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Interaction of p53 with the human Rad51 protein

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ABSTRACT

p53 is thought to function in the maintenance of genomic stability by modulating transcription and interacting with cellular proteins to influence the cell cycle, DNA repair and apoptosis. p53 mutations occur in >50% of human cancers, and cells which lack wild type p53 accumulate karyotypic abnormalities such as amplifications, deletions, inversions and translocations. We propose that p53 hinders these promiscuous recombinational events by interacting with cellular recombination and repair machinery. We recently reported that p53 can directly bind *in vivo* to human Rad51 (hRad51) protein and *in vitro* to its bacterial homologue RecA. We used GST-fusion and his-tagged protein systems to further investigate the physical interaction between p53 and hRad51, homologue of the yeast Rad51 protein that is involved in recombination and DNA double strand repair. The hRad51 binds to wild-type p53 and to a lesser extent, point mutants 135Y, 249S and 273H. This binding is not mediated by a DNA or RNA intermediate. Mapping studies using a panel of p53 deletion mutants indicate that hRad51 could bind to two regions of p53; one between amino acids 94 and 160 and a second between 264 and 315. Addition of anti-p53 antibody PAb421 (epitope 372–381 amino acids) inhibited the interaction with hRad51. In contrast, p53 interacts with the region between aa 125 and 220 of hRad51, which is highly conserved among Rad51 related proteins from bacteria to human. In *Escherichia coli* RecA protein, this region is required for homo-oligomerization, suggesting that p53 might disrupt the interaction between RecA and Rad51 subunits, thus inhibiting biochemical functions of Rad51 like proteins. These data are consistent with the hypothesis that p53 interaction with hRAD51 may influence DNA recombination and repair and that additional modifications of p53 by mutation and protein binding may affect this interaction.

INTRODUCTION

The p53 tumor suppressor gene product is a multifunctional molecule that influences the cell cycle, DNA repair and apoptosis by regulating transcription and interacting directly with other proteins (1–4). These functions enable p53 to contribute to the maintenance of genomic stability in the presence of a mutagenic environment. Following DNA damage, p53 upregulates the expression of p21[Waf1] protein to effect a G_1 cell cycle arrest (5–7) and G_2 arrest (16) in order to prevent the replication of damaged DNA (8,9). In addition, recent reports suggest that p53 may influence the repair machinery directly (10–13). For example, Wang *et al*. (11,14) demonstrated that p53 binds to and modulates the DNA repair activity of the nucleotide excision repair factors XPB and XPD. If these cell cycle arrest and DNA repair functions fail to restore the genome to a wild-type state, p53 may also direct the elimination of the damaged cell via apoptosis (15–17). Although current knowledge describes an intricate network of protective functions, there are likely additional mechanisms by which this protein with such critical importance in human cancer acts to prevent the accumulation of mutations (1,18–20).

Chromosomal abnormalities such as gene amplifications, translocations, inversions and deletions are often seen in tumor cells, suggesting that karyotype instability is involved in tumorigenesis (21). Common to the occurrence of these chromosomal defects is the formation and rejoining of DNA strand breaks (22–24). The fidelity of resolution of a break produced by either a DNA damaging agent, viral integration or normal cellular events such as recombination determines whether the wild type genome is restored. Thus, it is critical that the processes that repair DNA strand breaks are strictly regulated (25).

Since chromosomal abnormalities and the lack of p53 function seem to correlate, we hypothesized that p53 can suppress aberrant recombination. This novel function of p53 may occur by either direct or indirect mechanisms. The indirect route involves p53 regulation of cellular processes that secondarily influence recombination and is supported by a number of studies. Gene amplification is elevated in the absence of p53 function (26,27). In addition, Meyn and collegues (28) showed, via a chromosomal mitotic recombination assay, that homologous recombination is

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increased 20-fold in human fibroblasts with inactivated p53. These recombinational events in the absence of p53 function are thought to occur secondary to the loss of p53 regulated cell cycle control (8,9). Interestingly, emerging data indicate that p53 may also influence recombination directly. The p53 C-terminal basic domain can direct both DNA single strand annealing and strand transfer activities, events that occur during recombination (29,30). In addition, p53 is specifically expressed during the pachytene stage of meiosis during mouse spermatogenesis (31,32), and a subset of p53 knock out mice exhibit the testicular giant cell degenerative syndrome, suggesting a failure to progress through the pachytene stage of spermatocyte meiosis in the absence of p53 (32). It is possible that p53 is functionally involved in the synaptonemal complex formation and meiotic recombination that occur during the pachytene stage of meiosis. Evidence for direct involvement of p53 in homologous DNA recombination *in vitro* has been obtained recently (33).

In order to investigate this theoretically direct relationship between p53 and recombination, we sought to study the interaction between p53 and hRad51, a proposed member of the mammalian recombination machinery. Rad51, originally described in yeast, is closely related to the *Escherichia coli* RecA (34–36) protein that catalyzes DNA strand transfer and recombination. Yeast Rad51 mutants fail to repair double strand breaks, exhibit spontaneous and radiation induced chromosomal loss, and harbor a defect in both mitotic and meiotic recombination (34,36). Furthermore, yeast Rad51 appears to be required for conversion of double strand breaks to recombination intermediates, and localizes to presumptive foci of recombination in yeast meiosis (36,37). The human homologue of yeast Rad51 was recently cloned, and it shares 83% sequence homology with the yeast protein (35), while both Rad51 proteins share a common central DNA binding domain with RecA (35). Although no mammalian mutants exist, recent work suggests that hRad51 is part of the recombination machinery. It is nuclear localized, forms nucleoprotein filaments structurally similar to those formed by yeast Rad51, underwinds DNA in these filaments, possesses a DNA dependent ATPase activity, and promotes ATP-dependent homologous pairing and strand transfer reactions (38–40). Evidence for hRad51 involvement in class switch recombination also was obtained recently (41).

The *in vivo* interaction between p53 and the hRad51 proteins was recently described by Stürzbecher and colleagues (42). Since the modular nature of p53 enables assignment of specific functions to structural regions of the protein, definition of the binding domains of both p53 and hRad51 proteins may be useful for inferring functional consequences of this binding. We therefore used *in vitro* methods both to map further the domains of interaction between p53 and hRad51 and to investigate factors that affect the p53/hRad51 complex.

MATERIALS AND METHODS

Plasmids

GST-p53-WT encodes glutathione *S*-transferase (GST) fused to human wild-type p53. GST-p53-135Y, -249S and -273H encode GST fused to p53 mutated at codon 135 (H→Y), 249 (R→S) and 273 (R→H), respectively. These constructs were described elsewhere (43). p53 deletion mutants NC, N5, 25, 2C, 3C, 23, 24 and 35, inserted into the expression vector pET11GST and kindly provided by Bruce Stillman (Cold Spring Harbor Laboratory), encode GST fused to amino acids 2–393, 2–293, 94–293, 94–393, 155–393, 94–209, 94–269, 155–299 of p53, respectively (44). His-tagged p53 cDNAs were cloned into the pET19b prokaryotic expression plasmid (AGS GmbH, Heidelberg, Germany). p53 C-terminal fragments aa264–393, aa287–393, aa315–393, and aa334–393 were created by PCR-directed mutagenesis of wild-type human p53 as described previously (45). To construct the Core mutant, the p53 cDNA was released from pET11GST-NC by digestion with *Sal*I and *Bam*HI and inserted into the polylinker of the carrier plasmid pGEMEX-1 (Promega). pGEMEX-1-NC was digested with *Nco*I and *Afl*III to remove nucleotides encoding p53 amino acids 161–237. Ligation produced an in frame Core deletion mutant cDNA which was released from pGEMEX-1-p53 Core with *Sal*I and *Bam*HI and re-introduced into the same sites of the pET11-GST vector to produce pET11-GST-p53 Core. NTD was made by digesting pGEX-2T-p53 (46) with *Bam*HI and *Bsa*AI to release a fragment encoding p53 amino acids 2–124. The fragment was then inserted into the *Bam*HI and *Sma*I sites of pGEX-2T (Pharmacia LKB) to make pGEX-2T-p53 NTD. Authenticity of the Core and NTD mutant proteins was verified by molecular weight analysis and western blotting with a panel of anti-p53 specific monoclonal antibodies. The sequence map of the pET11GST-p53-NC plasmid was constructed from available references (47,48).

pCRII-hRad51 was used for *in vitro* translation of human Rad51. A cDNA of the hRad51 gene was amplified by PCR from a human testis cDNA library. The nucleotide sequence was verified by dideoxy nucleotide sequencing and cloned into the pCRII vector (Stratagene) under the control of the SP6 promoter. Deletion mutants of hRad51 N44 (aa44–339), N125 (aa125–339), N220 (aa220–339) and C220 (aa1–220) were created by PCR-directed mutagenesis of wild-type human Rad51 as described previously (45). pSelectp53 (14) was used for *in vitro* translation of human p53. pSPX46 (14) used for *in vitro* translation of the hepatitis B virus X protein (HBX) contains HBV nucleotides 1248–1718, the entire open reading frame of the HBX gene amplified by PCR and inserted into the *Bgl*II site of pSP72 (Promega).

Expression and purification of recombinant proteins

GST fusion proteins were produced in *E.coli* and purified on glutathione–Sepharose 4B beads (GSH beads) according to the manufacturers conditions (Pharmacia LKB) and as described elsewhere (14,43). The purified fusion proteins immobilized on the surface of GSH beads were stored at 4°C in phosphate buffered saline, pH 7.4, containing 1% Triton X-100 for up to 2 months. His-tagged proteins were produced in *E.coli* as above. The cell lysate supernatant was reacted overnight at 4°C with Ni^{2+}-NTA agarose resin. After extensive washing with 6 M guanidinium-hydrochloride, 50 mM Tris–HCl at pH 8.0, the resin was dialyzed stepwise against p53 buffer (49) (25 mM Tris–HCl, pH 8.0, 2 mM EDTA, 10 mM 2-mercaptoethanol, 0.1% Triton X-100, 7.5% glycerol, 300 mM NaCl) containing 1, 0.1 and 0 M guanidinium-hydrochloride. The resin was transferred to a column and washed twice with p53 buffer, pH 6.3. p53 proteins were eluted with p53 buffer, pH 5.0. The pH was immediately adjusted to 8.0 and the protein stored in aliquots under liquid nitrogen. Protein concentration and molecular weight was determined by comparing GST fusion and his-tagged proteins to

molecular weight and protein quantitation standards on Coomassie blue stained SDS/PAGE gels.

Labeled *in vitro* translated proteins were prepared in a one-step *in vitro* transcription and translation system (Promega TNT system) by incubating the appropriate plasmids for 90 min at room temperature (RT) in the presence of [^{35}S]cysteine (Dupont). *In vitro* translated proteins were prepared fresh for each binding assay. Purified RecA protein was obtained from Boehringer Mannheim.

In vitro protein binding and analysis of protein complexes

Binding assays with GST fusion proteins were carried out in 500 µl IP buffer (50 mM Tris–HCl, pH 8.0, 120 mM NaCl, 0.5% Nonidet P-40). Binding reactants (^{35}S-labeled, *in vitro* translated and GST fusion proteins synthesized in *E.coli*) were precipitated with either GSH beads or a combination of antibody plus protein A/protein G agarose. After incubation of the binding reaction at RT for 60 min, the bound proteins were washed five times with IP buffer, released by boiling of the beads in Laemmli buffer for 5 min, separated by SDS–PAGE and visualized by fluorography. For binding assays with his-tagged proteins, Ni^{2+}-NTA agarose resin containing 1 µg of p53 protein and equilibrated in NET-N buffer (20 mM Tris–HCl, pH 8.0, 100 mM NaCl, 1 mM EDTA, 0.5% Nonidet P-40) was washed twice with NET-N. Then either 5 µl of *in vitro* translated hRad51 or 2 µg of purified RecA protein (Boehringer Mannheim) was added. The reactions were incubated with shaking for 30 min at RT and washed three times with NET-N buffer. Bound proteins were released by boiling in Laemmli buffer for 5 min, separated by SDS–PAGE and visualized by fluorography or silver staining. Densitometric analysis of autoradiography was carried out on a Molecular Dynamics Computing Densitometer.

Antibodies and immunoprecipitation reagents

Anti-p53 antibody PAb421 and protein A/protein G agarose were obtained from Oncogene Science. Anti-hRad51 polyclonal antibody was described elsewhere (50). Anti-mouse control IgG was obtained from Southern Biotechnology Associates, Inc. Polyclonal anti-p53 antibody CM-1 was obtained from Signet labs.

RESULTS

Interaction between GST-p53 fusion proteins and *in vitro* translated hRad51

We used a GST fusion protein system to study the interaction between GST-p53 proteins synthesized in *E.coli* and *in vitro* translated, ^{35}S-labeled hRad51. As shown in Figure 1, hRad51 binds specifically to GST-p53 wild-type (WT) and to a lesser extent to a panel of p53 point mutants found commonly in human cancers. p53 does not bind to the GST protein alone. Pre-incubation of either hRad51 or the GST-fusion proteins with DNase I and RNase A did not alter binding (data not shown) demonstrating that the interaction is not mediated by a nucleic acid intermediate. Because p53 mutations do not eliminate binding to hRad51 completely, it is possible that the p53 point mutants may exert a dominant negative effect on binding, providing at least one mechanism by which certain mutant p53 proteins could disrupt the putative functions resulting from a productive p53/hRad51 interaction.

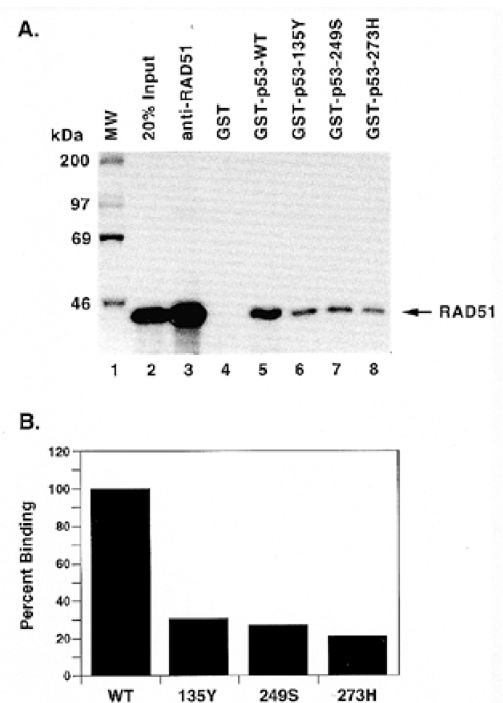

Figure 1. Binding between GST-p53 point mutants and *in vitro* translated hRad51. (**A**) GSH beads loaded with either 2 µg of GST (lane 4) or 2 µg of GST-p53-WT (lane 5), GST-p53-135Y (lane 6), GST-p53-249S (lane 7) or GST-p53-273H (lane 8) were mixed with 5 µl of ^{35}S-labeled, *in vitro* translated hRad51. Proteins which remained bound were analyzed by SDS–PAGE as described in Materials and Methods. As references, the hRad51 *in vitro* translation product was loaded to the gel as 1 µl directly (lane 2) or after immunoprecipitation of 5 µl of the ~37 kDa protein with polyclonal anti-hRad51 antibody (lane 3). Protein molecular weight markers are included (lane 1). (**B**) The percentage of input hRad51 protein bound to p53 was quantitated by densitometry. Results were an average of two independent experiments. WT displays ~12% binding of hRad51 protein.

Mapping of the hRad51 binding site on p53 polypeptide

In order to map the region of p53 to which hRad51 binds, we used a series of GST-p53 deletion mutants. Figure 2 provides both a schematic representation and Coomassie blue stained gel of these mutants that were used in the standard binding assay with *in vitro* translated hRad51 (Fig. 3). While equal amounts of these GST fusion proteins were used, each of the eight GST-p53 mutants bound to at least some degree with hRad51. Mutants 3C and 35 consistently showed diminished binding activity while the shortest mutant GST-p53-23 retained approximately WT binding capability. Since both 3C and 35 share a deletion of p53 N-terminal amino acids 1–154, we postulated that the hRad51 binding site likely resides within a region of p53 flanking amino acid 155 and wholly contained within mutant 23 (aa94–209).

In order to more precisely localize the binding site, we constructed two additional deletion mutants (Fig. 4). The mutants were created by deletional subcloning, with the identity of the purified protein products confirmed by molecular weight analysis and western blotting with a panel of anti-p53 monoclonal antibodies (data not shown). Mutant GST-p53 Core carries a deletion of core domain aa161–237 and mutant GST-p53-NTD retains aa2–124. The two mutants together delete approximately

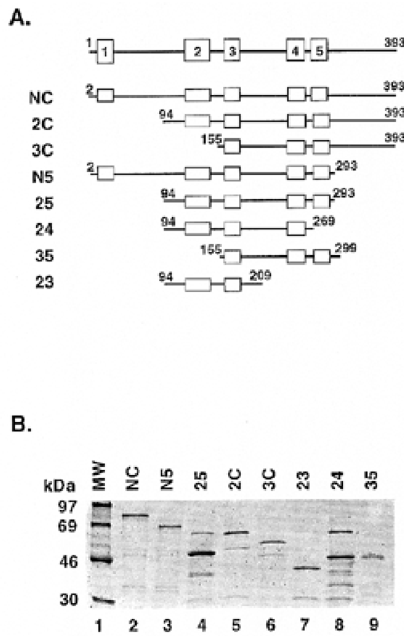

Figure 2. Coomassie blue stain of GST-p53 deletion mutant fusion proteins. (**A**) Schematic representation of wild-type p53 and deletion mutant fusion proteins. GST-p53-NC (retains p53 amino acids 2–393), N5 (2–293), 25 (94–293), 2C (94–393), 3C (155–393), 23 (94–209), 214 (94–269), 35 (155–299). Open boxes represent evolutionarily conserved domains of p53. (**B**) GST-p53 deletion mutant fusion proteins synthesized in bacteria were purified on GSH beads as described in Materials and Methods. 5 µl of the bead mixture was loaded into each lane as follows: NC (lane 2), N5 (lane 3), 25 (lane 4), 2C (lane 5), 3C (lane 6), 23 (lane 7), 24 (lane 8), 35 (lane 9). Proteins were separated by SDS–PAGE and stained with Coomassie blue. Each band of the molecular weight standards represents 1 µg of protein. Protein concentration was estimated by comparing to molecular weight standards and equal amounts of the fusion proteins were aliquoted and used for binding studies.

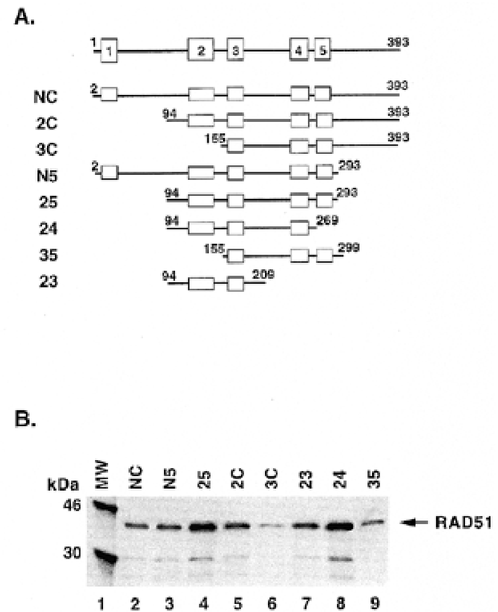

Figure 3. Binding site mapping using GST-p53 deletion mutant fusion proteins. (**A**) Schematic representation of p53 wild-type and deletion mutant fusion proteins. GST-p53-NC (retains p53 amino acids 2–393), N5 (2–293), 25 (94–293), 2C (94–393), 3C (155–393), 23 (94–209), 24 (94–269), 35 (155–299). Open boxes represent evolutionarily conserved domains of p53. (**B**) GSH beads loaded with 2 µg of GST-p53NC (lane 2), N5 (lane 3), 25 (lane 4), 2C (lane 5), 3C (lane 6), 23 (lane 7), 24 (lane 8) and 35 (lane 9) were mixed with 5 µl of ^{35}S-labeled, *in vitro* translated hRad51. Proteins which remained bound were analyzed by SDS–PAGE as described in Materials and Methods. The location of the ~37 kDa hRad51 protein is indicated by the arrow. Protein molecular weight markers are included in lane 1.

two-thirds of the amino acids within the GST-p53-23 mutant which likely contains the entire hRad51 binding site.

Both the Core and NTD mutants bind less hRad51 than WT p53 (Fig. 4). While the core mutant binds about half as well, mutant NTD has only 7% of the binding ability of WT. Thus, deletion of p53 sequence in the N-terminal direction from aa155 markedly affects binding, whereas deletion of a large region in the C-terminal direction has minimal effect. Collectively, these results suggest that the hRad51 binding site is localized somewhere between aa94 and 160 of p53.

The two binding site model

Experiments involving both the hRad51 protein and the homologous bacterial RecA protein suggest that an additional hRad51/RecA binding site exists between aa264 and 334 of p53 (42). In order to define this second binding site in more detail additional C-terminal p53 fragments were tested for hRad51 interaction. *In vitro* binding experiments between bacterially synthesized, histidine-tagged p53 protein fragments and *in vitro* translated hRad51 show that a p53 fragment retaining aa264–393 binds to hRad51 very efficiently while a fragment starting at aa287 shows ~75% of binding and a fragment starting at aa315 only 15% (Fig. 5), localizing the second site to aa264–315 of p53. These data suggest the presence of two hRad51 binding sites within p53: one between aa94 and 160 and another between aa264 and 315. Due to the nature of the panel of GST-p53 deletion mutants, we could not completely eliminate binding of these mutants to hRad51.

Mapping of the binding site for p53 on the Rad51 polypeptide

The open reading frames of vertebrates Rad51 genes specify proteins with high homology. Human protein differs by only 4 or 12 amino acid residues from those of mouse or chicken protein, respectively. The vertebrate proteins are highly homologous to yeast Rad51 protein with 73% identity. All of these proteins are homologous to the *E.coli* RecA protein, but only in the ‘homologous core’ region (51). Since both human Rad51 and *E.coli* RecA protein interact with p53, one would predict that the binding site for p53 on the Rad51 polypeptide should be part of this homologous core region. To identify the binding for p53 on hRad51 experimentally, a panel of N- and C-terminal truncation mutants of hRad51 were created by *in vitro* mutagenesis and the respective *in vitro* translated protein products were tested for binding to purified p53 protein. Figure 6 shows that C-terminal hRad51 fragments starting at aa44 (Fig. 6, lane c) and aa125 (Fig. 6, lane b), respectively, readily bind to p53 while the fragment starting at aa220 (Fig. 6, lane a) is incapable of interacting with p53. The N-terminal hRad51 fragment encompassing aa1–220 also binds very well to p53 protein (Fig. 6, lane d). Thus, the region of hRad51 protein involved in p53 interaction should include aa125–220. This region is part of the core region of hRad51 proteins highly conserved from bacteria to

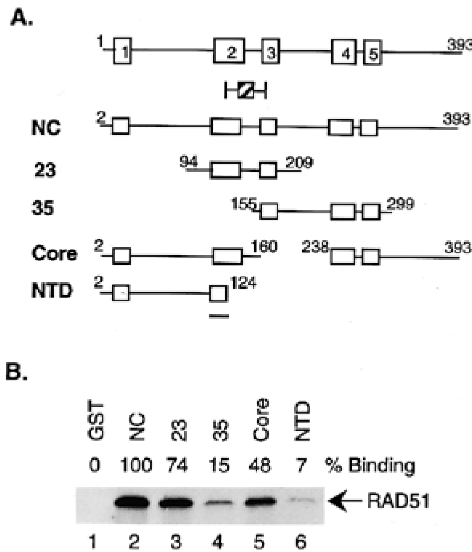

Figure 4. Binding site mapping using GST-p53 Core and NTD deletion mutant fusion proteins. (**A**) Schematic representation of p53 wild-type and deletion mutant fusion proteins used in this experiment. An approximation of the hRad51 binding site is represented by the hatched box with flanking regions. Open boxes represent evolutionarily conserved domains of p53. (**B**) GSH beads loaded with 2 µg of GST (lane 1), GST-p53-NC (lane 2), 23 (lane 3), 35 (lane 4), Core (lane 5) and NTD (lane 6) were mixed with 5 µl of ^{35}S-labeled, *in vitro* translated hRad51. Proteins which remained bound were analyzed by SDS–PAGE as described in Materials and Methods. The location of the ~37 kDa hRad51 protein is indicated by the arrow. Percent binding of input hRad51 to each GST fusion proteins is indicated above each lane.

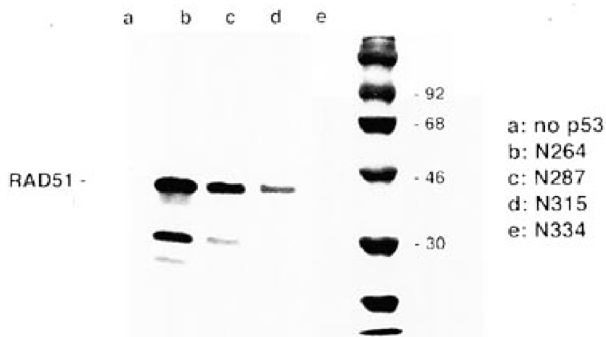

Figure 5. Binding site mapping using C-terminal fragments of p53. Ni^{2+}-NTA resin loaded with 1 µg of purified his-tagged C-terminal p53 protein fragments produced in *E.coli* was incubated with 5 µl of ^{35}S-labeled, *in vitro* translated human hRad51. As a control, 5 µl of *in vitro* translated hRad51 was reacted with Ni^{2+}-NTA resin (lane a). Lanes b–e, binding to C-terminal p53 fragments encompassing the following amino acids: lane b, aa264–393; lane c, aa287–393; lane d, aa315–393; lane e, aa334–393.

human. Consequently, as shown in the experiments described here, p53 interacts with Rad51 proteins from *E.coli* RecA to human Rad51. The p53 binding region in the model of the crystal structures of the RecA and p53 proteins includes one of the domains for RecA homo-oligomerization (51) (Fig. 7). In summary, the interaction between p53 and hRad51 is a highly conserved intrinsic property of both proteins and involves highly sensible regions at the center of biological functions of the proteins: the core domain of p53 required for sequence specific interaction of p53 with DNA on one hand, and the oligomerization domain of hRad51 necessary for nucleoprotein filament building and strand exchange on the other.

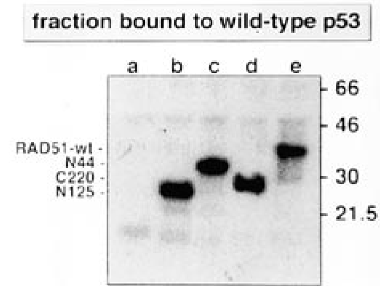

Figure 6. Mapping of the binding site for p53 on the hRad51 molecule using hRad51 deletion mutants. Ni^{2+}-NTA resin loaded with 1 µg of purified his-tagged wild-type p53 protein produced in *E.coli* was incubated with 5 µl of ^{35}S-labeled, *in vitro* translated deletion mutants of human hRad51. Lanes a–e, binding between full-length p53 protein and N- and C-terminal deletion mutants of hRad51 encompassing the following amino acids: lane a, aa220–339; lane b, aa125–339; lane c, aa44–339; lane d, aa1–220; lane e, full-length wild-type hRad51.

DISCUSSION

p53 function as a sequence specific DNA binding protein and transcriptional activator is exquisitely sensitive to single amino acid mutations in the DNA binding domain, phosphorylation and interaction with other proteins (1,19,52). Missense mutations cause p53 accumulation, presumably secondary to conformation changes, and eliminate sequence specific DNA binding and transactivation (53–56). Our binding data enable an analysis of the effect of such mutations upon the interaction between p53 and hRad51 protein. In contrast to complete loss of DNA binding, the point mutants retain a reduced amount of binding to hRad51. Thus, the alteration in p53 tertiary structure that accompanies missense mutations destroys DNA binding, but this protein–protein interaction is maintained to some extent. Although mutation of p53 does not eliminate binding to hRad51, the mutants could still exert a dominant negative effect upon the interaction of wild-type p53 and hRad51. Point mutants of p53 generally do not bind to DNA alone but eliminate DNA binding by poisoning the p53 tetramer. In contrast, since the mutants can bind to hRad51 directly, they would likely act via competitive inhibition of the WT p53/hRad51 complex or mutant p53 may directly dysregulate DNA recombination.

p53 tetramers produced in bacteria as well as temperature sensitive p53 isolated at the permissive temperature from Clone 6 cells require incubation with antibody PAb421 to expose a cryptic binding site and activate sequence specific DNA binding (57–59). Interestingly, either proteolytic removal or phosphorylation by casein kinase II of the C-terminus of p53 yielded a similar effect. While the endpoint of these studies was DNA binding and presumably the ensuing transactivation, our data indicate an effect on p53–protein interaction. Addition of antibody PAb421 equally reduced binding between hRad51 and both full length p53 and a fragment retaining aa264–393 (data not shown). This equivalent reduction of hRad51 binding to p53 containing one or both hRad51 binding sites suggests that antibody PAb421 binding to the C-terminal end of p53 influences both the proximal and distal hRad51 sites. Further, because the N-terminal hRad51

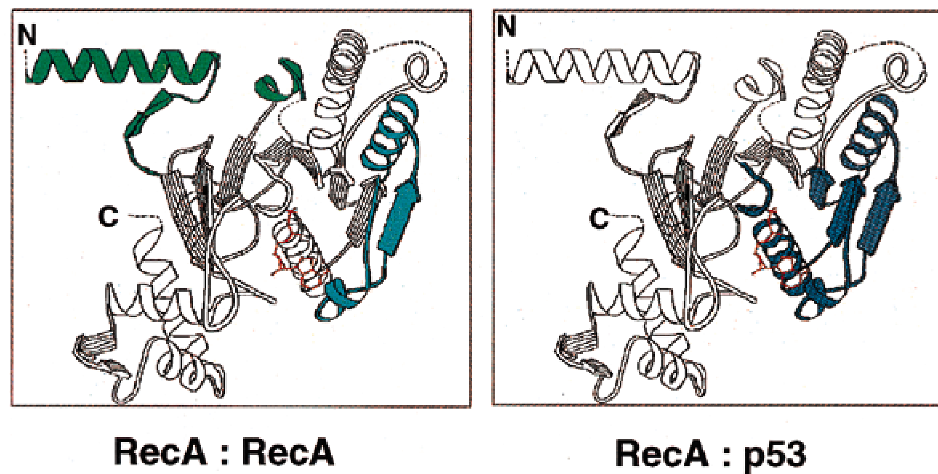

Figure 7. p53 binding site on the RecA protein. The cartoon shows a graphic representation of the *E.coli* RecA protein as determined by crystallization. The two binding sites required for RecA homo-polymerization are highlighted in green (aa1–28) and light blue (aa94–140). The p53 binding site is highlighted in dark blue (aa 66–144) (51).

binding site is distant from the PAb421 epitope, it is likely that the elimination of binding is due to PAb421 mediated conformational changes in p53 and not steric hindrance.

Lane and coworkers proposed an allosteric model for the activation of p53 (60). The key elements of this model are that the C-terminal end of p53 interacts with the core of the molecule. This interaction locks the core into a conformation that is inactive for specific DNA binding. Only after this C-terminus–core interaction is disrupted by covalent modification, non-covalent modification, or deletion, is the core then able to adopt the active form. We recently showed that both deletion of the last 40 amino acids from the C-terminal end of p53 and microinjection of the antibody Pab421, resulted in increased transcriptional transactivation activity of p53 but diminished apoptotic activity (61). Although these mechanisms have variable effects on a single endpoint, p53 sequence-specific DNA binding and transactivation, perhaps they could also affect the interaction of p53 with other proteins. The importance of p53 conformation for its interaction with hRad51 is underlined by the following observations. (i) Complex formation with hRad51 appears to be influenced by conformation as well as posttranslational modifications of p53. *Escherichia coli* produced unphosphorylated p53 might not display all the elements required for hRad51 binding in living mammalian cells (42). (ii) The capability to form homotetramers is a prerequisite for stabile Rad51 binding of full length p53. On the other hand, specific point mutations created by site directed mutagenesis with amino acid substitutions in the C-terminal basic region of p53 clearly enhance binding between p53 and hRad51 (42,45). These same variants display a striking increase in their activity as a sequence-specific transcriptional activator *in vivo* (62), presumably by allowing spontaneous activation of p53 during tetramer assembly in cells. Furthermore, these variants carry substitutions of exactly those basic amino acids shown to be involved in negative regulation of p53 specific DNA binding (60). Consequently, like specific DNA binding, the C-terminal basic domain appears to be implicated in regulating protein–protein interactions as well. (iii) The definition of two binding sites for hRad51 on the primary amino acid sequence of p53, one between aa94 and 160 the other between aa264 and 315, might turn out to represent the two more or less independent halves of one contact site between the two molecules considering the three dimensional structure of the core domain of p53 (52). The contact site between p53 and hRad51 might primarily consist of β-strands S2, S2′ and S3 (aa124–146) and S10 to helix H2 (aa264–286) of the core domain, which according to the crystallographic structure are part of the contact region of p53 with DNA (52). Insertion of four amino acids between aa286 and 287 of p53, on the other hand, causes complete loss of hRad51 binding (Buchhop and Stürzbecher, unpublished), again arguing for the requirement of a particular conformation of the core domain of p53 for stable interaction between the two partners.

In summary, p53 may exist in several structural states, (i) a mutant form unable to bind to DNA or induce G_1 arrest, (ii) a wild-type form that activates G_1 arrest and binds to hRad51 and (iii) an antibody PAb421 activated form that binds in a sequence-specific manner to DNA, with the particular function depending on the conformation of p53. Modulation of these conformational states of the wild-type sequence of p53 via phosphorylation or binding of other proteins may direct specific functions of p53, from protein–protein interactions to sequence-specific DNA binding transactivation. Whether or not the conformational state of p53 for specific DNA binding is comparable to the conformation favoring interaction with hRad51 awaits further analyses.

The corresponding binding site for p53 on the hRad51 molecule includes the highly conserved central part of the molecule. The crystal structure of RecA protein shows that this region is one of two sites required for homo-oligomerization of RecA to form nucleoprotein filaments around DNA. Functional analyses have shown that p53 severely inhibits RecA catalysed biochemical activities like DNA dependent ATPase and DNA strand exchange. Based on the mapping data presented here the underlying mechanism for this inhibition might include occupation of one of the required binding sites by p53 and consequently disruption of RecA (or Rad51, respectively) homo-oligomerization and nucleoprotein filament formation.

The model of conformational regulation of p53 function presented here may have significance in the role of p53 as a regulator of recombination and DNA repair. Since we and our

colleagues have shown a direct protein–protein interaction between p53 and a member of the recombination and DNA repair machinery, we propose this as a mechanism by which p53 may modulate these processes. Missense mutations of p53 or interaction with other undefined proteins could disrupt the p53/hRad51 interaction, thus leading to an increase in promiscuous recombination. Modulation of this interaction, by phosphorylation for example, during states of DNA damage or strand breakage (i.e. meiotic recombination), could serve to regulate and thus assure the fidelity of recombination and break repair.

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