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Characterization of Abasic Endonuclease Activity of Human Ape1 on Alternative Substrates, as Well as Effects of ATP and Sequence Context on AP Site Incision

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Human Ape1 is a multifunctional protein with a major role in initiating repair of apurinic/apyrimidinic (AP) sites in DNA by catalyzing hydrolytic incision of the phosphodiester backbone immediately adjacent to the damage. Besides in double-stranded DNA, Ape1 has been shown to cleave at AP sites in single-stranded regions of a number of biologically relevant DNA conformations and in structured single-stranded DNA. Extension of these studies has revealed a more expansive repertoire of model substrates on which Ape1 exerts AP endonuclease activity. In particular, Ape1 possesses the ability to cleave at AP sites located in (i) the DNA strand of a DNA/RNA hybrid, (ii) "pseudo-triplex" bubble substrates designed to mimic stalled replication or transcription intermediates, and (iii) configurations that emulate R-loop structures that arise during class switch recombination. Moreover, Ape1 was found to cleave AP-site-containing single-stranded RNA, suggesting a novel "cleansing" function that may contribute to the elimination of detrimental cellular AP-RNA molecules. Finally, sequence context immediately surrounding an abasic site in duplex DNA was found to have a less than threefold effect on the incision efficiency of Ape1, and ATP was found to exert complex effects on the endonuclease capacity of Ape1 on double-stranded substrates. The results suggest that in addition to abasic sites in conventional duplex genomic DNA, Ape1 has the ability to incise at AP sites in DNA conformations formed during DNA replication, transcription, and class switch recombination, and that Ape1 can endonucleolytically destroy damaged RNA.

Keywords: Ape1; DNA repair; polymerase stalling; class switch recombination; RNA cleavage

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Introduction

Apurinic/Apyrimidinic (AP) endonuclease 1 (Ape1) is a multifunctional enzyme of 318 amino acids (35.5 kDa) with redox-dependent regulation of transcription factors,[1] 3′ to 5′ exonuclease,[2] 3′ phosphodiesterase,[3] RNaseH,[4] and class II type AP endonuclease activities.[5] The endonuclease function entails incision of the phosphodiester bond immediately 5′ to an AP site, generating a single-strand break with 5′-deoxyribose phosphate and 3′-hydroxyl ends.[6] This action creates a substrate on which subsequent enzymes of the base excision repair (BER) pathway can act to fill the nucleotide gap and seal the nick in the DNA backbone to complete AP site

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Abbreviations used: AP, apurinic/apyrimidinic; Ape1, apurinic/apyrimidinic endonuclease 1; BER, base excision repair; ssDNA, single-stranded DNA; dsDNA, double-stranded DNA; CSR, class switch recombination; DSB, double-strand break; AID, activation-induced cytidine deaminase; TCR, transcription-coupled repair; TTP, thymidine 5′-triphosphate; CTP, cytidine 5′-triphosphate; GTP, guanosine 5′-triphosphate; dATP, deoxyadenosine triphosphate; (d)NTP, (deoxyribo)nucleotide triphosphate.

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repair (i.e., DNA polymerase β and XRCC1-DNA ligase IIIα). Ape1 is the major mammalian protein for initiating removal of abasic sites in DNA,[7] although a second Ape1-like protein that acts as a 3′ to 5′ exonuclease and possesses a weak AP endonuclease activity has been identified in mammalian cells.[8,9]

Abasic sites are estimated to arise spontaneously ~10,000 times per mammalian genome equivalent per day.[10] In addition to spontaneous base loss, abasic intermediates are formed via enzyme (DNA glycosylase)-catalyzed base release during the process of BER.[11] Unrepaired, noninstructional abasic sites have mutagenic and cytotoxic consequences to the cell.[12] For instance, elongating replicative DNA polymerases and RNA polymerases will pause or arrest at abasic products, leading to collapse of the synthetic process and possibly activation of cell death responses.[13] Abasic sites encountered during DNA replication can also lead to error-prone bypass synthesis by translesion DNA polymerases.[14] In *Saccharomyces cerevisiae*, enhanced mutagenesis and severe inhibition of transcription occur in strains defective in the removal of AP sites and that lack *RAD26*, a SWI/SNF family ATPase and a homolog of the human Cockayne syndrome B (*ERCC6/CSB*) gene.[15] In all, current experimental evidence supports the idea that (i) AP sites lead to enhanced DNA mutagenesis, (ii) natural and oxidized abasic forms are blocks to RNA polymerase progression, and (iii) CSB functions to promote AP-site processing, presumably by facilitating efficient transcription and/or preventing the production of mutant RNA templates.

The presence of damaged or mutant RNA molecules can have many consequences to the cell. For instance, damaged or miscoding ribosomal RNAs have the potential to poison an entire ribosome and lead to collapse of the translational machinery.[16] Damaged or miscoding tRNAs can lead to mutagenic protein synthesis.[17] Aberrant mRNAs have the capacity to do both, as a ribosome may stall upon encountering the damage or incorporate improper amino acids to produce mutant polypeptides.[18] Pathways for dealing with altered mRNAs exist in mammalian cells, such as nonsense-mediated decay and nonstop decay.[19] In addition to pathways that degrade improper mRNAs, enzymes have evolved to repair damage to RNA molecules. The AlkB family of oxidative demethylases removes 1-methyladenine, 3-methylcytosine, and 1-methylguanine from both DNA and RNA.[20] It is not currently known whether other types of RNA damage are subject to "repair," although oxidative RNA modifications have been shown to be increased in both Alzheimer's disease[21] and atherosclerosis.[22]

Complex nucleic acid structures form not only during the processes of DNA replication and RNA transcription, but also during other cellular events. Antibody class switch recombination (CSR) occurs at immunoglobulin (Ig) switch regions via the creation and joining of DNA double-strand breaks (DSBs).[23] The process of CSR is presumably mediated by transcription through the GC-rich switch regions, which leads to the formation of DNA/RNA hybrids within so-called R-loops.[24] The establishment of DSBs is thought to be initiated by the action of activation-induced cytidine deaminase (AID), which deaminates cytosine to uracil within these loop structures. The uracil bases are then

Table 1. Oligonucleotides used in this study

Name	Sequence (5′–3′)
DNA/DNA, DNA/RNA, ssDNA, ssRNA	
42F	CCGCTGAATTGCACCCTCGAFCTAGGTCGATGATCCTAAGCA
42bubbleComp	TGCTTAGGATCATCGAGGATCGAGCTCGGTGCAATTCAGCGG
42comp	TGCTTAGGATCATCGACCTAGGTCGAGGGTGCAATTCAGCGG
18DNA	TCGACCTAGATCGAGGGT
18RNA	rUrCrGrArCrCrUrArGrArUrCrGrArGrGrGrU
18RNAfor54comp	rUrGrGrGrArGrCrUrUrGrArUrCrCrArGrCrU
54Fendbubble18	CCGCTGCCGCTGAATTGCFCCCTCGATCTAGGTCGATGATCCTAAGCATAAGCA
54Fcenterbubble18	CCGCTGCCGCTGAATTGCACCCTCGAFCTAGGTCGATGATCCTAAGCATAAGCA
54bubble18comp	TGCTTATGCTTAGGATCAAGCTGGATCAAGCTCCCAGCAATTCAGCGGCAGCGG
26FDNA	AATTCACCGGTACGFACTAGAATTCG
26FRNA	rArArUrUrCrArCrCrGrGrUrArCrGFrArCrUrArGrArArUrUrCrG
34FDNA	CTGCAGCTGATGCGCFGTACGGATCCCCGGGTAC
34FRNA	rCrUrGrCrArGrCrUrGrArUrGrCrGrCFrGrUrArCrGrGrArUrCrCrCrCrGrGrGrUrArC
Sequence context	
GFA	AATTCACCGGTACGFACTAGAATTCG
GFA-C	CGAATTCTAGTCCGTACCGGTGAATT
GFA-G	CGAATTCTAGTGCGTACCGGTGAATT
CFT	AATTCACCGGTACCFTCTAGAATTCG
CFT-C	CGAATTCTAGACGGTACCGGTGAATT
CFT-G	CGAATTCTAGAGGGTACCGGTGAATT
GFT	AATTCACCGGTACGFTCTAGAATTCG
GFT-C	CGAATTCTAGACCGTACCGGTGAATT
GFT-G	CGAATTCTAGAGCGTACCGGTGAATT
CFA	AATTCACCGGTACCFACTAGAATTCG
CFA-C	CGAATTCTAGTCGGTACCGGTGAATT
CFA-G	CGAATTCTAGTGGGTACCGGTGAATT

F denotes synthetic abasic site analog tetrahydrofuran.