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Discoveries

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Human Apurinic/Apyrimidinic Endonuclease 1

Mengxia Li and David M. Wilson, III

Abstract

Significance: Human apurinic/apyrimidinic endonuclease 1 (APE1, also known as REF-1) was isolated based on its ability to cleave at AP sites in DNA or activate the DNA binding activity of certain transcription factors. We review herein topics related to this multi-functional DNA repair and stress-response protein. ***Recent Advances:*** APE1 displays homology to *Escherichia coli* exonuclease III and is a member of the divalent metal-dependent α/β fold-containing phosphoesterase superfamily of enzymes. APE1 has acquired distinct active site and loop elements that dictate substrate selectivity, and a unique N-terminus which at minimum imparts nuclear targeting and interaction specificity. Additional activities ascribed to APE1 include 3′–5′ exonuclease, 3′-repair diesterase, nucleotide incision repair, damaged or site-specific RNA cleavage, and multiple transcription regulatory roles. ***Critical Issues:*** APE1 is essential for mouse embryogenesis and contributes to cell viability in a genetic background-dependent manner. Haploinsufficient $APE1^{+/-}$ mice exhibit reduced survival, increased cancer formation, and cellular/tissue hyper-sensitivity to oxidative stress, supporting the notion that impaired APE1 function associates with disease susceptibility. Although abnormal APE1 expression/localization has been seen in cancer and neuropathologies, and impaired-function variants have been described, a causal link between an APE1 defect and human disease remains elusive. ***Future Directions:*** Ongoing efforts aim at delineating the biological role(s) of the different APE1 activities, as well as the regulatory mechanisms for its intra-cellular distribution and participation in diverse molecular pathways. The determination of whether APE1 defects contribute to human disease, particularly pathologies that involve oxidative stress, and whether APE1 small-molecule regulators have clinical utility, is central to future investigations. *Antioxid. Redox Signal.* 20, 678–707.

Introduction

SHORTLY AFTER DETERMINATION of the DNA structure, it became appreciated that our genetic material is susceptible to spontaneous hydrolytic decay, as well as reactions with endogenous and exogenous physical and chemical agents. Such events can lead to modification of the DNA composition and alter the coding content of the genome, potentially driving mutagenesis or activation of cell death responses. Through many decades of research, it has become apparent that there exist enzymatic processes which recognize DNA damage and restore genetic integrity. Importantly, defects in the efficiency or accuracy of these so-called DNA repair or DNA damage tolerance pathways have been found to result in developmental failings, immunological deficiencies, cancer predisposition, neurological abnormalities, and premature aging characteristics, to name a few.

One of the most common forms of DNA damage is the loss of the base moiety from the intact sugar phosphate backbone (Fig. 1). Early studies from Lindahl estimated that roughly 10,000 depurination/depyrimidination events occur spontaneously per mammalian genome per day [reviewed in Lindahl (122)]. Since the base residue provides the instructional information, loss of this component of DNA can lead to error-prone bypass synthesis or polymerase arrest, and, thus, problems during replication or transcription. Such events can cause mutagenesis, chromosome instability, and gene expression defects, which underlie the cellular dysfunction and pathologies commonly associated with a DNA repair defect.

Given the high frequency of apurinic/apyrimidinic (AP) sites in DNA, and their potential for promoting deleterious

Intramural Research Program, Laboratory of Molecular Gerontology, National Institute on Aging, National Institutes of Health, Baltimore, Maryland.

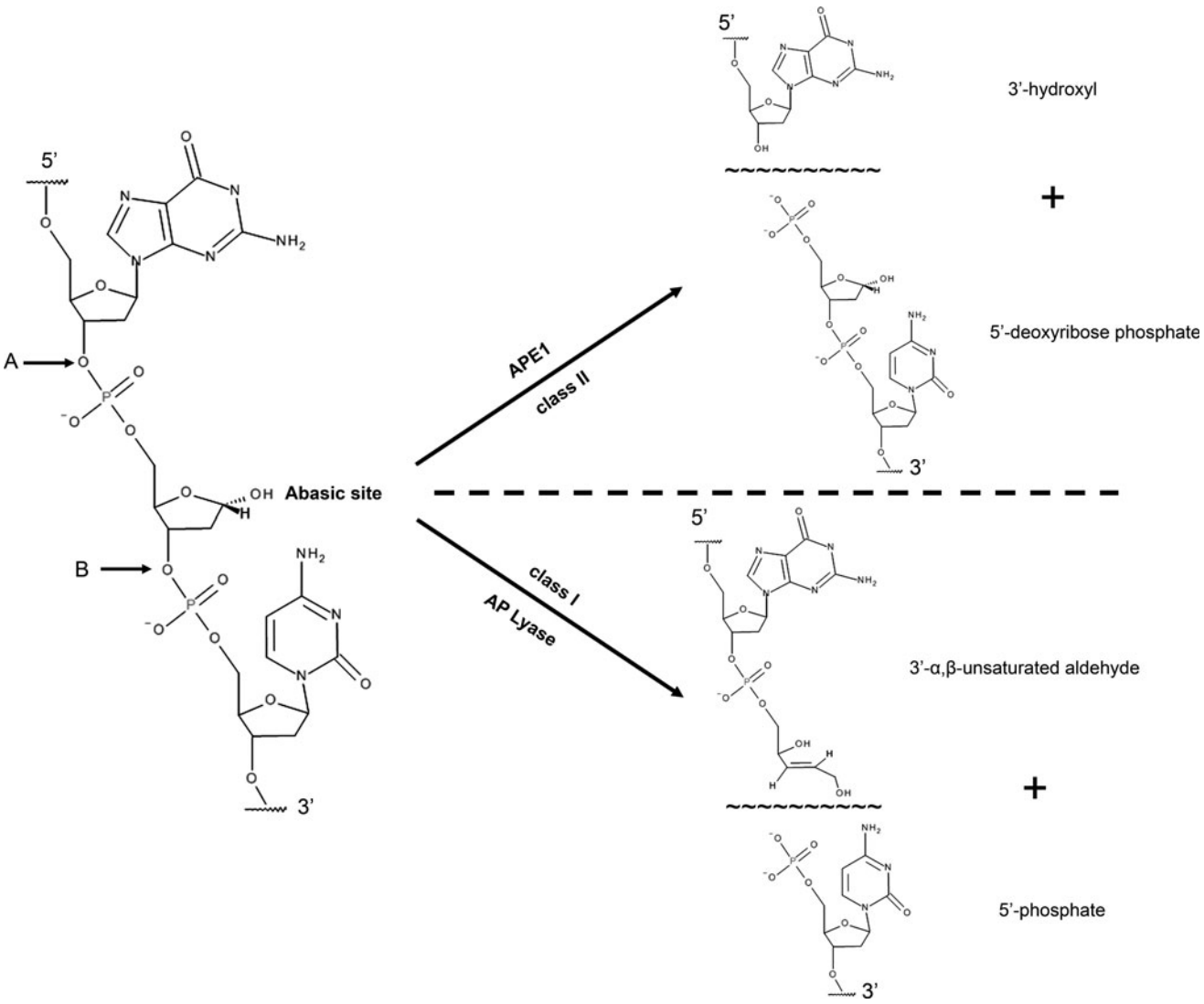

FIG. 1. Chemical structure of a hydrolytic abasic site and the cleavage position for the major classes of AP site incision enzymes. The phosphodiester bond cleavage site, immediately adjacent to an abasic lesion (see *arrows*), is shown for a class I AP lyase (site B) and a class II AP endonuclease (site A). Class I AP lyases cleave *via* β-elimination, generating a 3′-α,β-unsaturated aldehyde and a 5′-phosphate. Class II AP endonucleases, for example, APE1, incise the DNA backbone hydrolytically, leaving behind 5′-deoxyribose phosphate and 3′-hydroxyl termini. For simplicity, just the strand containing the AP site is shown, with two "random" flanking bases. Images were created using the Accelrys Draw 4.1 software (Accelrys, San Diego, CA). AP, apurinic/apyrimidinic; APE1, apurinic/apyrimidinic endonuclease 1.

outcomes, investigators searched for possible repair activities specific for these lesions. Reports in the 1970s [reviewed in Lindahl (121)], indeed, described protein fractions of varying purity from a range of organisms, including mammalian cell and tissue extracts, that exhibited the ability to cleave at abasic sites, which had been introduced into DNA by acid/heat hydrolysis or genotoxin-induced base release. Subsequent work by Linn and colleagues (147) revealed that there are two main classes of AP endonucleases: those that cleave 3′ to the abasic residue (class I) and those which cleave 5′ (class II) (Fig. 1). It was presumed that these incision activities represented an important step toward the eventual removal of abasic sites from chromosomal DNA.

Around the same time, a separate class of enzymes, termed DNA glycosylases, was discovered that had the capacity to release abnormal base residues from DNA in free form, producing an AP site intermediate. We now appreciate that there exist a collection of conserved DNA glycosylases that exhibit specificity for a broad range of substrate bases, including the deamination product of cytosine, uracil, the alkylation product 3-methyladenine, and the oxidation product 8-oxoguanine, to name a few (16, 177). Piecing together the information at the time, it was clear that organisms had evolved a DNA repair pathway which (i) excised a modified substrate base by breaking the N-glycosylic bond, (ii) cleaved the DNA backbone 5′ to the resulting AP site (class II incision), (iii) removed the 5′-abasic fragment *via* some form of nuclease degradation, (iv) carried out repair replication, and (v) sealed the remaining nick *via* DNA ligation. This pathway was designated base excision repair (BER), because unlike nucleotide excision repair, which involved the release of an oligonucleotide fragment, BER was initiated by direct base release (121).

Today, BER is considered the primary mechanism for coping with most forms of spontaneous hydrolytic, alkylative and oxidative DNA damage. BER has been sub-divided into two major pathways based on the number of nucleotides incorporated during repair synthesis, that is, single nucleotide and long patch, which involves incorporation of 2–13 nucleotides and strand-displacement polymerization. Single-strand break repair, which is a specialized process that is used for dealing with abnormal 3′- or 5′-termini that block DNA polymerase or ligase activity, also engages many of the core BER proteins. For a more detailed discussion of BER, its protein components, and its relationship to disease and other processes, the readers are directed to (39, 109) and the accompanying review by Izumi and colleagues.

Discovery and Cloning of Human AP Endonuclease 1

As alluded to earlier, early work from the Linn and Grossman laboratories described the purification of an endodeoxyribonuclease from HeLa cervical cancer cells and human placenta that acted on AP sites in substrate DNA molecules (64, 95, 187). This protein, which represented the predominant AP site incision activity in human cell extracts, was roughly 32–41 kDa in molecular weight and monomeric, was most active at a pH around 7.6 to 7.8, required the divalent metal Mg^{2+} (or to a lesser degree Mn^{2+}) for efficient cleavage, and exhibited class II endonuclease specificity, generating 3′-hydroxyl and 5′-deoxyribose-5-phosphate strand break termini (Fig. 1). Nearly a decade later, in the early 1990s, the transcript encoding the human AP endonuclease (at the time, termed APE, HAP1, and APEX, since named apurinic/apyrimidinic endonuclease 1 [APE1]/APEX1) was cloned by the Demple, Hickson, and Seki groups using one of two approaches: (i) a screen of a lambda phage expression library with an antibody against the purified protein (37) or (ii) a cross-hybridization screen of a human cDNA library using either the bovine or mouse AP endonuclease cDNA as a probe (171, 182). Strikingly, around the same time, human APE1 was independently identified (and the gene subsequently cloned) by Curran and colleagues as the major nuclear protein (termed REF-1) to simulate the DNA-binding activity of the AP-1 (Fos/Jun) transcription factor complex (233). This activation was shown to be mediated through reduction of a conserved cysteine residue within the DNA binding domain of the target protein, and was also observed with factors such as NF-κB, Myb, and members of the ATF/CREB family. Thus, it was recognized early on that APE1/REF-1 (from here on referred to as APE1) was a multifunctional protein, with roles in both DNA repair and transcriptional regulation. Figure 2 depicts a linear schematic of human APE1, identifying several of the key elements of the protein that will be discussed throughout.

APE1 Protein

The human *APE1* gene is composed of five exons, spans roughly 2.5 to 3 kb of DNA, and is located on chromosome 14q11.2 (3, 72, 73, 172, 247). The main mRNA transcript is ~1.5 kb in length, and appears to be ubiquitously expressed in all tissue and cell types from a housekeeping-like promoter that lacks a consensus TATA box, but harbors a CCAAT-like sequence and several putative CpG regulatory elements. The open reading frame is located within the last four exons, and it encodes a protein of 318 amino acids (theoretical molecular weight of 35.5 kDa). From residue ~60 through the end of the protein, human APE1 shares sequence homology with *Escherichia coli* exonuclease III (xth), but harbors a unique N-terminal region (Fig. 3A). Exonuclease III had been shown earlier to exhibit a range of nuclease activities consistent with what had been described for the mammalian protein,

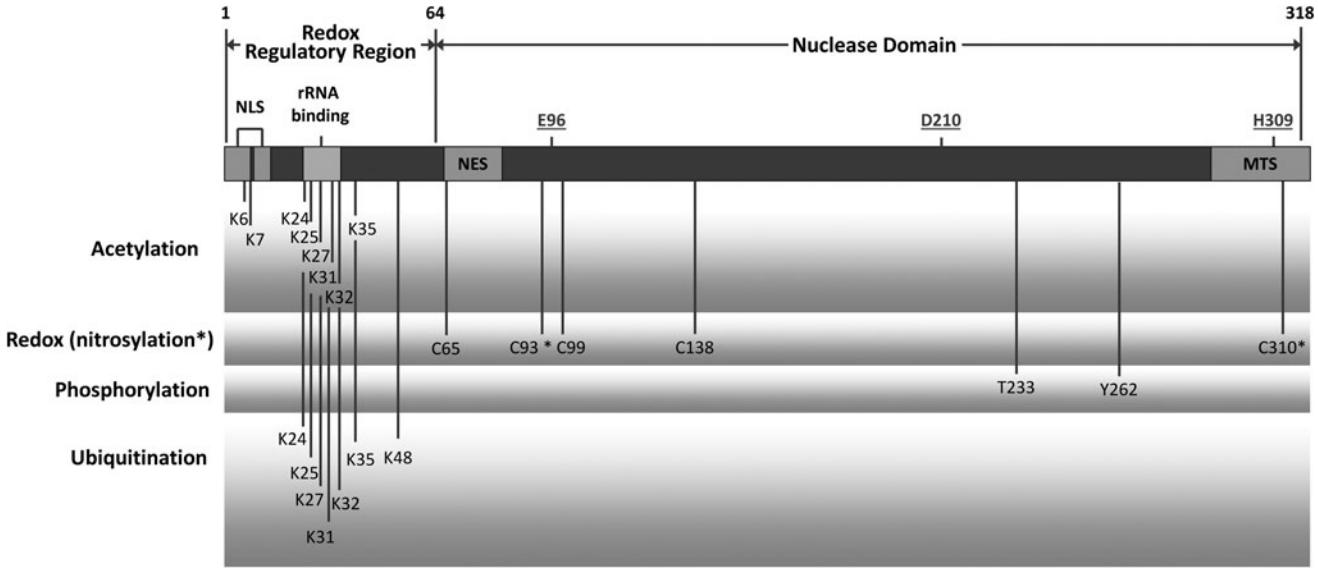

FIG. 2. Schematic of key elements in the human APE1 protein. The nuclease domain of APE1 spans roughly residues 64 to 318, with the N-terminal portion of the protein encompassing much of the transcriptional regulatory functions of APE1 (with some overlap between the two regions). Key active site residues for the nuclease activities of APE1 (*i.e.*, E96, D210, and H309) are identified *via* underline, and several PTM sites are depicted. A recently identified, lysine-rich, rRNA binding site is also designated. See text for further details. NLS, nuclear localization signal; NES, nuclear export signal; MTS, mitochondrial targeting sequence; PTM, post-translational modification.

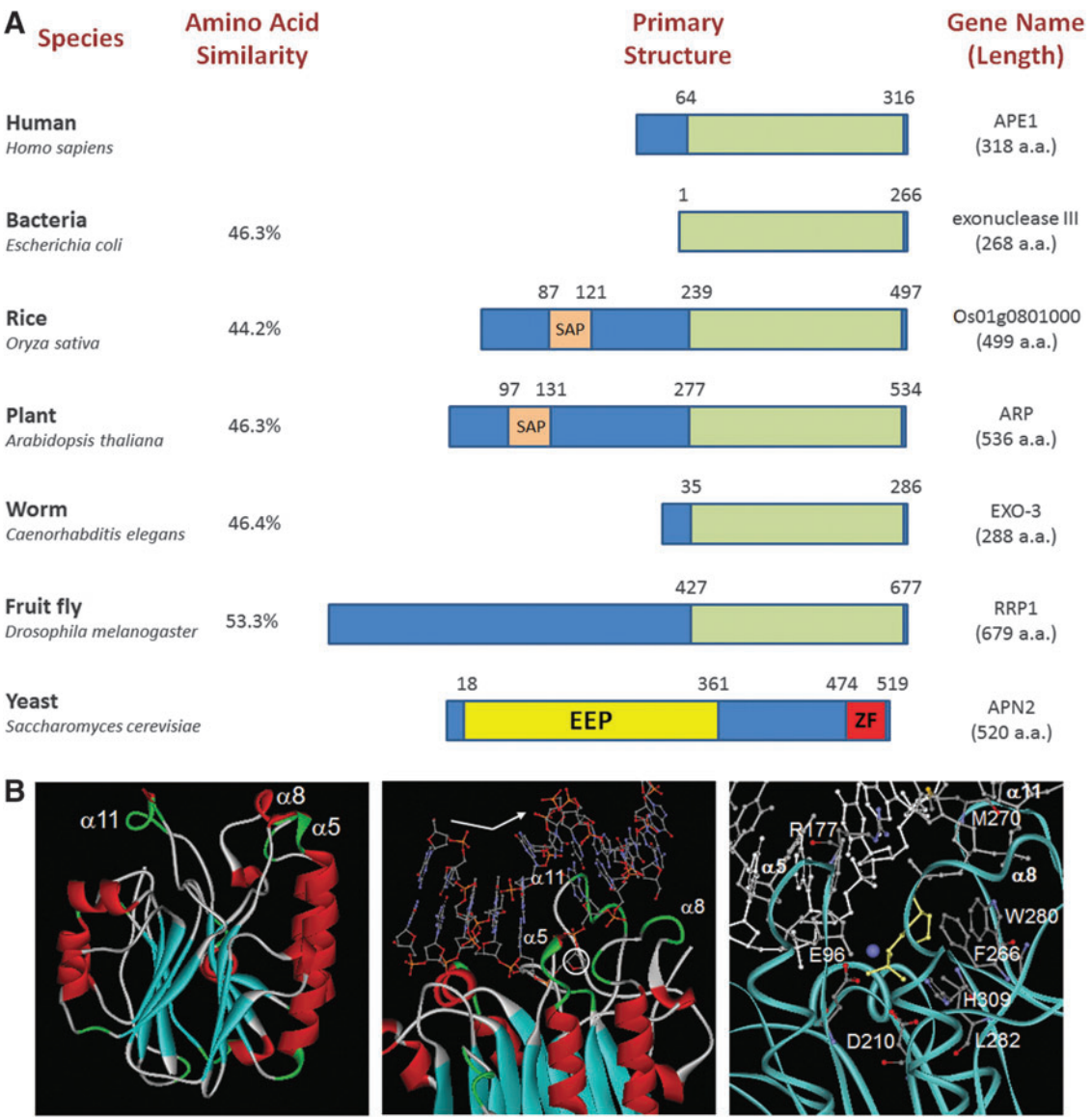

FIG. 3. Human APE1 orthologs and key protein structural elements. (A) APE1 orthologs. The particular species (*left*) and gene name (*right*) are indicated. Shown is the primary structure, with the AP endonuclease domain designated as a green box. Total amino-acid sequence length and percent similarity with human APE1 are specified. SAP, SAF-A/B, Acinus and PIAS motif, which is a putative DNA/RNA binding domain; EEP, Exonuclease-Endonuclease-Phosphatase domain; ZF, zinc finger. **(B)** APE1 structural features. All images were created using the DS ViewerPro software (Accelrys, San Diego, CA). Left: ribbon diagram of human APE1 [coordinates from 1BIX (63)]. The major recognition loops are designated. Middle: diagram of APE1 bound to un-incised AP-DNA [coordinates from 1DE8 (145)]. Recognition loops are indicated, and the abasic site is circled. Note the 35° kinking of the DNA backbone (emphasized by *arrow*). Right: diagram of the binding surface and active site of APE1 in complex with incised AP-DNA and Mn^{2+} [coordinates from 1DE9 (145)]. DNA strands are shown in white, with the 5′-abasic residue designated in yellow. The protein is shown as a cyan ribbon. The recognition pocket (comprising F266, W280, and L282) and several key active site residues (E96, D210, and H309) are highlighted. The recognition loops, and two key binding residues (R177 and M270), are also indicated. The catalytic metal ion is depicted as a purple sphere. To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/ars

including AP endonuclease, 3′-repair diesterase, and RNase H functions [reviewed in Demple and Harrison (36)]. Supportive of a critical biological role for these proteins, exonuclease III orthologs have been conserved throughout evolution and are present in many organisms (for examples, see Fig. 3A).

An initial analysis of the primary amino-acid sequence of human APE1 identified consensus nuclear localization signals (NLSs) within the acquired N-terminus (Fig. 2), which have since been confirmed to function in sub-cellular targeting (see "Intra-cellular targeting and mitochondrial function" section), as well as a number of putative phosphorylation sites throughout the length of the protein (see later). More recent, a comprehensive bioinformatic sequence analysis has revealed that APE1 is a part of a divalent cation-dependent phosphoesterase superfamily of proteins, which includes nucleases (*e.g.*, DNase I, RNase H, and the LINE-1 or L1

retrotransposon endonuclease), inositol (polyphosphate) and, possibly, protein phosphatases, and sphingomyelinases (40). These proteins, likely arising *via* divergent evolution, share a conserved four-layered α/β-sandwich structural core, and exhibit a wide range of substrate specificities, while maintaining a generally conserved catalytic mechanism for cleaving phosphoester bonds in nucleic acids, proteins, and phospholipids [see for example (225)]. X-ray crystallography studies indicate that the superfamily of enzymes, while preserving the common α/β fold, possess specific loop regions and active site elements that likely dictate substrate specificity [see for example (63, 209, 223)].

Crystal structures solved by Tainer and colleagues of human APE1 bound to both substrate (intact) and product (incised) forms of AP-DNA indicate that the protein uses a rigid, pre-formed positively charged surface to kink the DNA helix and engulf the AP site strand to facilitate targeted complex formation (145). Stabilization of the kinked abasic substrate is further mediated by residues emanating from four loops and one α-helix (*e.g.,* α5, α8, α11), with the "flipped-out" extrahelical AP site positioned within a pocket (comprising F266, W280, and L282) that excludes DNA bases (Fig. 3B). The importance of the α8 loop and the active site pocket residues in determining substrate specificity has been demonstrated by a biochemical analysis of site-specific mutant APE1 proteins (70, 188). A noteworthy feature of APE1 is that R177, which is located within the α5 loop, inserts into the major groove to provide a hydrogen bond to the AP site 3′ phosphate, and, in doing so, seems to lock the protein onto the incised DNA product. Since mutation of R177 improves the turnover rate of APE1, it would appear that the enzyme has evolved not for catalytic efficiency, but to facilitate coordination with the next enzyme in the BER pathway (145). This model of "passing the baton," while supported by biochemical and structural data, however, lacks biological evidence.

Despite our sound understanding of how APE1 locates, scanning DNA in a quasi-processive manner (24), and specifically recognizes AP sites, there remains controversy around how APE1 cleaves the phosphodiester backbone. Nonetheless, there is general consensus that E96 plays a role in divalent metal coordination and that D210 and H309 have critical functions in the hydrolytic reaction chemistry (Fig. 3B). Other important residues include N68, D70, Y171, N212, D283, and D308, which are generally conserved throughout the diverse members of the phosphoesterase superfamily. Still, further studies are needed to delineate the precise catalytic reaction mechanism, which is debated in several recent publications (123, 148, 152, 210).

Before proceeding with our more detailed discussion of human APE1, it is worth mentioning that in *E. coli* there are two prominent AP endonucleases: exonuclease III and endonuclease IV (nfo) [reviewed in Demple and Harrison (36) and Mol *et al.* (145)]. However, in mammals, there is no obvious functional ortholog of endonuclease IV. Instead, there is a second protein with homology to exonuclease III, termed APE2 (also known as APEX2) (71, 208). The AP endonuclease and 3′-damage excision activities of the human APE2 protein are generally weak, and, thus, its function as a DNA repair enzyme remains in question (18, 19, 70). It is not our intent to describe the current picture of APE2 in detail, but we point out that, while APE2-null mice exhibit growth retardation and dyshematopoiesis, APE2-deficient mouse embryonic stem cells or immortalized fibroblasts do not display increased sensitivity to several genotoxic agents, including hydrogen peroxide, bleomycin, or x-ray irradiation (86). Moreover, deletion of *APE2* in the CH12F3 mouse B cell line does not affect sensitivity to methylmethane sulfonate (MMS) (136). These data support the notion that APE2 is not a major player in DNA damage repair, and contend that APE1 is the predominant, if not sole, AP endonuclease in mammals. Given that APE2 is a member of the α/β-sandwich superfamily of enzymes that exhibit diverse signaling functions, it would be valuable to explore other substrate specificities of APE2, such as against proteins and phospholipids.

APE1 Biochemical Activities

3′-repair diesterase

The initial work on the purified mouse, bovine, and human AP endonuclease found that the mammalian protein exhibited not only a powerful class II AP site incision activity, but also the ability to excise 3′-damages, such as 3′-phosphates, 3′-phosphoglycolate esters, and 3′-deoxyribose fragments (27, 173, 182, 183). These 3′-end blocking groups prevent primer extension by a DNA polymerase or nick ligation by a DNA ligase, and, thus, have the potential to promote chromosome instability as unrepaired strand breaks. Such non-conventional groups arise as products of hydroxyl radical attack of the sugar phosphate backbone or as repair intermediates during DNA processing. Experiments using human whole cell extracts have shown that APE1 is rate limiting for the repair of DNA strand breaks induced by hydrogen peroxide (presumably 3′-phosphates) and bleomycin (3′-phosphoglycolates) (89), and is the predominant enzyme for the excision of 3′-phosphoglycolate residues from a single nucleotide gap (160). Biochemical studies also indicate that APE1 plays a prominent role in the removal of 3′-deoxyribose fragments that are generated by AP lyase β-elimination reactions, which are catalyzed by certain multi-functional DNA glycosylases (*e.g.,* 8-oxoguanine DNA glycosylase [OGG1] and endonuclease III homolog 1 [NTH1]) or are promoted "non-specifically" by basic polypeptides, such as polyamines or histones [reviewed in Doetsch and Cunningham (41) and Hegde *et al.* (75)]. It should be pointed out that the ability of human APE1 to excise 3′-damages appears to be largely restricted to single-strand breaks or 3′-recessed double strand break ends, as the enzyme is highly inefficient at removing 3′-phosphoglycolates from blunt or 3′-overhang (single-stranded) termini (199). Indeed, there exist other proteins; for example, polynucleotide kinase 3′-phosphatase (PNKP) and tyrosyl-DNA phosphodiesterase 1 (TDP1), which provide complementary 3′-damage repair activities [reviewed in Caldecott (23) and Wilson (227)].

3′ to 5′ exonuclease

Work by Seki *et al.* found that the mouse and human proteins possess 3′ to 5′ exonuclease activity, which is a prominent function of the *E. coli* exonuclease III protein (182, 183). The exonuclease activity of human APE1, however, is poorly processive and ≥100-fold less efficient than its AP endonuclease activity, although the former activity is somewhat influenced by reaction conditions, sequence/positional context (see comments in previous section), and thermal stability

of the duplex (28, 44, 45, 226, 231). Notably, APE1 displays the capacity to excise certain 3′-mispaired nucleotides with increased proficiency, and, in this setting, may act as an autonomous proofreading enzyme for DNA polymerases which lack editing activity (28, 226). Moreover, Chou *et al.* (29) isolated APE1 as the major protein that excises the clinically relevant, chain-terminating nucleoside analog, β-L-dioxolane-cytidine (also known as, troxacitabine), from the 3′-end of a 3′-recessed oligonucleotide substrate (see more in "APE1 as a Therapeutic Target"). We note that, at present, the crucial, biological role of the 3′ to 5′ exonuclease activity for the exonuclease III orthologs remains largely a mystery.

RNA cleavage

Similar to *E. coli* exonuclease III, human APE1 exhibits RNase H activity, albeit to a lesser extent than its bacterial counterpart (9). As with the 3′ to 5′ exonuclease activity, the biological significance of the ability of APE1 (or exonuclease III for that matter) to degrade an RNA molecule hybridized to a complementary DNA strand is unknown. We note that this activity in RNase H is used to remove the RNA primers during DNA replication, yet there is no evidence for a role of APE1 in this process. More recent work has found that APE1 can cleave abasic sites in RNA, and it has been proposed that the protein operates in RNA quality control, removing damaged RNA templates to prevent error-prone translation, although this model has not been sufficiently validated in cells (10, 215).

Lee and colleagues identified APE1 as a major endoribonuclease able to cleave the coding region determinant of the c-myc mRNA (preferentially in between UA and CA dinucleotides), thereby affecting transcript turnover (8). Subsequent *in vitro* experiments surprisingly found that APE1 catalyzes RNA incision in the absence of divalent metals (107). Since earlier work had demonstrated that APE1 can also incise acyclic AP sites in an Mg^{2+}-independent manner (47), the results suggest that flexibility/dynamics of the phosphodiester linkage, which is greater adjacent to an acyclic lesion, plays an important role in the establishment of the transition state intermediate. More extensive structure-function analyses revealed that APE1 engages several of the same active site residues used for AP-DNA cleavage to promote RNA incision (excluding D283), requires a 2′-hydroxyl group on the sugar moiety for catalysis, and generates products with a 3′-phosphate, indicating both conserved and distinct mechanisms for AP-DNA and RNA cleavage (106). Although the specific activity of APE1 to cleave target mRNAs is much lower than its AP endonuclease function *in vitro*, Barnes *et al.* (8) found that transient knockdown (KD) of APE1 in HeLa cells results in an increased half-life and steady-state level of c-myc mRNA, providing evidence for a biological role for its RNA cleavage function. The identification of potential separation-of-function mutations in APE1 [see the D283N mutant alluded to earlier, (106)] should permit additional studies to determine whether the enzyme contributes to mRNA turnover *in vivo*.

Nucleotide incision repair

Strikingly, APE1, thought to be limited to cleavage of abasic sites in intact, double-stranded DNA, has been reported to recognize and incise at certain base damages (*e.g.,* 5,6-dihydro-2′-deoxyuridine, 5,6-dihydrothymidine, 5-hydroxy-2′-deoxyuridine, α-2′-deoxyadenosine, and α-thymidine adducts), generating single-strand break ends with a 3′-hydroxyl and a 5′-dangling modified nucleotide (65). Distinct from its AP endonuclease function, this activity—proposed to initiate a corrective response termed nucleotide incision repair (NIR) that would serve as a back-up for the more classical glycosylase-initiated BER pathway—is most active at low $MgCl_2$ and KCl concentrations, at a pH range of 6.4 to 6.8. Such a nuclease activity is seemingly consistent with earlier work which found that APE1 can incise at benzene-derived base adducts, similarly creating 3′-hydroxyl and 5′-dangling modified nucleotide ends (67). Molecular modeling of a duplex harboring the benzetheno exocyclic adduct of cytosine (pBQ-C), and subsequent molecular dynamics simulations with APE1, implied that the pBQ-C adduct can be accommodated in the enzyme's active site on specific structural rearrangements of both the DNA and protein, and that APE1 would be able to execute a similar reaction mechanism for phosphodiester bond cleavage as used on AP-DNA. However, a precise understanding of how APE1 specifically binds DNA base adducts requires high resolution structural information, and further experiments are necessary to substantiate the biological role of this activity in human cells.

Redox regulation

As mentioned earlier, APE1 was independently purified based on its ability to stimulate the DNA-binding activity of several transcription factors through a redox regulatory mechanism (233). Since then, APE1 has been reported to modulate the redox status of both ubiquitous (*e.g.,* AP-1, Egr-1, NF-κB, p53, CREB, and HIF-1α) and tissue-specific transcription factors (*e.g.,* PEBP-2, Pax-5, and -8, TTF-1) with functions in stress responses and other cellular processes [reviewed in Kelley *et al.* (101)]. Although the redox regulatory and DNA repair nuclease activities can be disabled separately by site-specific mutagenesis (219, 234), the precise molecular details of the protein-facilitated activation step remain elusive (103). In particular, none of the cysteine residues within APE1 are found in a C-X-X-C motif that is common to most redox regulatory factors, such as thioredoxin, a cellular component which appears to be involved with APE1 in a redox regulatory cascade (81). In addition, disulfide bond formation is considered a necessary step in the resolving activity of redox factors, and while most evidence supports a role for C65 in the thiol-mediated redox reaction (61, 219), none of the other six cysteine residues in APE1 are located appropriately in the 3-dimensional structure to facilitate disulfide bond formation with C65. Finally, although some data imply a role for C93 in the redox reaction, both C65 and C93 are buried within the protein structure, while being positioned at a distance (9 Å) that is not compatible with disulfide bond formation. Based on the current experimental evidence, Georgiadis and colleagues have proposed a model in which (i) C65 serves as the nucleophilic residue for reduction of the disulfide bond in the target transcription factor; (ii) C93 operates as the resolving residue; and (iii) APE1 undergoes a significant conformational change (possibly unfolding) to reveal a third cysteine residue (possibly C99) to facilitate the redox reaction (130). While studies are accumulating which indicate that the redox function of APE1 is important to processes such as cell growth and differentiation (see more in

"Determination of cell fate" and "Small molecule inhibitors" sections), further experiments are needed to delineate the precise molecular mechanism of the reaction.

Trans-acting modulation

In addition to functioning as a transcriptional regulator through post-translational modification (PTM) of a target protein (see previous section), APE1 is also a component of nuclear protein complexes that bind to negative Ca^{2+} response elements (nCaREs) and modulate gene expression. In particular, on a rise in extracellular Ca^{2+}, Okazaki *et al.* observed an increase in APE1 transcript and protein levels that resulted in augmented complex assembly at the nCaREs (nCaRE-A and nCaRE-B) of the parathyroid hormone (*PTH*) gene that promoted transcriptional suppression (153). Subsequent work found that APE1 binds to the nCaRE *PTH* sequences in cooperation with, minimally, the two subunits of the Ku antigen, p70 and p80 (30), and that complex formation of APE1 with the promoter elements is controlled by lysine (K6 and K7) acetylation (11). A role for APE1 in nCaRE-dependent transcriptional suppression was also observed for the human renin gene in chorio-decidual cells as a part of a response to elevated intra-cellular Ca^{2+} levels (54) and for the *BAX* gene in gastric epithelial cells as a part of an elaborate response to *Helicobacter pylori* infection that involves APE1 acetylation and poly(ADP)ribose polymerase 1 (PARP1) (12). Interestingly, the upstream region of the *APE1* promoter contains three nCaRE-like sequences, at least one of which (nCaRE-B2) appears to be bound by an APE1-containing protein complex that negatively auto-regulates gene expression (90). A follow-up analysis revealed that heterogenous nuclear ribonucleoprotein L (hnRNP-L) was a component of the nCaRE-B2 binding complex (113). It would be worthwhile to determine the technical or biological (cell-dependent?) reasons for why an independent study did not identify these negative regulatory elements in the *APE1* promoter (72), as well as whether APE1 has a broader role in modulating gene expression *via* binding CaREs or other promoter sequence elements throughout the genome. A more exhaustive characterization of the composition of the APE1-specific, multi-protein transcriptional complexes would also be worthwhile.

We close this section by noting that Gillespie *et al.* have proposed a model in which "controlled" oxidative DNA damage in hypoxic regulatory elements in specific promoters (*e.g.*, of *VEGF*) may lead to BER processing events, such as binding and subsequent strand cleavage by APE1, which can induce changes in local sequence topology and DNA flexibility that could drive productive transcription [reviewed in Gillespie *et al.* (62)]. However, how such oxidative DNA damage is targeted and how repair may be strategically manipulated at the promoter site to foster transcription factor assembly and, ultimately, gene expression requires further clarification.

APE1 Biological Roles

Shortly after cloning of the *APE1* gene, studies were undertaken to elucidate the cellular functions of the protein. In particular, Walker *et al.* (218) showed that HeLa cells stably expressing antisense *APE1* RNA exhibited a similar doubling time, plating efficiency, and gross morphology, but were hyper-sensitive to killing by a range of DNA-damaging agents, including MMS, hydrogen peroxide, menadione, and paraquat, but not ultraviolet irradiation. Ono *et al.* (155) similarly found that C6 rat glioma cells expressing antisense *APE1* RNA displayed normal growth rates, but reduced survival when challenged with MMS or hydrogen peroxide. These data are consistent with a role for APE1 in repairing alkylative and oxidative DNA lesions, which are likely to include abasic sites and dirty 3′-strand break ends, respectively, that engage the AP endonuclease and 3′-repair diesterase activities of the enzyme. A more comprehensive discussion of the phenotypes of APE1-defective cells, specifically in the context of clinical DNA-damaging agent sensitivity, is presented later in "APE1 as a Therapeutic Target."

Complementation strategies were also employed to determine the potential biological functions of APE1. In particular, trans-complementation of *E. coli* strains which were deficient in the main AP endonucleases (exonuclease III and endonuclease IV) revealed that human APE1 is able to fully complement MMS sensitivity, but only partially correct hydrogen peroxide sensitivity (37, 171). A similar damaging agent rescue pattern was observed when the human protein was expressed in yeast-deficient in the major AP endonuclease, APN1 (72). Chinese hamster ovary cells designed to inducibly express a dominant-negative form of APE1 (termed ED, due to E96Q and D210N mutations), which displays better than wild-type binding affinity for substrate DNA, but is devoid of nuclease activity, exhibited pronounced hyper-sensitivity to MMS, yet a more mild increase in sensitivity to hydrogen peroxide (138). The combined data are consistent with APE1 maintaining a powerful AP endonuclease function that participates in the repair of AP sites formed by the direct and indirect action of MMS, and a lesser, albeit significant, 3′-repair activity for removal of 3′-blocking groups (*e.g.*, 3′-phosphates) generated by the oxidizing agent hydrogen peroxide. More extensive trans-complementation has not been undertaken to our knowledge, but could be a powerful strategy if employed creatively in bacteria or yeast to assess the other reported biochemical activities of APE1 (see "APE1 Biochemical Activities" section), such as its exonuclease function.

Recent work by Demple and colleagues has shown that strong, chronic down-regulation of APE1 in multiple human cell lines using a siRNA approach results in accumulation of total genomic abasic sites, inhibition of proliferation, a dramatic increase in the proportion of cells with sub-G1 DNA content, and activation of apoptotic cell death (55). Successful rescue of these cellular phenotypes *via* trans-complementation with the yeast AP endonuclease APN1, which has no sequence or structural homology to APE1 and lacks a redox regulatory function, suggests that the repair nuclease activities shared between these two proteins are required for cell survival (consistent with the observation of endogenous DNA damage accumulation). Studies by the Mitra group using nullizygous embryonic fibroblasts from $APE1^{-/-}$ mice that are transgenic with a "floxed" human *APE1* gene also found an essential role for the protein in cell viability, as Cre-mediated excision of the complementing gene resulted in rapid apoptosis (88). Trans-complementation experiments with various human *APE1* gene mutations revealed that the DNA repair nuclease activity (an H309N mutant) or its acetylation-mediated transcriptional regulatory function (a K6R/K7R mutant), but not its redox regulatory function (a C65S

mutant), was necessary for cell survival, although these studies should be interpreted with caution as expression of the complementing protein was not documented. More recently, the Tell laboratory created a stable, inducible KD HeLa cell line, in which APE1 expression is decreased to < 5% after 10 days of doxycycline treatment (214, 215). Although the KD cells eventually die, exhibiting the typical growth arrest and apoptosis, this model has been a valuable tool for delineating the contribution of specific APE1 functions. Consistent with the previous work, KD cells complemented with a nuclease-deficient human *APE1* cDNA harboring an H309N mutation did not survive, confirming the essential nature of the DNA repair activities of the enzyme (213). However, in contrast to what the Mitra laboratory observed (see above), the C65S redox regulatory mutant did not rescue the inviability of the KD cells; whereas the non-acetylatable K6R/K7R mutant provided near wild-type complementation for cell growth. While it remains unclear why opposite results were obtained in the two studies (possibly reflecting differences in strategies, cell types, *etc.*), the experiments provide evidence for biologically important roles for the redox regulatory function and acetylation of APE1.

Though it had generally become accepted that APE1 is required for mammalian cell viability, a recent report by Masani *et al.* (136) described the generation of an *APE1* knock-out (KO) CH12F3 mouse B cell line that displays normal cell proliferation, while exhibiting the expected hyper-sensitivity to MMS. Although the precise reason(s) for the normal growth of the APE1-deficient cells is uncertain, it is likely that a natural or induced up-regulation of a compensatory pathway, such as nucleotide excision repair, homologous recombination, or translesion DNA synthesis, mediates survival in the face of the high levels of endogenous DNA damage. Thus, by extension, we anticipate that in mammalian cells in which APE1 is deficient, the genetic and biological background will ultimately determine the cellular attributes, potentially explaining the contrasting viability results mentioned in the preceding paragraph with the different site-directed mutants. Of course, variability in technical aspects of the approaches cannot be excluded from contributing to the disparate outcomes. Addressing these issues would seem to be a worthwhile pursuit going forward. We close this section by pointing out that both *E. coli* and *S. cerevisiae* which lack their respective major AP endonuclease genes are able to survive, although they exhibit mild growth defects.

Intra-cellular targeting and mitochondrial function

APE1 has long been recognized as a prominent nuclear protein, where it would perform its DNA repair and transcriptional regulatory activities. It is, in fact, its nuclear functions that are often presumed to be essential for cell or organismal viability, although given its alternative compartmentalization (introduced below), this cannot be stated with absolute certainty at present. Using GFP-tagged truncated and mutated APE1 expression constructs, Izumi and colleagues found that APE1 harbors two independent segments within the first 20 N-terminal residues that direct its strong nuclear localization (Fig. 2): (i) residues 2–7, which contain a classic pat7 type NLS (PKRGKK), and (ii) residues 8–13, which harbor two critical acidic amino acids (E12 and D13), but lack a signature NLS (91). These authors also reported that the N-terminal portion of APE1 mediates a physical interaction with the nuclear importins, KAPα1 and KAPα2, which likely facilitate APE1 nuclear internalization. Notably, a normally cytoplasmic, mutant form of APE1 was found to be sequestered in the nucleus on treatment with leptomycin B, a nuclear export inhibitor, suggesting that the protein possesses a nuclear export signal (NES), although this sequence was not explicitly identified at the time (see more in next section).

Despite the strong nuclear targeting elements within APE1, the protein has been reported to be cytoplasmic in some cell types, such as those with high metabolic or proliferative rates, displaying co-localization with mitochondria and the endoplasmic reticulum [reviewed in Tell *et al.* (204)]. Using both standard and rigorous mitochondrial purification techniques, several groups have, indeed, reported the existence of AP endonuclease activity, and an APE1-like protein species, in the mitochondrial fraction (57, 203, 207). Mitochondria possess an extra-nuclear genome (mtDNA), which due to its proximity to the electron transport chain (ETC), is highly susceptible to attack by reactive oxygen species (ROS) generated as by-products of oxidative phosphorylation. Persistent mtDNA damage can lead to problems in expression of the essential ETC proteins, leading to a greater dysfunction in oxidative phosphorylation that drives further ROS production, a vicious cycle fundamental to the mitochondrial theory of aging [reviewed in Miquel *et al.* (141)]. Due to the high frequency of potentially deleterious oxidative modification, mitochondria have acquired their own protective DNA repair mechanisms to preserve genome integrity and maintain mitochondrial function. We now recognize that BER is the major DNA repair system in mitochondria, with many of the mitochondrial BER (mtBER) proteins arising from either alternative splicing or translation [reviewed in de Souza-Pinto *et al.* (35), Liu and Demple (125), and Van Houten *et al.* (212)].

How APE1 is targeted to mitochondria is still not completely clear, but several studies have shed insights on this issue. One report found that a 33 residue N-terminal truncated form of APE1 is present in mitochondria from both bovine liver and mouse NIH3T3 cells (26). The authors proposed that APE1 is directed to this organelle by removal of the N-terminus, which harbors the prominent NLS (Fig. 2), *via* a site-specific cleavage event that might engage a mitochondrial matrix peptidase. However, given the many studies which have reported full-length APE1 in the mitochondrial fraction [see for instance (203, 213)] and the evidence that the N-terminal portion of APE1 is susceptible to degradation (182, 184), it seems likely that non-specific proteolysis occurred during the fractionation steps, leading to the creation of the 33 amino-acid N-terminal truncated form. Since there is no consensus mitochondrial targeting sequence (MTS) in APE1 based on bioinformatics scrutiny, Li *et al.* (118) screened for interactions between a series of peptides that span the length of the APE1 protein and three translocases of the outer mitochondrial membrane. These binding assays uncovered a putative MTS in the C-terminus of APE1, encompassing residues 289–318. Site-directed mutagenesis of residues K299 and R301 confirmed the importance of this region in directing either the 289–318 peptide or full-length APE1 to the mitochondrial compartment. Moreover, a recent study found that full-length APE1 resides mainly in the mitochondrial intermembrane space (213), a localization pattern that appears distinct from other mtBER proteins, which are associated with

mtDNA in the matrix (198). In this work (213), APE1 was shown to interact, in a proximity ligation assay, with the oxidoreductase Mia40, which plays a key role in oxidative protein folding within the mitochondrial inter-membrane space. Thus, the current data suggest a model in which a presently unknown PTM of full-length APE1 is necessary to reveal the "masked" MTS within the C-terminus and enable mitochondrial-specific protein interactions that direct mitochondrial localization.

Relatively few studies have assessed the specific contributions of APE1 to the maintenance of mitochondrial integrity. This fact is due, in part, to the difficulty in separately examining the mitochondrial and nuclear functions of the protein. To determine the effects of increased APE1 in mitochondria, Li *et al.* expressed a recombinant fusion protein composed of the strong MTS of manganese superoxide dismutase and the repair domain of APE1 (residues 34–318) in human umbilical vein endothelial cells (119). Targeted APE1 mitochondrial over-expression was shown to enhance cell viability and suppress apoptosis after hydrogen peroxide-induced oxidative stress, presumably by enhancing mtDNA repair. This observation suggests that APE1 is rate limiting in mtBER, although this finding appears to be cell-type specific, as a separate study found that mitochondrial targeting of *E. coli* exonuclease III in the human malignant breast epithelial cell line, MDA-MB-231, results in impaired mtDNA repair and a decrease in long-term survival after oxidative stress (192). One message taken from these studies is that additional work is needed to better understand the coordination between the components of mtBER.

As noted earlier, Vascotto *et al.* showed that the redox mutant C65S is unable to rescue the inviability of the KD HeLa cell line (213). However, they also found that this mutant was unable to counteract the decreased mitochondrial membrane potential, that is, the mitochondrial dysfunction, of the APE1-deficient cells. A more comprehensive analysis revealed that the C65A mutation affected not only the global gene expression pattern, presumably due to defects in redox regulation of nuclear transcription factor binding, but also its redox-assisted protein folding, which adversely affected its protein interactions, AP endonuclease activity, and mitochondrial localization. Thus, the C65 residue, which has been proposed to play an important role in the redox chemistry (see "Redox regulation" section), also appears to mediate intra-cellular trafficking, such that reduced APE1 mitochondrial accumulation results in mitochondrial impairment and apoptotic cell death. Li *et al.* independently confirmed that reduced overall APE1 levels correlate with loss of mitochondrial membrane potential and apoptosis, yet presented evidence that much of this effect was mediated indirectly through reduced expression of several nuclear genes which encode mitochondrial proteins, including those that facilitate mitochondrial transmembrane transportation (117). The studies go on to identify the transcription factor NRF1, which plays an important role in modulating the expression of nuclear genes involved in respiration and mtDNA replication and transcription, as a key target of APE1-mediated redox regulation. Thus, in light of the complexity of APE1's functions, it is evident that more extensive studies employing separation-of-function, site-specific mutants will need to be performed to better clarify the cellular consequence(s) of disrupting a particular APE1 function, including its role in mitochondria.

Intra-cellular trafficking

Some of the early work indicating that APE1 localized to compartments other than the nucleus was reported by Kelley and colleagues. Using immunohistochemistry, they found that APE1 had both nuclear and cytoplasmic staining in certain brain and liver cells within paraffin-embedded autopsy specimens from a 70 year-old man (43). Since then, alternative localization patterns have been observed for APE1 that appear to be influenced by cell-type, environmental exposures, or disease status (see sections on "Cancer" and "Neuropathology" later). It would also appear that the intra-cellular distribution of APE1 can be regulated *via* an active process, although the precise molecular mechanisms are unclear [reviewed in Tell *et al.* (204)]. For example, Mitra and colleagues were one of the first to show that APE1 can translocate to the nucleus on the introduction of oxidative stress (168). Much research since has discovered that the APE1 distribution pattern can change on pro-oxidant injury, heavy metal or DNA-damaging agent exposure, or hormone or cytokine stimulation, to name a few [reviewed in Tell *et al.* (204, 205)].

While still in its infancy of understanding, there are some insights regarding the potential mechanisms for regulating APE1 intra-cellular distribution. For example, as described in the previous section, Vascotto *et al.* found that the redox state of C65 can control the mitochondrial localization of APE1 (213). In addition, one study showed that S-nitrosoglutathion, a nitric oxide donor and S-nitrosating agent, can induce APE1 nuclear export, with this nuclear to cytoplasmic translocation being mediated by S-nitrosation of residues C93 and C310 (165). The translocation process was reversible, could not be mimicked by hydrogen peroxide-induced oxidative stress, and involved, to some degree, reduced nuclear import. Deletion analysis revealed that residues 64–80 of APE1 likely harbor the NES and that there exists a "hidden" MTS in the final 69 amino acids of the protein [consistent with the results of (118); see the previous section]. The authors propose that S-nitrosation of C93 and C310 induces a protein comformational change that unmasks the NES, promoting cytosolic translocation. Collectively, the current data suggest a model in which unmodified, full-length APE1 is mainly targeted to the nucleus *via* its N-terminal elements, but on PTM (not fully defined to this point), the typically inaccessible NES or MTS is revealed (Fig. 2), driving re-distribution of the protein. A major emphasis of future work will be to unravel how protein interactions (see for example nucleophosmin below) and PTMs regulate APE1 activities and intra-cellular localization. We direct the readers to a recent review describing in greater detail potential PTMs of APE1 (21), and provide Table 1 as a summary of these alterations and their reputed effects, which are likely influenced in a cell type- or response-specific manner.

Participation in specific cellular processes

To this point, we have reviewed the major reported biochemical activities and the more general biological roles of APE1, but there are a few specific molecular processes that appear to engage the enzyme worth detailing:

First, APE1 appears to play a broad role in various aspects of RNA metabolism (see also section entitled "RNA cleavage"). Using a HeLa cell line in which the endogenous APE1 protein was depleted and a comparable amount of a

Table 1. Post-Translational Modification of Human APE1

PTM	*Modifying enzyme*	*Site(s) of modification*	*Impact on activity*	*Cellular aspects*	*Reference*
Phosphorylation	Cdk5 complex	T233 (T232 in mouse)	Nuclease: Decreased	Increased cell vulnerability to DNA damage Was elevated in post-mortem brain tissue of PD and AD patients	(84)
	ND	Y262	ND	Was detected in anaplastic large-cell lymphoma SU-DHL-1 cells	(175)
	CK II	ND	Nuclease: abolished	ND	(240)
	CK II	ND	Nuclease: normal Redox: increased	Was protective against MMS cytotoxicity	(53)
	CK I	ND	Nuclease: normal	ND	(240)
	PKC	ND	Nuclease: normal Redox: increased	Was elevated in response to oxidative stress	(82, 240)
Acetylation	p300 & SIRT1	K6/K7	Nuclease: decreased	Increased binding of APE1 to *PTH* nCaRE-B promoter sequence Affected YB-1 binding to *MDR1* promoter Decreased the interaction between APE1 and XRCC1 Promoted anti-cancer drug resistance	(11, 185, 241)
	SIRT1	K24/K25/K27/K31/K32	Nuclease: decreased	Stabilized the binding of APE1 to mRNA	(49)
	SIRT1	K27/K31/K32/K35	Nuclease: decreased	Promoted APE1 nucleolar accumulation Regulated K6/K7 acetylation	(124)
	ND	K35	ND	Was increased in triple negative breast cancer samples	(162)
Ubiquitination	MDM2	K24/K25/K27	ND	Promoted nuclear exclusion	(20)
	MDM2(Mono)	K48(Poly) K24/K25/K27(Mono)	ND	Poly-ubiquitination reduced APE1 protein stability Was enhanced by T233 phosphorylation	(22)
	UBR3	K24/K25/K27/K31/K32/K35	ND	Regulated APE1 protein level and MMS sensitivity	(140)
Nitrosylation	ND	C93/C310	ND	Regulated intra-cellular localization Was increased by nitrosative stress	(165)
Glutathionylation	None	C99	Nuclease: decreased	Was increased by oxidative stress	(108)

AD, Alzheimer disease; APE1, apurinic/apyrimidinic endonuclease 1; MMS, methylmethane sulfonate; ND, not determined; PD, Parkinson disease; PTM, post-translational modification.

Flag-tagged version of APE1 was re-introduced, Vascotto *et al.* discovered that many of the co-immunoprecipitating partners of the fusion protein operate in RNA processing and ribosome biogenesis (215). Most of these interactions, including one with nucleophosmin (NPM1), are mediated by the N-terminal 33 amino acids of APE1 and may involve stabilization by RNA molecules. In addition, a significant portion of nuclear APE1 was found to be concentrated within the nucleoli, co-localizing with both NPM1 and nucleolin. This nucleolar distribution was influenced by the cell cycle and dependent on active rRNA synthesis, supporting a role for APE1 in RNA metabolism. Based on a series of biochemical and cellular experiments, the authors argue that the NPM1-APE1 interaction functions to regulate the ability of the endonuclease to remove damaged RNA molecules from the transcript pool. Future investigations are needed to better define (i) the role of the interactions of APE1 with RNA and NPM1 in influencing intra-cellular distribution and (ii) the biological importance of APE1 in RNA quality control, transcript turnover, and RNA biogenesis (see accompanying review by Tell and colleagues for further discussion).

Second, APE1, and other components of BER, operate in the programmed processes of somatic hypermutation (SHM) and class switch recombination (CSR), which introduce genetic variability within the immunoglobulin genes of B cells [reviewed in Keim *et al.* (99)]. During the adaptive humoral immune response to foreign antigen, SHM introduces mutations into the variable region exons to promote antibody affinity maturation, while CSR promotes genetic re-arrangements in the constant region of the heavy chain to drive isotype switching and optimization of interactions between antibody and different effector molecules. Both SHM and CSR require the targeted action of activation-induced cytidine deaminase, a protein that is expressed restrictively in activated B cells and deaminates cytosine to uracil in DNA. These uracils serve as a critical launching point for the mutagenic and recombination events that take place during antibody diversification, and are excised by uracil-DNA glycosylase (UNG), which generates an abasic site product. Guikema *et al.*, using APE1 haploinsufficient mice ($APE1^{+/-}$), reported reduced CSR in the S region of splenic B cells due to a decrease in DNA double-strand break formation, consistent with a role for APE1 in the AP site cleavage step (66). The authors also found that CSR was reduced in APE2-deficient mice, implicating both exonuclease III-like proteins in the process of isotype switching and suggesting a possible specialized DNA processing function for APE2 in activated B cells. As noted earlier, Yu and colleagues were successful in generating an *APE1* KO CH12F3 mouse B cell line (136). Employing this resource, they found that CSR was drastically reduced in APE1-null cells, whereas the process was unaffected in CH12F3 cells deleted for APE2, whether APE1 was present or not. This research provides the strongest evidence to date of an essential role for APE1 in CSR, while implying that APE2 is not involved in this phenomenon, even as a back-up enzyme. Consistent with APE1 operating in CSR, purified recombinant APE1 protein can incise at AP sites in a synthetic oligonucleotide substrate designed to mimic the R-loop structure that is presumably needed for the recombination event (10). It is noteworthy that, to our knowledge, the contribution of APE1, or APE2 for that matter, in SHM has not been explicitly addressed.

Third, APE1 functions as a member of a 270–420 kDa multiprotein complex, termed the SET complex, to aid in the execution of the granzyme A (GzmA)-activated cell death response [reviewed in Lieberman (120)]. GzmA is an abundant serine protease in the granules (*i.e.,* specialized secretory lysosomes) of natural killer cells and cytotoxic T lymphocytes. On delivery to the target cell cytosol, GzmA interacts with the SET complex to initiate a caspase-independent cell death pathway that is characterized by DNA nicking and the production of large DNA fragments. This process is thought to be important in the immune defense against cancers and viruses that evade caspase-mediated apoptosis. Although the members of the SET complex have not been fully characterized, most of the functional components have been identified and include the nucleosome assembly protein SET, the DNA-binding protein HMGB2, the tumor suppressor protein pp32, the 5′–3′ exonuclease TREX1, the GzmA-activated DNase NM23-H1, and APE1. While the normal function of the SET complex is unknown, based on its protein composition, it likely operates to regulate chromatin structure, genomic integrity, and gene expression. The SET complex, in response to GzmA entry into the cell, translocates rapidly from the endoplasmic reticulum, where it usually resides, to the nucleus in a process that depends on mitochondrial damage and consequent ROS accumulation (135). Once in the nucleus, the complex presumably activates expression of key early response genes and carries out relevant DNA processing activities, such as DNA nicking. Lieberman and colleagues reported that a fraction of the total APE1 protein is a part of the SET complex, and that GzmA interacts with and cleaves APE1 at residue K31 (48). In addition, they found that GzmA inactivates both the AP endonuclease and redox regulatory functions of APE1. While the latter is consistent with the N-terminal region of APE1 playing a critical role in this activity, deletion of 61 residues from the N-terminus has little effect on its repair activity (89), implying an unknown mechanism for inactivation of its endonuclease function. Although the precise molecular task of APE1 in the caspase-independent cell death pathway remains unclear, given that silencing of APE1 enhances GzmA-induced cell death, the protein appears to play an important cellular role in the process. Moreover, overexpression of a non-cleavable version of APE1 had a protective effect against GzmA-activated cell death, indicating that degradation of the protein is important in the response. Recent studies also implicate APE1 in an analogous GzmK-mediated cell death pathway (68). Finally, Yan *et al.* reported that KD of any of the nucleases in the SET complex (*i.e.,* APE1, NM23-H1, or TREX1) increases auto-integration, while reducing chromosomal integration of the human immunodeficiency virus 1 (HIV-1), suggesting that this multi-protein complex can positively affect HIV-1 infection (242).

Determination of cell fate

As evident from the earlier presentation, APE1 can play a critical role in protecting cells from apoptotic cell death. Consistent with the findings of experiments using KD or genetic approaches (see "APE1 Biological Roles" section), expression of the dominant-negative form of APE1, that is, ED, results in genomic AP site accumulation, G1 arrest, and apoptosis in Chinese hamster ovary cells (137). Nevertheless, APE1 also appears to regulate the activation of cellular

senescence, a phenomenon by which normal diploid cells lose their capacity to divide (typically around 50 doublings in culture) and achieve a state of irreversible growth arrest, termed the "Hayflick Phenomenon," after its discoverer Leonard Hayflick. This outcome is thought to have evolved in certain organisms, such as mammals, to prevent the onset of cancer, with evidence suggesting that senescent cells accumulate with age and promote tissue aging. Heo *et al.* found that *in vitro* replicative or oxidative stress-induced senescence of bone marrow-derived human mesenchymal stem cells is closely related to a decrease in endogenous APE1 expression (78). Significantly, exogenous adenoviral-mediated APE1 over-expression was able to suppress the elevated superoxide levels and associated senescent phenotype. In addition, Karimi-Busheri *et al.* found that tumor-initiating cells isolated as mammospheres from MCF-7 breast cancer cells have approximately twofold higher APE1 expression and reduced cellular senescence in comparison with the bulk population of MCF-7 cells; they found no difference in the expression of other BER-related genes, namely DNA polymerase β (POLβ) and X-ray cross-complementing 1 (XRCC1), in mammospheres (97). Finally, Krutá *et al.* reported that prolonged maintenance of human embryonic stem cells in culture leads to significantly reduced APE1 expression, as well as a decreased overall BER capacity, with no changes in OGG1 or POLβ protein levels (112). Seeing that the data implicating APE1 in mediating cellular senescence is primarily corollary, experiments are needed to determine whether APE1 has a direct role in regulating this outcome, and if so, *via* what mechanism.

While exploring for a role of APE1 in hematopoiesis, Zou *et al.* discovered that KD of APE1 in mouse embryonic stem cells results in a significant decrease in the formation of hemangioblast and in primitive and definitive hematopoietic colony frequencies (248). Moreover, inhibition of the redox regulatory role of APE1 *via* the small molecule (E)-3-(2-(5,6-dimethoxy-3-methyl-1,4-benzoquinonyl))-2-nonyl propenoic acid (also known as, E3330; see also section "Small molecule inhibitors") caused impaired hemangioblast development, whereas the indirect AP site repair inhibitor, methoxyamine, had no effect. These data indicate a role for APE1 in positively regulating embryonic hematopoiesis, specifically through its redox function. Interestingly, Zou *et al.* found that KD of APE1 *via* siRNA in mouse embryoid bodies induced G1 arrest, but did not activate apoptotic cell death. Thus, APE1 has specific roles in regulating cellular responses, as well as cell fate, that involve both its DNA repair and transcriptional regulatory functions, and are likely dictated by cell type, environment, and genetic background.

APE1 in Disease

Similar to other core participants in mammalian BER, such as *POL*β and *XRCC1*, deletion of both alleles of *APE1* (also known as, *Apex1*) in mice leads to early lethality. The initial *APE1* KO mouse model employed a gene targeting strategy that deleted a 3.6 kb genomic fragment encompassing most of exon 1 (which is a non-coding exon) and all of exons 2–4, which cover the entire protein coding region (235). No homozygous KO ($APE1^{-/-}$) offspring were generated (out of 464 live births) when breeding $APE1^{+/-}$ parents, whereas heterozygous *APE1* KO mice ($APE1^{+/-}$) were reported to be normal in size, fertility, and behavior for approximately 9 months of age. $APE1^{-/-}$ blastocysts were obtained from the uteral lumen of pregnant females at around day E3.5, but no null embryos were explanted from the uterine tissue of heterozygous matings at day E6.5, indicating that embryonic death occurred between implantation and E6.5. At E5.5, a higher-than-expected percentage of deciduae from heterozygous matings contained embryos that appeared severely necrotic, characterized by disorganized patches of pyknotic cells, suggesting a severe consequence of APE1 absence at this stage of development. Since the redox status of preimplantation embryos is altered dramatically during development (58), it would appear that a sufficient amount of APE1 is required to protect embryos against physiological situations of oxidative stress.

In a separate study, Chen and colleagues, using a targeting strategy that deleted critical coding exons 2 and 3, yet left intact expression elements of neighboring genes, likewise did not observe homozygous KO pups; while heterozygous KO mice appeared generally healthy for at least 12 months (128). Genotyping revealed that no $APE1^{-/-}$ embryos survived beyond E9.5. However, unlike the original mouse model, embryonic epiblasts did not become progressively disorganized, pyknotic, and growth retarded until E7.5 to E9.5, well after implantation. The variability in survival relative to the previous study ($<$E6.5) could be explained by the nature of the genomic deletion, or differences in the exact mouse genetic background or the general breeding environment. Notably, explanted $APE1^{-/-}$ blastocysts showed increased sensitivity to γ-irradiation, which is consistent with a defect in DNA repair.

It is worth emphasizing that none of the mouse studies to date have determined which function of APE1 is required for animal viability. Toward this goal, Curran and colleagues created a C64A homozygous transgenic mouse model (C64 is equivalent to the C65 residue in the human protein thought to be critical for redox activity; see "Redox regulation" section), which was found to exhibit normal viability and life expectancy, and no overt phenotypic abnormalities (156). However, detailed analyses uncovered that these animals were normal for redox activity, raising uncertainty about the precise redox reaction mechanism and concerns about the relevance of this model for addressing the biological importance of this function. We note that in a separate attempt to identify the critical role of APE1 in organism development, no targeted *APE1* null mice, which harbored a human *APE1* genomic transgene that was mutated to determine the contribution of a specific function and flanked by loxP elements to permit eventual excision, were obtained, presumably due to improper ectopic expression of the complementing transgene during embryogenesis (88).

Since APE1 is essential for animal development, the relationship between APE1 deficiency and mammalian disease has been largely extrapolated from haploinsufficient mouse model studies. Consistent with the earlier work, Meira *et al.* found that heterozygous matings generated no viable homozygous KO progeny, but these authors observed a reduction in the number of heterozygous mutant embryos and young pups compared with that predicted by normal Mendelian inheritance (139). This trend could be countered by the addition of anti-oxidants in the diet, suggesting involvement of oxidative stress in the reduced survival. Moreover, a

detailed histopathological analysis found that *APE1*$^{+/-}$ mice, while having a normal life expectancy, displayed increased cardiac abnormalities (not defined) and spontaneous tumors, including lymphomas (two cases) and an adenocarcinoma and sarcoma (a single case each). *APE1*$^{+/-}$ embryonic fibroblasts and cerebellar granule cell neurons also exhibited increased sensitivity to oxidizing agents in culture, supporting the fundamental concept that reduced APE1 function can associate with elevated exposure-dependent susceptibility.

Huamani *et al.* found that heterozygous KO animals were similar to wild-type littermates for approximately 9 months for body and testis weight, and for testis, spleen, and liver histology (83). Consistent with the increased risk of carcinogenesis noted earlier, these authors observed a greater than twofold increase in spontaneous mutant frequencies in mixed spermatogenic cells from 9-month-old *APE1*$^{+/-}$ mice, and an approximately twofold increase in mutation frequency in the liver and spleen of 3-month-old *APE1*$^{+/-}$ mice, relative to wild-type littermates. A follow-up analysis by Walter and colleagues revealed that the heterozygous KO animals also accumulate more mtDNA damage with age relative to the wild-type control mice (217). In a separate study, Unnikrishnan *et al.* reported that the liver tissue of *APE1*$^{+/-}$ mice had increased genomic DNA damage in the form of aldehydic lesions and slightly increased apoptosis after treatment with the oxidizing agent and well-known hepato-carcinogen, 2-nitropropane (211). These data, in total, support the hypothesis that impaired function *APE1* alleles, should they exist in the human population, will give rise to exposure-dependent disease risk. However, as with the viability issue (see above), the studies to date have not clearly discerned whether the defects observed in the heterozygous animals are the result of a deficiency in DNA repair or a more general stress response. It is noteworthy that most studies conducted to date have concentrated mainly on haploinsufficient mice that are 9 months or younger, and it would be worthwhile to examine older animals, with a focus on tissues and organs which exhibit high oxygen consumption, such as the brain, incorporating relevant exposures where valuable.

Genetic variants

In humans, a firm linkage between an APE1 defect and the development of disease has not been documented. However, research initially pioneered by Mohrenweiser and colleagues, and since expanded by several investigative teams, indicates that there exist a large number of DNA repair gene nucleotide sequence variants within the normal and disease population (143, 144, 189). In fact, it has been extrapolated that for a representative DNA repair pathway comprising 20 genes, a typical individual will be variant for at least 5 of those genes. Thus, individuals who are wild type for all alleles in a single pathway will be rare, as will individuals with the same genotype. While in most instances it is unclear how the sequence variation affects DNA repair capacity, it has been hypothesized that the reported synonymous and non-synonymous (missense) genetic variants can lead to changes in transcript production, mRNA stability, translation efficiency, or protein structure function, which can ultimately affect DNA repair efficiency/efficacy and disease susceptibility. We focus our discussion here on *APE1* genetic variation that has been shown to alter the protein coding sequence or promoter activity.

Several years ago, studies began to identify and examine the consequence(s) of missense mutations in the *APE1* gene. The most common single nucleotide polymorphism (SNP) T1349G (rs1130409), which is present in the population at a roughly 45% frequency (dependent on race, ethnicity, *etc.*) and changes residue D148 to E148, did not alter the *in vitro* AP endonuclease efficiency of the encoded protein (69). D148E, as well as two other polymorphic variants (frequency of appearance >3%), Q51H (rs1048945) and I64V (rs2307486), have since been shown to exhibit normal thermodynamic stability of protein folding, abasic endonuclease, 3′–5′ exonuclease and REF-1 activities, coordination during the early steps of BER in a simple reconstituted assay, and intra-cellular distribution when expressed exogenously in HeLa cells (87). A few missense variants, some of which were reported to be uniquely associated with amyotrophic lateral sclerosis (ALS), such as L104R, E126D, and D283G, were estimated or experimentally demonstrated to exhibit 40 to 90% reductions in AP endonuclease activity (69); however, as is discussed later, the validity of these variants is in question. In addition to the identified population variants, a separate study found novel somatic *APE1* mutations in 3 out of 20 endometrial cancer cases; whereas no unique mutations were observed in 43 ovarian tumor samples (161). Two of the endometrial cancer-associated mutations resulted in an amino-acid substitution (P112L or R237C), with the other nucleotide change introducing a premature stop codon at residue W188 that would give rise to, minimally, a nuclease-inactive APE1 protein fragment. The P112L variant was found to exhibit normal activities in a range of biochemical and cell-based assays, whereas the R237C variant displayed reduced *in vitro* 3′ to 5′ exonuclease and 3′-damage processing activities (87), which could have contributed to the genome instability and carcinogenic process by reducing overall proofreading capacity and strand-break repair. While studies are ongoing to identify a causal relationship between an APE1 defect and human disease, we provide in Table 2 a summary of the current list of APE1 missense variants reported to date, many of which are rare and have not been functionally characterized. Lastly, we note that re-sequencing of the *APE1* exons in the 60 cancer cell lines within the NCI-60 cell line panel uncovered no novel non-synonymous mutations, suggesting that *APE1* variants are not frequent (87).

Though most research thus far has been focused on nucleotide substitutions which alter the coding sequence of DNA repair genes, one study found that an A-to-C SNP (rs1760944, ~35% frequency) in the *APE1* promoter associates with a decreased risk of lung cancer and results in reduced reporter gene expression in human embryonic lung fibroblasts and the H1299 non-small cell lung carcinoma cell line (127). The authors went on to demonstrate impaired binding of a transcription factor, presumed to be OCT-1, to the variant allele relative to the wild-type A allele. However, the mechanism by which lower APE1 expression would provide disease protection is unclear. In a separate study, Lo *et al.* (126) also found that the variant genotypes (A/C or C/C) associated with decreased lung cancer risk, yet they observed increased luciferase reporter gene expression with the C allele in comparison to the wild-type A allele in a panel of human lung adenocarcinoma cell lines (*i.e.*, A549, H1355, CL1-0 and H928). While the differences in the promoter response likely stem from disparate cellular backgrounds (*i.e.*, transcription

Table 2. APE1 Missense Variants

Variant	*rs*[a]	*Allele frequency (%)*	*Submitter*
G8R	rs202001645	N/A	1000Genome/EXOME_CHIP
P21L	rs150934075	Once	1000Genome
S26R	rs200630518	N/A	1000Genome
K35Q	rs61757709	Once	Multiple
G39E	rs34632023	1.3	APPLERA_GI|hCV25628237
A43V	rs146439344	N/A	1000Genome
Q51H[b]	rs1048945	2.5[a]	Multiple
I64T	rs61730854	1.3	CORNELL
I64V[b]	rs2307486	0.6[a]	Multiple
A88T	rs146768400	Once	NHLBI-ESP|ESP2500-chr14-20924842
E110G	rs200702900	N/A	1000Genome
C138Y	rs150356603	Once	NHLBI-ESP|ESP2500-chr14-20924993
I146V	rs201190560	N/A	EXOME_CHIP
D148E[b]	rs1130409	42.4	Multiple
D163E	rs149168435	0.1	NHLBI-ESP|ESP2500-chr14-20925199
R187H	rs148298598	Once	NHLBI-ESP|ESP2500-chr14-20925270
R221C	rs147110862	Once	NHLBI-ESP|ESP2500-chr14-20925371
N222H	rs201945833	0.1	EXOME_CHIP/CLINSEQ_SNP
R237H	rs189916038	N/A	1000Genome
G241R[b]	rs33956927	0.5	Multiple
P248L	rs201100630	N/A	EXOME_CHIP
P311S[b]	rs1803120	N/A	Multiple
T313A	rs113056798	Once	BUSHMAN|BUSHMAN-chr14-19995486
A317V[b]	rs1803118	N/A	Multiple

[a]Frequency varies between populations.
[b]Functionally characterized (see text).
N/A, not available (or unknown).

factor constellations) and/or construct designs, as with most epidemiology association analysis involving DNA repair SNPs, a causative role for the variant in disease development has not been firmly established. The issue of individual genetic variability and disease susceptibility is obviously highly complex, and will take several more years to unravel in the arena of APE1 and BER [reviewed in Simonelli *et al.* (195), Wallace *et al.* (220), and Wilson *et al.* (228)].

Cancer

There is accumulating evidence that indicates a possible relationship between APE1 alterations and cancer etiology. First, APE1 deficiency leads to increased mutagenesis and genotoxin/carcinogen susceptibility (see, for example, the mouse studies described earlier in this section), a cellular phenotype that is common to a DNA repair defect. Second, as discussed in the preceding section, one study reported unique somatic mutations in *APE1* in endometrial tumor samples that might have contributed to disease initiation or progression. Finally, although corollary, APE1 has often been seen to be over-expressed or to exhibit an atypical sub-cellular distribution pattern (with predominantly cytoplasmic localization) in many cancer types that is not observed in normal precancerous tissue. Since the topic of APE1 in cancer has been recently reviewed in detail (1), we only discuss the issue briefly here, and provide Table 3 as a glimpse of the current field.

APE1 expression has been primarily measured in fixed, paraffin-embedded, biopsy tissue sections from patients and matched controls using immunohistochemical techniques. Collectively, the studies have indicated that, in general, high expression or a cytoplasmic or cytoplasmic/nuclear distribution is associated with DNA-damaging agent resistance, tumor aggressiveness, or poor prognosis (summarized in Table 3). What causes the increase in protein levels or leads to the distinct sub-cellular localization pattern is largely unknown, but presumably involves changes in the intra-cellular environment and PTMs to the protein (see also "Intra-cellular trafficking" section). Since cancer cells are typically vigorously growing, they have an elevated demand for energy production and, thus, generate a high level of ROS as a result of enhanced mitochondrial oxidative phosphorylation. Moreover, in solid tumors, due to changes in angiogenesis, there are cycles of hypoxia/re-oxygenation that contribute to a state of oxidative stress. Thus, one hypothesis put forward is that the cytoplasmic localization of APE1 is a feature of the response to increased ROS, where the APE1 protein redistributes to the cytoplasm to maintain mtDNA integrity and function (see "Intra-cellular targeting and mitochondrial function" section) and/or to suppress Rac1-mediated oxidative stress and injury (6, 158). Elucidating the mechanisms and reasons for, as well as the consequences of, the altered APE1 expression patterns represents a critical step toward devising better treatment or management strategies for specific cancer patients.

While APE1 staining is a potential tool in cancer diagnosis, prognosis, and treatment prediction, it should be emphasized that not all disease samples exhibit distinct APE1 expression patterns, stressing the importance of developing individualized treatment plans. Moreover, in an effort to reduce experimental variability from lab to lab, as well as any artifacts, standardized methods for sample preparation and handling, and for immunodetection (*e.g.,* immunohistochemistry) that

Table 3. Studies of APE1 in Cancer

Cancer type	*Study highlights*	*Reference*
NSCLC	More cytoplasmic APE1 expression in tumor cells Higher APE1 nuclear staining associated with better overall survival	(93)
	Predominantly nuclear APE1 expression in tumor cells Cytoplasmic staining of APE1 associated with poor prognosis for adenocarcinoma or lymph node metastasis	(163)
	Increased APE1 expression in tumor cells Nuclear/cytoplasmic APE1 expression more prominent in tumor cells Mainly nuclear expression in normal cells High expression associated with cisplatin resistance, and poor disease-free and overall survival	(222)
	Nuclear/cytoplasmic APE1 expression more prominent in tumor cells Higher cytoplasmic APE1 expression associated with adenocarcinoma, stage II + III, and HPV E6-positive tumors High cytoplasmic APE1 is an independent, poor prognostic factor for NSCLC	(232)
	Nuclear/cytoplasmic APE1 expression more prominent in tumor cells Increased cytoplasmic expression associated with recurrence of adenocarcinoma	(96)
Ovarian	Nuclear/cytoplasmic APE1 expression more prominent in tumor cells High APE1 expression associated with advanced tumor (> grade 3) and poor prognosis	(157)
	Both nuclear and cytoplasmic APE1 expression patterns in tumor cells Nuclear expression associated with cancer type, optimal debulking, and overall survival	(4)
	Increased cytoplasmic APE1 expression in tumor cells Mainly nuclear APE1 expression in normal cells	(146)
	Cytoplasmic and nuclear/cytoplasmic expression patterns in tumor cells Cytoplasmic expression associated with low differentiation, poor overall, and disease-free survival	(190)
Breast	More cytoplasmic expression in tumor cells Mainly nuclear APE1 expression in normal cells Nuclear staining associated with low angiogenesis, negative LN status Cytoplasmic staining associated with high angiogenesis, positive LN status	(94)
	Predominantly nuclear APE1 expression in tumor cells Cytoplasmic staining associated with percentage of positive p53 staining Nuclear/cytoplasmic staining associated with poor survival APE1 is an independent prognostic factor for survival	(164)
	Increased nuclear APE1 expression in tumor cells Increased cytoplasmic $APE1^{K27–35Ac}$ in TNBC Exclusively nuclear $APE1^{K27–35Ac}$ in normal cells High overall expression and acetylation of APE1 are associated with TNBC	(162)
Cervical	Increased overall APE1 expression in tumor cells High expression associated with radio-resistance	(79)
Prostate	More cytoplasmic APE1 observed in PIN and malignant cancer Both nuclear and cytoplasmic staining increased in PIN and malignant cancer Predominantly nuclear APE1 expression in normal cells	(100)
Bladder	Predominantly nuclear APE1 expression in tumor cells High expression associated with radio-sensitivity, improved survival	(176)
Rectal	Nuclear/cytoplasmic APE1 expression more prominent in tumor cells Cytoplasmic expression associated with poor disease-free survival with pre-operative radio/chemotherapy	(105)
Gastro-oesophageal	Nuclear/cytoplasmic APE1 expression more prominent in tumor cells Nuclear expression inversely associated with overall survival with neo-adjuvant chemotherapy Cytoplasmic expression associated with cancer differentiation	(4)
Pancreatico-biliary	Nuclear/cytoplasmic APE1 expression more prominent in tumor cells Subcellular distribution varied among cancer types Absence of cytoplasmic expression associated with perineural invasion, vascular invasion and impaired differentiation	(4)
Hepatocellular	Increased cytoplasmic APE1 expression in tumor cells Cytoplasmic expression associated with poor differentiation, poor survival	(38)

(*continued*)

Table 3. (Continued)

Cancer type	*Study highlights*	*Reference*
Osteosarcoma	Increased overall APE1 expression in tumor cells High expression associated with poor survival	(221)
Multiple myeloma	High overall expression associated with recurrence and malignancy	(238)
Head & neck	Cytoplasmic and nuclear/cytoplasmic expression pattern more prominent in tumor cells Nuclear/cytoplasmic expression pattern in normal cells Nuclear staining associated with differentiation, positive LN status, and poor survival Nuclear APE1 staining inversely related to nuclear accumulation of p53 and response to chemotherapy	(111)
	Higher APE1 mRNA in advanced tumor (Stage III/IV) and large tumor (T3/T4) with LN metastasis	(132)
Gliomas	High AP endonuclease activity associated with malignancy	(13)
Pediatric ependymoma	Exclusively nuclear APE1 expression in tumor cells Low AP endonuclease activity associated with better disease-free survival with radiotherapy	(15)
Germ cell	Nuclear/cytoplasmic expression pattern more prominent in tumor cells Increased overall APE1 expression in tumor cells Predominantly nuclear APE1 expression in normal cells High expression associated with resistance to bleomycin and radiation	(170)

NSCLC, non-small cell lung cancer; HPV, human papillomavirus; LN, lymph node; TNBC, triple negative breast cancer; PIN, prostatic intraepithelial neoplasia.

employ the same antibodies and quantitative procedures to assess APE1 expression, are needed. The implementation of a high-volume tissue micro-array platform that spans multiple cancer types and controls represents a paradigm for future translational evaluations [see, for example, (4)], and hopefully will help provide a more definitive understanding of the molecular roles of APE1 in the carcinogenic process. Finally, it is noteworthy that elevated serum auto-antibodies against APE1 have recently been associated with the auto-immune disease, systemic lupus erythematosus, particularly those patients with psychiatric manifestations, and non-small cell lung carcinoma (33, 98). Future investigations will need to determine the contribution, if any, of the APE1 auto-antibodies to disease etiology and, more generally, whether APE1 auto-antibodies have utility in basic serological examinations.

Neuropathology

The central nervous system (CNS), consisting of the brain and spinal cord, is composed of some of the most metabolically active cells in the human body. As such, careful maintenance of ROS homeostasis and associated oxidative damage in cells of the CNS is vital for the well being of the organism. APE1 is, in general, highly expressed in the CNS, with some variability among the different cell types and regions of the human brain, including the existence of distinct nuclear and cytoplasmic distribution patterns [reviewed in Wilson and McNeill (229)]. Early investigations demonstrated that APE1 depletion by siRNA in primary rat hippocampal or sensory neuronal cell cultures results in reduced cell viability, as well as increased apoptosis and DNA damage, after hydrogen peroxide treatment (216). Jiang *et al.* found that inhibition of AP site repair using the inhibitor, methoxyamine, and not inhibition of the redox activity of APE1, induced hypersensitivity of differentiated (post-mitotic) SH-SY5Y neural cells to a range of oxidizing agents (92). APE1 protein levels were also shown to be correlated with the degree of neuroprotection against the deleterious effects of both transient focal and global cerebral ischemia in rodent models (104, 197), observations which are consistent with the emerging evidence that BER plays an important role in the defense against stroke [reviewed in Sykora *et al.* (201)]. In total, the experiments support the hypothesis that APE1, apparently in a DNA repair-dependent mechanism, and presumably other proteins which process oxidative DNA lesions, contributes to the maintenance of brain cell function, particularly when challenged with conditions of oxidative stress.

One of the first studies to suggest a relationship between an APE1 defect and human neuropathology found significantly lower APE1 protein and AP endonuclease activity in tissue extracts prepared from the frontal cortex of 11 patients suffering from sporadic ALS in comparison to six age-matched controls (110). ALS is a motor neuron disease that has been associated with oxidative stress, as pathogenic mutations have been identified in the radical scavenger superoxide dismutase gene (*SOD1*) in familial cases of the disorder. Consistent with APE1 contributing to the development of ALS, mutations, including the missense variants L104R, E126D, D148E, D283G, and G306A, were found in eight out of 11 patients with sporadic or familial ALS; while no mutations were detected in five healthy control subjects (154). In a separate, larger re-sequencing effort involving 153 ALS patients and 58 controls, Hayward *et al.* (74) observed three *APE1* nucleotide substitutions common among patients and controls (including the D148E polymorphism), as well as a silent base change (at residue Y315) unique to three sporadic ALS patients and a 4-bp deletion in a single sporadic ALS patient. The deletion mutation introduces a stop codon at amino acid 145, producing a truncated protein product that, if stable, would minimally lack nuclease activity; whether this variant

was inherited or sporadic, and whether it contributed to the disease, was unfortunately unable to be assessed. The fact that far fewer mutations were observed in this study was the first indication that APE1 plays a much smaller role in the etiology of ALS than insinuated by the apparent "jack-pot" of *APE1* sequence variants reported in the earlier work (154). Consistent with this notion, Tomkins *et al.* (206) found no novel *APE1* mutations in genomic DNA isolated from the cerebral cortex of 84 ALS patients, other than a 3′-UTR 4-bp deletion in a single case of ALS. They also observed that the SNPs related to Q51H and D148E are present at roughly equal frequencies among the ALS patients and controls. Coppede *et al.* (31) similarly observed no significant difference in the D148E allele frequency between 134 sporadic ALS patients and 129 matched controls, seemingly consistent with the normal biochemical properties of the variant enzyme (see "Genetic variants" section). In a separate study, however, APE1 protein was found to be increased or re-localized in CNS tissues of ALS patients, particularly within regions that contain motor neurons (186). It seems likely that these alterations are a consequence of the oxidative stress and increased DNA damage associated with ALS, and are not directly connected to disease initiation. While further studies to explore a possible role for APE1 in the different pathological stages of ALS might be worthwhile, it would seem to be most relevant to evaluate whether rare genetic mutations in *APE1* (or BER genes for that matter), such as the deletion mutants described earlier, contribute directly to disease manifestation in a subset of patients.

A similar, generally inconclusive picture has emerged regarding the potential contribution of APE1 to Alzheimer disease (AD), and, to a lesser extent, Parkinson disease (PD). AD is a degenerative dementia that involves cognitive and functional impairments due to the loss of neurons in the cerebral cortex, whereas PD is characterized by motor deficits and cell death in the substantia nigra. While the exact cause of either disorder is unclear, studies have suggested the involvement of oxidative stress in the disease pathology, prompting an interest in specific DNA repair systems, such as BER, as risk factors. Several studies have, indeed, found that oxidative DNA damage is higher in AD brain tissue [reviewed in Santos *et al.* (179)], and Weissman *et al.* (224) discovered that reduced BER activity, namely uracil DNA glycosylase and POLβ functions (although APE1 function was unchanged in AD samples), correlates with AD progression in a small set of patients. In addition, Tan *et al.* reported that APE1 immunostaining was relatively high in the hippocampus and surrounding temporal cortex of some AD sufferers relative to matched controls, a phenomenon that again may simply reflect a response to disease-induced oxidative stress (202). Further analysis revealed that APE1 was also present in a subset of senile plaques and plaque-like structures, suggesting a role for the protein in β-amyloid deposition, a marker for AD. Generally consistent with this earlier work, Davydov *et al.* (34) reported that APE1 protein levels tended to be higher in mid-frontal cortex nuclear extracts of AD patients, and Marcon *et al.* (134) observed more intense nuclear immunostaining of APE1 in the pathogenic regions of both sporadic and familial AD brains. Molecular epidemiology association studies, however, have indicated that the polymorphic variant of *APE1* (D148E) as well as common SNPs in *OGG1* and *XRCC1* are not independent risk factors of AD (159). With regard to PD, one recent study involving 60 case subjects and 108 normal controls found that the D148E variant of APE1 had a positive association as a risk factor for the disease (59), although it was acknowledged by the authors that more extensive follow-up experiments are needed to validate this observation. Finally, Huang *et al.* (84), in addition to confirming the importance of APE1 in neuronal cell viability, possibly in a mechanism involving Cdk5 phosphorylation of residue T232 (in the mouse protein, T233 in the human protein), which inactivates the enzyme's AP endonuclease activity (see Table 1), detected an increase in the T233-phosphorylated form of APE1 in post-mortem brain samples from PD and AD patients, uncovering a possible molecular connection between APE1 inactivation and neurodegenerative disease.

Despite the fact that strong evidence exists for a role of APE1 in protecting neuronal cells from conditions of oxidative stress, there is no firm evidence at present that indicates an involvement of the protein in human neurological disease. While global changes in protein expression or re-localization may be observed, these descriptive alterations likely reflect a response to the disease environment, presumably the state of oxidative stress, as also seen in cancer patient samples (see previous section). Moreover, it is important to point out that the large genome-wide association studies conducted on ALS, AD, and PD patients, to our knowledge, have not identified genes related to DNA repair pathways as major risk factors in any of these neurological disorders. Nevertheless, it remains plausible that rare *APE1* mutations, either inherited (which allow for organism survival) or sporadic, could directly contribute to disease initiation or susceptibility. In addition, it is possible that reduced BER capacity, particularly in a setting of oxidative stress that is typical of neurological disease, could exacerbate the pathologic process.

It is worth emphasizing that a set of inherited disorders with neurological deficits have been genetically linked to single-strand break DNA repair defects [reviewed in Reynolds and Stewart (169)]. In particular, spinocerebellar ataxia with axonal neuropathy-1 (SCAN1) arises from mutations in *TDP1*; ataxia oculomotor apraxia-1 (AOA1) results from mutations in *APTX* (Aprataxin); and microcephaly, early-onset, intractable seizures, and developmental delay (MCSZ) are caused by pathogenic mutations in *PNKP*. Since APE1 maintains a significant strand break 3′-repair diesterase activity, it will be of interest to examine whether APE1 deficiency can adversely affect end-points related to brain development and function, such as by using conditional KO mouse models, as has been done for other BER genes, including XRCC1 (116). Clearly, future studies are needed to more directly evaluate the contribution of APE1 and BER to various pathologies involving oxidative stress [reviewed in Hegde *et al.* (76)].

APE1 as a Therapeutic Target

Since APE1 has roles in both disease suppression and therapeutic agent resistance, it is presumed that if APE1 activities can be strategically regulated, the protein would be a favorable target in both preventative and curative treatment paradigms. Although the concept of DNA repair activators is of growing interest as a prospective mechanism for improving "healthspan" (*i.e.*, counter-acting the cumulative effects seen

during aging and in age-related disease), efforts to this point have focused mainly on strategies to reduce or block DNA repair function. In particular, the current emphasis has been on the design of small-molecule DNA repair inhibitors, pursuing the hypothesis that inactivation of a DNA damage response will increase the efficacy of a relevant anti-cancer agent, many of which induce cell death by introducing lethal DNA lesions. Since APE1 plays a central and critical role in the BER process, the protein has received significant attention as an attractive target. As will be expounded in the next sections, APE1 could be targeted in (i) combinatorial treatment schemes to enhance the cytotoxicity of a germane clinical DNA-damaging agent or (ii) mono-therapies, in which inhibition of APE1 function would be synthetically lethal with a genetic defect present uniquely in the disease (*e.g.*, cancer) tissue.

Protein depletion

As introduced earlier (see "APE1 Biological Roles" section), anti-sense-mediated depletion of APE1 increased cellular sensitivity to the alkylating agent MMS and the oxidizing agent hydrogen peroxide, providing an initial picture of the role of APE1 in resistance to DNA-damaging agent challenges (155, 218). On the advent of siRNA technologies, several groups began to look more exhaustively at the effects of APE1 depletion on sensitivity to a range of clinical DNA-interactive compounds. Some of the earliest work, published by Kelley and colleagues, revealed that APE1 provides resistance against not only MMS and hydrogen peroxide, but also the anti-cancer agents thiotepa, etoposide, and ionizing radiation (221). Through the efforts of many labs since, there is a general consensus emerging that indicates a role for APE1 in dictating cellular responsiveness to clinically relevant alkylators, most notably temozolomide (7, 14, 137, 142, 193), and a subset of chain-terminating nucleoside analogs, most prominently troxacitabine (114, 137, 180). Such a role is consistent with the biochemical activities of APE1, as alkylating agents, particularly mono-functional versions, induce a high number of abasic sites through both direct and indirect mechanisms, and certain anti-metabolites generate exonuclease 3′-end substrates. In addition, expression of the dominant-negative ED protein (introduced in "APE1 Biological Roles" section) in Chinese hamster ovary cells increases the toxicity of the anti-cancer agents, 5-fluorouracil and 5-fluorodeoxyuridine, with the latter causing greater cell death, presumably because its effects are targeted explicitly to DNA, and not to RNA as well (138). This observation is generally consistent with the recent findings that PARP1 and other BER enzymes, including APE1, play a role in resistance to 5-fluorodeoxyuridine more so than 5-fluorouracil, likely due to the higher levels of BER substrate base lesions, that is, 5-fluorouracil and uracil, introduced into genomic DNA by the former compound (60).

Hyper-sensitivity of APE1 KD cells has been reported for other clinical DNA-damaging agents, including ionizing radiation (32, 150, 237), bleomycin (56, 170, 218), gemcitabine (239), cisplatin (222, 246), etoposide (185, 221), and photodynamic therapy (236, 243). While in some cases it is not obvious what role APE1 would play in repairing the direct DNA damage (*e.g.*, for the crosslinking agent cisplatin), some level of oxidative stress is involved in most genotoxin exposures. Thus, APE1 may be required for oxidative DNA lesion processing or for a more general stress response. Moreover, Chattopadhyay *et al.* (25) have reported that acetylated APE1, through an interaction with the transcription factor YB-1, can activate expression of the multi-drug resistance gene *MDR1*. Increased levels of the MDR1 P-glycoprotein membrane transporter are associated with enhanced drug efflux and resistance in cancer cells, and could explain the sensitizing effect of APE1 depletion to compounds such as cisplatin. Hence, APE1 may have multiple roles in providing DNA-damaging agent protection, engaging its DNA repair and/or transcriptional regulatory functions. We note that for many of the anti-cancer agents mentioned earlier, an APE1-dependent influence on sensitivity has not been observed in all studies, suggesting presumably cell type- or experimental assay-specific responses/outcomes.

In addition to the pre-clinical KD studies performed in culture, the effects of APE1 down-regulation on cancer cell growth or sensitivity to therapeutic agents have been investigated in xenograft or tumor-bearing mouse models as well. Fishel *et al.* reported that human SKOV-3 ovarian cancer cells treated with APE1 siRNA *ex vivo* and then implanted into female nude mice exhibit a dramatic reduction in tumor volume and cell proliferation during the period of APE1 depletion (51). Moreover, Xiang *et al.* found that LOVO colon cancer cells injected subcutaneously into nude mice and subsequently treated with *APE1*-specific siRNA adenoviral particles display reduced tumor growth, particularly when challenged with X-ray irradiation, relative to the controls (237). Using this established approach, the Wang group has since shown that *in vivo* APE1 depletion sensitizes A549 non-small cell lung cancer xenografts to hematoporphyrin derivative-mediated photodynamic therapy (243) and human hepatocellular carcinoma cell lines to radio-therapy (32). The compilation of the studies strongly indicates that transient suppression of APE1 activity can have a beneficial effect when treating cancer with certain chemo- or radio-therapies. It is important to emphasize, however, that since KD strategies result in general suppression of target gene expression, it is uncertain in this scenario which activity of the multi-functional APE1 protein determines sensitivity to the different DNA-damaging agents.

Small-molecule inhibitors

Since DNA repair inhibitors have potential utility in combinatorial treatment paradigms with clinically relevant DNA-interactive agents and in mono-therapies under the premise of synthetic lethality (see more in next section), several investigators have focused on the development of small-molecule DNA repair protein inhibitors. In addition, function-specific inhibitors represent potentially powerful tools to address the contribution of the targeted activity to specific cellular end points, including DNA-damaging agent sensitivity. Since the topic of APE1 inhibitors has been extensively reviewed recently (5, 230), it is not our intent to revisit this issue in depth. Instead, we provide Table 4 as a summary of the efforts to design and apply APE1 inhibitors, and touch on the current state of the mission to develop a clinically useful, small-molecule inactivator of the AP endonuclease or redox regulatory function of APE1.

A number of laboratories have taken on the effort to identify and design potent inhibitors against the repair nuclease function(s) of APE1. These studies have generally relied on a high-throughput, fluorescence-based screening assay, in which inhibition of APE1 AP site incision activity prevents

Table 4. Studies of Direct APE1 Nuclease Inhibitors

Feature compound(s)	*Brief summary*	*Reference*
CRT0044876 (7-Nitroindole-2carboxylic acid)	First reported direct nuclease inhibitor, identified in a high-throughput screen of 5000 small molecules Inhibits AP endonuclease (IC_{50} ~3 μM), as well as 3′-phosphodiesterase (IC_{50} ~5 μM) and 3′-phosphatase, activities Inhibits AP endonuclease activity in cell extracts Sensitizes cells to MMS and TMZ	(131)
Several compounds (most potent contain two carboxylate functional groups in a 3D arrangement with hydrophobic groups)	Identified in an *in silico* screen of 365,000 compounds using 3D pharmacophore models IC_{50} in the low μM range Not evaluated in cell-based experiments	(244)
NCI-13755 NCI-13793	Arylstibonic acid compounds identified from a screen of the NCI 2000 Diversity Set IC_{50} 4–17 nM No effect on cellular sensitivity to DNA-damaging agents	(181)
6-Hydroxy-DL-DOPA Myricetin Reactive Blue 2	Identified from a screen of the Sigma LOPAC IC_{50} 0.11–0.32 μM Inhibit AP endonuclease activity in cell extracts Sensitize cells to MMS Multiple known biological targets	(194)
AR03 ([2,4,9-trimethylbenzo[b][1,8]-naphthyridin-5-amine)	Identified from a screen of 60,000 small molecules IC_{50} ~2 μM (no effect on redox function) Inhibits AP endonuclease activity in cell extracts Sensitizes glioblastoma cells to MMS and TMZ	(7)
Lucanthone Hycanthone	IC_{50} 5 μM and 80 nM, respectively (no effect on redox function) Sensitize cancer cells to MMS and TMZ Currently in clinical trials as an adjuvant in brain tumor radiotherapy	(129, 149)
Compounds containing 2-methyl-4-amino-6,7-dioxolo-quinoline structure	Identified from *in silico* screen IC_{50} in the μM range (no effect on redox function) Inhibits AP endonuclease activity in nuclear lysates Sensitize cancer cells to the methylating agent, MeLex	(196)
3-Carbamoylbenzoic acid and derivatives	SAR study around 3-carbamoylbenzoic acid scaffold IC_{50} in the low μM range Sensitize cancer cells to MMS, 5-fluorouracil and 5-fluorodeoxyuridine	(2)
Several compounds of different structures	Identified from the NIH MLSMR and additional public collections (352,498 total compounds) $IC_{50} < 30\ \mu M$ Sensitize HeLa cells to MMS	(42)
Compound 3 (N-(3-(benzo[d]thiazol-2-yl)-6-isopropyl-4,5,6,7-tetrahydrothieno[2,3-c] pyridin-2-yl)acetamide) and its analogue, compound 52	SAR study around compound 3 IC_{50} 2 and 3.3 μM, respectively Inhibit AP endonuclease activity in cell extracts Sensitize HeLa cells to MMS and TMZ Initial ADME profile: compound 3 crosses the blood-brain barrier more efficiently; compound 52 has a better general cytotoxicity profile, higher exposure levels, and a more favorable $t_{(1/2)}$ in the plasma	(167)

TMZ, temozolomide; SAR, structure-activity relationship.

release of a 5′-fluorophore-containing DNA fragment from its strand-opposite 3′-attached quench, and, thus, an associated increase in quantifiable signal [originally described in (131)]. As summarized in Table 4, quite a few reports have appeared detailing the identification and validation of initial hits that inactivate the AP endonuclease activity of APE1 *in vitro*. However, most of the reported inhibitors have affinities (typically μM) that are not compatible with a suitable pharmaceutical agent, are not amendable to extensive chemical modification, or have multiple biological targets. The "low affinity" attribute may stem from the fact that small molecules are unable to replicate the "high affinity" binding contacts seen in the APE1/AP-DNA complex, which spans roughly 7–8 bp in length. Indeed, in an extensive structure-function activity relationship study, the introduced modifications only slightly increased the effectiveness of the analogs against

APE1, either (i) supporting the notion that small molecules do not make sufficient contact with the protein for high affinity binding or (ii) indicating that the initial chemotype was a poor starting substrate. It is also noteworthy that none of the nuclease-targeted compounds identified to date have been demonstrated to have utility in pre-clinical animal cancer models. Nevertheless, in pre-clinical cell culture systems, APE1 repair inhibitors have been shown to enhance the cytotoxicity of temozolomide (7, 131, 142, 167), confirming that the nuclease function(s) of the protein plays a critical role in repairing alkylative DNA damage, presumably abasic sites. Future studies will need to examine the utility of the most promising compounds in both combinatorial and synthetic lethal treatment paradigms, and may need to employ covalently linked, small molecules that bind simultaneously multiple functional sites of APE1.

The design of APE1 redox inhibitors has been more limited, presumably due to the lack of a high-throughput screening assay to identify molecules that inactivate the redox function of the protein. Nevertheless, using a bead-based approach and nuclear cell extracts, Shimizu *et al.* (191) identified APE1 as a major binding protein for the quinone derivative, E3330 (introduced in "APE1 Biological Roles" section). E3330 had previously been shown to suppress NF-κB transactivational activity, and, in doing so, to promote an anti-inflammatory state, exhibiting potential utility in the treatment of various forms of hepatitis [(80) and references therein]. The APE1-E3330 interaction was validated using multiple biochemical and biological approaches, and was determined to have an apparent binding constant of 1.6 n*M* by surface plasmon resonance (191). Since this discovery, the ability of E3330 to selectively inactivate the redox activity of APE1 and suppress the activity of several transcription factor targets has been confirmed in multiple studies [reviewed in Kelley *et al.* (101)]. Moreover, E3330, and, in some instances, its synthesized chemical analogs, has been demonstrated to inhibit proliferation of human pancreatic and ovarian cancer cell lines in culture; angiogenesis or migration of cancer cells in *in vitro* models; and tumor growth of pancreatic cancer xenografts in mice (52, 102, 151, 249). Thus, despite the fact that there is controversy regarding the precise functionality of E3330 (133, 245), the findings with this compound and its analogs underscore the importance of further analyses around the molecular mechanisms and clinical value of targeting APE1 redox function in therapeutic paradigms. Indeed, an exciting development in recent years has been the observation that APE1 redox activity, through modulation of transcription factors such as HIF-1α (46, 85, 115), plays a significant role in regulating angiogenesis, and, as such, could represent a promising target in the control of tumor proliferation and metastasis. We close this section by mentioning that dietary agents, such as soy isoflavones (found in plants) and resveratrol (found in red wine), have been reported to have complex effects on the expression or the repair/redox activities of APE1, implying the potential for natural (intended or unintended) regulation of APE1 cellular functions [reviewed in Kelley *et al.* (101) and Raffoul *et al.* (166)].

Synthetic lethality

One of the most significant advances in recent years regarding DNA repair as a potential target in cancer therapeutics was the observation that PARP inhibitors can lead to reduced survival of cells which lack BRCA1 or BRCA2 (17, 50). PARP1 is a DNA damage sensor that facilitates efficient repair of DNA single-strand breaks. BRCA1 and BRCA2 are tumor suppressor gene products associated with hereditary breast/ovarian cancer that participate in resolving DNA double-strand breaks. Through a mechanism that engages protein and DNA interactions reviewed elsewhere (174), BRCA1 and BRCA2 facilitate RAD51-mediated homologous recombination, a molecular process which enables the faithful exchange of genetic information from an intact sister chromatid to resolve one-ended, DNA double-strand breaks formed on replication fork collapse. The fact that PARP inhibitors promote lethality of cells deficient in homologous recombination suggested that the pathways engaging PARP1 and the BRCA proteins are synthetically lethal (*i.e.*, simultaneous inactivation of both leads to cell death). Specifically, it was thought that inhibition of PARP1-mediated single-strand break repair would lead to increased levels of DNA double-strand breaks during copying of the genome, causing lethality in cells unable to efficiently resolve these damage-induced, replication intermediates. While the mechanism driving cell death has been modified in recent years, now involving potential trapping of PARP1 on DNA by the inhibitor (77), the enthusiasm for employing synthetic lethality in the treatment of repair-defective cancers has remained. Indeed, although issues and optimization phases remain, PARP inhibitors have shown promising results in select clinical trials [reviewed in Sandhu *et al.* (178)].

With regard to APE1, Sultana *et al.* observed synthetic lethality of BRCA1, BRCA2, or ATM double-strand break repair-deficient Chinese hamster lung or human cancer cell lines on treatment with an APE1 DNA repair inhibitor (200). In particular, exposure of these mutant cell lines to a direct or indirect endonuclease inhibitor resulted in reduced colony formation, elevated chromosomal AP sites and DNA single- and double-strand breaks, and G2/M cell cycle arrest. In the reverse direction, Chinese hamster ovary cells expressing the dominant-negative ED protein or human MDA-MB-231 breast cancer cells depleted for APE1 exhibited reduced survival in the presence of inhibitors (NU7441, KU55933, or Wortmannin) against the double-strand break repair signaling kinases, namely ATM and DNA-PKcs. Since the redox inhibitor E3330 did not induce synthetic lethality, it would appear that a defect in AP site repair induces replication fork collapse and the accumulation of DNA strand break intermediates that drive cell death. A more exhaustive search of the cellular pathways that are synthetically lethal with the disparate functions of APE1 would seem to be a worthwhile endeavor. Moreover, assuming effective and sufficiently validated APE1 inhibitors can be developed, future studies will need to assess the value of these small molecules in formal clinical trials.

Summary, Conclusions, and Future Perspectives

Abasic sites are a common form of DNA damage that are generated by spontaneous or damage-induced hydrolysis of the N-glycosylic bond, or *via* the action of DNA glycosylases that remove modified or substrate bases from DNA as a part of BER. In addition, single-strand breaks are created frequently by free radical attack of DNA or as intermediates of a DNA repair response. APE1 possesses a powerful AP

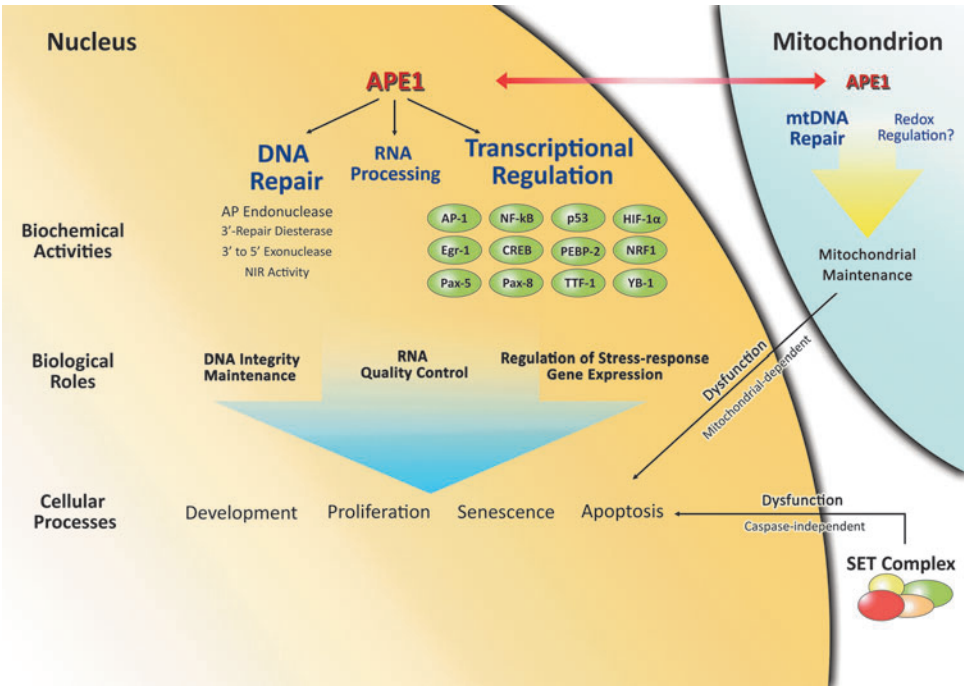

FIG. 4. The APE1 galaxy. As highlighted within, APE1 has multiple biochemical activities that impart multiple biological roles. These functions affect various cellular processes that can ultimately impact disease susceptibility, therapeutic response, and prognosis. In addition, complex mechanisms that involve protein interactions and PTMs regulate not only the activities of APE1, but also its intra-cellular compartmentalization. See text for details. To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/ars

endonuclease activity (around two orders of magnitude greater than its other nuclease activities), which is generally agreed to be a major biological function of the enzyme. Based on current work, its 3′-repair diesterase activity is also likely an important cellular function, minimally contributing to clean-up of DNA termini generated by β-elimination reactions at AP sites, and its 3′ to 5′ exonuclease activity appears to at least aid in the removal of certain chain-terminating nucleoside analogs in cells. Moreover, a great deal of biochemical and cell-based evidence supports the importance of the redox regulatory function of APE1 in moderating a range of cellular responses. The biological significance of the other functions, namely RNA cleavage and NIR, in our opinion, still requires further substantiation. Studies using genetically manipulated cell lines may help define which of the many activities of APE1 are important to the various biological end points discussed here. However, the difficulty in creating clean, separation-of-function mutants in APE1, particularly those that effectively distinguish the various nuclease activities of the enzyme, is likely to be a constraint.

Figure 4 provides an overview of the many proposed functions of APE1. Besides having prominent roles in the nucleus, APE1 also has important roles in mitochondria, at minimum in protecting the mitochondrial genome, and presumably in the endoplasmic reticulum as a part of the SET complex. As an extension of its formal biochemical activities, APE1 has general roles in a range of biological processes, including proliferation and growth, apoptosis, senescence, stress responses, angiogenesis, metabolism, and differentiation, to name a few. How the sub-compartmentalization and these various functions of APE1 are manipulated is just beginning to be understood, and presumably involve protein–protein interactions and PTMs. The unstructured, newly acquired N-terminal region of the protein also appears to play a key role in directing the participation of APE1 to specific molecular pathways. Teasing out the complexities that regulate the intra-cellular localization and the activities of this multi-functional protein will prove to be a real challenge, and should keep the APE1 aficionados out of trouble for many years to come.

It seems striking that at present there is no established link between an APE1 defect and human disease. This presumably stems from the fact that a severe APE1 deficit would be incompatible with life. Nevertheless, there is a collection of cell-based and animal model studies indicating that APE1 deficiency imparts reduced cell survival, increased sensitivity to DNA-damaging agent exposures, elevated mutagenesis, and an enhanced risk of disease development, to name a few. While studies continue to progress to explore the hypothesis that reduced BER capacity associates with increased disease susceptibility, the ongoing whole exome and whole genome re-sequencing efforts may reveal rare mutations in BER genes that contribute to disease manifestation. Those pathologies that are most likely to be caused or exacerbated by a BER defect are ones which involve oxidative stress and its associated DNA damage.

We close with an apology and a thank you. We have attempted to provide a comprehensive summary of the APE1 literature, and undoubtedly have either missed relevant publications or misinterpreted some of the reported data. While we aimed at delivering the content in an unbiased manner, there will always be some partiality in the presentation. For this we are sorry, but we hope that the review spurs on new ideas and identifies weaknesses in the APE1 field, and, of course, we thank the many investigators, both past and present, who have contributed to our understanding of this critical multi-functional protein.

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Address correspondence to:
Dr. David M. Wilson, III
Intramural Research Program
Laboratory of Molecular Gerontology
National Institute on Aging
National Institutes of Health
251 Bayview Boulevard
Baltimore, MD 21224

E-mail: wilsonda@mail.nih.gov

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Abbreviations Used

AD = Alzheimer disease
ALS = amyotrophic lateral sclerosis
APE1 = apurinic/apyrimidinic endonuclease 1
BER = base excision repair
CNS = central nervous system
CSR = class switch recombination
ETC = electron transport chain
KD = knock-down
KO = knock-out
MMS = methylmethane sulfonate
mtBER = mitochondrial BER
mtDNA = mitochondrial DNA
MTS = mitochondrial targeting sequence
nCaREs = negative Ca^{2+} response elements
NES = nuclear export signal
NIR = nucleotide incision repair
NLS = nuclear localization signal
PARP1 = poly(ADP)ribose polymerase 1
PD = Parkinson disease
PTM = post-translational modification
ROS = reactive oxygen species
SHM = somatic hypermutation
SNP = single nucleotide polymorphism