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The Processing of Holliday Junctions by BLM and WRN Helicases is Regulated by p53

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Running title: p53 modulates BLM and WRN helicase activities

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SUMMARY

BLM, WRN and p53 are involved in the homologous DNA recombination pathway. The DNA structure-specific helicases, BLM and WRN, unwind Holliday junctions (HJ), an activity that could suppress inappropriate homologous recombination during DNA replication. Here, we show that purified, recombinant p53 binds to BLM and WRN helicases and attenuates their ability to unwind synthetic HJ *in vitro*. The p53 248W mutant reduces abilities both to bind HJ and to inhibit helicase activities, whereas the p53 273H mutant losses these abilities. Moreover, full-length p53 and a C-terminal polypeptide (residues 373-383) inhibit the BLM and WRN helicase activities, but phosphorylation at Ser376 or Ser378 completely abolishes this inhibition. Following blockage of DNA replication, Ser15 phospho-p53, BLM and RAD51 colocalize in nuclear foci at sites likely to contain DNA replication intermediates in cells. Our results are consistent with a novel mechanism for p53-mediated regulation of DNA recombinational repair that involves p53 posttranslational modifications and functional protein-protein interactions with BLM and WRN DNA helicases.

[1] The abbreviations used are: BS, Bloom syndrome; WS, Werner syndrome; HR, Homologous recombination; HJ, Holliday junctions; APH, aphidicolin; LCL, lymphoblastoid cell line; PKC, Protein kinase C; PP1, Protein phosphatase 1; EMSA, Electrophoretic Mobility Gel Shift Assay.

INTRODUCTION

Bloom and Werner syndromes (BS and WS) 1 are autosomal recessive disorders characterized by immune deficiency, cancer predisposition and chromosomal instability (1). The products of the genes responsible for these disorders, BLM and WRN, are ATP-dependent DNA helicases that exhibit 3' to 5' polarity. Mutations in the *BLM* or *WRN* genes disrupt their helicase activity, which may be important for the phenotypic traits associated with these hereditary diseases (2).

Homologous recombination (HR) [1] is required for genetic exchange during meiosis, repair of complex lesions in DNA and the segregation of chromosomes at cell division. Expression of the BLM or WRN helicases in *S. cerevisiae* containing a mutation in *sgs1*, a BLM and WRN homolog, suppresses their increased rates of illegitimate recombination and HR (3). BLM and its yeast homologue, Sgs1, functionally interact with topoisomerase III (4) while the WRN interaction with DNA polymerase delta is apparently required for some aspect of DNA replication and/or repair (5,6). Recent reports indicate that both of these helicases recognize and disrupt alternative DNA structures, including G-quadruplex DNA and Holliday junctions (HJ) [1] (7-11). HJ arise as intermediates during HR and can occur spontaneously, or during DNA replication and the repair of DNA damage (12). BLM and WRN may promote ATP-dependent translocation of HJ to eliminate DNA recombination intermediates, thereby reducing inappropriate DNA recombination *in vivo* (10,11).

p53 suppresses genomic instability, particularly in response to DNA damage (13,14). p53 also has been implicated in HR. Evidence of p53 modulation of HR includes the following: (a) overexpression of wild-type p53 (WTp53) can down-regulate the rate of HR between SV40

molecules (15); (b) the rate of HR is increased in p53 mutant cell lines (16-18); (c) p53 has 3' to 5' exonuclease and DNA strand transfer activities (19); and (d) p53 can bind and inhibit human RAD51 and bacterial RecA, central components of the HR pathway (20,21). In vitro, p53 also can bind to the crossover region of HJ (22), positively or negatively supercoiled DNA (23,24), and DNA base mismatches (25); all of these structures can be associated with HR.

p53 physically and functionally interacts with BLM and WRN *in vivo* and *in vitro* (26-28). We hypothesize that p53 may regulate HR through its modulation of the BLM and WRN helicase activities. In this study, we present the first evidence that p53 modulates the ability of BLM and WRN helicases to disrupt HJ. This property can be altered by modifications to the p53 C-terminus at Ser376 or Ser378. These modifications decrease p53's modulation of recombination and abrogate its binding to BLM. p53-mediated inhibition of BLM or WRN helicase activity apparently occurs through direct binding to these helicases. In addition, we show that p53 colocalizes *in vivo* with BLM and RAD51 at putative sites of stalled DNA replication forks and HJ in cells arrested in S-phase by aphidicolin (APH) 1. These results indicate a possible physiological mechanism for the regulation of HR by the physical and functional interaction of p53 with the BLM and WRN DNA helicases as well as their DNA substrates.

Experimental Procedures

Cell culture, Western Blot Analyses and Immunoprecipitation—GM01310, a normal human lymphoblastoid cell line (LCL) [1], was maintained at a density greater than 3 X 10^5 cells/ml in RPMI medium supplemented with 15% fetal bovine serum, penicillin and streptomycin (Biofluids). Human WI-38 and GM08402 fibroblasts were used at early passage. Western blot analysis and immunoprecipitation were performed as described previously (27).

Proteins and Antibodies—Recombinant hexa-histidine tagged human BLM and WRN proteins were purified as described previously (29,30). Human WT, 248W and 273H p53 proteins were generated from recombinant baculoviruses in Sf9 insect cells, and purified using an anti-p53 (antibody PAb421, which recognizes the C-terminus of p53) immunoaffinity column, as described previously (31). The concentrations of dialyzed peak fractions of p53 were determined by silver staining and quantified with a Pantropic p53 Rapid Format Assay kit (Oncogene Research Products). RuvA protein was kindly provided by Dr. Michael M. Cox (University of Wisconsin-Madison). Protein kinase C (PKC) [1] and protein phosphatase 1 (PP1) [1] were from UBI. Anti-BLM and anti-WRN were from Santa Cruz (Santa Cruz). PAb421, DO-1, Ser15 phospho-p53 and anti-RAD51 were from Oncogene Research Products.

DNA Substrates—The synthetic X-junction (four-arm junction, blunt ends, X-12) was prepared by annealing four 50-mer oligonucleotides as described previously (10,32). Briefly, X12-1 was 5'-^{32}P-labeled and annealed with X12-2, X12-3 and X12-4. The product was then purified by separation through a 10% TBE gel, and recovered by electroelution and dialysis. The

linear blunt duplex DNA used as the nonspecific competitor was prepared by annealing X12-1 with its complement. The 28-mer M13mp18 partial duplex substrate was constructed with a 28-mer oligonucleotide complementary to position 3960-3987 in M13mp18. The substrate was labeled, annealed and purified as described previously (33).

Helicase assays—The BLM helicase assay reactions contained the ^{32}P-labeled X-junction in 20 mM Tris-HCl, pH 7.5, 1.25 mM $MgCl_2$, 2 mM ATP, 0.1 mg/ml BSA and 1 mM DTT. The WRN helicase assay reactions contained 40 mM Tris-Borate (pH 7.4), 5 mM $MgCl_2$, 5 mM DTT and 2 mM ATP. Reactions were initiated by the addition of BLM or WRN protein, and they were incubated at 37°C for 45 min. The products were separated by electrophoresis through 10% nondenaturing polyacrylaminde gels at 4°C and visualized using a PhosphorImager or film autoradiography and quantified using the ImageQuant software (Molecular Dynamics). Helicase data shown are representative of at least three independent experiments.

Electrophoretic Mobility Gel Shift Assay (EMSA)—The DNA binding reactions (20 μl) contained 20 mM triethanolamine-HCl, pH 7.5, 2 mM $MgCl_2$, 1 mM ATPγS, 0.1 μg/ml BSA and 1 mM DTT, and protein concentration as indicated in the figures. Reaction mixtures were incubated at room temperature for 20 min and fixed in the presence of 0.25% glutaraldehyde for 10 min at 37°C. The products were separated by electrophoresis through 4% nondenaturing polyacrylaminde gels at 4°C for 3 h, and visualized using a PhosphorImager or film autoradiography.

Electron Microscopic Visualization of p53-HJ interaction—HJ substrates (Hol575) for electron microscopy were prepared as described previously (22). p53-DNA complexes were assembled by incubating 50 ng of DNA in a 50-μl reaction containing 10 mM HEPES (pH 7.5) and 100 mM KCl for 20 min at room temperature using a 1:6 molar ratio of HJ DNAs to p53 tetramers. The complexes were fixed with 0.6% glutaraldehyde (v/v) for 10 min at room temperature followed by gel filtration using a 1 ml Biogel A5m (Bio-Rad) column to remove free protein and fixatives. The samples were prepared for electron microscopy as described previously (34). Briefly, the samples were adsorbed to thin carbon foils supported by 400-mesh copper grids in the presence of spermidine, then washed with a water/ethanol series, air dried and rotary shadowcast with tungsten. The grids were visualized in a Phillips CM12. Images for publication were scanned with a Nikon 4500 AF film scanner and the contrast was adjusted with Adobe Photoshop software.

ELISA and Far Western Blotting—BLM and WRN were diluted to a concentration of 2 nM in Carbonate buffer (0.016 M Na_2CO_3, 0.034 M $NaHCO_3$, pH 9.6) and were then used to coat appropriate wells of a 96-well ELISA plate. WTp53 or mutant p53 proteins were incubated at 0-20 nM in a binding buffer (50 mM Tris-HCl [pH7.4], 5 mM $MgCl_2$, 5 mM ATP, 100 μg/ml BSA and 50 mM NaCl) for 30 min at 24°C. Then, DO-1 and secondary antibodies were added sequentially. Phosphatase substrate (Sigma) was incubated for 1 h at 37°C. The OD_{405} values, corrected for background with BSA, were expressed as the mean of three independent experiments.

Far Western blotting was performed as described by Wu et al. (4). BLM or WRN (200 ng) was

run on SDS-PAGE and transferred to Hybond-ECL filters (Amersham). Filters were denatured and incubated with WT or mutant p53 proteins (200 ng/ml) for 1 h at 4°C. Western analysis was then conducted to detect p53 using DO-1 as the primary antibody.

Phosphorylation-dephosphorylation—Phosphorylation-dephosphorylation of p53 proteins was performed as described (35). p53 protein was incubated in a kinase reaction buffer with PKC (20 ng). The reactions were stopped by the addition of a peptide PKC inhibitor. Reaction mixture was then incubated in the presence or absence of phosphatase PP1 (0.02 U). Separate aliquots were analyzed by Western blot or helicase assay.

In vitro Protein Interaction—GST fusion p53 protein was produced in *E. coli* and purified on glutathion-Sepharose 4B beads according to the manufacturer (Pharmacia LKB). BLM and WRN proteins were prepared using TnT Quick Coupled Transcription/Translation System (Promega) in the presence of [^{35}S]methionine. *In vitro* binding assay was done in IP buffer with rotation at room temperature for 2 h. After washing, the samples were loaded on SDS-PAGE and separated by electrophoresis.

Indirect Immunofluorescence—Cells in 4-well glass chamber slides were cultured with 5 μg/ml APH for 14 h, fixed and stained, as described (4), using anti-BLM, anti-RAD51, and/or Ser15 phospho-p53 antibodies. Images were analyzed by Confocal Assistant software or Laser Sharp. Quantitation of nuclear foci was determined from 100 cells for each treatment. Data were obtained from at least three independent experiments.

RESULTS

Modulation of the BLM and WRN helicase activities by p53—Because the helicase activities of BLM and WRN are necessary for the promotion of HJ branch migration (10,11), a key step in HR, we investigated the possibility that p53 modulates the ability of BLM and WRN to disrupt a radiolabeled synthetic X-junction (X-12) substrate (blunt ends). This X-junction is a mimic of the HJ. Consistent with previous reports (9-11), both purified recombinant BLM and WRN disrupted the X-junction into one-armed (single-stranded DNA) and, to a lesser extent, into two-armed products (Fig. 1). To test the effect of p53 on BLM or WRN disrupting the X-junction, BLM or WRN was incubated with the X-junction in the presence of PAb421-immunopurified recombinant WTp53 (Fig. 1). WTp53 inhibited the activities of both BLM and WRN to similar extents. At 6 nM concentration, WTp53 inhibited both BLM and WRN helicase activities by about 80%. Two recombinant p53 mutant proteins (248W and 273H) that correspond to hotspot mutants found in human cancer also were tested. The 273Hp53 lacked this inhibitory activity, while the 248Wp53 mutant protein had less effect (about 50% inhibition) on BLM or WRN activity. In the absence of BLM or WRN, neither WTp53 nor mutants 248Wp53 and 273Hp53 showed intrinsic helicase activity (Fig. 1A).

To determine whether that inhibition of BLM and WRN helicases by p53 is structure-specific, an M13 28-bp partial duplex DNA substrate was incubated with BLM (6 nM) and increasing amounts of p53 (0, 5, 10, 20 nM monomer). As shown in Supplemental data 1, p53 did not inhibit BLM unwinding of the M13 partial duplex. Consistent with a previous report that p53 does not inhibit WRN unwinding of a partial duplex substrate (33), it suggests that inhibition of BLM and WRN helicases by p53 is structure-specific.

Specificity of BLM, WRN and p53 bound to HJ—Recombinant p53 protein produced in baculovirus exists in a tetrameric form and binds to its DNA consensus sites predominantly as a tetramer or as higher molecular weight complexes (36-40). We used electrophoretic mobility gel shift assays (EMSA) to determine the specific binding of BLM, WRN or p53 to the X-junction. Consistent with previous data (10,11,22), BLM induced a single-shifted band, while both WRN and p53 induced multiple-shifted bands (Fig. 2A). One possibility is that a single molecule of BLM binds to the X-junction, whereas WRN and p53 bind to this substrate either in different oligomeric states or at multiple sites on the DNA molecule. Whereas WTp53 bound efficiently to the X-junction, 248Wp53 bound to a lesser degree, and 273Hp53 showed no detectable binding activity (Fig. 2A). The shifted bands were competed efficiently by the unlabelled X-junction, but not by dsDNA (blunt ends). The simultaneous addition of both p53 and BLM to EMSA reactions resulted in an increased intensity of the shifted bands with different mobility. The presence of both p53 and BLM in these shifted bands was confirmed by Western blot analysis with either p53 or BLM antibody, indicating the presence of a p53-BLM-X-junction complex (Fig. 2B, band 7 and 8). Similar results were obtained when WRN and p53 were used on the EMSA analysis. Both proteins were detected in the shifted bands (Fig. 2C).

Electron Microscopic Visualization of p53-HJ interaction—To visualize HJ by electron microscopy, four-way junctions containing 500 bp arms (Hol575) were constructed as described previously (22). The Hol575 DNA was incubated with WTp53 as well as the 248Wp53 and 273Hp53 at room temperature for 20 min. The samples were fixed, processed through Biogel A5m to remove free proteins and fixatives, and prepared for EM. Examination of the WTp53 complexes

with Hol575 showed that a large number of the HJ DNAs had p53 bound at the crossover point (Fig. 3A). The DNA molecules were scored (n=300) and the results showed that 63% of the HJ had p53 bound somewhere on the DNA. Of these bound molecules, 70% contained p53 at the crossover point, 13% had protein bound along an arm of the HJ and 17% had p53 at an end of one arm. The 248Wp53 showed slightly lower binding affinity than the WTp53. Of the molecules scored, 40% of the molecules were bound by p53. In addition, this mutant had lower affinity for the crossover point of the junction. Of the bound molecules counted, only 45% had protein at the crossover junction when compared with 70% in WTp53. The remaining molecules had p53 bound either along the arm or at the termini of the arms. Finally, the 273Hp53 mutant showed very low binding to the Hol575 template. Only 12% of the DNA molecules were bound by the protein and, more interestingly, none of the bound molecules had p53 at the crossover point. When compared with the WTp53, this mutant not only had reduced binding to DNA, but also had lost the specificity of the crossover point.

The *E. coli* RuvA protein specific binds to HJ at the crossover junction (41,42). Here, it competed efficiently with WRN or p53 for binding to the X-junction (Fig. 3B). In the presence of a 3-fold excess of WRN or p53 over RuvA, the RuvA-X-junction complex predominated. This indicated that the affinity of WRN and p53 for the X-junction is less than that observed with RuvA. Consistent with a previous report (11), similar results were seen with BLM (data not shown). These data indicate that p53, BLM and WRN recognize the DNA crossover of the HJ.

Relationship between binding to HJ and inhibition of helicase activity by p53—Next, we compared the effects of various concentrations of WT, 248W or 273H p53 for their binding to the X-junction and their inhibition of BLM or WRN helicase activity. WTp53 bound to the X-

junction and inhibited the helicase activities of BLM and WRN in a dose-dependent manner (Fig. 4). Binding of the 248Wp53 to the X-junction and inhibition of BLM and WRN helicase activities were dose-dependent, but the magnitude of the effect was reduced significantly relative to that of WTp53. 273Hp53 did not significantly bind to the X-junction nor inhibit helicase activity.

In vitro and in vivo interaction of p53 with BLM or WRN—To determine whether protein-protein interactions were also responsible for p53-mediated modulation of the BLM and WRN helicase activities *in vivo*, cell lysates were prepared from either untreated or irradiated (5 Gy) normal lymphoblastoid cells (GM01310). The lysates were then subjected to immunoprecipitation with anti-BLM or anti-WRN antibody. The efficiency of the immunoprecipitation was assessed by analysing supernatants of the immunoprecipitation and straight loadings of the cell lysates by Western blotting with anti-BLM or anti-WRN antibody. Only about 20% of BLM or WRN proteins were immunoprecipitated by these antibodies (data not shown).

A dilution series of recombinant p53 was used as a standard for quantification of the amount of cellular p53. p53 increased to 3.3 x 10^4 molecules/cell 3 hr after irradiation (Fig. 5A), whereas the amount of BLM was unchanged under these experimental conditions. Assuming that there are 4,000 molecules of BLM/cell (43), the ratio of p53 to BLM is about 8:1. Previous studies have suggested that active forms of BLM and p53 are hexameric and tetrameric, respectively (30,36). Hence, the maximum calculated percentage of p53 that could be immunoprecipitated by anti-BLM antibodies is approximately 12%. We found that about 2% of the cellular p53 was immunoprecipitated by anti-BLM or anti-WRN antibody after exposure to irradiation (Fig. 5B).

The efficiency of BLM or WRN IP was only 20%, thus we conclude that approximate 10% of the cellular p53 binds to either BLM or WRN under our conditions. This is close to the theoretical maximum that could be immunoprecipitated by anti-helicase antibodies, indicating that both helicases bind saturating amounts of p53. Because p53 is in large excess, these results indicate that only a fraction of p53 is involved in binding to helicases. Competitive peptides blocked co-IP of p53 with anti-BLM or WRN excluding the possibility that the immunoprecipitation was nonspecific (data not shown).

Direct binding of p53 to both BLM and WRN was confirmed by Far Western analysis. BLM, WRN, or p53 was separated by SDS-PAGE and transferred to a nitrocellulose membrane, which was then incubated with WT or mutant p53 proteins. WTp53 and 248Wp53 exhibited strong binding to BLM or WRN, whereas 273Hp53 showed weaker, but detectable, binding. BSA was used as a negative control (Fig. 5C).

We next analyzed the binding affinities of WT and mutant p53 to BLM and WRN. Using an ELISA assay, WTp53 and 248Wp53 bound in a dose-dependent manner, and with similar affinities, to both BLM and WRN. In contrast, 273Hp53 showed a relatively weak level of binding to either BLM or WRN (Fig. 5D). The specificity of the interaction was demonstrated by the absence of detectable signals in wells that had been precoated with BSA only (data not shown).

Modifications to the p53 C-terminus attenuate its inhibition of BLM and WRN helicase activities—The p53 C-terminus is required for binding to both BLM or WRN (26-28) and may be posttranslationally modified (44,45). Therefore, we determined whether modification of the p53 C-terminus would alter its ability to modulate the helicase activities of BLM and WRN in a model

system. Protein kinase C (PKC) phosphorylates p53 *in vitro* at Ser378 within the PAb421 antibody epitope, thereby reducing PAb421 reactivity (35,46). Protein phosphatase 1 (PP1) dephosphorylates the PKC-reactive site in p53 (35). Consistent with these reports, phosphorylation of purified recombinant p53 protein by PKC significantly reduced the reactivity of p53 to PAb421, but not to DO-1, an antibody that targets the N-terminus of p53 (Supplemental data). Incubation of the PKC-treated p53 protein with phosphatase PP1 effectively restored PAb421 reactivity.

The PKC-phosphorylated p53 protein exhibited reduced inhibition of BLM or WRN helicase activity relative to unmodified p53, whereas dephosphorylation of PKC-treated p53 protein by PP1 restored its activity (Figs. 6A and B). With PKC-phosphorylated p53 protein at a concentration of 9 nM, approximately 28% inhibition of BLM and 21% inhibition of WRN helicase activities were observed. Approximately 93% inhibition of both BLM and WRN helicase activities was achieved using dephosphorylated PKC-treated p53, similar to the 94% inhibition seen when using unmodified WTp53. In addition, PAb421, but not DO-1, an antibody to the N-terminus of p53, also blocked the inhibitory effect of p53 on BLM and WRN helicase activities. Compared with 45% inhibition of BLM, the PAb421-p53 complex gave only 12% inhibition of WRN.

To examine whether modification of the p53 C-terminus altered binding to HJ, the binding affinities of PKC-phosphorylated p53 and dephosphorylated PKC-treated p53 to the X-junction were determined by EMSA. The PKC-phosphorylated p53 and dephosphorylated PKC-treated p53 had similar affinities toward the X-junction as unmodified WTp53 (Supplemental data). To explore the mechanism of p53 inhibition of BLM and WRN helicase activities, we examined binding affinities between C-terminal modified p53 and BLM or WRN protein. In agreement

with the results of the helicase assays, modification of the recombinant GST-p53 fusion protein, through either PAb421 antibody or PKC phosphorylation, attenuated its ability to bind to BLM or WRN protein (Fig. 6C).

To further determine the requirement of the p53 C-terminus for the modulation of BLM and WRN helicase activity, a synthetic p53 peptide corresponding to residues 373-383 was used in the helicase assay. This p53 peptide exhibited a concentration-dependent inhibition of BLM and WRN helicase unwinding of the X-junction (Figs. 7A and B). In contrast, the p53 peptide phosphorylated at Ser376 (P1) or Ser378 (P2) was noninhibitory, even at a high concentration (Figs. 7A and B). These three short peptides did not compete with p53 for binding to the X-junction, whereas the longer p53 peptide (319-393aa) competed efficiently (Supplemental data). Taken together, we conclude that a p53 C-terminal region containing residues 373-383 is required for the interaction with and modulation of the branch migration activities of the BLM and WRN helicases on HJ.

p53, BLM and RAD51 colocalize to nuclear foci—RAD51 protein catalyzes a key step in HR, and it accumulates in nuclear foci that are thought to correspond to sites of stalled replication forks and recombinational repair of DNA double-strand breaks (47). BLM and RAD51 form a complex and colocalize to nuclear foci in cells arrested in S-phase using either APH or hydroxyurea (47,48), suggesting that these proteins cooperate in the repair of breaks arising at stalled replication forks. The effects of p53 on BLM disruption of HJ presented here suggest that p53 may be involved in this process *in vivo*. To test this hypothesis, we examined the localization of p53, BLM and RAD51 in cells treated with APH. Cell cycle distribution was determined by flow cytometry (data not shown). Consistent with previous reports (47,48), BLM and RAD51 nuclear foci

increased and colocalized in S-phase cells after treatment with APH (Fig. 8). About 45% of the APH-treated cells displayed Ser15 phospho-p53 nuclear foci, but none of the untreated cells did. Quantitative analysis of the confocal microscopic pixels indicated that 63% and 39% of Ser15 phospho-p53 foci colocalized with BLM and RAD51, respectively (Fig 8B). Similarly, Ser15 phospho-p53, BLM and RAD51 were found to colocalize after S-phase arrest in U2OS cells, derived from a human osteogenic sarcoma that contains WTp53 (data not shown). These colocalization data provide *in vivo* evidence that p53 may play a role in a recombinational repair pathway that also includes BLM and RAD51.

DISCUSSION

p53-mediated inhibition of BLM and WRN helicase activities—We report evidence that p53 inhibits the helicase activities of both BLM and WRN. WTp53 inhibits BLM and WRN helicase disruption of the X-junction more efficiently than the 248Wp53, whereas the 273Hp53 lacks this activity. Mutations in p53 including codons 248 and 273 are observed frequently in human cancers (49,50). The influence of these missense mutations on DNA binding and on protein conformation is still unclear. Most of the mutants at codons 248 and 273 do not display any obvious change in their protein conformation, as determined by reactivity to antibodies PAb240 or PAb1620, or by binding to hsp70 protein (51). Analysis of the crystal structure of p53 reveals that both 248 and 273 residues contact the DNA directly (52). WTp53 exerts an inhibitory effect on the exonuclease activity of WRN, whereas the 273Hp53 mutant does not (33). Recent studies indicate that WTp53 inhibits recombinational processes when encountering mismatches in heteroduplexes, but 273Hp53 is significantly defective in this function (18). Our results indicate that WTp53 can regulate members of the RecQ helicase family involved in recombinational repair, but that the 273Hp53 mutant lacks this function. These data are consistent with the hypothesis that WTp53 plays a functional role in the helicase-HR pathway.

Effects of modifications to the p53 C-terminus on helicase activity—The p53 C-terminus contains several important phosphorylation sites that affect p53-mediated function (44,45). For example, p53 can be regulated positively or negatively by reversible PKC modifications *in vitro*, affecting the latent or active state of the protein (35,45), although it is uncertain whether or not PKC phosphorylates p53 *in vivo* (53-55). Certain types of cellular stress, e.g., ionizing irradiation, lead to

rapid dephosphorylation of p53 at Ser376 (46,56). Recent reports have shown that p53 binds to BLM or WRN *in vivo* and *in vitro* and that p53 lacking the C-terminus does not bind to these helicases (26-28). Based on the data presented here, the p53 C-terminus is involved in the inhibition of the BLM and WRN helicase activities. p53-mediated inhibition of BLM or WRN helicase activity is reduced by modification of p53 through either the binding of PAb421, a p53 specific antibody that binds to a C-terminal epitope, or the phosphorylation at Ser376 and Ser378, which inhibits its binding to BLM or WRN. Furthermore, a p53 C-terminal polypeptide (residues 373-383) is sufficient to inhibit BLM or WRN helicase activity, while a peptide phosphorylated at Ser376 or Ser378 lacks this activity. Taken together, our data provide direct evidence that posttranslational modification of the p53 C-terminus regulates its interaction with these DNA helicases. The fact that this small C-terminal peptide inhibits helicase activity and that the inhibition can be reversed by phosphorylation indicates that the p53 C-terminus contains an active site. Posttranslational modification of p53 in response to DNA strand breaks may be a molecular switch that regulates the functional interaction between p53 and DNA helicases.

Insight into the mechanism of p53-mediated BLM and WRN helicase activities—BLM specifically binds to HJ, but fails to form a stable complex with linear, blunt ended duplex DNA that contains a sequence identical to that of one of the ‘arms’ of HJ (11). This indicates that BLM binds strongly to the crossover region of HJ. WRN also binds to HJ (10). The activity of WRN on recombination intermediates is due, at least in part, to the recognition of the junction within the duplex DNA substrate.

Here, we report that the binding affinity of WTp53 to a mimic of the HJ is higher than that of the 248Wp53 mutant, and that the 273Hp53 lacks this binding ability. Since abilities of WTp53,

248Wp53 and 273Hp53 proteins to bind to the X-junction correlate with their capacity to inhibit BLM and WRN helicase activities, it is possible that p53 binding to the DNA substrate may be required for p53-mediated inhibition of the helicase activity. However, both helicases also interact physically with WTp53, as shown by Far Western blotting and ELISA assays, indicating that p53 also may modulate the helicase activity by binding directly to the BLM or WRN protein. Consistent with this latter hypothesis, modification of the p53 C-terminus leads to an attenuation of p53-mediated inhibition on BLM and WRN helicase activities, but does not impair its binding to the X-junction. These findings are strong evidence that inhibition of BLM and WRN helicase activities by p53 is mediated by direct interaction with BLM and WRN, and not with the HJ substrate.

p53 as a cofactor in BLM-RAD51 HR pathway—RAD51 is a central component of the HR pathway that is involved in DNA double-strand break repair (47). One major role for HR that has emerged in recent years is to facilitate the reinitiation of replication following replication fork collapse. Removal of HJ is necessary following such repair. Interaction between BLM and RAD51 may, therefore, serve to recruit BLM to the sites of recombinational repair (47,57). BLM disrupts HJ by branch migration, and the loss of BLM would give rise to excessive recombination, corresponding to the genome-wide hyper-recombination and genomic instability in BLM-deficient cells. p53 binds to RAD51 and is involved in recombinational repair (20) and p53-deficient cells show hyper-recombination (16-18,21). One model is that p53 or other proteins, e.g., RAD51 (47), recruit BLM and WRN to HJ and participate in the assembly of the multiprotein HR complex (28). The physical and functional interactions between these DNA helicases and p53 may be regulated either by its posttranslational modification, consistent with the data shown here, or by

other proteins in the HR complex. Further studies are needed to refine this model. Consistent with previous reports that WRN co-purifies with a DNA replication complex (58) and binds to p53 (26,27), p53 may regulate the anti-recombinase functions of the human RecQ helicase family members that are critical for the maintenance of genomic stability.

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Reference List

1. German, J., Ellis, N. A., and Proytcheva, M. (1996) *Clin. Genet.* **49,** 223-231

2. Chakraverty, R. K. and Hickson, I. D. (1999) *Bioessays* **21,** 286-294

3. Yamagata, K., Kato, J.-I., Shimamoto, A., Goto, M., Furuichi, Y., and Ikeda, H. (1998) *Proc. Natl. Acad. Sci. USA* **95,** 8733-8738

4. Wu, L., Davies, S. L., North, P. S., Goulaouic, H., Riou, J. F., Turley, H., Gatter, K. C., and Hickson, I. D. (2000) *J. Biol. Chem.* **275,** 9636-9644

5. Kamath-Loeb, A. S., Johansson, E., Burgers, P. M., and Loeb, L. A. (2000) *Proc. Natl. Acad. Sci. USA* **97,** 4603-4608

6. Szekely, A. M., Chen, Y. H., Zhang, C., Oshima, J., and Weissman, S. M. (2000) *Proc. Natl. Acad. Sci. USA* **97,** 11365-11370

7. Fry, M. and Loeb, L. A. (1999) *J. Biol. Chem.* **274,** 12797-12802

8. Sun, H., Karow, J. K., Hickson, I. D., and Maizels, N. (1998) *J. Biol. Chem.* **273,** 27587-27592

9. Mohaghegh, P., Karow, J. K., Brosh, J. R., Jr., Bohr, V. A., and Hickson, I. D. (2001) *Nucleic Acids Res.* **29,** 2843-2849

10. Constantinou, A., Tarsounas, M., Karow, J. K., Brosh, R. M., Bohr, V. A., Hickson, I. D., and West, S. C. (2000) *EMBO Reports* **1,** 80-84

11. Karow, J. K., Constantinou, A., Li, J. L., West, S. C., and Hickson, I. D. (2000) *Proc. Natl. Acad. Sci. USA* **97,** 6504-6508

12. Sharples, G. J., Ingleston, S. M., and Lloyd, R. G. (1999) *J. Bacteriol.* **181,** 5543-5550

13. Vogelstein, B., Lane, D., and Levine, A. J. (2000) *Nature* **408,** 307-310

14. Oren, M. (1999) *J. Biol. Chem.* **274,** 36031-36034

15. Wiesmuller, L., Cammenga, J., and Deppert, W. W. (1996) *J. Virol.* **70,** 737-744

16. Bertrand, P., Rouillard, D., Boulet, A., Levalois, C., Soussi, T., and Lopez, B. S. (1997) *Oncogene* **14,** 1117-1122

17. Mekeel, K. L., Tang, W., Kachnic, L. A., Luo, C. M., DeFrank, J. S., and Powell, S. N. (1997) *Oncogene* **14,** 1847-1857

18. Dudenhoffer, C., Kurth, M., Janus, F., Deppert, W., and Wiesmuller, L. (1999) *Oncogene* **18,** 5773-5784

19. Mummenbrauer, T., Janus, F., Muller, B., Wiesmuller, L., Deppert, W., and Grosse, F. (1996) *Cell* **85,** 1089-1099

20. Buchhop, S., Gibson, M. K., Wang, X. W., Wagner, P., Sturzbecher, H. W., and Harris, C. C. (1997) *Nucleic Acids Res.* **25,** 3868-3874

21. Sturzbecher, H.-W., Donzelmann, B., Henning, W., Knippschild, U., and Buchhop, S. (1996) *EMBO J.* **15,** 1992-2002

22. Lee, S., Cavallo, L., and Griffith, J. (1997) *J. Biol. Chem.* **272,** 7532-7539

23. Palecek, E., Vlk, D., Stankova, V., Brazda, V., Vojtesek, B., Hupp, T. R., Schaper, A., and Jovin, T. M. (1997) *Oncogene* **15,** 2201-2209

24. Mazur, S. J., Sakaguchi, K., Appella, E., Wang, X. W., Harris, C. C., and Bohr, V. A. (1999) *J. Mol. Biol.* **292,** 241-249

25. Degtyareva, N., Subramanian, D., and Griffith, J. D. (2001) *J. Biol. Chem.* **276,** 8778-8784

26. Blander, G., Kipnis, J., Leal, J. F., Yu, C. E., Schellenberg, G. D., and Oren, M. (1999) *J.Biol.Chem.* **274,** 29463-29469

27. Spillare, E. A., Robles, A. I., Wang, X. W., Shen, J. C., Schellenberg, G. D., and Harris, C. C. (1999) *Genes Dev.* **13,** 1355-1360

28. Wang, X. W., Tseng, A., Ellis, N. A., Spillare, E. A., Linke, S. P., Robles, A. I., Seker, H., Yang, Q., Hu, P., Beresten, S., Bemmels, N. A., Garfield, S., and Harris, C. C. (2001) *J. Biol. Chem.* **276,** 32948-32955

29. Brosh, R. M., Jr., Orren, D. K., Nehlin, J. O., Ravn, P. H., Kenny, M. K., Machwe, A., and Bohr, V. A. (1999) *J. Biol. Chem.* **274,** 18341-18350

30. Karow, J. K., Newman, R. H., Freemont, P. S., and Hickson, I. D. (1999) *Curr.Biol.* **9,** 597-600

31. Bargonetti, J., Friedman, P. N., Kern, S. E., Vogelstein, B., and Prives, C. (1991) *Cell* **65,** 1083-1091

32. McGlynn, P. and Lloyd, R. G. (1999) *Nucleic Acids Res.* **27,** 3049-3056

33. Brosh, R. M., Jr., Karmakar, P., Sommers, J. A., Yang, Q., Wang, X. W., Spillare, E. A., Harris, C. C., and Bohr, V. A. (2001) *J. Biol. Chem.* **276,** 35093-35102

34. Griffith, J. D. and Christiansen, G. (1978) *Annu. Rev. Biophys. Bioeng.* **7,** 19-35

35. Takenaka, I., Morin, F., Seizinger, B. R., and Kley, N. (1995) *J. Biol. Chem.* **270,** 5405-5411

36. Halazonetis, T. D. and Kandil, A. N. (1993) *EMBO J.* **12,** 5057-5064

37. Kern, S. E., Kinzler, K. W., Bruskin, A., Jarosz, D., Friedman, P., Prives, C., and Vogelstein, B. (1991) *Science* **252,** 1708-1711

38. Hainaut, P. and Milner, J. (1993) *Cancer Res.* **53,** 4469-4473

39. Hupp, T. R., Meek, D. W., Midgley, C. A., and Lane, D. P. (1993) *Nucleic Acids Res.* **21,** 3167-3174

40. Hupp, T. R., Meek, D. W., Midgley, C. A., and Lane, D. P. (1992) *Cell* **71,** 875-886

41. Adams, D. E. and West, S. C. (1995) *Mutat. Res.* **337,** 149-159

42. Rafferty, J. B., Sedelnikova, S. E., Hargreaves, D., Artymiuk, P. J., Baker, P. J., Sharples, G. J., Mahdi, A. A., Lloyd, R. G., and Rice, D. W. (1996) *Science* **274,** 415-421

43. Sanz, M. M., Proytcheva, M., Ellis, N. A., Holloman, W. K., and German, J. (2000) *Cytogenet. Cell Genet.* **91,** 217-223

44. Appella, E. and Anderson, C. W. (2001) *Eur. J. Biochem.* **268,** 2764-2772

45. Jayaraman, L. and Prives, C. (1999) *Cell Mol. Life Sci.* **55,** 76-87

46. Waterman, M. J., Stavridi, E. S., Waterman, J. L., and Halazonetis, T. D. (1998) *Nat. Genet.* **19,** 175-178

47. Wu, L., Davies, S. L., Levitt, N. C., and Hickson, I. D. (2001) *J. Biol. Chem.* **276**, 19375-19381

48. Bischof, O., Kim, S. H., Irving, J., Beresten, S., Ellis, N. A., and Campisi, J. (2001) *J. Cell Biol.* **153,** 367-380

49. Hollstein, M., Sidransky, D., Vogelstein, B., and Harris, C. C. (1991) *Science* **253,** 49-53

50. Hainaut, P. and Hollstein, M. (2000) *Adv. Cancer Res.* **77,** 81-137

51. Ory, K., Legros, Y., Auguin, C., and Soussi, T. (1994) *EMBO J* **13,** 3496-3504

52. Cho, Y., Gorina, S., Jeffrey, P., and Pavletich, N. P. (1994) *Science* **265,** 346-355

53. Chernov, M. V., Bean, L. J., Lerner, N., and Stark, G. R. (2001) *J. Biol. Chem.* **276,** 31819-31824

54. Milne, D. M., McKendrick, L., Jardine, L. J., Deacon, E., Lord, J. M., and Meek, D. W. (1996) *Oncogene* **13,** 205-211

55. Chernov, M. V., Ramana, C. V., Adler, V. V., and Stark, G. R. (1998) *Proc. Natl. Acad. Sci. USA* **95,** 2284-2289

56. Webley, K., Bond, J. A., Jones, C. J., Blaydes, J. P., Craig, A., Hupp, T., and Wynford-

Thomas, D. (2000) *Mol. Cell Biol.* **20,** 2803-2808

57. Wu, L. and Hickson, I. D. (2001) *Science* **292,** 229-230

58. Lebel, M., Spillare, E. A., Harris, C. C., and Leder, P. (1999) *J. Biol. Chem.* **274,** 37795-37799

FIGURE LEGENDS

Fig. 1. Effect of WT or mutant p53 on WRN (panel **A**) or BLM (panel **B**) helicase activity. The helicase assay used 6 nM BLM or WRN protein with the X-junction (1 fmol) in the absence (lane 3) or the presence (lanes 4-6) of 6 nM WT, 248W or 237H p53 protein. p53 was incubated simultaneously with BLM or WRN protein and the X-junction. The appearance of the faster migrating two-armed products (small amount) and ssDNA species indicates disruption of the X-junction. Δ, heat-denatured control.

Fig. 2. A. The binding of BLM, WRN, WT, 248W or 273H p53 to radiolabeled X-junction (2 fmol) was competed with double-stranded DNA or unlabelled X-junction. N, double-stranded DNA (20-fold excess over the radiolabeled X-junction). S, unlabelled X-junction (10-fold excess). **B** and **C**. Twenty fmols of the X-junction were incubated simultaneously with BLM or WRN (120 nM) and/or WTp53 (120 nM) and analyzed by EMSA. The shift bands were cut and separated by SDS-PAGE following by conventional Western blotting with anti-BLM, anti-WRN or anti-p53 (DO-1).

Fig. 3. A. Visualization of p53 bound to HJ. WTp53 (A), the 248Wp53 (B) and 273Hp53 proteins (C) were incubated with HJ containing 500 bp arms at a molar ratio of six p53 tetramers per DNA molecule. Shown in reverse contrast. Bar is equivalent to 500 bp. **B**. Competition for binding of WRN or p53 to the X-junction by RuvA using EMSA.

Fig. 4. WT or mutant p53 binding to the X-junction and its effect on BLM or WRN helicase

activity. **A**. EMSA was carried out with the X-junction (2 fmol) containing the indicated concentrations of WT, 248W or 273H p53 protein. The percent binding was calculated from the ratio of shifted bands to free probe. Percent DNA binding is expressed as a function of p53 concentration. **B**. The X-junction (1 fmol) was incubated with 6 nM BLM or WRN protein in the absence or the presence of the indicated concentrations of WT, 248W or 273H p53 under standard helicase assay conditions. Quantification of the products included two-armed and ssDNA species. The relative percent X-junction disruption is expressed as a function of p53 concentration.

Fig. 5. *In vivo* and *in vitro* interaction between p53 and BLM or WRN. **A**. Normal LCL (GM01310, NL) were treated with or without 5 Gy γ-radiation and incubated for 2 h. Cell lysates were analyzed by Western blotting with anti-p53 antibody (DO-1) to quantify the cellular p53 amount, using recombinant p53 as standards. **B**. Cell lysates (10-fold amounts of WCE) were subjected to immunoprecipitation with anti-BLM or anti-WRN antibody, followed by Western blotting with anti-p53 antibody (DO-1) to quantitate immunoprecipitated p53. WCE, whole cell extracts. **C**. Detection of p53 interaction with BLM and WRN by Far Western blotting. BLM or WRN was fixed to a nitrocellulose filter and incubated with purified WTp53, 248Wp53 or 273Hp53. The filter was then probed using the anti-p53 DO-1 antibody. WT and mutant p53 were loaded directly as positive controls (lanes 3, 7 and 11) and BSA was used as a negative control (lanes 4, 8 and 12). **D**. p53 binding to BLM or WRN was quantified by ELISA. BLM or WRN-precoated wells were incubated with WT or mutant p53 proteins. Bound p53 protein was detected using the DO-1 antibody. The OD_{405} values were corrected for background binding in the BSA-coated wells. WTp53-BLM: n, WTp53-WRN: o, 248Wp53-

BLM: l, 248Wp53-WRN: m, 273Hp53-BLM: s, 273Hp53-WRN: ³.

Fig. 6. A and **B**. Effect of modified p53 on BLM or WRN helicase activity. BLM or WRN protein (9 nM) was incubated with the X-junction (1 fmol) in the presence of WT p53 (9 nM), PAb421 (50 ng), DO-1 (50 ng, lane 5), PKC-phosphorylated p53 (9 nM p53, 20 ng PKC), and/or PP1-dephosphorylated p53 (9 nM p53, 20 ng PKC, 0.02 U) under standard helicase reaction conditions. **C**. Interaction of modified p53 with BLM or WRN. 2 μg GST-p53 fusion proteins were modified by PAb421, DO-1, PKC or PKC + PP1, as described as above, then incubated with 5 μl *in vitro*-translated BLM or WRN protein labeled with [^{35}S] methionine to determine the binding affinity of p53 with BLM and WRN. A 20% input of the BLM and WRN proteins is included in lane 6 (from the same blot). GST-p53 protein input was verified by Commassie blue staining.

Fig. 7. A and **B**. Effects of C-terminal peptides of p53 on BLM or WRN helicase activity. BLM or WRN protein was incubated with the X-junction (1 fmol) in the presence of C-terminal peptides of p53 under standard helicase reaction conditions.

Fig. 8. Increasing colocalization of p53 with BLM or RAD51 in cells treated with APH. **A**. WI-38 cells were stained with anti-BLM, anti-RAD51, and/or anti-Ser15 phospho-p53 antibodies, and nuclei were stained with DAPI. Untreated cells were used as controls. **B**. Quantitation of nuclear foci (mean±S.D.) was determined from 100 cells analyzed by Confocal Assistant software or Laser Sharp. Data were obtained from at least three independent experiments. Student's t-test was used for analyzing the statistical significance of colocalization between

p53-BLM and BLM-p53 ($P<0.5$), p53-RAD51 and RAD51-p53 ($P>0.5$), and BLM-RAD51 treated and untreated ($P<0.01$) groups.

Supplemental data 1. p53 does not inhibit BLM helicase activity on a 28-bp M13 partial duplex substrate. BLM protein with the M13 partial duplex in the presence of the indicated concentrations of WTp53 under the standard helicase reaction conditions. ΔH, heat-denatured control.

Supplemental data 2. A. Protein kinase C phosphorylates p53 protein and reduces p53 reactivity to PAb421. p53 protein was incubated with PKC and further incubated in the presence or absence of phosphatase PP1 (0.02 U). The immunoreactivity to antibodies PAb421and DO-1 was determined by Western blot analysis. **B**. Comparison of modified p53 by PKC and PP1 binding to the X-junction by EMSA. Student's t-test was used for analyzing the statistical significance between unmodified WTp53 and PKC-phosphorylated p53 or PP1-dephosphorylated p53 binding to the X-junction ($P>0.5$). **C.** Competition for binding of WTp53 to the X-junction by p53 peptides using EMSA.

Fig. 1

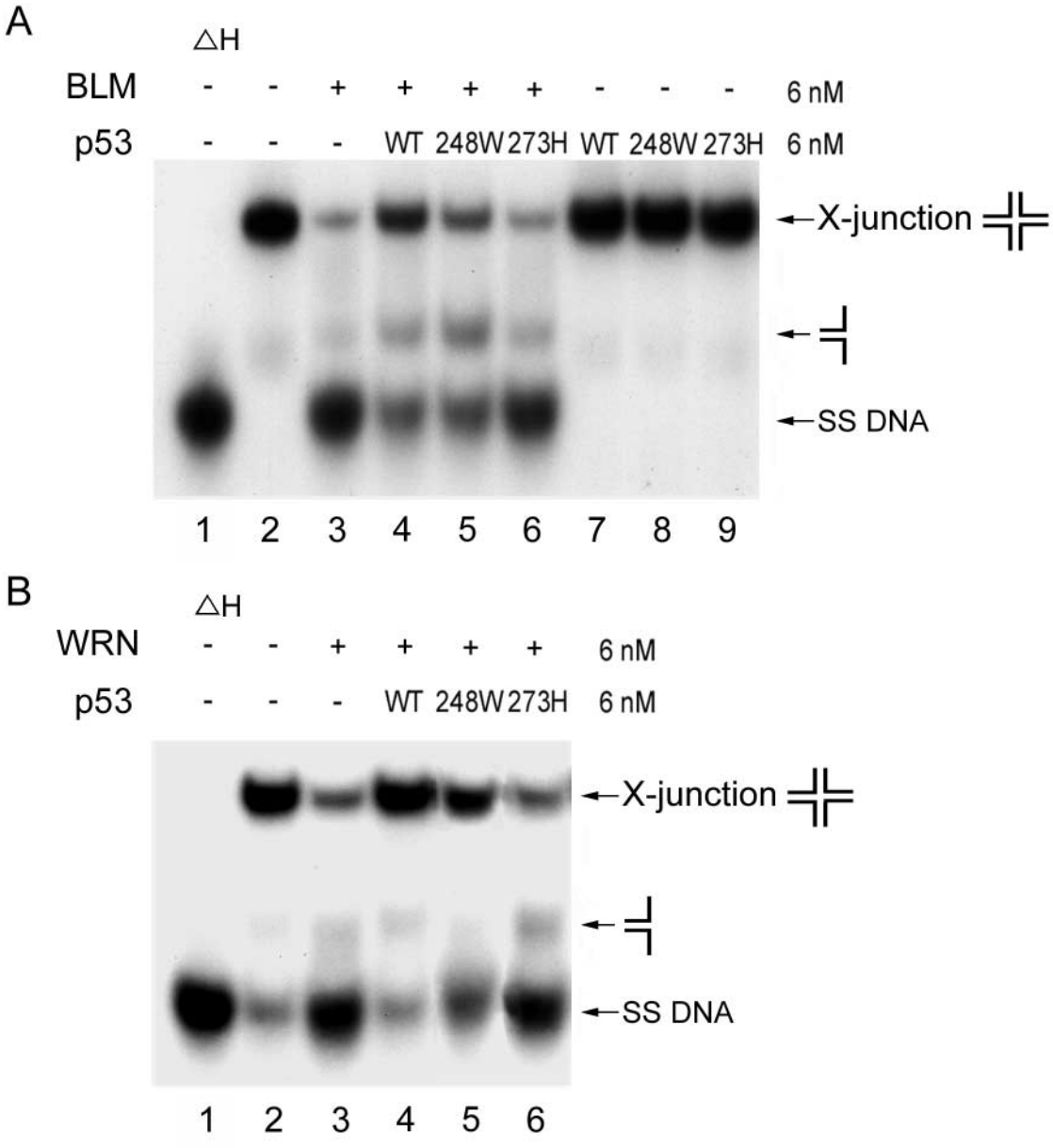

A
△H
BLM - - + + + + - - - 6 nM
p53 - - - WT 248W 273H WT 248W 273H 6 nM
X-junction
SS DNA
1 2 3 4 5 6 7 8 9
B
△H
WRN - - + + + + 6 nM
p53 - - - WT 248W 273H 6 nM
X-junction
SS DNA
1 2 3 4 5 6

Fig. 2

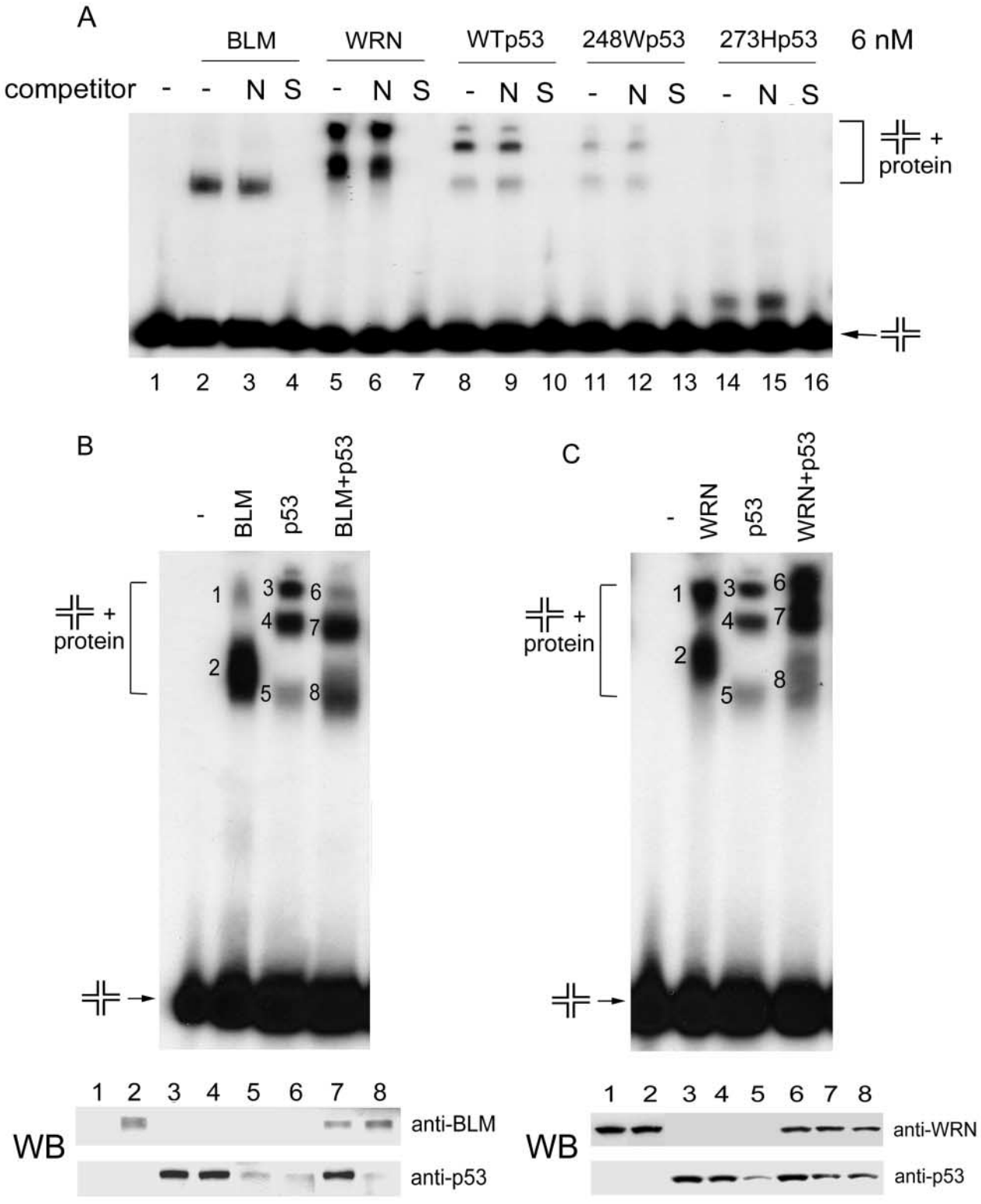
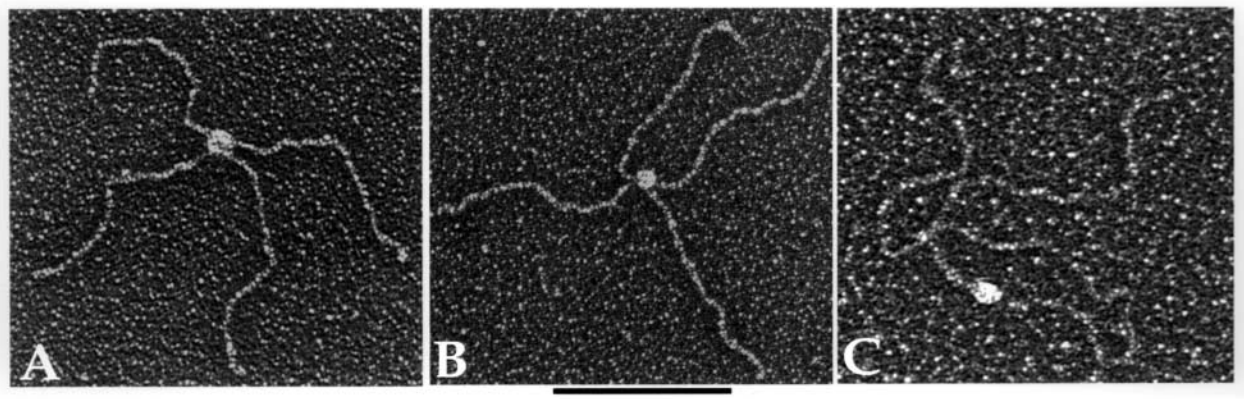

A
BLM
WRN
WTp53
248Wp53
273Hp53
6 nM
competitor - - N S - N S - N S - N S - N S
+ protein
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16
B
- BLM p53 BLM+p53
+ protein
1 2 3 4 5 6 7 8
WB
anti-BLM
anti-p53
C
- WRN p53 WRN+p53
+ protein
1 2 3 4 5 6 7 8
WB
anti-WRN
anti-p53

Fig. 3

A


A
B
C

B

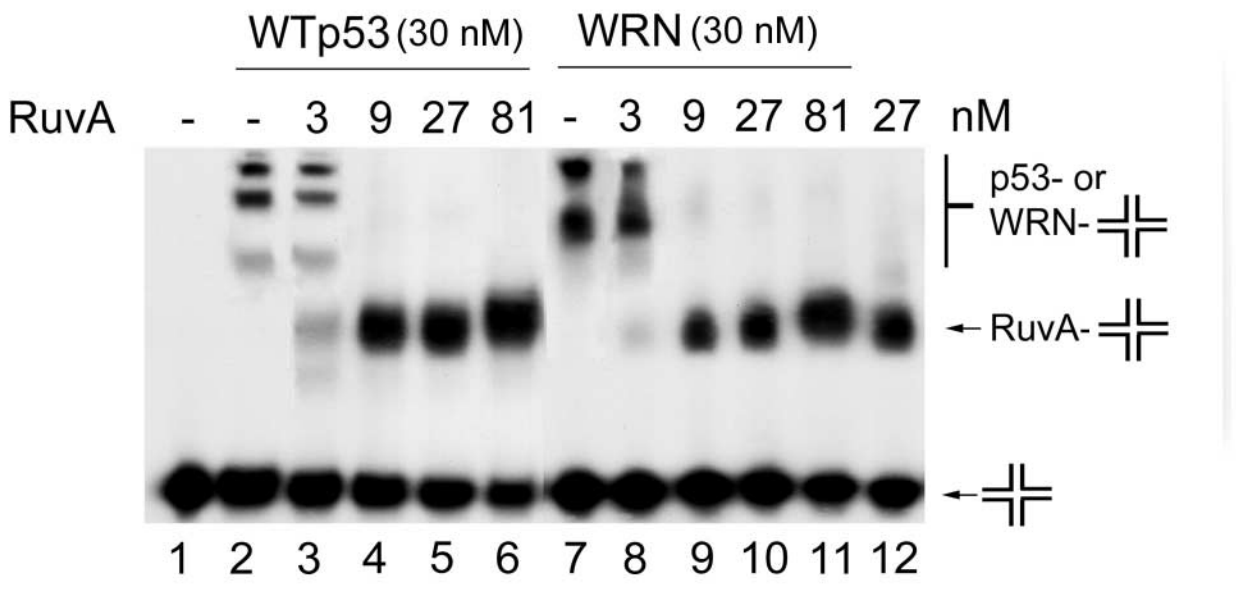

WTp53 (30 nM)
WRN (30 nM)
RuvA - - 3 9 27 81 - 3 9 27 81 27 nM
p53- or WRN-
RuvA-
1 2 3 4 5 6 7 8 9 10 11 12

Fig. 4

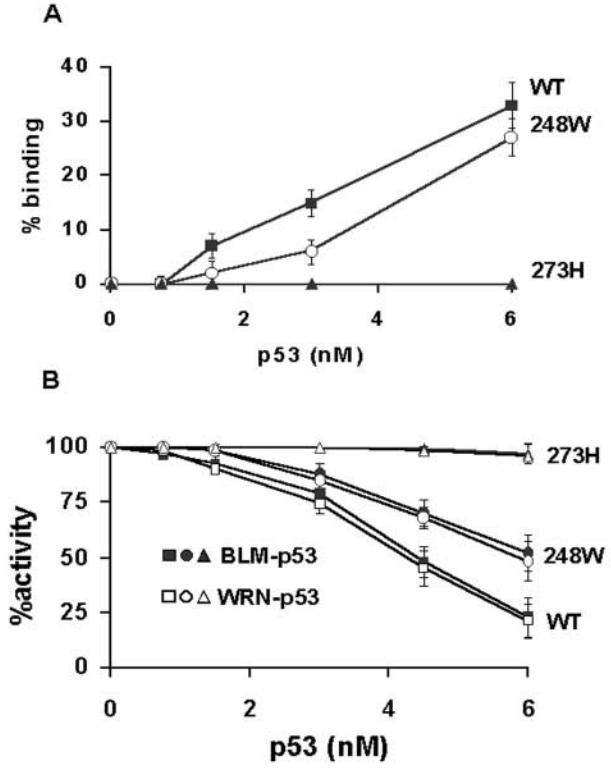

A
40
30
20
10
0
% binding
WT
248W
273H
0
2
4
6
p53 (nM)
B
100
75
50
25
0
%activity
273H
BLM-p53
WRN-p53
248W
WT
0
2
4
6
p53 (nM)

Fig. 5

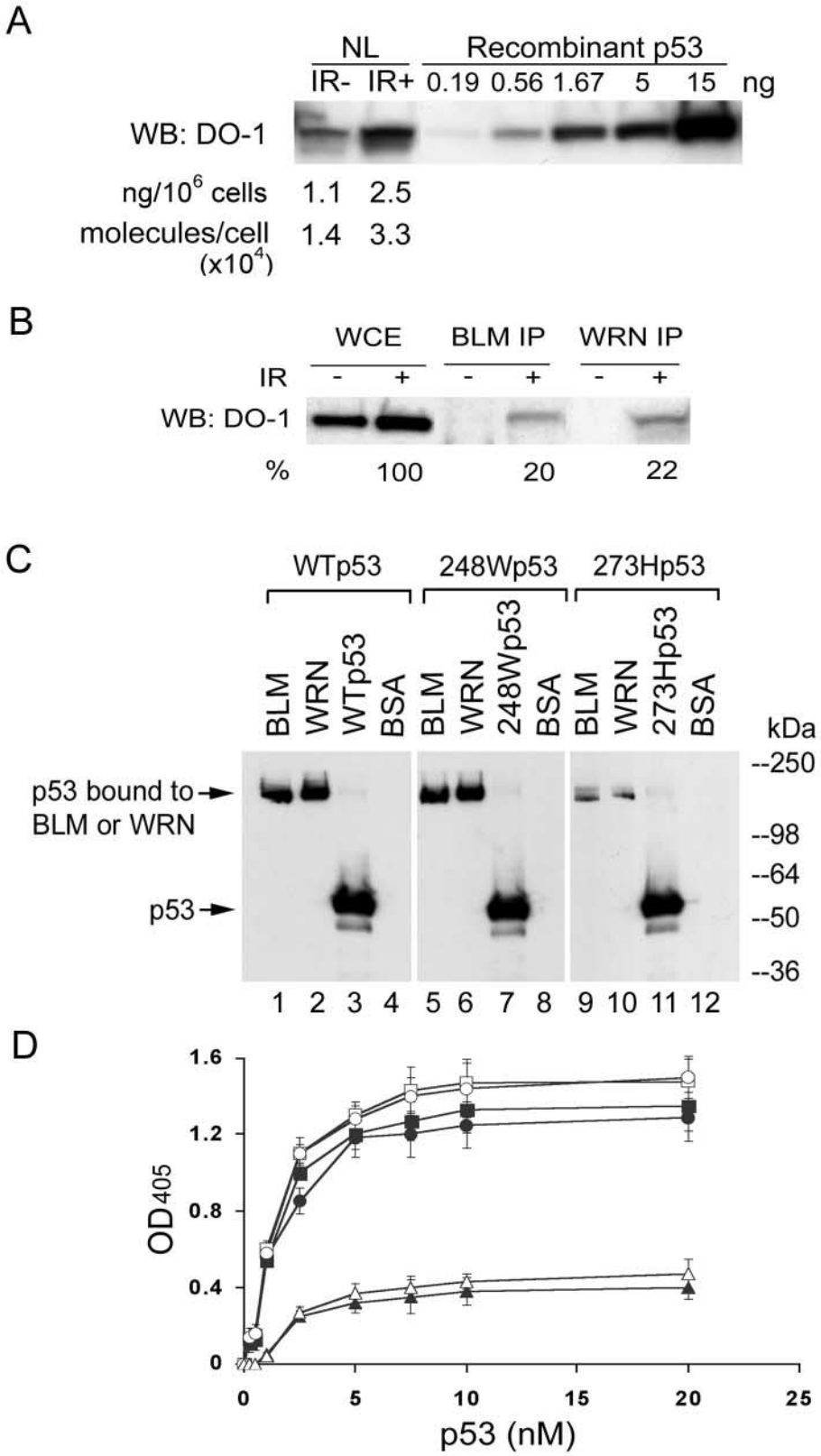

A
NL
Recombinant p53
IR- IR+ 0.19 0.56 1.67 5 15 ng
WB: DO-1
ng/10^6 cells 1.1 2.5
molecules/cell (x10^4) 1.4 3.3
B
WCE
BLM IP
WRN IP
IR - + - + - +
WB: DO-1
% 100 20 22
C
WTp53
248Wp53
273Hp53
BLM WRN WTp53 BSA BLM WRN 248Wp53 BSA BLM WRN 273Hp53 BSA
kDa
--250
--98
--64
--50
--36
p53 bound to BLM or WRN
p53
1 2 3 4 5 6 7 8 9 10 11 12
D
OD405
1.6
1.2
0.8
0.4
0
0 5 10 15 20 25
p53 (nM)

Fig. 6

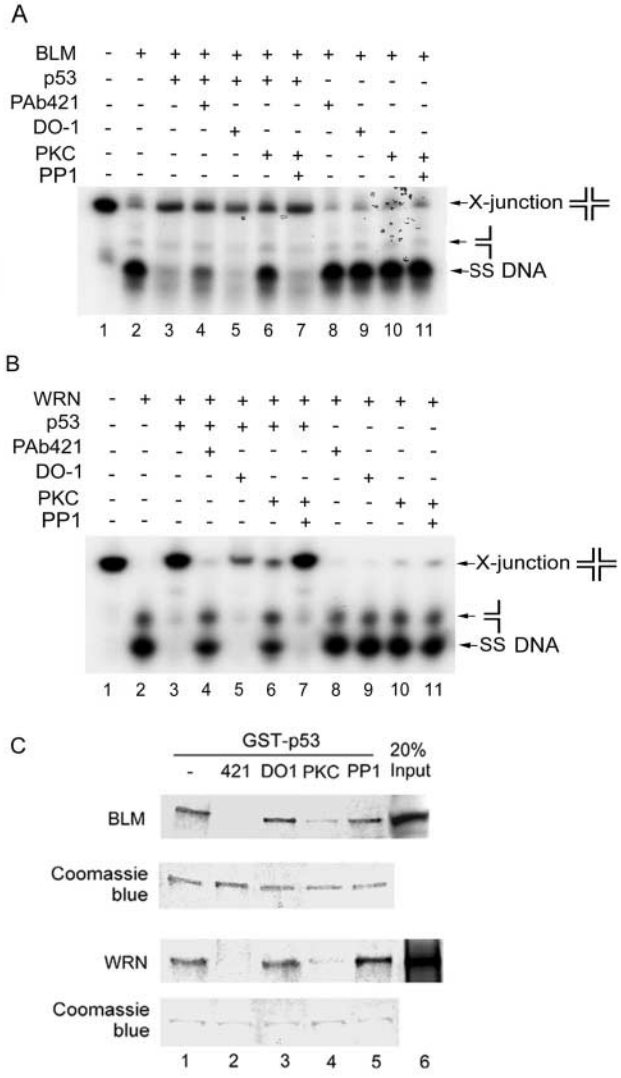
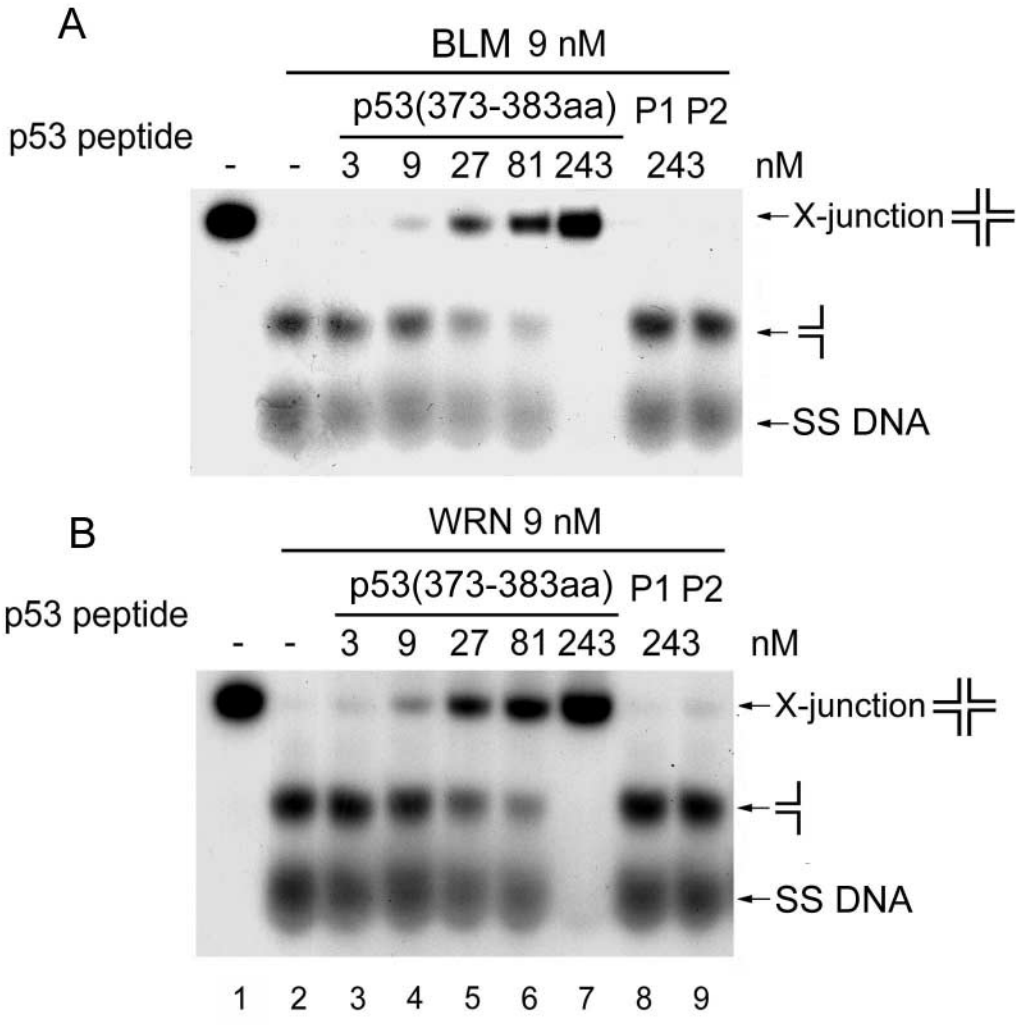
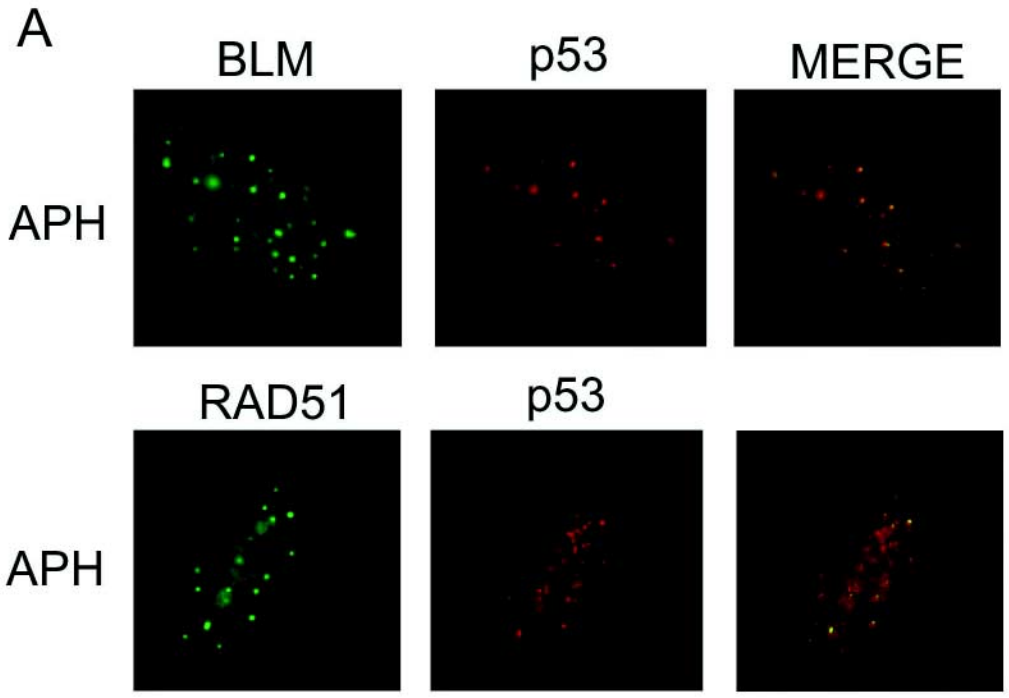

A
BLM
p53
PAb421
DO-1
PKC
PP1
X-junction
SS DNA
1 2 3 4 5 6 7 8 9 10 11
B
WRN
p53
PAb421
DO-1
PKC
PP1
X-junction
SS DNA
1 2 3 4 5 6 7 8 9 10 11
C
GST-p53
- 421 DO1 PKC PP1
20% Input
BLM
Coomassie blue
WRN
Coomassie blue
1 2 3 4 5 6

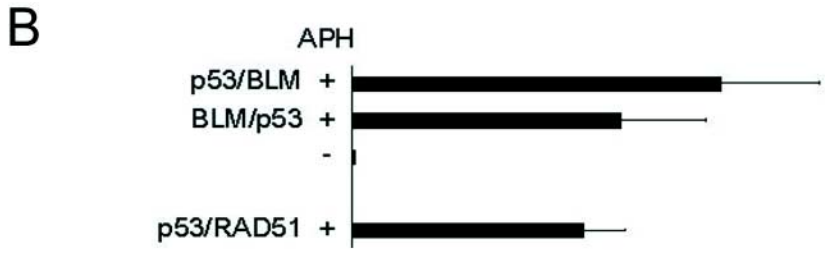
Fig. 7



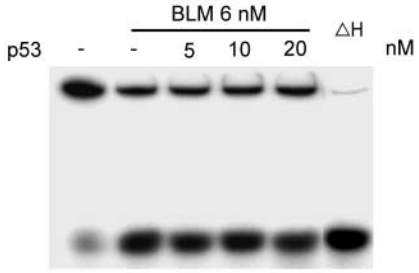
P1:371-381aa(376P)
P2:373-383aa(378P)

Fig. 8


A
BLM
p53
MERGE
APH
RAD51
p53
APH


B
APH
p53/BLM +
BLM/p53 +
-
p53/RAD51 +

Supplemental data 1



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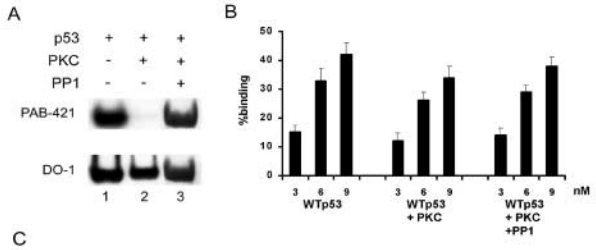

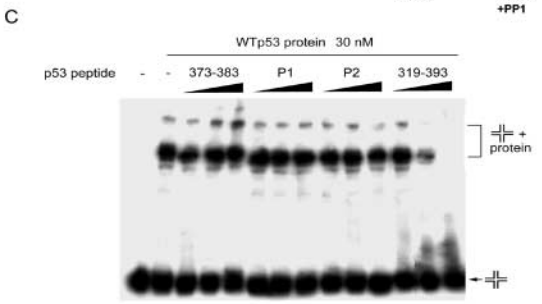

concentration of peptide: 50, 200, 800 nM.
P1:371-381(376P)
P2:373-383(378P)