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Forum Review

The Intracellular Localization of APE1/Ref-1: More than a Passive Phenomenon?

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ABSTRACT

Human apurinic/apyrimidinic endonuclease 1/redox effector factor-1 (APE1/Ref-1) is a perfect paradigm of the functional complexity of a biological macromolecule. First, it plays a crucial role, by both redox-dependent and –independent mechanisms, as a transcriptional coactivator for different transcription factors, either ubiquitous (*i.e.*, AP-1, Egr-1, NF-κB, p53, HIF) or tissue-specific (*i.e.*, PEBP-2, Pax-5 and -8, TTF-1), in controlling different cellular processes such as apoptosis, proliferation, and differentiation. Second, it acts, as an apurinic/apyrimidinic endonuclease, during the second step of the DNA base excision repair pathway, which is responsible for the repair of cellular alkylation and oxidative DNA damages. Third, it controls the intracellular reactive oxygen species production by negatively regulating the activity of the Ras-related GTPase Rac1. Despite these known functions of APE1/Ref-1, information is still scanty about the molecular mechanisms responsible for the coordinated control of its several activities. Some evidence suggests that the expression and subcellular localization of APE1/Ref-1 are finely tuned. APE1/Ref-1 is a ubiquitous protein, but its expression pattern differs according to the different cell types. APE1/Ref-1 subcellular localization is mainly nuclear, but cytoplasmic staining has also been reported, the latter being associated with mitochondria and/or presence within the endoplasmic reticulum. It is not by chance that both expression and subcellular localization are altered in several metabolic and proliferative disorders, such as in tumors and aging. Moreover, a fundamental role played by different posttranslational modifications in modulating APE1/Ref-1 functional activity is becoming evident. In the present review, we tried to put together a growing body of information concerning APE1/Ref-1's different functions, shedding new light on present and future directions to understand fully this unique molecule. *Antioxid. Redox Signal.* 7, 367–384.

INTRODUCTION

General considerations on APE1/Ref-1 biological and molecular functions

APE1/Ref-1 is a perfect example of the functional complexity of a biological macromolecule. Its acronym reflects its at least dual nature: human apurinic/apyrimidinic (AP) endonuclease, or APE1 (also HAP1 or APEX), is a major constituent of the base excision repair (BER) pathway of DNA lesions. Ref-1, the acronym for redox effector factor-1, refers to its redox abilities on different redox-regulated transcription factors (TFs). Interestingly, these two activities are split into two functionally independent domains of the protein itself: the N-terminus is principally devoted to the redox activity, whereas the C-terminus exerts enzymatic activity on the abasic sites of DNA (135). Different from the N-terminus, which is completely unconserved, the C-terminus is highly conserved from plant to man.

Both of these two activities seem to be fundamental in the control of the apoptotic process, as demonstrated by several

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works (14, 96, 126). APE1/Ref-1 expression is always inversely correlated with the onset of apoptosis, suggesting a role as an antiapoptotic molecule.

DNA-repair activity of APE1/Ref-1

APE1/Ref-1 is an essential protein (136) that contributes, through its participation in the BER pathways, to the regeneration of DNA damaged by products of cell metabolism and by environmental hazards. The two best characterized functions of APE1/Ref-1 in these pathways are production of a DNA primer for repair synthesis and coordination of the repair activities of other BER proteins (21, 28, 47, 63, 104, 129).

BER repairs DNA damage with a set of enzyme activities that sequentially remove the damaged base (glycosylases), incise the phosphodiester backbone 5′ adjacent to the abasic site (APE1/Ref-1), excise the abasic residue [APE1/Ref-1, β polymerase, or flap endonuclease 1 (FEN1)], polymerize the replacement nucleotide(s) [β polymerase, x-ray cross-species complementing 1 (XRCC1), or δ/ε polymerases with proliferating cell nuclear antigen (PCNA)], and ligate the final sequence (DNA ligases I and III, XRCC1) (Fig. 1).

BER repair synthesis requires a DNA primer with a 3′ hydroxyl end. APE1/Ref-1 generates this end in three ways. It binds specifically (102) and processively (13) to abasic sites, generated spontaneously or by glycosylases, and cuts the 5′ phosphodiester bond with its endonuclease activity to produce the primer. This endonuclease activity depends on the redox state of APE1/Ref-1, controlled in part by amino acid C310 (70). APE1/Ref-1 removes with its 3′-5′ exonuclease activity a 3′-phospho-α,β-unsaturated aldehyde, formed by complex glycosylases [such as the oxidative damage-repairing glycosylases 8-oxoguanine DNA glcosylase (Ogg1) and Nth] and by radiation. This activity may also contribute to the fidelity of repair synthesis by removing mispaired nucleotides (132). And, although less efficiently than PNK, APE1/Ref-1 removes with its 3′ phosphatase activity a 3′ terminal phosphate, produced by glycosylases NEIL1 and NEIL2 and by radiation (63). The repair function of APE1/Ref-1 requires 10 evolutionarily conserved amino acids, D70, D90, E96, Y171, D210, N212, D219, D283, D308, and H309 (35).

Coordination of these steps within the BER pathway (106) and with other DNA repair pathways is thought to be important for preventing the accumulation of toxic repair intermediates (105, 131) (each a type of DNA damage) and for increasing the overall efficiency of the pathways. Abasic sites produced spontaneously or by glycosylases can inhibit DNA and RNA polymerases, facilitate mutation, and promote single-strand breaks (129, 145). Single-strand breaks produced by complex glycosylases and by APE1/Ref-1 can inhibit polymerases, promote recombination, and become double-strand breaks during replication, which in turn can lead to chromosome rearrangements and cell death (11). Evidence from reconstituted systems and from cell extracts suggests that APE1/Ref-1 contributes to coordination by interacting directly or indirectly with other BER enzymes and with other repair pathways. The molecular basis of the specificity and of the stimulatory mechanisms and the biological significance of these interactions remain to be determined.

The glycosylase MYH (MutY DNA glycosylase homologue) removes adenine and 2-hydroxyadenine mismatched with guanine or 7,8-dihydro-8-oxodeoxyguanine (89, 90). APE1/Ref-1 increases MYH activity 10-fold in a reconstituted system by associating directly with MYH to promote the formation of efficient MYH·DNA complexes by decreasing the MYH·DNA substrate dissociation constant (142). Immunoprecipitation and affinity chromatography show that this association requires MYH amino acids 295–317 containing the conserved motif S/PGXZDV/I, where X and Z are any amino acids (89). APE1/Ref-1 may also bind directly to methylpurine glycosylase, as suggested by *in vitro* binding studies and by far western analysis of a nuclear ~30-kDa protein with APE1/Ref-1 in CHO cell extracts (35), although yeast two-hybrid analysis failed to detect this interaction (5).

Other glycosylases appear to interact with APE1/Ref-1 indirectly, through competition for DNA binding sites. Glycosylases Ogg1 (54), Nth1 (74), uracil DNA glycosylase (88), and thymine DNA glycosylase (128) display product inhibition, binding tightly to their processed DNA product. This binding may help recruit APE1/Ref-1 to the damaged site and protect the AP sites or single-strand breaks until APE1/Ref-1 continues the repair process. APE1/Ref-1 alleviates this inhibition by displacing the glycosylases through its stronger association with the DNA.

Yeast two-hybrid and gel supershift analyses suggest that a direct interaction between DNA-bound APE1/Ref-1 and DNA polymerase β (5) recruits polymerase β to the damaged site, stimulating fivefold the rate of removal of the abasic sugar (5′ dRp) by the lyase activity of polymerase β. This stimulation and consequent repair synthesis in turn increase APE1/Ref-1 endonuclease activity, by removing through repair synthesis the 3′ terminus that effects product inhibition of APE1/Ref-1 (77).

In addition to its role in single-strand break repair, XRCC1 may act as a scaffold to physically organize the BER process. XRCC1 binds directly to APE1/Ref-1 in yeast two-hybrid, far western, and affinity chromatography assays (122). This interaction requires the N-terminus of APE1/Ref-1, stimulates the rate of APE1/Ref-1 endonuclease activity fivefold, and stimulates the 3′ phosphodiesterase activity. Extracts prepared from CHO cells defective in XRCC1 activity show diminished APE1/Ref-1 endonuclease activity that is rescued by addition of XRCC1. Stimulation of this endonuclease activity may also decrease the lyase activity of Ogg1 to increase the efficiency of repair (75).

Coprecipitation and affinity chromatography show that PCNA, the sliding clamp processivity factor of DNA polymerases δ and ε, and FEN1, the flap endonuclease of long-patch BER, bind directly to APE1/Ref-1 (22). APE1/Ref-1 stimulates flap excision twofold in a reconstituted system, suggesting the APE1/Ref-1 interaction with FEN1 may be functional. Tom *et al.* (118) have suggested that the ability of APE1/Ref-1 to organize BER proteins in the presence and absence of PCNA may contribute to the differential regulation of BER and DNA replication during oxidative stress mediated by p21.

p53 binds directly to APE1/Ref-1 and stimulates BER, although the relationship of the binding and stimulation is unknown and p53 is not required for BER (105). Far western analysis of purified proteins and immunoprecipitation west-

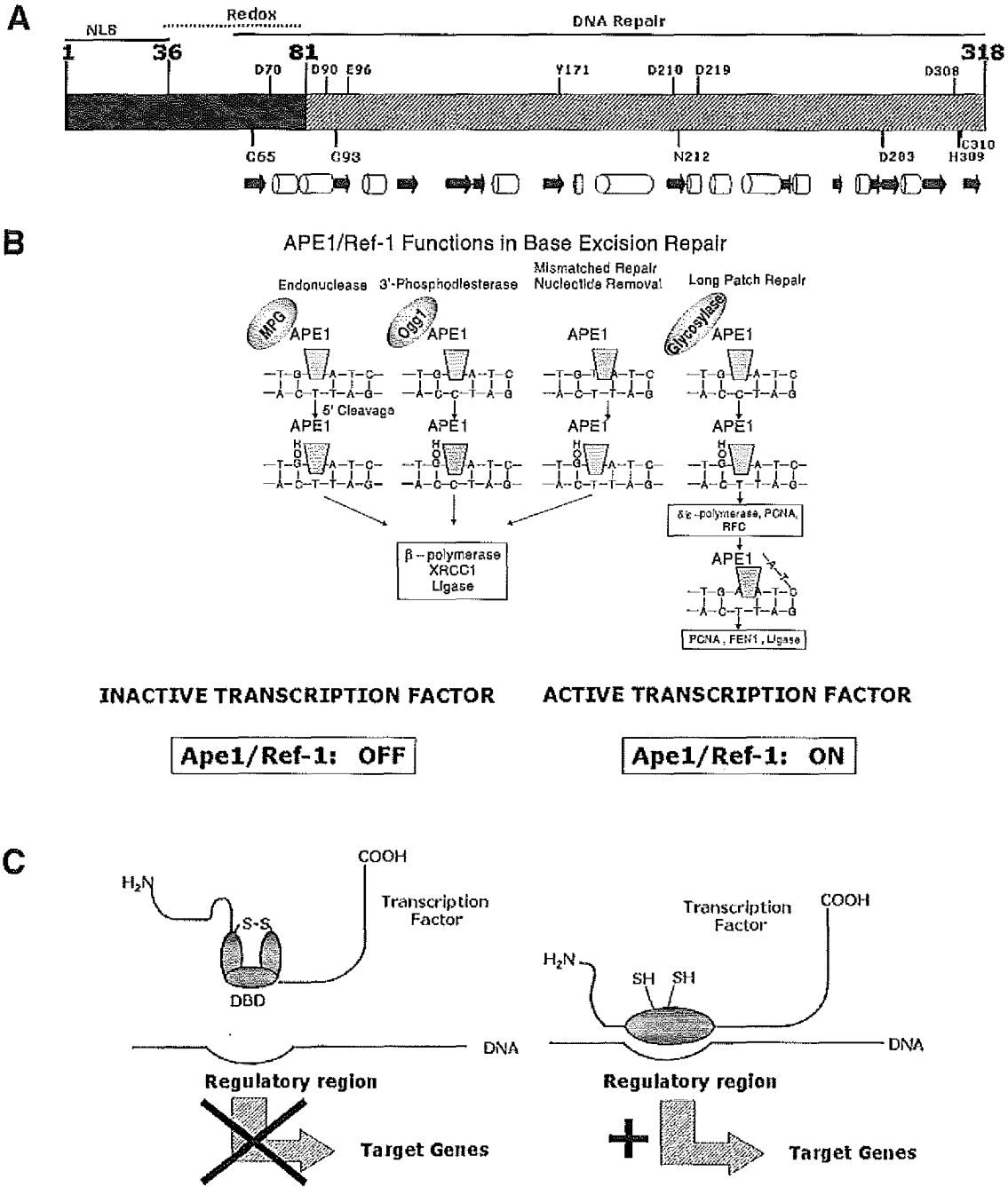

FIG. 1. Representations of APE1/Ref-1 structure and functions. (**A**) Schematic structure of APE1/Ref-1 with critical residues. NLS, nuclear localization signal. Cylinders represent α-helical regions, and arrows represent β-strands, as deduced by Gorman *et al.* (44). (**B**) APE1/Ref-1 functions in BER. MPG, methylpurine DNA glycosylase; RFC, replication factor C. (**C**) Theoretical molecular model of the redox function of APE1/Ref-1 as a transcriptional coactivator. DBD, DNA binding domain.

ern analysis of H24–14 cell extracts show that the tumor suppressor p53 binds directly to APE1/Ref-1 (41). p53 also stimulates BER in nuclear extracts and in reconstituted systems, dependent on its N-terminal transactivating region (146). Equal APE1/Ref-1-specific activities in extracts prepared from isogenic cell lines with wild-type and suppressed p53 suggest that the stimulation is not due to interaction between p53 and APE1/Ref-1 (101).

APE1/Ref-1 protein interactions may also influence DNA repair pathways other than BER. Affinity chromatography and gel mobility shift assays show that the single-strand break repair proteins Ku 70/80 bind APE1/Ref-1 (16).

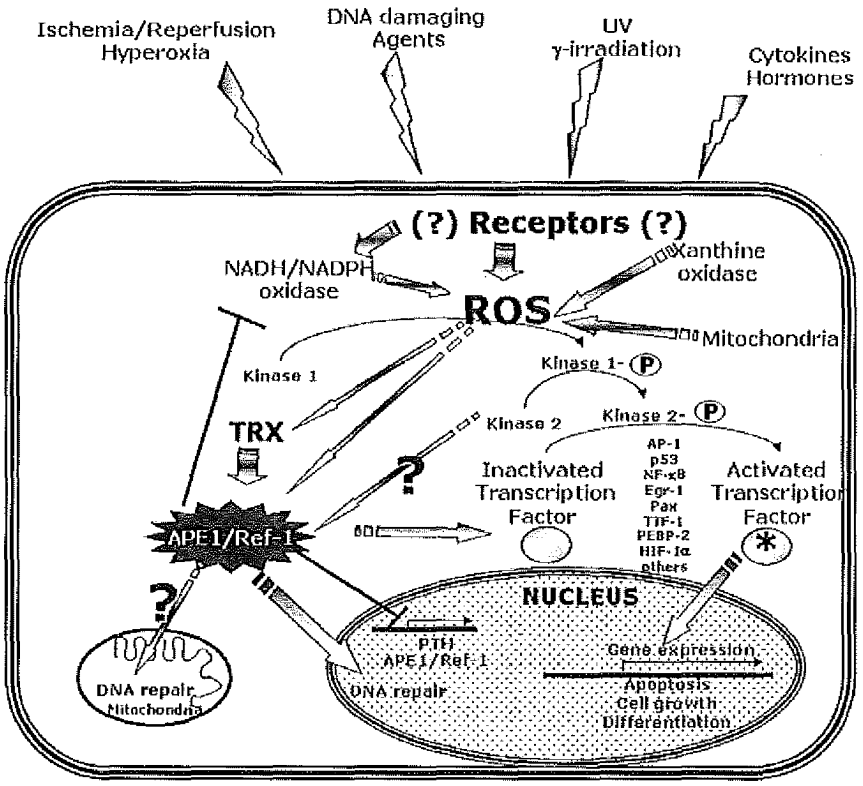

FIG. 2. Schematic representation of some of the stimuli known to activate APE1/Ref-1 expression and/or function.

Whether this interaction affects either BER or single-strand break repair is unknown.

Redox regulation of TFs activities

Redox regulation of cellular functions occurs as a consequence of the so-called "redox-cellular status," which is the result of a balance between the activity of antioxidant enzymatic cell systems (such as GSH/GSSG, superoxide dismutase, catalase, peroxidases, glutathione peroxidases, etc.) and the amount of reactive oxygen species (ROS) such as superoxide anion ($O_2^{-\bullet}$), hydrogen peroxide (H_2O_2), and hydroxyl radical ('OH). These last molecules can be produced in several ways: as by-products of respiration, thus being associated with cell proliferation rate; by external noxious agents, such as ionizing radiation (127); during pathological states in activated neutrophils (80); and as "second messengers" produced by intracellular enzymatic systems, such as NADPH oxidase regulated by the ubiquitous small GTPase Rac1 (20, 24, 43). It therefore represents a useful tuning device for intracellular signal transduction, as is the case in cascades induced by cytokines, such as tumor necrosis factor-α or interleukin (IL)-β (80).

This redox regulation ultimately affects gene expression. Recently, a great body of experimental evidence suggested that these outcomes are achieved through modulation of TFs activity. Up to now, several TFs containing specific Cys residues have been demonstrated to be the target of redox regulation. APE1/Ref-1 has been identified as a protein capable of nuclear redox activity, inducing the DNA-binding activity of several TFs, such as AP-1 (133), NF-κB (83), Myb (134), PEBP-2 (1), HLF (27), NF-Y (81), Egr-1 (60), HIF-1α (59), ATF/CREB family (134), p53 (41), Pax proteins (12, 110, 112). It accomplishes this through the control of the redox state of Cys residues located in the DNA-binding domains or within regulatory regions, such as the transactivation domain of the thyroid-specific transcription factor 1 (*i.e.*, TTF-1) of the TFs themselves (115). In order to properly bind specific DNA target sequences, these TFs require that critical Cys residues are in the reduced state. Therefore, by maintaining these cysteines in the reduced state, APE/Ref-1 provides a redox-dependent mechanism for regulation of target gene expression. APE/Ref-1 contains two cysteine residues located within the redox-active domain (Cys65 and Cys93), and previous studies show that Cys65 should be the redox-active site of the protein by using recombinant protein (123). In agreement with the molecular model describing redox regulation exerted by APE1/Ref-1, Cys65 should interact with the sensitive cysteine residues within the DNA-binding domains of TFs. However, Jayaraman *et al.* (64) suggest that the stimulatory role played by APE1/Ref-1 on p53 activity may also occur in a redox-independent way. This has been recently corroborated by the work of Ordway *et al.* (85) in which the authors provide first *in vivo* evidence that the Cys65 residue of APE1/Ref-1 is, unexpectedly, not essential for redox regulation of AP-1 DNA binding. However, these authors did not completely exclude a possible presence of compensatory phe-

nomena. In any case, this evidence challenges previous hypotheses about the molecular mechanisms by which APE1/Ref-1 exerts its redox-dependent activities of specific TFs.

According to the proposed redox-regulatory role on cellular functions played by APE1/Ref-1, both gene expression and protein levels are up-regulated by nontoxic levels of a variety of ROS and/or ROS-generating systems (Fig. 2).

REGULATION OF APE1/Ref-1 FUNCTIONS: AT THE GENE EXPRESSION LEVEL AND POSTTRANSLATIONAL (PT) MODIFICATIONS

Early evidence of APE1/Ref-1 regulation came from expression studies. It was immediately clear that APE1/Ref-1 expression was principally controlled at the transcriptional level (Table 1). Several studies, both *in vivo* and *in vitro*, clearly demonstrated that different oxidative agents efficiently and quickly (within minutes to hours) promote APE1/Ref-1 transient increase at both the mRNA and protein levels (Fig. 2). As it is blocked by cycloheximide, the latter process requires *de novo* protein synthesis (94, 112). Up-regulation of APE1/Ref-1 protein levels appears to have biological relevance. In fact, protein up-regulation is always associated with an increase in both redox and AP endonuclease activity, followed by an increase in cell resistance toward oxidative stress and DNA-damaging agents (46, 94). Therefore, an important issue was the identification of extracellular signals able to increase APE1/Ref-1 gene expression.

As experimental data grew, so did research aimed at identifying biological modulators of APE1/Ref-1 gene expression, such as hormones and cytokines. With respect to these observations, recent articles by our group (113, 116) together with that of Asai *et al.* (3) depicted a clear view of the thyrotropin (TSH)-induced APE1/Ref-1 gene expression in thyroid cells. It became immediately clear that functional triggering of membrane-bound receptors could be responsible for a positive regulation of APE1/Ref-1 gene expression itself for other cell systems. Such an example is represented by the human chorionic gonadotropin that has been previously demonstrated to induce APE1/Ref-1 mRNA synthesis in murine Leydig cells (107). In the case of the immune system, a physiological induction of APE1/Ref-1 gene expression has been recently demonstrated for at least two cell types. This is the case of human alveolar macrophages stimulated with granulocyte-monocyte colony-stimulating factor (31), which can be released by these cells during fibrotic processes in the lung, and of spleen B cells stimulated with CD40 ligand (78). In the first case, a functional role in AP-1 transcriptional activity has been proposed. In the latter case, the CD40 triggering is functional to the activation of *blk* promoter operated by Pax5a and EBF TFs. A role for IL-2-stimulated APE1/Ref-1 up-regulation has also been demonstrated in a murine pro-B cell line (140). Very recently, a functional role for this process has been suggested involving redox regulation of telomerase activity (137).

Other soluble mediators that have been investigated dealing with APE1/Ref-1 expression are dopamine and glutaredoxin (Grx2) in cerebellar granule neurons. Daily *et al.* (17) demonstrated that the endogenous neurotransmitter of the nigrostriatal pathway, *i.e.*, the proapoptotic dopamine, exerts an inhibitory effect on APE1/Ref-1 expression that is squelched by the antioxidant Grx2, thus leading to NF-κB activation and to cell protection from apoptosis.

APE1/Ref-1 levels seem to act as an intracellular signaling device. In fact, its protein levels correlate with the propensity of the cell to undergo apoptosis or proliferation. In practice, modulation of APE1/Ref-1 protein expression has been described for almost every cell type depending on the particular cellular redox status induced by exposure to an oxidative environment (Table 1). Moreover, physiological stimuli, such as those of cytokines, are able to promote APE1/Ref-1 up-regulation. Indeed, cell systems must be able to discriminate these different stimuli if APE1/Ref-1 behaves as a signaling molecule.

At present, it is not known what the *primum movens* responsible for APE1/Ref-1 gene expression upon oxidative stress is or the signaling pathways leading to its activation. A major role seems to be represented by cyclic AMP as a common second messenger in the case of both TSH and H_2O_2 stimulations (3, 116). However, from the comparative study of cytokine- and ROS-induced APE1/Ref-1 up-regulation, some conclusions could be drawn about the molecular switches responsible for its modulated expression. Moreover, as APE1/Ref-1 expression represents a molecular marker of oxidative stress, it would be important to understand the dissection of signaling pathways responsible for its role in regulating the mechanisms of ROS-induced cell responses.

The human *APE1/REF-1* gene is located on chromosome 14q11.2–12 and span 2.6 kb, and consists of four introns and five exons, the first of which is noncoding. Its *cis*-regulatory regions are composed of a proximal basal promoter of ~300 bp containing a CpG island (Fig. 3A). Within this area, several putative binding sites for redox-regulated (such as AP-1, Sp-1, and ATF) or cyclic AMP-regulated (such as CREB) transcriptional factors are present. At the distal promoter level, at least three negative calcium-responsive regulatory elements (nCAREs) were found: nCaRE-A, nCaRE-B1, and nCaREB2 (62). By binding to one of these regulatory elements (nCaRE-B2), APE1/Ref-1 is able to repress its own expression, thus providing a means to control its intracellular levels (62). Therefore, APE1/Ref-1 can also exert transcriptional control by binding to specific DNA sequences in cooperation with hnRNP-L, a member of the heterogeneous ribonucleoproteins (73). This has also been confirmed by findings demonstrating the ability of APE1/Ref-1 to repress the parathyroid hormone (PTH) gene expression (84). In fact, PTH promoter is characterized by the presence of a nCaRE binding motif that is specifically recognized by APE1/Ref-1.

Despite the fact that the APE1/Ref-1 gene was cloned several years ago (50) and its promoter characterized in detail (52), very few articles have addressed the molecular mechanisms responsible for its inducible regulation. Up to now, the unique ascertained contributors to APE1/Ref-1 transcriptional activation are that of Jun/ATF2 in an inducible way in response to oxidative stress (45) and that of Sp-1 required for both the basal (region −65 to −17 from the transcription start site) and the coordinate (region +99 to +183) expression of

TABLE 1. SUMMARY OF STUDIES DESIGNED TO INDUCE APE/REF-1 AT THE MRNA AND/OR THE PROTEIN LEVELS

Tissue/cells	*Treatment or pathological condition*	*Gene expression*	*Subcellular compartment*	*Reference*	*Proposed biological role*
HT-29	Hypoxia	↑		144	Involvement in detoxification of xenobiotics
HeLa	UV and hypoxia	↑		124	Protection against hypoxic stress
Rat dentate gyrus (granular cells)	Ischemia	↑	N	42	Neuronal protection against oxidative stress
Rat hippocampus (CA1 neurons)	Ischemia	↑	N	42	Neuronal protection against oxidative stress
	Hypoxic–ischemic insult	↓		125	Development of apoptosis
CHO	ROS	↑		46	Clastogenic adaptive response to oxidative stress
HeLa S3 and WI38	ROS	↑		94	Adaptive response to ROS causing enhanced repair of cytotoxic DNA lesions
Raji (B lymphocytes)	ROS	↑	N	112	Redox regulation of TFs activity
		↑	M	36	DNA repair
Rat hippocampus	Ischemia	↓		26	Development of apoptosis
Rat mesothelial cells	Asbestos	↑		39	DNA repair
FRTL-5 (rat thyroid cells)	TSH, ROS, elevation in intracellular Ca^{2+}	↑	N	3, 113, 116	Redox regulation of TFs activity
Rat SON and PVN cells	Hypertonic	=		95	DNA repair
Rat SCN cells	Light	=		95	DNA repair
Porcine epidermis	Wound healing	↑		51	DNA repair
MA-10	hCG	↑		107	Redox regulation of TFs activity
Liver	Ischemia/reperfusion	↑		87	Redox regulation of TFs activity, Control of intracellular ROS production
CPAEC and HUVEC	Hypoxia	↓		49	Redox regulation of TFs activity
RBL-2H3 mast cells	ROS	↑	N	37	Redox regulation of TFs activity
Spleen B cells	CD40 triggering	↑	N	78	Redox regulation of TFs activity
HT29 colon adenocarcinoma cells	Dithiolethione oltipraz	↑		143	Redox regulation of TFs activity
Alzheimer's disease	Chronic ROS	↑		19	DNA repair
Rat kidney	Aging	↑	N	15	Redox regulation of TFs activity
Human choriodecidual cells	Elevation in intracellular Ca^{2+}	↑	N	38	Transcriptional repression
Rat liver treated with WY14,643	ROS	↑	M	56	DNA repair
Rabbit spinal cord	Ischemia	↓		99	DNA repair, development of apoptosis
Human atherosclerotic plaques	ROS	↑		76	DNA repair
Human fibroblasts	Arsenic	↑		58	Redox regulation of TFs activity
HUVEC	Hypoxia/reoxygenation	↑	N	2	Redox regulation of TFs activity
Human macrophages	Asbestos	↑	N	32	Redox regulation of TFs activity
Human alveolar macrophages	GM-CSF	↑		31	Redox regulation of TFs activity
K562 human myeloid cell line	PMA, hypochlorite, MMS	↑		57	Redox regulation of TFs activity, DNA repair
BA/F3 murine pro-B cell line	IL-2	↑		140 137	Redox regulation of TFs activity Stimulation of telomerase activity

(continued)

TABLE 1. (*CONTINUED*)

Tissue/cells	*Treatment or pathological condition*	*Gene expression*	*Subcellular compartment*	*Reference*	*Proposed biological role*
Murine cerebellar granule neurons	Dopamine	↓		17	Redox regulation of TFs activity
Murine cerebellar granule neurons	GRx2	↑	N/C	18	Redox regulation of TFs activity, protection from apoptosis
Astrocyte primary cultures	Pb	↑	N	100	Redox regulation of TFs activity
CHO	ROS	↑		45	Redox regulation of TFs activity
Rat tamoxifen-induced hepatocarcinogenesis	Tamoxifen	↑		69	Redox regulation of TFs activity, DNA repair
Rat duodenal mucosa	Cysteamine-induced duodenal ulceration	↑	N	71	Redox regulation of TFs activity
Human placenta	Preeclampsia	↑		108	DNA repair

C, cytoplasm; CPAEC, calf pulmonary artery endothelial cells; GM-CSF, granulocyte-monocyte colony-stimulating factor; hCG, human chorionic gonadotropin; HUVEC, human umbilical vein endothelial cells; M, mitochondria; MMS, methyl methanesulfonate; N, nucleus; PMA, phorbol 12-myristate 13-acetate; PVN, paraventricular nuclei; SCN, suprachiasmatic nuclei; SON, supraoptic nuclei.

APE1/Ref-1 during cell cycle (40). These last results were obtained in the case of the mouse promoter. However, as a conserved Sp-1 binding site located downstream from the +1 transcription start site is present in the human promoter (region about +76 to +85), a similar behavior could be expected. Very recently, an important role in the control of APE1/Ref-1 gene expression has also been suggested for the signal transducer and activator of transcription-3 (Stat3) TF in liver (48), suggesting a new mechanism of protection against Fas-mediated liver injury. These findings confirm that much work remains to be done in order to elucidate, in detail, the molecular mechanisms of controlling APE1/Ref-1 gene expression.

Due to the fact that recombinant APE1/Ref-1 expressed in *E. coli* fully retains functional redox and endonuclease activity, it was erroneously thought for several years that APE1/Ref-1 was not subjected to PT modifications. The only functional exception to perfectly similar behavior between endogenous and recombinant APE1/Ref-1 was represented by a differential binding activity toward the nCaRE sites. In fact, recombinant APE1/Ref-1 alone was not able to bind to both nCaRE-A and nCaRE-B in the PTH gene promoter, indicating a requirement for additional factors in the complex (16, 73). These observations led to the identification of Ku70 (Ku86) and hnRNP-L involvement in the complex formation with nCaRE-A and nCaRE-B, respectively. Moreover, APE1/Ref-1 is an abundant protein in a eukaryotic cell. We have estimated that ~10–30,000 copies of the protein are present in a thyroid cell. Therefore, it is possible that many proteins for many different functions have to be "finely tuned" in order to coordinate specific biological activities. The best choice for a biological system to "recycle" the same protein for different biological activities is by means of PT modifications. First, *in silico* studies soon discovered that several different phosphorylation sites were scattered throughout the molecule. These potential phosphorylation sites included consensus sequences for casein kinase I and II (CKI and CKII), for protein kinase C (PKC), and for glycogen synthase kinase 3 (GSK3) (Fig. 3B) (34, 139). These two pioneering studies were, however, in disagreement regarding the potential phosphorylation sites determined and the functional consequences of *in vitro* phosphorylation.

These first studies were performed *in vitro* by using the recombinant APE1/Ref-1 protein expressed in *E. coli*. The first *in vivo* study was that of Hsieh *et al.* (57), which demonstrated for the first time the occurrence of a PKC-mediated phosphorylation on APE1/Ref-1 protein. Unfortunately, these authors did not determine the location of the specific phosphorylation site. The PKC-mediated phosphorylation was able to promote the redox activity of APE1/Ref-1 on AP-1 TF in response to phorbol 12-myristate 13-acetate treatment or to the oxidizing agent hypochlorite followed by methyl methane sulfonate treatment. However, this study did not address the question of whether endonuclease activity of APE1/Ref-1 is affected by PKC phosphorylation.

Another PT modification that was initially suspected and finally demonstrated *in vitro* was redox modification. Initial evidence came from studies of association with the dithiol-reducing enzyme thioredoxin (TRX) (55, 93, 121). Although the specific residues of APE1/Ref-1 involved in interaction with TRX are not known, Cys35 and Cys32 in the catalytic center of TRX have been demonstrated to be involved. As it was previously demonstrated that Cys65 and Cys93 of APE1/Ref-1 are redox-sensitive (123, 124, 135), it could be speculated that target residues of TRX interaction may include these two cysteine residues. This TRX-mediated redox regulation of APE1/Ref-1 is required for p53 and AP-1 functional activation (55, 121). Very recently, Kelley and Parsons (70) demonstrated that the repair activity is also redox-regulated with the specific involvement of C310 residue located immediately adjacent to the crucial histidine residue at position 309 within the DNA repair active site. Interestingly, oxidation occurring at other Cys residues located in the redox domain

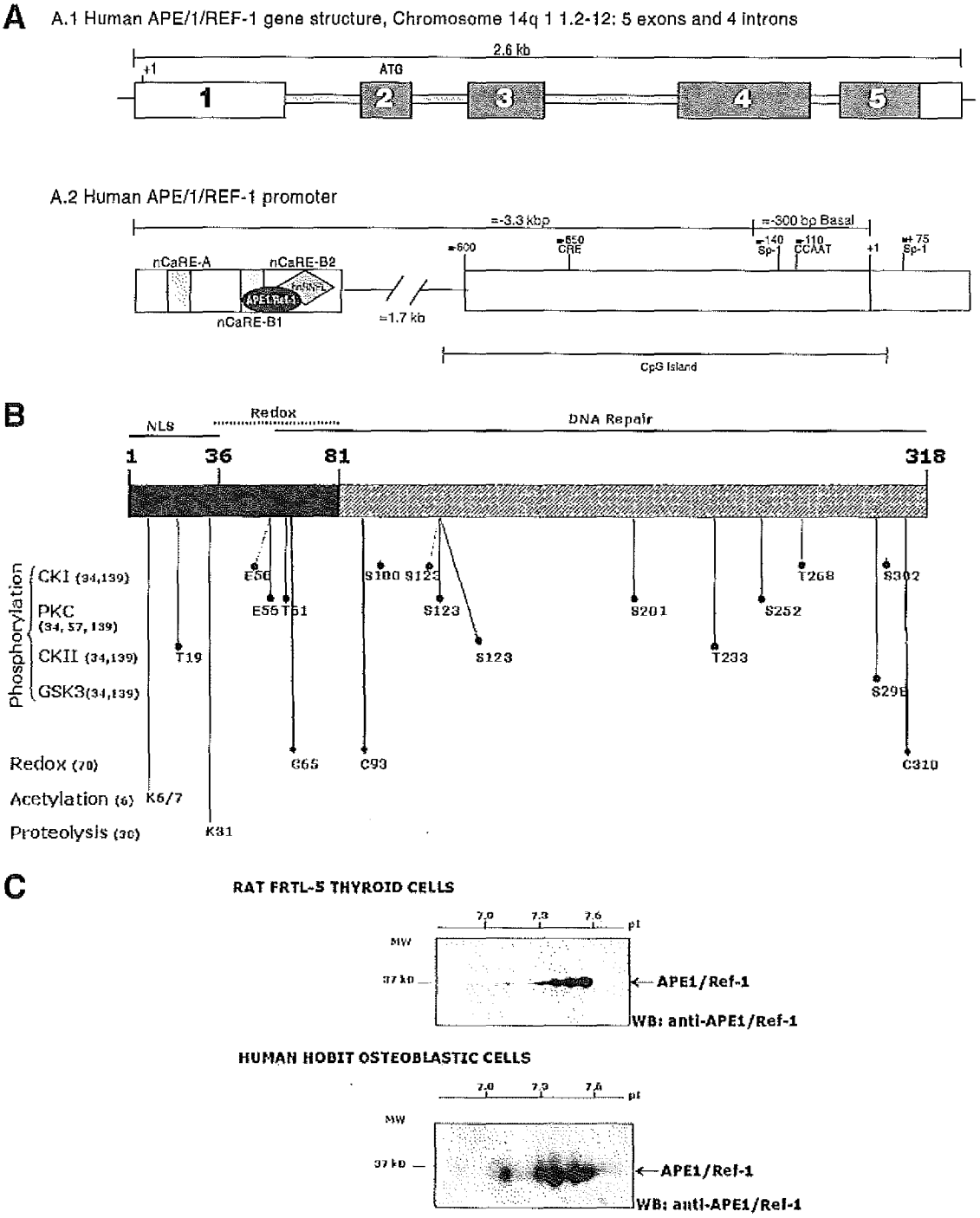

FIG. 3. Regulation of APE/Ref-1 functions: at the gene expression level and PT modifications. (A) Schematic structure of the APE1/Ref-1 gene (A.1) and regulatory region on its promoter (A.2), which is located approximately within ~3 kb upstream of the transcription start site. (B) Schematic diagram of putative or demonstrated PT modifications of APE1/Ref-1. (C) *In vivo* heterogeneity of APE1/Ref-1 protein associated with different PT modifications. Two-dimensional gel electrophoresis (2-DE) of 50 μg of nuclear extracts from (**upper**) rat FRTL-5 thyroid cells and (**lower**) HOBIT human osteoblastic cell line. After isoelectric focusing on an Immobiline dry strip (pH gradient 6–11) in an IPGphor electrophoresis system (Amersham Biosciences), the proteins were separated by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (10%) and APE1/Ref-1 was visualized by western blotting by using a specific rabbit polyclonal antibody (114).

does not affect the repair activity, confirming the functional independence of the two domains of APE1/Ref-1. The structural and functional modularity of APE1/Ref-1, together with this new evidence of different sensitivity toward different specific PT modifications, could explain the fine-tuning required for proper function of the protein.

PT regulation of APE1/Ref-1 protein is responsible for enhanced cell death mediated by granzyme A (GzmA) (30) in K562 cells. APE1/Ref-1 is associated with the endoplasmic reticulum (ER) in a macromolecular complex of 270–420 kDa containing evolutionarily conserved proteins called SET, pp32, and HMG2. GzmA cleaves APE1/Ref-1 after Lys31 and destroys its known DNA repair functions, forcing the cell to undergo apoptosis. These recently published data suggest that a complex array of interrelationships may control APE1/Ref-1 function.

Lastly, acetylation occurring on APE1/Ref-1 protein has very recently been found by Bhakat *et al.* (6). The transcriptional coactivator p300, which is activated by Ca^{2+}, is able to specifically acetylate APE1/Ref-1 both *in vitro* and *in vivo*. Acetylation at Lys6 or Lys7 enhances DNA binding of APE1/Ref-1 to nCaRE sequences, thus unraveling a means to down-regulate the PTH promoter by APE1/Ref-1 itself. These authors showed that APE1/Ref-1 protein is heterogeneously PT modified in HeLa cells used in this study. Interestingly, and more generally, this heterogeneity is not restricted to the cell line used in this work because our studies, performed on rat FRTL-5 cells and human HOBIT osteoblastic cells by using two-dimensional gel electrophoresis (2-DE) analysis coupled to western blot identification, confirmed that APE1/Ref-1 is heterogeneously present in cell nuclear extracts at basal conditions (see Fig. 3C).

Together, these recent observations have raised the possibility that subtle PT modifications provide a means for channeling the multifunctional APE1/Ref-1 to different activities and interactions and thus could act as a regulatory switch in performing different functions.

REGULATION OF APE1/Ref-1 FUNCTIONS: AT THE SUBCELLULAR LOCALIZATION LEVEL

Most reports have APE1/Ref-1 localized to the nucleus, but a growing body of evidence has shown that in some cell types, particularly those with high metabolic or proliferative rates, such as spermatocytes, thyrocytes, lymphocytes, hepatocytes, and hippocampal cells, APE1/Ref-1 can be cytoplasmic (25, 65, 67, 95, 112, 113, 130). Recent advances provided at least two functional explanations for the cytoplasmic expression of APE1/Ref-1. Previous evidence comes from our work on mitochondrial localization of APE1/Ref-1 (114), confirming previous findings by Fung *et al.* (39) and Tomkinson *et al.* (119). Due to occurrence of oxidative phosphorylation, mitochondria produce a large amount of ROS. Mitochondrial DNA (mtDNA), being located in the mitochondrial matrix, is extremely susceptible to oxidative damage, which represents a major mutational drive for mtDNA itself. The existence of DNA repair devices in mitochondria is still up for debate (8). However, the presence of the major abasic endonuclease repair enzyme APE1/Ref-1 in this subcellular compartment would account for the existence of a BER mechanism in mitochondria. The latter functional explanation of APE1/Ref-1 cytoplasmic location is related to its association with ER membranes. By using electron microscopy immunocytochemistry, we evidenced this association in FRTL-5 thyroid cells (114). However, more functional information about ER localization of APE1/Ref-1 protein came from recent work by Fan *et al.* (29, 30). APE1/Ref-1 associates with the tumor suppressor protein pp32 and with the nucleosome assembly protein SET in the so-called SET complex in K562 and HeLa cells. Within this context, APE1/Ref-1 together with the HMG2 architectural TF was demonstrated to be a physiologically relevant GzmA substrate in targeted cells. In so doing, GzmA may block cellular repair and force cells into apoptosis.

Moreover, cytoplasmic localization may be required in order to maintain newly synthesized TF in a reduced state while they are being translocated to the nucleus. In this respect, Duguid et al. (25) demonstrated an association of APE1/Ref-1 with ribosomes in the cytoplasm of hypoglossal neurons.

Another level of complexity in understanding the regulation/meaning of APE1/Ref-1 biological functions is represented by the widespread observation that the subcellular distribution of this protein is somewhat heterogeneous within cell subpopulations. This is the case of cerebellar granule cells. Their APE1/Ref-1 staining is mostly cytoplasmic, whereas adjacent cells show intense nuclear staining (67). The recent observation that APE1/Ref-1 is subjected to a cell cycle-dependent expression (40) could explain this. These data, paired with the demonstration of cell cycle-dependent hMYH glycosylase expression (9), suggest that coordinated expression of key DNA repair genes with the cell cycle is a general phenomenon. Moreover, as APE1/Ref-1 plays an important role in contributing to p53-mediated cell-cycle arrest (41) by regulating p53 TF activity through both redox-dependent and -independent mechanisms (64), and it is down-regulated during apoptosis in myeloid leukemia cells (96), then it is possible that the role of cell cycle-responsive APE1/Ref-1 expression relates to one of these other functions rather than to DNA repair.

Finally, in many cell types, APE1/Ref-1 displays both nuclear and cytoplasmic localization. These cell types include normal thyroid cells stimulated by TSH, transformed thyroid cells (113), mucosal and parietal cells of the stomach, cerebellar Purkinje cells, adrenal cortical cells, and some cervical cells (67, 95).

Therefore, to sum up, APE1/Ref-1 subcellular localization is quite varied. Some cell types exhibit only nuclear localization, others display only cytoplasmic, and some others show both nuclear and cytoplasmic localization. Such a complex staining pattern suggests that localization is not random but, on the contrary, is governed by a strictly regulated process. The understanding of the biological relevance of subcellular compartmentalization is now at its very beginning, but recent articles suggest that this field shows much promise.

APE1/Ref-1 FUNCTIONS, SUBCELLULAR MOVEMENT, AND TRAFFICKING

Generally, stimuli that promote APE1/Ref-1 expression are also able to promote its intracellular movement. The amount of evidence reporting APE1/Ref-1 subcellular relocalization upon a stimulus has grown exponentially in the last few years and is shown in Table 2. Most of the observed subcellular relocalizations are cytoplasm-to-nucleus APE1/Ref-1 translocations. Different cellular conditions are able to perturb the APE1/Ref-1 intracellular localization. Five categories of cellular stimuli can be identified: (a) prooxidant injuries, such as neuronal ischemia (42), hypoxia/reoxygenation in endothelial cells (2), cysteamine-induced duodenal ulceration (71), and aging (15); (b) heavy metals exposure, such as macrophages exposed to asbestos (32), and primary astrocyte cultures exposed to Pb (100); (c) direct ROS exposure, as in the case of B-lymphocytes (36, 112) or mast cells (37); (d) hormone or cytokine stimulation as in the case of TSH-stimulated thyroid cells (113), or CD40-triggering in splenic B-cells (78) and FcεRI signaling in mast cells (37); and (e) increase in intracellular Ca^{2+} concentration in both choriodecidual cells (38) and thyroid cells (116).

The question of how eukaryotic cells regulate the localization of APE1/Ref-1 with regard to the redox condition remains open. In many cases, it has been demonstrated that PT modifications, *i.e.*, phosphorylation at the level of nuclear localization signal (NLS), are responsible for the regulation of the protein levels present in the nuclear compartment. Computational analysis reveals that APE1/Ref-1 possesses a single consensus sequence for CKII at position 18–21, a region containing a NLS (TEPE). In particular, treatment of FRTL-5 thyroid cells with quercetin, a highly specific CKII inhibitor, is able to induce translocation of APE1/Ref-1 into the nucleus (unpublished observations), suggesting that the nuclear form of APE1 could be hypophosphorylated. Therefore, it is tempting to speculate that a dephosphorylating event is required for the nuclear localization of APE1/Ref-1 in thyroid cells, as in the case of the NF-AT TF (103).

As APE1/Ref-1 directly and physically interacts with redox-regulated TFs, this raises the possibility that nuclear relocalization could be, at least in part, due to a co-transportation mechanism by those TFs, such as NF-κB, that are specifically relocalized to the nucleus due to a stimulus and have been demonstrated to functionally interact with APE1/Ref-1 itself. This would not represent an isolated case of such a mechanism, being corroborated by recent reports for a role in cotransport of β-catenin by the LEF-1 TF (61).

Another interesting evidence is the case of the observed relocalization of APE1/Ref-1 into mitochondria of the B-lymphocyte Raji cell line following H_2O_2 activation (36). This relocalization is not associated with cytochrome *c* loss or with apoptosis induction, thus indicating that the APE1/Ref-1 translocation into mitochondria upon oxidative stress might exert a protective function toward mtDNA damages produced by both exogenous and endogenous ROS. Due to the relatively high molecular weight of APE1/Ref-1, which is not fully compatible with a passive mechanism of translocation through the outer membrane of mitochondria, the presence of a specific regulatory transport mechanism could be hypothesized. Although a large majority of proteins synthesized in the cytoplasm localize into mitochondria by means of an N-terminal presequence, or mitochondrial targeting sequence (MTS), a significant fraction of mitochondrial proteins lack this recognition signal. Little is known about other mitochondrial targeting signals, but there could be targeting signals within the molecule itself. This is the situation, for instance,

TABLE 2. SUMMARY OF STUDIES DESIGNED TO INDUCE APE1/REF-1 SUBCELLULAR RELOCALIZATION

Tissue/cells	*Treatment or pathological condition*	*Subcellular compartment*	*Translocation*	*Reference*	*Proposed biological role*
Rat hippocampus (CA1 neurons)	Ischemia	N	+	42	Neuronal protection against oxidative stress
Raji (B lymphocytes)	ROS	N	+	112	Redox regulation of TFs activity
		M	+	36	DNA repair
FRTL-5 (rat thyroid cells)	TSH, ROS, elevation in intracellular Ca^{2+}	N	+	113, 116	Redox regulation of TFs activity
Spleen B cells	CD40 triggering	N	+	78	Redox regulation of TFs activity
Rat kidney	Aging	N	+	15	Redox regulation of TFs activity
Human choriodecidual cells	Elevation in intracellular Ca^{2+}	N	+	38	Transcriptional repression
HUVEC	Hypoxia/reoxygenation	N	+	2	Redox regulation of TFs activity
Human macrophages	Asbestos	N	+	32	Redox regulation of TFs activity
Astrocyte primary cultures	Pb	N	+	100	Redox regulation of TFs activity
Rat duodenal mucosa	Cysteamine-induced duodenal ulceration	N	+	71	Redox regulation of TFs activity
RBL-2H3 mast cells	ROS and FcεRI-signalling	N	+	37	Redox regulation of TFs activity

HUVEC, human umbilical vein endothelial cells; M, mitochondria; N, nucleus.

in the heme lyases that have, in the third quarter of the molecule, a hydrophilic stretch of residues that represent a topogenic signal for protein sorting into the intermembrane space (23). APE1/Ref-1 does not present a classical mitochondrial N-terminal presequence while presenting, in the same region, a typical NLS in the first 20 residues. Therefore, it could be speculated that the mitochondrial localization signal may reside in another region of the molecule instead of the N-terminus. Obviously, identification of such a signal would represent an important discovery that would elucidate some of the functional roles of APE1/Ref-1. Recently, Tsuchimoto *et al.* (120) demonstrated the presence of MTS in APE2, which is a classical mammalian AP endonuclease enzyme belonging to the same functional category as APE1/ Ref-1. As opposed to APE1/Ref-1 and other members of this functional category, the presence of a 15-stretch amino acid N-terminal sequence resembling a canonical MTS is responsible for the localization of the protein within mitochondria. As in the case of APE1/Ref-1, APE2 is also localized within the nucleus of HeLa cells. Despite this evidence and opposed to APE1/Ref-1, APE2 does not possess a classical NLS that can justify nuclear localization. However, this subcellular localization seems to be ascribable to the presence of a PCNA binding motif in the C-terminus of the protein through which it physically interacts with PCNA itself.

A recent role that has been proposed for the cytoplasmic APE1/Ref-1 came from studies on endothelial and liver oxidative stress by hypoxia/reoxygenation-induced oxidative stress (2, 87). From these studies, a new role for APE1/Ref-1 in protecting cells from apoptosis induced by oxidative stress was suggested. In fact, APE1/Ref-1 is able to inhibit oxidative stress by inhibiting ROS generation by the cytoplasmic small GTPase Rac1, therefore accounting for a new and unexplored extranuclear role of APE1/Ref-1.

ALTERED EXPRESSION/DISTRIBUTION OF APE1/Ref-1 AND HUMAN PATHOLOGY

The multifunctional properties of APE1/Ref-1 closely parallel the differential expression pattern found in a wide spectrum of cells first realized by Kakolyris *et al.* in 1998 (67). In the following years, many articles observed that this heterogeneity of expression pattern is also linked to different pathological conditions ranging from metabolic to differentiative disorders. Early studies by Kakolyris *et al.* showed that different kinds of human tumors were characterized by alterations in subcellular distribution of APE1/Ref-1 with respect to normal tissue. This is the case, for instance, in colorectal carcinoma. In normal colorectal mucosa, the predominant staining is nuclear in the less differentiated cells of the lower part of the crypt, but is cytoplasmic in the more differentiated and superficial colonic epithelium. This distribution is completely disrupted during tumorigenesis because the nuclear-restricted pattern is lost in both adenoma and carcinoma, which display nuclear and cytoplasmic localization with a predomination of the latter, in front of a prominent nuclear localization in the normal tissue (65). A similar pattern has been described for breast cancer. In normal tissue, the APE1/Ref-1 localization is eminently nuclear, whereas in carcinomas, nuclear, cytoplasmic, and nuclear/cytoplasmic stainings were observed. This peculiar distribution correlates well with aggressiveness and prognosis of the tumor: nuclear localization was always associated with a better prognostic feature being related to better differentiation, low angiogenesis, and negative lymph node status. In contrast, both the cytoplasmic and the nuclear/cytoplasmic stainings were associated with poor prognostic factors, such as angiogenesis together with node and p53 positivities (66, 92). Interestingly, there seems to be no functional relationship between alterations in subcellular distribution of APE1/Ref-1 and the ability of cancerous tissue to repair abasic sites, suggesting that, at least in breast cancer, DNA repair by BER is not affected (98). As an aside, Bobola *et al.* (7) demonstrated that even an increase in AP endonucleasic activity occurs in human gliomagenesis with a concomitant elevation of APE1/Ref-1 protein expression level. Similar observations have also been made by Robertson *et al.* (97). In different germ cell tumors, such as seminomas, yolk sac tumors, and malignant teratomas, APE1/Ref-1 expression levels and DNA repair ability correlated and conferred a proportional level of protection from bleomycin treatment. These outcomes suggest that a consequence of the increase in AP activity accompanying tumorigenesis could be enhanced resistance to radiotherapy and chemotherapy.

A disregulation in nuclear versus cytoplasmic ratio toward a more cytoplasmic staining was also observed in thyroid carcinomas (113) and epithelial ovarian cancers (79) with respect to normal tissue.

In other studies, a quantitative evaluation of APE1/Ref-1 expression was taken into consideration. In cervical (138), non–small cell lung cancer (68, 91), rhabdomyosarcomas (117), and squamous cell head-and-neck cancer (72), a strong up-regulation at the nuclear level of APE1/Ref-1 was always observed. Some studies suggested that APE1/Ref-1 levels and/or subcellular disregulation may be used as a therapeutic index to indicate the sensitivity of the tumor toward radio- or chemotherapy. Koukourakis *et al.* (72) found that nuclear expression of APE1/Ref-1 in head-and-neck cancer was associated with resistance to cisplatin chemoradiation therapy and poor outcome. Herring *et al.* (53) reported the existence of an inverse relationship between intrinsic radiosensitivity and the levels of APE1/Ref-1 in cervical carcinoma. However, these are not general features of all tumors. In fact, although it has been clearly demonstrated that a subcellular disregulation was associated with the onset of the tumorigenic process in ovarian cancer (79), APE1/Ref-1 expression was ubiquitous between different epithelial ovarian cancers and was unaltered during the metastatic process (33). Moreover, APE1/Ref-1 proved not to be a useful biomarker for platinum resistance, because there was no difference in its expression among platinum-sensitive and platinum-refractory ovarian cancers. These recent outcomes highlight the fact that much work needs to be done in order to understand the real possibility of using APE1/Ref-1 as a therapeutic target.

Probably, the right direction in which to investigate the role played by APE1/Ref-1 in tumorigenesis is that indicated by recent results on PT modifications of APE1/Ref-1 itself. In particular, Orii *et al.* (86) investigated the role played by

APE1/Ref-1 in uterine leiomyomas; the most common benign smooth muscle tumors in the myometrium, by evaluating its expression at both the mRNA and protein levels. Despite the presence of a unique mRNA transcript, these authors were able to detect at least two forms of the protein by using a combination of three antibodies raised against different epitopes of the protein itself. The large form of the protein, prominent in leiomyoma extracts with respect to myometrial tissue extracts, was correlated with PCNA levels, suggesting an association with increased proliferation (86). These observations on PT modifications of APE1/Ref-1, coupled with those cited in the previous paragraph on acetylation and phosphorylation, confirm a new investigative direction for the comprehension of APE1/Ref-1 multifaceted function and for its therapeutic potential.

Cellular oxidative stress is a common pathogenic event in different disorders, therefore, it was expected that APE1/Ref-1 expression and/or subcellular localization could be affected in those pathologies where oxidative stress is a shared feature. Other than in proliferative disorders mentioned above, APE1/Ref-1 disregulation has also been demonstrated for other pathologies, particularly degenerative disorders. Gillardon *et al.* (42) observed that transient global ischemia induced by cardiac arrest was able to activate both APE1/Ref-1 mRNA expression and protein nuclear accumulation in granular cells of the ischemia-resistant dentate gyrus at 6 h after injury. In contrast, these effects where absent in CA1 pyramidal neurons of the postischemic hippocampus. Moreover, the decrease in APE1/Ref-1 protein, but not in its mRNA, was followed by an increase in the apoptotic rate, suggesting the presence of an opposite relationship existing among APE1/Ref-1 expression and apoptosis. The lack of correspondence between mRNA and protein levels emphasizes the importance of studying protein PT modifications to understand the functional roles and regulation of APE1/Ref-1 protein. Evidence published by Gillardon *et al.* (42) suggests that, in the case of CA1 pyramidal neurons, these discrepancies could be due to the presence of a posttranscriptional block during the translation process or to an increased degradation of the protein upon ischemia. Interestingly, it was also demonstrated that APE1/Ref-1 may undergo ubiquitination by Ubc9 enzyme (141). A similar decrease of APE1/Ref-1 protein levels was recently observed in motor neurons in a rabbit model of spinal cord ischemia by Sakurai *et al.* (99). It is noteworthy that this reduction preceded oxidative DNA damage and may constitute one of the factors responsible for the delay in neuronal death after spinal cord ischemia. It is probably not casual that cells characterized by a high rate of metabolic activities, such as neurons, are also vulnerable when DNA repair or redox controlling systems are defective. In fact, these cells are a particular target of the noxious effects of ROS at both the DNA and protein levels. Another important neurological disorder with which APE1/Ref-1 has been associated is Alzheimer's disease. Hippocampi of affected patients show an increase in APE1/Ref-1 levels in senile plaques and plaque-like structures (109). This evidence accounts for a wider involvement of the DNA repair mechanisms in Alzheimer's disease, as recently suggested by Davydov *et al.* (19).

Recent evidence also points to a role of APE1/Ref-1 in cardiovascular disease. Analysis of endomyocardial biopsies from patients with dilated cardiomyopathy demonstrated an association between left ventricular wall stress and up-regulation of APE1/Ref-1 expression associated with active DNA repair (4). Blood vessels are also subjected to disorders with an oxidative stress-based etiopathogenesis. In fact, ROS formation is a crucial event in atherosclerosis because it causes oxidation of low-density lipoprotein, hyperproliferation, and, when damage is excessive, apoptosis. In this light, it is not surprising that several DNA repair systems involved in BER, such as APE1/Ref-1, are associated with this pathology (76).

Finally, two other pathologies in which APE1/Ref-1 alteration is involved are inclusion body myositis (IBM) and preeclampsia. In the first case, APE1/Ref-1 localized in paired-helical filaments, amyloid-like fibrils, and amorphous material in muscle of IBM patients suggested a role played by APE1/Ref-1 in IBM pathogenesis (10). In the second case, an up-regulation of APE1/Ref-1 and TRX was associated with preeclampsia and intrauterine growth restriction in complicated pregnancies, definitively suggesting that cellular redox regulation is affected in the pathophysiology of this disorder (108).

CONCLUDING REMARKS AND PERSPECTIVES

APE1/Ref-1 is a perfect paradigm of the functional complexity of a biological macromolecule. First, as a transcriptional coactivator, it impacts on a wide variety of cellular functions ranging from the control of proliferation to apoptosis, cytokines, and hormone signaling where ROS are mainly produced and function as second messengers. Second, as a DNA-repair enzyme, it plays a fundamental role in the BER pathways of DNA lesions caused by oxidants and/or alkylating agents. Therefore, as APE1/Ref-1 is a master player of the above-mentioned cellular decisions, it is not surprising that (a) it is critical to the survival of animals (136); (b) it is ubiquitously expressed in every cell type, and (c) generally, it is quite abundantly expressed. However, for a coordinated and strictly integrated functioning of APE1/Ref-1 in the proper biological context, it is necessary that its different functions must be highly regulated. It is known that APE1/Ref-1 is an inducible protein whose expression is controlled mainly at the transcriptional level by the action of APE1/Ref-1 itself, which functions as a suppressor of its own promoter activity (62), thus constituting an autoregulatory loop. Moreover, it is coming to light that compartmentalization of the protein in different subcellular districts, such as nucleus, ER, or mitochondria, may explain the different functional specificities. This is particularly reinforced by its complex and heterogeneous staining pattern, which is typical of each cell type, and by the fact that the peculiar subcellular distribution is usually lost during cancer progression, or a cytoplasm to nucleus translocation occurs upon oxidative stimuli in different cell types. All this evidence suggests that subcellular localization and trafficking of APE1/Ref-1 should be highly regulated processes. However, they open another problem by shifting the molecular question to the mechanisms responsible for the control of a so highly regulated process. Hopefully, convinc-

ing answers will come from the studies on PT modifications occurring on APE1/Ref-1 and responsible for the coordinated control of its different functions. Recent evidence suggests that at least three kinds of PT modifications affect APE1/Ref-1 primary structure, *i.e.,* phosphorylation, redox, and acetylation, and condition its function. This way of looking at the problem of APE1/Ref-1 functioning holds the promise of representing the "Rosetta stone" for the comprehension of this unique biological molecule.

Further improvement in unraveling the "world" of APE1/Ref-1 will also come from new proteomics approaches devoted to the investigation of a biological problem as a whole. The identification of APE1/Ref-1 "interactomes" under different biological conditions will lead to a deeper understanding of the role played by this multifaceted protein in different biological systems. These outcomes will be of help in developing new strategies based on different APE1/Ref-1 functions as therapeutic targets for the several pathologies in which the protein plays a role, such as cancer, neurodegenerative, and immunological disorders. The recent chemogenomic identification of APE1/Ref-1 as a therapeutic target for asthma is suggestive for a promising scenario in the near future (82).

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ABBREVIATIONS

AP, apurinic/apyrimidinic; AP-1, activator protein-1; APE1, apurinic/apyrimidinic endonuclease 1; BER, base excision repair; CKI and CKII, casein kinase I and II; 2-DE, two-dimensional gel electrophoresis; Egr-1, early growth response protein-1; ER, endoplasmic reticulum; FEN1, flap endonuclease 1; Grx2, glutaredoxin 2; GSK3, glycogen synthase kinase 3; GzmA, granzyme A; HIF-1α, hypoxia-inducible factor-1α; HLF, HIF-1α-like factor; hnRNP-L, heterogeneous nuclear ribonucleoprotein L; H_2O_2, hydrogen peroxide, IBM, inclusion body myositis; IL, interleukin; MTS, mitochondrial targetin sequence; MH, MutY DNA glycosylase homologue; nCaRE, negative calcium-responsive regulatory element; NF-κB, nuclear factor-κB; NLS, nuclear localization signal; Ogg1, 8-oxoguanosine DNA glycosylase; Pax, paired box contaning genes; PCNA, proliferating cell nuclear antigen; PEBP-2, polyoma virus enhancer-binding protein-2; PKC, protein kinase C; PT, posttranslational; PTH, parathyroid hormone; Ref-1, redox effector factor-1; ROS, reactive oxygen species; TF, transcription factor; TRX, thioredoxin; TSH, thyrotropin; TTF-1, thyroid transcription factor-1; XRCC1, x-ray cross-species complementing 1.

REFERENCES

1. Akamatsu Y, Ohno T, Hirota K, Kagoshima H, Yodoi J, and Shigesada K. Redox regulation of the DNA binding activity in transcription factor PEBP2. The roles of two conserved cysteine residues. *J Biol Chem* 272: 14497–14500, 1997.
2. Angkeow P, Deshpande SS, Qi B, Liu YX, Park YC, Jeon BH, Ozaki M, and Irani K. Redox factor-1: an extranuclear role in the regulation of endothelial oxidative stress and apoptosis. *Cell Death Differ* 9: 717–725, 2002.
3. Asai T, Kambe F, Kikumori T, and Seo H. Increase in Ref-1 mRNA and protein by thyrotropin in rat thyroid FRTL-5 cells. *Biochem Biophys Res Commun* 236: 71–74, 1997.
4. Bartunek J, Vanderheyden M, Knaapen MW, Tack W, Kockx MM, and Goethals M. Deoxyribonucleic acid damage/repair proteins are elevated in the failing human myocardium due to idiopathic dilated cardiomyopathy. *J Am Coll Cardiol* 40: 1097–1103, 2002.
5. Bennett RA, Wilson DM 3rd, Wong D, and Demple B. Interaction of human apurinic endonuclease and DNA polymerase beta in the base excision repair pathway. *Proc Natl Acad Sci U S A* 94: 7166–7169, 1997.
6. Bhakat KK, Izumi T, Yang SH, Hazra TK, and Mitra S. Role of acetylated human AP-endonuclease (APE1/Ref-1) in regulation of the parathyroid hormone gene. *EMBO J* 22: 6299–6309, 2003.
7. Bobola MS, Blank A, Berger MS, Stevens BA, and Silber JR. Apurinic/apyrimidinic endonuclease activity is elevated in human adult gliomas. *Clin Cancer Res* 7: 3510–3518, 2001.
8. Bogenhagen DF. Repair of mtDNA in vertebrates. *Am J Hum Genet* 64: 1276–1281, 1999.
9. Boldogh I, Milligan D, Lee MS, Bassett H, Lloyd RS, and McCullough AK. hMYH cell cycle-dependent expression, subcellular localization and association with replication foci: evidence suggesting replication-coupled repair of adenine:8-oxoguanine mispairs. *Nucleic Acids Res* 29: 2802–2809, 2001.
10. Broccolini A, Engel WK, Alvarez RB, and Askanas V. Redox factor-1 in muscle biopsies of patients with inclusion-body myositis. *Neurosci Lett* 287: 1–4, 2000.
11. Caldecott KW. Mammalian DNA single-strand break repair: an X-ra(y)ted affair. *Bioessays* 23: 447–455, 2001.
12. Cao X, Kambe F, Ohmori S, and Seo H. Oxidoreductive modification of two cysteine residues in paired domain by Ref-1 regulates DNA-binding activity of Pax-8. *Biochem Biophys Res Commun* 297: 288–293, 2002.
13. Carey DC and Strauss PR. Human apurinic/apyrimidinic endonuclease is processive. *Biochemistry* 38: 16553–16560, 1999.
14. Chiarini LB, Freitas FG, Petrs-Silva H, and Linden R. 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, 2000.
15. Cho CG, Kim HJ, Chung SW, Jung KJ, Shim KH, Yu BP, Yodoi J, and Chung HY. Modulation of glutathione and thioredoxin systems by calorie restriction during the aging process. *Exp Gerontol* 38: 539–548, 2003.
16. Chung U, Igarashi T, Nishishita T, Iwanari H, Iwamatsu A, Suwa A, Mimori T, Hata K, Ebisu S, Ogata E, Fujita T, and Okazaki T. The interaction between Ku antigen and REF1 protein mediates negative gene regulation by extracellular calcium. *J Biol Chem* 271: 8593–8598, 1996.
17. Daily D, Vlamis-Gardikas A, Offen D, Mittelman L, Melamed E, Holmgren A, and Barzilai A. Glutaredoxin protects cerebellar granule neurons from dopamine-induced apoptosis by activating NF-kappa B via Ref-1. *J Biol Chem* 276: 1335–1344, 2001.
18. Daily D, Vlamis-Gardikas A, Offen D, Mittelman L, Melamed E, Holmgren A, and Barzilai A. Glutaredoxin protects cerebellar granule neurons from dopamine-induced apoptosis by dual activation of the ras-phosphoinositide 3-kinase and Jun N-terminal kinase pathways. *J Biol Chem* 276: 21618–21626, 2001.
19. Davydov V, Hansen LA, and Shackelford DA. Is DNA repair compromised in Alzheimer's disease? *Neurobiol Aging* 24: 953–968, 2003.
20. Deshpande SS, Angkeow P, Huang J, Ozaki M, and Irani K. Rac1 inhibits TNF-alpha-induced endothelial cell apoptosis: dual regulation by reactive oxygen species. *FASEB J* 14: 1705–1714, 2000.
21. Dianov GL, Sleeth KM, Dianova II, and Allinson SL. Repair of abasic sites in DNA. *Mutat Res* 531: 157–163, 2003.
22. Dianova II, Bohr VA, and Dianov GL. 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, 2001.
23. Diekert K, Kispal G, Guiard B, and Lill R. An internal targeting signal directing proteins into the mitochondrial intermembrane space. *Proc Natl Acad Sci U S A* 96: 11752–11757, 1999.
24. Droge W. Free radicals in the physiological control of cell function. *Physiol Rev* 82: 47–95, 2002.
25. Duguid JR, Eble JN, Wilson TM, and Kelley MR. Differential cellular and subcellular expression of the human multifunctional apurinic/apyrimidinic endonuclease (APE/ref-1) DNA repair enzyme. *Cancer Res* 55: 6097–6102, 1995.
26. Edwards M, Kent TA, Rea HC, Wei J, Quast M, Izumi T, Mitra S, and Perez-Polo JR. APE/Ref-1 responses to ischemia in rat brain. *Neuroreport* 9: 4015–4018, 1998.
27. Ema M, Hirota K, Mimura J, Abe H, Yodoi J, Sogawa K, Poellinger L, and Fujii-Kuriyama Y. Molecular mechanisms of transcription activation by HLF and HIF1alpha in response to hypoxia: their stabilization and redox signal-induced interaction with CBP/p300. *EMBO J* 18: 1905–1914, 1999.
28. Evans AR, Limp-Foster M, and Kelley MR. Going APE over ref-1. *Mutat Res* 461: 83–108, 2000.
29. Fan Z, Beresford PJ, Zhang D, and Lieberman J. HMG2 interacts with the nucleosome assembly protein SET and is a target of the cytotoxic T-lymphocyte protease granzyme A. *Mol Cell Biol* 22: 2810–2820, 2002.
30. Fan Z, Beresford PJ, Zhang D, Xu Z, Novina CD, Yoshida A, Pommier Y, and Lieberman J. Cleaving the oxidative repair protein Ape1 enhances cell death mediated by granzyme A. *Nat Immunol* 4: 145–153, 2003.
31. Flaherty DM, Monick MM, Carter AB, Peterson MW, and Hunninghake GW. GM-CSF increases AP-1 DNA binding and Ref-1 amounts in human alveolar macrophages. *Am J Respir Cell Mol Biol* 25: 254–259, 2001.
32. Flaherty DM, Monick MM, Carter AB, Peterson MW, and Hunninghake GW. Oxidant-mediated increases in redox factor-1 nuclear protein and activator protein-1 DNA binding in asbestos-treated macrophages. *J Immunol* 168: 5675–5681, 2002.
33. Freitas S, Moore DH, Michael H, and Kelley MR. Studies of apurinic/apyrimidinic endonuclease/ref-1 expression in epithelial ovarian cancer: correlations with tumor progression and platinum resistance. *Clin Cancer Res* 9: 4689–4694, 2003.
34. Fritz G and Kaina B. Phosphorylation of the DNA repair protein APE/REF-1 by CKII affects redox regulation of AP-1. *Oncogene* 18: 1033–1040, 1999.
35. Fritz G, Grosch S, Tomicic M, and Kaina B. APE/Ref-1 and the mammalian response to genotoxic stress. *Toxicology* 193: 67–78, 2003.
36. Frossi B, Tell G, Spessotto P, Colombatti A, Vitale G, and Pucillo C. H_2O_2 induces translocation of APE/Ref-1 to mitochondria in the Raji B-cell line. *J Cell Physiol* 193: 180–186, 2002.
37. Frossi B, De Carli M, Daniel KC, Rivera J, and Pucillo C. Oxidative stress stimulates IL-4 and IL-6 production in mast cells by an APE/Ref-1-dependent pathway. *Eur J Immunol* 33: 2168–2177, 2003.
38. Fuchs S, Philippe J, Corvol P, and Pinet F. Implication of Ref-1 in the repression of renin gene transcription by intracellular calcium. *J Hypertens* 21: 327–335, 2003.
39. Fung H, Kow YW, Van Houten B, Taatjes DJ, Hatahet Z, Janssen YM, Vacek P, Faux SP, and Mossman BT. Asbestos increases mammalian AP-endonuclease gene expression, protein levels,and enzyme activity in mesothelial cells. *Cancer Res* 58: 189–194, 1998.
40. Fung H, Bennett RA, and Demple B. 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, 2001.
41. Gaiddon C, Moorthy NC, and Prives C. Ref-1 regulates the transactivation and pro-apoptotic functions of p53 in vivo. *EMBO J* 18: 5609–5621, 1999.
42. Gillardon F, Bottiger B, and Hossmann KA. Expression of nuclear redox factor ref-1 in the rat hippocampus following global ischemia induced by cardiac arrest. *Brain Res Mol Brain Res* 52: 194–200, 1997.
43. Gorlach A, Brandes RP, Nguyen K, Amidi M, Dehghani F, and Busse R. A gp91phox containing NADPH oxidase selectively expressed in endothelial cells is a major source of oxygen radical generation in the arterial wall. *Circ Res* 87: 26–32, 2000.
44. Gorman MA, Morera S, Rothwell DG, de La Fortelle E, Mol CD, Tainer JA, Hickson ID, and Freemont PS. 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, 1997.
45. Grosch S and Kaina B. Transcriptional activation of apurinic/apyrimidinic endonuclease (Ape, Ref-1) by oxidative stress requires CREB. *Biochem Biophys Res Commun* 261: 859–863, 1999.
46. Grosch S, Fritz G, and Kaina B. Apurinic endonuclease (Ref-1) is induced in mammalian cells by oxidative stress and involved in clastogenic adaptation. *Cancer Res* 58: 4410–4416, 1998.
47. Hadi MZ, Ginalski K, Nguyen LH, and Wilson DM 3rd. Determinants in nuclease specificity of Ape1 and Ape2, human homologues of *Escherichia coli* exonuclease III. *J Mol Biol* 316: 853–866, 2002.
48. Haga S, Terui K, Zhang HQ, Enosawa S, Ogawa W, Inoue H, Okuyama T, Takeda K, Akira S, Ogino T, Irani K, and Ozaki M. Stat3 protects against Fas-induced liver injury by redox-dependent and -independent mechanisms. *J Clin Invest* 112: 989–998, 2003.
49. Hall JL, Wang X, Van Adamson, Zhao Y, and Gibbons GH. Overexpression of Ref-1 inhibits hypoxia and tumor necrosis factor-induced endothelial cell apoptosis through nuclear factor-kappaB-independent and -dependent pathways. *Circ Res* 88: 1247–1253, 2001.
50. Harrison L, Ascione G, Menninger JC, Ward DC, and Demple B. Human apurinic endonuclease gene (APE): structure and genomic mapping (chromosome 14q11.2–12). *Hum Mol Genet* 1: 677–680, 1992.
51. Harrison L, Galanopoulos T, Ascione AG, Antoniades HN, and Demple B. Regulated expression of APE apurinic endonuclease mRNA during wound healing in porcine epidermis. *Carcinogenesis* 17: 377–381, 1996.
52. Harrison L, Ascione AG, Takiguchi Y, Wilson DM 3rd, Chen DJ, and Demple B. Comparison of the promoters of the mouse (APEX) and human (APE) apurinic endonuclease genes. *Mutat Res* 385: 159–172, 1997.
53. Herring CJ, West CM, Wilks DP, Davidson SE, Hunter RD, Berry P, Forster G, MacKinnon J, Rafferty JA, Elder RH, Hendry JH, and Margison GP. Levels of the DNA repair enzyme human apurinic/apyrimidinic endonuclease (APE1, APEX, Ref-1) are associated with the intrinsic radiosensitivity of cervical cancers. *Br J Cancer* 78: 1128–1133, 1998.
54. Hill JW, Hazra TK, Izumi T, and Mitra S. Stimulation of human 8-oxoguanine-DNA glycosylase by AP-endonuclease: potential coordination of the initial steps in base excision repair. *Nucleic Acids Res* 29: 430–438, 2001.
55. Hirota K, Matsui M, Iwata S, Nishiyama A, Mori K, and Yodoi J. AP-1 transcriptional activity is regulated by a direct association between thioredoxin and Ref-1. *Proc Natl Acad Sci U S A* 94: 3633–3638, 1997.
56. Holmes EW, Bingham CM, and Cunningham ML. Hepatic expression of polymerase beta, Ref-1, PCNA, and Bax in WY 14,643-exposed rats and hamsters. *Exp Mol Pathol* 73: 209–219, 2002.
57. Hsieh MM, Hegde V, Kelley MR, and Deutsch WA. Activation of APE/Ref-1 redox activity is mediated by reactive oxygen species and PKC phosphorylation. *Nucleic Acids Res* 29: 3116–3122, 2001.
58. Hu Y, Jin X, and Snow ET. Effect of arsenic on transcription factor AP-1 and NF-kappaB DNA binding activity and related gene expression. *Toxicol Lett* 133: 33–45, 2002.
59. Huang LE, Arany Z, Livingston DM, and Bunn HF. Activation of hypoxia-inducible transcription factor depends primarily upon redox-sensitive stabilization of its alpha subunit. *J Biol Chem* 271: 32253–32259, 1996.
60. Huang RP and Adamson ED. 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, 1993.
61. Huber O, Korn R, McLaughlin J, Ohsugi M, Herrmann BG, and Kemler R. Nuclear localization of beta-catenin by interaction with transcription factor LEF-1. *Mech Dev* 59: 3–10, 1996.
62. Izumi T, Henner WD, and Mitra S. Negative regulation of the major human AP-endonuclease, a multifunctional protein. *Biochemistry* 35: 14679–14683, 1996.
63. Izumi T, Wiederhold LR, Roy G, Roy R, Jaiswal A, Bhakat KK, Mitra S, and Hazra TK. Mammalian DNA base excision repair proteins: their interactions and role in repair of oxidative DNA damage. *Toxicology* 193: 43–65, 2003.
64. Jayaraman L, Murthy KG, Zhu C, Curran T, Xanthoudakis S, and Prives C. Identification of redox/repair protein Ref-1 as a potent activator of p53. *Genes Dev* 11: 558–570, 1997.
65. Kakolyris S, Kaklamanis L, Engels K, Turley H, Hickson ID, Gatter KC, and Harris AL. Human apurinic endonuclease 1 expression in a colorectal adenoma-carcinoma sequence. *Cancer Res* 57: 1794–1797, 1997.
66. Kakolyris S, Kaklamanis L, Engels K, Fox SB, Taylor M, Hickson ID, Gatter KC, and Harris AL. Human AP endonuclease 1 (HAP1) protein expression in breast cancer correlates with lymph node status and angiogenesis. *Br J Cancer* 77: 1169–1173, 1998.
67. Kakolyris S, Kaklamanis L, Giatromanolaki A, Koukourakis M, Hickson ID, Barzilay G, Turley H, Leek RD, Kanavaros P, Georgoulias V, Gatter KC, and Harris AL. 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, 1998.
68. Kakolyris S, Giatromanolaki A, Koukourakis M, Kaklamanis L, Kanavaros P, Hickson ID, Barzilay G, Georgoulias V, Gatter KC, and Harris AL. Nuclear localization of human AP endonuclease 1 (HAP1/Ref-1) associates with prognosis in early operable non-small cell lung cancer (NSCLC). *J Pathol* 189: 351–357, 1999.
69. Kasahara T, Kuwayama C, Hashiba M, Harada T, Kakinuma C, Miyauchi M, and Degawa M. The gene expression of hepatic proteins responsible for DNA repair and cell proliferation in tamoxifen-induced hepatocarcinogenesis. *Cancer Sci* 94: 582–588, 2003.
70. Kelley MR and Parsons SH. Redox regulation of the DNA repair function of the human AP endonuclease Ape1/ref-1. *Antioxid Redox Signal* 3: 671–683, 2001.
71. Khomenko T, Deng X, Jadus MR, and Szabo S. Effect of cysteamine on redox-sensitive thiol-containing proteins in the duodenal mucosa. *Biochem Biophys Res Commun* 309: 910–916, 2003.
72. Koukourakis MI, Giatromanolaki A, Kakolyris S, Sivridis E, Georgoulias V, Funtzilas G, Hickson ID, Gat-

ter KC, and Harris AL. Nuclear expression of human apurinic/apyrimidinic endonuclease (HAP1/Ref-1) in head-and-neck cancer is associated with resistance to chemoradiotherapy and poor outcome. *Int J Radiat Oncol Biol Phys* 50: 27–36, 2001.
73. Kuninger DT, Izumi T, Papaconstantinou J, and Mitra S. 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, 2002.
74. Marenstein DR, Chan MK, Altamirano A, Basu AK, Boorstein RJ, Cunningham RP, and Teebor GW. Substrate specificity of human endonuclease III (hNTH1). Effect of human APE1 on hNTH1 activity. *J Biol Chem* 278: 9005–9012, 2003.
75. Marsin S, Vidal AE, Sossou M, Menissier-de Murcia J, Le Page F, Boiteux S, de Murcia G, and Radicella JP. Role of XRCC1 in the coordination and stimulation of oxidative DNA damage repair initiated by the DNA glycosylase hOGG1. *J Biol Chem* 278: 44068–44074, 2003.
76. Martinet W, Knaapen MW, De Meyer GR, Herman AG, and Kockx MM. Elevated levels of oxidative DNA damage and DNA repair enzymes in human atherosclerotic plaques. *Circulation* 106: 927–932, 2002.
77. Masuda Y, Bennett RA, and Demple B. Dynamics of the interaction of human apurinic endonuclease (Ape1) with its substrate and product. *J Biol Chem* 273: 30352–30359, 1998.
78. Merluzzi S, Moretti M, Altamura S, Zwollo P, Sigvardsson M, Vitale G, and Pucillo C. CD40 stimulation induces PAX5/BSAP and EBF activation through a APE/REF-1-dependent redox mechanism. *J Biol Chem* 279: 1777–1786, 2004.
79. Moore DH, Michael H, Tritt R, Parsons SH, and Kelley MR. Alterations in the expression of the DNA repair/redox enzyme APE/ref-1 in epithelial ovarian cancers. *Clin Cancer Res* 6: 602–609, 2000.
80. Nakamura H, Nakamura K, and Yodoi J. Redox regulation of cellular activation. *Annu Rev Immunol* 15: 351–369, 1997.
81. Nakshatri H, Bhat-Nakshatri P, and Currie RA. Subunit association and DNA binding activity of the heterotrimeric transcription factor NF-Y is regulated by cellular redox. *J Biol Chem* 271: 28784–28791, 1996.
82. Nguyen C, Teo JL, Matsuda A, Eguchi M, Chi EY, Henderson WR Jr, and Kahn M. Chemogenomic identification of Ref-1/AP-1 as a therapeutic target for asthma. *Proc Natl Acad Sci U S A* 100: 1169–1173, 2003.
83. Nishi T, Shimizu N, Hiramoto M, Sato I, Yamaguchi Y, Hasegawa M, Aizawa S, Tanaka H, Kataoka K, Watanabe H, and Handa H. Spatial redox regulation of a critical cysteine residue of NF-kappa B in vivo. *J Biol Chem* 277: 44548–44556, 2002.
84. Okazaki T, Chung U, Nishishita T, Ebisu S, Usuda S, Mishiro S, Xanthoudakis S, Igarashi T, and Ogata E. A redox factor protein, ref1, is involved in negative gene regulation by extracellular calcium. *J Biol Chem* 269: 27855–27862, 1994.
85. Ordway JM, Eberhart D, and Curran T. Cysteine 64 of Ref-1 is not essential for redox regulation of AP-1 DNA binding. *Mol Cell Biol* 23: 4257–4266, 2003.
86. Orii A, Masutani H, Nikaido T, Zhai YL, Kato K, Kariya M, Konishi I, Yodoi J, and Fujii S. Altered posttranslational modification of redox factor 1 protein in human uterine smooth muscle tumors. *J Clin Endocrinol Metab* 87: 3754–3759, 2002.
87. Ozaki M, Suzuki S, and Irani K. Redox factor-1/APE suppresses oxidative stress by inhibiting the rac1 GTPase. *FASEB J* 16: 889–890, 2002.
88. Parikh SS, Mol CD, Slupphaug G, Bharati S, Krokan HE, and Tainer JA. Base excision repair initiation revealed by crystal structures and binding kinetics of human uracil-DNA glycosylase with DNA. *EMBO J* 17: 5214–5226, 1998.
89. Parker A, Gu Y, Mahoney W, Lee SH, Singh KK, and Lu AL. 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, 2001.
90. Parker AR and Eshleman JR. Human MutY: gene structure, protein functions and interactions, and role in carcinogenesis. *Cell Mol Life Sci* 60: 2064–2083, 2003.
91. Puglisi F, Aprile G, Minisini AM, Barbone F, Cataldi P, Tell G, Kelley MR, Damante G, Beltrami CA, and Di Loreto C. Prognostic significance of Ape1/ref-1 subcellular localization in non-small cell lung carcinomas. *Anticancer Res* 21: 4041–4049, 2001.
92. Puglisi F, Barbone F, Tell G, Aprile G, Pertoldi B, Raiti C, Kelley MR, Damante G, Sobrero A, Beltrami CA, and Di Loreto C. Prognostic role of Ape/Ref-1 subcellular expression in stage I–III breast carcinomas. *Oncol Rep* 9: 11–17, 2002.
93. Qin J, Clore GM, Kennedy WP, Kuszewski J, and Gronenborn AM. The solution structure of human thioredoxin complexed with its target from Ref-1 reveals peptide chain reversal. *Structure* 4: 613–620, 1996.
94. Ramana CV, Boldogh I, Izumi T, and Mitra S. 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 U S A.* 95: 5061–5066, 1998.
95. Rivkees SA and Kelley MR. 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, 1994.
96. Robertson KA, Hill DP, Xu Y, Liu L, Van Epps S, Hockenbery DM, Park JR, Wilson TM, and Kelley MR. Down-regulation of apurinic/apyrimidinic endonuclease expression is associated with the induction of apoptosis in differentiating myeloid leukemia cells. *Cell Growth Differ* 8: 443–449, 1997.
97. Robertson KA, Bullock HA, Xu Y, Tritt R, Zimmerman E, Ulbright TM, Foster RS, Einhorn LH, and Kelley MR. 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, 2001.
98. Rossi O, Carrozzino F, Cappelli E, Carli F, and Frosina G. Analysis of repair of abasic sites in early onset breast cancer patients. *Int J Cancer* 85: 21–26, 2000.

99. Sakurai M, Nagata T, Abe K, Horinouchi T, Itoyama Y, and Tabayashi K. Oxidative damage and reduction of redox factor-1 expression after transient spinal cord ischemia in rabbits. *J Vasc Surg* 37: 446–452, 2003.
100. Scortegagna M and Hanbauer I. Increased AP-1 DNA-binding activity and nuclear REF-1 accumulation in lead-exposed primary cultures of astrocytes. *Neurochem Res* 25: 861–866, 2000.
101. Seo YR, Fishel ML, Amundson S, Kelley MR, and Smith ML. Implication of p53 in base excision DNA repair: in vivo evidence. *Oncogene* 21: 731–737, 2002.
102. Shen JC and Loeb LA. Mutations in the alpha8 loop of human APE1 alter binding and cleavage of DNA containing an abasic site. *J Biol Chem* 278: 46994–47001, 2003.
103. Shibasaki F, Price ER, Milan D, and McKeon F. Role of kinases and the phosphatase calcineurin in the nuclear shuttling of transcription factor NF-AT4. *Nature* 382: 370–373, 1996.
104. Slupphaug G, Kavli B, and Krokan HE. The interacting pathways for prevention and repair of oxidative DNA damage. *Mutat Res* 531: 231–251, 2003.
105. Sobol RW, Kartalou M, Almeida KH, Joyce DF, Engelward BP, Horton JK, Prasad R, Samson LD, and Wilson SH. Base excision repair intermediates induce p53-independent cytotoxic and genotoxic responses. *J Biol Chem* 278: 39951–39959, 2003.
106. Sokhansanj BA, Rodrigue GR, Fitch JP, and Wilson DM 3rd. A quantitative model of human DNA base excision repair. I. Mechanistic insights. *Nucleic Acids Res* 30: 1817–1825, 2002.
107. Suzuki S, Nagaya T, Suganuma N, Tomoda Y, and Seo H. Inductions of immediate early genes (IEGS) and ref-1 by human chorionic gonadotropin in murine Leydig cell line (MA-10). *Biochem Mol Biol Int* 44: 217–224, 1998.
108. Takagi Y, Nikaido T, Toki T, Kita N, Kanai M, Ashida T, Ohira S, and Konishi I. Levels of oxidative stress and redox-related molecules in the placenta in preeclampsia and fetal growth restriction. *Virchows Arch* 444: 49–55, 2004.
109. Tan Z, Sun N, and Schreiber SS. Immunohistochemical localization of redox factor-1 (Ref-1) in Alzheimer's hippocampus. *Neuroreport* 9: 2749–2752, 1998.
110. Tell G, Scaloni A, Pellizzari L, Formisano S, Pucillo C, and Damante G. Redox potential controls the structure and DNA binding activity of the paired domain. *J Biol Chem* 273: 25062–25072, 1998.
111. Tell G, Pellizzari L, Cimarosti D, Pucillo C, and Damante G. Ref-1 controls pax-8 DNA-binding activity. *Biochem Biophys Res Commun* 252: 178–183, 1998.
112. Tell G, Zecca A, Pellizzari L, Spessotto P, Colombatti A, Kelley MR, Damante G, and Pucillo C. An "environment to nucleus" signaling system operates in B lymphocytes: redox status modulates BSAP/Pax-5 activation through Ref-1 nuclear translocation. *Nucleic Acids Res* 28: 1099–1105, 2000.
113. Tell G, Pellizzari L, Pucillo C, Puglisi F, Cesselli D, Kelley MR, Di Loreto C, and Damante G. TSH controls Ref-1 nuclear translocation in thyroid cells. *J Mol Endocrinol* 24: 383–390, 2000.
114. Tell G, Crivellato E, Pines A, Paron I, Pucillo C, Manzini G, Bandiera A, Kelley MR, Di Loreto C, and Damante G. Mitochondrial localization of APE/Ref-1 in thyroid cells. *Mutat Res* 485: 143–152, 2001.
115. Tell G, Pines A, Paron I, D'Elia A, Bisca A, Kelley MR, Manzini G, and Damante G. 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, 2002.
116. Tell G, Pines A, Pandolfi M, D'Elia AV, Donnini D, Lonigro R, Manzini G, Russo D, Di Loreto C, and Damante G. APE/Ref-1 is controlled by both redox and cAMP-dependent mechanisms in rat thyroid cells. *Horm Metab Res* 34: 303–310, 2002.
117. Thomson B, Tritt R, Davis M, and Kelley MR. Histology-specific expression of a DNA repair protein in pediatric rhabdomyosarcomas. *J Pediatr Hematol Oncol* 23: 234–239, 2001.
118. Tom S, Ranalli TA, Podust VN, and Bambara RA. Regulatory roles of p21 and apurinic/apyrimidinic endonuclease 1 in base excision repair. *J Biol Chem* 276: 48781–48789, 2001.
119. Tomkinson AE, Bonk RT, and Linn S. Mitochondrial endonuclease activities specific for apurinic/apyrimidinic sites in DNA from mouse cells. *J Biol Chem* 263: 12532–12537, 1988.
120. Tsuchimoto D, Sakai Y, Sakumi K, Nishioka K, Sasaki M, Fujiwara T, and Nakabeppu Y. Human APE2 protein is mostly localized in the nuclei and to some extent in the mitochondria, while nuclear APE2 is partly associated with proliferating cell nuclear antigen. *Nucleic Acids Res* 29: 2349–2360, 2001.
121. Ueno M, Masutani H, Arai RJ, Yamauchi A, Hirota K, Sakai T, Inamoto T, Yamaoka Y, Yodoi J, and Nikaido T. Thioredoxin-dependent redox regulation of p53-mediated p21 activation. *J Biol Chem* 274: 35809–35815, 1999.
122. Vidal AE, Boiteux S, Hickson ID, and Radicella JP. XRCC1 coordinates the initial and late stages of DNA abasic site repair through protein–protein interactions. *EMBO J* 20: 6530–6539, 2001.
123. Walker LJ, Robson CN, Black E, Gillespie D, and Hickson ID. 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, 1993.
124. Walker LJ, Craig RB, Harris AL, and Hickson ID. A role for the human DNA repair enzyme HAP1 in cellular protection against DNA damaging agents and hypoxic stress. *Nucleic Acids Res* 22: 4884–4889, 1994.
125. Walton M, Lawlor P, Sirimanne E, Williams C, Gluckman P, and Dragunow M. Loss of Ref-1 protein expression precedes DNA fragmentation in apoptotic neurons. *Brain Res Mol Brain Res* 44: 167–170, 1997.
126. Wang N and Stemerman MB. Ref-1 and transcriptional control of endothelial apoptosis. *Circ Res* 88: 1223–1225, 2001.
127. Ward JF. The complexity of DNA damage: relevance to biological consequences. *Int J Radiat Biol* 66: 427–432, 1994.

128. Waters TR, Gallinari P, Jiricny J, and Swann PF. Human thymine DNA glycosylase binds to apurinic sites in DNA but is displaced by human apurinic endonuclease 1. *J Biol Chem* 274: 67–74, 1999.
129. Wilson DM 3rd and Barsky D. The major human abasic endonuclease: formation, consequences and repair of abasic lesions in DNA. *Mutat Res* 485: 283–307, 2001.
130. Wilson TM, Rivkees SA, Deutsch WA, and Kelley MR. Differential expression of the apurinic/apyrimidinic endonuclease (APE/ref-1) multifunctional DNA base excision repair gene during fetal development and in adult rat brain and testis. *Mutat Res* 362: 237–248, 1996.
131. Wilstermann AM and Osheroff N. Base excision repair intermediates as topoisomerase II poisons. *J Biol Chem* 276: 46290–46296, 2001.
132. Wong D, DeMott MS, and Demple B. Modulation of the 3′→5′-exonuclease activity of human apurinic endonuclease (Ape1) by its 5′-incised Abasic DNA product. *J Biol Chem* 278: 36242–36249, 2003.
133. Xanthoudakis S and Curran T. Identification and characterization of Ref-1, a nuclear protein that facilitates AP-1 DNA-binding activity. *EMBO J* 11: 653–665, 1992.
134. Xanthoudakis S, Miao G, Wang F, Pan YC, and Curran T. Redox activation of Fos-Jun DNA binding activity is mediated by a DNA repair enzyme. *EMBO J* 11: 3323–3335, 1992.
135. Xanthoudakis S, Miao GG, and Curran T. The redox and DNA-repair activities of Ref-1 are encoded by nonoverlapping domains. *Proc Natl Acad Sci U S A* 91: 23–27, 1994.
136. Xanthoudakis S, Smeyne RJ, Wallace JD, and Curran T. The redox/DNA repair protein, Ref-1, is essential for early embryonic development in mice. *Proc Natl Acad Sci U S A* 93: 8919–8923, 1996.
137. Xu W, Yan M, Sun L, Zheng Z, and Liu X. Ref-1 protein enhances the IL-2-stimulated telomerase activity. *J Cell Biochem* 88: 1120–1128, 2003.
138. Xu Y, Moore DH, Broshears J, Liu L, Wilson TM, and Kelley MR. The apurinic/apyrimidinic endonuclease (APE/ref-1) DNA repair enzyme is elevated in premalignant and malignant cervical cancer. *Anticancer Res* 17: 3713–3719, 1997.
139. Yacoub A, Kelley MR, and Deutsch WA. The DNA repair activity of human redox/repair protein APE/Ref-1 is inactivated by phosphorylation. *Cancer Res* 57: 5457–5459, 1997.
140. Yan M, Xu W, Lu L, Sun L, Liu X, and Zheng Z. Induction of ref-1 ensures AP-1 activation in intracellular oxidative environment of IL-2-stimulated BA/F3beta cells. *Biochem Biophys Res Commun* 278: 462–469, 2000.
141. Yan MD, Xu WJ, Lu LR, Sun LY, Liu XY, and Zheng ZC. Ubiquitin conjugating enzyme Ubc9 is involved in protein degradation of redox factor-1 (Ref-1). *Sheng Wu Hua Xue Yu Sheng Wu Wu Li Xue Bao (Shanghai)* 32: 63–68, 2000.
142. Yang H, Clendenin WM, Wong D, Demple B, Slupska MM, Chiang JH, and Miller JH. Enhanced activity of adenine-DNA glycosylase (Myh) by apurinic/apyrimidinic endonuclease (Ape1) in mammalian base excision repair of an A/GO mismatch. *Nucleic Acids Res* 29: 743–752, 2001.
143. Yao KS and O'Dwyer PJ. Role of the AP-1 element and redox factor-1 (Ref-1) in mediating transcriptional induction of DT-diaphorase gene expression by oltipraz: a target for chemoprevention. *Biochem Pharmacol* 66: 15–23, 2003.
144. Yao KS, Xanthoudakis S, Curran T, and O'Dwyer PJ. Activation of AP-1 and of a nuclear redox factor, Ref-1, in the response of HT29 colon cancer cells to hypoxia. *Mol Cell Biol* 14: 5997–6003, 1994.
145. Yu SL, Lee SK, Johnson RE, Prakash L, and Prakash S. The stalling of transcription at abasic sites is highly mutagenic. *Mol Cell Biol* 23: 382–388, 2003.
146. Zhou J, Ahn J, Wilson SH, and Prives C. A role for p53 in base excision repair. *EMBO J* 20: 914–923, 2001.

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