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Redox Signalling and Transition Metals in the Control of the p53 Pathway

Catherine Méplan, Marie-Jeanne Richard† and Pierre Hainaut*‡*

*Unit of Mechanisms of Carcinogenesis, International Agency for Research on Cancer, 69372 Lyon Cedex 08 and †Laboratoire de Biochimie C, Centre Hospitalier et Universitaire Albert Michallon, Grenoble Cedex 9, France

ABSTRACT. The p53 tumour suppressor protein exerts multiple, antiproliferative effects in response to genotoxic exposures. Reactive oxygen intermediates (ROI) play several distinct roles in the p53 pathway. First, they are important activators of p53 through their capacity to induce DNA strand breaks. Second, they regulate the DNA-binding activity of p53 by modulating the redox status of a critical set of cysteines in the DNA-binding domain, which are also involved in the coordination of zinc. Third, they play a role in the signalling pathways regulated by p53, as several genes encoding redox effectors are transcriptionally controlled by p53. In this review, we summarize the evidence for the involvement of ROI at these three levels. Emphasis is placed on the role of metals and ROI as potential regulators of p53 protein conformation and functions, and on the putative toxicological consequences of such a regulation. BIOCHEM PHARMACOL **59**;1:25–33, 2000. © 1999 Elsevier Science Inc.

KEY WORDS. p53; free radicals; zinc; cadmium; cancer; cell cycle; apoptosis

The p53 tumour suppressor protein is a 53 kilodalton transcription factor constitutively expressed in most cells and tissues. *TP53* (MIM 1911170), the gene encoding p53, is located on human chromosome 17p13.1 and is frequently mutated in a wide variety of human neoplasms. Many of these mutations are missense mutations scattered in the region of the gene that encodes the DNA-binding domain of p53. The major mutation sites ("hotspots") correspond to residues with structurally well-defined roles in protein–DNA contacts. This observation underlines the role of sequence-specific DNA binding as a crucial property for the tumour suppressive activities of p53 [1]. The p53 protein has a rapid intracellular turnover and does not accumulate in most normal cells. The protein is, however, inducible by a variety of stress-related signals. Upstream, p53-activating signals share the property of representing a form of cellular or genotoxic stress (DNA-damaging chemicals, irradiation, temperature, depletion of ribonucleotides, hypoxia). The common denominator of downstream, p53-regulated effectors is that they are involved in overlapping, antiproliferative pathways modulating cell-cycle progression, apoptosis, DNA repair, differentiation, and senescence (Fig. 1) [2, 3].

ROI§ are involved at several levels in the p53 signalling pathways. First, upstream of p53, ROI represent a major source of DNA damage that activates p53, and hypoxia is a potent inducer of p53 in a pathway that differs from the one elicited by DNA damage [4, 5]. Second, the p53 protein is redox-sensitive and contains a structure which depends upon the binding of zinc to critical cysteines [6, 7]. Third, p53 regulates several genes involved in ROI metabolism, including the inducible forms of NOS (NOS2) and cyclooxygenase (COX2) (which are both transcriptionally repressed by p53), and GPx (which is transcriptionally activated by p53) [8–10]. Moreover, p53 also activates the expression of several genes that control or regulate the production of ROI during apoptosis, including quinone oxidoreductase (PIG3) and proline oxidase (PIG6) homologues, as well as glutathione transferase (PIG12) [11]. In this review, we summarize the role of ROI in the p53 signalling pathways, with particular emphasis on how redox and metal factors cooperate to control the DNA-binding capacity of the p53 protein.

STRUCTURE AND CONFORMATIONAL FLEXIBILITY OF THE P53 PROTEIN

The p53 protein has the general anatomy of a transcription factor, with an N-terminal, acidic domain containing a minimal transactivation domain, a central, DNA-binding region, and a C-terminal domain that contains multiple

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‡ Corresponding author: Pierre Hainaut, Ph.D., Unit of Mechanisms of Carcinogenesis, International Agency for Research on Cancer, 150 Cours Albert Thomas, 69372 Lyon Cedex 08, France. Tel. 33 4 72 73 85 32; FAX 33 4 72 73 83 22; E-mail: hainaut@iarc.fr

§ *Abbreviations:* ROI, reactive oxygen intermediates; NOS2, nitric oxide synthase 2; COX2, cyclooxygenase 2; GPx, glutathione peroxidase; ATM, ataxia-telangiectasia, mutated; DNAPK, DNA-dependent protein kinase; CBP, CREB-binding protein; NFkappaB, nuclear factor kappa B; Ref-1, redox factor 1; TPEN,*N,N,N′,N′*-tetrakis(2-pyridymethyl)ethylenediamine; MT, metallothioneins; DTT, dithiothreitol; NO, nitric oxide; AP 1, activator protein 1; and mdm-2, murine double minute 2.

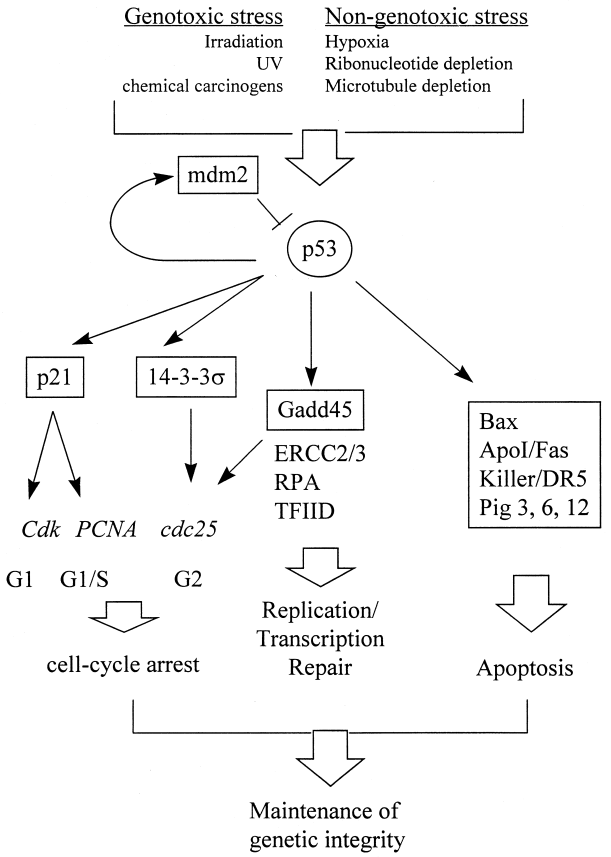

FIG. 1. An overview of the p53 signalling pathway. The p53 protein is induced by several forms of genotoxic and non-genotoxic stresses. After induction, p53 can regulate cell-cycle progression, DNA repair, and apoptosis by several mechanisms, in particular transcriptional activation or repression and direct binding to heterologous proteins. Some of the downstream effectors of p53 are indicated (boxed effectors correspond to genes regulated by p53 at the transcriptional level). All these effects contribute to a set of antiproliferative responses to maintain genetic integrity in stressed cells. The mdm-2 protein binds to p53 and inhibits its activity in an autoregulatory feedback loop. Cdk, cyclin-dependent kinase; Gadd45, growth arrest and DNA damage-inducible protein; RPA, replication protein A; DR5, death receptor 5; PCNA, proliferating cell nuclear antigen; TFII D, transcription factor II.

regulatory signals and promotes the assembly of p53 into a tetramer. The structure of the DNA-binding domain has been elucidated by x-ray crystallography (as a p53-DNA complex) [1]. This domain is made up of an array of two beta-sheets supporting large loop-helix structures directly involved in contacting DNA. These loops are bridged together by the tetrahedral co-ordination of a divalent zinc atom on three cysteines (residues 176, 238, and 242) and one histidine (residue 179) (Fig. 2A). This structure differs from the classical "zinc finger" of many transcription factors and has no known equivalent in other proteins. Primary sequence data suggest that this structure is conserved in the recently identified, p53-related proteins p73 and p63 [12]. *In vitro*, the DNA-binding domain of p53 shows a high degree of conformational flexibility, as shown by reactivity with several conformation-specific monoclonal antibodies (summarized in Fig. 2B). The so-called human "wild-type" form is reactive with the antibody PAb1620, which binds to a denaturation-sensitive epitope within the DNA-binding surface [13]. Reactivity with PAb1620 is at least partially hindered when the protein is bound to DNA [14, 15]. The so-called "mutant" form reacts with, among other antibodies, PAb240, which recognizes a primary epitope which is cryptic in the "wild-type" form [16]. This form owes its name to the fact that many common cancer mutants were found to be negative with PAb160 and positive with PAb240 [17]. This is, however, not a general rule, and it is now clear that these two forms correspond to folded (PAb1620+) and unfolded (PAb240+) forms of the protein. Several cancer mutants retain reactivity with PAb1620 (as for example mutants at codon 273, which specifies a residue in direct contact with target DNA but not involved in the p53 protein architecture) [18]. Alternatively, wild-type p53 adopts a PAb240-positive phenotype after denaturation (as for example after mild heat shock at 45°) [19]. Mutants of p53 which are temperature-sensitive for function in intact cells oscillate between these two immunological phenotypes *in vitro* by a simple temperature switch between 37° ("mutant", unfolded phenotype) and 32° ("wild-type", folded phenotype) [20].

In addition to the DNA-binding domain, the extreme C-terminus (residues 365–393) also shows conformational flexibility *in vitro* and in cultured cells. This region contains a domain which exerts a negative regulation on DNA binding. In normal cells, the C-terminus is thought to be packed against the DNA-binding domain, thus preventing stable interactions of the protein with target DNA. This conformation corresponds to the "latent" form of p53 which is present in most cells under normal conditions. After exposure to inducing factors (e.g. irradiation or genotoxic chemicals), the C-terminus adopts an extended conformation, releasing the DNA-binding domain for interaction with target DNA. This conformation corresponds to "active" p53 (Fig. 2B). *In vitro*, conversion of p53 from "latent" to "active" forms is achieved by biochemical modification of the C-terminus (acetylation, phosphorylation), by binding of heterologous proteins, or by reaction with C-terminal-specific monoclonal antibodies such as PAb421 [21, 22].

UPSTREAM OF P53: SIGNALLING OF DNA DAMAGE

The observation that p53 is inducible in response to DNA damage has led to the hypothesis that p53 is a protective factor activated by genotoxic stress to mediate a set of antiproliferative responses in order to maintain genomic integrity (Fig. 1). Agents that induce p53 include physical or chemical DNA-damaging agents (x- or gamma-irradiation, UV rays, oxidizing agents, cytotoxic drugs, and cancer-causing chemicals) (reviewed in [3]). Many of these agents induce production of ROI that can damage the

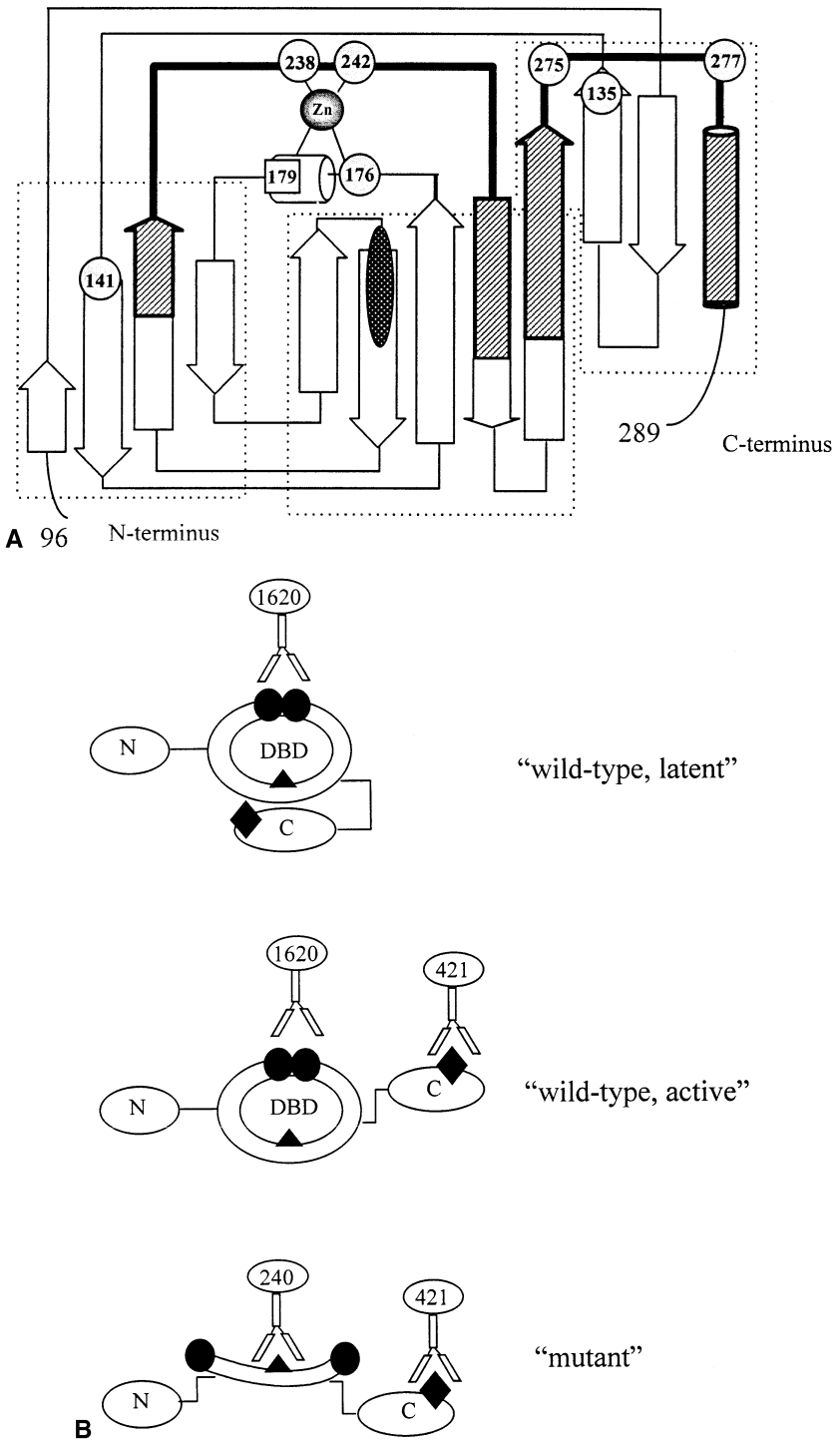

FIG. 2. (A) Topological relationships between secondary structure elements in the DNA-binding domain of p53 (residues 96–292, from Ref. [1], with modifications). The DNA-binding surface is underlined in bold. Boundaries of the two β-sheets forming the β-sandwich are shaded in grey. The position of important cysteine (circles) and of one histidine involved in the binding of zinc (square) are shown. The position of the epitope recognized by PAb240, which is cryptic in the "wild-type" form of p53, is shown as a shaded oval. (B) Representation of different conformations of the p53 protein. These conformations are defined by their reactivity with specific monoclonal antibodies. DBD, DNA-binding domain.

genome in a number of ways, in particular DNA double- and single- strand breaks, a type of damage that induces a rapid and highly specific p53 response [23]. However, p53 is also activated in response to non-genotoxic damage such as hypoxia [4, 5], depletion of microtubules [24, 25] or nucleotides [26], cytokines of the tumour necrosis factor-alpha family [27], modulation of polyamine metabolism by synthetic polyamines [28], and modulation of cell adhesion [29]. Thus, p53 behaves as a sensor of multiple forms of physical and chemical stress rather than a specific DNA

damage response factor. The time–course, extent, and biological consequences of p53 induction vary with the nature and intensity of the stress and with the type of cells or tissue considered. For example, after γ-irradiation, p53 is induced within a few hours in most cultured epithelial cells and transactivates target genes (such as the cyclin-dependent kinase inhibitor $p21^{waf-1}$) within 6 to 12 hr, leading to cell-cycle arrest in G1 (and, to a lesser extent, in G2/M). In other cell types such as fibroblast and lymphoid cells, induction of p53 by irradiation promotes apoptosis with a similar time scale. In contrast, agents that require extensive cellular metabolization before the formation of DNA damage, such as polycyclic aromatic hydrocarbons, induce p53 with a much slower time–course (within 12 to 24 hr) [30].

Induction of p53 is mostly, but not exclusively, a post-translational process by which the protein accumulates and is turned from "latent" to "active" form by conformational modifications (Fig. 3). This mechanism involves sequential, covalent, and non-covalent modifications at the N- and C-termini of the protein. It is important to realize that this process involves quantitative (protein stabilization and accumulation) and qualitative changes (acquisition of a conformation with high affinity for DNA). Two factors are crucial for the maintenance of p53 in the "latent" form. The first is the mdm-2 protein, a binding partner of p53 that targets p53 for rapid, proteasome-mediated degradation and is considered as the universal regulator of p53 protein stability [31, 32]. The second is the folding of the C-terminal domain of p53, which maintains p53 in a form with low affinity for specific DNA (see above). We have recently proposed the following three-step model to describe how p53 may be converted from the "latent" to the "active" form [3]. However, this model is probably not applicable to all pathways of p53 activation and should be taken only as an example of how multiple signals may cooperate to transmit DNA damage signals to p53.

The first step requires phosphorylation of p53 within the N-terminus to dissociate complexes with the mdm-2 protein, which conceals the transcriptional activation domain of p53 [33]. Kinases that phosphorylate p53 in this domain include the ATM protein (the product of the ataxia-telangiectasia gene) and DNAPK. These two kinases share phosphorylation sites on p53 at Ser-15 and perhaps Ser-37 [34, 35]. Both kinases are involved in the direct recognition of several forms of DNA damage, including DNA strand breaks. ATM-deficient cells are unable to activate p53 in response to γ-irradiation but not to other forms of DNA damage. Conflicting results have been reported in cells lacking DNAPK activity. These kinases are components of a network of factors which also includes Atr (AT-related, a close homologue of ATM), the chk-1 kinase (the human homologue of a yeast mitotic checkpoint kinase) and tyrosine kinase c-Abl (which binds to Atm and DNAPK and has also been reported to interact with p53 as well as with the p53 homologue p73 in irradiated cells) [36, 37]. The second step consists in an exchange of signals between the N-terminus and the C-terminus of p53. Dissociation of

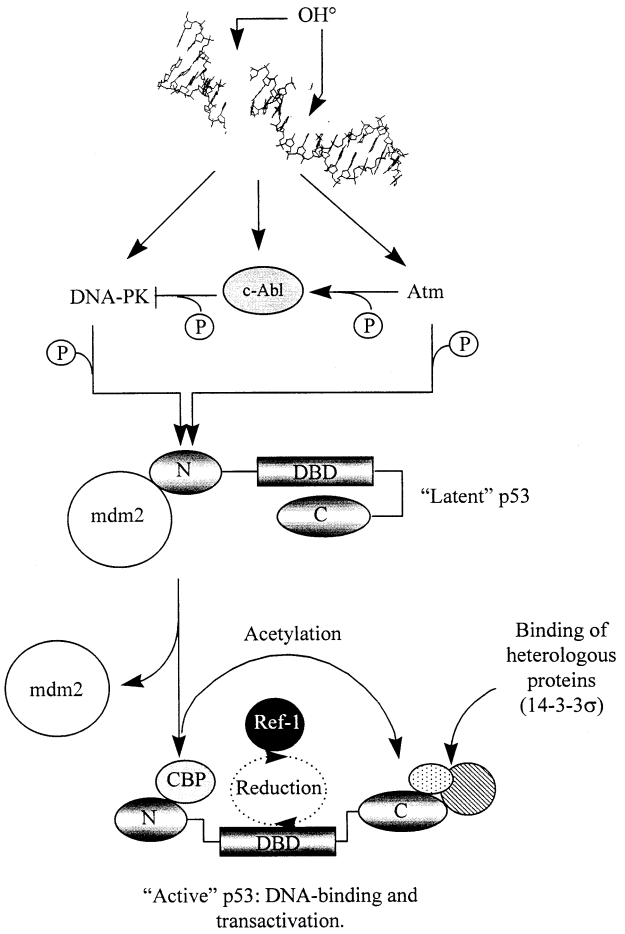

FIG. 3. Induction of p53 in response to DNA strand breaks generated by ROI. Strand break damage leads to the activation of a network of factors including the kinases DNAPK and Atm. Both kinases interact with and are regulated by the tyrosine kinase c-Abl. DNAPK and Atm phosphorylate p53 in N-terminus and contribute to dissociate p53 from mdm-2, thus stabilizing the p53 protein. Binding of acetyltransferases of the CBP/p300 family results in covalent and non-covalent modifications of the C-terminus, including acetylation, changes in phosphorylation, and binding of specific proteins such as 14-3-3 σ. The Ref-1 protein may be involved in the reduction of cysteines that play an important role in the conformation of the DNA-binding domain. For explanations and references, see text. DBD, DNA-binding domain.

mdm-2 results in p53 protein stabilization by escape from proteasome-mediated degradation [38, 39] and makes room for binding of the transcriptional co-activators of the CBP/p300 family. CBP is an acetyl transferase which acetylates conserved lysines in the C-terminus of p53 (Lys-370, -372, -373, -381, and -382) [22, 40–43]. Acetylation initiates a conformation change in the C-terminus that allows further, covalent, and non-covalent modifications. Dephosphorylation of Ser-378 creates a binding site for the signal transduction protein 14-3-3 sigma, and this association (as well as interactions with other proteins) contributes to further modify the C-terminus and to neutralize its negative regulatory effect on DNA binding [44]. The third step is hypothetical and is deduced mostly from *in*

vitro experiments using recombinant p53. In this step, we postulate that regulation by transition metals and/or by oxidation/reduction may play a role in the "fine tuning" of DNA binding. This may be achieved by reduction of cysteines of the DNA-binding domain by Ref-1, a 37 kDa redox/DNA repair enzyme that binds to the C-terminus of p53 [45]. The core domain and the C-terminus of Ref-1 bind to apurine/apyrimidine sites in damaged DNA, whereas the N-terminus has a redox activity catalyzing the reduction of sensitive cysteines in the Jun/Fos heterodimer, thereby activating the DNA-binding complex AP1 [46–48]. Ref-1 also stimulates the DNA ATF-CREB binding of Myb, activating transcription factor, CREB, early growth response-1 protein and, to a lesser extent, NFκB. Oxidized Ref-1 is thought to be regenerated by reduced thioredoxin and thus behaves as a physical link between the cellular redox machinery, DNA repair, and activation of transcription factors. We have obtained preliminary evidence that p53 may bind to Ref-1 in intact cells, but whether this association is modulated in response to DNA damage remains to be established.*

Additional factors are required in at least some pathways of p53 activation. Poly(ADP-ribose) polymerase, an enzyme that uses NAD^+ as a substrate to catalyze the formation of polymers of poly(ADP-ribose) in response to DNA strand interruptions, has been implicated as a cofactor in p53 stabilization, since its inhibition or absence partially prevents p53 accumulation in several cell types [49, 50]. Depletion of NAD^+ may affect p53 by perturbing the intracellular redox homeostasis. The hypoxia-inducible factor Hif-1 alpha binds to p53, stabilizes the protein, and stimulates the transcription of p53-dependent reporter genes [51]. In turn, p53 represses Hif-1-stimulated transcription [52]. Hypoxia causes nuclear accumulation and activation of p53 in several cell types. It has been proposed that hypoxia may act as a selection pressure for the expansion of cells with p53 mutations in the progression of cancer [5, 51].

INTRINSIC METAL/REDOX SENSITIVITY OF P53

Regulation of p53 In Vitro

Zinc binding is crucial for the stabilization of p53 in the "wild-type", folded form *in vitro*. Exposure of wild-type p53 synthesized *in vitro* to metal chelators such as EDTA or orthophenanthroline results in a rapid switch to the unfolded, "mutant-like" form [6, 53]. This effect is accompanied by loss of DNA-binding activity. Metal chelation also induces the oxidation of thiols into dissulfides and the cross-linking of p53 in high molecular weight aggregates. Although these complexes can be solubilized using thiol reductants such as DTT, renaturation to the folded, "wild-type" form by addition of zinc has not been observed *in vitro* [7, 54]. Using buffers containing EDTA and DTT to eliminate trace amounts of transition metals and to control the oxidation of cysteines, we have recently produced a form of wild-type p53 which spontaneously adopts the unfolded, "mutant-like" phenotype (PAb240-positive) and is unable to bind with high affinity to DNA *in vitro*. Upon addition of micromolar amounts of Zn^{2+}, this protein undergoes a conformational change to the PAb1620+ form and acquires DNA-binding competence. Using the radioactive isotope ^{65}Zn, we have shown that this effect is correlated with incorporation of Zn^{2+} within the protein. Of the various transition metals tested (including Cu^{2+}, Fe^{2+}, Cd^{2+}, Ni^{2+}), Zn^{2+} is the only one to induce such an effect. However, increasing the concentration of zinc results in a dose-dependent inhibition of the protein.†

Studies by other groups have also found that excess zinc alters p53 protein conformation and down-regulates binding to supercoiled DNA *in vitro* [55, 56]. Metals that compete with Zn^{2+} for binding to cysteines alter p53 conformation and DNA-binding activity *in vitro*. Cd^{2+} abrogates DNA binding in a dose-dependent manner and this effect is not reversed by a 25-fold excess of Zn^{2+}, whereas Cu^{2+} exerts complex effects which may be interpreted as a consequence of redox cycling [6, 57].

Binding of p53 to specific DNA *in vitro* also depends upon oxidation–reduction. Binding requires addition of DTT in a concentration-dependent manner (up to 10 mM). Thiol oxidants such as diamide abrogate DNA binding [7, 58]. These redox effects may be mediated by the same cysteines as those involved in the binding of zinc. However, the DNA-binding domain also contains four conserved cysteines other than those involved in the binding of zinc (residues 135, 141, 275, and 277) (Fig. 2A). 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 an hydrogen bond to bases in the major groove of DNA. Site-directed mutagenesis of the residues corresponding to Cys-275 and Cys-277 in murine p53 has shown that these residues play a role in the redox regulation of DNA binding *in vitro* [59]. These residues may be more accessible for redox modifications than those involved in the binding of zinc. Cys-135 belongs to a beta-sheet located just beneath the loop containing Cys-275 and Cys-277. The orientation of these residues makes it unlikely that they are involved in the stable coordination of a structural metal ion. However, it is possible that they may represent a secondary site for transient metal interactions.

Regulation in Cultured Cells

The metal chelator TPEN has been shown to cross plasma and nuclear membranes and to decrease the availability of intracellular zinc. In cultured cells expressing wild-type p53, we have shown that TPEN induces p53 to accumulate in a "mutant-like" form, unable to bind DNA. Removal of

* Méplan C, unpublished data.

† Méplan C, Richard MJ and Hainaut P, manuscript in preparation.

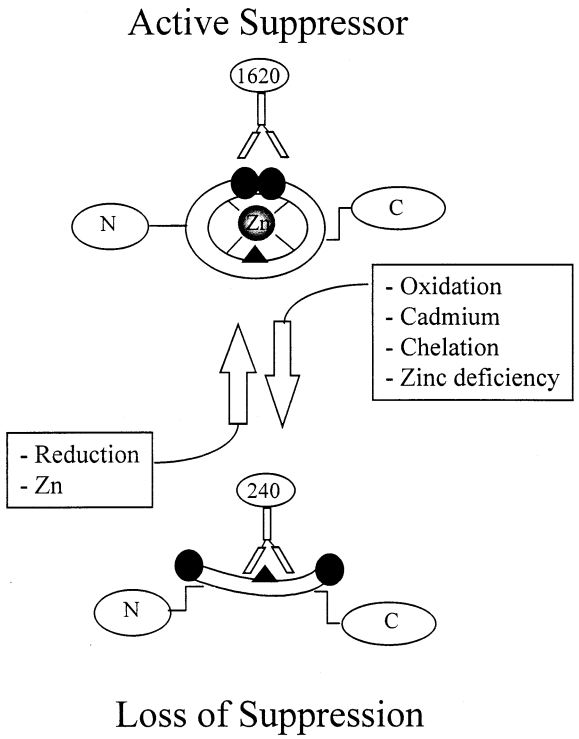

FIG. 4. Role of metals and oxidation–reduction in the control of p53 conformation. This model proposes that metal binding and oxidation of cysteines regulate the conformation of the DNA-binding domain of p53. Factors that affect this metal and redox-dependent equilibrium are indicated.

TPEN from the culture medium allows p53 to fold back into the native, "wild-type" form and to recover DNA-binding activity [60]. Refolding into the "wild-type" form requires the presence of micromolar amounts of Zn^{2+} in the culture medium and is not observed under metal-free conditions (Méplan *et al.*, see footnote page 5) (Fig. 4). Additional evidence for a role of transition metals in the control of p53 was obtained with pyrollidine dithiocarbamates (PDTC), a class of antioxidants which has been shown to regulate the activity of several redox-dependent transcription factors. Similar to TPEN, PDTC inhibits the DNA-binding activity of p53 [61, 62]. This inhibition is correlated with intracellular accumulation of copper ions, a well-known effect of PDTC. Blocking copper uptake with bathocuproine disulfonic acid (BCS), a chelator that does not penetrate into cells, prevents inhibition of p53 by PDTC [62]. These results are in agreement with *in vitro* data showing that copper ions bind to p53 and disrupt its native conformation [57] and further suggest that small changes in intracellular transition metals such as zinc or copper may drastically alter p53 protein activity.

Intracellular metal transfer reactions involving zinc and copper are tightly regulated by the MTs, a class of inducible proteins that bind zinc with high affinity (up to seven Zn^{2+} ions per molecule of MT) and contribute to protect cells against toxic metal stress. MTs are expressed in several isoforms, including the ubiquitous MT I and MT II forms [63]. *In vitro*, incubation of p53 with recombinant MT turns the p53 protein into an inactive, "mutant-like" form. In intact cells, transfection of MTs has biphasic effects on p53. When transfected at high copy numbers (p53:MT plasmid ratio of 1:10), human MT IIa inhibits p53 protein activity, consistent with a metal-chelating effect. However, at lower concentrations (p53:MT plasmid ratio of 1:1), MT IIa stimulates p53 transcriptional activity by a factor of 2–3 (Méplan *et al.*, see footnote page 5). The latter effect suggests that MT acts as a metal chaperone to regulate metal supply to p53 and to control the fine tuning of specific DNA binding.

DOWNSTREAM OF P53: REGULATION OF ROI PRODUCTION

Once activated, p53 acts as a transcription factor to promote or repress the expression of genes containing p53-binding sequences in their promoter or regulatory regions. Several of these genes are involved in the production or control of reactive oxygen species (see Table 1). First, p53 transrepresses the promoters of two inducible genes, *COX2* [10] and *NOS2* [8]. COXs are key enzymes in the conversion of arachidonic acid to prostaglandins and other eicosanoids. NOS is the enzyme that catalyzes the formation of NO, a regulator of vascular permeability, from L-arginine. NO is a putative endogenous mutagen which induces p53 protein accumulation and activation as a result of the formation of oxidative DNA damage. Expression of wild-type p53 in a variety of human tumour cell lines, as well as in murine fibroblasts, results in down-regulation of NOS2 expression through inhibition of the NOS2 promoter. These results suggest the existence of a negative feedback loop in which p53 safeguards against NO-induced DNA damage through transrepression of NOS2. As both enzymes induce the formation of basic mediators of angio-

TABLE 1. Redox effectors regulated by p53

Factor	Activity	Mode of regulation	Reference
COX2	Inducible cyclooxygenase	Transcriptional repression	10
GPx	Glutathione peroxidase	Transcriptional activation	9
NOS2/iNOS	Inducible nitric oxide synthase	Transcriptional repression	8
Pig-12	Glutathione Transferase homologue	Transcriptional activation	11
Pig-3	Quinone oxidase homologue	Transcriptional activation	11
Pig-6	Proline oxidase homologue	Transcriptional activation	11

genesis, repression by p53 may be part of an anti-angiogenic pathway contributing to tumour suppression. Loss of p53-dependent repression may explain the enhanced expression of NOS2 and COX2 in many cancers. Second, p53 up-regulates GPx through a p53-binding element localized in the promoter [9]. GPx is a primary antioxidant enzyme that scavenges hydrogen peroxide or organic hydroperoxides. Although this mechanism is expected to contribute to reduce intracellular levels of potentially damaging ROI, its role in tumour suppression by p53 is not understood. Third, p53 specifically regulates a number of redox enzymes during the induction of apoptosis [11]. PIG 3, 6, and 12 are three cDNA isolated by serial analysis of gene expression in colon cancer cells undergoing p53-dependent apoptosis [11]. PIG 3 encodes an NADPH quinone oxidoreductase homologue, PIG 6 is a proline oxidase homologue, and PIG 12 is a homologue of microsomal glutathione transferase. Although the exact role of these genes in the p53 pathways is not elucidated, there is good evidence that they participate in a set of responses that promotes apoptosis through the production of intracellular ROI. Overexpression of p53 in cultured colon cancer cells induces the production of intracellular ROI and the depletion of GSH levels, resulting in mitochondrial damage. Furthermore, apoptosis induced by p53 is at least partially prevented by antioxidants or by agents that compensate for depletion of GSH such as *N*-acetyl cysteine. These observations suggest that ROI act as second messengers in some of the p53-mediated pathways of apoptosis.

TOXICOLOGICAL SIGNIFICANCE OF METAL/REDOX REGULATION OF P53

The observation that p53 is intrinsically sensitive to oxidative stress and to transition metals has raised the hypothesis that p53 activity may be transiently or permanently defective in zinc-deficient cells or in cells undergoing excessive exposure to toxic metals and/or free radicals. Substantial direct and indirect evidence of such a mechanism has been obtained in experimental systems. Drugs that deplete pools of glutathione, for example, down-regulate the transcriptional activity of p53. However, there is no proof to date that this effect is due to oxidation of p53, and it should be noted that depletion of glutathione also activates the WAF-1 promoter, indicating that $p21^{waf\text{-}1}$ is activated in a p53-independent pathway of response to oxidative stress [64]. In a recent study, Mann and co-workers have shown that hydrogen peroxide added in the medium of cultured cells induces the inactivation of wild-type p53 by direct oxidation of the protein [65]. Synthetic nitric oxide donors (such as *S*-nitroso-*N*-acetylpeniciuamine and *S*-nitrosoglutathione) have been shown to induce a conformational switch of "wild-type" p53 to the unfolded, "mutant-like" form, with loss of DNA-binding activity *in vitro* [66]. Such a mechanism may enhance the carcinogenic potential of free radicals by switching off the tumour-suppressive activity of p53 (Fig. 4).

We have proposed that perturbation of the metal-dependent folding of p53 could play a role in the carcinogenicity of cadmium, a metal which is chemically close to zinc. Cadmium is a widespread environmental pollutant which binds with high affinity to cysteine thiolate clusters and is a demonstrated carcinogen in humans and animals. Exposure of cells expressing wild-type p53 to Cd^{2+} induced a dose-dependent disruption of "wild-type" conformation, with loss of DNA binding, attenuation of transcriptional activity, and inhibition of p53-dependent responses to DNA damage induced by γ-irradiation. Full inhibition of p53 was observed at toxic concentrations of Cd^{2+} (30 μM), but non-toxic concentrations (10 μM) were sufficient to reduce by more than 50% the extent of p53 protein induction after exposure to DNA-damaging agents [67]. Overall, inhibition of p53 by Cd^{2+} would allow cells to proliferate under genotoxic stress conditions that normally trigger a protective, cell-cycle arrest response.

CONCLUSIONS

ROI play important regulatory roles in the signalling pathways upstream and downstream of p53, as well as in the modulation of the activity of the p53 in itself. ROI participate in p53 protein induction through their capacity to create DNA strand breaks as well as other forms of DNA damage. In turn, p53 activates genes that regulate ROI production. In this context, the intrinsic redox and metal sensitivity of the p53 protein may be seen as part of a complex, autoregulatory feedback network that modulates the fine tuning of p53 activity. Many transcription factors are redox targets. The best described examples are the Fos/Jun heterodimeric complex AP1 and the ubiquitous transcription factor NFκB (see papers by Bours *et al.*, Bowie and O'Neill, and Herrlich and Böhmer in this issue). Both are highly inducible factors important for the cellular response to oxidative stress. As in the case of p53, ROI play several, distinct roles in the control of AP1 and NFκB [68, 69]. First, ROI activate specific signalling cascades upstream of AP1 and NFκB. In the case of AP1, ROI initiate a cascade of molecular events leading to the activation of the mitogen-activated protein kinase pathway and to phosphorylation of Fos/Jun proteins. In the case of NFκB, ROI directly or indirectly contribute to trigger the IκB kinase, leading to the phosphorylation and degradation of IκB and to the translocation of the active, p50–p65 NFκB complex to the nucleus. Second, both protein complexes contain regulatory cysteines at the surface of the proteins in contact with DNA. These cysteines need to be reduced for high-affinity binding to DNA. Third, both AP1 and NFκB control the expression of genes involved in antioxidant responses, such as glutathione *S*-transferase in the case of AP1 and manganese-dependent superoxide dismutase in the case of NFκB. Thus, the three levels of ROI involvement that we have defined for p53 can also be identified for AP1 and NFκB.

The data summarized here have three important impli-

cations. First, metal ions such as zinc might behave as signalling molecules in the control of p53 and of other factors. Interestingly, MT levels have been shown to vary during cell-cycle progression, with a sharp peak at the G1 to S transition [70]. This observation suggests that changes in metal supply may have a signalling or regulatory role during cell cycle. Second, disruption of metal/redox control (e.g. by exposure to NO or Cd^{2+}) may represent a mechanism for inactivation of p53 function in some cancer or precancer conditions. Third, the fine tuning of p53 activity as a function of ROI and metal levels may modulate the affinity of the p53 for promoters containing p53-binding sites, thereby helping the protein to discriminate among subsets of target genes after activation of p53. Although these hypotheses require further experimental evaluation, these putative effects of metals and ROI on the p53 protein may have implications for cancer prevention and therapy.

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