Cell. Mol. Life Sci. (2010) 67:3589–3608
DOI 10.1007/s00018-010-0486-4

Cellular and Molecular Life Sciences

MULTI-AUTHOR REVIEW

Understanding different functions of mammalian AP endonuclease (APE1) as a promising tool for cancer treatment

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Received: 28 July 2010 / Accepted: 28 July 2010 / Published online: 13 August 2010

Abstract The apurinic endonuclease 1/redox factor-1 (APE1) has a crucial function in DNA repair and in redox signaling in mammals, and recent studies identify it as an excellent target for sensitizing tumor cells to chemotherapy. APE1 is an essential enzyme in the base excision repair pathway of DNA lesions caused by oxidation and alkylation. As importantly, APE1 also functions as a redox agent maintaining transcription factors involved in cancer promotion and progression in an active reduced state. Very recently, a new unsuspected function of APE1 in RNA metabolism was discovered, opening new perspectives for this multifunctional protein. These observations underline the necessity to understand the molecular mechanisms responsible for fine-tuning its different biological functions. This survey intends to give an overview of the multifunctional roles of APE1 and their regulation in the context of considering this protein a promising tool for anticancer therapy.

Keywords Base excision repair · Oxidative stress · Redox signalling · Nucleolus · Cancer

Abbreviations

8-OHG	8-Hydroxyguanine
AP	Apurinic/apyrimidinic
AP-1	Activating Protein-1
APE1	Apurinic apyrimidinic endonuclease 1
BER	Base excision repair
CKI and CKII	Casein kinase I and II
CREB	cAMP-responsible element binding protein
Egr-1	Early growth response protein-1
FEN1	Flap endonuclease I
GSK3	Glycogen synthase kinase 3
GzmA	Granzyme A
GzmK	Granzyme K
HIF-1α	Hypoxia inducible factor-1α
MTS	Mitochondrial targeting sequence
MPG	Methylpurine DNA glycosylase
MYH	Human MutY glycosylase homolog
NF-κB	Nuclear factor-kappaB
nCaRE	Negative calcium responsive elements
NLS	Nuclear localization signal
NPM1	Nucleophosmin 1
OGG-1	8-Oxoguanine DNA glycosylase
PARP-1	Poly(ADP-ribose) polymerase
Pax	Paired box-containing proteins
PCNA	Proliferating cell nuclear antigen
PEBP-2	Polyoma virus enhancer-binding protein-2
PKC	Protein kinase C
Polβ	Polymerase β
PTEN	Phosphatase and tensin homolog
PTH	Parathyroid hormone
PTM	Post-translational modification
RFC	Replication factor C
ROS	Reactive oxygen species
Trx	Thioredoxin
XRCC1	X-ray cross-species complementing 1

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Introduction

APE1/Ref-1 (also called APEX1 or Ref-1 and here referred to as APE1), the mammalian ortholog of *Escherichia coli*

Xth (Exo III), is a master regulator of cellular response to oxidative stress and plays a central role in the maintenance of genome stability acting as the major apurinic/apyrimidinic (AP)-endonuclease in the DNA base excision repair (BER) pathway that copes with DNA damage caused by both endogenous and exogenous agents including chemotherapeutic agents (see the reviews by Hazra et al. and Wilson III et al. in this issue for details). Upon removal of the damaged base, APE1 cleaves the abasic site to facilitate repair. The vital effects of APE1 are attributed to this role [1]. However, APE1 has at least another major cellular function, since it works as a redox protein which stimulates the DNA binding activity of several transcription factors that are involved in cancer promotion and progression, such as nuclear factor-kappaB (NF-κB), early growth response protein-1 (Egr-1), p53, HIF-1α, CREB, AP-1 and paired box-containing proteins (Pax) in different cell systems [2]. This function is accounted for by Cys65 residue. Interestingly, through this redox-mediated activation of NF-κB-driven IL-8 expression, APE1 has recently been suggested to play a role in inflammatory processes [3]. A third secondary and poorly characterized APE1 function is represented by its transcriptional repressor activity (on PTH and APE1 promoters) through binding to the negative calcium responsive elements (nCaRE) [4, 5]. The two major functions of APE1, redox and in BER, are completely independent: the N-terminus, which contains a bipartite nuclear localization signal (NLS), is principally devoted to redox-mediated transcriptional co-activation activity while the C-terminus exerts the enzymatic activity on the abasic sites of DNA mainly through the residue H309 in the catalytic site [6].

We have recently discovered a new unsuspected function of APE1 in RNA metabolism which is controlled by the first 33N-terminal aminoacids. In particular, we demonstrated that APE1 is able to bind RNA and may act as a cleansing factor of abasic RNA ([7, 8], and unpublished data). Another recent paper demonstrated that APE1 may control *c-Myc* expression by cleaving its mRNA [9]. This evidence points to a new function of APE1 in regulating gene expression through post-transcriptional mechanisms and opens new perspectives in understanding APE1 multifunctional roles in cancer cells.

APE1 is essential for cell viability [1, 10] and, therefore, a detailed comprehension of the molecular targets of APE1 functions has been very difficult. Conditional knock-out and knock-down strategies [1, 11] confirmed its essentiality and allowed establishment of cell models to inspect and characterize in better detail the major functions of APE1. However, knowledge of the molecular effectors regulated by APE1 in determining its biological essentiality is still scanty.

Recently, proteolysis occurring at Asn33 (giving rise to a form called NΔ33APE1) has been described, suggesting that removal of NLS may constitute a general mechanism for redirecting APE1 towards non-canonical subcellular compartments, such as mitochondria [12]. Unfortunately, the specific protease responsible for this cleavage has not yet been identified. The first 42 amino acids of APE1 appear highly unordered in the crystallographic structure [13], while the remainder have a globular fold [14]. It is therefore plausible, as is becoming evident, that the protein N-terminus is used for interacting with other partners, thus modulating the different APE1 functions. Interestingly, a similar bipartite arrangement for Rrp1, the *Drosophila* homologue of mammalian APE1, has been described, indicating a functional role of the unstructured N-terminal domain in modulating protein–protein interactions ([15], and Hazra et al. this issue).

Accumulating evidence has demonstrated that the heterogeneity of APE1 expression pattern is linked to different pathological conditions ranging from metabolic to differentiative disorders and, in particular, to cancer, and that elevated levels of APE1 have been linked to resistance to chemotherapy, poor prognosis and poor survival. Different kinds of human tumors are characterized by alterations in subcellular distribution of APE1 with respect to non-tumoral tissue [16]. Furthermore, alteration in subcellular distribution of APE1 is not functionally related to the ability of cancerous tissue to repair abasic sites, suggesting that BER may not be affected [17–19]. Even though it is not clear at present whether APE1 extranuclear localization is responsible for or only associated with tumor progression, its function in RNA metabolism is a possible antitumor candidate target.

The essentiality of mammalian APE1 cannot be ascribed only to its DNA repair function, since attempts to restore this activity in APE1-deficient cells with the yeast homologous Apn1 [1], lacking the redox-activation domain, or with an APE1 mutant lacking the acetylation sites or the first 33N-terminal domain, required for its function in RNA metabolism [7, 8] but not the DNA repair activity [11], were unsuccessful. It is also known that the specific block of the APE1 redox activity on NF-κB with E3330 impairs hemangioblast development in vitro [20]. Therefore, it appears that the essentiality of this protein is due to its pleiotropic effects rather than merely to its DNA repair activity: this implies that post-translational modifications (PTMs) and modulation of the interactome networks of APE1 may fine-tune its different biological functions. While some PTMs may have a functional role (i.e. K6/K7 acetylation by p300) [21, 22], poor information is available on APE1 protein interacting partners [23], particularly under genotoxic stress conditions. We characterized an APE1 interactome map in HeLa cells under basal conditions comprising about 20 different protein species ([7], and unpublished observations). Modulation of

this network by PTMs under different stress conditions may target APE1 towards a specific function. Our recent work is actually devoted to fill in this gap [7, 8].

APE1 structure and domain organization

The 318-amino-acid-long human APE1 protein acts as a monomer and is organized into three functionally independent domains (Fig. 1): (1) the first 33–35N-terminal unstructured sequence is essentially involved in protein–protein interaction and is important for the RNA-binding activity of the protein [7] and the modulation of its catalytic activity on abasic DNA ([24], and unpublished observations); (2) the redox domain which spans the region between amino acids 35 and 127; and (3) the DNA repair domain which spans the C-terminal domain of the protein from about residue 161 onwards.

The structure of the DNA repair domain of APE1 consists of two symmetrically related portions with similar topology and has a significant structural similarity to both bovine DNase I and its *Escherichia coli* homologue Exonuclease III. It is important to note that all information about the structure of the APE1 were retrieved by X-ray crystallography studies on a truncated form of the enzyme which lacks the first 35N-terminal amino acids, thus precluding complete understanding of the structural–functional relationships in light of the most recent findings on the very N-terminal region of the protein. 3-D structure of human APE1 shows a globular α/β protein consisting of two domains (N-terminal domain core: residues 44–136 and 295–318; C-terminal domain core: 137–260), with overall dimensions of $40 \times 45 \times 40$ Å^3. Both domains display similar topologies, and each of them comprises a six-stranded β-sheet surrounded by α-helices, which pack together to form a four-layered α/β sandwich [14].

The N-terminal domain of APE1 is responsible for redox regulation of transcription factors activities

Recent data pointing to the first 33–35 amino acid region as an independent APE1 functional and structural subdomain are discussed below; thus, in this section, the classical

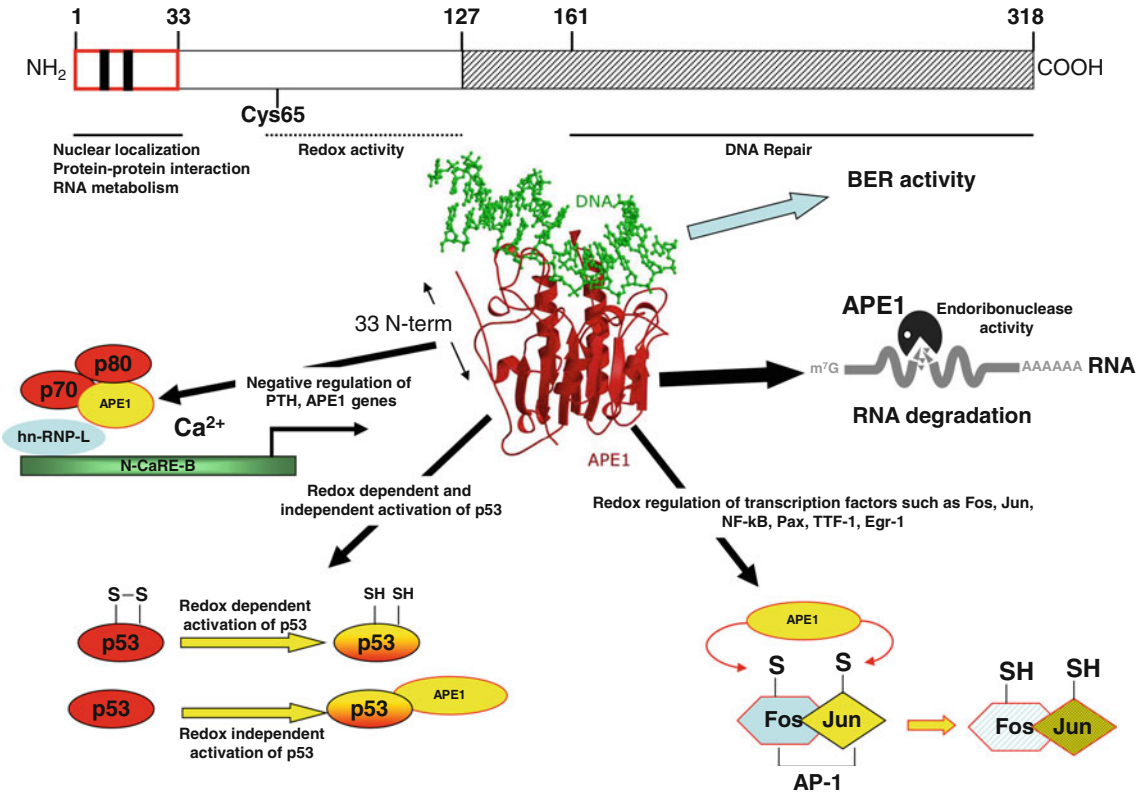

Fig. 1 Schematic organization of human APE1 structure, based on functional studies reported in Refs. [26, 46, 136], with some critical residues. Ribbon representation of the APE1 structure. An α/β-sandwich is formed by the packing of two–six-stranded β-sheets surrounded by α-helices, with strand order $\beta3$-$\beta4$-$\beta2$-$\beta1$-$\beta1$-$\beta13$ and $\beta5$-$\beta6$-$\beta7$-$\beta8$-$\beta12$-$\beta9$ from domains 1 and 2, respectively [14]. The main functions of APE1 are those involved in: (1) BER pathway of DNA lesions; (2) transcriptional repressor through binding to negative calcium responsive elements (N-CaRE); (3) redox-dependent and -independent regulation of different transcription factors activities; (4) RNA metabolism

function as redox co-activator by APE1 is examined. By using in vitro assays, it has been demonstrated that the N-terminal region comprised between residues 43 and 65 is necessary for APE1 to act in a redox mode, reductively activating oxidized proteins like p53 [25], AP-1 and Myb [26, 27]. The structural data indicate that this region forms an extended loop which lies across the β-strands β13 and β14 making a number of hydrogen bond and salt bridge interactions with the globular core of the molecule. Cys65 is implicated in the redox activity of the APE1 [27] and is located on β1 with the side chain pointing into a hydrophobic pocket and away from the central β sheet. Solvent accessibility calculations show that Cys65 is inaccessible to solvent and would therefore be unable to directly interact with residues from other proteins. Since the structure of the C65A mutated APE1 [28] appears similar to that of the wild-type protein, it is likely that the crystallized structure does not represent the molecule in a redox-active state. It was proposed that APE1 may undergo a conformational change, which involves exposure of Cys65 and creates a binding site that will accommodate the different transcription factors [28]. However, further work is required to test this hypothesis and to understand which are the factors capable of stimulating this conformational rearrangement. A redox-independent mode of action by APE1 on transcription factors activities has also been hypothesized, as in the case of p53 [25], subsequently extended to AP-1 [29].

The C-terminal domain of APE1 is responsible for the DNA repair activity

This domain harbors APE1's catalytic site responsible for AP endonuclease activity. Before protein structure was solved, some of the key residues involved in catalysis had already been determined by site directed mutagenesis studies or analysis of sequence conservation between human APE1 and EXOIII [30]. Initially, the active site structure was described by Gorman et al. [14]; further details were provided by the work of Mol et al. [31] and Beernink et al. [13]. The active site lies in a pocket at the top of the α/β sandwich and is surrounded by loop regions. The active site is defined by several residues (His309, Glu96, Asp283, Thr265, Tyr171, Asn68, Asp210, Asp70, Asn212), most of which are involved in a hydrogen bonding network. Within the active site, at least one divalent ion (Mg^{2+}) is present [32], probably bound to Glu96. APE1 orients a rigid pre-formed DNA binding face and penetrates the DNA helix from both the major and the minor grooves, stabilizing an extra-helical conformation for the target abasic deoxyribose and excluding any normal DNA nucleotide: APE1 thus seems to be preformed for abasic site recognition. APE1-bound DNA is severely distorted (bent $\approx$35°). Even if APE1 interacts with both strands of DNA, most of interactions involve the strand containing the abasic site. APE1 binds to phosphates at 5′ and 3′ of the AP site. Met270 inserts through the minor groove to pack against the orphan base. Meanwhile, Arg177 inserts through the DNA major groove and delivers a hydrogen bond to the AP site 3′ phosphate. Together, Arg177 and Met270 effectively lock APE1 onto AP-DNA. There is not a unified view concerning the hypothetical catalytic mechanism (see Refs. [13, 14, 31] for details), thus confirming that further work is needed along these lines.

APE1 is a multifunctional protein

DNA repair activity of APE1 in base excision repair

BER is a molecular pathway devoted to removal of bases with small, non-helix distorting lesions that could otherwise cause mutations during replication or lead to strand breaks in DNA molecules [33]. The importance of BER is underlined by the fact that its defects have been associated with cancer susceptibility and neurodegenerative disorders. As for the mechanism, BER funnels diverse base lesions into a common intermediate, the apurinic/apyrimidinic (AP) site, which is then handled by APE1. Subsequent steps follow either of two distinct sub-pathways, called short patch- or long patch-DNA repair [34]. In mammalian cells, APE1 hydrolyzes the 5′ phosphodiester bond of the AP site to generate a DNA intermediate that contains a single strand break with 3′-hydroxyl and deoxy-ribose-5′-phosphate (5′-dRp) termini. However, APE1 can generate a 3′-hydroxyl terminus even in other ways: APE1 can remove through its 3′–5′ exonuclease activity a 3′-phospho-α,β-unsaturated aldehyde, formed by complex glycosylases and by radiation [35]. APE1 can also remove a 3′ terminal phosphate (through its 3′ phosphatase activity), produced by glycosylases NEIL1 and NEIL2 [36]. Therefore, APE1 is the key enzyme responsible for the incision of the AP sites and the generation of a 3′-OH, which represents a primer for Polβ (for details refer to Wilson III et al. this issue). BER involves several other proteins that, even if not acting directly on DNA, can recruit other factors, coordinate them or stimulate their activities. Among these are the scaffold protein X-ray cross-species complementing 1 (XRCC1), proliferating cell nuclear antigen (PCNA), poly(ADP-ribose) polymerase (PARP-1), immediately binding to an incised AP site, recruiting other BER proteins and increasing overall process efficiency [37]. Interestingly, evidence from reconstituted systems and from cell extracts suggests that, besides providing the AP endonuclease activity, APE1 also contributes to coordination of

the different BER steps by interacting directly or indirectly with other BER enzymes and with other repair pathways. APE1 can interact with different glycosilases (OGG-1, MYH, MPG), with Polβ, with XRCC1, which in turn stimulates APE1 endonuclease activity, or with FEN1 (Flap endonuclease 1), directly stimulating its activity [2]. Due to its ability to recruit different protein factors, APE1 can be considered as one of the key elements capable of forming large DNA repair factories. The molecular basis of the specificity of these interactions, as well as their biological significance, remains to be largely determined [8].

Redox regulation of transcription factors activity by APE1

Oxidative stress represents a common threat and danger for all aerobic organisms. Any enzyme capable of metabolizing oxygen generates reactive oxygen species (ROS), such as superoxide ion ($O_2^{\cdot-}$), hydrogen peroxide (H_2O_2) or hydroxyl radical ($OH^{\cdot}$) [38]. ROS produced in vivo at levels that cannot be adequately dealt with by endogenous antioxidant systems, damage lipids, proteins, carbohydrates and nucleic acids [39], leading to functional inhibition of these molecules. Therefore, cellular redox balance is maintained by an array of enzymatic (such as catalase, peroxidases, superoxide dismutase) and non-enzymatic (such as glutathione, L-ascorbic acid, α-tocopherol) systems that ensure that an overall reducing condition prevails [2]. More recently, however, interest has focused on the ROS formation at sub-toxic levels and on their potential to act as biological signaling molecules. Sub-toxic ROS production can lead to alterations in cellular and extracellular redox state, and these alterations have been shown to signal changes in cell functions. Thiol groups, for their ability to be reversibly oxidized, may act as redox sensitive switches, thereby providing a common trigger for a variety of ROS mediated signaling events [39].

It has been demonstrated that redox regulation affects gene expression. Recently, a great body of experimental evidence suggests that this outcome is achieved through direct modulation of transcription factors activity. Up to now, several transcription factors have been demonstrated to be targets of redox regulation, which is mediated by the redox state of thiol groups exposed by critical Cys residues. APE1 has been identified as a protein capable of nuclear redox activity inducing the DNA binding activity of several transcription factors, such as AP-1 [26], NF-κB [40], Myb [41], Egr-1 [42], p53 [43], and Pax proteins [44]. APE1 accomplishes this activity through the control of the redox state of the Cys residues located either in the DNA-binding domains or within regulatory regions of the transcription factor itself [45, 46]. Therefore, by maintaining these Cys in the reduced state, through the action of its redox-sensitive Cys65, APE1 provides a redox-dependent mechanism for regulation of target gene expression.

Recently, a novel APE1 activity, termed "redox chaperone activity", was discovered, by which APE1 may regulate the DNA binding activity of various transcription factors by promoting reduction of their critical Cys residues by means of third reducing molecules, such as GSH and thioredoxin [47] (Fig. 2). The redox-chaperone activity seems to be mediated by direct interactions between APE1 and the target transcription factor, and does not require high concentrations of APE1 as does its redox activity. It is therefore plausible that APE1 may regulate redox-sensitive transcription factors essentially through this redox-chaperone activity in living cells. The authors proposed three possible mechanisms for explaining this activity. First, APE1 may facilitate reduction of target transcription factors by bridging between the target and the reducing molecule (recruitment model). Second, APE1 could facilitate reduction by inducing conformational changes of the target protein such that the redox-sensitive residues may become accessible to reducing molecules (conformational change model). Third, APE1 may stabilize the reduced states of the target transcription factors by preventing oxidation, possibly through hydrogen bond formation with the thiol groups (oxidation barrier model). These models are not mutually exclusive.

In conclusion, the redox and the redox-chaperone activities are leading functions of APE1, and may contribute to preserve the cell from the genotoxic insults due to increased ROS concentration. However, further work is needed to characterize in detail the mechanisms responsible

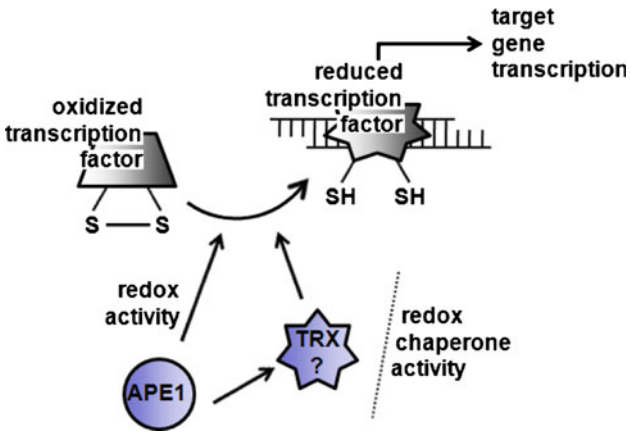

Fig. 2 Schematic representation of APE1 redox and redox-chaperone activities. APE1 is responsible for the reduction of oxidized cysteines within DNA-binding domain or regulatory domain of a number of transcription factors. The exact mechanism by which APE1 achieves this thiol reduction is not very well understood. In addition, the state of oxidation of the transcription factor in the nucleus is also poorly characterized (adapted from Ref. [38])

for targeting this APE1 activity in response to a specific stimulus.

Extra-nuclear functions of APE1: inhibition of Rac1-mediated intracellular ROS production

APE1 has an important extra-nuclear function, which is the suppression of intracellular ROS production in endothelial cells, through modulation of Rac1-regulated NADPH oxidase [48] (Fig. 3). The NADPH oxidase complex is a multisubunit enzyme involved in the innate immune response by phagocytic cells [49]. Its activation generates ROS, which are important in the host defense against infection [49]. ROS are also important modulators of vascular cell function and are generated by the NADPH oxidase complex [50] in response to a number of ligands such as angiotensin II [51], vascular endothelial growth factor (VEGF) [52], EGF [53], PDGF [54], and endothelin I [55] and to mechanical stimuli [56]. Rac1 has been shown to play a critical role in the assembly and activation of NADPH oxidase. Rac1 is a 21-kDa GTP binding protein belonging to the Ras superfamily. Rac1 cycles between an inactive GDP-bound form and, upon stimulation, an active GTP bound form. This cycle is regulated by GDP exchange factors (GEF) and GTPase activating proteins (GAP). The exact mechanism by which Rac1 is involved in the activation of the vascular NADPH oxidase remains unclear [57]; however, it seems that one of the oxidase complex subunits, p67phox, is sequestered by p40phox. Rac1 binding to p67phox could result in the disruption of p67phox/p40phox association, allowing NADPH oxidase activation [58]. Endothelial cells subjected to TNFα treatment or hypoxia/reoxygenation (H/R), respond by activating NADPH oxidase in a Rac1-dependent manner. Angkeow et al. [48] discovered that APE1 overexpression, in an endothelial cell line, prevents oxidative stress induced by TNFα or H/R, even if the molecular mechanism was not characterized. The authors hypothesized that APE1 interferes with Rac1 activation, presumably perturbing either an activation (GEF) or inactivation (GAP) process of Rac1. These results suggest once more that the control of APE1 subcellular localization is a finely regulated phenomenon and that subcellular trafficking of the protein could have a dramatic impact on cell fate [48].

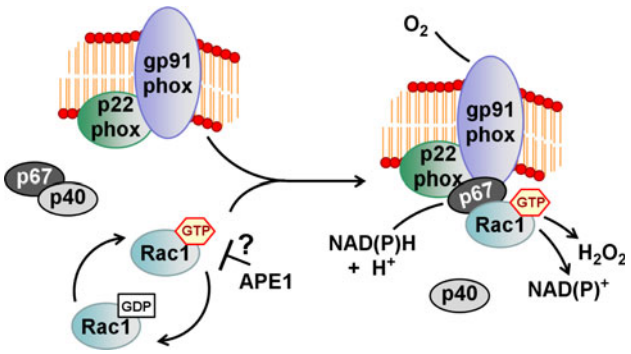

Fig. 3 Role of APE1 in intracellular ROS production control. APE1 is capable of inhibiting intracellular H_2O_2 production, through modulation of a Rac1-regulated NADPH oxidase. The molecular mechanism underlying this APE1 activity is not clear; however, a functional interaction between APE1 and Rac1 was demonstrated (adapted from Ref. [48])

NADPH oxidase, Rac1 and oxidative stress also play an important role in regulation of cell shape and motility, cell–cell contacts and cell adhesion, which are processes mediated by the rapid re-organization of the cytoskeletal actin network. In endothelial cells, for example, it has been demonstrated that actin cytoskeleton responds to an increased ROS concentration with enhanced motility and that Rac1 inhibitors can influence both ROS levels and actin cytoskeleton-based motility. Thus, by controlling Rac1-mediated ROS production, APE1 could also play a fundamental role in regulating actin network organization in endothelial cells. Further experiments are required to understand these aspects that may devise a possible role of APE1 in the tumorigenic process.

Recently, E3330, a specific inhibitor of APE1 redox activity [59], was found to be able to increase ROS levels in human pancreatic cancer PANC1 cell line [60]. The authors did not investigate the molecular mechanism; however, pancreatic cells express NADPH oxidase [61], thus it is likely that E3330 influences the pathway involving Rac1 [48]. This evidence offers at least two possible explanations. E3330 could inhibit the ability of APE1 to block Rac1-mediated NADPH oxidase activation. A second possibility is that APE1 could also exert a redox-like activity in the cytoplasm or that the Rac1 inhibition could be attributable to APE1 redox activity, therefore one of its substrates should be a factor involved in Rac1 activation/inactivation cycle. Further experiments are required to shed light on these mechanisms.

Transcriptional repressor activity by APE1 through binding to nCaRE elements

APE1 was identified as a component of a trans-acting complex with transcriptional repressor activity which binds to the negative calcium responsive elements, nCaRE-A and nCaRE-B, first discovered in the promoter of the human parathyroid hormone (PTH) gene [62], and later in the APE1 promoter itself. An increase in hematic PTH concentration mobilizes Ca^{2+} from the storage tissues [63]: high levels of extracellular calcium inhibit PTH transcription with a negative feedback mechanism which involves the binding of the trans-acting complex to the nCaRE sequences [62, 64]. More recently, other nCaRE

elements were identified on the promoter sequences of other genes, like renin [65] and APE1 itself [66]. Thus, experimental observations suggest that APE1 could down-regulate its own gene and this negative feedback mechanism would represent the first example of such a transcriptional self-regulatory mechanism for a DNA repair enzyme [5]. However, experimental results show that APE1 is not able to directly bind nCaRE elements; other protein factors are indeed required like the heterogeneous ribonucleoprotein-L (hnRNP L) [5], raising the question of how APE1 protein may represent a key factor in specifically regulating nCaRE function. More recently, Bhakat et al. [22] showed that an increase of extracellular calcium concentration can induce p300 dependent acetylation of APE1 at lysine residues 6 and 7 and that acetylated APE1 has an increased capability of binding nCaRE elements. The discovery of calcium-induced APE1 acetylation and its effect on DNA binding activity suggested that specific post-translational modifications may provide a means for challenging this multi-functional protein to different activities and interactions. Indeed, APE1 acetylation seems to be an important mechanism, capable of switching specific functions of the protein either on or off [22].

Roles of APE1 in RNA metabolism: a new paradigm

The first evidence hypothesizing the APE1 involvement in RNA metabolism dates back to 1995, when Barzilay et al. [67] demonstrated its RNase H-activity. However, their experimental data show that APE1 binds with relatively low affinity to undamaged single- and double-stranded nucleic acid substrates (RNA and DNA) but does not exhibit general nuclease activity against them.

Later on, it has been suggested that APE1 can also bind to RNA molecules in vivo: in fact, APE1 was found to be associated with ribosomes in the cytoplasm of different cell types [2]. Besides the direct interaction with RNA molecules, APE1 was also found to be able to interact with other protein factors involved in RNA metabolism, like YB-1 [68] and hnRNP-L [5]. Altogether, these observations suggest a direct involvement of APE1 in RNA metabolic pathways, but until recently it was not clear which kind of activity APE1 could exert on RNA molecules.

Very recently, by performing a number of in vitro experiments, Berquist et al. [69] proposed a possible role for APE1 in RNA metabolism. Starting from the observations that APE1 also exerts a powerful AP endonuclease activity on AP-site containing ssDNA molecules [70], they discovered the same ability on abasic ssRNA molecules, which highly depended on the molecule conformation rather than on the nucleotide sequence context. This ability implies that APE1 can remove damaged RNA templates from the endogenous pool, thus affecting, to an ultimate extent, general protein synthesis. It is known that RNA is oxidized to a greater extent than cellular DNA by treatment with H_2O_2 [71] and that oxidized RNA molecules are present in huge amounts in brains from Alzheimer patients [72]. Although the biological relevant question is whether the formation of abasic RNA molecules is a spontaneous or guided process, the results propose APE1 as an ideal candidate for a novel RNA cleansing process [7, 8, 69]. Our data, discussed later in this review, demonstrate that APE1 affects cell growth by directly acting on RNA quality control mechanisms, thus affecting gene expression through posttranscriptional mechanisms [7, 8].

Very recently, Barnes et al. [9] identified APE1 as the endonuclease capable of cleaving *c-myc* mRNA in vitro. The APE1 catalyzed cleavage occurs on a specific sequence located in a loop portion of a complex stem and loop structure and shares the same active site with the DNA-endonuclease activity of the protein, because both E96A and H309N APE1 mutants were almost inactive in cleaving the RNA template [9].

Altogether, these evidences support the existence of a direct, previously unknown, role of APE1 in RNA metabolism, presumably in RNA decay or RNA quality control pathways.

General biological role of APE1 within cells: a pro-survival factor required for cellular defense against oxidative stress and other genotoxicants

Due to the activities exerted by APE1 within mammalian cells, the protein is essential for human cells viability and for mouse embryo development [1, 11, 73].

Several experimental studies suggest that APE1 is a pro-survival factor and a reduced APE1 level usually occurs before the onset of the apoptotic process [74]; other studies showed that APE1 gene silencing directly induces apoptosis [75]. Moreover, APE1 is often overexpressed in several human tumors [2, 76, 77] leading to a reduced tumor sensitivity towards anti-neoplastic drugs, especially towards alkylating agents [78]. Even though APE1 overexpression seems to be insufficient to promote cell transformation or cell cycle control alteration, it can render cells more resistant to different genotoxic treatments [79]. Interestingly, APE1 requires both redox and endonuclease activities to act as a pro-survival factor [80].

Despite the above evidence, some authors suggested that, under some circumstances, APE1 could act as a 'permissive factor' for pro-apoptogenic signalling, by exerting its redox function over transcription factors such as p53 or Egr-1, or for cell cycle promotion, by activating transcription factors such as AP-1, NF-κB or NF-Y (for reviews, see [2, 46]). These apparently contrasting observations could be explained under the assumption that APE1

may act as a 'molecular switch', capable of integrating different signal transduction cascades and of activating either a set of pro-survival or pro-apoptotic transcription factors in response to different conditions. Indeed, as pointed out before, it is reasonable to think that APE1 could reduce and co-activate different factors with different efficiencies, and that PTMs could play a major role in determining the final outcome. Unfortunately, until now, no convincing evidence has been adduced to confirm this hypothesis.

Modulation of APE1 different functions

The regulatory functions of the different APE1 activities can be fine-tuned and implemented via three different mechanisms: (1) increase in APE1's level after transcriptional activation; (2) re-localization of APE1 from the cytoplasm to the nucleus; and (3) modulation of APE1's PTMs.

To date, no examples of editing or alternative splicing have been reported for APE1 (see, e.g., [81]). Moreover, no reports concerning the control of APE1 mRNA stability, either miRNA-mediated or miRNA-independent, are available in current scientific literature. All examples of reduction in APE1 mRNA levels can indeed be explained in terms of a transcriptional repression [82, 83].

Knowledge of PTMs affecting APE1 function has grown exponentially in the last decade and at least six different kinds have been described occurring in vivo: acetylation, phosphorylation, ubiquitination, S-nitrosation, proteolytic cleavage of the N-terminal 33 amino acids domain and redox regulation, which is at the basis of APE1 redox activity.

Together with PTMs, changes in the interactome of APE1 could target it towards a specific biological function. Information about interactome of APE1 is very limited, due to the poor sensitivity of available techniques. Moreover, interactions were mainly analyzed under basal conditions, while most interesting results are expected when APE1 interactome variations are analyzed after specific stimulations, such as during oxidative stress. To date, the only systematic attempt to characterize the APE1 interactome in HeLa cells under basal condition was performed in our laboratory and led to the identification of several novel APE1 interacting proteins [7], most of which are factors involved in RNA metabolism, suggesting this activity as a significant one in non-stressed cells.

Transcriptional regulation of APE1 gene

APE1 gene maps on chromosome 14q11.2-q12 [84] spans 2.6 kbp and consists of five exons and four small introns. The first exon is untranslated [85]. Consistent with the constitutive expression of APE1 observed in other studies, the 0.5 kbp of DNA sequence upstream of APE1 gene revealed only a possible CCAAT box and no TATA box. Like many TATA-less genes [86], APE1 displays multiple transcription start sites, which are clustered around 130 bp upstream from the first splice junction [85] (Fig. 4).

In silico analyses revealed the existence of several potential recognition sites for the glucocorticord receptor, for Sp1 and for *c-myc*-like proteins such as USF and AP-1 [2, 87]. However, AP-1 putative binding site seems not to exert any effect, as suggested by results of independent groups [87, 88]. Fung et al. [89] showed that APE1 transcription is tightly coordinated with the cell cycle: the mRNA levels begin to increase before the onset of DNA synthesis, continue to rise during the S-phase of cell cycle and depend on a specific DNA element, which can be bound by Sp1 transcription factor. APE1 promoter bears two distinct Sp1 binding sites, the first located close to the CCAAT box (upstream Sp1 site, or Sp1-1), and the second located downstream of the transcription start site (downstream Sp1 site, or Sp1-2). Sp1-1 site is involved in basal gene expression, while the Sp1-2 site is required for cell cycle coordinated APE1 gene transcription [89].

More recently, downregulation of APE1 expression by p53 has been suggested and the responsive element has been located upstream of the transcription start site and close to the CCAAT box [82]. However, no p53 binding sites were revealed by in silico analysis of this region nor the recombinant p53 protein is able to bind an oligonucleotide with the same sequence. Therefore, it was

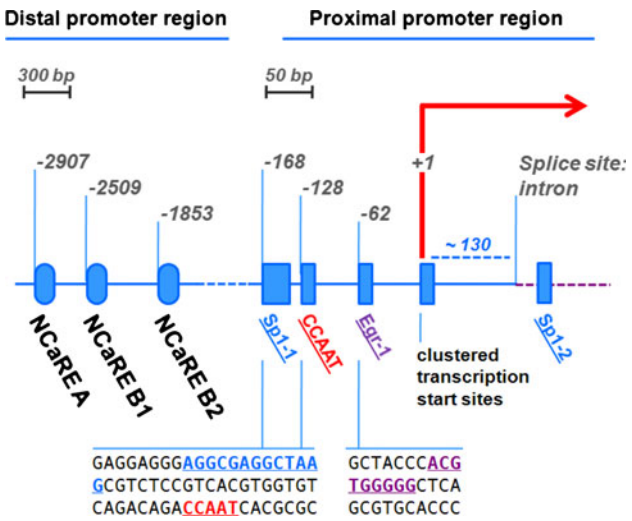

Fig. 4 Schematic representation of APE1 gene promoter region. In the distal promoter region (between −3,000 and −1,000 bp relative to the transcription start site) there are three nCaRE elements; in the proximal promoter region (between −500 and +200 relative to the transcription start site), there are two Sp1 binding elements, an Egr-1 binding site and a CCAAT box. Moreover, several other putative binding sites for other transcription factors are also present

hypothesized that p53 was indirectly recruited to this region. The exact mechanism is still not clear. However, Zaky et al. [82] suggested that p53 could physically interact with Sp1 bound to Sp1-1 element, inhibiting basal gene transcription. This study suggests an intriguing hypothesis concerning an inverse relationship between nuclear protein levels of p53 (a pro-apoptotic factor) and APE1 expression (a pro-survival factor), especially in tumors: in fact, malignancies in which p53 gene is mutated or downregulated, could be associated with an increased APE1 gene expression, and this mechanism could be at the basis of tumor resistance to chemotherapy.

The mechanisms of inducible activation of APE1 gene are well documented. Among them, cell stimulation with hormones, such as TSH [90], or upon oxidative stress.

Subtoxic doses of ROS strongly increase APE1 mRNA levels in different cell lines [79, 91, 92]. For example, Ramana et al. showed that HeLa S3 cells subjected to low doses of HOCl show an increase in APE1 mRNA levels, due to a transcriptional regulation (and not to an increased mRNA stability). Moreover, ROS-stimulated cells become more resistant to a second treatment with oxidants, thanks to APE1 increased expression. Ramana et al. [79] referred to this phenomenon as "adaptive response". Since that time, much effort has been spent in order to understand the molecular mechanisms underlying ROS-induced expression of APE1 and the onset of "adaptive response".

However, the molecular mechanism of ROS-induced transcriptional activation of APE1 is still not so clear. As NF-κB, C/EBP, AP1 and Egr-1 have been implicated in activation of several ROS-responsive genes, many attempts were carried out to discover whether any of them were also involved in ROS-induced APE1 expression.

Grosch and Kaina [93] identified a CREB binding site (CRE) within the human APE1 gene promoter, approximately 600 bp upstream of the transcription start site. By performing transfection experiments on CHO cells, they discovered that this element is required for APE1 induction by ROS. They showed that CRE is bound by a complex of ATF2 and c-Jun, which is also induced by H_2O_2.

Some years later, we demonstrated the involvement of Egr-1 in ROS inducible APE1 gene transcription [94]. We characterized a specific sequence on APE1 promoter, which could be directly bound by Egr-1. Moreover, by using a cell transfection approach in the human osteoblastic HOBIT cell line, we showed that ROS induce DNA binding activity of Egr-1, which in turn can activate APE1 promoter [94].

Even if these two reports give us some important indications about APE1-inducible transcriptional regulatory mechanisms, much work still remains to be done. In particular, the presence of *cis*-acting elements placed several kbp downstream or upstream of the transcription start site should be considered. For example, three nCaRE elements have been located 2 kbp upstream of the start site [66]. One of them is similar to the PTH gene promoter nCaRE-A element, while the others are similar to nCaRE-B element. Since APE1 was identified as one of the protein factors bound to nCaRE elements on the PTH promoter, it may also regulate its own expression by binding to these sequences, in particular to the nCaRE B2 element. Such autoregulation, if confirmed, would be the first example of such a regulatory mechanism of a mammalian DNA repair gene [66].

Post-translational modifications of APE1

PTMs of a protein represent a quick and usually reversible way to modulate its activities or functions. Concerning APE1 protein, several PTMs have been described in the last decade (refer to [46] for a comprehensive review and to Busso and Izumi in this issue) (Fig. 5). Their study is extremely important to understand which APE1 specific activity is switched on or off by the specific modification and which stimuli are eventually required. Several APE1 PTMs have been described occurring in vitro, but only a few having a function in vivo.

A detailed description of APE1 phosphorylation, S-nitrosation, acetylation and ubiquitination is extensively reported in Busso et al. (this issue). A very peculiar APE1 PTM was first evidenced in vivo by Yoshida et al. [95], who purified a 34-kDa, Mg^{2+}-dependent endonuclease, from apoptotic human promyelocytic leukemia HL-60 cells treated with etoposide. Five years later, the same group discovered that this enzyme was just a truncated form of APE1 enzyme, lacking a N-terminal portion spanning 33 amino acids (NΔ33APE1) and obtained by an unidentified trypsin-like enzyme, activated by caspase-3 during apoptosis [81].

A truncated APE1 form was also discovered during a granzyme-induced caspase-independent type of apoptosis. Granzymes are serine proteases that are released by cytotoxic T and natural killer cells and that kill virus-infected cells [96]. Granzyme A (GzmA) activates a caspase-independent cell death pathway with morphological features of apoptosis, including accumulation of single-strand breaks. Single-stranded DNA damage is initiated when the endonuclease NM23-H1 is activated, and this event takes place after GzmA has cleaved the endonuclease inhibitor, called SET. SET and NM23-H1 are part of an endoplasmic reticulum-associated complex (the SET complex), that translocates to the nucleus in response to superoxide generation by GzmA [97]. Fan et al. [98] showed that APE1 is also present in the SET complex bound to GzmA, which in turn cleaves the APE1 after Lys31. The authors concluded that this proteolytic PTM of APE1 could have a role in

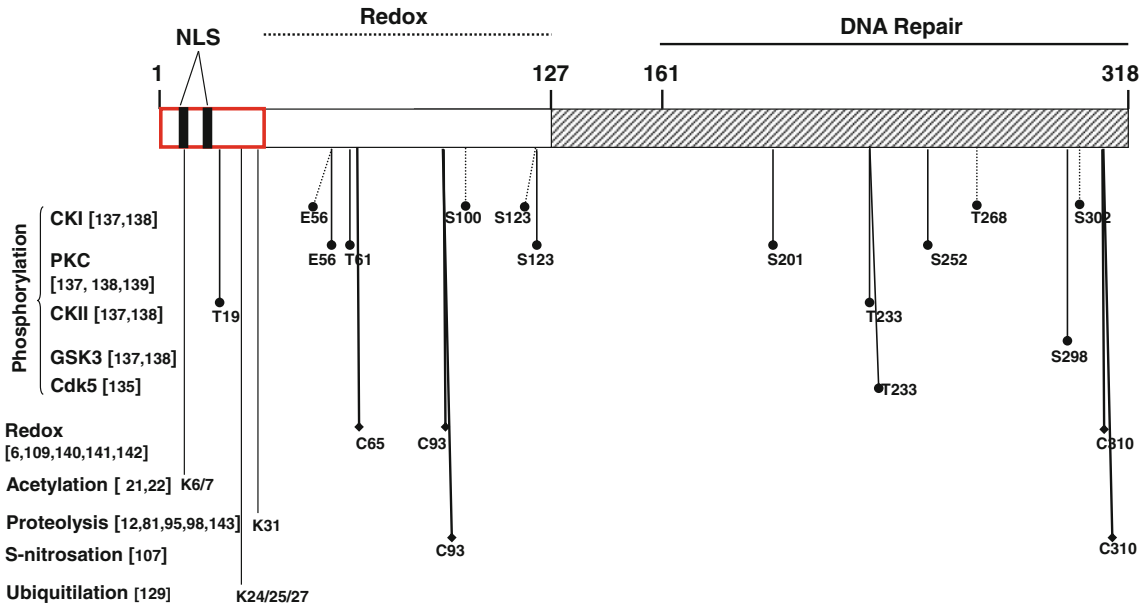

Fig. 5 Schematic representation of the PTM acceptor sites on APE1 sequence. Although several putative phosphorylation sites have been mapped, it is still unknown which are the real targets of phosphorylation in vivo except for T233 [135]. In contrast, the sites of acetylation, S-nitrosation and ubiquitination of the protein have been precisely mapped on APE1 sequence (adapted from Ref. [2])

GzmA-induced cell death [98]. An APE1 role within the SET complex in preventing HIV autointegration was also recently demonstrated [99].

Interestingly, it has been suggested that the production of the truncated APE1 protein could be induced during triggering of different cell death pathways, and could exert a nuclear function by contributing to the accumulation of single-strand breaks on DNA molecules. This is a very interesting hypothesis; however, some concerns still exist, since it is known from early studies on different APE1 deletion mutants that the NΔ33APE1 keeps both redox and AP endonuclease activities [27], without acquiring a general nuclease activity. Moreover, the truncated protein lacks both nuclear localization signals, previously identified by Jackson et al. [100]. Thus, it would be necessary to understand which molecular mechanisms could bring NΔ33APE1 to the nucleus of the apoptotic cell. More recently, truncated APE1 protein was detected in the cytoplasm of some cell types, and it was recognized as the predominant APE1 form present inside mitochondria. These observations suggested the existence of a hypothetical physiological role for the NΔ33APE1 as the AP endonuclease enzyme involved in mitochondrial BER [12]. However, other observations showed that, at least in some cell types, the prevalent mitochondrial form of APE1 is the full length one (Vascotto et al., unpublished observation; [101]), thus indicating that APE1 internalization into the mitochondria should follow a non-conventional mechanism, as recently suggested [102].

In conclusion, the proteolytic cleavage of the N-terminal portion of APE1 is a peculiar, irreversible PTM, which appears extremely intriguing. Since it has been associated with cell death processes, it could represent an important regulatory switch for APE1 functions; thus, non-physiological alterations of this PTM, could lead to anomalous control of apoptosis, such as that previously seen in some examples of human pathologies, like cancer or neurodegenerative diseases. Unfortunately, many unsolved questions remain concerning the identification of the protease, the determination of the stimuli capable of inducing such a PTM, the analysis of the cellular compartment where the cleavage takes place, and the effects of the modification on the localization and the activities of the truncated APE1 protein.

Intracellular trafficking and localization of APE1 protein

Subcellular localization of a protein plays an important role in controlling its functions. For example, segregation of an enzyme in a cellular compartment, which is different from the one where its substrate is localized, is an effective way to inhibit its function. For a multifunctional protein like APE1, which exerts both nuclear and extra-nuclear functions, as described before, it is extremely important to understand how subcellular trafficking is regulated in different cell types and under different conditions. For example, oxidant stimuli or even other signal molecules like TSH or Ca^{2+} have been shown to trigger nuclear translocation of APE1 [79, 92]. In agreement with its

endonuclease and redox functions, APE1 is a nuclear protein in many cell types. This protein presents two known independent nuclear localization signals. There is a canonical nuclear localization signal (NLS) comprising the first seven residues at the N-terminal of the protein. The second signal is defined by the amino acid stretch comprised between residues 8 and 13 [100]. Since lysine residues K6 and K7 are part of the first NLS, but are also target of acetylation by p300, Jackson et al. investigated whether this PTM could influence APE1 subcellular localization; however, they found that lysines K6 and K7 and their acetylation status do not influence nuclear import of APE1 protein [100].

It is important to again point out that APE1 can also undergo a proteolytic PTM, which generates the truncated protein NΔ33APE1 lacking both NLS sequences. This modification could represent a mechanism to prevent APE1 nuclear import. However, it is clear that deletion of the known NLS sequences does not completely abolish APE1 nuclear accumulation [7]; thus additional mechanisms should be involved in regulating APE1 subcellular distribution.

A second kind of intracellular protein trafficking is the intra-nuclear one.

From a historical perspective, the concept of nuclear subdivisions in functional compartments initially focused on the nucleolus, as the site of ribosomal gene expression, and on nuclear pores, as gates for bidirectional exchange of RNAs and proteins between the nucleus and cytoplasm. We now recognize that specific mechanisms might coordinate the spatial organization of genes, transcripts and proteins within the nucleus; moreover, there are several large specialized subnuclear domains, such as PML nuclear bodies, Cajal bodies, nucleoli, splicing speckles and paraspeckles, that have been characterized, even if their precise functions often remain elusive [103].

Thus, besides a nuclear localization signal (NLS), many nuclear proteins often expose at least another sequence, which is able to mediate interactions with other molecular partners (proteins, but also RNAs or chromatin/DNA) and target them to specific intra-nuclear compartments [104]. Intra-nuclear compartmentalization of a protein may represent a way to regulate different functions. Unfortunately, very little information concerning intra-nuclear localization of APE1 is available at present. Besides our recent work [7], just one additional report indicates that APE1 could localize within nucleoli [105]. Interestingly, two known APE1 interacting proteins involved in BER were also found in this subnuclear compartment, XRCC1 and FEN1 [106], raising the question whether the nucleolus may be a storage site for accumulation of DNA repair proteins or whether DNA repair proteins are active in this subnuclear compartment. Further work is currently ongoing in our laboratory to address this issue.

Though APE1 is a nuclear protein in many cell types, in other cell types or under some circumstances APE1 can be found prevalently in the cytoplasm [16]. Since cell treatment with leptomycin-B (specific nuclear export inhibitor) may induce a nuclear accumulation of APE1 in some of these cell types, it is likely that the protein presents a cytoplasm export signal, even though no canonical signals were detected within the APE1 sequence. A recent work by Qu et al. [107] showed that APE1 can be translocated to the cytoplasm upon S-nitrosation of Cys93 and Cys310; however, this mechanism does not explain the results obtained with leptomycin-B treatment, since it was found that S-nitrosation-mediated APE1 export was not abrogated by treatment with this drug. Moreover, also in this case, the physiological conditions capable of stimulating such a PTM are not yet known; and also, the role played by APE1 in cytoplasm still remains elusive. As discussed above, cytoplasmic APE1 is able to inhibit Rac1-induced NADPH-mediated ROS production [48]. However, other roles were hypothesized for cytoplasmic APE1. One possibility is that APE1 is maintained in the cytoplasm just to prevent its nuclear activities (DNA repair, redox regulation of transcription factors), perhaps under conditions in which cell is triggering apoptosis; a second possibility was suggested by Fan et al. [98], who showed the presence of APE1 on endoplasmic reticulum and hypothesized that APE1 could be important for keeping neo-synthesized transcription factors in a reduced and thus active state. Another more intriguing possibility is that APE1 could be present within mitochondria to preserve mitochondrial DNA (mtDNA) from oxidative damage during ROS-generated oxidative phosphorylation through mitochondrial BER [108].

In conclusion, subcellular localization of APE1 is a dynamic and very precisely regulated phenomenon [2]. However, comprehension of the mechanisms regulating APE1 intracellular trafficking is still fragmentary and requires further analyses.

APE1 interactome network

Biological processes within cells are carried out by multiprotein molecular machineries rather than by isolate enzymes; discovering the constituents of the different protein assemblies in which a specific protein of interest is integrated is an effective way to understand its role, its functions and the processes in which it is involved. By using a powerful cell model developed in our laboratory, in which endogenous APE1 was replaced by an ectopic flag-tagged protein [10], we recently characterized the APE1 interactome network under basal conditions and tested the role of the unstructured 33N-terminal domain in stabilization of this network. By an unbiased functional proteomic

approach, we have recently identified and characterized several novel APE1 protein partners which, unexpectedly, include a number of proteins involved in ribosome biogenesis and RNA processing. In particular, a novel interaction between Nucleophosmin (NPM1) and APE1 was characterized [7, 8] (Table 1). We observed that the APE1 33N-terminal residues are required for stable interaction with the NPM1 oligomerization domain and with RNA. As a consequence of the interaction with NPM1 and RNA, APE1 is localized within the nucleolus and this localization depends on cell cycle and active rDNA transcription, being predominant during the S-phase. NPM1 stimulates APE1 endonuclease activity on abasic dsDNA but decreases APE1 endonuclease activity on abasic ssRNA by masking the N-terminal region of APE1 required for stable RNA binding. In the APE1 knocked-down cells, pre-rRNA synthesis and rRNA processing were not affected, but an inability to remove 8-OHG rRNA upon oxidative stress, impaired translation, lowered intracellular protein content and decreased cell growth rate were found [7, 8]. Genotoxic stress may reduce NPM1 affinity for APE1, through modulation of the acetylation status of the above-mentioned K residues, or decrease NPM1 binding to RNA, thus freeing APE1 to exert its cleansing function on abasic RNA [7, 8]. Following APE1 cleavage, the exosome complex and XRN1 exoribonuclease may operate to degrade the resulting RNA fragments. An additional interpretation, currently under evaluation, is that NPM1 interaction within nucleoli may promote storaging of APE1. These new data may well explain the cytoplasmic accumulation of APE1 observed in different tumors [2]. Thus, we believe that modulation of the new function of APE1 in the RNA quality control process, through inhibition of its interaction with NPM1, may represent a powerful candidate for development of new anticancer drugs because it is expected that tumor cells will be more sensitive to inhibition of this activity than normal cells.

Below, we detail the main protein partners of APE1, which were identified by us (Table 1) following the described approach, or even earlier by other groups (Table 2) and using different approaches.

A detailed description of each of these interacting partners goes far beyond the aim of this review. However, some general considerations may be drawn from the analysis of the main functions exerted by the proteins listed in the tables. In particular, it is possible to cluster APE1 interacting partners in at least six different groups.

Group 1 Enzymes involved in BER or in other DNA repair pathways (downstream of APE1): this group includes all enzymes already described before, such as XRCC1, PCNA, FEN1. Also Polβ can be inserted in this group. Moreover, the group includes two protein factors (Ku70, described by Chung et al. [4], and PRP19, identified in our laboratory) involved in DNA double-strand break repair pathway.

Group 2 Enzymes or proteins capable of recognizing damaged nucleic acid molecules (DNA, RNA or both). This group includes BER glycosylases OGG1, hMYH and MPG; however, it also includes hS3 and YB-1. Interestingly, both these proteins are able to specifically bind 8-oxo-G-containing ssRNA molecules.

Group 3 Proteins involved in redox or redox-chaperone activities of APE1: the proteins belonging to this group are Trx (interaction characterized by Wei et al. [109]) and peroxiredoxin 6 (PRDX 6) (identified in our laboratory).

Group 4 Protein targets of Redox regulation mediated by APE1: even if many transcription factors were described as

Table 1 List of 10 novel proteins which can interact with APE1

	Protein	Function
1	NPM1	One of the hub proteins inside nucleolus: it may organize and interact with many other nucleolar proteins; involved in ribosomal protein assembly and transport; involved in control of centrosome duplication, and in the regulation of the tumor suppressor ARF
2	TCPA	Molecular chaperone
3	K2C8	Keratin, type II: part of the cytoskeleton
4	PRP19	DNA double-strand break (DSB) repair and pre-mRNA splicing
5	RSSA	Belongs to the ribosomal protein S2P family
6	MEP50	Component of the 20S PRMT5-containing methyltransferase complex, which modifies specific arginines to dimethylarginines in several spliceosomal Sm proteins
7	RLA0	Acidic ribosomal protein, rich in hydrophobic amino acid residues
8	PRPS1	Phosphoribosylpyrophosphate synthetase 1: catalyzes the phosphoribosylation of ribose 5-phosphate to 5-phosphoribosyl-1-pyrophosphate, which is necessary for the de-novo and salvage pathways of purine and pyrimidine biosynthesis
9	PRPS2	Phosphoribosylpyrophosphate synthetase 2: ribose metabolism
10	PRDX6	Redox regulation of several biological processes and in protection under oxidative injuries

These proteins were identified in our laboratory using an unbiased proteomic approach (adapted from [7])

Table 2 List of APE1 interacting proteins retrieved from literature

	Protein	Function	References
11	Ku70/80	Double-strand breaks repair of DNA	[4]
12	Polβ	DNA Polymerase involved in Base Excision DNA Repair	[125]
13	p53	Pro-apoptotic transcription actor; genome stability control	[43]
14	Thioredoxin	Cytoplasmic redox-sensitive signaling factor, capable of facilitating protein–nucleic acid interactions of several protein factors	[109]
15	FLAP-1	Base Excision DNA Repair	[126]
16	PCNA	Base Excision DNA Repair	[126]
17	hMYH	DNA glycosylase involved in Base Excision DNA Repair	[127]
18	XRCC1	Base Excision DNA Repair	[128]
19	hnRNP-L	Pre-mRNA splicing; role in regulating chromatin modification	[5]
20	MDM2	Main ubiquitin E3 ligase for p53	[129]
21	SET complex	Function in cells is unknown; associated mostly with the endoplasmic reticulum (ER); involved in the nuclear single-stranded DNA nicking in GZMA induced apoptosis	[98]
22	GZMA	Released by T- and NK- cells; induces a caspase-independent cell death pathway with morphological features of apoptosis	[98]
23	MPG	Methylpurine glycosylase, involved in Base Excision DNA Repair	[130]
24	HDAC	Histone deacetylases, involved in transcriptional gene repression	[22]
25	hS3	Binds to 8-oxoG residues on DNA	[131]
26	OGG1	DNA glycosylase involved in Base Excision DNA Repair	[132]
27	Rad9/Rad1 Hus1	DNA damage sensing and signaling pathways; the complex plays also a direct role in various DNA repair processes	[133]
28	YB-1	Member of the cold-shock domain (CSD) protein superfamily; it may bind Y-box elements on DNA (transcription factor); it is involved in DNA repair pathways; it may bind ssDNA, and RNA molecules as well; it protects mRNA against degradation; it binds to 8-oxo-G containing RNAs	[68]
29	SIRT-1	Class III histone deacetylase; it is a stress-response and chromatin-silencing factor, involved in various nuclear events such as transcription, DNA replication, and DNA repair	[122]
30	ERα	Transcription factor; hormonal ligand, 17β-estradiol facilitates the interaction of the receptor with estrogen-response elements (EREs) on DNA	[134]
31	Cdk5	Pro-directed Ser/Thr kinase belonging to cyclin-dependent kinase family playing a crucial role in neuronal death	[135]

A brief description of each protein is shown, together with the bibliographic reference

targets of APE1 redox activity, only a few were found to physically interact with the protein. In this group are p53 and ERα.

Group 5 Enzymes involved in APE1 modification: among them are MDM2, SIRT-1 and HDACs proteins. It would be interesting to study whether MEP50, a component of a multi-protein complex capable of modifying specific arginines to dimethylarginines in several spliceosome Sm proteins, could also be able to methylate APE1.

Group 6 A large set of proteins involved in RNA metabolism. This group shares some factors with other groups (for example, the proteins YB-1 and hS3 already described in group 2). Within this group, we can distinguish proteins involved in rRNA metabolism or in ribosome biogenesis (NPM1, RSSA, RLA0), proteins involved in pre-mRNA maturation or splicing (hnRNP L, PRP19, MEP50), and enzymes involved in general ribonucleotides metabolism and synthesis pathways (PRPS1 and PRPS2). All these newly discovered interactions of APE1 strongly suggest that its role in RNA quality control processes or, more generally, in RNA metabolism, could represent one of its major functions in mammals.

Notably, APE1 also acts as a "hub" at the transcriptome level. Through an unbiased approach, combining both gene expression array and proteomics analysis to identify genes directly or indirectly regulated by APE1 [7, 8], we recently analyzed the molecular networks in which APE1 is involved (Fig. 6). APE1 silencing induces apoptosis through mitochondrial pathway and p53 activation. Differentially expressed genes in our study strongly indicate significant perturbation of the cellular stress response signaling pathway. Proteins of the HSP70 complex are significantly

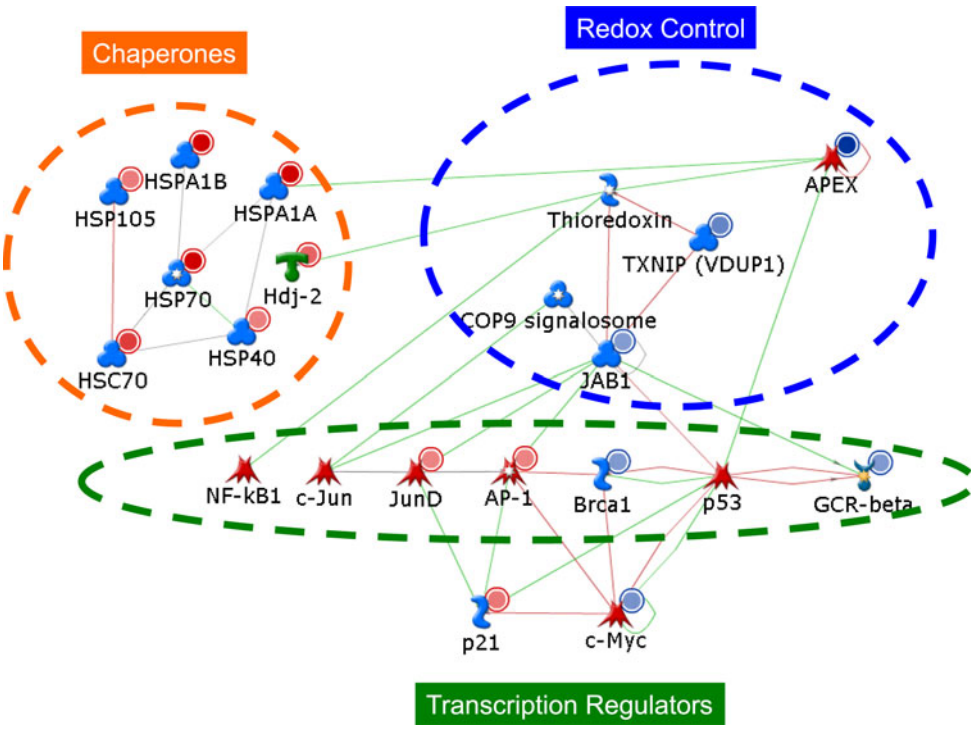

Fig. 6 Different model networks for APE1-mediated signaling as derived by gene expression and proteomic data. Direct interaction network for genes dysregulated upon APE1 knock-down, involved in redox control, stress response and in transcription factors-signaling. *Colors* of the *symbols* indicate inhibition (*blue*) and activation (*red*). Here, the name *APEX* is used to refer to APE1. This model network is accessible at this site: https://portal.genego.com/pub/network/n-860173808.html

up-regulated (HSPA1A, HSPA1B and HSPA8). The overall picture confirmed the dual-role nature of APE1, as a prototypical example of an apparent biological paradox. In fact, while a number of papers clearly demonstrated its anti-apoptotic roles as well as its positive effect on cell proliferation (for a review, see Refs. [1, 2, 11, 110]), some works underlined its potential role in controlling proapoptotic functions through p53-mediated activation [43, 111, 112] of p21, leading to cell cycle arrest by inhibiting cyclin-dependent kinase function [113], and cyclin G, having pro-apoptotic activity [114]. Thus, since the DNA repair and/or RNA-cleansing functions of APE1 seems to explain its anti-apoptotic roles [1], the APE1 function as transcriptional coactivator for different transcription factors factors (i.e. p53 and Egr-1) ensuring efficient cell cycle arrest, may act in concert with the previous (repair of DNA damage) to protect cells from accumulation of oxidative damage. Obviously, for a proper modulation of these two interconnected functions, a fine-tuning regulation of APE1 activities is required. Thus, it is mandatory that a full understanding of the processes controlling (1) APE1 subcellular distribution, (2) protein post-translational modifications, (3) protein half-life, and (4) the different interacting partners recruited as a function of cellular response, is required to fully address this paradoxical issue. Interestingly, this dual nature in transcriptional regulation and DNA repair for proteins involved in the five major DNA repair pathways, i.e. homologous recombinational repair (HRR), non-homologous end joining (NHEJ), nucleotide excision repair (NER), base excision repair (BER) and mismatch repair (MMR), is generally shared, as in the cases of BRCA1, ATM and p53 itself (for review, see Ref. [115]). The existence and correct regulation of a switching mechanism shifting cells from DNA repair to apoptosis is central to avoid progression to cancer, preventing clonal expansion of cells in which unrepaired damage would lead to mutation and to carcinogenesis. In this regard, it is interesting to note that a number of DNA repair genes (i.e. GADD45, BRCA1) were down-regulated as a consequence of APE1 silencing, thus suggesting the existence of a cross-regulation, in terms of expression, between individual partners of different pathways and underlining the leading role of APE1 in DNA repair processes with a different role besides the well-known AP-endonuclease activity. This hypothesis will require further investigation in the future.

APE1 in human pathologies and as a potential new pharmacological target

As discussed before, APE1 is a vital protein for mammalian cells [10, 11, 73]. The reason is the abrogation of both main activities of APE1 (AP endonuclease and redox activities) [80], and probably the abolition of more recently discovered functions, such as that on RNA metabolism. Thus, its involvement in human pathologies is not unexpected. Oxidative stress is, for example, a common feature

of several degenerative disorders, including neurodegenerative diseases such as Alzheimer disease (AD), Parkinson disease (PD) or amyotrophic lateral sclerosis (ALS) and of cancer development. Alteration of APE1 regulation is, in particular, a well-established common feature in different neoplastic diseases [2]. In many tumors, APE1 is overexpressed; however, this could often be easily explained as an alteration of transcriptional regulation of APE1 gene, due to inactivation of p53 or altered SP1 expression. In these tumors, increased nuclear levels of APE1 usually correlate with reduced sensitivity to antineoplastic drugs, especially alkylating agents [78].

However, even fewer evident alterations of APE1 regulation, such as a perturbation in specific PTMs regulatory mechanisms, like an anomalous intracellular localization, could be correlated with human cancer. Generally, APE1 localization is eminently nuclear while in several carcinomas a nuclear, cytoplasmic, and nuclear/cytoplasmic staining were observed (for a comprehensive review, see Ref. [2]). This peculiar broader distribution correlates well with the aggressiveness and prognosis of the tumor, as nuclear localization was always associated with a better prognostic feature together with a higher degree of cellular differentiation, low angiogenesis and negative lymph node status. Because in hepatocellular carcinoma (HCC), a cytoplasmic localization of APE1 has been associated with a significantly lower degree of differentiation and with a shorter survival time, the localization of APE1 in liver biopsy is of prognostic value [116]. Interestingly, in normal cell types characterized by high metabolic rates, like Leydig cells [117], APE1 is also prevalently localized within cytoplasm. The reasons of this unusual localization is still not clear; presumably those cell types depend heavily on a extra-nuclear function of APE1 for their survival, and perhaps mitochondrial functions of APE1 could be involved.

Therefore, APE1 also represents a promising target for pharmacological treatment in some cancer types. An ideal pharmacological target should be a biomolecule expressed only in the pathological tissue, and should be involved in the pathogenesis of the disease, so that, by its blockage, it would be possible to also inhibit cancer growth. APE1, as a whole, is not an ideal target, because it is expressed in all human tissues and is essential for all mammalian cells. However, it could still be considered as an ideal pharmacological target in specific kinds of cancers, provided that the specific activity required for that cancer cell is known. On this basis, development of small compounds specifically targeting the DNA repair rather than the redox activity of the protein is growing more and more (refer to Wilson III and Simeonov, this issue, for details).

In solid tumors, characterized by high levels of oxidative stress and increased nuclear APE1 levels, APE1 redox function could be crucial for maintaining pro-survival transcription factors in a reduced, active state [2]. In these cases, inhibition of redox function of APE1 could compromise neoplastic cell viability, while normal cells, which are not usually subjected to high levels of oxidative stress, could be unaffected by the treatment. Also, in other kinds of tumors, such as those showing a higher cytoplasmic than nuclear APE1 staining, the inhibition of extra-nuclear activities could kill neoplastic cells in a very specific way.

Since APE1 extra-nuclear activities are not yet very well understood and characterized, most of the research concerning compounds able to inhibit APE1 functions is focused on the discovery of molecules capable of targeting its redox or DNA repair activities. Inhibitors could be used alone or, more frequently, in combination with a conventional chemotherapeutic agent, such as bleomycin, temozolomide or gemcitabine [118], to enhance the cytotoxic effect.

Though this is a novel field in APE1 research, a few compounds have already been reported, such as APE1 redox inhibitors [119]; moreover, results obtained so far suggest that at least one of these compounds could be a promising lead drug. Soy isoflavones, a component of soybeans, have been investigated for their potential as chemopreventive agents in prostate cancer [120]. Though it is not clear whether APE1 redox activity is the only activity affected by these molecules, it is clear that they block the redox signaling through APE1 and NF-κB, dramatically increasing prostate cancer cells' sensitivity to radiotherapy.

Resveratrol is another natural occurring compound, with reported effects on the redox activity of APE1 [121]. Treating cells with resveratrol does not impair APE1 DNA repair activity; Yang et al. [121] claimed a reduction of APE1 redox activity, but other groups [59] were not able to confirm these observations. Thus, further experiments investigating the efficacy of resveratrol are required. Moreover, since resveratrol could increase the activity of class III HDAC SIRT1, a deacetylase acting on APE1 [122], the existence of indirect effects of this compound mediated by APE1 cannot be excluded.

E3330 is a quinone-derivative compound having clinical potential as a redox inhibitor of APE1. Previous studies demonstrated that E3330 selectively inhibits NF-κB-mediated gene expression without affecting degradation of IκB alpha. Within APE1, there are three E3330-binding sites, and all these are located within the redox domain [123]. E3330 was found to be a specific inhibitor of APE1 redox function, without interfering with AP endonuclease activity [59]. Moreover, binding constant between APE1 and E3330 was estimated, and confirmed that the drug can specifically interact with its target [123]. E3330 was found to inhibit growth and migration of human pancreatic cancer cells, while not interfering with the growth of human

pancreatic epithelial cells [60]. However, tests with E3330 showed an important side effect: the compound inhibits hematopoiesis [20]. This side effect may limit its potential use as an anticancer agent in pancreatic cancer. However, a combination of E3330 and hematopoietic stimulatory factors could represent a promising implementation of the therapeutical protocol.

An extensive review of novel inhibitors of APE1 DNA repair activity is by Simeonov and Wilson in this issue.

In conclusion, APE1 biological importance is reflected by its involvement in cancer, and, for this reason, therapeutical strategies aiming at specific inhibition of different APE1 activities seem to be very promising.

Conclusions and future directions

Recent evidence clearly shows that bifunctional therapy, including DNA damaging agents (such as treatment with alkylating agents, ionizing radiation or radiomimetics treatment) and inhibition of DNA repair enzymes activities, may represent a promising direction in the field of cancer treatment [124]. Therefore, knowledge of the molecular mechanisms regulating key enzymes in DNA repair is of fundamental importance for developing new anticancer strategies. APE1 is a central enzyme in BER and a multifunctional protein with both gene expression regulatory and redox capabilities within eukaryotic cells. In addition to basic research aimed at the understanding the molecular mechanisms responsible for fine-tuning the different APE1 functions, development of small molecule inhibitors of each function will be necessary to ultimately conclude which activity is required in normal and cancer cell function. Recent findings on redox inhibition of APE1 have potential clinical translational significance such that a redox inhibitor could be used as a single agent, in combination with current treatments or as a potential anti-growth, cytostatic agent. To this aim, we are currently working on identification of a panel of small molecules able to specifically inhibit APE1/NPM1 interaction as an effective tool for mechanistic investigations and as a potential starting point for development of therapeutic agents.

Obviously, for a proper modulation of different interconnected functions of multifunctional proteins as APE1, a fine-tuned regulation of APE1 activities is required. Thus, a better understanding of the processes controlling APE1 subcellular distribution, of the post-translational modifications occurring on the protein itself, of the mechanisms controlling protein half-life, and of the different interacting partners recruited as a function of cellular response, is required to fully address this complex issue for translating this unique protein from benchtop to bedside.

Acknowledgments We thank Dr. Mark R. Kelley for helpful discussions. This work was supported by grants from: MIUR (FIRB RBRN07BMCT_008 and PRIN 2008CCPKRP_003) and from MAE (Joint Mobility Projects Program 2008–2010) to G.T.

References

1. Fung H, Demple B (2005) A vital role for Ape1/Ref1 protein in repairing spontaneous DNA damage in human cells. Mol Cell 17:463–470
2. Tell G, Damante G, Caldwell D, Kelley MR (2005) The intracellular localization of APE1/Ref-1: more than a passive phenomenon? Antioxid Redox Signal 7:367–384
3. O'Hara AM, Bhattacharyya A, Bai J, Mifflin RC, Ernst PB, Mitra S, Crowe SE (2009) Tumor necrosis factor (TNF)-alpha-induced IL-8 expression in gastric epithelial cells: role of reactive oxygen species and AP endonuclease-1/redox factor (Ref)-1. Cytokine 46:359–369
4. Chung U, Igarashi T, Nishishita T, Iwanari H, Iwamatsu A, Suwa A, Mimori T, Hata K, Ebisu S, Ogata E, Fujita T, Okazaki T (1996) The interaction between Ku antigen and REF1 protein mediates negative gene regulation by extracellular calcium. J Biol Chem 271:8593–8598
5. Kuninger DT, Izumi T, Papaconstantinou J, Mitra S (2002) Human AP-endonuclease 1 and hnRNP-L interact with a nCaRE-like repressor element in the AP-endonuclease 1 promoter. Nucleic Acids Res 30:823–829
6. Xanthoudakis S, Miao GG, Curran T (1994) The redox and DNA-repair activities of Ref-1 are encoded by nonoverlapping domains. Proc Natl Acad Sci USA 91:23–27
7. Vascotto C, Fantini D, Romanello M, Cesaratto L, Deganuto M, Leonardi A, Radicella JP, Kelley MR, D'Ambrosio C, Scaloni A, Quadrifoglio F, Tell G (2009) APE1/Ref-1 interacts with NPM1 within nucleoli and plays a role in the rRNA quality control process. Mol Cell Biol 29:1834–1854
8. Tell G, Wilson DM III, Lee CH (2010) Intrusion of a DNA repair protein in the RNome world: is this the beginning of a new era? Mol Cell Biol 30:366–371
9. Barnes T, Kim WC, Mantha AK, Kim SE, Izumi T, Mitra S, Lee CH (2009) Identification of Apurinic/apyrimidinic endonuclease 1 (APE1) as the endoribonuclease that cleaves c-myc mRNA. Nucleic Acids Res 37:3946–3958
10. Vascotto C, Cesaratto L, Zeef LA, Deganuto M, D'Ambrosio C, Scaloni A, Romanello M, Damante G, Taglialatela G, Delneri D, Kelley MR, Mitra S, Quadrifoglio F, Tell G (2009) Genome-wide analysis and proteomic studies reveal APE1/Ref-1 multifunctional role in mammalian cells. Proteomics 9:1058–1074
11. Izumi T, Brown DB, Naidu CV, Bhakat KK, Macinnes MA, Saito H, Chen DJ, Mitra S (2005) Two essential but distinct functions of the mammalian abasic endonuclease. Proc Natl Acad Sci USA 102:5739–5743
12. Chattopadhyay R, Wiederhold L, Szczesny B, Boldogh I, Hazra TK, Izumi T, Mitra S (2006) Identification and characterization of mitochondrial abasic (AP)-endonuclease in mammalian cells. Nucleic Acids Res 34:2067–2076
13. Beernink PT, Segelke BW, Hadi MZ, Erzberger JP, Wilson DM III, Rupp B (2001) Two divalent metal ions in the active site of a new crystal form of human apurinic/apyrimidinic endonuclease, Ape1: implications for the catalytic mechanism. J Mol Biol 307:1023–1034
14. Gorman MA, Morera S, Rothwell DG, de La Fortelle E, Mol CD, Tainer JA, Hickson ID, Freemont PS (1997) The crystal structure of the human DNA repair endonuclease HAP1 suggests the recognition of extra-helical deoxyribose at DNA abasic sites. EMBO J 16:6548–6558

15. Takeuchi R, Ruike T, Nakamura R, Shimanouchi K, Kanai Y, Abe Y, Ihara A, Sakaguchi K (2006) Drosophila DNA polymerase zeta interacts with recombination repair protein 1, the Drosophila homologue of human abasic endonuclease 1. J Biol Chem 281:11577–11585
16. Kakolyris S, Kaklamanis L, Giatromanolaki A, Koukourakis M, Hickson ID, Barzilay G, Turley H, Leek RD, Kanavaros P, Georgoulias V, Gatter KC, Harris AL (1998) Expression and subcellular localization of human AP endonuclease 1 (HAP1/Ref-1) protein: a basis for its role in human disease. Histopathology 33:561–569
17. Bobola MS, Blank A, Berger MS, Stevens BA, Silber JR (2001) Apurinic/apyrimidinic endonuclease activity is elevated in human adult gliomas. Clin Cancer Res 7:3510–3518
18. Robertson KA, Bullock HA, Xu Y, Tritt R, Zimmerman E, Ulbright TM, Foster RS, Einhorn LH, Kelley MR (2001) Altered expression of Ape1/ref-1 in germ cell tumors and overexpression in NT2 cells confers resistance to bleomycin and radiation. Cancer Res 61:2220–2225
19. Rossi O, Carrozzino F, Cappelli E, Carli F, Frosina G (2000) Analysis of repair of abasic sites in early onset breast cancer patients. Int J Cancer 85:21–26
20. Zou GM, Luo MH, Reed A, Kelley MR, Yoder MC (2007) Ape1 regulates hematopoietic differentiation of embryonic stem cells through its redox functional domain. Blood 109:1917–1922
21. Fantini D, Vascotto C, Deganuto M, Bivi N, Gustincich S, Marcon G, Quadrifoglio F, Damante G, Bhakat KK, Mitra S, Tell G (2008) APE1/Ref-1 regulates PTEN expression mediated by Egr-1. Free Radic Res 42:20–29
22. Bhakat KK, Izumi T, Yang SH, Hazra TK, Mitra S (2003) Role of acetylated human AP-endonuclease (APE1/Ref-1) in regulation of the parathyroid hormone gene. EMBO J 22:6299–6309
23. Parlanti E, Locatelli G, Maga G, Dogliotti E (2007) Human base excision repair complex is physically associated to DNA replication and cell cycle regulatory proteins. Nucleic Acids Res 35:1569–1577
24. Yu E, Gaucher SP, Hadi MZ (2010) Probing conformational changes in Ape1 during the progression of base excision repair. Biochemistry 49:3786–3796
25. Jayaraman L, Murthy KG, Zhu C, Curran T, Xanthoudakis S, Prives C (1997) Identification of redox/repair protein Ref-1 as a potent activator of p53. Genes Dev 11:558–570
26. Xanthoudakis S, Curran T (1992) Identification and characterization of Ref-1, a nuclear protein that facilitates AP-1 DNA-binding activity. EMBO J 11:653–665
27. Walker LJ, Robson CN, Black E, Gillespie D, Hickson ID (1993) Identification of residues in the human DNA repair enzyme HAP1 (Ref-1) that are essential for redox regulation of Jun DNA binding. Mol Cell Biol 13:5370–5376
28. Georgiadis MM, Luo M, Gaur RK, Delaplane S, Li X, Kelley MR (2008) Evolution of the redox function in mammalian apurinic/apyrimidinic endonuclease. Mutat Res 643:54–63
29. Ordway JM, Eberhart D, Curran T (2003) Cysteine 64 of Ref-1 is not essential for redox regulation of AP-1 DNA binding. Mol Cell Biol 23:4257–4266
30. Rothwell DG, Hickson ID (1996) Asparagine 212 is essential for abasic site recognition by the human DNA repair endonuclease HAP1. Nucleic Acids Res 24:4217–4221
31. Mol CD, Izumi T, Mitra S, Tainer JA (2000) DNA-bound structures and mutants reveal abasic DNA binding by APE1 and DNA repair coordination [corrected]. Nature 403:451–456
32. Barzilay G, Mol CD, Robson CN, Walker LJ, Cunningham RP, Tainer JA, Hickson ID (1995) Identification of critical active-site residues in the multifunctional human DNA repair enzyme HAP1. Nat Struct Biol 2:561–568
33. Hegde ML, Hazra TK, Mitra S (2008) Early steps in the DNA base excision/single-strand interruption repair pathway in mammalian cells. Cell Res 18:27–47
34. Fortini P, Pascucci B, Parlanti E, D'Errico M, Simonelli V, Dogliotti E (2003) The base excision repair: mechanisms and its relevance for cancer susceptibility. Biochimie 85:1053–1071
35. Flaherty DM, Monick MM, Carter AB, Peterson MW, Hunninghake GW (2002) Oxidant-mediated increases in redox factor-1 nuclear protein and activator protein-1 DNA binding in asbestos-treated macrophages. J Immunol 168:5675–5681
36. Izumi T, Wiederhold LR, Roy G, Roy R, Jaiswal A, Bhakat KK, Mitra S, Hazra TK (2003) Mammalian DNA base excision repair proteins: their interactions and role in repair of oxidative DNA damage. Toxicology 193:43–65
37. Caldecott KW, Aoufouchi S, Johnson P, Shall S (1996) XRCC1 polypeptide interacts with DNA polymerase beta and possibly poly (ADP-ribose) polymerase, and DNA ligase III is a novel molecular 'nick-sensor' in vitro. Nucleic Acids Res 24:4387–4394
38. Liu H, Colavitti R, Rovira II, Finkel T (2005) Redox-dependent transcriptional regulation. Circ Res 97:967–974
39. Moran LK, Gutteridge JM, Quinlan GJ (2001) Thiols in cellular redox signalling and control. Curr Med Chem 8:763–772
40. Nishi T, Shimizu N, Hiramoto M, Sato I, Yamaguchi Y, Hasegawa M, Aizawa S, Tanaka H, Kataoka K, Watanabe H, Handa H (2002) Spatial redox regulation of a critical cysteine residue of NF-kappa B in vivo. J Biol Chem 277:44548–44556
41. Xanthoudakis S, Miao G, Wang F, Pan YC, Curran T (1992) Redox activation of Fos-Jun DNA binding activity is mediated by a DNA repair enzyme. EMBO J 11:3323–3335
42. Huang RP, Adamson ED (1993) Characterization of the DNA-binding properties of the early growth response-1 (Egr-1) transcription factor: evidence for modulation by a redox mechanism. DNA Cell Biol 12:265–273
43. Gaiddon C, Moorthy NC, Prives C (1999) Ref-1 regulates the transactivation and pro-apoptotic functions of p53 in vivo. EMBO J 18:5609–5621
44. Tell G, Pellizzari L, Cimarosti D, Pucillo C, Damante G (1998) Ref-1 controls pax-8 DNA-binding activity. Biochem Biophys Res Commun 252:178–183
45. Tell G, Pines A, Paron I, D'Elia A, Bisca A, Kelley MR, Manzini G, Damante G (2002) Redox effector factor-1 regulates the activity of thyroid transcription factor 1 by controlling the redox state of the N transcriptional activation domain. J Biol Chem 277:14564–14574
46. Tell G, Quadrifoglio F, Tiribelli C, Kelley MR (2009) The many functions of APE1/Ref-1: not only a DNA repair enzyme. Antioxid Redox Signal 11:601–620
47. Ando K, Hirao S, Kabe Y, Ogura Y, Sato I, Yamaguchi Y, Wada T, Handa H (2008) A new APE1/Ref-1-dependent pathway leading to reduction of NF-kappaB and AP-1, and activation of their DNA-binding activity. Nucleic Acids Res 36:4327–4336
48. Angkeow P, Deshpande SS, Qi B, Liu YX, Park YC, Jeon BH, Ozaki M, Irani K (2002) Redox factor-1: an extra-nuclear role in the regulation of endothelial oxidative stress and apoptosis. Cell Death Differ 9:717–725
49. Bokoch GM, Diebold BA (2002) Current molecular models for NADPH oxidase regulation by Rac GTPase. Blood 100:2692–2696
50. Mohazzab KM, Wolin MS (1994) Properties of a superoxide anion-generating microsomal NADH oxidoreductase, a potential pulmonary artery PO2 sensor. Am J Physiol 267:L823–L831
51. Griendling KK, Sorescu D, Lassegue B, Ushio-Fukai M (2000) Modulation of protein kinase activity and gene expression by reactive oxygen species and their role in vascular physiology

and pathophysiology. Arterioscler Thromb Vasc Biol 20: 2175–2183
52. Ushio-Fukai M, Alexander RW (2004) Reactive oxygen species as mediators of angiogenesis signaling: role of NAD(P)H oxidase. Mol Cell Biochem 264:85–97
53. Wassmann S, Laufs U, Muller K, Konkol C, Ahlbory K, Baumer AT, Linz W, Bohm M, Nickenig G (2002) Cellular antioxidant effects of atorvastatin in vitro and in vivo. Arterioscler Thromb Vasc Biol 22:300–305
54. Marumo T, Schini-Kerth VB, Fisslthaler B, Busse R (1997) Platelet-derived growth factor-stimulated superoxide anion production modulates activation of transcription factor NF-kappaB and expression of monocyte chemoattractant protein 1 in human aortic smooth muscle cells. Circulation 96:2361–2367
55. Duerrschmidt N, Wippich N, Goettsch W, Broemme HJ, Morawietz H (2000) Endothelin-1 induces NAD(P)H oxidase in human endothelial cells. Biochem Biophys Res Commun 269:713–717
56. Hwang J, Saha A, Boo YC, Sorescu GP, McNally JS, Holland SM, Dikalov S, Giddens DP, Griendling KK, Harrison DG, Jo H (2003) Oscillatory shear stress stimulates endothelial production of O2- from p47phox-dependent NAD(P)H oxidases, leading to monocyte adhesion. J Biol Chem 278:47291–47298
57. Patil S, Bunderson M, Wilham J, Black SM (2004) Important role for Rac1 in regulating reactive oxygen species generation and pulmonary arterial smooth muscle cell growth. Am J Physiol Lung Cell Mol Physiol 287:L1314–L1322
58. Rinckel LA, Faris SL, Hitt ND, Kleinberg ME (1999) Rac1 disrupts p67phox/p40phox binding: a novel role for Rac in NADPH oxidase activation. Biochem Biophys Res Commun 263:118–122
59. Fishel ML, Kelley MR (2007) The DNA base excision repair protein Ape1/Ref-1 as a therapeutic and chemopreventive target. Mol Aspects Med 28:375–395
60. Zou GM, Maitra A (2008) Small-molecule inhibitor of the AP endonuclease 1/REF-1 E3330 inhibits pancreatic cancer cell growth and migration. Mol Cancer Ther 7:2012–2021
61. Lee JK, Edderkaoui M, Truong P, Ohno I, Jang KT, Berti A, Pandol SJ, Gukovskaya AS (2007) NADPH oxidase promotes pancreatic cancer cell survival via inhibiting JAK2 dephosphorylation by tyrosine phosphatases. Gastroenterology 133:1637–1648
62. Okazaki T, Chung U, Nishishita T, Ebisu S, Usuda S, Mishiro S, Xanthoudakis S, Igarashi T, Ogata E (1994) A redox factor protein, ref1, is involved in negative gene regulation by extracellular calcium. J Biol Chem 269:27855–27862
63. Mallette LE (1989) Regulation of blood calcium in humans. Endocrinol Metab Clin North Am 18:601–610
64. Yamamoto M, Igarashi T, Muramatsu M, Fukagawa M, Motokura T, Ogata E (1989) Hypocalcemia increases and hypercalcemia decreases the steady-state level of parathyroid hormone messenger RNA in the rat. J Clin Invest 83:1053–1056
65. Fuchs S, Philippe J, Corvol P, Pinet F (2003) Implication of Ref-1 in the repression of renin gene transcription by intracellular calcium. J Hypertens 21:327–335
66. Izumi T, Henner WD, Mitra S (1996) Negative regulation of the major human AP-endonuclease, a multifunctional protein. Biochemistry 35:14679–14683
67. Barzilay G, Walker LJ, Robson CN, Hickson ID (1995) Site-directed mutagenesis of the human DNA repair enzyme HAP1: identification of residues important for AP endonuclease and RNase H activity. Nucleic Acids Res 23:1544–1550
68. Chattopadhyay R, Das S, Maiti AK, Boldogh I, Xie J, Hazra TK, Kohno K, Mitra S, Bhakat KK (2008) Regulatory role of human AP-endonuclease (APE1/Ref-1) in YB-1-mediated activation of the multidrug resistance gene MDR1. Mol Cell Biol 28:7066–7080
69. Berquist BR, McNeill DR, Wilson DM III (2008) Characterization of abasic endonuclease activity of human Ape1 on alternative substrates, as well as effects of ATP and sequence context on AP site incision. J Mol Biol 379:17–27
70. Marenstein DR, Wilson DM III, Teebor GW (2004) Human AP endonuclease (APE1) demonstrates endonucleolytic activity against AP sites in single-stranded DNA. DNA Repair (Amst) 3:527–533
71. Hofer T, Badouard C, Bajak E, Ravanat JL, Mattsson A, Cotgreave IA (2005) Hydrogen peroxide causes greater oxidation in cellular RNA than in DNA. Biol Chem 386:333–337
72. Shan X, Chang Y, Lin CL (2007) Messenger RNA oxidation is an early event preceding cell death and causes reduced protein expression. FASEB J 21:2753–2764
73. Xanthoudakis S, Smeyne RJ, Wallace JD, Curran T (1996) The redox/DNA repair protein, Ref-1, is essential for early embryonic development in mice. Proc Natl Acad Sci USA 93:8919–8923
74. Fujimura M, Morita-Fujimura Y, Kawase M, Chan PH (1999) Early decrease of apurinic/apyrimidinic endonuclease expression after transient focal cerebral ischemia in mice. J Cereb Blood Flow Metab 19:495–501
75. Guan Z, Basi D, Li Q, Mariash A, Xia YF, Geng JG, Kao E, Hall JL (2005) Loss of redox factor 1 decreases NF-kappaB activity and increases susceptibility of endothelial cells to apoptosis. Arterioscler Thromb Vasc Biol 25:96–101
76. Evans AR, Limp-Foster M, Kelley MR (2000) Going APE over ref-1. Mutat Res 461:83–108
77. Al-Attar A, Gossage L, Fareed KR, Shehata M, Mohammed M, Zaitoun AM, Soomro I, Lobo DN, Abbotts R, Chan S, Madhusudan S (2010) Human apurinic/apyrimidinic endonuclease (APE1) is a prognostic factor in ovarian, gastro-oesophageal and pancreatico-biliary cancers. Br J Cancer 102: 704–709
78. Silber JR, Bobola MS, Blank A, Schoeler KD, Haroldson PD, Huynh MB, Kolstoe DD (2002) The apurinic/apyrimidinic endonuclease activity of Ape1/Ref-1 contributes to human glioma cell resistance to alkylating agents and is elevated by oxidative stress. Clin Cancer Res 8:3008–3018
79. Ramana CV, Boldogh I, Izumi T, Mitra S (1998) Activation of apurinic/apyrimidinic endonuclease in human cells by reactive oxygen species and its correlation with their adaptive response to genotoxicity of free radicals. Proc Natl Acad Sci USA 95:5061–5066
80. Chiarini LB, Freitas FG, Petrs-Silva H, Linden R (2000) Evidence that the bifunctional redox factor/AP endonuclease Ref-1 is an anti-apoptotic protein associated with differentiation in the developing retina. Cell Death Differ 7:272–281
81. Yoshida A, Urasaki Y, Waltham M, Bergman AC, Pourquier P, Rothwell DG, Inuzuka M, Weinstein JN, Ueda T, Appella E, Hickson ID, Pommier Y (2003) Human apurinic/apyrimidinic endonuclease (Ape1) and its N-terminal truncated form (AN34) are involved in DNA fragmentation during apoptosis. J Biol Chem 278:37768–37776
82. Zaky A, Busso C, Izumi T, Chattopadhyay R, Bassiouny A, Mitra S, Bhakat KK (2008) Regulation of the human AP-endonuclease (APE1/Ref-1) expression by the tumor suppressor p53 in response to DNA damage. Nucleic Acids Res 36:1555–1566
83. Heo JY, Jing K, Song KS, Seo KS, Park JH, Kim JS, Jung YJ, Hur GM, Jo DY, Kweon GR, Yoon WH, Lim K, Hwang BD, Jeon BH, Park JI (2009) Downregulation of APE1/Ref-1 is involved in the senescence of mesenchymal stem cells. Stem Cells 27:1455–1462
84. Robson CN, Hochhauser D, Craig R, Rack K, Buckle VJ, Hickson ID (1992) Structure of the human DNA repair gene

HAP1 and its localisation to chromosome 14q 11.2–12. Nucleic Acids Res 20:4417–4421
85. Harrison L, Ascione G, Menninger JC, Ward DC, Demple B (1992) Human apurinic endonuclease gene (APE): structure and genomic mapping (chromosome 14q11.2–12). Hum Mol Genet 1:677–680
86. Konecki DS, Wang Y, Trefz FK, Lichter-Konecki U, Woo SL (1992) Structural characterization of the 5′ regions of the human phenylalanine hydroxylase gene. Biochemistry 31:8363–8368
87. Harrison L, Ascione AG, Wilson DM III, Demple B (1995) Characterization of the promoter region of the human apurinic endonuclease gene (APE). J Biol Chem 270:5556–5564
88. Rivkees SA, Kelley MR (1994) Expression of a multifunctional DNA repair enzyme gene, apurinic/apyrimidinic endonuclease (APE; Ref-1) in the suprachiasmatic, supraoptic and paraventricular nuclei. Brain Res 666:137–142
89. Fung H, Bennett RA, Demple B (2001) Key role of a downstream specificity protein 1 site in cell cycle-regulated transcription of the AP endonuclease gene APE1/APEX in NIH3T3 cells. J Biol Chem 276:42011–42017
90. Asai T, Kambe F, Kikumori T, Seo H (1997) Increase in Ref-1 mRNA and protein by thyrotropin in rat thyroid FRTL-5 cells. Biochem Biophys Res Commun 236:71–74
91. Grosch S, Fritz G, Kaina B (1998) Apurinic endonuclease (Ref-1) is induced in mammalian cells by oxidative stress and involved in clastogenic adaptation. Cancer Res 58:4410–4416
92. Tell G, Pellizzari L, Pucillo C, Puglisi F, Cesselli D, Kelley MR, Di Loreto C, Damante G (2000) TSH controls Ref-1 nuclear translocation in thyroid cells. J Mol Endocrinol 24:383–390
93. Grosch S, Kaina B (1999) Transcriptional activation of apurinic/apyrimidinic endonuclease (Ape, Ref-1) by oxidative stress requires CREB. Biochem Biophys Res Commun 261:859–863
94. Pines A, Bivi N, Romanello M, Damante G, Kelley MR, Adamson ED, D'Andrea P, Quadrifoglio F, Moro L, Tell G (2005) Cross-regulation between Egr-1 and APE/Ref-1 during early response to oxidative stress in the human osteoblastic HOBIT cell line: evidence for an autoregulatory loop. Free Radic Res 39:269–281
95. Yoshida A, Pourquier P, Pommier Y (1998) Purification and characterization of a Mg^{2+}-dependent endonuclease (AN34) from etoposide-treated human leukemia HL-60 cells undergoing apoptosis. Cancer Res 58:2576–2582
96. Bots M, Medema JP (2006) Granzymes at a glance. J Cell Sci 119:5011–5014
97. Chowdhury D, Beresford PJ, Zhu P, Zhang D, Sung JS, Demple B, Perrino FW, Lieberman J (2006) The exonuclease TREX1 is in the SET complex and acts in concert with NM23–H1 to degrade DNA during granzyme A-mediated cell death. Mol Cell 23:133–142
98. Fan Z, Beresford PJ, Zhang D, Xu Z, Novina CD, Yoshida A, Pommier Y, Lieberman J (2003) Cleaving the oxidative repair protein Ape1 enhances cell death mediated by granzyme A. Nat Immunol 4:145–153
99. Yan N, Cherepanov P, Daigle JE, Engelman A, Lieberman J (2009) The SET complex acts as a barrier to autointegration of HIV-1. PLoS Pathog 5:e1000327
100. Jackson EB, Theriot CA, Chattopadhyay R, Mitra S, Izumi T (2005) Analysis of nuclear transport signals in the human apurinic/apyrimidinic endonuclease (APE1/Ref1). Nucleic Acids Res 33:3303–3312
101. Tell G, Crivellato E, Pines A, Paron I, Pucillo C, Manzini G, Bandiera A, Kelley MR, Di Loreto C, Damante G (2001) Mitochondrial localization of APE/Ref-1 in thyroid cells. Mutat Res 485:143–152
102. Li M, Zhong Z, Zhu J, Xiang D, Dai N, Cao X, Qing Y, Yang Z, Xie J, Li Z, Baugh L, Wang G, Wang D (2010) Identification and characterization of mitochondrial targeting sequence of human apurinic/apyrimidinic endonuclease 1. J Biol Chem 285:14871–14881
103. Nunez E, Fu XD, Rosenfeld MG (2009) Nuclear organization in the 3D space of the nucleus—cause or consequence? Curr Opin Genet Dev 19:424–436
104. Stein GS, van Wijnen AJ, Stein JL, Lian JB, Montecino M, Zaidi K, Javed A (2000) Subnuclear organization and trafficking of regulatory proteins: implications for biological control and cancer. J Cell Biochem Suppl Suppl 35:84–92
105. Andersen JS, Lam YW, Leung AK, Ong SE, Lyon CE, Lamond AI, Mann M (2005) Nucleolar proteome dynamics. Nature 433:77–83
106. Leung AK, Trinkle-Mulcahy L, Lam YW, Andersen JS, Mann M, Lamond AI (2006) NOPdb: nucleolar proteome database. Nucleic Acids Res 34:D218–D220
107. Qu J, Liu GH, Huang B, Chen C (2007) Nitric oxide controls nuclear export of APE1/Ref-1 through S-nitrosation of cysteines 93 and 310. Nucleic Acids Res 35:2522–2532
108. Pinz KG, Bogenhagen DF (1998) Efficient repair of abasic sites in DNA by mitochondrial enzymes. Mol Cell Biol 18:1257–1265
109. Wei SJ, Botero A, Hirota K, Bradbury CM, Markovina S, Laszlo A, Spitz DR, Goswami PC, Yodoi J, Gius D (2000) Thioredoxin nuclear translocation and interaction with redox factor-1 activates the activator protein-1 transcription factor in response to ionizing radiation. Cancer Res 60:6688–6695
110. Mitra S, Izumi T, Boldogh I, Bhakat KK, Chattopadhyay R, Szczesny B (2007) Intracellular trafficking and regulation of mammalian AP-endonuclease 1 (APE1), an essential DNA repair protein. DNA Repair (Amst) 6:461–469
111. Seo YR, Kelley MR, Smith ML (2002) Selenomethionine regulation of p53 by a ref1-dependent redox mechanism. Proc Natl Acad Sci USA 99:14548–14553
112. Hanson S, Kim E, Deppert W (2005) Redox factor 1 (Ref-1) enhances specific DNA binding of p53 by promoting p53 tetramerization. Oncogene 24:1641–1647
113. Brugarolas J, Chandrasekaran C, Gordon JI, Beach D, Jacks T, Hannon GJ (1995) Radiation-induced cell cycle arrest compromised by p21 deficiency. Nature 377:552–557
114. Okamoto K, Prives C (1999) A role of cyclin G in the process of apoptosis. Oncogene 18:4606–4615
115. Bernstein C, Bernstein H, Payne CM, Garewal H (2002) DNA repair/pro-apoptotic dual-role proteins in five major DNA repair pathways: fail-safe protection against carcinogenesis. Mutat Res 511:145–178
116. Di Maso V, Avellini C, Croce LS, Rosso N, Quadrifoglio F, Cesaratto L, Codarin E, Bedogni G, Beltrami CA, Tell G, Tiribelli C (2007) Subcellular localization of APE1/Ref-1 in human hepatocellular carcinoma: possible prognostic significance. Mol Med 13:89–96
117. Duguid JR, Eble JN, Wilson TM, Kelley MR (1995) Differential cellular and subcellular expression of the human multifunctional apurinic/apyrimidinic endonuclease (APE/ref-1) DNA repair enzyme. Cancer Res 55:6097–6102
118. Bobola MS, Finn LS, Ellenbogen RG, Geyer JR, Berger MS, Braga JM, Meade EH, Gross ME, Silber JR (2005) Apurinic/apyrimidinic endonuclease activity is associated with response to radiation and chemotherapy in medulloblastoma and primitive neuroectodermal tumors. Clin Cancer Res 11:7405–7414
119. Luo M, He H, Kelley MR, Georgiadis MM. Redox regulation of DNA repair: implications for human health and cancer therapeutic development (2010) Antioxid Redox Signal 12:1247–1269
120. Messina M, Kucuk O, Lampe JW (2006) An overview of the health effects of isoflavones with an emphasis on prostate cancer

risk and prostate-specific antigen levels. J AOAC Int 89: 1121–1134
121. Yang S, Irani K, Heffron SE, Jurnak F, Meyskens FL Jr (2005) Alterations in the expression of the apurinic/apyrimidinic endonuclease-1/redox factor-1 (APE/Ref-1) in human melanoma and identification of the therapeutic potential of resveratrol as an APE/Ref-1 inhibitor. Mol Cancer Ther 4:1923–1935
122. Yamamori T, DeRicco J, Naqvi A, Hoffman TA, Mattagajasingh I, Kasuno K, Jung SB, Kim CS, Irani K (2010) SIRT1 deacetylates APE1 and regulates cellular base excision repair. Nucleic Acids Res 38:832–845
123. Shimizu N, Sugimoto K, Tang J, Nishi T, Sato I, Hiramoto M, Aizawa S, Hatakeyama M, Ohba R, Hatori H, Yoshikawa T, Suzuki F, Oomori A, Tanaka H, Kawaguchi H, Watanabe H, Handa H (2000) High-performance affinity beads for identifying drug receptors. Nat Biotechnol 18:877–881
124. Helleday T, Petermann E, Lundin C, Hodgson B, Sharma RA (2008) DNA repair pathways as targets for cancer therapy. Nat Rev Cancer 8:193–204
125. Bennett RA, Wilson DM III, Wong D, Demple B (1997) Interaction of human apurinic endonuclease and DNA polymerase beta in the base excision repair pathway. Proc Natl Acad Sci USA 94:7166–7169
126. Dianova II, Bohr VA, Dianov GL (2001) Interaction of human AP endonuclease 1 with flap endonuclease 1 and proliferating cell nuclear antigen involved in long-patch base excision repair. Biochemistry 40:12639–12644
127. Parker A, Gu Y, Mahoney W, Lee SH, Singh KK, Lu AL (2001) Human homolog of the MutY repair protein (hMYH) physically interacts with proteins involved in long patch DNA base excision repair. J Biol Chem 276:5547–5555
128. Vidal AE, Boiteux S, Hickson ID, Radicella JP (2001) XRCC1 coordinates the initial and late stages of DNA abasic site repair through protein–protein interactions. EMBO J 20:6530–6539
129. Busso CS, Iwakuma T, Izumi T (2009) Ubiquitination of mammalian AP endonuclease (APE1) regulated by the p53-MDM2 signaling pathway. Oncogene 28:1616–1625
130. Fritz G, Grosch S, Tomicic M, Kaina B (2003) APE/Ref-1 and the mammalian response to genotoxic stress. Toxicology 193: 67–78
131. Hegde V, Wang M, Deutsch WA (2004) Human ribosomal protein S3 interacts with DNA base excision repair proteins hAPE/Ref-1 and hOGG1. Biochemistry 43:14211–14217
132. Sidorenko VS, Nevinsky GA, Zharkov DO (2007) Mechanism of interaction between human 8-oxoguanine-DNA glycosylase and AP endonuclease. DNA Repair (Amst) 6:317–328
133. Gembka A, Toueille M, Smirnova E, Poltz R, Ferrari E, Villani G, Hubscher U (2007) The checkpoint clamp, Rad9-Rad1-Hus1 complex, preferentially stimulates the activity of apurinic/apyrimidinic endonuclease 1 and DNA polymerase beta in long patch base excision repair. Nucleic Acids Res 35:2596–2608
134. Curtis CD, Thorngren DL, Ziegler YS, Sarkeshik A, Yates JR, Nardulli AM (2009) Apurinic/apyrimidinic endonuclease 1 alters estrogen receptor activity and estrogen-responsive gene expression. Mol Endocrinol 23:1346–1359
135. Huang E, Qu D, Zhang Y, Venderova K, Haque ME, Rousseaux MW, Slack RS, Woulfe JM, Park DS (2010) The role of Cdk5-mediated apurinic/apyrimidinic endonuclease 1 phosphorylation in neuronal death. Nat Cell Biol 12:563–571
136. Izumi T, Mitra S (1998) Deletion analysis of human AP-endonuclease: minimum sequence required for the endonuclease activity. Carcinogenesis 19:525–527