The p53 Tumor Suppressor Stimulates the Catalytic Activity of Human Topoisomerase IIα by Enhancing the Rate of ATP Hydrolysis*

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DNA topoisomerase II is an essential nuclear enzyme for proliferation of eukaryotic cells and plays important roles in many aspects of DNA processes. In this report, we have demonstrated that the catalytic activity of topoisomerase IIα, as measured by decatenation of kine-toplast DNA and by relaxation of negatively supercoiled DNA, was stimulated 2–3-fold by the tumor suppressor p53 protein. In order to determine the mechanism by which p53 activates the enzyme, the effects of p53 on the topoisomerase IIα-mediated DNA cleavage/religation equilibrium were assessed using the prototypical topoisomerase II poison, etoposide. p53 had no effect on the ability of the enzyme to make double-stranded DNA break and religate linear DNA, indicating that the stimulation of the enzyme catalytic activity by p53 was not due to alteration in the formation of covalent cleavable complexes formed between topoisomerase IIα and DNA. The effects of p53 on the catalytic inhibition of topoisomerase IIα were examined using a specific catalytic inhibitor, ICRF-193, which blocks the ATP hydrolysis step of the enzyme catalytic cycle. Clearly manifested in decatenation and relaxation assays, p53 reduced the catalytic inhibition of topoisomerase IIα by ICRF-193. ATP hydrolysis assays revealed that the ATPase activity of topoisomerase IIα was specifically enhanced by p53. Immunoprecipitation experiments revealed that p53 physically interacts with topoisomerase IIα to form molecular complexes without a double-stranded DNA intermediary in vitro. To investigate whether p53 stimulates the catalytic activity of topoisomerase IIα in vivo, we expressed wild-type and mutant p53 in Saos-2 osteosarcoma cells lacking functional p53. Wild-type, but not mutant, p53 stimulated topoisomerase IIα activity in nuclear extract from these transfected cells. Our data propose a new role for p53 to modulate the catalytic activity of topoisomerase IIα. Taken together, we suggest that the p53-mediated response of the cell cycle to DNA damage may involve activation of topoisomerase IIα.

Eukaryotic DNA topoisomerase IIα is a nuclear enzyme that modulates the topological states of DNA via transient double

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Recently, it has been shown that expression of wild-type, but not mutant, p53 is able to decrease substantially the activity of the topoisomerase IIα gene promoter (26, 27). Inactivation of wild-type p53 may reduce normal regulatory suppression of topoisomerase IIα gene expression, resulting in accelerated cell proliferation, chromosomal rearrangements, and gene amplification seen in tumor cells. These data suggest that topoisomerase IIα is one of the downstream targets for p53-dependent regulation of cell cycle progression. The tumor suppressor protein p53 is a nuclear phosphoprotein that functions as a negative regulator of cell proliferation (28, 29). Inactivation of p53 by a deletion or mutation in the p53 gene or by the selective interaction with certain viral or cellular proteins leads to a selective growth advantage, resulting in tumor progression and therefore is strongly correlated with human cancer (30, 31). Although wild-type p53 protein acts as a transcriptional activator of a number of downstream effector genes containing p53-binding sites (32–34), it is also capable of repressing the activity of a variety of genes lacking the p53 consensus binding site (35–37). Genotoxic lesions leading to DNA strand breaks result in a rapid increase of p53 protein levels (38). Depending on cell type, the microenvironment of a cell, and p53 levels, the induction of wild-type, but not mutant, p53 might lead to cell cycle arrest or apoptosis (28, 29). p53-induced cell cycle arrest at the G<sub>1</sub> phase has been associated with increased expression of p21/WAF1/CIP1 gene, which encodes a potent inhibitor of cyclin-dependent kinases (39, 40). It has been also reported that expression of p53 has been associated with growth arrest in the G<sub>2</sub>/M phase of the cell cycle (41, 42).

Recent studies on mammalian cells using topoisomerase II inhibitors that do not stabilize covalent cleavable complexes have shown that topoisomerase II is required for complete chromosome condensation and for entry into mitosis (43, 44). Although wild-type p53 protein acts as a transcriptional activator of a number of downstream effector genes containing p53-binding sites (32–34), it is also capable of repressing the activity of a variety of genes lacking the p53 consensus binding site (35–37). Genotoxic lesions leading to DNA strand breaks result in a rapid increase of p53 protein levels (38). Depending on cell type, the microenvironment of a cell, and p53 levels, the induction of wild-type, but not mutant, p53 might lead to cell cycle arrest or apoptosis (28, 29). p53-induced cell cycle arrest at the G<sub>1</sub> phase has been associated with increased expression of p21/WAF1/CIP1 gene, which encodes a potent inhibitor of cyclin-dependent kinases (39, 40). It has been also reported that expression of p53 has been associated with growth arrest in the G<sub>2</sub>/M phase of the cell cycle (41, 42).

**EXPERIMENTAL PROCEDURES**

**Enzymes and Chemicals**—Purified human topoisomerase IIα, and kinetoplast DNA (k-DNA),<sup>1</sup> were obtained from Topogen, Inc. Etoposide was purchased from Sigma. ICRF-193 was a generous gift from Dr. R. Ishida, Aichi Cancer Center Research Institute, Nagoya, Japan. Supercoiled plasmid DNA was purified using standard methods. Restriction enzymes and other DNA-modifying enzymes were purchased from Promega. Radioactive nucleotides were from Amersham Pharmacia Biotech.

**Generation and Purification of p53 Recombinant Proteins**—Full-length human p53 cDNA was cloned into the BamHI and SalI sites of pGEX-5X-3 vector (Amersham Pharmacia Biotech). The fusion protein GST-p53 was expressed in Escherichia coli DH5α cells by adding 0.1 mM isopropyl-1-thio-β-D-galactopyranoside and purified on glutathione-Sepharose 4B (Amersham Pharmacia Biotech) according to the manufacturer’s recommendation. GST protein itself was produced from bacteria carrying an empty pGEX-5X-3 vector. To generate polyhistidine-tagged proteins, full-length p53 cDNA was ligated into pQE32 vector (Qiagen), in-frame with a 6× polyhistidine amino-terminal tag. The recombinant proteins were expressed in E. coli M15 cells by adding 1 mM isopropyl-1-thio-β-D-galactopyranoside and purified using nickel-agarose affinity chromatography (Qiagen) according to the manufacturer’s recommendation. The final protein preparation was dialyzed against TNE buffer (10 mM Tris-HCl, pH 7.5, 50 mM NaCl, 0.5 mM EDTA, 10% glycerol), and stored at –80 °C.

**Topoisomerase II Catalytic Activity Assays**—Topoisomerase II activity was assayed either by the ATP-dependent decatenation of k-DNA or by relaxation of negatively supercoiled pBluescript in the presence or absence of recombinant p53. The decatenation reactions were performed in a total volume of 20 μl of assay buffer (50 mM Tris-HCl, pH 7.6, 60 mM KCL, 8 mM MgCl<sub>2</sub>, 1 mM ATP, 15 mM 2-mercaptoethanol, and 30 μg/ml bovine serum albumin) containing 0.2 μg of k-DNA and the indicated amounts of topoisomerase IIα. After incubation for 15 min at 37 °C, the reactions were stopped by the addition of 5 μl of 5% Sarkosyl, 0.025% bromphenol blue, 25% glycerol, and the products were analyzed on a 1% agarose gel containing 0.5 μg/ml ethidium bromide. Enzyme activity is expressed in units, where 1 unit is defined as amount of the enzyme required to fully decatenate 0.2 μg of k-DNA in 15 min at 37 °C. The relaxation reactions were performed in a total volume of 20 μl of assay buffer (30 mM Tris-HCl, pH 7.6, 60 mM KCl, 8 mM MgCl<sub>2</sub>, 15 mM 2-mercaptoethanol, 30 μg/ml bovine serum albumin) containing 0.2 μg of negatively supercoiled pBluescript, and the indicated amounts of topoisomerase IIα. The reactions were incubated for 15 min at 37 °C and stopped by the addition of 5 μl of 5% Sarkosyl, 0.1 M EDTA, and 0.5 μl of RNase. DNA samples were then analyzed on a 1.2% agarose gel. The amounts of DNA products were quantified by densitometric analysis using the Eagle Eye II imaging system (Stratagene).

**Topoisomerase II Cleavage Assay**—Topoisomerase II cleavage reactions were performed in a total volume of 20 μl of cleavage buffer (30 mM Tris-HCl, pH 7.6, 60 mM KCl, 8 mM MgCl<sub>2</sub>, 15 mM 2-mercaptoethanol, 30 μg/ml ATP, 30 μg/ml bovine serum albumin) containing 0.2 μg of negatively supercoiled pBluescript and 50 μM etoposide in the presence or absence of recombinant p53. The reactions were initiated by the addition of the indicated amounts of topoisomerase IIα. After incubation for 15 min at 37 °C, the cleavage complexes were trapped by addition of 2 μl of 10% SDS followed by topoisomerase II digestion with proteinase K (50 μg/ml) for 30 min at 45 °C. The reaction products were purified with phenol/chloroform extraction and electrophoresed on a 1.2% agarose gel containing 0.5 μg/ml ethidium bromide. For mapping cleavage sites, topoisomerase II cleavage reactions were performed on a whole plasmid DNA, linearized with HindIII, and run into a 1.2% agarose gel containing 0.5 μg/ml ethidium bromide. The DNA was transferred onto nylon membrane and hybridized with 32P-labeled HindIII-PstI fragment and autoradiographed.

**Topoisomerase II Religation Assay**—Topoisomerase II religation reactions were performed in a total volume of 120 μl of cleavage buffer containing 1.2 μg of negatively supercoiled pBluescript, 12 units of purified topoisomerase IIα, 50 μM etoposide, and GST or GST-p53. After incubation for 15 min at 37 °C, the enzyme-mediated religation was induced by shifting the temperature from 37 to 65 °C. Aliquots (20 μl) were withdrawn at various times and stopped by addition of SDS and proteinase K (100 μg/ml) for 30 min at 45 °C, followed by addition of 5 μl of 5% Sarkosyl, 0.1 M EDTA, and 0.5 μl of RNase. DNA samples were then run on a 1% agarose gel and ethidium bromide stained. The amounts of DNA products were quantified by densitometric analysis.

**Hydrolysis of ATP by Topoisomerase II**—ATPase assays were performed in a total volume of 20 μl of relaxation assay buffer containing 0.3 μg of negatively supercoiled pBluescript, 2 units of topoisomerase IIα, and 0.1 mM [γ-32P]ATP (2 Ci/mole) reaction, and 0.3 mM polyhistidine-tagged p53. Reaction mixtures were incubated at 37 °C, and aliquots were removed at various intervals up to 30 min and quenched by the addition of an equal volume of 50 μl EDTA and 1% sodium dodecyl sulfate to each reaction. Each quenched sample (1 μl) was chromatographed in triplicate on polyethyleneimine-cellulose plates (Merck). Plates were developed by chromatography in fleshly made 400 mM NH<sub>4</sub>HCO<sub>3</sub>. The dried plates were exposed to imaging plates, and the exposed screens were scanned using a Bio-Imaging analyzer (Fuji, BAS-2500). The concentration of ATP hydrolyzed was determined by dividing the free inorganic phosphate counts by the total number counts per lane and multiplying that fraction by the starting ATP concentration.

**Immunoprecipitation of p53**—Forty units of purified topoisomerase IIα were mixed with 1.8 μg GST or GST-p53 in a total volume of 600 μl of assay buffer (30 mM Tris-HCl, pH 7.6, 60 mM KCl, 8 mM MgCl<sub>2</sub>, 15 mM 2-mercaptoethanol, 30 μg/ml bovine serum albumin) for 15 min on ice. Reaction mixtures were incubated for 1 h on ice in the presence of a...
p53 Stimulates the Topoisomerase IIα Activity

Fig. 1. Effects of GST-p53 on the k-DNA decatenation activity of topoisomerase IIα. Decatenation reactions were performed in a total volume of 20 μl and analyzed on a 1% agarose gel containing 0.5 μg/ml ethidium bromide, as described under “Experimental Procedures.” The positions of catenated k-DNA, decatenated open circular (oc), and decatenated relaxed DNA (rel) are indicated. A, titration of topoisomerase IIα against a fixed concentration of GST-p53. Decatena-

tion reactions contained 0.2 μg of k-DNA, the indicated amounts of topoisomerase IIα, and 0 or 300 nM GST-p53. Lane 1 contained catenated k-DNA alone. B, titration of GST-p53 against a fixed amount of topoisomerase IIα. Decatenation reactions contained 0.2 μg of k-DNA, 0.5 unit of topoisomerase IIα, and the indicated concentrations of GST-p53. C, a large reaction mixture (100 μl) containing 1 μg of k-DNA and 300 nM GST or GST-p53 or buffer were incubated with 2.5 unit of topoisomerase IIα. Aliquots (20 μl) were withdrawn at various times, and analyzed on an agarose gel. D, decatenation reactions contained 0.2 μg of k-DNA, 0.5 unit of topoisomerase IIα, and wild-type p53 or mutant p53-22/23 as indicated.

RESULTS

Effects of p53 on the Catalytic Activity of Human Topoisomerase IIα—In order to determine the effects of p53 on the catalytic activity of topoisomerase IIα, we assayed enzyme activity by the decatenation of k-DNA in the presence of GST-p53 fusion protein. The experiment shown in Fig. 1A reveals the effect of increasing amounts of topoisomerase IIα on enzyme-catalyzed DNA decatenation in the presence or absence of GST-p53. The amounts of decatenated k-DNA products rose proportionally with the enzyme levels. Decatenation activity was ~2–3-fold higher in the presence of GST-p53 than in the absence of GST-p53 as determined by comparison of the band intensities of the decatenated k-DNA molecules in several enzyme dilutions. For further comparison of the stimulatory effect of GST-p53, k-DNA was incubated with topoisomerase IIα at a concentration where approximately 50% of catenated k-DNA molecules were converted to decatenated molecules in a few enzyme dilutions. For this purpose, the stimulatory effect of GST-p53 was attributed to p53, catenated k-DNA was mixed with topoisomerase IIα at a concentration selected to give a detectable amount of decatenated k-DNA. After incubation for 16 min, a full decatenation activity was detected by addition of GST-p53 (Fig. 1B), whereas only a partial increase in amount of decatenated k-DNA was observed by addition of either GST or buffer. Since inactivation of p53 by treatment with cold phosphate-buffered saline containing 1 mM phenylmethylsulfonylfluoride, and centrifuged at 1,000 × g for 4 min. The cell pellet was resuspended in hypotonic buffer, and the nuclear proteins were extracted as described previously (46). Protein concentrations were determined by the Bio-Rad protein assay (Bio-Rad). The proteins were stored in aliquots at −80 °C.

Cell Culture and Transfection—The human p53 expression plasmids and the empty vector used in this study have been described previously (30). All of the p53 constitutive expression constructs were produced with a cytomegalovirus promoter-enhancer expression vector. The Saos-2 human osteosarcoma cells were obtained from the American Type Culture Collection and maintained in Dulbecco’s modified Eagle’s medium supplemented with 10% fetal bovine serum, 100 units of penicillin/ml, and 100 μg of streptomycin/ml in a humidified incubator at 37 °C with 5% CO2. Transfections were carried out using LipofectAMINE (Life Technologies) as described previously (45). 5 × 105 Saos-2 cells were transfected with 5 μg of p53 expression plasmids. After 24 h, cells were trypsinized from the plate, washed once with ice-cold phosphate-buffered saline containing 1 mM phenylmethylsulfonylfluoride, and centrifuged at 1,000 × g for 4 min. The cell pellet was resuspended in hypotonic buffer, and the nuclear proteins were extracted as described previously (46). Protein concentrations were determined by the Bio-Rad protein assay (Bio-Rad). The proteins were stored in aliquots at −80 °C.

Decatenation reactions were performed in a total volume of 20 μl and analyzed on a 1% agarose gel containing 0.5 μg/ml ethidium bromide, as described under “Experimental Procedures.” The positions of catenated k-DNA, decatenated open circular (oc), and decatenated relaxed DNA (rel) are indicated. A, titration of topoisomerase IIα against a fixed concentration of GST-p53. Decatena-

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Monoclonal antibody against p53, Pab1801, or Pab240 (Santa Cruz Biotechnology), under gentle agitation. 10 μl of protein G-Sepharose CL-4B (Amersham Pharmacia Biotech) prepared in 50 mM Tris-HCl, pH 7.5, 1 mM EDTA, 150 mM NaCl, and 10 mM 2-mercaptoethanol, were added to the mixtures and further incubated on ice for 1 h under gentle agitation. Protein G-Sepharose was sedimented, washed three times, and resuspended in a 20 μl of assay buffer. The presence of topoisomerase IIα in the immunoprecipitated complexes was determined by immuno blot analysis. The supernatants and immunoprecipitants (20 μl each) were electrophoresed on a 7% SDS-polyacrylamide gel electrophoresis and transferred to Hybond-ECL membrane (Amersham Pharmacia Biotech). Topoisomerase IIα was detected with an antibody against human topoisomerase IIα (Topogen, Inc.).
Effects of p53 on the DNA Cleavage Activity of Topoisomerase IIα—Since the formation of covalent cleavable complexes between topoisomerase II and DNA is one of the crucial steps in enzyme activity, the effects of p53 on the cleavage of supercoiled DNA mediated by topoisomerase IIα were determined. Topoisomerase II cleavage reactions were performed in the presence of GST or GST-p53 by using the antitumor drug etoposide, which stabilizes the covalent topoisomerase II-associated DNA complexes. The resulting DNA samples were analyzed on an agarose gel containing ethidium bromide. As shown in Fig. 3A, increasing amounts of topoisomerase IIα in the presence of a fixed concentration (50 μM) of etoposide induced a dose-dependent formation of double-stranded breaks in DNA. Addition of GST-p53 to cleavage reactions did not alter the amount of linear DNA at all enzyme dilutions. However, relaxation activity of topoisomerase IIα was stimulated by GST-p53 as compared with the reactions containing GST. To examine the effects of GST-p53 on the site-specific DNA cleavage mediated by topoisomerase IIα, the cleavage sites were mapped in pBluescript DNA using indirect end labeling (47). Topoisomerase IIα induced dose-dependent DNA cleavage, as indicated by the appearance of specific DNA cleavage bands (Fig. 3B). In the presence of GST-p53, no significant increase in topoisomerase IIα cleavage was observed for all dilutions of enzyme tested as compared with GST or buffer containing reactions. Furthermore, no new cleavage site was observed by the inclusion of GST-p53 in the cleavage reactions. Taken together, these data indicate that the stimulation of the topoisomerase IIα catalytic activity by p53 was not due to alteration in the formation of covalent cleavable complexes.

Effects of p53 on the DNA Religation Activity of Topoisomerase IIα—The formation of covalent cleavable complexes can be readily reversed by adding EDTA or salt to the reaction mixture or by elevating the temperature prior to the addition of SDS (48–52). To determine whether p53 has an effect on the topoisomerase IIα-mediated DNA religation activity, we performed DNA religation reactions using a heat-induced religation assay. DNA religation induced by temperature shift relies on the fact that religation activity of topoisomerase II remains less sensitive to variations in temperature than DNA cleavage activity. By shifting the temperature from 37 °C to 65 °C before termination with SDS and proteinase K, linear DNA molecules generated by topoisomerase IIα-mediated DNA cleavage in the presence of etoposide were reconverted to covalently closed circular DNA in a time-dependent manner (Fig. 3C). These data revealed that GST-p53 did not alter the ability of the enzyme to religate linear DNA. Similar effects were observed when the polyhistidine-tagged p53 was used (data not shown).

Effects of p53 on the Catalytic Inhibition of Topoisomerase IIα by ICRF-193—Unlike the topoisomerase IIα poison, some antitumor drugs such as ICRF-193, merbarone, aclarubicin, quinobenoxazines, and staurosporine have been shown to inhibit topoisomerase IIα activity without significantly stabilizing cleavable complexes (17–20, 53). The effects of p53 on the catalytic inhibition of topoisomerase IIα were examined using ICRF-193 (Fig. 4A). In the absence of GST-p53, ICRF-193 partially inhibited decatenation activity of topoisomerase IIα at 1 μM concentration and strongly inhibited at higher concentrations in a dose-dependent manner under the conditions employed in this assay. However, GST-p53 reduced the catalytic inhibition of topoisomerase IIα by ICRF-193 as compared with the reactions without GST-p53. For example, in the presence of GST-p53, catenated k-DNA was almost completely converted to decatenated products at 1 μM concentration of ICRF-193. Similar results were obtained when DNA religation activity was assayed (Fig. 4B). These results suggest that p53 may enhance...
the catalytic activity of topoisomerase II through the mechanism by which ICRF-193 inhibits enzyme activity.

Stimulation of Topoisomerase IIα-catalyzed ATP Hydrolysis by p53—ICRF-193 has been shown to inhibit the catalytic activity of topoisomerase II by stabilizing the closed-clamp form of the enzyme and preventing its conversion to the open-clamp form (54). ICRF-193 was found to inhibit the ATPase activity of topoisomerase II. Since p53 reduced the enzyme catalytic inhibition by ICRF-193, we examined the effects of p53 on the ATPase activity of topoisomerase IIα. In the ATP hydrolysis assays, polyhistidine-tagged p53 was used because unwanted bacterial ATPase activity was copurified with GST-p53 on glutathione-Sepharose affinity chromatography. As shown by data in Fig. 5, polyhistidine-tagged p53 stimulated the ATPase activity of topoisomerase IIα; 2-fold as compared with the reactions without p53. This increase in the rate of ATP hydrolysis is comparable to the stimulation of the enzyme’s overall catalytic activity by GST-p53 (see Fig. 1). Reactions containing only polyhistidine-tagged p53 without topoisomerase IIα gave near base-line levels of ATPase activity. These results strongly suggest that p53 modulates the catalytic activity of topoisomerase IIα by specifically enhancing the ATPase activity of the enzyme.

Physical Interaction between p53 and Topoisomerase IIα—Previously, it was reported that topoisomerase II can form a molecular complex with wild-type p53 without a double-stranded DNA intermediary (55). However, it is unclear whether other proteins could mediate the binding between topoisomerase II and p53. To address this issue directly, purified topoisomerase IIα was incubated with GST or GST-p53 in relaxation assay buffer, and the reaction mixtures were immunoprecipitated with p53-specific monoclonal antibody. The resulting proteins in supernatants (S) and immunoprecipitants (IP) were resolved on a 7% SDS-polyacrylamide gel electrophoresis and were subjected to Western blot using an affinity-purified antibody specific for topoisomerase IIα. As shown in Fig. 6A, most of the topoisomerase IIα was found in the supernatant fraction, but some of the topoisomerase IIα was detected in the immunoprecipitants recovered by a p53-specific mono-

![Fig. 3. Effects of GST-p53 on the ability of topoisomerase IIα to mediate DNA cleavage and religation. A, DNA cleavage reactions contained 0.2 μg of negatively supercoiled pBluescript, 50 μM etoposide, and 300 nM GST or GST-p53. The reactions were initiated by addition of the indicated amounts of topoisomerase IIα. The positions of open circular (oc), linear (lin), supercoiled (sc), and relaxed DNA (rel) are indicated. Lanes 1 and 8 contained supercoiled and linear DNA, respectively. B, cleavage reactions were performed on a whole pBluescript in the presence of GST or GST-p53 or buffer. The cleavage products were linearized with HindIII and analyzed on a 1.2% agarose gel containing 0.5 μg/ml ethidium bromide. The DNA was transferred onto nylon membrane and hybridized with radiolabeled probe as described under “Experimental Procedures.” C, a large reaction mixture (120 μl) containing 50 μM etoposide and 300 nM GST or GST-p53 was incubated with 12 units of topoisomerase IIα. The reaction mixture was then heated to 65 °C, and aliquots (20 μl) were withdrawn at various times after heat treatment. The reactions were terminated by addition of SDS and proteinase K as described under “Experimental Procedures.”

![Fig. 4. Effects of GST-p53 on the catalytic inhibition of topoisomerase IIα by ICRF-193. A, decatenation reactions contained 0.2 μg of k-DNA, 1.5 unit of topoisomerase IIα, the indicated concentrations of ICRF-193, and 0 or 300 nM GST-p53. The positions of catenated k-DNA, decatenated open circular (oc), and decatenated relaxed DNA (rel) are indicated. Lane 1 contained catenated k-DNA alone. B, relaxation reactions contained 0.2 μg of negatively supercoiled pBluescript, 1.5 unit of topoisomerase IIα, and the indicated concentrations of ICRF-193, and 0 or 300 nM GST-p53. The positions of supercoiled DNA (sc) and relaxed DNA (rel) are indicated.

The catalytic activity of topoisomerase II through the mechanism by which ICRF-193 inhibits enzyme activity.

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clonal antibody Pab1801. However, topoisomerase IIα was absent in the immunoprecipitants recovered from reaction mixtures containing topoisomerase IIα and GST protein. When another p53-specific monoclonal antibody Pab240 was used for immunoprecipitation, similar results were obtained. Supernatant and immunoprecipitant fractions were also tested for the presence of k-DNA decatenation activity (Fig. 6B). The results clearly indicate that decatenation activity was present in the p53-immunoprecipitants but absent in the immunoprecipitants recovered from the reaction mixtures containing topoisomerase IIα and GST protein. These results suggest that p53 binds directly to topoisomerase IIα in the absence of DNA.

Expression of Wild-type p53 in Saos-2 Cells Stimulates Topoisomerase IIα Activity—All the results described above are obtained with p53 produced in bacteria. It is well known that there is no post-translational modification of p53 in bacteria. Moreover, the structural conformation of p53 produced in bacteria might differ from that of the protein synthesized in mammalian cells. In order to investigate whether p53 stimulates the catalytic activity of topoisomerase IIα in vivo, we transiently expressed wild-type p53 and mutant p53-22/23 in Saos-2 human osteosarcoma cells which lack functional p53 and compared the topoisomerase II activity in nuclear extracts by decatenation of k-DNA (Fig. 7A). As indicated by amounts of trapped catenated k-DNA in the well, decatenation activity was ~2-fold higher in nuclear extracts of cells expressing wild-type p53 (lanes 2–4) as compared with extracts from cells expressing mutant p53-22/23 (lanes 5–7) or mock-transfected cells (lanes 8–10). Immunoblots of nuclear extracts with topoisomerase IIα and GST protein. When p53 was precipitated with a monoclonal antibody against p53, Pab1801, or Pab240 and protein G-Sepharose, as described under “Experimental Procedures,” A, the supernatants (S) and immunoprecipitants (IP) (20 μl each) were separated on a 7% SDS-polyacrylamide gel electrophoresis and immunostained with an antibody against topoisomerase IIα. B, the supernatant and immunoprecipitants (10 μl each) were assayed for k-DNA decatenation activity. The positions of catenated k-DNA, decatenated open circular (oc), and decatenated relaxed DNA (rel) are indicated.

were detected. These results clearly indicate that wild-type, but not mutant, p53 stimulates the catalytic activity of topoisomerase IIα in vivo. Stimulation of topoisomerase II activity by wild-type p53 in vivo was underestimated since topoisomerase IIα protein level was slightly decreased in cells transfected with wild-type p53.

DISCUSSION

Eukaryotic DNA topoisomerase II is the essential enzyme to remove the catenations formed between the DNA molecules of sister chromatids during replication and hence permit their faithful segregation during mitosis (43, 44). Here, we report that p53 has a pronounced stimulatory effect of DNA catalysis by topoisomerase IIα as measured by decatenation of k-DNA and by relaxation of supercoiled DNA. The results of the ATP hydrolysis assays revealed that p53 stimulates the catalytic activity of topoisomerase IIα by specifically enhancing the ATPase activity of the enzyme. We also show that p53 physically associates with topoisomerase IIα without a double-stranded DNA intermediary in vitro. Transient transfection experiments with Saos-2 cells showed that topoisomerase II activity was stimulated by ectopically expressed wild-type p53 but not by mutant p53-22/23. In this report, we propose that p53 could be a catalytic regulator of topoisomerase IIα.

A number of mechanistic studies have shown that the catalytic cycle of topoisomerase II can be divided into several discrete reaction steps (56). The physiological regulation of DNA
topoisomerase II requires a coordinated function of the all steps of the enzymes catalytic cycle. To determine the mechanism by which p53 activates topoisomerase IIa, the effects of p53 on the DNA cleavage/religation reaction steps of the catalytic cycle were examined. The results showed that GST-p53 had no effect on the formation of covalent cleavable complexes in the presence of etoposide. Cleavage sites induced by topoisomerase IIa remained unaffected by GST-p53. Furthermore, the religation step, during which the double-stranded break is resealed, was not altered by GST-p53. These results provide the evidence that p53 does not enhance the rate of enzyme catalysis at cleavage/religation steps. Previous studies showed that p53 also physically associates with topoisomerase I and enhances its DNA relaxation activity (57, 58). Unlike topoisomerase IIa, both cleavage and religation steps in the catalytic cycle of topoisomerase I were stimulated by p53.

A specific catalytic inhibitor of topoisomerase II, ICRF-193, blocks the final step of the catalytic cycle, ATP hydrolysis (54). This drug traps topoisomerase II on the DNA in its closed clamp form and prevents both enzyme release and regeneration. Clearly manifested in decatenation and relaxation assays, p53 reduced the catalytic inhibition of topoisomerase IIa by ICRF-193. In conjunction with the results of the ATPase assays, these findings strongly suggest that ATP hydrolysis is the control step for the modulation of topoisomerase IIa catalytic activity by p53. Although topoisomerase II has been known to be an essential enzyme for proliferation of eukaryotic cells and play important roles in many aspects of DNA processes, little is understood concerning the mechanism by which its catalytic activity is regulated in eukaryotic cells except for a few examples. The catalytic activity of topoisomerase II is stimulated 2–3-fold following phosphorylation by either casein kinase II or protein kinase C (56, 59). Like p53 action on topoisomerase II activity, both protein kinases enhance enzyme activity by specifically stimulating the ability of topoisomerase II to hydrolyze its ATP cofactor. More recently, it was reported that casein kinase II increases the activity of topoisomerase IIb by stabilization against thermal inactivation of the enzyme (60). This activation of topoisomerase IIb is apparently independent of any phosphorylation. However, the functional significance of this regulation in vivo is unclear. Topoisomerase IIa appeared to physically interact with Rb protein, and wild-type, but not mutant, Rb inhibited topoisomerase II activity (61).

The critical question that remains to be answered is how p53 modulates the catalytic activity of topoisomerase IIa. The most obvious mechanism is via direct association of p53 with topoisomerase IIa. Our data showing the co-immunoprecipitation of purified topoisomerase IIa with p53 and the presence of k-DNA decatenation activity in the immunoprecipitated complexes are consistent with this suggestion. Furthermore, it is unlikely that other factors could mediate the binding between topoisomerase IIa and p53. Yuwen and co-workers (55) reported that topoisomerase IIa can form a complex with wild-type p53. Interaction between p53 and topoisomerase II was confirmed by co-immunoprecipitation of p53 protein by a monoclonal antibody to topoisomerase II in p53-overexpressed HeLa cell lysates. This binding was shown to occur in the absence of DNA. These data, together with our results presented in this study, strongly suggest that the two proteins may form molecular complexes in vivo. Recently, Cowell et al. (62) reported that both topoisomerase IIa and IIb interact with p53 in vivo and in vitro, and the regulatory COOH-terminal basic region of p53 (residues 364–393) is necessary and sufficient for interaction with topoisomerase II (62). These data suggest that mutant p53 (p53-22/23, p53-248, and p53-273) used in this study can physically associate with topoisomerase IIa, resulting in similar levels of stimulatory effect in vitro on the decatenation activity with wild-type p53. However, in vivo transient transfection experiments revealed that decatenation activity was higher in nuclear extracts of cells expressing wild-type p53 as compared with extracts from cells expressing mutant p53-22/23 or mock-transfected cells. These results suggest that in vivo stimulation of topoisomerase II catalytic activity by p53 may additionally require leucine and tryptophan residues at amino acids 22 and 23.

Since decatenation function of topoisomerase IIa is required at mitosis for high chromosome condensation and chromosome segregation, the level and activity of the enzyme are tightly controlled during cell cycle. mRNA levels of topoisomerase IIa are virtually absent in the G1 phase and accumulate to high levels during late S phase (63). The protein levels are maximal in the G2/M phase. It would appear that topoisomerase IIa is activated only when its decatenation function is required over the cell cycle. Our data propose a new role for p53 as a regulator of topoisomerase IIa. Modulation of the catalytic activity of topoisomerase IIa by p53 might contribute to tight regulation of cell cycle progression when DNA has been damaged. Increased expression of wild-type p53 in response to DNA damage would arrest cells in G1 phase by stimulating the p21 gene expression, to allow time for damaged DNA to be repaired before continuation of cell cycle (64). A significant G1 arrest function has also been reported for p53 (41, 42), p53 can lead to arrest of cell growth at a G2/M phase of the cell cycle in the absence of DNA-damaging treatments. In the experiments using a topoisomerase II inhibitor, ICRF-193, that does not stabilize cleavable complexes, Downes and co-workers (43) showed that topoisomerase II-dependent G2 checkpoint is distinct from the G2-damage checkpoint. This topoisomerase II-dependent G2 checkpoint could be sensitive to decatenation state of DNA or...
the decatenation activity of topoisomerase II. Thus, the main function of the G2 phase is to allow adequate decatenation of replicated DNA. A previous report has indicated that expression of wild-type p53 severely inhibits topoisomerase II gene promoter activity (27). Inactivation of p53 may reduce normal regulatory repression of topoisomerase II gene expression and contribute to abortive G2 cycle checkpoints. These results also suggest that the p53-induced G2 arrest could be linked to the topoisomerase II-dependent G2 cycle checkpoint through transcriptional repression of topoisomerase II by p53. Our data provide strong evidence that p53 stimulates the catalytic activity of topoisomerase II by specifically enhancing the ATPase activity. We therefore propose that wild-type p53 contributes to the proper regulation of topoisomerase II levels required in the G2 checkpoint by at least two modes: via repression of topoisomerase II gene expression (27) and/or via stimulation of topoisomerase II catalytic activity by the formation of molecular complexes. Future experiments will be required to understand the functional significance of p53 regulation in topoisomerase II activity in vivo.

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