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Forum Review

Zinc Binding and Redox Control of p53 Structure and Function

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ABSTRACT

The p53 protein is a tumor suppressor often inactivated in cancer, which controls cell proliferation and survival through several coordinated pathways. The p53 protein is induced in response to many forms of cellular stress, genotoxic or not. p53 is a zinc-binding protein containing several reactive cysteines, and its key biochemical property, sequence-specific DNA binding, is dependent upon metal and redox regulation *in vitro*. In this review, we describe the main features of p53 as a metalloprotein and we discuss how metal binding and oxidation–reduction may affect p53 activity *in vivo*. In particular, we stress the possible involvement of thioredoxin, Ref-1 (redox factor 1), and metallothionein in the control of p53 protein conformation and activity. Furthermore, we also review the available evidence on the role of p53 as a transactivator or transrepressor of genes involved in the production and control of reactive oxygen intermediates. Overall, these data indicate that p53 lies at the center of a network of complex redox interactions. In this network, p53 can control the timely production of reactive oxygen intermediates (*e.g.*, to initiate apoptosis), but this activity is itself under the control of changes in metal levels and in cellular redox status. This redox sensitivity may be one of the biochemical mechanisms by which p53 acts as a "sensor" of multiple forms of stress. Antioxid. Redox Signal. 3, 611–623.

INTRODUCTION: p53, A PROTEIN AT THE HEART OF A NETWORK OF REDOX INTERACTIONS

THE P53 PROTEIN is a transcription factor (molecular mass 53,000) constitutively expressed in most cells and tissues. TP53 (MIM no. 1911170), the gene encoding p53, is located on human chromosome 17p13.1 and is frequently mutated in a wide variety of human cancers. Most of these mutations are missense mutations scattered in the region of the gene that encodes the DNA binding domain of p53, emphasizing the role of sequence-specific DNA-binding as the main biochemical property of p53 in tumor suppression. The p53 protein is weakly expressed in most normal cells. However, after activation by a variety of stress-related signals, the protein is stabilized by escape from proteosome-mediated degradation, accumulates in the nucleus, binds specifically to DNA, and regulates (positively or negatively) the transcription of several dozen downstream effector genes. In addition, p53 also binds to a number of proteins involved in DNA replication, transcription, and repair. Together, these various DNA and protein targets mediate a set of antiproliferative responses, including cell-cycle arrest, apoptosis, DNA repair, or differentiation (for reviews, see 29 and 41).

Reactive oxygen intermediates (ROI) are in-

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volved in a direct or indirect manner at several levels in the p53 signaling pathways (Fig. 1). First, ROI induce the formation of DNA strand breaks, one of the most potent signals leading to p53 induction (55). Strand break damage is signaled to p53 through a complex cascade involving among others, ATM (the product of the ataxia-telangiectasia gene) and Chk-2 (a cell-cycle regulatory kinase) (5, 33). Second, hypoxia induces p53 in a pathway that differs from the one elicited by DNA damage (26). This pathway involves an interaction of the hypoxia-inducible factor (HIF-1α) with p53, but the mechanisms of this control are still poorly understood (2, 7). Recently, it has been shown that ROI released from mitochondria regulate the

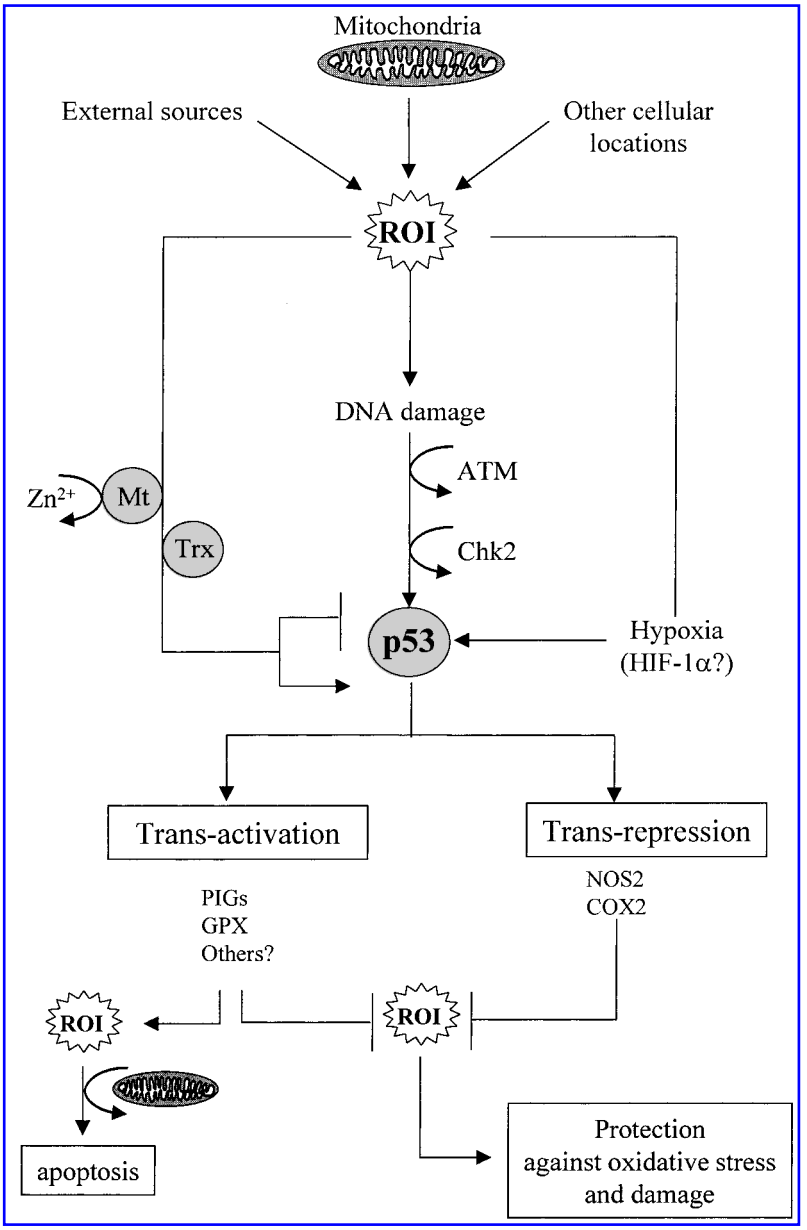

FIG. 1. Multiple roles of ROI in the p53 pathway. The p53 protein is pictured at the center of a network of redox interactions. Upstream of p53, ROI produced from intracellular or extracellular sources may induce DNA damage. This damage is signaled to p53 through a signaling cascade involving ATM, the product of the ataxia-telangiectasia gene, and the cell-cycle checkpoint kinase Chk-2. The p53 protein is also induced by hypoxia in a redox-dependent manner. Finally, the protein is intrinsically redox-sensitive and its conformation is modulated by metallothioneins (Mt) and by thioredoxin (Trx). Downstream of p53, the protein transactivates or transrepresses genes involved in the production and control of ROI. Down-regulation of ROI may participate in a protective response against DNA damage. Up-regulation of ROI may play a role in the signaling pathways of apoptosis.

cytosolic redox state and are required for stabilization of p53 protein levels in response to hypoxia (10). Third, the p53 protein is itself redox-sensitive and contains several critical cysteines located within the DNA-binding domain (for review, see 49). Three of these cysteines, together with a histidine, are involved in the tetrahedral coordination of zinc to form a protein structure that interacts with the minor groove of target DNA. Several other cysteines are located in the regions that bind within the major groove of target DNA (13). Fourth, p53 regulates the transcription of genes involved in ROI metabolism, such as the inducible forms of nitric oxide synthase (NOS2) (22) and cyclooxygenase (COX2) (64) (which are both transcriptionally repressed by p53), and glutathione peroxidase (GPX) (which is transcriptionally activated by p53) (66). Moreover, p53 also transactivates several genes known as PIGs (p53-induced genes), suspected to be involved in the production and control of ROI (62).

These observations show that ROI play several overlapping roles, both as upstream regulators and downstream effectors of p53 (Fig. 1). In this respect, the p53 protein shows functional similarities with ubiquitous, redox-sensitive transcription factors such as activating protein 1 (AP1) and nuclear factor-κB (NF-κB) (52, 61). In this review, we summarize the structural and regulatory roles of metals and ROI in the p53 signaling pathway, with particular emphasis on how these factors cooperate to control the DNA-binding capacity of the p53 protein.

p53 AS A METALLOPROTEIN

The p53 protein has the general anatomy of a transcription factor, with an N-terminal acidic domain containing a minimal transactivation domain, a central sequence-specific DNA-binding region, and a C-terminal domain that contains multiple regulatory signals and promotes the assembly of p53 into a tetramer. The C-terminus also contains a domain that binds DNA in a non–sequence-specific manner, but the role of this activity is not well understood.

The notion that p53 is a metal-dependent protein was established in 1993 when it was found that metal chelators, as well as zinc competitors, could unfold the structure of the DNA-binding domain of the protein (30, 59). These studies were based on the use of sets of monoclonal antibodies to distinguish between the correctly folded (zinc-containing) or misfolded forms of the DNA-binding domain (16, 53). The presence of tetrahedrally coordinated zinc in the DNA-binding domain was demonstrated by x-ray crystallography by Pavletich and colleagues in 1994 (13).

The DNA-binding domain is made up of an array of two β-sheets supporting large loop/helix structures directly involved in contacting DNA. These loops are bridged together by the tetrahedral coordination of a divalent zinc atom by three cysteines (residues 176, 238, and 242) and one histidine (residue 179) (Fig. 2) (13, 25). Primary sequence data suggest that this structure is conserved in the recently identified p53-related proteins, p73 and p63 (39). A comparison with other metal-dependent transcription factors indicates that the metal structure in p53 differs from the "zinc finger" structures found in many transcription factors (15). In p53, the two sets of bridging amino acids (176–179 and 238–242) are located >50 residues apart and belong to two structurally distinct entities (helix 1 and loop 3, respectively), separated by two short β-strands. Rather than forming a finger-like structure that inserts into the major groove of DNA, the bridging of zinc confers some stability to an otherwise very loose structure that contains a crucial residue, Arg^{248}, which forms the main contact between p53 and the minor groove of DNA. Another difference is that, in the "classical" zinc fingers, invariant hydrophobic residues contribute to an interior hydrophobic core in which the zinc atom is buried, forming a very stable structure (23). Such a stable structure does not exist in p53 and, to our knowledge, the p53 zinc structure has no equivalent in other proteins (other than members of the p53 family).

The structure of the DNA-binding domain of p53 shows similarities to the one of NF-κB. The latter is made of two distinct parts, each with a general architecture similar to the p53 DNA binding domain (a sandwich of two β-sheets

connected by loose loop/helix structures) (17). Although it does not contain structural zinc, several studies have shown that zinc regulates the binding of NF-κB to its target DNA (14, 40). NF-κB binds to a consensus sequence corresponding to repeats of the motif 5′-GGGRNNYYCC-3′, quite similar to the 5′-RRRC(A/T)(T/A)GYYY-3′ motif bound by p53. Actually, there are instances where the two proteins can recognize the same DNA motif as, for example, in the mouse p53 promoter (18, 73).

p53 AS A REDOX- and METALLO-REGULATED TRANSCRIPTION FACTOR

Effect of zinc and other metals on transcriptional regulation by p53

Experiments with metal chelators have provided evidence that p53 is dependent upon the coordination of zinc for both correct folding and correct binding to specific DNA in intact cells (for review see 49). Exposure of wild-type p53 synthesized *in vitro* to metal chelators such as EDTA or *o*-phenanthroline results in a rapid switch to a form immunologically similar to that of mutant p53, with loss of DNA-binding activity. This form is thought to correspond to a partially unfolded state of the DNA-binding domain, exposing an epitope recognized by the monoclonal antibody PAb240. Metal chelation results in oxidation of thiols into disulfides and cross-linking of p53 into high molecular weight aggregates (19, 30, 31).

Using recombinant p53 prepared in defined, metal-free conditions, we have shown that incorporation of zinc (measured by detection of incorporated ^{65}Zn) was required for the protein to adopt the DNA-binding capacity. Of the various transition metals tested (including Cu^{2+}, Fe^{2+}, Cd^{2+}, and Ni^{2+}), Zn^{2+} is the only one to induce such an effect. However, excess Zn^{2+} alters protein conformation and down-regulates binding to specific and nonspecific DNA (50, 57). Metals that compete with Zn^{2+} for binding to cysteines, such as Cd^{2+} and Cu^{2+}, alter p53 conformation and DNA-binding activity *in vitro* (32, 48). Cd^{2+} abrogates DNA binding in a dose-dependent manner, even in the presence of a 25-fold excess of Zn^{2+}. Copper ions exert complex effects. They bind to p53 as Cu^{1+}, but may undergo redox cycling between the Cu^{1+} and Cu^{2+} forms and induce drastic changes in p53 protein conformation (32).

The role of zinc on p53 activity in intact cells was probed using the metal chelator *N,N,N′,N′*-tetrakis(2-pyridylmethyl)ethylenediamine (TPEN), which crosses both plasma and nuclear membranes and decreases the intracellular availability of zinc. In cultured cells expressing wild-type p53, TPEN induces p53 to accumulate in the "mutant" form, unable to bind DNA. Removal of TPEN from the culture medium and addition of zinc allow p53 to fold back into the native, "wild-type" form and to recover DNA-binding activity. The restoration of wild-type p53 is not observed when cells are plated in metal-free medium after removal of the chelator, indicating that addition of exogenous zinc is essential for p53 renaturation (50, 71). Different effects have been reported with another metal chelator, *o*-phenanthroline. Exposure of cultured cells to the latter chelator increases p53 levels and DNA binding activity (65). We have shown that this effect is due to the capacity of *o*-phenanthroline to mediate the accumulation within the cell of redox-active metals, such as copper and iron, resulting in the formation of DNA strand breaks that induce p53 protein accumulation. Thus, in contrast with *in vitro* experiments, the effect of *o*-phenanthroline on p53 in intact cells is not the consequence of an alteration of zinc binding (49, 70).

Intracellular movements of zinc and copper are tightly regulated by metallothioneins (MTs), a class of inducible proteins that can bind up to seven zinc equivalents. MTs have protective roles against toxic metal stress and control physiological metal transfer reactions (45, 69). Incubation of *in vitro* translated p53 with thionein (apo-MT) shifts the p53 protein into the "mutant" form, unable to bind DNA. This effect is consistent with zinc chelation by thionein. However, different effects are observed when p53 and MT are cotransfected in p53-null cells. When p53 and MT expression plasmids are transfected in equimolar ratios, MT induces a threefold increase in p53 tran-

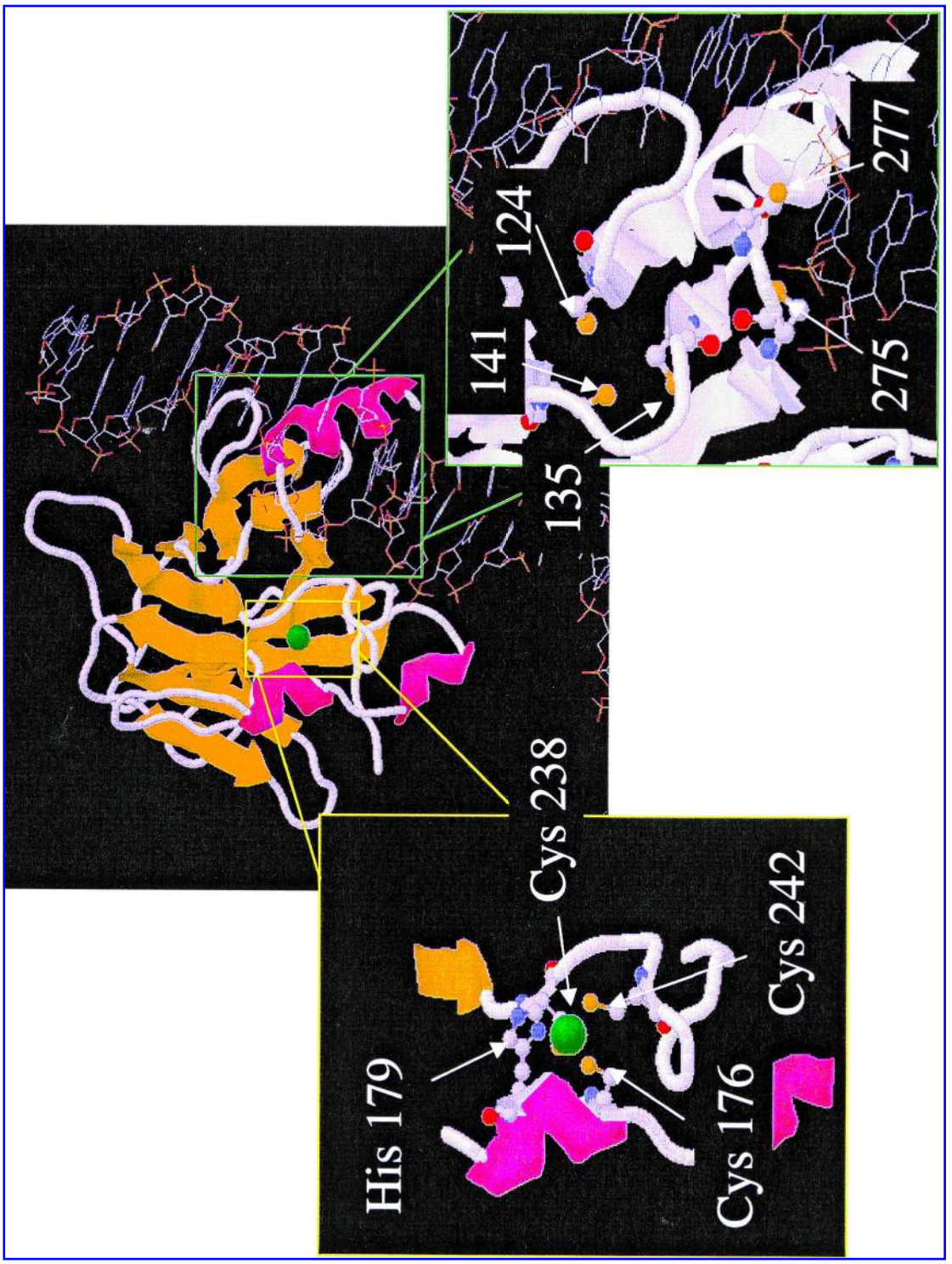

FIG. 2. Zinc-binding site and reactive cysteines within the p53 protein structure. The structure of the DNA-binding domain of the protein, in complex with its target DNA, is shown (13), with β-sheets colored in yellow, α-helixes in magenta, and loops in white. The zinc atom is shown as a green sphere. **Left inset** (yellow) is a close-up of the zinc-binding site, showing the four residues involved in the tetrahedral coordination of the metal. **Right inset** (green) is a close-up of the loops and helixes in the domain of the protein that binds in the major groove of target DNA, showing the position of five potentially reactive cysteines. The sulfur atoms are colored yellow. Pictures were generated using RasMol 2.6 software from the Protein Brookhaven Database file 1TSR, with modifications.

scriptional activity. When expressed in excess relative to p53, MT inhibits transactivation by p53 (50). These observations suggest that MT can act as a regulator of zinc binding by p53, depending on its concentrations. At low levels, MT may help p53 to fold in the "wild-type," DNA-binding conformation. At high levels, however, MT acts as a chelator to sequester zinc and thus prevents the protein from folding into its active conformation (Fig. 3). Such a regulatory role of MT has also been proposed for several zinc-binding proteins (46) and for NF-κB (1).

Effect of oxidation–reduction on transcriptional regulation by p53

Binding of p53 to DNA *in vitro* requires the addition of thiol reducing agents. The protein does not bind to DNA in the absence of β-mercaptoethanol or dithiothreitol, but can be induced to bind a target oligonucleotide in a concentration-dependent manner in their presence (up to 10 m*M* of thiol equivalents). Moreover, binding is abrogated by thiol oxidants such as diamide (19, 31, 63). The redox state of p53 affects binding to target DNA in such a way that consensus DNA sequences are recognized by reduced p53 only and not by oxidized p53, whereas nonspecific DNA is recognized equally well by both oxidized and reduced p53 (58).

It is likely that redox changes modify the cysteines involved in the binding of zinc, but there is evidence that other conserved cysteines in the DNA-binding domain may also be affected. The DNA-binding domain contains five such cysteines (residues 124, 135, 141, 275, and 277). Residues 275 and 277 form a C-X-C motif located within a loop that binds in the major groove of DNA. Cys^{277} is exposed at the protein surface and donates a hydrogen bond to bases in the major groove of DNA (13). Oxidation of Cys^{277} would prevent the formation of this hydrogen bond. It also would induce conformational and steric changes at the DNA-binding surface. Site-directed mutagenesis of the residues corresponding to Cys^{124}, Cys^{135}, Cys^{141}, and Cys^{275} in murine p53 has shown that these cysteines have a role in the redox regulation of DNA binding *in vitro* (63). These residues may be more accessible for redox modifications than those involved in the binding of zinc. Cys^{135} and Cys^{141} belong to a β-sheet located just beneath the loop containing Cys^{275} and Cys^{277} (Fig. 2). Although they are not in direct contact with DNA, their experimental mutation also decreases DNA binding. The orientation of these residues makes it unlikely that they are involved in the stable coordination of a metal ion. However, the possibility remains that several of these residues represent a secondary site for transient metallic interactions.

There is evidence to suggest that p53 function is redox-regulated *in vivo*. Treatment of cells with the thiol-antioxidant compound pyrrolidine dithiocarbamate (PDTC) has multiple effects on the p53 pathway. Several studies have shown that PDTC inhibits nuclear translocation of p53, induces a shift in conformation from "wild-type" to "mutant" form, down-regulates the DNA-binding activity of p53, and prevents transactivation of murine double minute 2 (MDM2). These multiple effects are the consequence of an increase in p53 thiol oxidation. This increased oxidation may, in turn, result from the capacity of PDTC to bind extracellular copper and induce its accumulation within the cell (70, 74, 75).

Various oxidizing agents have been shown to affect p53 transcriptional activity. Although many strong oxidants can activate p53 through a DNA-damage-dependent pathway, there is evidence that exposure of cells to high levels of hydrogen peroxide results in decreased transactivation by p53 of a target reporter construct *in vivo*. Simultaneous treatment of the cells with *N*-acetylcysteine protects the transactivation ability of p53 from the oxidative effects of hydrogen peroxide (58). A similar, paradoxical situation has been reported with nitric oxide (NO). Although nitric oxide (NO) is an inducer of DNA strand break damage and activates p53 (22), there is evidence that exposure of cells to high doses of NO donors actually turns p53 into an oxidized form similar to mutant p53 (8, 12). The recent demonstration that NO nitrates tyrosine residues in p53 protein in MCF-7 cells gives further substance to the notion that NO can directly modify the p53 protein (11).

Studies in yeast have provided perhaps the

best evidence so far for a physiological role of redox regulation in the control of p53 function. Although the existence of a yeast homologue of p53 is not established, this organism is a convenient model system for functional expression of mammalian p53. Studies by Casso and Beach (9) and by Pearson and Merrill (60) show that mutation or deletion of TRR1, the yeast gene encoding thioredoxin reductase, inhibits p53-regulated expression of a reporter gene. This observation implies that reduction of disulfides within p53 is essential for its function as a transcription factor. This finding is supported by studies in cultured mammalian cells showing that cotransfection of the genes encoding p53 and thioredoxin (Trx) enhances the transcription of p21, whereas a dominant-negative mutant TRX suppresses this effect (67). We have recently observed that p53 and Trx can be immunoprecipitated together as a complex, but whether this interaction is direct or requires other protein partners remains to be determined (unpublished data). There is also evidence that Trx and p53 colocalize in the nucleus of cells after exposure to cisplatin (68).

Trx is known to control the redox status of several transcription factors, including NF-κB and AP1 (3, 34, 35, 76). This redox regulation involves a third protein partner, redox factor 1 [Ref-1; also called apurinic-apyrimidinic endonuclease 1 (APE-1)] (20). Ref-1 is a bifunctional enzyme, with a redox and a DNA repair activity (binding to apurinic/apyrimidinic sites, followed by endonuclease removal) encoded by nonoverlapping domains (34, 78). Ref-1 stimulates p53 DNA binding *in vitro* (37) and regulates p53 transcriptional activity in intact cells (24). It is interesting to note that, through its dual function, Ref-1 can provide a molecular bridge between two pathways of p53 regulation, namely DNA-damage sensing and redox modulation. However, the exact nature of the interactions between p53 and Ref-1 remain to be determined (Fig. 3).

Ref-1 has been shown to bind to a negative regulatory region located within the 30 C-terminal residues of p53 (37). Moreover, studies in yeast indicate that deletion of the same region allows p53 to escape the requirement for thioredoxin reductase (24, 51). This region of p53 is known as one of the most important regulatory sites for the control of DNA-binding activity. Typically, specific binding of p53 to DNA *in vitro* requires the neutralization of this negative regulatory region using a specific antibody, PAb421, which recognizes an epitope in the C-terminus of p53 (36). Without neutralization of this region, thiol reducing agents cannot activate DNA binding *in vitro*. It is therefore tempting to speculate that Ref-1 may have a double function with respect to p53 activation: binding to the C-terminus to neutralize negative regulation and, in doing so, making the protein accessible to redox regulation.

Additional evidence for a role of redox regulation in the control of p53 studies has been recently obtained in cells exposed to dicoumarol, an inhibitor of the NADH quinone oxidoreductase 1. Dicoumarol was found to induce strong reduction in the levels of p53 pro-

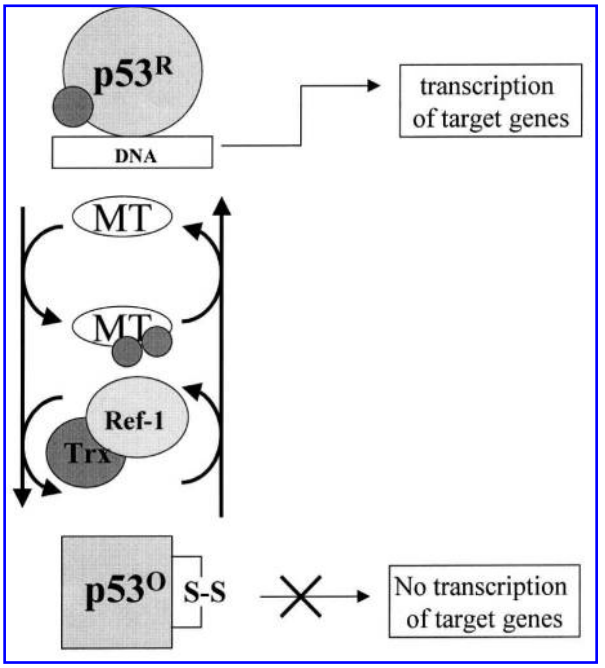

FIG. 3. MT and thioredoxin (Trx)/Ref-1 in the control of p53 protein conformation and activity: a hypothesis. The p53 protein is shown as being able to oscillate between two conformations, one corresponding to reduced active protein ("wild-type" form, circle) and the other corresponding to oxidized inactive protein ("mutant" form, square). Zinc atoms are represented as small dark circles. This model proposes that the transition of p53 from the oxidized to reduced form and conversely from the reduced to oxidized form is regulated by Ref-1 and Trx, which can either alone or in complex control the reduction state of reactive cysteines, and by MT, which controls zinc supply to p53.

tein in both normal and γ-irradiated cells, and to suppress p53-dependent apoptosis in irradiated mouse thymocytes. Whether these effects involve direct, redox modifications of the p53 protein is not known (4).

p53 AS A TRANSCRIPTIONAL REGULATOR OF REDOX EFFECTORS

Acting as either a transactivator or a transrepressor, p53 is capable of regulating a number of genes known or suspected to affect the cellular redox state. Genes specifically up-regulated in a p53-dependent manner include GPX (66) and a gene called p53-induced gene 3 (PIG3) (62). Both of these genes contain consensus p53 binding sequences in their promoter. GPX is one of the primary antioxidant enzymes that scavenges hydrogen peroxide and organic hydroperoxides with glutathione as the hydrogen donor. Using DNA chips, GPX was found to be induced in wild-type p53-containing human cells treated with etoposide (72) or exposed to γ-irradiation (42). PIG3 encodes a NADPH quinone oxidoreductase homologue, and was initially identified in a screen for genes induced by p53 before the onset of apoptosis (62). A recent study shows that the PIG3 protein is localized to the cytoplasm and induced in primary, nontransformed, and transformed cell cultures after exposure to genotoxic agents (21). It has been proposed that PIG3 is involved in the production of damaging radicals that signal the induction of apoptosis. However, expression of PIG3 is not restricted to the apoptotic response, but also occurs during p53-mediated growth arrest, with delayed kinetics as compared with other p53 targets such as p21 and MDM2 (21).

p53 also induces other PIGs with known redox function, but in which the regulatory sequences bound by p53 have not yet been isolated. These PIGS include PIG1, a member of the galectin family involved in superoxide production; PIG6, a homologue of proline oxidoreductase, a mitochondrial enzyme involved in the conversion of proline to glutamate; and PIG12, a member of the microsomal glutathione *S*-transferase gene family (62). The family of redox-regulated genes induced by p53 can be further extended to include genes activated in response to oxidative stress in cells, but for which functions have not yet been identified. These genes are likely to be involved in modulating, either directly or indirectly, the redox status of the cell. They include PIG4, which encodes a serum amyloid protein; PIG7, a gene induced by tumor necrosis factor-α; and PIG8, the human homologue of murine ei24 (42, 43, 62). Although the function of PIG8/ei24 is not known, it is important to note that its ectopic expression is strongly antiproliferative and induces the morphological features of apoptosis. Furthermore, this gene is located within a chromosomal region often altered in human tumors (11q23) (27, 28).

The p53 protein also acts as a transcriptional repressor for genes involved in redox metabolism, including the inducible forms of cyclooxygenase (COX2) (64) and of nitric oxide synthase (NOS2) (22). COX2 is up-regulated in various cancers and catalyzes the synthesis of prostaglandins from arachidonic acid. NOS2 is responsible for the production of NO, which in turn induces p53 accumulation. It is possible that down-regulation of these two genes contributes to cellular mechanisms of protection against oxidative stress.

It is somewhat paradoxical that p53 may be able to transactivate both prooxidant (for example, PIG3) and antioxidant (for example, GPX) enzymes, and also to repress enzymes generating damaging species such as NOS2. How these various and sometimes opposite activities are coordinated is not clearly understood. One hypothesis is that these genes are differentially regulated in a time-dependent manner. Indeed, there is evidence that whereas PIG3 is induced rather late in response to p53 induction (21), GPX and NOS2 are part of an earlier response (62, 66). This distribution into two groups implies that p53 may regulate an initial protective response against oxidative stress, and a secondary response in which ROI production is activated.

Such a time compartmentalization of p53-dependent gene expression may have several physiological consequences. First, the primary antioxidant response may help p53 fold into a "wild-type" active conformation during the induction phase, whereas the secondary prooxi-

dant response may be part of a feedback loop in which ROI can inactivate p53 function. Second, it is important to keep in mind that p53 plays a dual role within the cell, both as a regulator of cell-cycle arrest under conditions of mild genotoxic damage and as a trigger for apoptosis under conditions of severe genotoxic damage. The primary antioxidant response may occur preferentially in cells undergoing cell-cycle arrest, whereas the secondary prooxidant response is required for the induction of ROI as second messengers of the apoptotic cascade. Infection of HeLa cells with an adenoviral vector containing human wild-type p53, as opposed to β-galactosidase, results in a transient increase in ROI followed by alterations in the mitochondrial membrane potential and apoptosis (44). Likewise, transfection of HeLa cells with wild-type p53 results in an increase in the intracellular ROI level followed by apoptosis, whereas transfected mutant p53 (R173H) is defective in inducing both ROI production and apoptosis. Cotransfection of the cells with wild-type p53 and peroxiredoxin V, coding for an antioxidant thioredoxin peroxidase, results in a lower ROI level and in decreased apoptosis (79).

PERSPECTIVES: IS p53 A REDOX REGULATOR?

The evidence summarized in this review indicates that p53 is subject to modulation by redox and by metal changes, and that this modulation determines the activity of p53 as a transcriptional regulator of redox effectors, both antioxidants and prooxidants. The balance of reductants to oxidants within the cell, as well as the levels of physiological metals, must be critical in determining the structure and the function of p53. Factors that affect the fine tuning of this regulation may therefore have an impact on p53 function. In this respect, it is interesting to note that MT is regulated in a cell-cycle-dependent manner and accumulates in rapidly growing cells in tissues. In a colon cancer cell line, MT levels increase during G1 to reach a maximum in late G1 and at the G1/S transition (54). Such a transient increase in MT levels may affect intracellular fluxes of zinc and participate in the control of p53 activity at a crucial cell-cycle checkpoint.

The intracellular localization of p53 is also likely to play a role in controlling the redox state and thereby the function of p53. It is known that relocation of the protein to the nucleus or the cytoplasm varies according to stages of the cell cycle and to conditions to which the cells are subjected (for review, see 38). As concentrations of the redox effectors glutathione and Ref-1 are higher in the nucleus (6, 77), the redox state of p53 may be differentially affected depending on whether the protein is located in the nucleus or cytoplasm of the cell.

The sensitivity of p53 to redox modulation offers perspectives for the pharmacological modulation of p53 function. Recently, we have shown that the aminothiol WR1065 is a potent activator of p53 in cultured cells (56). The mechanism of activation of p53 by WR1065 is not known, but data from our laboratory suggest that it is not dependent on a DNA-damage pathway (O. Pluquet and P. Hainaut, unpublished data). WR1065 is a demonstrated antioxidant (47) and is currently used (in the form of a phosphorylated pro-drug, WR2721) as a cytoprotectant of normal cells in a number of cancer therapeutic protocols. It is tempting to speculate that the cytoprotective effects of WR1065 may be due at least in part to its capacity to redox modulate p53 and thereby activate a physiological cell-cycle and DNA repair checkpoint.

In addition to being at the center of a network of pathways affecting the redox balance within the cell, p53 may actually participate directly in redox reactions. As previously mentioned, when TRR1 is deleted in yeast, p53 is no longer able to stimulate reporter expression, presumably because the protein is in an oxidized state (60). Recently, Pearson and Merrill have shown that Trx is predominantly in the reduced form in wild-type yeast and in the oxidized form in TRR1-deleted yeast. Surprisingly, however, when both the TRX and TRR1 genes are deleted in *Saccharomyces cerevisiae*, p53 activates the expression of a reporter gene (Pearson and Merrill, unpublished observations). Thus, the accumulation of oxidized Trx in the absence of TRR1 induces p53 oxidation

and inactivation, but the presence of reduced Trx itself is not essential for p53 function (Fig. 4). These results suggest that p53 may play an active role in thiol interchanges with thioredoxin and, possibly, with other proteins.

These observations led us to investigate the crystal structure of p53 in search of a potential redox active site. The three cysteines that bind zinc (Cys^{176}, Cys^{238}, and Cys^{242}) are unlikely candidates, as their role is in essence a structural one. However, two other cysteines, Cys^{135} and Cys^{275}, form an interesting motif as a result of being brought in close contact with each other by the tertiary folding of the protein, even though located far apart in the primary structure of the protein (Fig. 2) (13). Mutation of the murine equivalents of Cys^{135} and Cys^{275} suggests that these two cysteines are involved in redox regulation of p53 function (63). Examination of the crystal structure of p53 shows that these two cysteines are the ones most likely to modulate the presentation of the loop– sheet–helix region of p53 to target DNA. Whether these cysteines are part of a redox-active site, or are involved in interactions with metal ions, remains to be tested experimentally.

In summary, control of zinc binding and control of redox status of p53 are two interdependent factors that play a major role in the regulation of both structure and function of this key tumor suppressor protein. Clearly, p53 belongs to the family of oxidative stress response factors found within eukaryotic cells. We postulate that p53 is the critical protein at the core of a network of redox interactions essential for the survival of those cells that are capable of DNA repair and for the apoptosis of those cells that are incapable of repair of severe genotoxic damage.

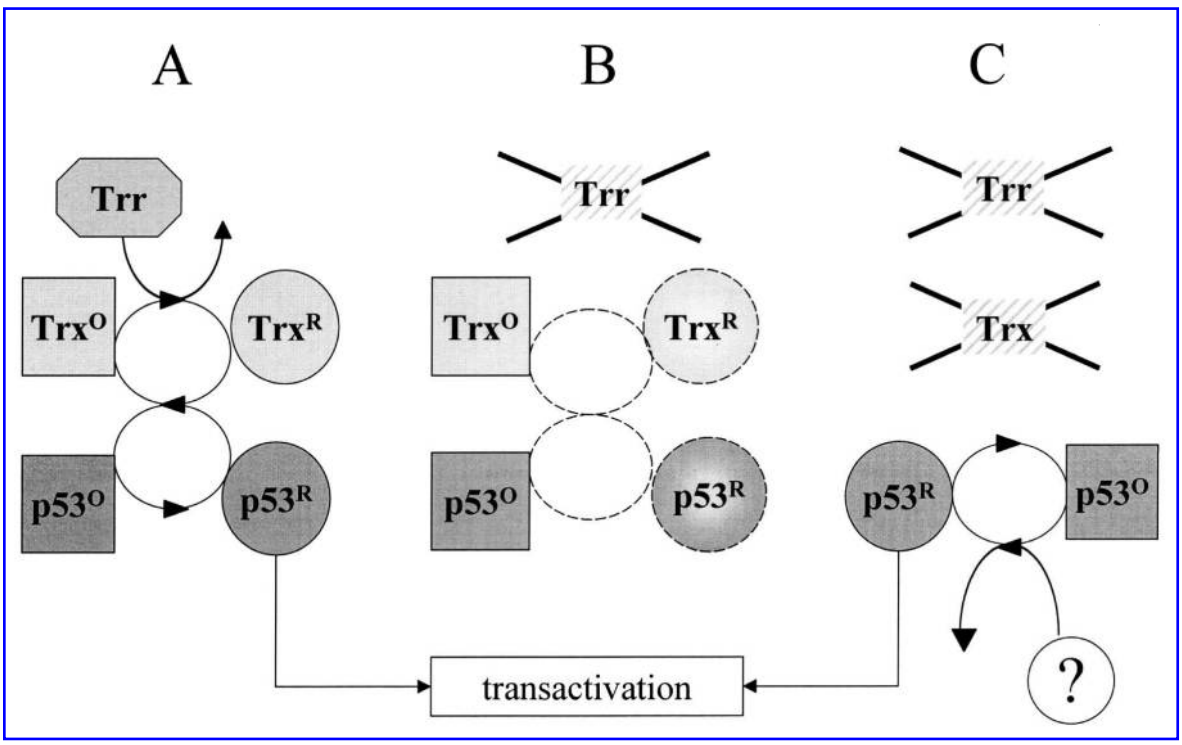

FIG. 4. Possible roles of thioredoxin reductase and thioredoxin in the control of the redox state of p53. A diagrammatic interpretation of the findings of Pearson and Merrill (60) is presented as sequential redox reactions, with thioredoxin reductase represented by a diamond (Trr), the oxidized forms of thioredoxin (Trx) and p53 represented by squares, and the reduced forms of each represented by circles. Deletions of genes, and absence of corresponding gene products, are illustrated by crossed lines, whereas dashed lines and fading shades indicate breaks in the redox pathway and the resulting absence of reduced forms of Trx and p53. (**A**) The normal situation. (**B**) The situation when the Trr1 gene is deleted, (**C**) The situation when both the Trr1 and Trx genes are deleted. In C, it is proposed that an agent (indicated by a question mark) other than Trx can maintain p53 in a reduced form.

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ABBREVIATIONS

AP1; activating protein 1 (Fos-Jun complex); ATM, ataxia telangiectasia mutated; Chk-2, cell-cycle checkpoint kinase 2; COX2, inducible cyclooxygenase; GPX, glutathione peroxidase; HIF-1α, hypoxia-induced factor 1α; MDM2, murine double minute 2; MT, metallothionein; NF-κB, nuclear factor-κB; NO, nitric oxide; NOS2, inducible nitric oxide synthase; PDTC, pyrrolidone dithiocarbamate; PIG, p53-induced gene; Ref-1, redox factor 1; ROI, reactive oxygen intermediates; TPEN, *N*,*N*,*N*′,*N*′-tetrakis(2-pyridylmethyl)ethylenediamine; TRRI, (yeast) thioredoxin reductase; Trx, thioredoxin.

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