DNA Damage-induced Association of ATM with Its Target Proteins Requires a Protein Interaction Domain in the N Terminus of ATM*

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Received for publication, October 25, 2004, and in revised form, December 22, 2004 Published, JBC Papers in Press, February 15, 2005, DOI 10.1074/jbc.M412065200

The ATM protein kinase regulates the response of the cell to DNA damage by associating with and then phosphorylating proteins involved in cell cycle checkpoints and DNA repair. Here, we report on deletion studies designed to identify protein domains required for ATM to phosphorylate target proteins and to control cell survival following exposure to ionizing radiation. Deletion studies demonstrated that amino acids 1–150 of ATM were required for the ATM protein to regulate cellular radiosensitivity. Additional deletions and point mutations indicated that this domain extended from amino acids 81–106 of ATM, with amino acid substitutions located between amino acids 91 and 97 inactivating the functional activity of ATM. When ATM with mutations in this region (termed ATM90) was expressed in AT cells, it was unable to restore normal radiosensitivity to the cells. However, ATM90 retained normal kinase activity and was autophosphorylated on serine 1981 following exposure to DNA damage. Furthermore, wild-type ATM displayed DNA-damage induced association with p53, brca1, and LKB1 in vivo, whereas ATM90 failed to form productive complexes with these target proteins either in vivo or in vitro. Furthermore, ATM90 did not phosphorylate p53 in vivo and did not form nuclear foci in response to ionizing radiation. We propose that amino acids 91–97 of ATM contain a protein interaction domain required for the DNA-damage-induced association between ATM and its target proteins, including the brca1, p53, and LKB1 proteins. Furthermore, this domain of ATM is required for ATM to form nuclear foci following exposure to ionizing radiation.

Mammalian cells respond to DNA damage by activating signal transduction pathways that arrest cell cycle progression and initiate DNA repair (1). A key regulator of the cells response to DNA strand breaks is the ATM protein. Mutations in the ATM gene give rise to the inherited disease AT. AT is characterized by immunodeficiency, cerebellar ataxia, increased incidence of cancer, and extreme sensitivity to ionizing radiation (2, 3). Cells derived from AT patients exhibit radioreistant DNA synthesis, loss of cell cycle checkpoints, radiosensitivity, and defective DNA repair (2, 4, 5). The ATM protein is therefore an essential regulator of the cells ability to respond to genotoxic damage.

The ATM protein is a 3056-amino acid nuclear protein containing a C-terminal kinase domain (6). All of the functions of ATM are dependent on its kinase activity (7), indicating that the ATM-dependent DNA damage response is mediated through phosphorylation. The activated ATM protein phosphorylates multiple proteins involved in the DNA damage response, including nbs1, (8, 9), brca1 (10), the p53 tumor suppressor gene (11, 12), the checkpoint kinase chk2 (13–15), SMC1 (16, 17) BLM (18), FANCD2 (19) and Pin2/Trrf1 (20). These phosphorylated proteins, in turn, regulate the 2 key responses to DNA damage, the activation of cell cycle checkpoints and the initiation of DNA repair.

The activation of ATM in response to DNA damage occurs through the conversion of inactive ATM dimers to active ATM monomers (21). The activation of ATM may be triggered by changes in chromatin structure brought about by DNA strand breaks, leading to the autophosphorylation of serine 1981 within the FAT domain of ATM (21). This autophosphorylation of ATM causes a conformation change in the inactive ATM dimer, leading to the dissociation of the active, monomeric ATM. Several studies have demonstrated that the MRN complex also plays an essential role in ATM activity. The MRN complex contains the mre11, rad50, and nbs1 proteins and is a DNA binding complex involved in the detection and repair of DNA damage (22). Nbs1 and mre11 are required for ATM to efficiently phosphorylate several ATM target proteins, including chk2 (23), SMC1 (16, 17), and others. MRN is thought to function as an adaptor protein, bringing activated ATM and certain ATM substrates together to allow productive phosphorylation (24–27). Furthermore, some of these studies demonstrate that activation of the kinase activity of ATM in cells lacking functional MRN complex is compromised (25–27), leading to suggestions that the MRN complex is an upstream activator of ATM. However, other studies demonstrated that activation of the kinase activity of ATM proceeds normally in MRN defective cells (24). The complexity of the interactions between the MRN complex and ATM implies that MRN may participate in both ATM activation as well as facilitating the efficient phosphorylation of many target proteins by ATM. Furthermore, the nbs1 component of the MRN complex is directly phosphorylated by ATM (8, 28), adding an additional level of regulation to this system. These results indicate that the ability of ATM to form productive complexes with adaptor proteins, such as nbs1, and with key target proteins, such as p53 or chk2, is critical for correct ATM function. However, the protein do-
mains within ATM, which mediate these interactions, are not known.

To explore the mechanism of ATM activation, we have examined the relationship between the protein structure and function of ATM. Previously, we demonstrated that the N terminus of ATM was essential for correct ATM function (7). Using a combination of deletion analysis and mutagenesis we have now localized this domain to amino acids 91–97 of ATM. When ATM with point mutations in this region (termed ATM90) was expressed in AT cells, it was unable to restore normal radiosensitivity to the cells. However, the kinase activity of ATM90 was still activated by DNA damage, and ATM90 was still able to undergo autophosphorylation on serine 819. Furthermore, whereas wild-type ATM displayed DNA-damage-induced association with p53, brca1, and LKB1 in vivo, ATM90 failed to form productive complexes with these target proteins. We propose that the N terminus of ATM, centered on amino acids 91–97, contains an essential protein interaction domain for binding to ATM target proteins, including the brca1, p53, and LKB1 proteins.

**EXPERIMENTAL PROCEDURES**

**Cells and Transfection—**GM367 (normal fibroblast cells), GM5849 (AT fibroblast cells), and GM02814 (normal lymphoblast cells) were obtained from the Coriel Institute, Camden, NJ. Cells were cultured in minimal Eagle’s medium with 10% fetal bovine serum. Transfection of cells and measurement of clonogenic cell survival were as described previously by us (7, 30).

**Western Blot and Kinase Assays—**Preparation of cell extracts, immunoprecipitation, and immunokininase were carried out as described previously by us (7, 30). For co-immunoprecipitation experiments, the high salt wash was omitted. The following ATM antibodies were used: PC116 (Oncogene Science), 5C2 (Genetex) or anti-ATMpS1981 (Rockland Immunochemicals). Antibodies to p53 and brca1 were from Oncogene Science. (Oncogene Science), followed by IgG-Texas Red (Santa Cruz Biotechnology, Santa Cruz, CA). Slides were mounted with Fluoromount-G (Southern Biotech) and visualized with a Nikon Eclipse TE 2000.

**Plasmid Construction—**Deletions were constructed by PCR using plasmid pCC1, which contains amino acids 2–767 of ATM ligated into the KpnI/EcoRI sites of pcDNA3.1/HisA (Invitrogen). Internal ATM deletions are generated by PCR. For mutagenesis, primers contained at least 2 mismatched bases at their 5’-ends to generate four point mutations per reaction. pCC1 was amplified by PCR (1 min at 94 °C, 2 min at 58 °C, 8 min at 72 °C) for 9 cycles using Pfu polymerase. The PCR products were digested with dpm1 to remove methylated pCC1, self-ligated, transformed into competent E. coli, and positive clones sequenced. Deletions or mutations were transferred to ATM by digesting pCC1 with SacI, which cuts pcDNA/HisA (position 675). The SacI fragment containing the ATM by digesting pCC1 with SacI, which cuts pcDNA/HisA (position 60–124) and ATM (position 675). The fraction of cells surviving a 4-Gy dose (SF4Gy) is shown. However, the results were essentially identical at all doses used. In Fig. 1C, deletion of amino acids 1–70 did not significantly affect the ability of ATM to restore normal sensitivity to AT cells, whereas deletion of amino acids 60–124 or 114–177 blocked ATM function. Further deletions demonstrated that deletion of amino acids 60–81 or 82–109 abolished ATM activity, whereas deletion of amino acids 131–150 had minimal impact on ATM function (Fig. 1C). Deletion of amino acids 110–130 gave an intermediate level of ATM activity. Since ATM1–70 retains normal activity, but ATM Δ60–81 is inactive, the start of this domain must be located at amino acid 71 or higher. ATM Δ82–109 is also inactive, whereas ATM Δ110–130 retains significant functional activity. We interpret this to mean that the end of this domain is located close to amino acid 110 but may extend upstream from amino acid 110. Fig. 1 demonstrates that ATM contains an essential domain, including amino acids 71–110, which is required for ATM function.

To identify the key amino acids that form this essential N-terminal domain of ATM, a series of point mutations were introduced into amino acids 70 and 110. Amino acids 70–110 do not contain any protein motifs with homology to previously characterized domains nor do they contain sequences that could be predicted to have functional relevance. Furthermore, no point mutations have been reported in this region of the ATM protein in either AT patients or human malignancies. Since the domain covers at least 40 amino acids, we reasoned that this region of ATM may be a site for protein docking and may extend over tens of amino-acids. If this was the case, single amino acid substitutions may not significantly impact ATM function. Accordingly, a “randomized” semiconservative mutagenesis approach was used. Four different ATM proteins, each containing four closely linked point mutations, were used to analyze the region between amino acids 70 and 110 (Fig. 2A). By clustering point mutations in a small area, we aimed to define the boundaries of the domain. The serine residues at positions 83 and 85, and the glutamine at position 87, were targeted due to their similarity to the ATM consensus phosphorylation site (TQ). Additional mutations were designed to

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2. B. D. Price, unpublished observation.
remove charge (e.g. lysines 79, 92, 102, and 106 were mutated to glutamine or aspartate). The overall aim was to inactivate the domain through multiple mutations.

Mutations (Fig. 2A) were introduced between amino acids 73–79 (ATM70), amino acids 81–87 (ATM80), amino acids 91–97 (ATM90), and amino acids 100–106 (ATM100). Stable cell lines expressing each construct were derived and examined for ATM expression. Each of the ATM constructs was expressed at similar levels to the wild-type ATM protein, although ATM70 protein levels were consistently lower than wild-type ATM levels (Fig. 2B). Next, we examined the ability of each construct to complement the increased radiosensitivity of AT cells when compared with the wild-type ATM protein. Cells were exposed to 2 or 4 Gy and the surviving fraction measured (Fig. 2C). At 2 Gy, wild-type ATM increased cell survival, and ATM70, ATM80, and ATM100 retained significant functional activity. However, ATM90 was completely inactive. At a higher dose of 4 Gy, ATM80 retained significant activity, but ATM70, ATM90, and ATM100 were all essentially inactive. Several conclusions can be made from these observations. First, since ATM90 was inactive at all doses examined, this implies that the essential domain is centered on amino acids 91–97. Furthermore, since ATM80 retained significant activity at both 2- and 4-Gy doses, the N terminus of this domain is probably located within the ATM80 domain. The C-terminal limit of the domain must extend into ATM100, since this construct retained limited activity at low doses but was inactive at higher doses. The results for ATM70 are more difficult to interpret since ATM70 was expressed at reduced levels compared with wild-type ATM (Fig. 2B). Although ATM70 retained significant activity at 2 Gy, it was inactive at 4 Gy. The inability of ATM70 to alter cellular radiosensitivity may therefore stem from reduced expression levels rather than mutational effects on its function. Overall, we interpret Fig. 2C to demonstrate that ATM contains an essential functional domain, centered on amino acids 91–97, but extending over amino acids 81–106, which is required for cells to survive DNA damage.

Amino acid substitutions within the ATM protein may have either localized effects on the targeted sequence or may have long range effects on overall protein function due to changes in conformation. In AT patients, many point mutations alter both the stability and the kinase activity of the ATM protein. To eliminate this possibility, we next explored whether the mutations altered the activation of the kinase activity of ATM. The activation of ATM involves the autophosphorylation of serine 1981 of ATM, leading to dimer-monomer transition and phosphorylation of ATM target proteins (21). To determine whether the ATM constructs were correctly activated in response to DNA damage, cells were exposed to the radiomimetic agent bleomycin, and the in vivo kinase activity of ATM as well as ATM autophosphorylation determined. In Fig. 3A, GM637 cells, expressing endogenous ATM, serve as a control for the kinase assay. GM637 cells immunoprecipitated with IgG or incubated without the ATM substrate displayed minimal phosphorylation. In contrast, GM637 cells immunoprecipitated with ATM antibody yielded high levels of kinase activity when incubated with ATM substrate (Fig. 3A). GM637 cells transfected with vector had minimal kinase activity, whereas wild-type ATM and ATM70 and ATM80 and ATM90 had equivalent levels of kinase activity. Interestingly, ATM100 had slightly reduced kinase activity. In Fig. 3B, the autophosphorylation of serine 1981 of ATM was examined. GM5849 ATM cells, which do not express ATM, did not display any DNA-damage inducible autophosphorylation of serine 1981. GM5849 ATM cells expressing ATM showed strong phosphorylation of the transfected ATM protein in response to bleomycin, which was similar to that seen in HeLa cells. ATM70 showed increased autophosphorylation following exposure to bleomycin; however, the level of activation was reduced compared with wild-type ATM. This may reflect the reduced levels of expression of the ATM70 protein. In contrast, the autophosphorylation of ATM80, ATM90, and ATM100 following exposure to bleomycin was similar to wild-type ATM and to endogenous ATM in HeLa cells. Thus the mutations in ATM do not impair the activation...
ATM's N-terminal catalytic domain is required for the interaction of ATM with the p53 tumor suppressor protein. In vitro studies using purified protein components have shown that the N terminus of ATM interacts with several substrates of ATM, including p53 and BLM (18, 33). However, the *in vivo* relevance of these observations has not been examined. In Fig. 4, the ability of two ATM substrates, p53 and LKB1, to interact with the N terminus of ATM was analyzed. The p53 protein and the LKB1 protein kinase are both phosphorylated by ATM in response to DNA damage (11, 34), and p53 can interact with ATM *in vivo* (33). ATM was *in vitro* translated as four distinct protein fragments, covering amino acids 1–806 (N-terminal) and 769–1436, 1437–2138, and 2139–3056 of ATM were immobilized to glutathione-Sepharose columns. [35S]Methionine labeled *in vitro* translated proteins were allowed to interact with the immobilized proteins for 60 min. Following extensive washing, bound [35S]-labeled protein was eluted in SDS-sample buffer, separated by SDS-PAGE, and visualized by autoradiography.

ATM regulates the cells response to DNA damage. For example, ATM90 does not complement the increased radiosensitivity of AT cells, indicating that activation of the kinase activity of ATM is insufficient to regulate the cells response to DNA damage.

**Fig. 2.** Amino-acids 90–110 contain an essential domain. A, N-terminal mutations. Sequence of wild-type and mutated ATM constructs. B, GM5849 AT cells stably expressing vector (Vec), ATM, ATM70, ATM80, ATM90, or ATM100 constructs were immunoprecipitated with IgG (lane 1) or ATM antibody PC116 (lanes 2–7) and ATM levels identified by Western blotting. C, GM5849 cells expressing vector, full-length ATM, or the indicated mutation were irradiated at 2 or 4 Gy and the surviving fraction (SF) measured. Results + S.E. (n = 6).

**Fig. 3.** Kinase activity of ATM. A, immunokinase activity of ATM. GM637 or GM5849 cells expressing the indicated construct were immunoprecipitated with IgG (Ab: −) or ATM antibody PC116 (Ab: +). Washed immunoprecipitates were incubated with GST protein (cds1: −) or GST-cds11–89 (cds1: +) and [γ-32P]ATP in kinase buffer. Phosphorylated proteins were separated by SDS-PAGE and identified by autoradiography. B, autophosphorylation of ATM. Cells were exposed to solvent (−) or bleomycin (5 μM: +) for 60 min. ATM was immunoprecipitated and ATM phosphorylated on serine 1981 identified with antiphosphoserine 1981 antibody.

**Fig. 4.** Binding of ATM to p53-GST. GST, p53-GST, or LKB1-GST were immobilized to glutathione-Sepharose columns. [35S]Methionine labeled *in vitro* translated proteins were allowed to interact with the immobilized proteins for 60 min. Following extensive washing, bound [35S]-labeled protein was eluted in SDS-sample buffer, separated by SDS-PAGE, and visualized by autoradiography. IT = 0.1× of input *in vitro* translate. A, amino acids 1–806, 769–1436, 1437–2138, or 2139–3056 of ATM were *in vitro* translated and allowed to interact with GST, p53-GST, or LKB1-GST. B, amino acids 1–806 of ATM or amino acids 1–806 with internal deletions of amino acids 60–81, 82–109, 110–130, or 131–150 were *in vitro* translated and allowed to interact with either GST or p53-GST. C, amino acids 1–806 of ATM, or amino acids 1–806 with the mutations described in the legend to Fig. 2A were *in vitro* translated and allowed to interact with either GST or p53-GST.
activity (Fig. 4C) and retained significant functional activity in the cell survival assay (Fig. 2C). Although ATM70 retained the ability to interact with p53 in vitro, the altered mobility of this protein (Fig. 4C), and the lower levels of expression (Fig. 2B), indicate that its functional activity may be compromised. Overall, we interpret Figs. 1–4 to indicate that there is a p53 binding site centered on amino acids 91–97 that is essential for ATM function.

Next, we determined whether amino acids 91–97 of ATM were required for the interaction between ATM and p53 in vivo. Furthermore, we extended this analysis to determine whether ATM90 was also defective in the ability to form productive complexes with other ATM target proteins in addition to p53. Accordingly, we examined the interaction of ATM with three ATM substrates: p53 (33), LKB1 (34), and brca1 (35). brca1 forms nuclear foci in response to DNA damage (36), whereas p53 and LKB1 remain mobile in the nucleoplasm (24). AT cells expressing either vector, ATM or ATM90, were untreated or exposed to bleomycin. In untreated cells, no interaction between ATM and either p53, LKB1, or brca1 was detected (Fig. 5). In contrast, exposure to bleomyccin stimulated the binding of p53, LKB1, and brca1 to ATM. No inducible interactions were detected in cells that did not express ATM. When ATM90 was immunoprecipitated, bleomyccin did not stimulate the formation of complexes between ATM90 and either p53, LKB1, or brca1. These experiments demonstrate that the association between ATM and three of its known in vivo targets is stimulated upon DNA damage and that this interaction requires the protein interaction domain, located between amino acids 91 and 97 of ATM.

The activated form of ATM is autophosphorylated on serine 1981 (21) and can directly phosphorylate serine 15 of the p53 protein (11, 12). The previous results predict that the inability of ATM90 to associate with p53 should prevent the ATM-dependent phosphorylation of ATM on serine 15. In Fig. 6, the phosphorylation of p53 by ATM was examined by immunofluorescent staining. GM5849 cells expressing ATM or ATM90 showed high levels of nuclear ATM expression, whereas the vector-transformed cells had no detectable ATM signal. ATM and ATM90 are therefore expressed in the nucleus, excluding the possibility that mutations in the ATM90 protein affect the cellular location of ATM90. When cells were irradiated, p53 was phosphorylated on serine 15 in ATM-positive cells, whereas no p53 phosphorylation was detected in AT cells or ATM90 cells (Fig. 6). Similar results were obtained when cell extracts were examined by Western analysis for phosphorylation of serine 15 of p53. These results are consistent with the inability of ATM90 to associate productively with p53 in vitro.

Previous studies have also demonstrated that ATM phosphorylated on serine 1981 forms specific nuclear foci following exposure to ionizing radiation (21). Furthermore, the formation of these foci requires the presence of functional brca1 protein.
lish that the ATM protein contains an essential protein interaction domain centered around amino acids 91–97 of ATM.

Amino acids 81–106 of ATM has no significant homology to previously characterized domains, nor is there a similar conserved sequence in related proteins, including Atr and DNA-PK (6). This is somewhat surprising as DNA-PK, Atr, and ATM phosphorylate a common set of proteins, including p53; however, they do not share a common protein interaction motif, implying that the protein interaction domain of ATM is unique to the ATM protein. Furthermore, if the ATM protein interaction domain is utilized for binding multiple target proteins, the small size of the domain (26 amino acids) suggests that it may be occupied by only 1 protein at any given moment. This, in turn, indicates that the cell may contain many different ATM protein complexes at a given time (1).

ATM target proteins can be divided into two broad groups based upon their location within the nucleus. The first group, including brca1 (10), nbs1 (9, 37), and SMC1 (17, 24), is recruited to sites of DNA damage within the chromatin, where it forms distinct ionizing radiation-induced foci (24). The second group, including p53 and LKB1 (34), is present in the nucleoplasm and remains mobile without being incorporated into nuclear foci. Following activation, a fraction of the activated ATM protein redistributes into nuclear foci, and this correlates with the phosphorylation of nbs1, brca1, and SMC1 (21, 24). In cells in which either nbs1 or brca1 is inactivated, ATM-dependent phosphorylation of SMC1, brca1, and nbs1 is greatly reduced (24, 25, 27), and ATM does not redistribute into nuclear foci. However, the phosphorylation of the p53 protein by activated ATM is independent of brca1 or nbs1, presumably because p53 is not localized to nuclear foci but remains mobile in the nucleoplasm (24, 25). In our experiments, we tested ATM target proteins from both groups for the ability to associate with the protein interaction domain. All three proteins (brca1, p53, and LKB1) failed to interact with ATM90, implying that the protein interaction domain is required for ATM to associate with both nucleoplasmic proteins (p53 and LKB1) as well as proteins within the nuclear foci (brca1). In addition, ATM90, despite being activated by ionizing radiation, did not redistribute into discrete nuclear foci but rather exhibited a diffuse nuclear staining pattern following ionizing radiation. This diffuse nuclear staining of activated ATM is similar to that seen in both nbs1 and brca1 negative cells (24) and in cells exposed to nonspecific modulators of chromatin structure (21). Thus we conclude that the association of ATM with its target proteins in vivo requires the protein interaction domain and that this domain targets activated ATM to nuclear foci, possibly through its ability to associate with the brca1 protein. Furthermore, since the phosphorylation of brca1, nbs1, and other ATM substrates requires the formation of ATM foci, the phosphorylation of these proteins will also be defective in ATM90 cells. The failure of ATM90 to correct the increased radiosensitivity of AT cells is therefore due to the inability of ATM90 to associate with and then phosphorylate its target proteins.

We also examined whether the nbs1 protein associates with the protein interaction domain of ATM, since DNA damage-inducible binding of nbs1 to ATM has been reported (9). However, we were unable to detect any stable association between ATM and nbs1 or nbs1 and the protein interaction domain either in vivo or in vitro.2 It is possible that the association of nbs1 with ATM is transient or that nbs1 is too tightly associated with DNA to allow isolation of ATM-nbs1 complexes in our system. However, we note that an additional study demonstrated that, in vitro, nbs1 does not associate with the N terminus of ATM (28). Thus we are unable to address whether nbs1 binds to the protein interaction domain or some other region of ATM.

The presence of a protein interaction domain at the extreme N terminus of ATM has several predicted consequences for ATM function. ATM activation occurs through the autophosphorylation of serine 1981 of ATM in response to DNA damage. This autophosphorylation of ATM converts the inactive dimeric ATM complex into an active monomer (21). The binding of p53, brca1, and LKB1 to ATM was only detected after activation of ATM by DNA damage. The protein interaction domain is therefore inaccessible to p53 and other substrates until ATM is activated. The dimer-monomer transition of ATM may therefore function to uncover the substrate binding domain and allow formation of ATM-substrate complexes. Furthermore, when p53 binds to the N terminus, ATM monomers must be folded in a complex manner to bring the C-terminal kinase domain and N-terminal protein interaction domain together to allow phosphorylation to proceed. Alternatively, ATM protein complexes may form in a head-to-tail manner, in which the N terminus of 1 ATM monomer associates with the C-terminal kinase domain of an adjacent ATM monomer, using two p53 molecules to bridge the two ATM monomers. In support of this, we note that the C-terminal kinase domain of ATM also binds to p53 and LKB1 in vitro (Fig. 4). These two models may not be mutually exclusive, and the cells may contain discrete ATM complexes, some located within tightly bound chromatin structures, while others may be more mobile. These differences may reflect the different locations of ATMs target proteins within the nucleus of the cell.

In conclusion, we have identified an essential protein interaction domain located between amino acids 91 and 97 of the ATM protein. This domain is required for ATM to associate with several target proteins, including p53, LKB1, and brca1. Furthermore, this domain of ATM is required for the formation of nuclear foci following exposure of cells to ionizing radiation.

REFERENCES
1. Bakkenist, C. J., and Kastan, M. B. (2004) Cell 118, 9–17
2. Meyn, M. S. (1999) Clin. Genet. 55, 289–304
3. Luo, C. M., Tang, W., Mekhel, K. L., DeFranco, J. S., Anne, P. R., and Powell, S. N. (1996) J. Biol. Chem. 271, 4497–4503
4. Bradbury, J. M., and Jackson, S. P. (2003) Curr. Biol. 13, R468
5. Shiloh, Y. (2003) Nat. Rev. Cancer 3, 155–168
6. Sirotkina, K., Slez, S., Tagliabracci, V., Y., N., Sartiel, A., Collins, P., Shiloh, Y., and Rotman, G. (1995) Hum. Mol. Genet. 4, 2025–2032
7. Turetta, G. A., Paup, P., Laflair, L., and Price, B. D. (2001) Oncogene 20, 5100–5110
8. Lim, D. S., Kim, S. T., Xu, B., Maser, R. S., Lin, J., Petrini, J. H., and Kastan, M. B. (2000) Nature 404, 613–617
9. Zhao, S., Weng, Y. C., Yuan, S. S., Lin, Y. T., Hsu, H. C., Lin, S. C., Gerbino, K., Song, M. H., Zdkien, A., Z., Gotti, R. A., Shay, J. W., Ziv, Y., Shiloh, Y., and Lee, Y. E. (2000) Nature 405, 473–477
10. Cortez, D., Wang, Y., Qin, J., and Elledge, S. J. (1999) Science 286, 1162–1166
11. Camann, C. E., Lim, D. S., Cimprich, K. A., Taya, Y., Tamai, K., Sakaguchi, K., Appella, E., Kastan, M. B., and Siliciano, J. D. (1998) Science 281, 1677–1679
12. Banin, S., Moyal, L., Shieh, S., Taya, Y., Anderson, C. W., Chessa, L., Smorodinsky, N. I., Priev, C., Reiss, Y., Shiloh, Y., and Zvy, Y. (1998) Science 281, 1674–1677
13. Blazina, A., de Weyer, I. V., Laus, M. C., Luyten, W. H., Parker, A. E., and Mccowan, C. H. (1999) Curr. Biol. 9, 1–10
14. Matsuo, S., Rotman, G., Ogawa, A., Shiloh, Y., Taya, K., and Elledge, S. J. (2000) Proc. Natl. Acad. Sci. U. S. A. 97, 10389–10394
15. Blazina, A., Price, B. D., Turetta, G. A., and Mcgowan, C. H. (1999) Curr. Biol. 9, 1135–1138
16. Yazdi, P. T., Wang, Y., Zhao, S., Patel, N., Lee, E. Y., and Qin, J. (2002) Genes Dev. 16, 571–582
17. Kim, S. T., Xu, B., and Kastan, M. B. (2002) Genes Dev. 16, 560–570
18. Beamish, H., Kedar, P., Kaneko, H., Chen, P., Fukao, T., Peng, C., Beresten, S., Gueven, N., Purdie, D., Ellis, N., Kondo, N., and Lavin, M. F. (2002) J. Biol. Chem. 277, 30515–30523
19. Taniguchi, T., Garcia-Higuera, I., Xu, B., Andreassen, P. R., Gregory, R. C., Kim, S. T., Lane, W. S., Kastan, M. B., and D'Andrea, A. D. (2003) Cell 109, 459–472
20. Kishi, S., Zhou, X. Z., Ziv, Y., Khoos, C., Hilt, D. E., Shiloh, Y., and Lu, K. P. (2001) J. Biol. Chem. 276, 29282–29291
21. Bakkenist, C. J., and Kastan, M. B. (2003) Nature 421, 499–506
22. Tauchi, H., Matsuura, S., Kobayashi, J., Sakamoto, S., and Komatsu, K. (2002) Oncogene 21, 8986–8980
23. Bussem, G., Savio, C., Zannini, L., Mucic, F., Masada, D., Nakanishi, M., Tauchi, H., Komatsu, K., Mizutani, S., Khanna, K., Chen, P., Concanan,
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24. Kitagawa, R., Bakkenist, C. J., McKinnon, P. J., and Kastan, M. B. (2004) *Genes Dev.* **18**, 1423–1438
25. Mochan, T. A., Venere, M., DiTullio, R. A., Jr., and Halazonetis, T. D. (2003) *Cancer Res.* **63**, 8586–8591
26. Lee, J. H., and Youmell, M. B. (1996) *Cancer Res.* **56**, 246–250
27. Chen, S., Paul, P., and Price, B. D. (2003) *Oncogene* **22**, 6332–6339
28. Turenne, G. A., and Price, B. D. (2001) *BMC Cell Biol.* **2**, 12
29. Price, B. D., and Youmell, M. B. (1996) *Cancer Res.* **56**, 246–250
30. Chen, S., Paul, P., and Price, B. D. (2003) *Oncogene* **22**, 6332–6339
31. Taylor, A. M., Harmend, D. G., Arlett, C. F., Harcourt, S. A., Lehmann, A. R., Stevens, S., and Bridges, B. A. (1975) *Nature* **258**, 427–429
32. Khanna, K. K., Keating, K. E., Kozlov, S., Scott, S., Gatei, M., Hobson, K., Taya, Y., Gabrielli, B., Chan, D., Lees-Miller, S. P., and Lavin, M. F. (1998) *Nat. Genet.* **20**, 398–400
33. Sapkota, G. P., Deak, M., Kieloch, A., Morrice, N., Goodarzi, A. A., Smythe, C., Shiloh, Y., Lees-Miller, S. P., and Alessi, D. R. (2002) *Biochem. J.* **368**, 507–516
34. Gatei, M., Scott, S. P., Filipovitch, I., Soroniko, N., Lavin, M. P., Weber, B., and Khanna, K. K. (2000) *Cancer Res.* **60**, 3299–3304
35. Zhong, Q., Chen, C. F., Li, S., Chen, Y., Wang, C. C., Xiao, J., Chen, P. L., Sharp, Z. D., and Lee, W. H. (1999) *Science* **285**, 747–750
36. Lukas, C., Falck, J., Bartkova, J., Bartek, J., and Lukas, J. (2003) *Nat. Cell Biol.* **5**, 255–260