p53 is a tumor suppressor protein that controls cell proliferation by regulating the expression of growth control genes. In a previous study, we identified two proteins, 53BP1 and 53BP2, that are able to bind to wild type but not to mutant p53 via the DNA-binding domain of p53. We isolated cDNAs expressing a full-length human 53BP1 clone, which predicts a protein of 1972 residues that can be detected in the H358 human lung carcinoma cell line. The 53BP1 and 53BP2 genes were mapped to chromosomes 15q15-21 and 1q41-42, respectively. Immunofluorescence studies showed three types of staining patterns for 53BP1 as follows: both cytoplasmic and nuclear, homogeneous nuclear, and a nuclear dot pattern. In contrast, 53BP2 localized exclusively to the cytoplasm, and this pattern did not change upon coexpression of wild type p53. Although our previous study revealed that p53 is not able to bind simultaneously to either 53BP1 or 53BP2 and to DNA carrying a consensus binding site, both 53BP1 and 53BP2 enhanced p53-mediated transcriptional activation and induced the expression of a p53-dependent protein, suggesting that these proteins might function in signal transduction pathways to promote p53 activity.

The p53 protein is the product of a tumor-suppressor gene (1, 2), with mutations in this protein being the most common genetic change in human cancer (3, 4). The observations that introduction of the wild type (wt)9 p53 gene into cells leads to growth arrest (5–8) or apoptosis (9, 10) and that DNA damage leads to increases in the level of p53 (11, 12) suggest that the protein acts at a checkpoint to regulate cell cycle arrest in the G1 (13), G2/M (14), and G0 phases (15). The cell cycle arrest in G1 phase is mediated, at least in part, by the trans-activation function of p53 (16, 17), which can induce the expression of p21 (WAF1/CIP1) (18–21), a cyclin-dependent kinase inhibitor. However, the signal from p53 to the Gas1 gene product, which can result in a G0 arrest, does not require the trans-activation function of p53 (15). Furthermore, in some cell types, a mutant p53 that lacks this function can induce apoptosis (22).

We have used the yeast two-hybrid system to identify two cellular proteins that bind to wt but not to mutant p53, designated p53-binding protein 1 and 2 (53BP1 and 53BP2) (23). Both 53BP1 and 53BP2 bind to the central domain of p53 which is required for site-specific DNA binding. Although neither 53BP1 and 53BP2 has extensive homology to other known proteins, recent sequence analysis revealed that the C terminus of 53BP1, which is sufficient for binding to p53, has homology both to the C terminus of BRCA1, a tumor suppressor specific for breast and ovarian cancer, and to Rad9, a yeast cell cycle checkpoint protein (24). This BRCT (BRCA1 C terminus) domain is found in other proteins involved in a checkpoint that responds to DNA damage (25, 26), suggesting that it may mediate protein-protein interactions involved in this process. 53BP2 has four ankyrin repeats and a single Src homology-3 domain in its C terminus (23). Structural analysis indicated that 53BP2 binds to wt p53 via its fourth ankyrin repeat and the Src homology-3 domain (27). 53BP2 also interacts with BCL2, an apoptosis inhibitor, indicating a possible role for 53BP2 in apoptosis (28). To analyze further these p53-binding proteins, we have cloned and sequenced a full-length 53BP1 cDNA. Both 53BP1 and 53BP2 have been expressed in mammalian cells, and their cellular localization has been determined. Assaying a transfected p53-dependent reporter gene as well as endogenous p21 expression, we show that both 53BP1 and 53BP2 enhance p53-mediated transcriptional activation. For 53BP2, this effect may be responsible for its partial suppression of oncogene-mediated cell transformation.

EXPERIMENTAL PROCEDURES

Isolation of 53BP1 cDNA—Rapid amplification of cDNA ends (RACE) experiments (29, 30) were carried out using the 5’-AmpliFINDER RACE kit (CLONTECH) following the manufacturer’s suggestions. Two antisense primers were used: A7, 5’-TCGCGCTCGCCAGGTTGAACTGCAAAGACTCTTCACTC-3’, and A6, 5’-TGGCAACAGACTCAGCAACAGACAGTATGCC-3’. These primers hybridize to sites on the sense strand of the reported partial 53BP1 cDNA clone, A70 (23), 186 and 242 bases downstream from the 5’ end, respectively, of this clone (Fig. 1A). Human skeletal muscle mRNA (CLONTECH) was used to generate cDNA with primer A6. After the mRNA was hydrolyzed with 0.375 M NaOH, an anchor oligonucleotide was ligated to the 3’ end of cDNA. PCR amplification with the anchor primer and primer A7 resulted in amplification of a 450-bp fragment (R1), containing an
**Transcriptional Activation by 53BP1 and 53BP2**

additional 260 bp of 53BP1 cDNA sequence (Fig. 1A). R1 was then used to screen a human skeletal muscle cDNA library (CLONTECH) according to standard procedures (31). Clone, P7, which was identified, was also used to screen this library, resulting in the isolation of clone P45 (Fig. 1A). A 1.8-kb fragment was amplified from clone P45 by PCR with primer P7a containing restriction sites for EcoRI and 53BP1 fragment, and the clone P7a was isolated. A 1.8-kb fragment from clone P45 to generate pL8 fragment was deleted from pL6-2 and replaced by a 2.6-kb BamHI fragment of the 53BP1 cDNA sequence (Fig. 1A). Finally, a Jurkat cDNA library (a gift of Dr. P. Enrietto, State University of New York, Stony Brook) was screened with this 1.8-kb fragment, and the clone P7a was isolated.

**Sequencing**—The 53BP1 cDNA sequence was obtained by sequencing overlapping cDNA inserts on both strands. All cDNA fragments were sequenced by the dideoxy chain termination method using the Sequenase Kit Version 2.0 (U. S. Biochemical Corp.). The sequencing of hybridization signals were analyzed in 15–20 well spread, well banded metaphases for each plasmid.

**Cell Culture**—NCI-H358, human lung carcinoma cells (37) (a gift of Dr. Y. Yoshitake, Kanazawa Medical University), HepG2, Saos-2, and WI38 cells (RIKEN Cell Bank, Japan) were used. REFS, MCF7, HepG2, and WI38 cells contain wt p53, whereas H358 and Saos-2 cells lack p53 alleles. All cells except WI38 cells were grown in Dulbecco’s modified essential medium containing 10% fetal calf serum. WI38 cells were grown in minimal essential medium containing 10% fetal calf serum. Cells were grown at 37 °C in a humidified, 5% CO2 incubator.

**Western Blot Analysis**—H358, MCF7, and COS-1 cells that had been transfected with various expression plasmids were washed once with PBS, scraped, and pelleted by centrifugation. H358 and COS-1 cells were lysed with SDS-sample buffer, boiled for 3 min, and applied to a 7% SDS-polyacrylamide gel. MCF7 cells were lysed with PBS supplemented with 2% SDS and boiled for 3 min. Chromosomal DNA was sheared by passage repeatedly through a 26-gauge needle. The supernatant was obtained by centrifugation at 14,000 rpm for 15 min at 4 °C. The DNA was extracted by the phenol/chloroform method.

**Immunochemical Localization**—COS-1 and H358 cells were seeded on coverslips and transfected with expression vectors expressing HA-tagged 53BP1, 53BP2, or p53. At 36 h post-transfection, cells were fixed with 3% formaldehyde and permeabilized with cold methanol acetone (50: 50) for 3 min at room temperature. After a wash in PBS (31), cells were incubated with the anti-HA monoclonal antibody, 12CA5 (Boehringer Mannheim) at 2 μg/ml for 1 h at room temperature, followed by incubation with 2.4 μg/ml fluorescein isothiocyanate-conjugated goat anti-mouse antibodies (BioSource International) at room temperature for 1 h in the dark. The coverslips were washed and mounted on slides, and the cells were examined and photographed with Kodak Tmax 400 film.

**Nuclear/Cytoplasmic Fractionation**—Two transiently transfected NCI-H358 cells were pooled 40 h after transfection. Cells were lysed with PBS supplemented with 2% SDS and boiled for 3 min. Chromosomal DNA was sheared by passage repeatedly through a 26-gauge needle. The supernatant was obtained by centrifugation at 14,000 rpm for 15 min at 4 °C. The DNA was extracted by the phenol/chloroform method.

**Isolation of Human Genomic DNA for 53BP1 and 53BP2**—A 1.3-kb PvuII fragment of the 53BP1 cDNA and a 1.3-kb HindIII-XbaI fragment of the 53BP2 cDNA were used to screen 1.2 million plaques of a human genomic library (a gift of Dr. P. Enrietto, State University of New York, Stony Brook) according to standard procedures (31). Inserts of positive phage clones were excised into plasmid II KS+ (Stratagene), and a panel of primers that hybridized on the 53BP1 or the 53BP2 DNA was used to obtain sequences of the corresponding genomic clones. Clones that were confirmed to contain 53BP1 or 53BP2 genomic DNA were used for fluorescence in situ hybridization.

**Fluorescence in Situ Hybridization**—Plasmid DNAs were biotinylated by nick translation, prehybridized in the presence of human Cot1DNA, and hybridized (at 10 ng/μl) to metaphase spreads of a normal male following procedures described in detail elsewhere (35). After hybridization and washing, the hybridization sites were labeled with fluorescein-conjugated avidin, and the chromosomes, which had previously been released from an early S-methotrexate block in the presence of colcemid, were counterstained with 5′-muridine-2-phenylindole to produce a QF-like banding pattern. Digital image processing was performed as described elsewhere (36). The locations of hybridization signals were analyzed in 15–20 well spread, well banded metaphases for each plasmid.

**Mapping**—To screen a human skeletal muscle cDNA library (CLONTECH) according to standard procedures (31). Clone, P7, which was identified, was also used to screen this library, resulting in the isolation of clone P45 (Fig. 1A). A 1.8-kb fragment was amplified from clone P45 by PCR with primer P7a containing restriction sites for EcoRI and 53BP1 fragment, and the clone P7a was isolated. A 1.8-kb fragment from clone P45 to generate pL8 fragment was deleted from pL6-2 and replaced by a 2.6-kb BamHI fragment of the 53BP1 cDNA sequence (Fig. 1A). Finally, a Jurkat cDNA library (a gift of Dr. P. Enrietto, State University of New York, Stony Brook) was screened with this 1.8-kb fragment, and the clone P7a was isolated.

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**Nuclear/Cytoplasmic Fractionation**—Two transiently transfected NCI-H358 cells were pooled 40 h after transfection. Cells were lysed, and both nuclear and cytoplasmic fractions were recovered following the protocol of Gashter et al. (38). The nuclear fraction was lysed in 100 μl of lysis buffer (100 mm Tris-HCl, pH 9.0, 150 mm NaCl, 1% Nonidet P-40, 0.2 μm phenylmethylsulfonyl fluoride, 5 μg/ml pepstatin, 2 μg/ml leupeptin). Both cytoplasmic and nuclear fractions were incubated with Ni-NTA beads that were equilibrated in the same lysis buffer. After incubation at 4 °C for 2 h, the beads were washed and boiled in SDS-sample buffer. Supernatants were fractionated by SDS-polyacrylamide gel electrophoresis and analyzed by Western blotting with the 12CA5 monoclonal antibody.

**Transfection Assay**—The transfection assay was performed as described by Reed et al. (33). REFS were prepared by passing Fisher rat embryos three times. For transfection, a mixture of DNA, 2.5 μg of pBS72-RAS, and 2.5 μg of pBS-E1A, 50 μl of Lipofect (Boehringer Mannheim), and 50 μl of 2× HEPS buffer (150 mM NaCl, 20 mM HEPES, pH 7.4) was added to a 10-cm dish containing 5 × 106 cells/dish. For the transfection suppression assay, either 5 μg of
pCMH6Kp53 or 22.5 μg of pCMH6K53BP2 or both were included. Transfected REFs were incubated and refed 20 h post-transfection and every 3 days. At 14 days post-transfection, cells were washed with PBS, fixed with methanol, and stained for 2 h with the Coomassie Brilliant Blue solution for protein detection. Plates were rinsed with water, and foci were counted. Duplicate samples were assayed each time, and each set of assays was repeated at least three times.

**RESULTS**

Isolation of cDNAs Encoding 53BP1—Northern blotting experiments indicated that 53BP1 is expressed in all tissues assayed with two transcripts of 11 and 6.6 kb (23). As the initial 53BP1 cDNA clones contained only 3.6 kb of sequence, we sought to identify a full-length 53BP1 cDNA. By using the 5′-RACE method (29, 30) with human skeletal muscle mRNA, we cloned a 450-bp fragment (R1), containing 260 bp of new sequence (Fig. 1 A). The R1 fragment was used to screen a cDNA library, followed by subsequent screenings using newly obtained sequences of two different cDNA libraries (see “Experimental Procedures”) to result in the isolation of cDNAs that together spanned 6.6 kb (Fig. 1 A). The sequence of the assembled 53BP1 cDNA is available, and the predicted open reading frame is shown in Fig. 1 B. The likely translation initiation codon of 53BP1 is preceded by an in-frame stop codon located 18 bp upstream. Although the nucleotide sequence 5′ of the

FIG. 1. A, schematic representation of isolated 53BP1 cDNA fragments. Top, the assembled 53BP1 cDNA with key restriction enzyme sites and position of the open reading frame (white box) is shown. Below, the relevant cDNA fragments are depicted from top to bottom in the order in which they were isolated. Arrows represent primers used in the 5′-RACE experiment or PCR amplifications (see “Experimental Procedures”). B, deduced protein sequence of 53BP1. The BRCT domain is underlined.
first ATG (GAGCAGATG) does not resemble the consensus initiation sequence (39), as more than 90% of translation in vertebrates starts from the first methionine (39), we designate this ATG as the putative translation start site. The predicted open reading frame of the 6.6-kb 53BP1 cDNA encompasses 1972 amino acids, a protein with a molecular mass of 217 kDa.

Recent sequence analysis revealed two BRCT domains in the C-terminal 247 residues of 53BP1. However, analysis of the entire protein by BLAST (40) did not show any extensive homology to other known proteins.

**Chromosomal Localization of the 53BP1 and 53BP2 Genes**—The interactions between 53BP1 and 53BP2 with wt but not mutant p53 raise the possibility that 53BP1 and 53BP2 are involved in some aspect of carcinogenesis in humans. Furthermore, it is possible that 53BP1 is a tumor suppressor based on its sequence homology to BRCA1. Therefore, it was of interest to map the chromosomal locations of these genes to determine whether they are located near cytogenetic locations known or suspected to harbor oncogenes or tumor suppressor genes. To obtain probes long enough for *in situ* hybridization, we screened a human genomic library with probes derived from the cDNAs of 53BP1 and 53BP2, and we obtained 5 phage clones for 53BP1 and 9 for 53BP2. Two of these clones, clone A2–3 for 53BP1 and clone B1–2 for 53BP2, were confirmed to contain the appropriate DNA and were used for the chromosome localization experiments.

53BP1 and 53BP2 were mapped to their chromosomal location by fluorescence *in situ* hybridization. Plasmids A2–3 and B1–2 were biotinylated and hybridized to metaphase spreads of a normal male donor. 53BP1 (clone A2–3) maps to 15q15–21, and 53BP2 (clone B1–2) maps to 1q41–42 (Fig. 2). Neither of these regions contains a known tumor suppressor gene or oncogene.

**Expression of Full-length 53BP1 and 53BP2 and Detection of Endogenous 53BP1 in Mammalian Cells**—To identify the protein expressed by the full-length 53BP1 cDNA we had assembled, we transfected H358 cells with a plasmid expressing HA-tagged 53BP1 (pCMH6K53BP1). In parallel, we also transfected expression plasmids for HA-tagged 53BP2 (pCMH6K53BP2) and p53 (pCMH6Kp53). Cells lysates were subjected to Western blot analysis with the 12CA5 monoclonal antibody which recognizes the HA tag. In cells transfected with pCMH6K53BP2, a protein larger than the 220-kDa marker was detected (Fig. 3A, lane 2), indicating that the 53BP1 protein migrates significantly slower than its predicted size. Proteins with apparent sizes of 150 and 53 kDa were detected in cells transfected with the 53BP2 or p53 plasmids, respectively (Fig. 3A, lanes 3 and 4). A construct that expresses native non-tagged 53BP2 was reported to express a protein of 150 kDa, as determined by Western blot using rabbit anti-53BP2 antibodies (28). A background of proteins was detected by 12CA5 in all cells, including those transfected with the expression plasmid lacking an insert (Fig. 3A, lane 1).

We also examined the time course of expression of the transfected 53BP1 and 53BP2 in H358 cells so that cells with peak level expression of these proteins could be used for the reporter assays (see below). Both 53BP1 and 53BP2 were expressed at 30 h post-transfection, and this expression continued at least until 40 h post-transfection (Fig. 3B). Based on these time courses, we used cells 40 h after transfection in our transcriptional activation assays.

In order to detect the endogenous 53BP1 protein in mammalian cells, we raised polyclonal antibodies against the C-terminal 270 residues of this protein (41). These antibodies detected the HA-tagged full-length (Fig. 3C, lane 2) and HA-tagged C-terminal half (Fig. 3C, lane 4) but not the HA-tagged N-terminal half (Fig. 3C, lane 3) of 53BP1 expressed in COS-1 cells. The expression of each HA-tagged protein in COS-1 cells was confirmed by Western blot analysis of the same blot with 12CA5 (Fig. 3C, lanes 7–9). Cell extracts from the human fibroblast cell line, WI38, and from the human cancer cell lines Saos-2, H358, and HepG2 were subjected to Western blot analysis with anti-53BP1 polyclonal antibodies. We detected a band that has the same molecular weight as the protein produced by the full-length 53BP1-expressing plasmid only in H358 cells (Fig. 3C, lane 1), indicating that the assembled 53BP1 cDNA encodes the endogenous 53BP1 protein.

**Modulation of the Transcriptional Activation Function of p53 by 53BP1 and 53BP2**—Previously, we showed that both 53BP1 and 53BP2 bind to the DNA-binding domain of p53 and that p53 bound to 53BP1 or 53BP2 was not able to bind simultaneously to DNA carrying a consensus p53-binding site (23). These data suggested that the interactions of 53BP1 and 53BP2 with p53 might interfere with the activity of p53 as a sequence-specific transcriptional activator, which is required for its function in tumor suppression. To understand the biological consequences of these protein-protein interactions in...
cell culture, we assayed the transcriptional activation function of p53 in the presence of overexpressed p53-binding proteins. A CAT reporter gene under the control of p53-binding sites (pCAB-PG26TATA) was transfected into H358 cells together with an expression plasmid for wt p53 (pCMH6Kp53) or a combination of expression plasmids for p53 and 53BP1 or 53BP2. p53-induced transactivation of the CAT reporter gene increased approximately 25-fold in cells transfected with the reporter plasmid and the p53 plasmid (2100 cpm) compared with cells transfected with the reporter alone (80 cpm) (Fig. 4A). When increasing amounts of the 53BP1 plasmid (pCMH6K53BP1) were cotransfected in combination with the p53 plasmid and the reporter, CAT expression increased further in a dose-dependent manner, up to an approximately 10-fold stimulation by 8 μg of the 53BP1 plasmid (23,000 cpm) (Fig. 4A). 53BP2 also stimulated p53-mediated transcriptional activation in a dose-dependent manner. Activity is represented by the radioactive cpm of 14C-labeled acetylated chloramphenicol. C, induction of the p21 protein by 53BP1 and 53BP2. Extracts were prepared from MCF7 cells left untreated (lane 1), irradiated with 8 Gy (lane 2), or transfected with 1 μg of pCMH6K vector (lane 3), pCMH6Kp53 (lane 4), pCMH6K53BP1 (lane 5), and pCMH6K53BP2 (lane 6) and the Western blot probed with pAB421 which detects p53 and F5 which detects p21. Cells were lysed after 4 h for the radiation experiment (lanes 1 and 2) and 24 h after transfection (lanes 3–6).
the trans-activation of an endogenous target of p53 activity, the p21 gene. MCF7 human breast carcinoma cells that have wt p53 were transfected with plasmids containing either the 53BP1, 53BP2, or mouse p53 gene and harvested 24 h later. As a control for p21 induction, MCF21 cells were treated with 8 Gy γ-irradiation and lysed 4 h later. Western blot analysis was performed with a combination of monoclonal antibodies pAb421, which recognizes mouse and human p53, and F5, which recognizes p21. The antibody mixture detected p21 protein in the irradiated (Fig. 4C, lane 2) but not untreated cells (Fig. 4C, lane 1). Although p53 was detected in cells harvested 2 h post-irradiation (data not shown), it had already disappeared at the 4 h time point when p21 induction was apparent. While cells transfected with the vector alone contained only the basal level of p21 (Fig. 4C, lane 3), both the 53BP1 and 53BP2 plasmids behaved similarly to the p53 plasmid in inducing the expression of the p21 protein (Fig. 4C, lanes 4–6). In contrast to the high level of p53 protein observed upon transfection of the p53 plasmid, neither the 53BP1 nor 53BP2 plasmid led to detectable p53 protein (Fig. 4C, lanes 4–6), indicating that both 53BP1 and 53BP2 induce endogenous p21 expression without an apparent change in the level of p53 protein. These enhancements of the trans-activation function of p53 may reflect a direct role for 53BP1 or 53BP2 in the transcription process or their ability to render the p53 protein more competent for transcription.

53BP2 Partially Suppresses Cell Transformation by Oncogenes—Since 53BP1 and 53BP2 stimulate at least one activity of p53 (trans-activation), they may be capable of stimulating its overall tumor suppression function. Wild type p53 can reduce the efficiency of cooperating oncogenes such as ras and E1A to transform primary REFs in culture (42, 43), whereas oncogenic mutant p53 cooperates with these oncogenes to transform primary cells (44, 45). We tested the possibility that overexpressed 53BP2 could enhance transformation suppression either alone or in the presence of excess p53. REFs, which express a low level of wt p53, were transfected with expression plasmids for Ras and E1A. These cells gave rise to transformed foci 14 days after transfection (Table I). As expected, overexpressed wt murine p53 suppressed this transformation, whereas cotransfection of 53BP2 with the oncogenes and wt p53 further suppressed transformation only slightly in one experiment (Table I). However, in three separate experiments, cotransfection of 53BP2 without p53 resulted in an approximately 30% decrease in the number of foci induced by the oncogenes (Table I). These data indicate that 53BP2 overexpression alone partially suppresses cellular transformation by oncogenes. This effect may be achieved through the tumor suppressor function of p53. By interacting with the endogenous wt p53 in REFs, 53BP2 may stimulate its trans-activation function to activate growth control genes, which in turn suppress transformation.

**Subcellular Localization of 53BP1 and 53BP2**—Although

![Figure 5](image)

**Fig. 5. Subcellular localization of p53, 53BP1 and 53BP2.** COS-1 cells were transfected with pCMH6K, pCMH6K53BP1 (A–C), pCMH6K53BP2 (D), and pCMH6Kp53 (E). At 36 h post-transfection, cells were fixed, permeabilized, and probed with 12CA5. Anti-mouse antibodies conjugated to fluorescein isothiocyanate were used for detection. F, cellular fractionation assay. H358 cells were transfected with 22.5 μg of pCMH6Kp53, pCMH6K53BP2, or both pCMH6K and pCMH6K53BP2 as indicated. Cells were harvested at 40 h post-transfection, and cytosol and nuclear fractions were isolated as described under "Experimental Procedures." Each fraction was incubated with Ni-NTA beads to concentrate the histidine-tagged 53BP2 and p53, and the fractions were subjected to Western blot analysis with 12CA5.

### Table I

**Suppression of oncogene-mediated transformation by p53 and 53BP2**

Plasmids encoding Ras, E1A, p53, and 53BP2 were transfected into rat embryo fibroblasts in different combinations as indicated. Fourteen days after transfection, cells were fixed with methanol and stained with Coomassie Brilliant Blue.

| Transfected genes | No. of foci |
|-------------------|------------|
|                   | Exp. 1 | Exp. 2 | Exp. 3 |
| Vectors           | 0     | 0      | 0      |
| EIA + ras         | 73    | 68     | 45     |
| EIA + ras + p53   | 35    | 36     | 34     |
| EIA + ras + p53 + 53BP2 | 20   | 25     | ND*    |
| EIA + ras + 53BP2 | 49    | 48     | 30     |

* ND, not determined.
the stage of the cell cycle at the time of staining. 53BP1 showed more complex staining patterns, being present in both cytoplasm and nucleus in some cells (Fig. 5A) and only in the nucleus in others (Fig. 5, B and C). In addition, there are two nuclear patterns for 53BP1, one homogeneous staining (Fig. 5B) and the other dot staining (Fig. 5C). Possibly the cellular localization of 53BP1 changes during the cell cycle or under different conditions. When H35B cells, which lack the p53 gene, were transfected with the 53BP1 plasmid, 53BP1 showed the same three types of staining pattern (data not shown), suggesting that the translocation of 53BP1 between the cytoplasm and nucleus does not require its binding to p53. In contrast to p53 and 53BP1, 53BP2 was detected only in the cytoplasm of both COS-1 (Fig. 5D) and H35B (data not shown) cells.

We determined whether the subcellular localization of p53 and 53BP2 changed as a result of the interaction between these two proteins using a cellular fractionation assay. We transiently transfected H35B cells with p53 and 53BP2 plasmids that express proteins containing an N-terminal tag of the HA epitope and six histidines. The cytosol and nucleus of the transfected cells were separated, and histidine-tagged p53 and/or 53BP2 proteins were enriched with Ni-NTA beads. The proteins bound to Ni-NTA beads were assayed in Western blot with 12CA5. p53 was detected in both nuclear and cytosol fractions (Fig. 5F, lanes 1 and 2); a protein comigrating with p53 was nonspecifically recognized by 12CA5 (Fig. 5F, lanes 3 and 4). In agreement with the immunofluorescence data, the HA-tagged 53BP2 protein was found only in the cytosol fraction (Fig. 5F, lanes 3 and 4). In the cells expressing both p53 and 53BP2 plasmids, the localization of these proteins did not change (Fig. 5F, lanes 5 and 6).

DISCUSSION

We have further characterized the 53BP1 and 53BP2 proteins, which bind to the DNA-binding domain of wt p53. A 6.6-kb cDNA for 53BP1 was cloned and sequenced, predicting a protein of 1972 residues. Polyclonal antibodies raised against a C-terminal domain of this protein detected a protein in a human cell line of similar molecular weight to that expressed by the cloned cDNA, indicating that we isolated a cDNA that encodes at least one form of the authentic 53BP1 protein. Previously, the 53BP2 protein had been characterized as the BCL2-binding protein BBP of 1005 residues (28). We mapped the chromosomal location of the 53BP1 and 53BP2 genes to 15q15–21 and 1q41–42, respectively, which do not correspond to known regions harboring tumor suppressor genes or oncogenes. The 53BP1 protein shows a complex pattern of cellular localization, present either in both the nucleus and cytoplasm or in the nucleus only as homogeneous or dot staining. By contrast, the 53BP2 protein is only present in the cytoplasm. The two p53-binding proteins are able to enhance the transcriptional activation function of p53, suggesting that they may function in a signaling pathway to promote p53 activity.

The 53BP1 protein shows no significant homology to other proteins apart from two copies of a region that is similar to the C-terminal domain of the BRCA1 protein (BRCT domain) (24). The BRCT domain is essential for BRCA1 function, as a deletion of this domain results in loss of tumor suppression by this protein (46). Recently, the family of proteins with the BRCT domain has expanded to nearly 40 members (25, 26), including the yeast checkpoint protein Rad9 and transcription factor Rap1 and vertebrate terminal deoxynucleotidyltransferases. Although these proteins have diverse functions, their common involvement in cell cycle checkpoints and DNA damage response suggests a similar possible role for 53BP1. The presence of BRCT domains in 53BP1 and BRCA1 also raises the possibility that BRCA1 interacts with p53. Additionally, both p53 and BRCA1 bind to Rad51 (47, 48), a protein with strand exchange activity that is involved in recombinational DNA repair. These interactions could allow the formation of a BRCA1, Rad51, and p53 complex or, alternatively, a series of sequential interactions, perhaps in response to DNA damage.

Another striking parallel between the 53BP1 protein and the BRCA1 and Rad51 proteins is a cellular localization typified by nuclear dot staining (47). In addition, BRCA1 localization undergoes dynamic change after DNA damage, with the dots becoming dispersed and the protein relocating to DNA replicating structures where it may play a role in repair of damaged DNA (49). Besides staining as nuclear dots, 53BP2 also appeared cytoplasmic and homogeneously nuclear, which may correlate with different cell cycle stages or responses to various stresses such as DNA damage.

Both 53BP1 and 53BP2 enhanced p53-mediated transcriptional activation, and this activity may account for the ability of 53BP2 to suppress transformation of fibroblasts by activated oncogenes. Another study has shown that an interferon-induced protein, p202, inhibits trans-activation by p53 and that 53BP1 can bind p202 and relieve this inhibition (41). Thus, a possible mechanism for the 53BP1 stimulatory activity is displacement of an inhibitor bound to p53, resulting in p53 free to participate in the transcription process. The failure of overexpressed 53BP1, as well as 53BP2, to increase the level of p53 is consistent with this idea. 53BP2 appears to localize exclusively to the cytoplasm, as previously noted by others using a different expression system and antibody (28). A possible mechanism for the stimulatory activity of 53BP2 is to promote the nuclear transport of p53. In some tumors, p53 is sequestered in the cytosol (50, 51) where it may be bound to an anchoring protein (52), and 53BP2 could release p53 from such an inhibitor. Finally, these proteins might play a role in converting a latent form of p53 into an active one. Recent reports indicated possible regulation of the DNA binding activity of p53 by its redox state (53) or by allosteric change (54). For example, Ref-1, a redox/repair protein, is a potent activator of latent p53 (53), and p300, a transcriptional coactivator, activates the sequence-specific DNA binding activity of p53 by acetylating its C-terminal domain (55).

The signaling pathway from damaged DNA to p53 activation is still poorly defined. The ability of 53BP1 and 53BP2 to bind to the conformationally sensitive central domain of p53 and to stimulate p53-mediated transcriptional activation suggests that one or both of these proteins play roles in this pathway. Continued analysis of these p53-binding proteins may thus further clarify the key processes by which p53 is regulated.

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