Oncogene (2007) 26, 7741–7748

www.nature.com/onc

REVIEW

Activation and regulation of ATM kinase activity in response to DNA double-strand breaks

J-H Lee and TT Paull

Department of Molecular Genetics and Microbiology, Institute of Cellular and Molecular Biology, University of Texas at Austin, Austin, TX, USA

The ataxia–telangiectasia-mutated (ATM) protein kinase is rapidly and specifically activated in response to DNA double-strand breaks in eukaryotic cells. In this review, we summarize recent insights into the mechanism of ATM activation, focusing on the role of the Mre11/Rad50/Nbs1 (MRN) complex in this process. We also compare observations of the ATM activation process in different biological systems and highlight potential candidates for cellular factors that may participate in regulating ATM activity in human cells.
Oncogene (2007) **26,** 7741–7748; doi:10.1038/sj.onc.1210872

Keywords: ATM; MRN; DNA repair; checkpoints

Ataxia–telangiectasia mutated (ATM) is a serine–threonine kinase that is activated when cells are exposed to DNA double-strand breaks (DSBs) (Shiloh, 2006). ATM phosphorylates a number of proteins involved in cell cycle checkpoint control, apoptotic responses and DNA repair, including p53, Chk2, BRCA1, RPAp34, H2AX, SMC1, FANCD2, Rad17, Artemis and Nbs1. Phosphorylation of these and other substrates by ATM initiates cell-cycle arrest at G_1/S, intra-S and G_2/M checkpoints and also promotes DNA repair. Mutations in the ATM gene are responsible for the rare autosomal recessive disorder ataxia–telangiectasia (A–T), characterized by cerebellar degeneration, immunodeficiency and an increased risk of cancer. Cells from individuals with A–T exhibit defects in DNA damage-induced checkpoint activation, radiation hypersensitivity and an increased frequency of chromosome breakage (Shiloh, 1997; Petrini, 2000).

The role of the MRN complex in ATM activation

The Mre11/Rad50/Nbs1 (MRN) complex has been shown by several groups to be required for the ATM signaling pathway (Lim *et al.*, 2000; Buscemi *et al.*, 2001; Girard *et al.*, 2002; Nakanishi *et al.*, 2002; Yazdi *et al.*, 2002; Uziel *et al.*, 2003). Most of these studies were performed using cells derived from patients with Nijmegen Breakage Syndrome (NBS) or Ataxia–Telangiectasia-Like-Disorder (ATLD), which are caused by hypomorphic alleles of the Nbs1 or Mre11 genes, respectively (Carney *et al.*, 1998; Varon *et al.*, 1998; Stewart *et al.*, 1999). The MRN complex was first considered to be in the same pathway with ATM because both NBS and ATLD patients exhibit similar clinical and cellular phenotypes compared to A–T patients, including chromosomal instability, radiation sensitivity and defects in cell cycle checkpoints. Girard *et al.* (2002) showed that phosphorylation of p53 on Ser15 was impaired in NBS cells following exposure to low doses of ionizing radiation (IR), suggesting that Nbs1 may be required for ATM-dependent phosphorylation of substrates in cells. Uziel *et al.* (2003) also showed that ATM-dependent phosphorylation of p53, Chk2 and Mdm2 was defective in ATLD cells after treatment with neocarzinostatin (NCS), a radiomimetic agent. A recent study confirmed that Nbs1 is required for ATM autophosphorylation as well as the association of active ATM with chromatin after the induction of endonuclease-generated DSBs (Berkovich *et al.*, 2007). These findings suggest that a functional MRN complex is required for ATM activation after DNA damage.

The functional relationship between ATM and the MRN complex has also been demonstrated using virus-infected cells. By studying the ATM signaling pathway after infection with adenovirus, Weitzman and co-workers found that the MRN complex functions upstream of ATM in mammalian cells (Stracker *et al.*, 2002). They showed that the cellular DNA damage response through ATM and ATR is blocked by wild-type adenovirus, which relocalizes and degrades the MRN complex through the action of the viral proteins E1b55K/E4orf6 and E4orf3. As a result, ATM autophosphorylation and chromatin retention after treatment with NCS is abrogated (Carson *et al.*, 2003; Araujo *et al.*, 2005). Their findings show that the MRN complex acts upstream of ATM during adenovirus infection and that the combined actions of the MRN complex and ATM in DNA repair and checkpoint control block virus replication and infection. In support of this, Wu *et al.* (2004) also showed that SV40 large T antigen can interact with Nbs1 and that this interaction disrupts Nbs1-mediated suppression of SV40 replication. Recently, infection of cells with herpes simplex virus (HSV) was also shown to

Correspondence: Dr TT Paull, Department of Molecular Genetics and Microbiology, Institute of Cellular and Molecular Biology, University of Texas at Austin, 1 University Station, A4800, Austin, TX 78712, USA.
E-mail: tpaull@mail.utexas.edu

induce an ATM- and MRN-dependent DNA-damage response, including ATM autophosphorylation and phosphorylation of ATM target substrates. Phosphorylation of Nbs1, p53 and Chk2 were not observed in A–T cells and were delayed in NBS cells, suggesting that a functional MRN complex is required for efficient ATM activation (Shirata *et al*., 2005).

Significant insight into the mechanism of ATM activation was generated by Bakkenist and Kastan (2003), which demonstrated that ATM exists as an inactive dimer under normal conditions and converts into active monomers after DNA damage. They also demonstrated that autophosphorylation of ATM on Ser1981 accompanied ATM activation and was required for monomerization.

In agreement with numerous studies of the MRN complex and ATM in mammalian cells, we found that ATM interacts with the MRN complex directly *in vitro*, using purified recombinant proteins. The MRN complex also stimulates the kinase activity of monomeric ATM toward p53 and Chk2 by 12 to 15-fold. The mechanism of this stimulation appeared to be primarily through an increase in substrate recruitment by ATM (Lee and Paull, 2004). From these findings, we proposed that the MRN complex binds to ATM, inducing conformational changes that facilitate an increase in the affinity of ATM toward its substrates.

We subsequently purified dimeric ATM, which, in contrast to monomeric ATM was inactive even in the presence of the MRN complex. Purified dimeric ATM also required linear DNA as well as the MRN complex for activity and under these conditions ATM activity was stimulated 80 to 200-fold over ATM alone (Lee and Paull, 2005). These results suggest that the MRN complex acts as a sensor for DNA breaks and is required for DSB-induced ATM signaling. We also showed that the unwinding of DNA ends by the MRN complex is essential for the ATM stimulation. This was based on our analysis of the Rad50 S1202R mutant, which is deficient in ATP-dependent DNA unwinding (Moncalian *et al*., 2004) as well as in adenylate kinase activity (Bhaskara *et al*., 2007). In agreement with this finding, experiments using *Xenopus* egg extracts also showed that the wild-type MRN complex restores ATM kinase activity in Mre11-depleted Xenopus extracts but not the MR(S1202R)N-mutant complex (Costanzo *et al*., 2004).

Functions of Nbs1 in the MRN complex

The Nbs1 protein is essential in mammals (Zhu *et al*., 2001), as are Mre11 and Rad50 (Xiao and Weaver, 1997; Luo *et al*., 1999). But cells from NBS patients can be used to analyse the importance of Nbs1 for ATM signaling since these cells do not have wild-type Nbs1 and the MRN complex they have is present at a significantly lower level compared to normal cells. Most NBS patients carry a deletion mutation, 657del5 and contain low levels of a 70-kDa N-terminally truncated form of Nbs1 (p70) made by internal translation initiation upstream of the deletion site (Maser *et al*., 2001). Nbs1 (p70) forms a complex with Mre11 and Rad50 because it contains the Mre11 binding domain; however, this mutant does not contain the FHA or the BRCT protein–protein interaction domains present in the N-terminus of the wild-type protein. Although MRN (p70) is present at very low levels in NBS cells, this hypomorphic Nbs1 mutant has some residual functions of the MRN complex (Maser *et al*., 2001; Lee *et al*., 2003a; Lee and Paull, 2004). In a mouse model of the human NBS disorder, B cells expressing low levels of p70 exhibit partial defects in ATM autophosphorylation and phosphorylation of some downstream targets (including SMC1 and Chk2), although p53 phosphorylation is normal after IR exposure (Difilippantonio *et al*., 2005).

The Nbs1 protein plays many roles in the functions of the MRN complex, some of which are still being elucidated. It is required for nucleotide-dependent DNA binding by the complex and ATP-dependent DNA unwinding, suggesting that Nbs1 regulates MR catalytic functions (Lee *et al*., 2003a). In NBS cells, which contain very low levels of the p70 form of Nbs1, Mre11 and Rad50 were shown to be distributed in the cytoplasm, suggesting that Nbs1 is required for nuclear localization of these proteins (Carney *et al*., 1998; Difilippantonio *et al*., 2005). Mapping of the domains within the Nbs1 protein showed that a 101 amino-acid domain in the Nbs1 C-terminus interacts with a 319 amino-acid domain in the N-terminus of Mre11 (Desai-Mehta *et al*., 2001). Furthermore, the C-terminal half of Nbs1 is sufficient to localize in the nucleus with the MR complex, although these interactions were not sufficient to result in nuclear focus formation. These findings suggest that one of the primary functions of Nbs1 is to localize Mre11 and Rad50 in the nucleus. Cerosaletti *et al*. (2006) later showed that insertion of a nuclear localization signal on Mre11 resulted in nuclear localization of MR in the absence of Nbs1, but that the complex could not induce ATM autophosphorylation or phosphorylation of its downstream targets. We also found *in vitro* that the MR complex was unable to activate dimeric ATM (Lee and Paull, 2005), even though phosphorylation of p53 by monomeric ATM was stimulated by MR in the absence of Nbs1 (Lee and Paull, 2004). These results suggest that Nbs1 has a very specific role in activating dimeric ATM, in addition to recruiting MR to DSB sites.

Functions of the N-terminus of Nbs1

Nbs1 contains a forkhead-associated (FHA) domain (aa 24–108), a breast cancer C-terminal (BRCT) domain (aa 108–196) and a second putative BRCT domain (aa 221–330) in its N-terminus (Becker *et al*., 2006) (Figure 1). These domains have been shown to be essential for nuclear foci formation and also contribute to chromatin association and IR-induced responses of the MRN complex (Tauchi *et al*.,

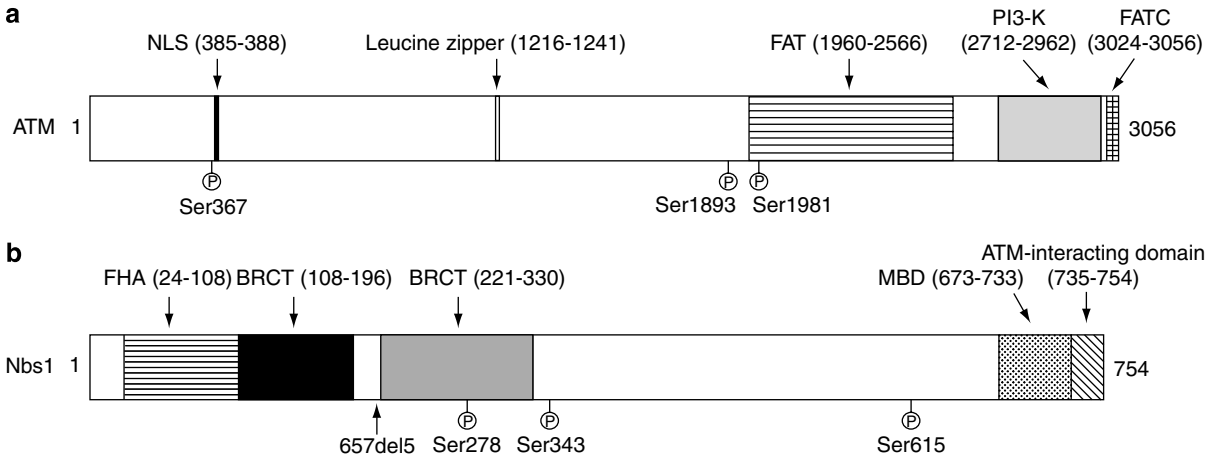

Figure 1 A schematic diagram of the ATM and Nbs1 proteins. (**a**) ATM is a 350 kDa protein, consisting of 3056 amino acids. The phosphorylation sites (P) indicate the positions of serine residues that are autophosphorylated (Bakkenist and Kastan, 2003; Kozlov *et al*., 2006). The identified domains within ATM consist of the nuclear localization signal (NLS) (Young *et al*., 2005), the leucine zipper (LZ) (Savitsky *et al*., 1995b), the FRAP/ATM/TRRAP (FAT) domain (Bosotti *et al*., 2000), the Kinase domain (PI3-K) (Savitsky *et al*., 1995a), and the FAT c-terminal (FATC) domain (Bosotti *et al*., 2000). (**b**) Nbs1 is a 90 kDa protein, consisting of 754 amino acids. The phosphorylation sites (P) show the serine residues that are phosphorylated by ATM (Gatei *et al*., 2000; Lim *et al*., 2000; Stewart *et al*., 2001; Kim *et al*., 2002; Lee *et al*., 2003b). The domains within Nbs1 include the forkhead-associated (FHA) domain (Featherstone and Jackson, 1998), the breast cancer C-terminal (BRCT) domains (Becker *et al*., 2006), the Mre11-binding domain (MBD) (Desai-Mehta *et al*., 2001) and the ATM-interacting domain (Falck *et al*., 2005).

2001; Kobayashi *et al*., 2002; Zhao *et al*., 2002; Cerosaletti and Concannon, 2003). Concannon and co-workers also showed that the expression of Nbs1 mutants containing point mutations in either the FHA or BRCT domains in NBS cells partially restored ATM kinase activity toward Chk2 and SMC1 after exposure to 10 Gy IR (Cerosaletti and Concannon, 2003), suggesting that the N-terminus of Nbs1 contributes to but is not essential for ATM activation. A recent study from Nussenzweig and co-workers also showed that B cells from mice expressing p70 Nbs1 or Nbs1 containing H45A, a missense mutation in a critical residue in the FHA domain, exhibit mild defects in Chk2 and Chk1 phosphorylation, G2/M and S-phase checkpoint arrest and radiation sensitivity after IR (Difilippantonio *et al*., 2007).

Using *in vitro* assays, we showed that the MRN(p70) complex, which lacks the N-terminus of Nbs1, exhibits normal MRN catalytic activities including DNA-binding and nuclease activity (Lee *et al*., 2003a). However, this complex was only partially effective in stimulating Chk2 phosphorylation by ATM (Lee and Paull, 2004). These results suggest that some characteristics exhibited by NBS cells may be due to the loss of both FHA and BRCT domains on Nbs1, in addition to the effects of the overall reduction in MRN complex levels.

Role of the C-terminus of Nbs1

The interaction domain of Nbs1 with ATM was initially suggested in *Saccharomyces cerevisiae* (Nakada *et al*., 2003). Nakada and co-workers found that the C-terminus of Xrs2 (the budding yeast homolog of Nbs1) interacts with Tel1 (the budding yeast homolog of ATM) by two-hybrid analysis and confirmed this by showing that a C-terminal 162 amino-acid deletion mutant of Xrs2 was not immunoprecipitated with Tel1. Studies with human MRN and ATM have shown that Nbs1 contains a phosphoinositide-3-kinase-related protein kinase (PIKK) interaction motif in its C-terminus that binds specifically to ATM (Falck *et al*., 2005). Nbs1 lacking this C-terminal 20 amino-acid domain (ΔC) did not interact with ATM and did not form nuclear foci with phosphorylated ATM on Ser1981 after IR. Consistent with this finding, Russell and co-workers also showed that the FXFG motif (part of the proposed PIKK interaction domain) in the C-terminus of Nbs1 interacts with a specific region of HEAT repeats on ATM in fission yeast (You *et al*., 2005). Russell also showed that the interaction between Mre11 and Nbs1 is required for ATM activation since the deletion of this domain abrogated ATM activation. Addition of a C-terminal fragment of Nbs1 that contains both Mre11- and ATM-binding domains could restore the ATM autophosphorylation in Nbs1-depleted *Xenopus* egg extracts, while a C-terminal Nbs1 fragment containing only the ATM-binding domain could not activate ATM (You *et al*., 2005). This also suggests that Nbs1 has to form a complex with MR in order to activate ATM, a result consistent with our finding with purified components showing that Nbs1 by itself is incapable of stimulating ATM *in vitro* (Lee and Paull, 2005).

Despite the importance of the Nbs1 PIKK interaction motif documented in human cells, *Xenopus* egg extracts and fission and budding yeast, mice expressing a C-terminal truncated Nbs1 mutant exhibit relatively subtle phenotypes (Difilippantonio *et al*., 2007; Stracker *et al*., 2007). In mouse B cells expressing C-terminal truncated Nbs1, ATM interaction with Nbs1 and colocalization of ATM with γ-H2AX was similar to normal cells (Difilippantonio *et al*., 2007). In mice expressing this mutant Nbs1, there was a defect in DNA damage-induced intra-S checkpoint activation in mouse

embryonic fibroblasts (MEFs) and a defect in apoptosis in thymocytes (Stracker *et al*., 2007). Disruption of the ATM binding domain in Nbs1 reduced ATM-dependent phosphorylation of Chk2 (Thr68), SMC1 (Ser966), Nbs1 (Ser343) and proapoptotic BID protein (Ser61 and Ser78) although p53 and H2AX phosphorylation was not influenced by this disruption (Falck *et al*., 2005; Difilippantonio *et al*., 2007; Stracker *et al*., 2007). It is not yet clear why mouse cells appear to tolerate the loss of the C-terminus of Nbs1 while human cells show a much stronger dependence on this domain.

Although several studies have shown that ATM interacts with the C-terminus of Nbs1, we have found that ATM can associate with the MR complex even in the absence of Nbs1 (Lee and Paull, 2004). This suggests that ATM makes multiple direct contacts with the MRN complex—one through Nbs1 and the other through MR. The C-terminal 20 amino acids of Nbs1 must therefore have an additional role in ATM activation in addition to recruiting ATM. This idea is consistent with the findings of Gautier and co-workers who have proposed a two-step mechanism for the activation of ATM (Dupre *et al*., 2006). Their findings using *Xenopus* egg extracts suggest that MRN-dependent DNA tethering, in conjunction with ATM recruitment, first induces ATM monomerization through an increase in the local concentration of DNA. In their experiments, this first role of the MRN complex could be replaced by the addition of large amounts of linear DNA. In a second step, they show that monomeric ATM can be further activated by interacting with Nbs1. These results also suggest that direct interaction of Nbs1 is connected to ATM kinase activity. The mechanism of ATM activation through interaction with the C-terminus of Nbs1 is still unclear, but it is possible that the binding of this small domain to ATM causes conformational changes in ATM that facilitate activation.

Recent studies of ATM and Rad3-related kinase (ATR) activation by TopBP1 and Rad9 may also be informative since ATR is a related PIKK. ATR phosphorylation of Chk1 after hydroxyurea (HU) treatment was found to be stimulated by the interaction of ATR with the small active domain (AD) of TopBP1, which is recruited to chromatin through Rad9 phosphorylation (Delacroix *et al*., 2007; Lee *et al*., 2007). They also showed that fusion of the TopBP1 AD with other chromatin-binding proteins including PCNA and H2B induces Chk1 phosphorylation after HU treatment. The PIKK-interaction domain on Nbs1 was originally shown by Jackson and co-workers to be analogous to a similar domain on ATR–interacting protein (ATRIP) that is important for the association with ATR (Falck *et al*., 2005). ATRIP does not seem to function in the same manner as Nbs1, however, because ATRIP is constitutively bound to ATR and seems to primarily function in the recruitment of ATR to RPA-bound DNA (Cortez *et al*., 2001).

Phosphorylation of Nbs1

Nbs1 is phosphorylated in an ATM-dependent manner after DNA damage on residues Ser278, Ser343 and Ser615 (Gatei *et al*., 2000; Lim *et al*., 2000; Stewart *et al*., 2001; Kim *et al*., 2002; Lee *et al*., 2003b). Several studies showed that the expression of S343A mutant Nbs1 in NBS cells resulted in partial defects in IR-induced S-phase checkpoint activation, radiosensitivity and partial defects in ATM-dependent phosphorylation events including Chk2, SMC1 and FANCD2 (Lim *et al*., 2000; Buscemi *et al*., 2001; Nakanishi *et al*., 2002; Yazdi *et al*., 2002). Our studies also showed that the MRN complex containing mutant Nbs1 (S343A) failed to stimulate the phosphorylation of Chk2 by ATM although p53 phosphorylation was normal *in vitro*. These results suggest that phosphorylation of Nbs1 is not absolutely required for ATM activation but may contribute to the recruitment of specific substrates by ATM.

ATM autophosphorylation

ATM autophosphorylation has been considered to be an indicator of its kinase activity since ATM was shown to be autophosphorylated on Ser1981 *in vivo* after treatment of IR or NCS (Bakkenist and Kastan, 2003). As already mentioned, Bakkenist and Kastan (2003) showed that autophosphorylation of ATM after DNA damage is required for monomerization. Immunoprecipitated ATM was also shown to be autophosphorylated *in vitro* during incubation with γ^{32}-ATP, and the preincubation with ATP correlated with higher levels of kinase activity *in vitro* (Kozlov *et al*., 2003). Later, other phosphorylation sites on ATM were found in addition to Ser1981, including Ser367 and Ser1893 (Kozlov *et al*., 2006). Mutation of these autophosphorylation sites to alanine and expression in A–T cells resulted in defects in ATM-dependent phosphorylation and increased radiosensitivity. A recent study also confirmed that autophosphorylation is required for monomerization and chromatin association of ATM in human cells by immunoprecipitation and western blotting after co-transfection with Flag- and HA-ATM expression plasmids and induction of DSBs by the expression of the endonuclease IPpoI (Berkovich *et al*., 2007). These results suggest that ATM autophosphorylation is important for monomerization and activation of ATM *in vivo*.

In contrast to the findings from human cells, studies in other organisms and *in vitro* have shown that autophosphorylation is dispensable for ATM monomerization and activation. Monomerization of ATM was observed in the absence of autophosphorylation in Mre11-depleted *Xenopus* egg extracts when high levels of linear DNA were used to circumvent the requirement for the MRN complex (Dupre *et al*., 2006). These results suggest that ATM autophosphorylation is not required for ATM monomerization but that the MRN complex and DNA are required. Nussenzweig and co-workers also showed that expression of an S1987A (analogous to S1981A in humans) allele of ATM in the mouse yielded normal ATM-dependent phosphorylation of substrates after DNA damage and normal localization of ATM at DSB sites (Pellegrini *et al*., 2006). It is still unclear why the function of ATM autophosphorylation appears to

differ so dramatically between humans and mice. The originally identified autophosphorylation site, Ser1981, is not conserved in all ATM homologs (absent in *Arabidopsis*, *Drosophila*, budding yeast and fission yeast), also suggesting that ATM autophosphorylation, while important for regulation, may not be essential for the fundamental process of activation by DSBs.

In our *in vitro* system, we observed a very low level of autophosphorylation, which increased only threefold with the addition of DNA and the MRN complex. Under these conditions, we found that ATM-kinase activity increased 80- to 200-fold by the addition of both linear DNA and the MRN complex (Lee and Paull, 2005). These findings suggest that ATM autophosphorylation does not correlate with kinase activity in our *in vitro* conditions. In addition, we also showed that the S1981A-dimeric ATM was fully activated and monomerized efficiently *in vitro*, similar to wild-type ATM. Therefore, using purified components *in vitro*, there appears to be no requirement for autophosphorylation (Lee and Paull, 2005).

Considering the close relationship between ATM autophosphorylation and activation in human cells, however, it is likely that there are cellular factors that make ATM autophosphorylation essential *in vivo*. Several proteins have been shown to regulate ATM autophosphorylation and ATM-dependent phosphorylation events in human cells, thus one or more of these may affect ATM activity in a manner that depends on autophosphorylation. We envision that there could be two general and not necessarily mutually exclusive models to explain this: inhibitor vs activator hypotheses. An inhibitor may be present in human cells that actively prevents ATM activation in the absence of autophosphorylation. Conversely, autophosphorylation may mediate a direct interaction with an activator protein(s) that facilitates its activation in cells. The activator hypothesis seems less likely since the magnitude of activation we observed *in vitro* with purified components is similar to that observed *in vivo*; however, an autophosphorylation-dependent activation mechanism may occur in combination with inhibitory factor in human cells, which would give rise to a greater dependency on autophosphorylation *in vivo*.

Likely candidates for inactivators and activators of ATM

Recent articles show that two phosphatases, protein phosphatase 2A (PP2A) and protein phosphatase 5 (PP5), both affect ATM activity *in vivo* (Ali *et al*., 2004; Goodarzi *et al*., 2004). PP2A is a member of the protein serine/threonine phosphatase family and is composed of a catalytic (C) subunit, a scaffolding A subunit and a regulatory B subunit. Guo *et al*. (2002) showed that the B55α regulatory subunit dissociates from the A and C subunits after DNA damage in ATM-dependent manner. A later study also showed that ATM interacts directly with the A subunit, and that the A and C subunits co-immunoprecipitate with ATM in unirradiated human cells but dissociate after IR exposure (Goodarzi *et al*., 2004). They also showed that overexpression of a dominant-negative form of the C subunit-induced autophosphorylation of ATM on Ser-1981 in the absence of IR. This suggests that autophosphorylation of ATM is regulated by PP2A and that PP2A may constitutively dephosphorylate ATM in human cells. Recently, McConnell *et al*. (2007) identified a type 2A interacting protein (TIP), which interacts with the catalytic subunit of PP2A and inhibits PP2A phosphatase activity. They also showed that overexpression of TIP causes an increase in phosphorylation of ATM/ATR substrates, and depletion of TIP by siRNA causes a reduction in phosphorylation of ATM substrates. These data suggest that ATM activation is regulated by PP2A, although the mechanism of this regulation and the consequences of the ATM-dependent subunit reorganization are not understood.

PP5 is also a member of the serine/threonine phosphatase family. Ali *et al*. (2004) showed that PP5 coimmunoprecipitates with ATM from human cells, but unlike PP2A, this interaction increases after exposure to either NCS or IR. Disruption of PP5 activity by siRNA resulted in defects in ATM activation and radioresistant DNA synthesis. In addition, the phosphatase activity of PP5 was shown to be required for ATM autophosphorylation and activation (Ali *et al*., 2004). These findings suggest that PP5 might control ATM kinase activity by dephosphorylating ATM, the MRN complex, or other factors involved in ATM activation. One possibility is that ATM is phosphorylated on non-autophosphorylation sites in untreated cells and is then dephosphorylated by PP5 after DNA damage. An alternate possibility is that PP5 could act in conjunction with another yet to be identified protein factor to activate ATM by dephosphorylating this factor. The A subunit of PP2A has been shown to interact with PP5, suggesting that PP5 may require the A subunit of PP2A for its function (Lubert *et al*., 2001). A third possibility is that PP5 may dephosphorylate PP2A, as a result activating ATM. Further experiments are required to determine which, if any, of these possibilities are correct.

Interestingly, another phosphatase, wild-type p53-induced phosphatase (Wip1), is recently reported to be a negative regulator of ATM. Wip1 is a member of the protein phosphatase 2C (PP2C) family and is a negative regulator of p38 mitogen-activated protein kinase (MAPK) (Takekawa *et al*., 2000). Shreeram *et al*. (2006) showed that ATM autophosphorylation and phosphorylation of p53 was slightly increased in Wip1-null MEFs compared to wild-type MEFs, as well as in Wip1-siRNA-treated human cells after IR exposure. They also showed that overexpression of Wip1 in mouse cell or human cells reduced the levels of ATM autophosphorylation, and that phosphorylated Ser1981 on ATM is directly dephosphorylated by Wip1 (Shreeram *et al*., 2006). These results suggest that Wip1 controls ATM kinase activity through its phosphatase activity.

One of the candidates for a coactivator of ATM is p53-binding protein 1 (53BP1), which colocalizes with γ-H2AX at DNA DSBs (Schultz *et al*., 2000; Anderson *et al*., 2001; Rappold *et al*., 2001; Xia *et al*., 2001; Shang *et al*., 2003). Disruption of 53BP1 by siRNA causes defects in intra-S-phase and G2-M checkpoint arrest

after IR, decreases in ATM autophosphorylation and ATM substrate phosphorylation (Chk2 and SMC1) (DiTullio *et al*., 2002; Wang *et al*., 2002). A separate study showed that these deficiencies in ATM-mediated signaling after treatment with siRNA specific to 53BP1 were more pronounced in NBS cells (Mochan *et al*., 2003). This suggests that the role of 53BP1 is more critical in the absence of functional Nbs1 protein. Although the mechanisms of ATM activation by 53BP1 are still unclear, recent studies of 53BP1 and its ortholog in fission yeast, Crb2, suggest that 53BP1 directly interacts with dimethylated Lys20 of Histone H4 (Botuyan *et al*., 2006). These findings suggest that DNA damage could cause changes in chromatin structure in a manner that recruits 53BP1 to pre-existing dimethylated Lys20 of H4 analogous to the recruitment of Crb2 to H4-K20 methylated sites in fission yeast.

The BRCA1−/− human breast cancer line, HCC1937, exhibits defects in S-phase and G2/M checkpoint arrest similar to that of A–T cells after exposure to IR, thus BRCA1 is considered to be in the same pathway as ATM (Scully *et al*., 1999). BRCA1 is also an ATM substrate and is phosphorylated on Ser1387, Ser1423 and Ser1524 after DNA damage (Cortez *et al*., 1999; Gatei *et al*., 2001). Phosphorylation of BRCA1 on these sites is closely linked to S-phase and G2/M checkpoints (Xu *et al*., 2001, 2002). Several studies also showed that BRCA1 is required for optimal levels of ATM-dependent phosphorylation of p53, c-Jun, Nbs1, Chk2, CtIP and SMC1, although the BRCA1 dependence of some of these phosphorylation events is controversial (Foray *et al*., 2003; Fabbro *et al*., 2004; Kitagawa *et al*., 2004). Kitagawa *et al*. (2004) also showed that BRCA1 is required for focus formation by autophosphorylated ATM. These results suggest that BRCA1 is required for localization of already activated ATM at DNA DSBs and may mediate ATM-kinase activity toward several of its substrates.

Interestingly, Nbs1-independent ATM activation was reported recently through a novel factor, ATM interacting protein (ATMIN), when cells were treated with chloroquine under hypotonic conditions (Kanu and Behrens, 2007). ATMIN was shown to interact with ATM through a C-terminal ATM interaction motif, which shows some similarity to the PIKK interaction motif in Nbs1. ATMIN colocalizes with autophosphorylated ATM after treatment of cells with chloroquine or in hypotonic conditions, but ATMIN dissociates from ATM after exposure to IR. ATM-dependent phosphorylation of p53 and Chk2 was reduced in ATMIN-depleted MEF cells after treatment with chloroquine and hypotonic conditions, suggesting that ATMIN regulates ATM activation in the absence of DNA breaks. These results are consistent with previous findings showing that chromatin-modifying agents induce autophosphorylation of ATM on Ser1981 in the absence of DNA breaks (Bakkenist and Kastan, 2003).

Another potential ATM coactivator is p18 (AIMP3), which is a component of a translation complex and shows sequence homology to elongation factor subunit (EF-1) (Quevillon and Mirande, 1996), yet appears to function in the DNA damage response. Park *et al*. (2005) showed that p18 regulates ATM-dependent p53 induction after DNA damage, and that ATM and ATR directly interact with p18 after UV and/or adriamycin treatment. ATM autophosphorylation was increased by the overexpression of p18, and disruption of p18 using antisense RNA caused a reduction in p53 phosphorylation after adriamycin treatment. These findings suggest that p18 is required for efficient ATM autophosphorylation and activation, but the mechanism of this relationship is unknown.

Histone acetyltransferases are also possible cofactors for ATM autophosphorylation. Price and co-workers showed that ATM is acetylated by the Tip60 complex after DNA damage and that disruption of Tip60 by siRNA causes defects in ATM autophosphorylation, suggesting that the activity of Tip60 is required for ATM autophosphorylation (Sun *et al*., 2005, 2006). They also showed that Tip60 is required for ATM-dependent phosphorylation of p53 and Chk2 in bleomycin-treated cells and interacts with the C-terminal FATC domain on ATM. Disruption of the interaction between ATM and Tip60 by mutations of the FATC domain in ATM caused defects in DNA damage-induced ATM signaling. These findings suggest that Tip60-mediated acetylation of ATM is required for autophosphorylation and activation.

hMOF, which is also a histone acetyltransferase, was also shown to have effects on ATM autophosphorylation and ATM activity similar to Tip60. Disruption of hMOF by siRNA or expression of dominant-negative forms of hMOF caused a decrease in ATM autophosphorylation and phosphorylation of Chk2 and p53 (Gupta *et al*., 2005). Although both Tip60 and hMOF acetylate histones, which may alter chromatin structure and affect ATM indirectly, Tip60 is unique in its direct acetylation of ATM. Both histone and ATM acetylation are likely to play important roles in the regulation of ATM activity *in vivo*.

The mechanisms of ATM activation by these cofactors are still unclear. Since ATM has many substrates, these cofactors may be required for substrate-specific effects on ATM function. It is also possible that these are required for ATM activation by different stimuli. It will be interesting to find out if these cofactors work in the same pathway as the MRN complex or they are involved in MRN-independent pathways of ATM activation by different forms of cellular stress.

References

Ali A, Zhang J, Bao S, Liu I, Otterness D, Dean NM *et al*. (2004). Requirement of protein phosphatase 5 in DNA-damage-induced ATM activation. *Genes Dev* **18**: 249–254.

Anderson L, Henderson C, Adachi Y. (2001). Phosphorylation and rapid relocalization of 53BP1 to nuclear foci upon DNA damage. *Mol Cell Biol* **21**: 1719–1729.

Araujo FD, Stracker TH, Carson CT, Lee DV, Weitzman MD. (2005). Adenovirus type 5 E4orf3 protein targets the Mre11 complex to cytoplasmic aggresomes. *J Virol* **79**: 11382–11391.

Bakkenist CJ, Kastan MB. (2003). DNA damage activates ATM through intermolecular autophosphorylation and dimer dissociation. *Nature* **421**: 499–506.

Becker E, Meyer V, Madaoui H, Guerois R. (2006). Detection of a tandem BRCT in Nbs1 and Xrs2 with functional implications in the DNA damage response. *Bioinformatics* **22**: 1289–1292.

Berkovich E, Monnat Jr RJ, Kastan MB. (2007). Roles of ATM and NBS1 in chromatin structure modulation and DNA double-strand break repair. *Nat Cell Biol* **9**: 683–690.

Bhaskara V, Dupre A, Lengsfeld B, Hopkins BB, Chan A, Lee JH *et al*. (2007). Rad50 adenylate kinase activity regulates DNA tethering by Mre11/Rad50 complexes. *Mol Cell* **25**: 647–661.

Bosotti R, Isacchi A, Sonnhammer EL. (2000). FAT: a novel domain in PIK-related kinases. *Trends Biochem Sci* **25**: 225–227.

Botuyan MV, Lee J, Ward IM, Kim JE, Thompson JR, Chen J *et al*. (2006). Structural basis for the methylation state-specific recognition of histone H4-K20 by 53BP1 and Crb2 in DNA repair. *Cell* **127**: 1361–1373.

Buscemi G, Savio C, Zannini L, Micciche F, Masnada D, Nakanishi M *et al*. (2001). Chk2 activation dependence on Nbs1 after DNA damage. *Mol Cell Biol* **21**: 5214–5222.

Carney JP, Maser RS, Olivares H, Davis EM, Le Beau M, Yates III JR *et al*. (1998). The hMre11/hRad50 protein complex and Nijmegen breakage syndrome: linkage of double-strand break repair to the cellular DNA damage response. *Cell* **93**: 477–486.

Carson CT, Schwartz RA, Stracker TH, Lilley CE, Lee DV, Weitzman MD. (2003). The Mre11 complex is required for ATM activation and the G2/M checkpoint. *EMBO J* **22**: 6610–6620.

Cerosaletti K, Wright J, Concannon P. (2006). Active role for nibrin in the kinetics of ATM activation. *Mol Cell Biol* **26**: 1691–1699.

Cerosaletti KM, Concannon P. (2003). Nibrin forkhead-associated domain and breast cancer C-terminal domain are both required for nuclear focus formation and phosphorylation. *J Biol Chem* **278**: 21944–21951.

Cortez D, Guntuku S, Qin J, Elledge SJ. (2001). ATR and ATRIP: partners in checkpoint signaling. *Science* **294**: 1713–1716.

Cortez D, Wang Y, Qin J, Elledge SJ. (1999). Requirement of ATM-dependent phosphorylation of BRCA1 in the DNA damage response to double-strand breaks. *Science* **286**: 1162–1166.

Costanzo V, Paull T, Gottesman M, Gautier J. (2004). Mre11 assembles linear DNA fragments into DNA damage signaling complexes. *PLoS Biol* **2**: E110.

Delacroix S, Wagner JM, Kobayashi M, Yamamoto K, Karnitz LM. (2007). The Rad9-Hus1-Rad1 (9-1-1) clamp activates checkpoint signaling via TopBP1. *Genes Dev* **21**: 1472–1477.

Desai-Mehta A, Cerosaletti KM, Concannon P. (2001). Distinct functional domains of nibrin mediate Mre11 binding, focus formation, and nuclear localization. *Mol Cell Biol* **21**: 2184–2191.

Difilippantonio S, Celeste A, Fernandez-Capetillo O, Chen HT, Reina San Martin B, Van Laethem F *et al*. (2005). Role of Nbs1 in the activation of the ATM kinase revealed in humanized mouse models. *Nat Cell Biol* **7**: 675–685.

Difilippantonio S, Celeste A, Kruhlak MJ, Lee Y, Difilippantonio MJ, Feigenbaum L *et al*. (2007). Distinct domains in Nbs1 regulate irradiation-induced checkpoints and apoptosis. *J Exp Med* **204**: 1003–1011.

DiTullio Jr RA, Mochan TA, Venere M, Bartkova J, Sehested M, Bartek J *et al*. (2002). 53BP1 functions in an ATM-dependent checkpoint pathway that is constitutively activated in human cancer. *Nat Cell Biol* **4**: 998–1002.

Dupre A, Boyer-Chatenet L, Gautier J. (2006). Two-step activation of ATM by DNA and the Mre11–Rad50–Nbs1 complex. *Nat Struct Mol Biol* **13**: 451–457.

Fabbro M, Savage K, Hobson K, Deans AJ, Powell SN, McArthur GA *et al*. (2004). BRCA1-BARD1 complexes are required for p53Ser-15 phosphorylation and a G1/S arrest following ionizing radiation-induced DNA damage. *J Biol Chem* **279**: 31251–31258.

Falck J, Coates J, Jackson SP. (2005). Conserved modes of recruitment of ATM, ATR and DNA-PKcs to sites of DNA damage. *Nature* **434**: 605–611.

Featherstone C, Jackson SP. (1998). DNA repair: the Nijmegen breakage syndrome protein. *Curr Biol* **8**: R622–R625.

Foray N, Marot D, Gabriel A, Randrianarison V, Carr AM, Perricaudet M *et al*. (2003). A subset of ATM- and ATR-dependent phosphorylation events requires the BRCA1 protein. *EMBO J* **22**: 2860–2871.

Gatei M, Young D, Cerosaletti KM, Desai-Mehta A, Spring K, Kozlov S *et al*. (2000). ATM-dependent phosphorylation of nibrin in response to radiation exposure. *Nat Genet* **25**: 115–119.

Gatei M, Zhou BB, Hobson K, Scott S, Young D, Khanna KK. (2001). Ataxia telangiectasia mutated (ATM) kinase and ATM and Rad3 related kinase mediate phosphorylation of BRCA1 at distinct and overlapping sites. *In vivo* assessment using phospho-specific antibodies. *J Biol Chem* **276**: 17276–17280.

Girard PM, Riballo E, Begg AC, Waugh A, Jeggo PA. (2002). Nbs1 promotes ATM dependent phosphorylation events including those required for G1/S arrest. *Oncogene* **21**: 4191–4199.

Goodarzi AA, Jonnalagadda JC, Douglas P, Young D, Ye R, Moorhead GB *et al*. (2004). Autophosphorylation of ataxia–telangiectasia mutated is regulated by protein phosphatase 2A. *EMBO J* **23**: 4451–4461.

Guo CY, Brautigan DL, Larner JM. (2002). ATM-dependent dissociation of B55 regulatory subunit from nuclear PP2A in response to ionizing radiation. *J Biol Chem* **277**: 4839–4844.

Gupta A, Sharma GG, Young CS, Agarwal M, Smith ER, Paull TT *et al*. (2005). Involvement of human MOF in ATM function. *Mol Cell Biol* **25**: 5292–5305.

Kanu N, Behrens A. (2007). ATMIN defines an NBS1-independent pathway of ATM signalling. *EMBO J* **26**: 2933–2941.

Kim ST, Xu B, Kastan MB. (2002). Involvement of the cohesin protein, SMC1, in ATM-dependent and independent responses to DNA damage. *Genes Dev* **16**: 560–570.

Kitagawa R, Bakkenist CJ, McKinnon PJ, Kastan MB. (2004). Phosphorylation of SMC1 is a critical downstream event in the ATM–NBS1–BRCA1 pathway. *Genes Dev* **18**: 1423–1438.

Kobayashi J, Tauchi H, Sakamoto S, Nakamura A, Morishima K, Matsuura S *et al*. (2002). NBS1 localizes to gamma-H2AX foci through interaction with the FHA/BRCT domain. *Curr Biol* **12**: 1846–1851.

Kozlov S, Gueven N, Keating K, Ramsay J, Lavin MF. (2003). ATP activates ataxia–telangiectasia mutated (ATM) *in vitro*. Importance of autophosphorylation. *J Biol Chem* **278**: 9309–9317.

Kozlov SV, Graham ME, Peng C, Chen P, Robinson PJ, Lavin MF. (2006). Involvement of novel autophosphorylation sites in ATM activation. *EMBO J* **25**: 3504–3514.

Lee J, Kumagai A, Dunphy WG. (2007). The Rad9-Hus1-Rad1 checkpoint clamp regulates interaction of TopBP1 with ATR. *J Biol Chem* **282**: 28036–28044.

Lee J-H, Ghirlando R, Bhaskara V, Hoffmeyer MR, Gu J, Paull TT. (2003a). Regulation of Mre11/Rad50 by Nbs1: effects on nucleotide-dependent DNA binding and association with ATLD mutant complexes. *J Biol Chem* **278**: 45171–45181.

Lee JH, Paull TT. (2004). Direct activation of the ATM protein kinase by the Mre11/Rad50/Nbs1 complex. *Science* **304**: 93–96.

Lee JH, Paull TT. (2005). ATM activation by DNA double-strand breaks through the Mre11-Rad50-Nbs1 complex. *Science* **308**: 551–554.

Lee JH, Xu B, Lee CH, Ahn JY, Song MS, Lee H *et al*. (2003b). Distinct functions of Nijmegen breakage syndrome in ataxia–telangiectasia mutated-dependent responses to DNA damage. *Mol Cancer Res* **1**: 674–681.

Lim DS, Kim ST, Xu B, Maser RS, Lin J, Petrini JH *et al*. (2000). ATM phosphorylates p95/nbs1 in an S-phase checkpoint pathway. *Nature* **404**: 613–617.

Lubert EJ, Hong Y, Sarge KD. (2001). Interaction between protein phosphatase 5 and the A subunit of protein phosphatase 2A: evidence for a heterotrimeric form of protein phosphatase 5. *J Biol Chem* **276**: 38582–38587.

Luo G, Yao MS, Bender CF, Mills M, Bladl AR, Bradley A *et al*. (1999). Disruption of mRad50 causes embryonic stem cell lethality, abnormal embryonic development, and sensitivity to ionizing radiation. *Proc Natl Acad Sci USA* **96**: 7376–7381.

Maser RS, Zinkel R, Petrini JH. (2001). An alternative mode of translation permits production of a variant NBS1 protein from the common Nijmegen breakage syndrome allele. *Nat Genet* **27**: 417–421.

McConnell JL, Gomez RJ, McCorvey LR, Law BK, Wadzinski BE. (2007). Identification of a PP2A-interacting protein that functions as a negative regulator of phosphatase activity in the ATM/ATR signaling pathway. *Oncogene* **26**: 6021–6030.

Mochan TA, Venere M, DiTullio Jr RA, Halazonetis TD. (2003). 53BP1 and NFBD1/MDC1-Nbs1 function in parallel interacting pathways activating ataxia-telangiectasia mutated (ATM) in response to DNA damage. *Cancer Res* **63**: 8586–8591.

Moncalian G, Lengsfeld B, Bhaskara V, Hopfner KP, Karcher A, Alden E *et al*. (2004). The rad50 signature motif: essential to ATP binding and biological function. *J Mol Biol* **335**: 937–951.

Nakada D, Matsumoto K, Sugimoto K. (2003). ATM-related Tel1 associates with double-strand breaks through an Xrs2-dependent mechanism. *Genes Dev* **17**: 1957–1962.

Nakanishi K, Taniguchi T, Ranganathan V, New HV, Moreau LA, Stotsky M *et al*. (2002). Interaction of FANCD2 and NBS1 in the DNA damage response. *Nat Cell Biol* **4**: 913–920.

Park BJ, Kang JW, Lee SW, Choi SJ, Shin YK, Ahn YH *et al*. (2005). The haploinsufficient tumor suppressor p18 upregulates p53 via interactions with ATM/ATR. *Cell* **120**: 209–221.

Pellegrini M, Celeste A, Difilippantonio S, Guo R, Wang W, Feigenbaum L *et al*. (2006). Autophosphorylation at serine 1987 is dispensable for murine Atm activation *in vivo*. *Nature* **443**: 222–225.

Petrini JH. (2000). The Mre11 complex and ATM: collaborating to navigate S phase. *Curr Opin Cell Biol* **12**: 293–296.

Quevillon S, Mirande M. (1996). The p18 component of the multisynthetase complex shares a protein motif with the beta and gamma subunits of eukaryotic elongation factor 1. *FEBS Lett* **395**: 63–67.

Rappold I, Iwabuchi K, Date T, Chen J. (2001). Tumor suppressor p53 binding protein 1 (53BP1) is involved in DNA damage-signaling pathways. *J Cell Biol* **153**: 613–620.

Savitsky K, Bar-Shira A, Gilad S, Rotman G, Ziv Y, Vanagaite L *et al*. (1995a). A single ataxia–telangiectasia gene with a product similar to PI-3 kinase. *Science* **268**: 1749–1753.

Savitsky K, Sfez S, Tagle DA, Ziv Y, Sartiel A, Collins FS *et al*. (1995b). The complete sequence of the coding region of the ATM gene reveals similarity to cell cycle regulators in different species. *Hum Mol Genet* **4**: 2025–2032.

Schultz LB, Chehab NH, Malikzay A, Halazonetis TD. (2000). p53-binding protein 1 (53BP1) is an early participant in the cellular response to DNA double-strand breaks. *J Cell Biol* **151**: 1381–1390.

Scully R, Ganesan S, Vlasakova K, Chen J, Socolovsky M, Livingston DM. (1999). Genetic analysis of BRCA1 function in a defined tumor cell line. *Mol Cell* **4**: 1093–1099.

Shang YL, Bodero AJ, Chen PL. (2003). NFBD1, a novel nuclear protein with signature motifs of FHA and BRCT, and an internal 41-amino acid repeat sequence, is an early participant in DNA damage response. *J Biol Chem* **278**: 6323–6329.

Shiloh Y. (1997). Ataxia–telangiectasia and the Nijmegen breakage syndrome: related disorders but genes apart. *Annu Rev Genet* **31**: 635–662.

Shiloh Y. (2006). The ATM-mediated DNA-damage response: taking shape. *Trends Biochem Sci* **31**: 402–410.

Shirata N, Kudoh A, Daikoku T, Tatsumi Y, Fujita M, Kiyono T *et al*. (2005). Activation of ataxia telangiectasia-mutated DNA damage checkpoint signal transduction elicited by herpes simplex virus infection. *J Biol Chem* **280**: 30336–30341.

Shreeram S, Demidov ON, Hee WK, Yamaguchi H, Onishi N, Kek C *et al*. (2006). Wip1-phosphatase modulates ATM-dependent signaling pathways. *Mol Cell* **23**: 757–764.

Stewart GS, Last JI, Stankovic T, Haites N, Kidd AM, Byrd PJ *et al*. (2001). Residual ataxia telangiectasia mutated protein function in cells from ataxia telangiectasia patients, with 5762ins137 and 7271T—>G mutations, showing a less severe phenotype. *J Biol Chem* **276**: 30133–30141.

Stewart GS, Maser RS, Stankovic T, Bressan DA, Kaplan MI, Jaspers NG *et al*. (1999). The DNA double-strand break repair gene *hMre11* is mutated in individuals with an ataxia-telangiectasia-like disorder. *Cell* **99**: 577–587.

Stracker TH, Carson CT, Weitzman MD. (2002). Adenovirus oncoproteins inactivate the Mre11-Rad50-NBS1 DNA repair complex. *Nature* **418**: 348–352.

Stracker TH, Morales M, Couto SS, Hussein H, Petrini JH. (2007). The carboxy terminus of NBS1 is required for induction of apoptosis by the MRE11 complex. *Nature* **447**: 218–221.

Sun Y, Jiang X, Chen S, Fernandes N, Price BD. (2005). A role for the Tip60 histone acetyltransferase in the acetylation and activation of ATM. *Proc Natl Acad Sci USA* **102**: 13182–13187.

Sun Y, Jiang X, Chen S, Price BD. (2006). Inhibition of histone acetyltransferase activity by anacardic acid sensitizes tumor cells to ionizing radiation. *FEBS Lett* **580**: 4353–4356.

Takekawa M, Adachi M, Nakahata A, Nakayama I, Itoh F, Tsukuda H *et al*. (2000). p53-inducible wip1 phosphatase mediates a negative feedback regulation of p38 MAPK-p53 signaling in response to UV radiation. *EMBO J* **19**: 6517–6526.

Tauchi H, Kobayashi J, Morishima K, Matsuura S, Nakamura A, Shiraishi T *et al*. (2001). The forkhead-associated domain of NBS1 is essential for nuclear foci formation after irradiation but not essential for hRAD50/hMRE11/NBS1 complex DNA repair activity. *J Biol Chem* **276**: 12–15.

Uziel T, Lerenthal Y, Moyal L, Andegeko Y, Mittelman L, Shiloh Y. (2003). Requirement of the MRN complex for ATM activation by DNA damage. *EMBO J* **22**: 5612–5621.

Varon R, Vissinga C, Platzer M, Cerosaletti KM, Chrzanowska KH, Saar K *et al*. (1998). Nibrin, a novel DNA double-strand break repair protein, is mutated in Nijmegen breakage syndrome. *Cell* **93**: 467–476.

Wang B, Matsuoka S, Carpenter PB, Elledge SJ. (2002). 53BP1, a mediator of the DNA damage checkpoint. *Science* **298**: 1435–1438.

Wu X, Avni D, Chiba T, Yan F, Zhao Q, Lin Y *et al*. (2004). SV40 T antigen interacts with Nbs1 to disrupt DNA replication control. *Genes Dev* **18**: 1305–1316.

Xia Z, Morales JC, Dunphy WG, Carpenter PB. (2001). Negative cell cycle regulation and DNA damage-inducible phosphorylation of the BRCT protein 53BP1. *J Biol Chem* **276**: 2708–2718.

Xiao Y, Weaver DT. (1997). Conditional gene targeted deletion by Cre recombinase demonstrates the requirement for the double-strand break repair Mre11 protein in murine embryonic stem cells. *Nucleic Acids Res* **25**: 2985–2991.

Xu B, Kim S, Kastan MB. (2001). Involvement of BRCA1 in S-phase and G(2)-phase checkpoints after ionizing irradiation. *Mol Cell Biol* **21**: 3445–3450.

Xu B, O'Donnell AH, Kim ST, Kastan MB. (2002). Phosphorylation of serine 1387 in BRCA1 is specifically required for the Atm-mediated S-phase checkpoint after ionizing irradiation. *Cancer Res* **62**: 4588–4591.

Yazdi PT, Wang Y, Zhao S, Patel N, Lee EY, Qin J. (2002). SMC1 is a downstream effector in the ATM/NBS1 branch of the human S-phase checkpoint. *Genes Dev* **16**: 571–582.

You Z, Chahwan C, Bailis J, Hunter T, Russell P. (2005). ATM activation and its recruitment to damaged DNA require binding to the C terminus of Nbs1. *Mol Cell Biol* **25**: 5363–5379.

Young DB, Jonnalagadda J, Gatei M, Jans DA, Meyn S, Khanna KK. (2005). Identification of domains of ataxia-telangiectasia mutated required for nuclear localization and chromatin association. *J Biol Chem* **280**: 27587–27594.

Zhao S, Renthal W, Lee EY. (2002). Functional analysis of FHA and BRCT domains of NBS1 in chromatin association and DNA damage responses. *Nucleic Acids Res* **30**: 4815–4822.

Zhu J, Petersen S, Tessarollo L, Nussenzweig A. (2001). Targeted disruption of the Nijmegen breakage syndrome gene NBS1 leads to early embryonic lethality in mice. *Curr Biol* **11**: 105–109.