracil), αT (alpha-Thymine), 5OH-Hy (5-hydroxyhydrindan), 5OH-5Me-Hy (5-methyl-5-hydroxyhydrindan), 5-hydroxy-2′-deoxyctydine (5OHc), α-anomeric 2′-deoxyctydine (αdC), dTt (2′,3′-dideoxythymidine), AZT (3′-azido-3′-deoxythymidine), D4T(2,3-didehydro-2′, 3′-dideoxythymidine), L-OddC (b-L-Dioxolane-cytidine), dOddC (b-D-Z′, 2′-difuoro-dodeoxyctydine), araC (b-D-arabinofuranosylcytosinate), dOddC (b-D-Z′, 3′-dideoxyctydine), L-SddC (b-L-Z′, 3′-dideoxyctydine), L-Fd4c (b-L-Z′, 3′-dideoxy-2′, 3′-didehydro-5-fluorocytidine), 8-oxoG (8-oxoguanine), cdA (8,5′-cyco-2′-deoxyadenosine), cdG (8,5′-cyco-2′-deoxyguanosine), cdT (8,5′-cyco-2′-deoxyctydine).

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 endonucleolytic 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 (exocl, 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 [19,20]. Interestingly, another E. coli enzyme, exonuclease IV (endonu, 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 exoII and APE1 do [22]. In E. coli, exoII 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 Aprn2 [30] have been identified to be the homologs of exoII. On the other hand, C. elegans APN-1 [31] and S. cerevisiae Aprn1 [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*, exonull is the major AP-site specific endonuclease in DNA repair, but the main player becomes the endoIV homolog APN1 in yeast and endoV in *Mycobacterium tuberculosis*[^33,34]. A mystery is thus the respective roles of exonull and endoV types of nucleases in the cell. A potential origin for their overlapping functionalities is one serving as a backup system for the other. However, the corresponding levels of endonuclease and exonuclease activities and the base preference of these two nuclease types are somewhat different[^35]. For example, only exonull 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[^11,13,38]. 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 exonuclease 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* exonull and mammalian APE2 for example, greater preference toward exonuclease substrates is observed[^25,26,44]. Unlike the endonucleolytic 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.

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**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, Thr280 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 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 causing 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 exonuclease cleavage against a variety of nucleotide analogues as the 3’-end substrate, including 2’-3’-dideoxythymidine (dTd), 3’-azido-3’-deoxythymidine (AZT), 2,3-dideoxy-2,3-dideoxythymidine (d4T), L-D-2’,3’-dideoxyxytidine (d4DC), L-D-2’,3’-dideoxyxytidine (d4D), L-D-2’,3’-dideoxythymidine (d4TC), L-D-2’,3’-dideoxythymidine (TdT), 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 nuclease 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).
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 exonucleaseolytically 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. 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.](image-url)
disorders like cancer, cardiovascular, and brain dysfunction\cite{67,68}.
The oxidative lesions repaired by NIR are listed in Table 1\cite{69–72}.
Unlike BER, NIR is a DNA glycosylase-independent pathway and requires both the endonuclease and exonuclease activities of APE1\cite{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\cite{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)\cite{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\cite{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 \cite{74–76}.

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).
distort the double-helix structure, block replication and transcrip-
tion, 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 exci-
sion repair (NER) pathway [36,49]. The actual route for the in-
volvement 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 ter-
minate 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]. Exonucle-
olytic 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
shown to be able to process matched blunt-ended dsDNA and
amino acids 1–35)[82]. The exonuclease activity of AN34 APE1 was
strates without the 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 [36,84,87,88]. On the other hand, the lack of base preference in APE1 enables it to process the DNA sub-
strates 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 cer-
tainly valuable to further test this hypothesis.

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 sub-
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APE1 work together in cellular responses to DNA damage. It is cer-
tainly 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 binding the
dsDNA substrates. Along the same line, the HMGB2 homolog
HMGB1 enhances the abilities of APE1 and FEN1 in substrate inci-
son [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 mediat-
ing 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 dis-
torted 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 mis-
matched 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 nucleases 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 cytoplasm 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 influenced the work reported in this paper.

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