Potential Role for 53BP1 in DNA End-joining Repair through Direct Interaction with DNA*

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Upon DNA damage, p53-binding protein 1 (53BP1) relocalizes to sites of DNA double-strand breaks and forms discrete nuclear foci, suggesting its role in DNA damage responses. We show that 53BP1 changed its localization from the detergent soluble to insoluble fraction after treatment of cells with x-ray, but not with ultraviolet or hydroxurea. Either DNase or phosphatase treatment of the insoluble fraction released 53BP1 into the soluble fraction, showing that 53BP1 binds to chromatin in a phosphorylation-dependent manner after X-irradiation of cells. 53BP1 was retained at discrete nuclear foci in X-irradiated cells even after detergent extraction of cells, showing that the chromatin binding of 53BP1 occurs at sites of DNA double-strand breaks. The minimal domain for focus formation was identified by immunofluorescence staining of cells ectopically expressed with 53BP1 deletion mutants. This domain consisted of conserved Tudor and Myb motifs. The Tudor plus Myb domain possessed chromatin binding activity in vitro and bound directly to both double-stranded and single-stranded DNA in vitro. This domain also stimulated end-joining by DNA ligase IV/Xrc4c, but not by T4 DNA ligase in vitro. We conclude that 53BP1 has the potential to participate directly in the repair of DNA double-strand breaks.

A DNA double-strand break (DSB) is one of the most serious threats to cells because it can result in loss or rearrangement of genetic information, that leads to cell death or carcinogenesis.

DSBs can be induced by ionizing radiation (IR) and arise in endogenous normal processes of cells such as DNA replication, meiosis, and V(D)J recombination. The induction of DSBs activates various sophisticated pathways, termed cell-cycle checkpoints, which monitor DNA damage and transduce signals to coordinate repair and cell cycle progression (reviewed in Refs. 1 and 2).

DSBs are repaired by two major pathways, homologous recombination (HR) and non-homologous end joining (NHEJ) (reviewed in Ref. 3). HR uses a DNA molecule with significant length of sequence homology to prime the repair DNA synthesis, allowing for accurate repair of the lesions. In contrast, NHEJ rejoins DNA ends with the use of little or no sequence homology, leading to imprecise joining. The repair of DSBs by NHEJ requires at least five proteins (reviewed in Ref. 4). Three are components of the DNA-dependent protein kinase (DNA-PK) complex, the two DNA end-binding subunits of Ku (Ku70 and Ku80), and the catalytic subunit termed DNA-PKcs. The remaining two are DNA ligase IV and Xrc4c. It is thought that broken termini are recognized by the Ku heterodimer, which then recruits DNA-PKcs, thereby activating its kinase activity. This large complex serves to protect the DNA ends from nucleolytic attack, while also facilitating the recruitment of the DNA ligase IV/Xrc4c heterodimer. Although it is not at present clear how end-bridging is achieved, these reactions result in the rejoining of the DSBs, restoring the integrity of the DNA.

53BP1 was originally identified in a yeast two-hybrid screen as a protein that interacts with p53 (6, 7), through the 53BP1 C-terminal BRCA1 C terminus (BRCT) domain. The BRCT domain is found in a large number of proteins involved in the cellular responses to DNA damage (11–13), suggesting the role of 53BP1 in these aspects. Consistent with this, upon exposure of cells to IR, 53BP1 has been shown to relocalize rapidly to sites of DNA DSBs forming discrete nuclear foci and to be hyperphosphorylated in an Ataxia Telangiectasia Mutated (ATM)-dependent manner (14–17). ATM is a central signaling kinase in the response for DSBs, and upon activation in response to IR, ATM phosphorylates many proteins involved in checkpoint or DNA repair (reviewed in Ref. 18).

On the basis of its high similarity to Saccharomyces cerevisiae Rad9 in the BRCT motifs (8, 10), 53BP1 is thought to be a candidate for a human counterpart of Rad9 (2). Rad9, an essential protein for the cell cycle arrest induced by DNA damage (19), becomes phosphorylated in response to DNA damage dependent on Mec1, the functional S. cerevisiae homologue of ATM (20, 21). The phosphorylated Rad9 interacts physically

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The abbreviations used are: DSB, double-strand break; ds, double-stranded; ss, single-stranded; IR, ionizing radiation; GY, gray; UV, ultraviolet; HU, hydroxurea; GST, glutathione S-transferase; HA, hemagglutinin; NLS, nuclear localization signal; NHEJ, non-homologous end joining; PBS, phosphate-buffered saline; KBD, kinetochore-binding domain.

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with Rad53, the \(\text{Rad53}^\text{p53BP1}\) has been shown to bind to Chk2, although this interaction is required for subsequent phosphorylation and activation of Rad53 (20–22). Recently, like Rad9 and Rad53, 53BP1 has been shown to be involved in the regulation of chromatin structure.

Although the roles of 53BP1 in DNA damage checkpoint have been elucidated, it is unclear whether 53BP1 has roles in the repair of DNA DSBs. Here we report that 53BP1 binds to chromatin in vivo after treatment of cells with x-ray, but not with ultraviolet (UV) or hydroxyurea (HU). The minimal domain for focus formation consists of conserved Tudor and Myb motifs. The Tudor plus Myb domain possesses chromatin binding activity in vivo and binds directly to both double-stranded (ds) and single-stranded (ss) DNA in vitro. This domain also stimulates end-joining by DNA ligase IV/Xrcc4, which indicates that 53BP1 is a central mediator of the DNA damage checkpoint.

EXPERIMENTAL PROCEDURES

Antibodies—A monoclonal anti-53BP1 antibody, 2B6, was prepared using a recombinant protein consisting of the 361 N-terminal residues of 53BP1 fused to glutathione S-transferase (GST) as an antigen. Monoclonal antibodies recognizing BRCA1 (clone MS110) and Rho A (plasmid 26C4) were obtained from Oncogene Research Product and Santa Cruz Biotechnology, respectively. Anti-Actin and anti-Orc2 antibodies were obtained from Sigma and BD Pharmingen, respectively. A monoclonal antibody recognizing the hemagglutinin (HA) epitope tag (clone 12CA5) was obtained from Roche Applied Science.

Plasmids—Plasmids expressing HA-tagged full 53BP1, the C-terminal half of 53BP1 (53BP1-C) and the N-terminal half of 53BP1 (53BP1-N), pcMH6K53BP1, pcMH6K53BP1-C and pcMH6K53BP1-N respectively, were described previously (7). The HA1 fragment of pcMH6K53BP1 was filled-in with the Klenow fragment of DNA polymerase I. This fragment was then ligated between the NheI site of pcMH6K (7) and the BamHI site of pGEX-3X (Amersham Biosciences). In the second gel, the lane with pCMH6KNLSC was washed with renature buffer (200 g/ml RNase (Sigma) or 200 g/ml DNase with 1% bovine serum albumin) with 70 fmol of the BPB site of the first gel.

Preparations of Whole Cell Extracts, Soluble, and Insoluble Fractions—The 53BP1 site of the HpaI site of pcMH6K (7) to produce pcMH6KNLSS53BP1-N. 53BP1cDNA insert coding residues 1617–1850 was obtained from Oncogene Research Product and Santa Cruz Biotechnology, respectively. Anti-Actin and anti-Orc2 antibodies were obtained from Sigma and BD Pharmingen, respectively. A monoclonal antibody recognizing the hemagglutinin (HA) epitope tag (clone 12CA5) was obtained from Roche Applied Science.

Plasmids—Plasmids expressing HA-tagged full 53BP1, the C-terminal half of 53BP1 (53BP1-C) and the N-terminal half of 53BP1 (53BP1-N), pcMH6K53BP1, pcMH6K53BP1-C and pcMH6K53BP1-N respectively, were described previously (7). The HA1 fragment of pcMH6K53BP1 was filled-in with the Klenow fragment of DNA polymerase I. This fragment was then ligated between the NheI site of pcMH6K (7) and the BamHI site of pGEX-3X (Amersham Biosciences). In the second gel, the lane with pCMH6KNLSC was washed with renature buffer (200 g/ml RNase (Sigma) or 200 g/ml DNase with 1% bovine serum albumin) with 70 fmol of the BPB site of the first gel.

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FIG. 1. 53BP1 binds to chromatin in vivo in response to IR-induced DNA damage. A, 53BP1 binds to chromatin in vivo after X-irradiation of cells. MCF7 cells were untreated or irradiated with 8 Gy. At 2 h post-treatment, cells were lysed with Nonidet P-40 containing buffer in the presence or absence of the indicated enzymes. The detergent-soluble and insoluble fractions were obtained as described in "Experimental Procedures." The resultant fractions were resolved on SDS-PAGE and immunoblotted with the indicated antibodies. B, phosphatase treatment releases 53BP1 from chromatin. MCF7 cells, untreated or irradiated with 8-Gy of X-rays, were lysed with Nonidet P-40 containing buffer. The resultant cell lysates were untreated or treated with phosphatase as described in "Experimental Procedures." Soluble fractions were analyzed by immunoblotting with the indicated antibodies. C, X-ray, but not UV or HU treatment, induces chromatin binding of 53BP1. MCF7 cells were untreated or exposed to 4 Gy of x-ray or 50 J/m² of UV 2 h before cell lysis. Alternatively, cells were treated for 1 h with 1 mM of HU. Soluble fractions were analyzed by immunoblotting with the indicated antibodies. D, correlation between chromatin binding, hyperphosphorylation and focus formation of 53BP1. Top panel, soluble fractions and whole cell extracts were obtained from MCF7 cells at indicated time points after 2-Gy X-irradiation and then immunoblotted for 53BP1. Actin is included as a protein loading control for soluble fractions. Whole cell extracts were prepared by lysing cells in 2% SDS-PBS. Bottom panel, MCF7 cells were immunostained with anti-53BP1 antibody at 0.5, 3 h and at 3-h intervals until 24 h post-exposure to 2 Gy. Percentages of 53BP1 focus-positive cells are plotted against time after irradiation. Over 300 cells were counted at each time point.

FIG. 2. 53BP1 binds to chromatin at sites of DNA double-strand breaks. MCF7 cells were untreated (0 Gy) or irradiated with x-ray (4 Gy). At 2 h post-irradiation, cells were extracted in situ with the Nonidet P-40 (+NP40) or not extracted (−NP40). Cells were stained with anti-53BP1 antibody (left) and counterstained with 4’,6’ diamino-2-phenylindole (right). Signals for 53BP1 were obtained in the same exposing time for all samples.
53BP1 Binds to Chromatin in Vivo at Sites of DSBs after X-irradiation of Cells—As 53BP1 forms foci at sites of DSBs (14, 15, 17), we asked whether 53BP1 bound to chromatin after exposure of cells to IR. To determine this possibility, we examined the amount of 53BP1 in the soluble and insoluble fractions of cell lysates prepared in 0.5% Nonidet-P 40 (see “Experimental Procedures”). If 53BP1 binds to chromatin it should change its localization, like human Rad9 (27), from the soluble to insoluble fraction, the latter being enriched for chromatin. A soluble protein (Rho A) and a chromatin-binding protein (Orc2) were well separated by the fractionation (Fig. 1A, left panel). We found that the level of soluble 53BP1 was dramatically reduced in response to X-irradiation (Fig. 1A, left panel and right panel, lanes 1 and 2). Concomitant with the decrease of soluble 53BP1 protein, the amount of insoluble 53BP1 increased after irradiation (Fig. 1A, left panel and right panel, lanes 6 and 7).

To determine whether 53BP1 in the detergent-insoluble fraction was bound to chromatin, we examined the effect of DNA digestion. Irradiated cells were permeabilized in the presence of RNase, DNase with or without EDTA, or no treatment. DNA digestion almost fully restored the amount of soluble 53BP1 in X-irradiated cells (Fig. 1A, right panel, lane 3). In accord with this, 53BP1 in the DNA-treated insoluble fraction decreased to an undetectable level (Fig. 1A, right panel, lane 8). Treatment with RNase or DNase plus EDTA did not result in an increase of soluble 53BP1 in irradiated cells, ruling out non-specific effects of the DNase preparation. X-irradiation did not alter significantly the amount of a control protein, BRCA1, in both the soluble and insoluble fractions. These results indicate that 53BP1 is bound to chromatin after X-irradiation of cells.

53BP1 has been shown to be phosphorylated in asynchronous cultures and becomes hyperphosphorylated after IR treatment of cells (14–16). To determine whether phosphorylation was necessary for 53BP1 to bind to chromatin, we treated cell lysates from X-irradiated cells with phosphatase. Phosphatase treatment released 53BP1 from the insoluble fraction into the soluble fraction (Fig. 1B). This treatment also converted the slower migrating phosphorylated forms of BRCA1 to the faster migrating form, indicating that the enzyme was active. Thus, phosphorylation was necessary for 53BP1 to bind to chromatin in cells treated with x-ray.

In contrast to x-ray treatment, neither UV nor HU treatment resulted in reduction of the soluble 53BP1 (Fig. 1C), suggesting that 53BP1 binding to chromatin was specifically correlated with induction of DSBs. To ask whether the 53BP1 chromatin binding activity was correlated with the formation of IR-induced 53BP1 foci, we carried out a time-course experiment after X-irradiation. When MCF7 cells were irradiated with 2 Gy, the reduction of soluble 53BP1 was observed at the earliest time point measured (30 min after irradiation), reached a maximum level at 4 h, and decreased at 15 h after irradiation (Fig. 1D, top panel, soluble fractions). Importantly, hyperphosphorylated forms of 53BP1, which migrate slower in SDS-polyacrylamide gel, were not present in the soluble fraction after treatment of cells with x-ray. In a parallel experiment, appearance of hyperphosphorylated forms of 53BP1 was monitored by 53BP1 immunoblotting with whole cell extracts prepared by lysing cells with 2% SDS-PBS buffer. Hyperphosphorylated forms of 53BP1 were observed at the same time course as that of the reduction of soluble 53BP1 in X-irradiated cells (Fig. 1D, top panel, soluble fractions and whole cell extracts). These data indicate that hyperphosphorylated forms of 53BP1 were bound to chromatin. 53BP1 foci were evident at 30 min, with nearly all nuclei displaying foci, and declined in number from 12 to 27 h (Fig. 1D, bottom panel). The time point (15 h) when 53BP1 was released from chromatin coincided with the time point of extensive 53BP1 focus dispersion. Collectively, these results show that the focus-formation, phosphorylation and chromatin binding of 53BP1 are different manifestations of the same cellular response specifically activated by DNA DSBs.

To determine whether 53BP1 was bound to chromatin at sites of IR-induced 53BP1 foci, we adapted a protocol for in situ detergent extraction of attached cells (see “Experimental Procedures”) (Fig. 2). In non-irradiated cells, 53BP1 showed diffuse nuclear staining with the exception of the nucleoli that were not stained. Some cells showed a bright large nuclear dot...
as well as diffuse nuclear staining as reported previously (14). However, 53BP1 was lost from the nuclei of non-irradiated cells after extraction with Nonidet P-40. Whereas, 53BP1 localized at the foci in x-ray-irradiated cells was still retained in the foci after the detergent extraction (Fig. 2). These data indicate that 53BP1 is loosely tethered and readily extracted from the nucleus by permeabilization in non-irradiated cells, and that, upon IR treatment, 53BP1 binds to chromatin and forms extraction-resistant foci at sites of DSBs.

Identification of the Minimal Focus-forming Domain of 53BP1—To identify the region of 53BP1 required for focus formation, we ectopically expressed a series of HA-tagged 53BP1-deletion mutants in MCF7 cells (Fig. 3A). Cells were subjected to immunofluorescence staining with anti-HA antibody before or after X-irradiation. All mutants except NLS53BP1-N, the N-terminal half of 53BP1 fused to a 53BP1-NLS, were localized in the nucleus before irradiation (data not shown). The signals from the NLS53BP1-N mutant were seen in both the nucleus and cytoplasm (data not shown). After irradiation, all mutants that contained residues 1052 to 1709 showed focus formation (Fig. 3B), indicating that this region was sufficient for 53BP1 focus formation. This region was named 53BP1-CΔBRCT.

Recently, it was reported that the mouse 53BP1 homologue localizes at the kinetochore during mitosis (28). The kinetochore-binding domain (KBD) mapped to residues 1235 to 1716 of human 53BP1 (human KBD) and we noted that this domain lies within the 53BP1 focus-forming fragment (53BP1-CΔBRCT) (Fig. 4A). To aid in the identification of specific focus-forming domains within human KBD, we searched the protein sequence data base, using iterative PSI-BLAST (29) data base searches, for proteins with significant homology to the human, mouse, and Xenopus 53BP1 KBDs. Intriguingly, these searches indicated that KBD contains “Tudor” and “Myb” domains (Fig. 4, A and B). Tudor domains were first identified as a 10-fold repeat in the Drosophila Tudor protein (30, 31) and have subsequently been found in many proteins that co-localize with ribonucleoproteins or bind to chromatin in the nucleus, such as lamin B (32). Currently, it is not known whether these domains bind directly to polynucleotides or interact with other proteins within nucleoprotein complexes. In contrast, Myb domains, first identified in the retroviral v-myb oncogene, have been shown to bind directly to DNA (33, 34).

To assess the functional importance of the Tudor and Myb domains, we assayed the KBD and six KBD-deletion mutants for the focus-forming activity (Fig. 5A). KBD and KBD mutants were expressed in MCF7 cells as a fusion with both HA-tag and 53BP1-NLS. Cells were subjected to immunofluorescence staining with anti-HA antibody. These assays revealed that KBD and KBD-C (the Tudor plus Myb domain), but not the other mutants, formed foci in response to X-irradiation of cells.
These data indicate that the Tudor plus Myb domain is the minimal region for focus formation.

To test whether a polypeptide of the Tudor plus Myb domain (KBD-C) was bound to chromatin, cells expressing KBD-C fused to HA tag and 53BP1-NLS were subjected to immunofluorescence staining with anti-HA antibody before or after in situ detergent extraction. In contrast to endogenous 53BP1, KBD-C expressed in non-irradiated cells was retained in the nuclei after the detergent extraction. X-ray-induced foci formed by KBD-C were not lost after the detergent extraction (Fig. 5C).

These data indicate that the Tudor plus Myb domain possesses the chromatin-binding activity.

53BP1 Possesses at Least Two DNA-binding Domains—
Given that 53BP1 bound to chromatin in vivo, we asked whether 53BP1 had potential activity to bind to DNA in vitro. In a screening experiment, six overlapping 53BP1 fragments fused to GST including GST-53BP1-CΔBRCT (Fig. 6A) were tested for DNA binding activity using a DNA probe-based assay (Fig. 6B), in which GST fusion proteins and GST were separated by SDS-polyacrylamide gel and 32P-labeled linear dsDNA was electrophoresed on the gel after removing SDS from the gel by washing in renature buffer (see "Experimental Procedures"). The dsDNA probe bound to 53BP1-CΔBRCT and HCV core (positive control) but not to the other 53BP1 fragments or GST alone (Fig. 6B, right panel). Note that the 32P signals were present at the appropriate positions for HCV core and 53BP1-CΔBRCT (Fig. 6B, left and right panel). A weak signal was detected where the largest degradation product of 53BP1-CΔBRCT fusion protein migrated. These data show that the 53BP1-CΔBRCT fragment has activity to bind DNA in vitro, although the possibility remains that the 53BP1 fragments that failed to bind DNA were not renatured properly.

To further confirm that 53BP1-CΔBRCT domain directly binds to DNA, and to test whether 53BP1 binds only to DNA ends, we assayed the DNA-binding activities of recombinant 53BP1-CΔBRCT domain with linearized- and closed-circular (cc) dsDNA in electrophoretic mobility shift assays (EMSA). 53BP1-CΔBRCT bound avidly to both DNA substrates (Fig. 6C) suggesting that binding of 53BP1 to DNA did not require dsDNA ends. These data indicate that 53BP1 binds in vitro to DNA directly through the 53BP1-CΔBRCT region, which includes the Tudor plus Myb, the minimal focus-forming domain.

To ask whether focus-forming activity was correlated with the DNA binding activity, we assayed the KBD and six KBD-deletion mutants for the DNA binding activity (Figs. 5A and 6D). EMSA assays revealed that recombinant KBD, KBD-N, KBD-M, and KBD-C polypeptides bound avidly to both linear...
ds and ssDNA substrates (Fig. 6D). In contrast, the Tudor, KBD-NN, and KBD-CC polypeptides showed no affinity for any DNA substrates (Figs. 5A and 6D and data not shown). These data indicate that 53BP1 has at least two independent DNA binding domains, KBD-M and KBD-C. These results also suggest that the DNA binding activity of the KBD-C domain is closely coupled with 53BP1 focus-formation.

The Focus-forming Domain of 53BP1 Promotes DNA End-joining by DNA Ligase IV—It is known that many factors that bind directly to DNA, such as Ku 70/80, participate in the repair process (4). To determine if the DNA binding domains of 53BP1 directly promoted DNA end-joining in vitro, we tested the ability of KBD and sub-domains of KBD to stimulate ligation by the NHEJ ligase complex, DNA ligase IV/Xrcc4. The DNA ligation activity of this complex was significantly stimulated by the addition of KBD, KBD-N, KBD-C, KBD-M, Tudor, and KBD-CC to linearized plasmid DNA and lower panel, binding of 53BP1 domains to ss M13mp18.

B cell populations (35). Processes for the maturation of T cell receptors and immunoglobulins during V(D)J recombination are known to utilize end-joining DNA repair machinery (4). To determine if the DNA binding domains of 53BP1 directly promoted DNA end-joining in vitro, we tested the ability of KBD and sub-domains of KBD to stimulate ligation by the NHEJ ligase complex, DNA ligase IV/Xrcc4. The DNA ligation activity of this complex was significantly stimulated by the addition of KBD, KBD-C and KBD-N but not by the Tudor domain (Fig. 7A). T4 DNA ligase activity was also stimulated by the addition of KBD and KBD-N (Fig. 7B) but notably, while KBD-C stimulated end-joining by DNA ligase IV/Xrcc4, it did not stimulate...
In this report we show a line of evidence suggesting that 53BP1 participates directly in the repair of DNA DSBs. Firstly, 53BP1 binds to chromatin in vivo after treatment of cells with x-ray, but not with UV or HU. Secondly, the Tudor and Myb domain (KBD-C), which is sufficient for focus formation, possesses chromatin binding activity in vivo and binds directly to both ds and ssDNA in vitro. Thirdly, and most importantly, the KBD-C domain also promotes specifically the DNA end-joining by DNA ligase IV/Xrcc4 complex, which is involved in the NHEJ pathway of DSB repair in mammalian cells.

Chromatin binding of 53BP1 occurred after X-irradiation of cells. We propose two mechanisms to explain how 53BP1 binds to chromatin only after induction of DSBs. First, a change in the chromosomal structure around the sites of DNA DSBs may occur, rather than a change in 53BP1 structure. The localized change in the structure of the chromatin may be induced by histone H2AX phosphorylation. Histone H2AX has been shown to be phosphorylated on chromosomal regions encompassing megabase lengths of DNA adjacent to break sites (36). Second, the focus-forming/chromatin-binding domain (the Tudor plus Myb domain) of 53BP1 may be sequestered in the absence of DSBs and be unable to bind to chromatin. After the induction of DNA DSBs, 53BP1 phosphorylation by ATM may induce a conformational change in the protein, thereby exposing the focus-forming/chromatin-binding domain. 53BP1 is then targeted to sites of DSB, possibly through protein-protein interactions between the Tudor domain and proteins at the site of DSBs (e.g. Ku70/80 or γ-H2AX, a phosphorylated form of histone H2AX), allowing 53BP1 to bind to chromatin at DSBs. Consistent with this model, γ-H2AX has been shown to interact physically with 53BP1 in irradiated cells (15), and mouse embryonic fibroblasts from H2AX(--/--) mice show impaired recruitment of 53BP1 to IR-induced foci (37). Of course, these two mechanisms are not mutually exclusive.

DiTullio et al. (24) proposed that 53BP1 function as a scaffold by recruiting ATM substrates at sites of DNA DSBs. This model...
is consistent with the current understanding of *S. cerevisiae* Rad9 function. Chromatin binding of 53BP1 at sites of DSBs may be important not only for signaling checkpoint pathway by recruiting ATM substrates but also for binding to DNA by 53BP1 itself and for recruiting proteins that are involved in the repair of DSBs. As 53BP1 significantly stimulates the ligase activity of DNA ligase IV/Xrcc4 complex, this complex is one of the candidates that are recruited to sites of DSBs by 53BP1.

It has been reported for many proteins involved in the repair of DNA DSBs interact directly with DNA. ATM has been reported to bind only linearized DNA in *vitro* (38) and the Ku70/Ku80 heterodimer stimulates end-joining by binding to DNA ends (39, 40). The Mre11/Nbs1/Rad50 complex has been shown to tether the ends of two linear dsDNAs (41). In contrast, the 53BP1 fragment bound both linearized, circular plasmids and ss DNA in *vitro*. 53BP1 may bind DNA close to, but not at, the ends of DSBs. Although KBD-M did not show the focus-formation activity, this domain might function in tethering the two ends of DSBs after chromatin binding of 53BP1. This was supported by the observation for KBD-M containing polyepitide (KBD-N) to stimulate ligation by both T4 ligase and DNA ligase IV. In contrast, the Tudor plus Myb domain (KBD-C) specifically stimulated the ligase activity of DNA ligase IV/ Xrcc4 complex, suggesting that 53BP1 may be one of the components of the NHEJ machinery. The Ku70/Ku80 heterodimer, the DNA end binding components in NHEJ pathway, stimulates ligation in *vitro* by not only ligase IV but also ligase I, III (40) and T4 ligase (42), through Ku protein’s end-bridging activity. However, the Ku complex binds specifically to ligase IV but not to ligase I and III (42), and it is this recruitment that leads to the specificity of the Ku complex for ligase IV end-joining. 53BP1, like Ku, may have a dual mode of action. Firstly, the DNA binding activity of KBD-M may bridge DNA ends, and secondly, the KBD-C domain may bind to DNA in the vicinity of the break and recruit the ligase IV/Xrcc4 complex to the DSB thus specifically stimulating end-joining.

53BP1 has been shown to be hyperphosphorylated in mitotic cells and to undergo an even higher level of phosphorylation in response to spindle disruption with colcemid (28). Furthermore, 53BP1 has been shown to be bound to the fibrous corona of the kinetochore and to colocalize with CENP-E, one of the components that directly tether spindle microtubules to the kinetochore (28). These data suggest that 53BP1 plays a role in checkpoint signaling during mitosis. The kinetochore-binding domain has been mapped to a portion of murine 53BP1 from residues 1220–1601. Although the corresponding region of *human* 53BP1 (KBD) contains the domain for 53BP1 DNA binding, it is unlikely that the binding of 53BP1 to the kinetochore is due to the DNA binding activity of 53BP1, since the region of the kinetochore to which 53BP1 localizes does not contain detectable DNA (28, 43). The roles of 53BP1 in mitosis remain to be resolved.

In conclusion, our data demonstrate that 53BP1 has the potential to participate in the NHEJ repair of DNA DSBs, probably through a direct interaction with damaged DNA. The precise mechanism by which 53BP1 functions in the repair machinery remains to be resolved. However, recently it has been reported that mice with truncated or disrupted 53BP1 gene show abnormality in both T and B cell populations, and cells from these mice exhibit chromosomal abnormalities (35, 44), supporting the conclusion of this report. Since DNA repair mechanisms are important to prevent oncogenesis, it will be interesting to examine whether 53BP1 is mutated in tumor cells.
