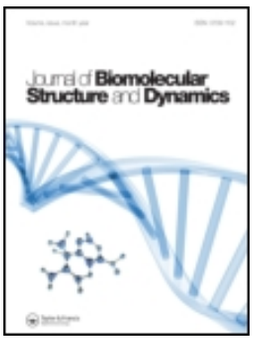

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Summary

Wild type human full length (f.l.) tumor suppressor p53 protein binds preferentially to supercoiled (sc) DNA in vitro both in the presence and absence of the p53 consensus sequence (p53CON). This binding produces a ladder of retarded bands on the agarose gel. Bands revealed by immunoblotting with antibody DO-1 corresponded to the ethidium stained retarded bands. The intensity and the number of bands of p53-scDNA complex were decreased by physiological concentrations of unchelated zinc ions. Nickel and cobalt ions inhibited binding of p53 to scDNA and to p53CON in linear DNA fragments less efficiently than zinc. Compared to the intrinsic zinc strongly bound to Cys 176, Cys 238, Cys 242 and His 179 in the p53 core domain, binding of additional Zn^{2+} to p53 was much weaker as shown by an easy removal of the latter ions by low concentrations of EDTA. Oxidation of the protein with diamide resulted in a decrease of the number of the retarded bands. Under the same conditions, no binding of oxidized p53 to p53CON in a linear DNA fragment was observed. In agreement with the literature oxidation of f.l. p53 with diamide was irreversible and was not reverted by an excess of DTT. We showed that in the presence of 0.1 mM zinc ions, oxidation of p53 became reversible. Other divalent cations tested (cadmium, cobalt, nickel) exhibited no such effect. We suggested that the irreversibility of p53 oxidation was due, at least in part, to the removal of intrinsic zinc from its position in the DNA binding domain (after oxidation of the three cysteines to which the zinc ion is coordinated in the reduced protein) accompanied by a change in the p53 conformation. Binding of C-terminal anti-p53 antibody also protected bacterially expressed protein against irreversible loss of activity due to diamide oxidation. Binding the human p53 core domain (segment 94-312) to scDNA greatly differed from that observed with the full-length p53. The core domain did not posses the ability to bind strongly to many sites in scDNA regardless of the presence or absence of p53CON suggesting involvement of some other domain (probably C-terminal) in binding of the full-length p53 to scDNA. Supershift experiments using antibodies against p53 N- or C-terminus suggested that in oxidized p53, scDNA binding through the C-terminus gained importance.

Introduction

Protein p53 was discovered twenty years ago as a 53-kDa protein overexpressed in cells transformed with SV40 virus (reviewed in (1); (2-4)). The protein tightly bound to SV40 large T - antigen and was originally considered to be product of an oncogene. However, later extensive studies of this molecule lead to conclusion that normal biological role of p53 is to act as a tumor suppressor (reviewed in (5-12)). Protein p53 is a stress-inducible transcription effector that activates expression of genes involved in cell cycle arrest (p21/Waf1/Cip1), apoptosis (Bax) or DNA repair (GADD45). One of the p53-inducible genes is also Mdm2, a negative regulator of p53 involved in p53 nuclear export, degradation in proteosomes and, consequently, maintaining of low p53 levels under normal conditions (13,14). Genotoxic stresses such as DNA damage induce activation of p53 function by increasing the protein level, its stabilization and activation by posttranslational modification (15-21). Subsequently, expression of p53 downstream genes is activated. This mechanism

prevents the cell to accumulate damaged DNA in further generations: the p53 pathway either arrests cell cycle in G1 or G2 phases (10,22), allowing the repair machinery to fix DNA damage prior to replication or mitosis, or triggers apoptosis (23,24). Mutations in the p53 gene that prevent normal function of the p53 protein frequently result in genomic instability and malignant transformation of the cells (12,25).

A crucial feature of p53 protein is its ability to bind DNA (19, 26-33). The protein consists of four structure-functional domains two of which possess DNA-binding properties (Figure 1) (19,32,34). The core domain (amino acids 102-292 in human protein) contains a DNA binding site that recognizes p53 consensus sequence (p53CON) consisting of two copies of a decanucleotide 5′-PuPuPuC(A/T)(T/A)GPyPyPy-3′ separated by 0-13 base pairs (35). X-ray crystallography identified a structure of two large loops held together by a tetrahedrally coordinated zinc ion and a loop-sheet-helix motif representing the DNA-binding surface of the p53 core (26). The same domain was shown to interact also non-specifically with internal segments of long single- and double-stranded DNA molecules (31). Another DNA-binding site of p53 is located in the carboxy-terminal domain, in a basic region close to the extreme C-terminus. This site binds non-sequence-specifically to single-stranded DNA ends, and to mismatched or damaged DNA (36-40). This C-terminal DNA binding site coincides with region that negatively regulates DNA binding by the core domain in non-activated (latent) p53 (38). It was observed that small nucleic acid molecules activates p53 core domain through blocking of the inhibition function of the C-terminus, and that the two DNA-binding activities of p53 interfere reciprocally (31,37). It has been proposed that recognition of damaged DNA by the p53 C-terminus may trigger p53 activation.

Preferential Binding of Full Length Wild Type p53 to Supercoiled DNA

Until recently, the relation between DNA superhelicity and p53 DNA binding was not considered. We have found that human wild type p53 strongly binds to negatively supercoiled (sc) plasmid DNA, regardless of the presence or absence of p53CON (41-43). Depending on the protein/DNA ratio, complexes of p53 with scDNA produced a ladder of bands in agarose gel (Figure 2). After immunoblotting of the electrophoresed p53-DNA complexes, ladder of p53 bands was obtained identical to the retarded DNA bands on ethidium-stained gel (42,43) (Figure 2). Recently, several groups have addressed the questions of the influence of the p53 sequence-specific DNA binding on DNA bending and twisting (44,45), and of the interaction of p53 with non-B DNA structures that can be stabilized by negative DNA superhelicity (46-48).

Modulation of p53 Binding to scDNA and to p53CON by Metal Ions

We have shown that physiological concentrations of zinc ions efficiently inhibit binding of f.l. p53 to scDNA (42). 5 μM Zn^{2+}

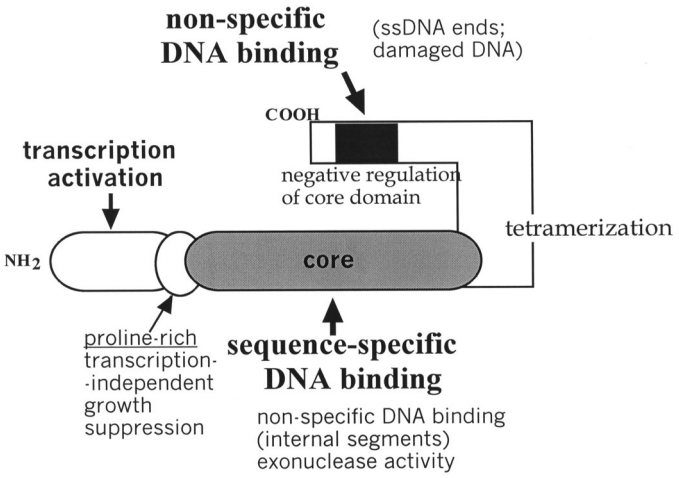

Figure 1: Scheme of structure-functional domains of p53 protein. DNA-binding sites are located in the protein core and in the basic C-terminal domain.

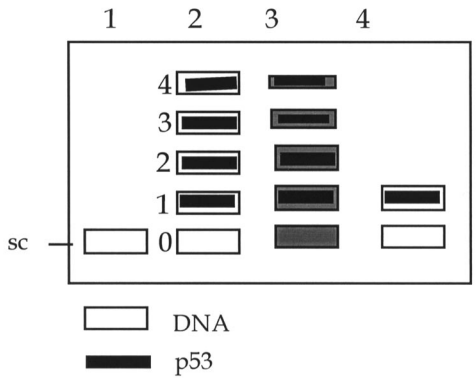

Figure 2: Schematic representation of scDNA ethidium staining in agarose gel and DO-1 immunoblot of p53. Sc, supercoiled DNA of (lanes 1,2,4) pBluescript $SK_{(-)}$ (without p53 consensus sequence, p53CON) or (lane 3) pPGM1 (with p53CON). Lane 1, mobility of free sc DNA in agarose gel. Complexes of f.l. p53 with scDNAs yield a ladder of bands, regardless of the presence or absence of p53CON (lanes 2,3). Due to p53 oxidation, number of the retarded bands decreases (lane 4).

partially inhibited binding to scDNA while 20 μM exhibited full inhibition. This inhibition was cancelled by relatively low concentrations of EDTA or DTT suggesting that binding of additional Zn^{2+} ions to p53 is substantially weaker than that of the intrinsic zinc in the core domain. Sequence-specific DNA binding was influenced by zinc ions in a similar manner (42). The latter results are at variance with the results of Coffer and Knowles (Coffer & Knowles, 1994) that 100 μM Zn^{2+} has no effect on p53 binding to p53CON in a 30-mer oligonucleotide. Although the reason for this discrepancy is not completely clear, it cannot be excluded that the presence of EDTA (and/or DTT) might have influenced the results reported by Coffer and Knowles.

Inhibition of p53 binding to DNA by zinc ions was discussed in terms of the interaction of zinc ions with different sites in the p53 molecule (other than the site formed by Cys 176, Cys 238, Cys 242 and His 179), switching the p53 conformation to an inactive (PAb1620-negative) immunological phenotype (49) and possibly modifying the p53 DNA binding surface. The findings that level

Table I
Effects of metal ions on binding of p53 to DNA.

	scDNA		p53CON in DNA fragment	
ion	partial	full	partial	full
Zn^{2+}	5	20	5	20
Ni^{2+}	100	300	50	100
Co^{2+}	300	800	100	300

Minimal concentrations (μM) of metal ions required for partial or full inhibition of the f.l. wild type p53 binding to scDNA and p53CON in DNA fragments. Inhibition was tested at p53/DNA=10, in absence of EDTA and concentration of DTT <100 μM.

of zinc may play a role in signalling *in vivo* (50,51) and the role of zinc ions in stabilization of p53 under oxidative conditions (see above) suggest that modulation of p53 activity by zinc can be considered as a physiologically relevant mechanism. Cobalt, nickel and iron ions also inhibited p53 binding to scDNA and to p53CON but at least an order of magnitude higher concentrations (far above physiological levels, Table I) of these metals were required to obtain the same effect as with zinc (42).

Redox Modulation of p53 Binding to Supercoiled DNA and to p53CON in a Linear DNA Fragment

p53 core domain contains ten cysteine residues, including three cysteines (amino acids 176, 238 and 242) coordinating zinc atom (together with histidine 179) stabilizing proper conformation of p53 DNA binding site (26,52). Thiol groups can readily be oxidized by variety of agents, including oxygen, hydroxyl radicals, or specific agents such as diamide (azodicarboxylic acid bis[dimethylamide]). Several groups (20,40,52-57) have studied redox regulation of p53 binding to DNA. It was established that the p53 cysteines must be in a reduced state for sequence-specific DNA binding (20,40,53). Loss of p53 binding activity due to oxidation was accompanied by a conformational change of the core domain from "wild type" (PAb1620+) to "mutant" (PAb1620-) immunological phenotype (53).

We have found that binding of f.l. p53 to scDNA was influenced by the protein redox state (43), and was partially decreased upon oxidation of the protein with diamide (43) or with 1 mM hydrogen peroxide (Figure 3). The decreased ability of oxidized p53 to bind scDNA was manifested by a decrease in the number of retarded bands on the gel and on the immunoblot (43) (Figures 3, 5). On the contrary, binding of p53 to p53CON was fully lost under the same conditions (Figure 4). Oxidized protein was, however, able to bind linear DNA non-specifically, as manifested by an increased signal on the blot corresponding to p53 complex with the 2.5 kb fragment of the pPGM1 plasmid (Figure 4, lane 3). Parks et al. (40) concluded that the redox state of p53 does not regulate non-specific binding of the protein to 49-mer double-stranded and mismatched DNAs. This conclusion is in accordance with the expected preferential role of the C-terminal domain (lacking redox-active cysteine residues) in p53 binding to short non-specific DNA molecules (31,37). Although the amounts of labelled DNA bound by reduced and oxidized p53 in the Parks' experiments appear to be equal (40), the different mobility shifts observed in the two cases suggest that the number of bound p53 molecules per DNA molecule may be influenced by protein oxidation. Interestingly, an *increase* of binding of p53 to the ds 49-mer can be observed in the results of Parks et al. (40) when (probably supercoiled) Bluescript DNA was used as a competitor. This observation is in agreement with our results revealing decreased p53 binding to supercoiled DNA upon protein oxidation (43).

Irreversibility of p53 Oxidation by Diamide and Protection by Zinc Ions

Redox modulation has been taken into account as a possible mechanism of p53 regulation *in vivo*. Such a model requires reversible interconversion between reduced (active) and oxidized (inactive) state of the protein (20,40,53,55). Oxidation of f.l. baculovirus-expressed p53 by diamide resulted in an irreversible loss of DNA binding activity that could not be reverted by addition of an excess of DTT (Figure 4). On the other hand, p53 oxidized by ambient oxygen (e.g. when the protein was purified in the absence of DTT, (40,53)) was easily reactivated by addition of DTT. We observed similar behaviour with f.l. p53 oxidized

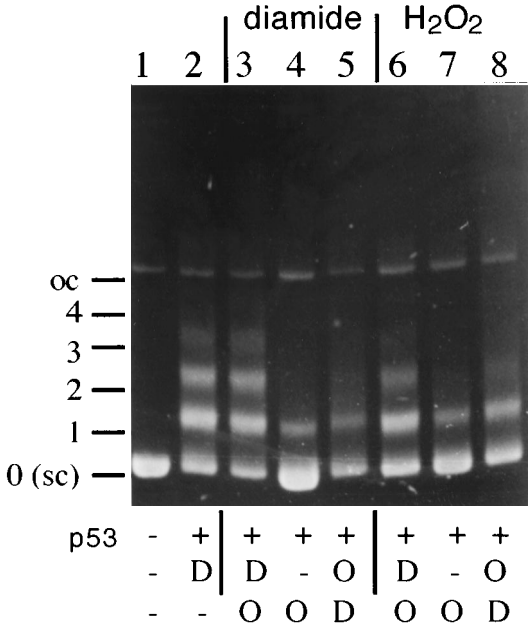

Figure 3: Effects of oxidation of f.l. wild type p53 with diamide and with hydrogen peroxide followed by reduction with DTT on its binding to sc Bluescript DNA. Protein p53 was incubated in different combinations with 1 mM diamide (lanes 3-5) or 1 mM H_2O_2 (lanes 6-8) for 15 min followed by 4 mM DTT for 15 min in 50 mM KCl, 5 mM Tris-HCl (pH 7.8) at 0°C. Next, EDTA (final concentration 1 mM) and 200 ng of scDNA were added. After 30 min incubation at 0°C, the samples were loaded on 1 % agarose gel and electrophoresed. Order of incubations with the reagents: lane 2, DTT - none; lane 3, DTT - diamide; lane 4, none - diamide; lane 5, diamide - DTT; lane 6, DTT - H_2O_2; lane 7, none - H_2O_2; lane 8, H_2O_2 - DTT; lane 1, control DNA. Table below the panel indicates order of addition of the reagents: D, DTT; O, oxidant; p53/DNA = 5. Protein oxidized with hydrogen peroxide yielded only retarded band 1 in agarose gel, displaying the same behavior as diamide-oxidized p53 (lanes 7 and 4, respectively). After addition of an excess of DTT, however, p53 binding to scDNA significantly increased, yielding more intense band 1 and weaker bands 2 and 3 (lane 8), suggesting partial reversibility of p53 oxidation with H_2O_2. No distinct retarded bands except band 1 appeared after DTT treatment of diamide-oxidized p53 (lane 5).

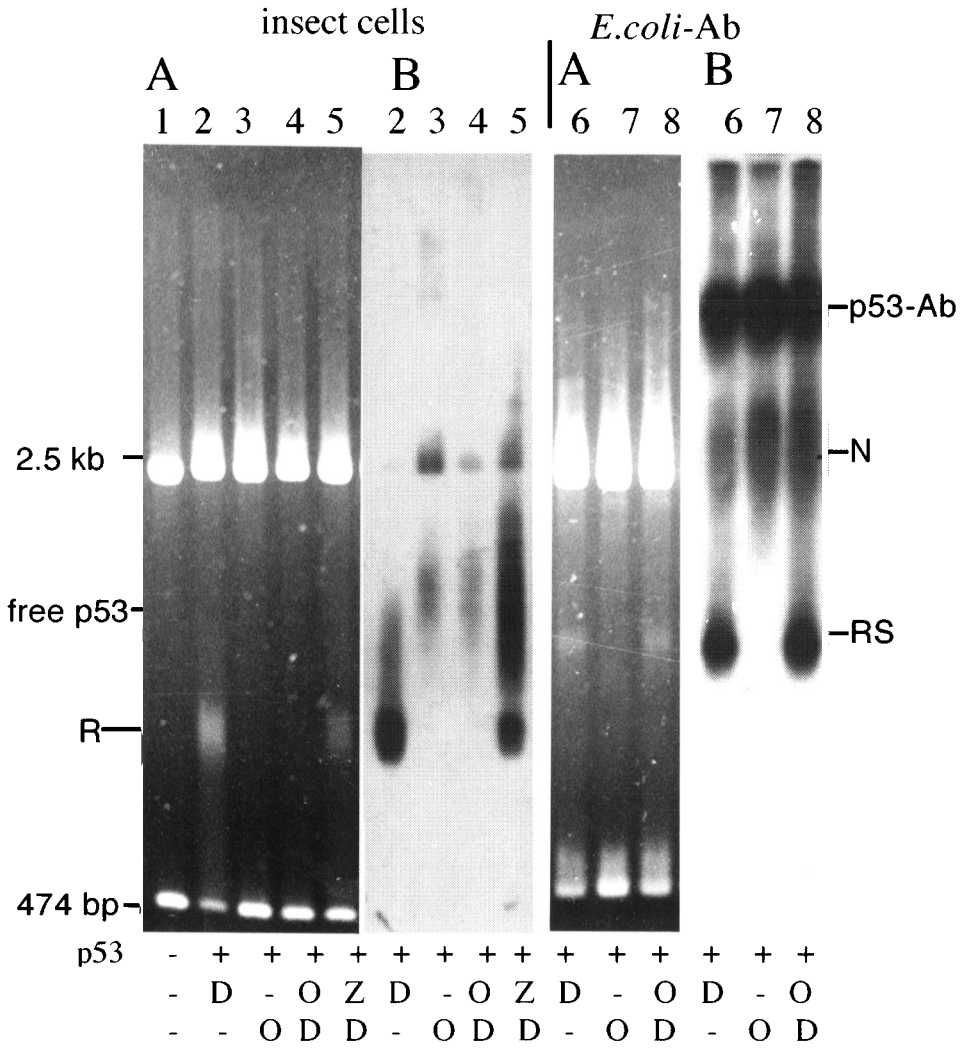

Figure 4: Comparison of the reversibility of redox modulation of sequence-specific binding of p53 (lanes 1-5) expressed in insect cells and (lanes 6-8) *E. coli*-expressed p53 activated by antibody Bp53-30. Protein p53 was oxidized by 1 mM diamide (lanes 4-5, 8) followed by reduction by 2 mM DTT; (lane 5), oxidation of p53 in presence of 100 μM Zn^{2+}; (lane 6-7), monoclonal antibody Bp53-30 was added to p53 prior to oxidation; (lanes 2, 6), reduced p53 incubated with 2 mM DTT; (lane 1), control DNA (pPGM1 cleaved with *Pvu* II). R; band of sequence-specific complex of p53 with the 474 bp fragment; RS, band R supershifted by the antibody; N, band due to non-specific p53-DNA binding; p53-Ab, spot a complex of p53 with Bp53-30 not containing DNA. (A), ethidium-stained agarose gel; (B), DO-1 immunoblot. Table below the panels indicates order of addition of the reagents (as in Figure 3); Z, zinc added together with diamide. For other details see Figure 3. Antibody-activated p53 expressed in *E. coli* was reversibly modulated by diamide oxidation followed by DTT reduction (lanes 7,8). On the contrary, baculovirus-expressed p53 (not treated by the antibody) was irreversibly inactivated by the same oxidation procedure (lane 4). Partial reversibility was attained when 100 μM zinc was present in the solution during oxidation of this protein (lane 5).

with 1 mM hydrogen peroxide (Figure 3). We assumed that this reversibility might be due to partial oxidation of p53 which does not result in the removal of the intrinsic zinc ion from its position in the 53 core domain. In agreement with this assumption we have found that zinc ions at concentrations of 20-200 μM protect diamide-oxidized baculovirus-expressed p53 against irreversible inactivation (43) (Figure 4). This protection was observed only when zinc was present in the medium during oxidation (zinc displayed no protective effect on already oxidized protein). Other divalent transition metals (cadmium, cobalt, nickel) at 100 μM concentration did not protect p53; instead, they promoted deeper structural changes in the oxidized protein, resulting in loss of reactivity towards N-terminal domain-binding antibodies DO-1, DO-13 and DO-14 (43). We suggest that irreversibility of diamide oxidative inactivation of f.l. p53 is, at least partially due to release of the intrinsic zinc ion from its binding site in p53 core domain, accompanied by a conformational change. These changes result in loss of p53 binding activity. Abundant zinc ions in the solution can be expected to act against zinc removal (43). Rainwater et al. (52) observed that removal of trace amounts of external zinc from the p53 solution resulted in an increased sensitivity of the protein towards air oxidation, as manifested in loss of its DNA binding activity. Removal of the p53 intrinsic zinc by o-phenanthroline was associated with oxidation of the thiol groups in the protein (53,58).

Recently we have observed that diamide oxidation (in the absence of zinc) of bacterially expressed p53 activated by antibodies Bp53-30 (Figure 4) and Bp53-10 (antibodies similar to PAb421 (59) recognizing epitopes in the p53 C-terminus) resulted in reversible loss of sequence-specific binding. Furthermore, binding of isolated p53 core 94-312 to p53CON, to scDNA and

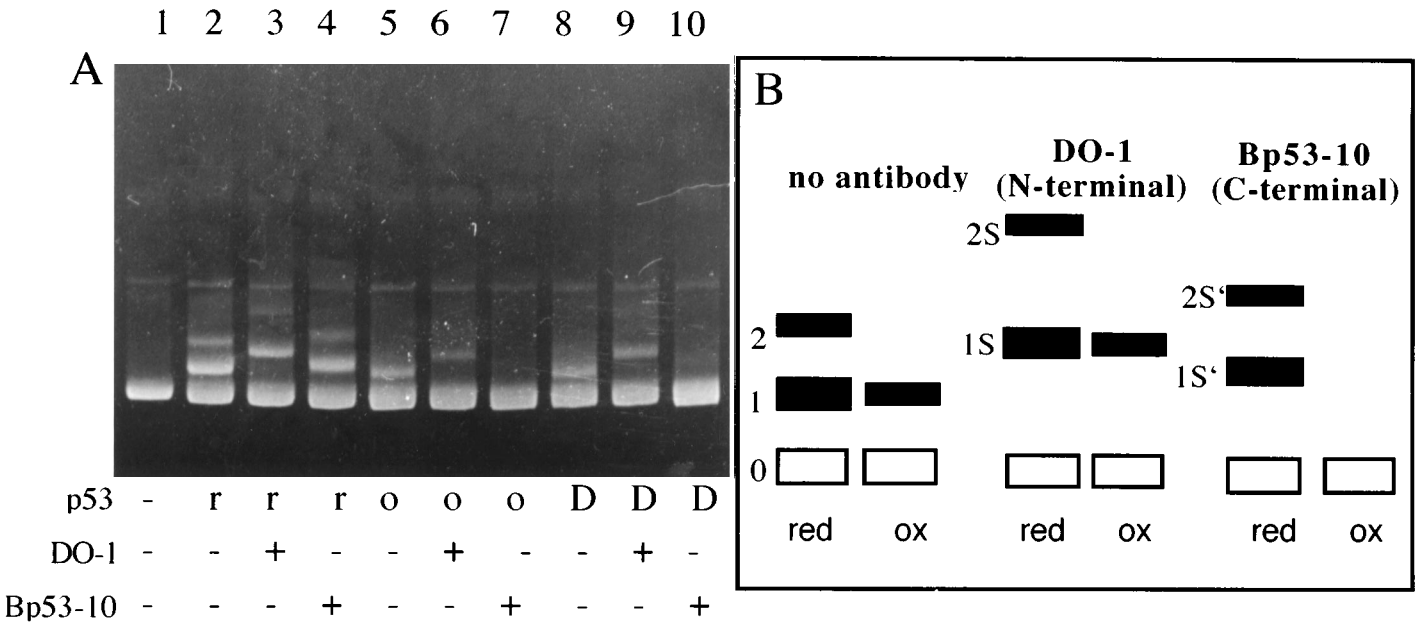

Figure 5: Supershifting of scDNA complexes with reduced or oxidized p53 by monoclonal antibodies DO-1 (recognizing epitope in the p53 N-terminal domain) and Bp53-10 (C-terminal). Protein p53 was preincubated with (lanes 2-4), 2 mM DTT; (lanes 5 - 7), 1 mM diamide; (lanes 8 - 10), 1 mM diamide followed by 2 mM DTT as in Figure 3. Then, 200 ng of sc Bluescript DNA was added together with 100 ng of affinity-purified antibodies DO-1 (lanes 3, 6 and 9) or Bp53-30 (lanes 4, 7 and 10); (lanes 2, 5 and 8), no antibody; (lane 1), control DNA. (A), ethidium stained gel; (B), schematic superimposition of gel and blot: empty rectangles, free DNA; filled rectangles, DNA-p53 complexes; 1S, 2S, 1S′, 2S′, supershifted bands 1, 2. For other details, see Figure 3. Monoclonal antibody DO-1 (mapping p53 amino acids 20-25 in p53 N-terminus) supershifted scDNA complexes with both reduced and oxidized p53 (lanes 3, 6) suggesting that binding of this antibody to p53 did not prevent scDNA binding. On the contrary, antibody Bp53-10 (mapping amino acids 371-380 in the p53 C-terminus) supershifted only scDNA complexes with reduced p53 (lane 4) showing no supershift with oxidized p53 (lane 7). Addition of an excess of DTT was without significant effect (lanes 8-10), confirming irreversibility of the changes in p53 molecules due to diamide oxidation.

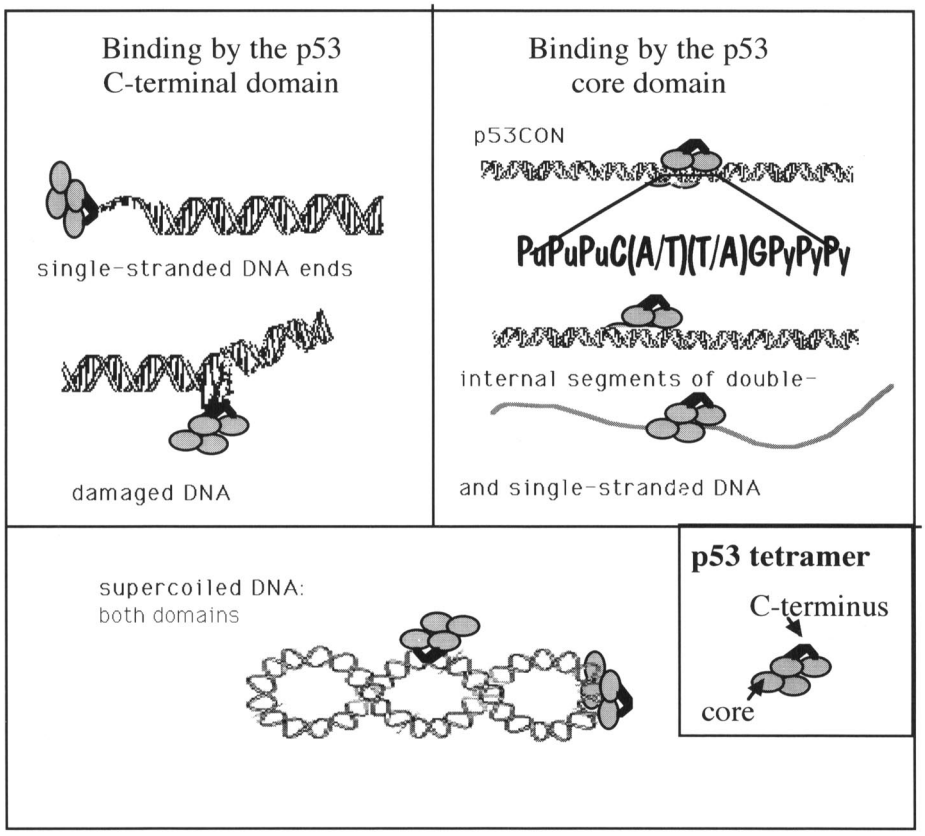

Figure 6: Schematic representation of different modes of interactions of f.l. wild type p53 with DNA. Sequence-specific binding of p53 to its response elements in DNA fragments is maintained by the p53 core domain. The same p53 domain binds also to internal segments of long molecules of double- as well as single-stranded DNA. C-terminal DNA-binding site of p53 interacts with single-stranded ends of DNA molecules and with mismatched or otherwise damaged DNA. In binding of p53 to supercoiled DNA both the core and the C-terminal p53 domains can be involved.

to linear non-specific DNA was (at least partially) reversibly modulated *in vitro* by oxidation with diamide and reduction with DTT (M. Fojta, M. Brazdova, T. Jovin, V. Subramaniam and E. Palecek, unpublished results). Presence of 1 mM EDTA resulted in loss of reversibility of the process (but did not influence DNA binding of reduced core domain in the presence of 1-5 mM DTT) supporting the idea of zinc release due to oxidation. Analogous results were previously reported by Hupp et al. (29) who observed reversible modulation of sequence-specific DNA binding of PAb421-activated or C-terminally truncated p53 upon treatments with diamide and DTT. Interestingly, Hansen et al. (60) observed that deletion of 30 amino acids from the p53 C-terminus significantly enhanced thermostability of p53, suggesting that the C-terminus contributes to instability of p53 core.

Reversible Redox Modulation of p53 in vivo. The possibility of reversible redox modulation of p53 DNA binding activity *in vivo* was earlier considered by other authors (20,40,52-57). Oxidants such as oxygen, hydrogen peroxide or radicals derived from these species may occur *in vivo* and effect the p53 redox state. Moreover, *in vivo* p53 is expected to interact with variety of proteins. Some of them act as p53 regulators switching off the inhibiting and destabilizing effect of the C-terminus (60) ("activating" antibodies such as PAb421 or Bp53-10 probably mimic these natural effectors). These effectors may modulate or protect p53 in response to different situations, including oxidative stress, temperature shock, etc. For example, redox-repair protein Ref-1 related to cellular response on oxidative stress was shown to activate p53 by redox-dependent and redox-independent mechanisms (20,55). Molecular chaperones such as DnaK or Hsp70 (proteins protecting or restoring proper tertiary folding of their specific target molecules), also activated p53 sequence-specific DNA binding. These chaperones interact with the p53 core and dramatically enhance stability of p53 (60,61). In context of the protective effect of zinc ions, it may be noted that zinc metallothioneins have been shown to act (in redox-dependent manner) as active zinc donors for other zinc metalloproteins (62,63). Such mechanisms might be involved in fine regulation of p53 activity *in vivo*.

Involvement of p53 Domains in Binding to scDNA

We have shown that f.l. p53 binds preferentially to scDNA (41,64). The affinity of this binding is comparable to the sequence-specific binding to p53CON in linear DNA fragments and substantially higher than that of nonspecific binding to dsDNA. In addition to the known sequence-specific and non-specific binding we may thus consider a new p53 DNA binding mode which might be tentatively called supercoiled-specific (SCS) DNA binding. Strong SCS binding is observed with f.l. p53 but not with its isolated core 94-312 (64). These findings in combination with the observed effects of metal ions (42) and p53 redox states (43) (Figures 3-5) allow us to make some tentative conclusions about the involvement of the p53 domains in SCS binding of f.l. p53 to DNA.

It was shown that two domains of p53 are involved in DNA binding: p53 core domain and C-terminus (19,34,37). We suggested that both these p53 domains might be involved in binding of reduced p53 to scDNA. Oxidized p53 is inactivated for the DNA sequence-specific binding (due to oxidation of cysteine residues in the core domain) while the non-specific binding by the C-terminus (not containing any cysteine) remains uninfluenced (40) (Figure 6). Binding to scDNA is significantly influenced but not abolished by the p53 oxidation (43) (Figures 3, 5). These results suggest that in p53 binding to scDNA both the core and the C-terminal domains may be involved.

Quite recently we observed that oxidized isolated core 94-312 does not bind to scDNA (M. Fojta, M. Brazdova, V. Subbramaniam, T. Jovin and E. Palecek, unpublished) strongly supporting the idea that the remaining scDNA binding activity of oxidized f.l. p53 is localized outside the core domain. We have shown that p53-DNA complexes can be supershifted by anti-p53 monoclonal antibodies in agarose gels (65). Antibody Bp53-10 (59) mapping amino acids 371-380 in the p53 C-terminus supershifted complexes of reduced f.l. p53 with scDNA. On the other hand, the same antibody inhibited binding of oxidized p53 to scDNA (Figure 5) suggesting that the C-terminus is critical for the residual binding of the oxidized f.l. p53 to scDNA. In contrast to the C-terminus binding Bp53-10, the DO-1 antibody, binding to the N-terminal domain, showed the ability to supershift the p53-scDNA complexes regardless of the p53 redox state. These observations suggest that the core domain is involved in binding of f.l. p53 to scDNA and that the C-terminal domain contributes to this binding. In the oxidized protein, binding by the C-terminal domain gains importance.

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References and Footnotes

1. M. Oren and V. Rotter, *Cell. Mol. Life Sci. 55*, 9-11 (1999).
2. M. Kress, E. May, R. Cassingena and P. May, *J .Virol.31*, 472-483 (1979).
3. D. P. Lane and L. V. Crawford, *Nature 278*, 261-263 (1979).
4. D. I. Linzer and A. J. Levine, *Cell 17*, 43-52 (1979).
5. M. E. Anderson and P. Tegtmeyer, *BioEssays 17*, 3 - 7 (1995).
6. L. S. Cox and D. P. Lane, *BioEssays 17*, 501 - 508 (1995).
7. R. M. Elledge and W.-H. Lee, *BioEssays 17*, 923 - 930 (1995).
8. L. J. Ko and C. Prives, *Genes Dev. 10*, 1054-1072 (1996).
9. A. J. Levine, *Cell 88*, 323-331 (1997).
10. F. Janus, N. Albrechtsen, I. Dornreiter, L. Wiesmuller, F. Grosse and W. Deppert, *Cell. Mol. Life Sci. 55*, 12-27 (1999).
11. R. R. WallaceBrodeur and S. W. Lowe, *Cell. Mol. Life Sci. 55*, 64-75 (1999).
12. L. D. Attardi and T. Jacks, *Cell. Mol. Life Sci. 55*, 48-63 (1999).
13. Y. Haupt, R. Maya, A. Kazaz and M. Oren, *Nature 387*, 296-299 (1997).
14. D. A. Freedman, L. Wu and A. J. Levine, *Cell. Mol. Life Sci. 55*, 96-107 (1999).
15. M. V. Chernov, C. V. Ramana, V. V. Adler and G. R. Stark, *Proc. Natl.*

Acad.Sci. 95, 2284-2289 (1998).
16. C. E. Canman, D. S. Lim, K. A. Cimprich, Y. Taya, K. Tamai, K. Sakaguchi, E. Appella, M. B. Kastan and J. D. Siliciano, *Science 281,* 1677-1679 (1998).
17. S. Banin, L. Moyal, S. Y. Shieh, Y. Taya, C. W. Anderson, L. Chessa, N. I. Smorodinsky, C. Prives, Y. Reiss, Y. Shiloh and Y. Ziv, *Science 281,* 1674-1677 (1998).
18. J. D. Siliciano, C. E. Canman, Y. Taya, K. Sakaguchi, E. Appella and M. B. Kastan, *Genes Dev. 11,* 3471-3481 (1997).
19. T. R. Hupp, *Cell. Mol. Life Sci. 55,* 88-95 (1999).
20. L. Jayaraman and C. Prives, *Cell. Mol. Life Sci. 55,* 76-87 (1999).
21. D. W. Meek, *Cellular Signalling 10,* 159-166 (1998).
22. M. B. Kastan and S. J. Kuerbitz, *Environmental Health Perspectives 101,* 55-58 (1993).
23. K. Polyak, Y. Xia, J. L. Zweier, K. W. Kinzler and B. Vogelstein, *Nature 389,* 300-305 (1997).
24. S. Bates and K. H. Vousden, *Cell. Mol. Life Sci. 55,* 28-37 (1999).
25. W. S. ElDeiry, *Cancer J. 11,* 229-236 (1998).
26. Y. Cho, S. Gorina, P. D. Jeffrey and N. P. Pavletich, *Science 265,* 346-355 (1994).
27. T. D. Halazonetis, L. J. Davis and A. N. Kandil, *EMBO J. 12,* 1021-1028 (1993).
28. T. R. Hupp, D. W. Meek, C. A. Midgley and D. P. Lane, *Cell 71,* 875-886 (1992).
29. T. R. Hupp, D. W. Meek, C. A. Midgley and D. P. Lane, *Nucleic Acids Res. 21,* 3167-3174 (1993).
30. J. Jayaraman and C. Prives, *Cell 81,* 1021-1029 (1995).
31. G. Bakalkin, G. Selivanova, T. Yakovleva, E. Kiseleva, E. Kashuba, K. P. Magnusson, L. Szekely, G. Klein, L. Terenius and K. G. Wiman, *Nucleic Acids Res. 23,* 362-369 (1995).
32. C. Prives, *Cell 78,* 543-546 (1994).
33. R. Srinivasan, J. A. Roth and S. A. Maxwell, *Cancer Res. 53,* 5361-5364 (1993).
34. P. Wang, M. Reed, Y. Wang, G. Mayr, J. E. Stenger, M. E. Anderson, J. F. Schwedes and P. Tegtmeyer, *Mol. Cell. Biol. 14,* 5182-5191 (1994).
35. W. S. El-Deiry, S. E. Kern, J. A. Pietenpol, K. W. Kinzler and B. Vogelstein, *Nature Genet 1,* 45-49 (1992).
36.1. J. H. Bayle, B. Elenbaas and A. J. Levine, *Proc. Natl. Acad. Sci. 92,* 5729-5733 (1995).
37. M. E. Anderson, B. Woelker, M. Reed, P. Wang and P. Tegtmeyer, *Mol. Cell. Biol.17,* 6255-6264 (1997).
38. G. Selivanova, V. Iotsova, E. Kiseleva, M. Strom, G. Bakalkin, R. C. Grafstrom and K. G. Wiman, *Nucleic Acids Res. 24,* 3560-3567 (1996).
39. M. Reed, B. Woelker, P. Wang, Y. Wang, M. E. Anderson and P. Tegtmeyer, *Proc. Natl. Acad. Sci. 92,* 9455-9459 (1995).
40. D. Parks, R. Bolinger and K. Mann, *Nucleic Acids Res. 25,* 1289-1295 (1997).
41. E. Palecek, D. Vlk, V. Stankova, V. Brazda, B. Vojtesek, T. R. Hupp, A. Schaper and T. M. Jovin, *Oncogene 15,* 2201-2209 (1997).
42. E. Palecek, M. Brazdova, H. Cernocka, D. Vlk, V. Brazda and B. Vojtesek, *Oncogene 18,* 3617-3625 (1999).
43. M. Fojta, T. Kubicarova, B. Vojtesek and E. Palecek, *274,* 25749-25755 (1999).
44. A. K. Nagaich, V. B. Zhurkin, S. R. Durell, R. L. Jernigan, E. Appella and R. E. Harrington, *Proc.Natl. Acad.Sci.96,* 1875-1880 (1999).
45. A. K. Nagaich, E. Appella and R. E. Harrington, *J. Biol. Chem. 272,* 14842-14849 (1997).
46. E. Palecek, *Crit. Rev. Biochem. Mol. Biol. 26,* 151-226 (1991).
47. E. Kim, N. Albrechtsen and W. Deppert, *Oncogene 15,* 857-869 (1997).
48. B. Tiedt, G. Rohaly, S. Heinrichs, E. Kim and W. Deppert, Workshop "P53: Twenty Years On" Trieste - Italy 20 - 22 May 1999, Lawrence Banks, ICGEB, Trieste, Italy (1999).
49. A. I. Coffer and P. P. Knowles, *Biochim. Biophys. Acta 1209,* 279-285 (1994).
50. J. M. Berg and Y. Shi, *Science 271,* 1081-1085 (1996).
51. G. W. Verhaegh, M.-O. Parat, M.-J. Richard and P. Hainaut, *Mol. Carcinog. 21,* 205-214 (1998).
52. R. Rainwater, D. Parks, M. E. Anderson, P. Tegtmeyer and K. Mann, *Mol. Cell .Biol.15,* 3892-3903 (1995).
53. P. Hainaut and J. Milner, *Cancer Res. 53,* 4469-4473 (1993).
54. P. Hainaut, N. Rolley, M. Davies and J. Milner, *Oncogene 10,* 27-32 (1995).
55. L. Jayaraman, K. G. Murthy, C. Zhu, T. Curran, S. Xanthoudakis and C. Prives, *Genes Dev. 11,* 558-570 (1997).
56. Y. Sun, J. Bian, Y. Wang and C. Jacobs, *Oncogene 14,* 385-393 (1997).
57. G. W. Verhaegh, M. J. Richard and P. Hainaut, *Mol. Cell. Biol. 17,* 5699-5706 (1997).
58. P. Hainaut and J. Milner, *Cancer Res. 53,* 1739-1742 (1993).
59. S. Pospisilova, V. Brazda, E. Palecek and B. Vojtesek, *submitted* (1999).
60. S. Hansen, T. R. Hupp and D. P. Lane, *J. Biol.Chem. 271,* 3917-3924 (1996).
61. A. M. Fourie, T. R. Hupp, D. P. Lane, B. C. Sang, M. S. Barbosa, J. F. Sambrook and M. J. Gething, *J. Biol.Chem. 272,* 19471-19479 (1997).
62. L. J. Jiang, W. Maret and B. L. Vallee, *Proc. Natl. Acad. Sci. 95,* 3483-3488 (1998).
63. C. Jacob, W. Maret and B. L. Vallee, *Proc. Natl. Acad. Sci. 95,* 3489-3494 (1998).
64. E. Palecek, M. Brazdova,V. Brazda,J. Palecek, V. Subramaniam and T. M. Jovin *in preparation* (1999).
65. V. Brazda, S. Pospisilova, B. Vojtesek and E. Palecek, *submitted* (1999).