The FATC Domains of PIKK Proteins Are Functionally Equivalent and Participate in the Tip60-dependent Activation of DNA-PKcs and ATM*\(^{1,5}\)

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Members of the phosphatidylinositol 3-kinase-related kinase (PIKK) family, including the ATM, DNA-PKcs, Atr, and Trrap proteins, function in signal transduction pathways that activate the DNA damage response. PIKK proteins contain a conserved C-terminal FAT/kinase domain/FATC domain structure. The FATC domain of ATM mediates the interaction between ATM and Tip60, a histone acetyltransferase that regulates activation of ATM. Here, we examined whether the FATC domains of DNA-PKcs, Atr, and Trrap were also able to interact with Tip60. Deletion of the FATC domain of ATM blocked the interaction between ATM and Tip60 and suppressed the activation of ATM kinase activity by DNA damage. Replacement of the FATC domain of ATM with the FATC domains of DNA-PKcs, Atr, or Trrap restored the activation of ATM and its association with Tip60. These results indicate that the FATC domains of DNA-PKcs, Atr, Trrap, and ATM are functionally equivalent. Immunoprecipitation experiments demonstrated that Tip60 is constitutively associated with DNA-PKcs and that the histone acetyltransferase activity associated with DNA-PKcs is up-regulated by DNA damage. When Tip60 expression was suppressed by small interfering RNA, the activation of DNA-PKcs (measured by autophosphorylation of DNA-PKcs at serine 2056 and threonine 2609) was inhibited, demonstrating a key role for Tip60 in the activation of DNA-PKcs by DNA damage. The conserved FATC domains of PIKK proteins may therefore function as a binding domain for the Tip60 histone acetyltransferase. Further, the ability of Tip60 to regulate the activation of both ATM and DNA-PKcs in response to DNA damage demonstrates that Tip60 is a key component of the DNA damage-signaling network.

The phosphatidylinositol 3-kinase-related kinase family (PIKK)\(^3\) regulates a diverse array of cellular pathways, including both transcription (1) and cellular responses to genotoxic or environmental stress (2, 3). The ATM, Atr, and DNA-PKcs PIKK proteins are activated in response to distinct types of DNA damage (2, 4) and subsequently activate cell cycle checkpoints and regulate DNA repair through phosphorylation of key protein targets. For example, ATM kinase activity is rapidly activated when cells are exposed to ionizing radiation (4–6). Subsequently, ATM undergoes autophosphorylation and is located at sites of DNA damage (7). ATM then phosphorylates multiple DNA damage response proteins, including p53 (5, 6), nbs1 (8), and SMC1 (9). In addition to functioning in cellular responses to DNA damage, members of the PIKK family are also involved in the control of cell growth (mTor) (3) and in regulating chromatin structure (Trrap) (1).

PIKK proteins are defined by the presence of several conserved protein domains. The N terminus of each is predicted to consist of multiple HEAT domains, a protein domain involved in mediating protein-protein interactions (10, 11). The C-terminal of PIKK proteins contains the conserved FAT/kinase domain/FATC structure that is present as a single unit in all known PIKK proteins (12). The FAT domain is weakly conserved between family members, whereas the kinase domain is highly conserved and is essential for the function of these proteins (2). The exception is Trrap. Trrap is the core component of NuA4, a multisubunit chromatin-remodeling complex containing both histone acetyltransferase (HAT) and ATPase activity (13, 14). Trrap retains the conserved kinase domain but lacks the conserved amino acids required for ATP binding and catalytic activity (15). The extreme C-terminal of the PIKK proteins also contains the 33-amino acid FATC domain. This domain is required for the kinase activity of the mTor (16) and DNA-PKcs proteins (17, 18), indicating it plays a critical role in regulating kinase activity. Structural studies on DNA-PKcs indicate that the kinase domain forms a central domain from which the FAT and FATC domains protrude (19). However, the exact function of the FAT and FATC domains of PIKK proteins is unknown.

Recently, we demonstrated that the ATM protein forms a complex with the Tip60 histone acetyltransferase (20). Following exposure to ionizing radiation, Tip60 acetylates ATM, leading to the activation of ATM kinase activity. Tip60 is therefore a key upstream regulator of the ATM protein. The interaction between ATM and Tip60 is mediated by the FATC domain of ATM, and the introduction of point mutations into the FATC domain of ATM inhibits this interaction (20). One potential function for the FATC domain of ATM is to mediate the association between ATM and Tip60. Because the FATC domain is conserved between PIKK proteins, this predicts that the FATC domain of the PIKK proteins DNA-PKcs, Atr, and Trrap may also mediate association with Tip60. Here, we report on studies designed to test this possibility.

**EXPERIMENTAL PROCEDURES**

*Cells and Antibodies—Culture conditions for 293T, HeLa, and GM5849 AT cells and generation and selection of transfected cell lines is described in Ref. 21. siRNAs T3 (GGAAGCUGCUGAUCGAGUUU),
Function of FATC Domains

T4 (GACGUAAGAACAAGAGUUUAU), and green fluorescent protein were obtained from Dharmacon and were transfected into cells with Lipofectamine 2000 (Invitrogen). Antibodies used were ATM antibodies 5C2 or 2C1 (Genetex), anti-phosphoserine 1981 (Rockland Biochemicals), anti-acetyl lysine or Tip60 antibody (Upstate Biotech), ATM antibody PC116 (Oncogene Science). DNA-PKcs antibody (clone 25–4), Ku70 (clone N3H10), and Ku80 (clone GE2.9.5) were purchased from Neomarkers. Cell survival was as previously described (22). Immunofluorescence was carried out using ATM antibody 5C2 (Genetex) and signal detected with Texas Red secondary antibody as previously described (20).

Immunoprecipitation, Kinase Assays, and HAT Assays—Cells were lysed in lysis buffer (20 mM Hepes, pH 7.4, 150 mM NaCl, 0.2% Tween 20, 1.5 mM MgCl2, 1 mM EGTA, 2 mM dithiothreitol, 50 mM NaF, 500 μM Na3VO4, 1 mM phenylmethylsulfonyl fluoride, 3 μg/ml of aprotinin, 3 μg/ml of leupeptin). Immunoprecipitates were washed three times in lysis buffer and once in base buffer (10 mM Hepes, pH 7.4, 10 mM MgCl2, 50 mM NaCl, 1 mM dithiothreitol, 1 mM phenylmethylsulfonyl fluoride). For immunokinase assays, immunoprecipitates were washed twice in kinase buffer [10 mM Hepes, (pH 7.4), 10 mM MgCl2, 50 mM NaCl, 10 mM MnCl2] and incubated in 50 μl of kinase buffer supplemented with 50 μM ATP, p53 peptide (2 μg of EPPLSQEAFADLWKK), and 10 μCi of [γ-32P]ATP for 30 min at 30 °C. Reactions were terminated with 30% acetic acid (20 μl) and an aliquot spotted onto P81 paper. Filters were washed five times in 15% acetic acid, air dried, and counted. For HAT assays, immunoprecipitates were washed twice in HAT assay buffer (50 mM Tris, pH 8, 10% glycerol, 0.1 mM EDTA, 1 mM dithiothreitol) and incubated in 60 μl of HAT assay buffer supplemented with acetyl-CoA (100 μM) and biotinylated histone H4 peptide (0.5 μg) for 30 min at 30 °C. An aliquot of the reaction was immobilized onto streptavidin plates and acetylation detected using a HAT enzyme-linked immunosorbent assay according to the manufacturer’s instructions (Upstate Biotechnology).

**ATM Constructs**—A BlpI-XhoI fragment encoding the C-terminal and 3′-untranslated region of ATM was subcloned into pBluescript. Point mutations were inserted by site-directed mutagenesis to create restriction sites for SpeI (nucleotide 9279: A9281T/G9282A) and EcoR1 (nucleotide 9373: A8378C). The nucleotide sequence encoding the FATC domain of ATM was replaced with the FATC domain of Atr, DNA-PKcs, or Trrap, each of the chimeric constructs was expressed in the ATM-deficient GM5849 cell line, derived from an ataxia telangiectasia patient. ATMAtr, ATM DNA-PK, and ATMTrrap constructs were expressed at similar levels to ATM, whereas ATM DNA-PKcs was expressed at significantly lower levels.

**Function of FATC Domains**

The FATC domain spans the C-terminal 33 amino acids of the ATM, Atr, DNA-PKcs, and Trrap proteins (Fig. 1a), and is conserved in the yeast Tor1 (3) and mec1 (23) proteins. The FATC domain contains a group of invariant amino acids that constitute the conserved core of this domain. Mutation of these highly conserved residues in the FATC domain of ATM prevents the formation of ATM-Tip60 complexes in vivo (20), implying that the FATC domain may mediate the interaction between ATM and Tip60. Here, we examined whether the FATC domains of ATM, Atr, DNA-PKcs, and Trrap were functionally equivalent. The last 24 amino acids, including the most highly conserved region of the FATC domain, were deleted from ATM, creating ATMΔFATC. In addition, 3 chimeric constructs, in which the FATC domain of ATM was replaced with the FATC domain of either Atr, Tip60, or DNA-PKcs, were constructed. Each of these chimeric constructs was expressed in the ATM-deficient GM5849 cell line, derived from an ataxia telangiectasia patient. ATMAtr, ATM DNA-PK, and ATM Tip60 constructs were expressed at similar levels to ATM, whereas ATM DNA-PKcs was present at lower levels compared with ATM (Fig. 1b). Deletion or replacement of the FATC domain of ATM did not, therefore, significantly destabilize the protein. To examine whether the interaction between ATM and Tip60 required the FATC domain, ATM cells expressing either wild-type ATM, ATMΔFATC, or the chimeric ATM proteins were immunoprecipitated with ATM antibody and then examined for the presence of co-precipitating Tip60. In ATM cells that lack ATM expression no coprecipitation of Tip60 was detected (Fig. 1b, Vec). When ATM was expressed in AT cells, Tip60 was readily detected in association with ATM (Fig. 1b). Further, the interaction between ATM and Tip60 was not altered following exposure to the DNA-damaging agent bleomycin. When the FATC domain of ATM was deleted, the interaction between ATM and Tip60 was abolished, indicating that the FATC domain is required to mediate this interaction. However, when the FATC domain of ATM was replaced with the FATC domain of Atr, DNA-PKcs, or Trrap, the interaction between ATM and Tip60 was restored. This demonstrates that the FATC domains of Atr, DNA-PKcs, and Trrap are functionally interchangeable.

We next examined whether the chimeric ATM constructs retained the ability to be activated in response to DNA damage. Previously, we showed that the Tip60-dependent acetylation of ATM was essential for the activation of ATM kinase activity (20). The activation of ATM kinase activity was measured by monitoring the autophosphorylation of ATM on serine 1981 (7), and the acetylation of ATM was monitored using a pan-specific acetyl-lysine antibody. No ATM acetylation or autophosphorylation was detected in AT cells (Fig. 1b, Vec). Introduction of exogenous ATM into AT cells restored both acetylation and autophosphorylation of ATM in response to bleomycin. Deletion of the FATC domain of ATM, which prevents interaction with Tip60, abolished both the autophosphorylation and acetylation of ATM. When the FATC domain of ATM was replaced with the FATC domain of Atr, DNA-PKcs, or Trrap, each of the chimeric proteins was acetylated and autophosphorylated in response to bleomycin. Fig. 1b demonstrates that the FATC domain of ATM is required for the DNA damage-
duced autophosphorylation and acetylation of the ATM protein. Further, the FATC domains of the Atr, DNA-PKcs, and Trrap proteins can functionally replace the FATC domain of ATM.

To ensure that the chimeric ATM proteins retained functional activity and were correctly localized to the nucleus of cells, the cellular location, intrinsic kinase activity, and the ability of each to complement the increased radiosensitivity of A7 cells were measured. All of the chimeric constructs, including ATM \( ^{\text{FATC}} \), were localized to the nucleus of the cell (supplemental Fig. S1). In Fig. 2a, immunokinase assays were carried out to monitor intrinsic ATM kinase activity. A7 cells immunoprecipitated with ATM antibody displayed no significant ATM kinase activity (Fig. 2a, Vec). A7 cells expressing ATM exhibited ATM kinase activity that was increased in response to bleomycin. ATM \( ^{\text{FATC}} \) had similar basal kinase activity to full-length ATM; however, ATM \( ^{\text{FATC}} \) did not exhibit DNA damage-induced activation of its kinase activity in response to bleomycin (Fig. 2a). Mutations in ATM frequently lead to loss of ATM kinase activity (24). The observation that the ATM \( ^{\text{FATC}} \) protein retained basal kinase activity suggests that deletion of the FATC domain (which is adjacent to the kinase domain) did not significantly disrupt the overall protein structure. The inability of ATM \( ^{\text{FATC}} \) to increase its kinase activity in response to DNA damage is therefore consistent with the observation that the association of Tip60 with the FATC domain of ATM is required for activation of ATM kinase activity (20). When the FATC domains Atr, DNA-PK, or Trrap were inserted into ATM \( ^{\text{FATC}} \), the activation of ATM kinase activity by bleomycin was restored (Fig. 2a). The ability of ATM to phosphorylate and activate key target proteins is required for ATM to regulate the cell response to ionizing radiation (21). Because the FATC domain of ATM is adjacent to the kinase domain, the FATC domain may influence the ability of ATM to phosphorylate key target proteins. That is, replacing the FATC domain of ATM with the FATC domain of Atr, Trrap, or DNA-PK may restore activation of ATM kinase activity but prevent ATM from efficiently phosphorylating downstream effector proteins. The failure of the chimeric proteins to correctly phosphorylate downstream effector proteins would prevent ATM from regulating the ability of the cells to survive exposure to ionizing radiation. To test this possibility, we examined whether the chimeric ATM proteins could complement the increased radiosensitivity of A7 cells. In Fig. 2b, A7 cells were sensitive to ionizing radiation and re-expression of ATM increased cell survival. In contrast, expression of ATM \( ^{\text{FATC}} \) had no effect on radiosensitivity, consistent with the failure of this protein to exhibit either increased kinase activity or autophosphorylation of serine 1981 of ATM in response to bleomycin-induced DNA damage (Figs. 1b and 2a). ATM \( ^{\text{FATC}} \), ATM \( ^{\text{DNAPK}} \), and ATM \( ^{\text{Trrap}} \) were all able to complement the increased sensitivity of A7 cells to ionizing radiation, although they were all slightly less effective then ATM. Figs. 1 and 2 demonstrate that the FATC domain of ATM is required for the formation of ATM-Tip60 complexes, the activation of ATM kinase activity, and the subsequent phosphorylation and acetylation of ATM. Further, the FATC domains of Trrap, Atr, and DNA-PK are functionally equivalent, implying that the FATC domains of these proteins mediate association with Tip60.

Both ATM (Fig. 1b) and Trrap (1, 25, 26) have been previously found in association with the Tip60 protein. Here, we examined whether DNA-PKcs were also associated with Tip60. To identify the potential association between DNA-PKcs and Tip60, cell extracts were immunoprecipitated with Tip60 antibody and co-precipitating DNA-PKcs detected by Western blot analysis (Fig. 3a). DNA-PKcs was co-precipitated with Tip60 antibody, but not IgG (Fig. 3a, lanes 1 and 2). The levels of DNA-PKcs in whole cell extracts (lane 5) and immunoprecipitated with DNA-PKcs (lane 4) are shown for comparison. To further confirm this interaction between Tip60 and DNA-PKcs, we utilized HeLa cells that stably express HA-Tip60 (described in Refs. 20, 27). Fig. 3b demonstrates that immunoprecipitation of HeLa cells expressing HA-Tip60 with DNA-PKcs antibody yielded both DNA-PKcs and HA-Tip60. The reciprocal experiment, in which cells were immunoprecipitated with HA antibody, also yielded both HA-Tip60 and DNA-PKcs. Furthermore, this interaction between Tip60 and DNA-PKcs was constitutive, because there was no detectable change in the interaction between DNA-PKcs and HA-Tip60 after exposure to bleomycin (Fig. 3b). The Tip60-DNA-PKcs immune complex also contained associated Ku70 protein (supplemental Fig. 2a), a regulatory subunit of DNA-PKcs. Further, the Tip60-DNA-PKcs complex could not be dissociated by treatment with ethidium bromide (supplemental Fig. S2, panel b), demonstrating that DNA is not required for the interaction between Tip60 and DNA-PK. DNA-PKcs and Tip60 are therefore components of a complex containing the Ku70 regulatory protein in vivo. We also examined whether Atr could also associate with Tip60. However, no significant interaction between endogenous Atr and Tip60 was detected (data not shown). Whether this is a technical problem related to antibody specificity or availability of epitopes, or because Atr does not interact with Tip60 in vivo, is not known.

We previously demonstrated that the HAT activity of the Tip60 bound to ATM was specifically activated in response to DNA damage (20). Because DNA-PKcs is constitutively associated with Tip60, the HAT activity of Tip60 associated with DNA-PK may also be activated in response to DNA damage. Trrap is a key component of NuA4, a chro-
Function of FATC Domains

![Diagram](https://example.com/diagram.png)

**FIGURE 3.** ATM, DNA-PKcs, and Trrap associate with Tip60. a, HeLa cells were immunoprecipitated with IgG (lanes 1 and 2), DNA-PKcs antibody (DPK, lane 4), or Tip60 antibody (lane 2). Whole cell extract (WCE) was added to assess the fraction of DNA-PKcs associated with Tip60. Immunoprecipitates were separated by SDS-PAGE and DNA-PKcs measured by Western blot analysis. b, HeLa cells stably expressing HA-Tip60 were exposed to solvent (−) or bleomycin (+) (5 μM for 30 min) and immunoprecipitated with IgG, DNA-PKcs antibody, or HA antibody (to detect HA-Tip60). Immunoprecipitates were then examined for the presence of DNA-PKcs and co-precipitating HA-Tip60 by Western blot analysis (WB). c and d, HeLa cells were treated with solvent (−) or bleomycin (+) (5 μM for 30 min). Cell extracts were prepared and individually immunoprecipitated with either IgG or antibodies to Tip60, ATM, DNA-PKcs, or Trrap. c, the presence of Tip60, ATM, DNA-PKcs, and Trrap in each immunoprecipitate was confirmed by Western blot. d, immunoprecipitates were resuspended in HAT assay buffer and allowed to acetylate a peptide derived from the N-terminal of histone H4. HAT activity associated with each protein was then measured by enzyme-linked immunosorbent assay. Results ± S.D. (n = 3).

matin-remodeling complex that contains the Tip60 protein (13). In yeast, NuA4 is recruited to sites of DNA damage where it may acetylate histones at sites of DNA strand breaks (28). Thus, the Tip60 bound to NuA4 (via Trrap) may also be up-regulated in response to DNA damage. We therefore examined whether the HAT activity of the ATM-Tip60, DNA-PKcs-Tip60, and Trrap-Tip60 complexes was increased in response to bleomycin treatment.

Tip60, ATM, DNA-PKcs, or Trrap were immunoprecipitated and the associated HAT activity monitored using an N-terminal peptide derived from histone H4 that includes lysine residues specifically acetylated by Tip60 (25). Fig. 3c demonstrates the efficient immunoprecipitation of Tip60, ATM, DNA-PKcs, and Trrap from cell extracts, and Fig. 3d details the associated HAT activity. Cells immunoprecipitated with IgG displayed minimal HAT activity (Fig. 3d). To examine the total cellular pool of Tip60, cells were immunoprecipitated with Tip60 antibody, which potentially isolates all cellular Tip60 complexes. Significant amounts of Tip60 HAT activity were detected in unstimulated cells, and the HAT activity of this Tip60 was increased 2.5-fold in cells exposed to bleomycin (Fig. 3d). As we previously reported, ATM has significant amounts of Tip60 HAT activity associated with it, and this activity is increased 6-fold in response to bleomycin (Fig. 3d). DNA-PKcs immunoprecipitated from untreated cells contained minimal amounts of HAT activity; however, exposure to bleomycin caused a significant increase in the HAT activity associated with DNA-PKcs. Because we did not detect any change in the interaction between DNA-PKcs and Tipp60 (Fig. 3a), this change presumably reflects activation of the HAT activity of Tip60. The level of DNA-PKcs-associated HAT activity is only a small percentage of the total cellular Tip60 HAT activity and is significantly less than that detected in association with ATM (Fig. 3d). Because DNA-PKcs is a highly abundant cellular protein compared with ATM, this implies that only a small fraction of the DNA-PKcs protein is associated with Tip60, a conclusion supported by the low levels of Tip60 that coprecipitate with DNA-PKcs during immunoprecipitation (Fig. 3, a and b). The effect of bleomycin on the HAT activity of the Trrap complex was also measured. The yeast NuA4 complex contains both Trrap and Tip60 (1) and is recruited to sites of DNA damage (28). However, although the Trrap-Tip60 complex contained significant amounts of HAT activity, this activity was not increased by exposure to the DNA-damaging agent bleomycin (Fig. 3d). Fig. 3d therefore demonstrates that the majority of the increase in Tip60 HAT activity in response to bleomycin is due to activation of the Tip60 associated with ATM. The DNA-PKcs complex makes a small contribution to the increased HAT activity, whereas the Trrap complex does not contribute any significant increase in HAT activity. However, the high levels of basal HAT activity associated with Trrap are consistent with its known role in histone acetylation during normal cell cycle functions (1, 13).

The presence of a DNA-PKcs-Tip60 complex in cells and the activation of this DNA-PKcs-associated HAT activity implies that Tip60 activation may be required for the activation of the DNA-PKcs protein. Recent studies have shown that DNA-PKcs undergoes autophosphorylation at multiple sites in response to DNA damage (29). At least seven sites have been identified, with the majority clustered in a 38-amino acid domain located between amino acids 2609 and 2638 (29, 30). Mutations in these sites have been shown to impair double strand break repair and increase cellular sensitivity to ionizing radiation (30–35). Although the exact role of these sites in regulating DNA-PKcs is still under investigation, autophosphorylation at serine 2056 and threonine 2609 has now been demonstrated to occur in vivo (32, 34). Further, mutations in these amino acids affect repair of DNA strand breaks and the ability of cells to survive exposure to ionizing radiation (32, 34). To test whether Tip60 was required for the autophosphorylation of DNA-PKcs, we examined whether silencing of Tip60 expression with siRNA blocked the autophosphorylation of DNA-PKcs at serine 2056 and threonine 2609. In Fig. 4, Tip60 expression was reduced by expression of Tip60 siRNA but not by control green fluorescent protein siRNA. The role of Tip60 in the autophosphorylation of DNA-PKcs and the interaction of DNA-PKcs with the Ku70/Ku80 heterodimer was examined next. Immunoprecipitated DNA-PKcs underwent rapid autophosphorylation on both serine 2056 and threonine 2609 in response to bleomycin (Fig. 4). In cells depleted of Tip60 by siRNA, autophosphorylation of serine 2056 and threonine 2609 of DNA-PKcs was greatly reduced. This demonstrates a requirement for the Tip60 protein for the autophosphorylation...
Silencing of Tip60 expression in whole cell extracts (WCE) was confirmed by Western blotting with Tip60 antibody.

Although serine 2056 of DNA-PKcs is a well-defined autophosphorylation site, autophosphorylation of these residues has been reported to be essential for DNA-PKcs to regulate both DNA repair and ATM. This implies that the FATC domains of PIKK proteins function to recruit the Tip60 HAT to these protein complexes. This conclusion is supported by the demonstration that the ATM (20), DNA-PKcs (Fig. 3), and Trrap proteins (25) have all been found in association with Tip60 in vivo. Although the FATC domain of Atr could mediate recruitment of Tip60 when inserted into the ATM protein, we were unable to detect any interaction between Atr and Tip60. Whether this is due to technical problems in detecting this interaction or due to an alternative function for the FATC domain of Atr is not currently known. Interestingly, recent work indicates that the FATC domain of Mec1 (the yeast homologue of Atr) is required for Mec1 to associate with RPA (23). This suggests that the FATC domain of Atr may share some common functions with the FATC domains of ATM, DNA-PKcs and Trrap as well as associated with specific proteins such as RPA.

Our results provide several lines of evidence that indicate a crucial role for the Tip60 HAT in the activation of DNA-PKcs kinase activity. DNA-PKcs was constitutively associated with Tip60 in vivo, and the HAT activity associated with DNA-PKcs was increased ~5-fold in response to bleomycin treatment. Further, silencing of Tip60 expression blocked the autophosphorylation of DNA-PKcs at serine 2056 and threonine 2609. Autophosphorylation of these residues has been reported to be essential for DNA-PKcs to regulate both DNA repair and cell survival (32, 34). We interpret our results to indicate that the activation of DNA-PKcs kinase activity proceeds through a mechanism involving the Tip60 histone acetyltransferase and may involve the acetylation of key components of the DNA-PK complex by Tip60. Several other proteins, including the Ku70/Ku80 heterodimer (34) and the MDC1 protein (38), are also required for the recruitment of DNA-PKcs to sites of DNA damage and for the activation of its kinase activity. Whether the Ku70, Ku80, or Mdc1 proteins mediate the activation of Tip60 bound to DNA-PKcs is not known. Finally, both ATM and DNA-PKcs are activated in response to genotoxic agents that give rise to DNA strand breaks. The finding that DNA-PKcs and ATM share a common activating step suggests that Tip60 plays a key role in the early detection and activation of both PIKK proteins in response to DNA strand breaks.

Trapp is the key organizing component of the NuA4 chromatin-remodeling complex (I), a 16-subunit complex containing both ATPase activity and HAT activity. The HAT activity of NuA4 is supplied by Tip60 (25), and the C-terminal of Trapp (including the FATC domain) is essential for the Trapp complex to recruit HAT activity to NuA4 (26). These results are consistent with the FATC domain of Trapp recruiting Tip60 to the NuA4 complex. In yeast, NuA4 has been shown to be recruited to sites of DNA damage through association with γH2AX, where it may regulate chromatin structure during DNA repair (28, 39). However, unlike the ATM-Tip60 and DNA-PKcs-Tip60, the HAT activity of the NuA4-Tip60 complex was not measurably increased in response to DNA damage. The recruitment of the active NuA4-Tip60 complex to sites of DNA damage, rather than up-regulation of the associated Tip60 HAT activity, may therefore be the main mechanism for NuA4-Tip60 function during DNA repair.

Several studies have examined the function of the FATC domain of PIKK proteins. In ataxia telangiectasia (AT) patients, truncating mutations within the C-terminus that delete part or all of the FATC domain are associated with decreased kinase activity (40). Mutations (18) or truncations (17) in the FATC domain of DNA-PKcs lead to decreased expression of DNA-PKcs protein and reduced intrinsic kinase activity. In mec1, the yeast homolog of Atr (23), and mTor (16), deletion or mutation of the FATC domain also reduces the intrinsic kinase activity of these proteins. Here, deletion of the FATC domain of ATM resulted in a small reduction in ATM protein levels and loss of the DNA damage-
Function of FATC Domains

induced increase in ATM kinase activity. These observations demonstrate that the FATC domain of PIKK proteins is essential for the kinase activity of PIKK proteins. Further, the FATC domains of Trp1 (26), ATM (20), and DNA-PKcs (this report) can function as sites to recruit HAT activity. Overall, this indicates two key functions for the FATC domain of PIKK proteins: to provide a binding domain for regulatory proteins such as Tip60 (ATM, Trp1, DNA-PKcs) or RPA (Atr) and to regulate the activity of the kinase domain. However, whether this association between Tip60 and the FATC domains of PIKK proteins is direct or is mediated by an intermediary protein is not known.

Structural analysis of DNA-PKcs has provided a low resolution three-dimensional structure for DNA-PKcs that has been used to predict the architectural of the ATM protein (19). In this model, the FAT and FATC domains protruding from this central core region (19). Additional studies have shown that the FATC domain of Tor1 exists as a highly flexible α-helical structure (19, 41). Taken together, these structural studies have led to the proposal that changes in the conformation of the FAT and FATC domains can influence the catalytic activity of ATM (19, 41). These structural studies are consistent with reports that disruption of the FATC domain (either by mutation or truncation) greatly reduces the intrinsic kinase activity of DNA-PKcs (17, 18), Mec1 (23), Tor1 (16), and ATM (this study). The FATC domain may therefore function as a highly flexible detector that regulates the kinase domain through conformational changes. The association of Tip60 with the FATC domain may mediate this conformational effect through acetylation within the FAT/kinase domain/FATC structure, resulting in conformational changes in the FATC domain that are then communicated to the kinase domain of ATM or DNA-PKcs. Taken together, our results indicate that the FATC domain of PIKK proteins is an essential regulatory domain that can mediate the association of these proteins with Tip60 or other regulatory proteins. Further studies on the three-dimensional structure of FATC domain of these proteins will provide crucial insights into these functions.

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