ATM regulates DNA synthesis and binds to the replication machinery.

**Significance:** ATM kinase inhibition affects DNA replication in a different manner to ATM protein disruption.

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**Background:** The role of ATM kinase activity in DNA replication was unknown.

**Results:** ATM kinase inhibition, but not ATM protein disruption, impedes DNA replication, and ATM physically and functionally interacts with proliferating cell nuclear antigen to regulate DNA synthesis.

**Conclusion:** ATM regulates DNA synthesis and binds to the replication machinery.

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Ataxia telangiectasia (A-T) is a pleiotropic disease, with a characteristic hypersensitivity to ionizing radiation that is caused by biallelic mutations in A-T mutated (ATM), a gene encoding a protein kinase critical for the induction of cellular responses to DNA damage, particularly to DNA double strand breaks. A long known characteristic of A-T cells is their ability to synthesize DNA even in the presence of ionizing radiation-induced DNA damage, a phenomenon termed radioresistant DNA synthesis. We previously reported that ATM kinase inhibition, but not ATM protein disruption, blocks sister chromatid exchange following DNA damage. We now show that ATM kinase inhibition, but not ATM protein disruption, also inhibits DNA synthesis. Investigating a potential physical interaction of ATM with the DNA replication machinery, we found that ATM co-precipitates with proliferating cell nuclear antigen (PCNA) from cellular extracts. Using bacterially purified ATM truncation mutants and in vitro translated PCNA, we showed that the interaction is direct and mediated by the C terminus of ATM. Indeed, a 20-amino acid region close to the kinase domain is sufficient for strong binding to PCNA. This binding is specific to ATM, because the homologous regions of other PIKK members, including the closely related kinase A-T and Rad3-related (ATR), did not bind PCNA. ATM was found to bind two regions in PCNA. To examine the functional significance of the interaction between ATM and PCNA, we tested the ability of ATM to stimulate DNA synthesis by DNA polymerase δ, which is implicated in both DNA replication and DNA repair processes. ATM was observed to stimulate DNA polymerase activity in a PCNA-dependent manner.

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**Conclusion:** Radioresistant DNA synthesis is caused by a defect in the inhibition of late origin firing in A-T cells following ionizing radiation (2). The contribution of DNA chain elongation arrest to the intra-S-phase checkpoint has been difficult to establish, because the DNA lesions that activate the checkpoint also directly arrest replication fork progression. However, early reports suggest that A-T cells have a slightly longer S-phase and contain an increase in replication intermediates relative to controls (3, 4).

Several observations suggest that mere checkpoint abrogation is unlikely to be the cause for the cellular radiosensitivity of A-T cells. For instance, holding irradiated A-T cells in prolonged periods of either G1 or G2 and thus giving them ample time to repair the damage (as would occur under wild-type conditions), still leads to increased levels of chromosome aberrations in A-T cells (5). The requirement of A-T mutated (ATM) for the repair of a small fraction of double strand breaks after γ-irradiation (6) or after cleavage by the endonuclease I-Ppol (7) directly implicate ATM in signaling to the DNA-repair machinery. It was proposed that ATM is particularly needed for the repair of double strand breaks in heterochromatin (8).
The gene defective in A-T patients, ATM, encodes a serine/threonine protein kinase that is critical for the induction of cellular responses to DNA damage, particularly to DNA double strand breaks (9). ATM kinase activity is increased following exposure to as little as 0.05 gray (Gy) of ionizing radiation and following the introduction of just two DNA double strand breaks (10). More than 1000 substrates of ATM and the related kinase A-T and Rad3-related (ATR) have been identified (11, 12). Although gene ontology analyses identified groups of substrates implicated in biological processes as diverse as immunity and defense, cell proliferation and differentiation, intracellular protein traffic, cell structure and motility, and the cell cycle, the largest group of 202 substrates was categorized as nucleoside, nucleotide, and nucleic acid metabolism (11). Of these 202 protein substrates, 46 are proteins implicated in DNA replication, recombination, and repair, and these include the large and fourth subunit of DNA polymerase ɛ as well as DNA polymerase θ and DNA polymerase λ (11). Neither the effects of a selective ATM kinase inhibitor on DNA replication nor the functional significance of these phosphorylations on DNA polymerase substrates have been reported. To our knowledge, whether small molecule ATM kinase inhibitors induce radioresistant DNA synthesis has not been investigated.

We previously reported that ATM kinase inhibition, but not ATM protein disruption, blocks sister chromatid exchange, a phenotype attributed to the repair of damaged DNA replication forks, following DNA damage (13). Here we show that ATM kinase inhibition, but not ATM protein disruption, also inhibits DNA synthesis. Investigating a potential physical interaction of ATM with the DNA replication machinery, we found that ATM interacts with proliferating cell nuclear antigen (PCNA) both in vivo and in vitro. PCNA was originally characterized as an essential component of the eukaryotic DNA replication machinery wherein it functions as a DNA sliding clamp that enhances the processivity of replicative DNA polymerases (14). Subsequently PCNA was found to recruit other DNA-modifying enzymes, including repair proteins as diverse as DNA ligase 1, XP-G, Fen1, and MSH2 and -6 (15).

Using purified ATM truncation mutants we show that the interaction between ATM and PCNA is direct and mediated by the C terminus of ATM. A 20-amino acid region close to the ATM kinase domain was found to be sufficient for strong binding to PCNA. The peptide sequence mediating the interaction (ATM PBP) is distinct from previously identified PCNA-interacting motifs such as the PIP and KA boxes, and the observed binding is specific to ATM, because the homologous regions of other PIKK members, including the closely related kinase A-T and Rad3-related, do not bind PCNA. Because PCNA is known to stimulate DNA synthesis by DNA polymerase δ (polδ), in both DNA replication and several DNA repair processes, we tested the effect of ATM PBP in an in vitro DNA synthesis assay. We show that ATM stimulates DNA polymerase δ activity in a PCNA-dependent manner.

**EXPERIMENTAL PROCEDURES**

**Cell Culture, Transfection, and Expression Vectors**—H460 large cell lung cancer cells were cultured in RPMI, and IMR90 lung fibroblasts, 293T embryonic kidney cells, and U2OS osteosarcoma cells were kept in DMEM, both supplemented with 10% fetal calf serum. Transfections were conducted according to the manufacturers’ instructions using FuGENE6 (Roche Applied Science) for U2OS cells and Lipofectamine (Invitrogen) for 293T cells. Expression vectors for ATM without the 3′-untranslated region (UTR) were constructed by cutting a previously described ATM expression vector containing the 3′-UTR (16) with Bsu36I and XhoI and inserting an ATM C-terminal DNA sequence lacking the 3′-UTR, obtained by amplification with the appropriate primers.

**In Vivo DNA Synthesis Assays**—Cellular DNA synthesis was measured by subsequent incubation with medium containing 14C- or 3H-labeled thymidine as described (2). Incubation of cells with 14C was for 16 h, with 3H for 30 min. In the case of reconstitution experiments ATM knockdown cells were labeled with 14C before transfection with the indicated ATM expression vector. Tritium labeling was done 24 h after transfection.

**Antibodies, Inhibitors, and Irradiation**—Antibodies against ATM were purchased from Sigma; those against PCNA and heat shock cognate 70 (HSC70) were from Santa Cruz Biotechnology. KU60019 (Kudos Pharmaceuticals) was used at 1 μM concentration. Cells were γ-irradiated in a Shepherd Mark I Model 68 137Cs irradiator (J. L. Shepherd & Associates).

**In Vivo Interaction Assays**—Whole cell lysates of H460 or U2OS cells were prepared by washing cells in PBS, lysing in TGN buffer (150 mM NaCl, 5 mM NaF, 1% Tween 20, 0.5% Nonidet P-40, 50 mM Tris-HCl, pH 7.5, protease inhibitors) on ice for 30 min and twice clearing by centrifugation. For immunoprecipitation of endogenous PCNA, lysates were incubated with antibodies against PCNA for 5 h and precipitated after four washes with TGN buffer. Anti-rabbit immunoglobulins served as the negative control. The immunoprecipitates with Protein A/G-agarose beads were tested for PCNA and ATM by immunoblots. Alternatively, in the case of exogenous PCNA, FLAG-tagged PCNA or hemagglutinin (HA)-tagged ATM was expressed in U2OS cells. 48 h after transfection the cells were washed, and the lysate was cleared by centrifugation and incubated with M2-agarose for 8 h. After washes with BC buffer (20 mM Tris-HCl 7.9, 20% glycerol, 0.2 mM EDTA, 0.5 mM PMSF, 1 mM DTT) with 150 mM KCl, the beads were boiled in reducing SDS buffer for elution. Inputs and eluates were examined by immunoblotting with antibodies against PCNA and ATM. In the case of the reciprocal immunoprecipitation, 293T cells were transfected with FLAG-tagged ATM and co-precipitation of ATM and PCNA was assessed in the same way. When investigating DNA dependence on the co-immunoprecipitations, lysates were incubated with M2-agarose in the presence or absence of 20 μg/ml ethidium bromide (Invitrogen) or 100 units of DNase I (Roche Applied Science).

**In Vitro Interaction Assays**—GST-fused proteins were expressed in Rosetta(DE3)pLysS cells at 30 °C and harvested 4–4.5 h after induction with 0.4 mM isopropyl 1-thio-β-d-galactopyranoside in bacterial lysis buffer (20 mM HEPES (pH 7.9), 500 mM NaCl, 1 mM EDTA, 10% glycerol, 0.1% Nonidet P-40, 1 mM DTT, 0.5 mM PMSF). Lysates were obtained by sonicating the bacterial resuspension and were cleared by ultracentrifugation (Sorvall T647.5, 35,000 rpm, 30 min, 4 °C).
Appropriate amount of lysates were incubated with 10 μl of glutathione-Sepharose for at least 6 h and washed three times with BC buffer with 300 mM KCl and once with BC buffer with 100 mM KCl. Proteins were labeled with 35S-labeled methionine using TsT reticulate lysates, following the standard protocol provided by Promega (Madison, WI). In vitro translated proteins were incubated with resin-bound proteins (10 μg) by rotating at 4 °C for 4 h in BC buffer containing 100 mM KCl and 0.05 μg/μl BSA. After several washes with BC buffer containing 100 mM KCl and 300 mM KCl, the resin was boiled in SDS loading buffer before subjecting to SDS-PAGE. Gels were incubated in AmplifyTM (Amersham Biosciences) before drying and autoradiography to enhance the signal.

In Vitro DNA Synthesis Assays—DNA synthesis assays were carried out as described previously (17) with minor modifications. Briefly, a 5'-end-labeled primer (41 nucleotides: 0.05 pmol) annealed to a template (94 nucleotides) was extended by DNA polymerase δ (pol δ: 0.1 pmol) in the absence or presence of PCNA (0.25 pmol of trimer), replication factor C (RFC) (0.05 pmol), and indicated peptides for 15 min at 37 °C in a 40-μl reaction buffer containing 20 mM HEPES (pH 7.5), 100 mM NaCl, 10 mM MgCl2, 2 mM ATP, 40 μM dNTP mix, 0.1 mg/ml BSA, and 0.01% Triton X-100. All the peptides used in the DNA synthesis assays were diluted in DMSO, and all the reactions were adjusted to a final concentration of 2.5% DMSO, which did not interfere with DNA synthesis by pol δ. Reaction products were resolved by electrophoresis in denaturing 12.5% polyacrylamide gels and analyzed by using a PhosphorImager. ATM peptides consisted of a fluorescein-labeled tat-fused peptide from ATM, GRKKRRQRRRPPQVLTIQSFKAERFLAGGVNLPK, and its mutant derivative, GRKKRRQRRRPPQVLHEQFKAERFLAGGVNLPK.

RESULTS

Acute ATM Kinase Inhibition Suppresses Cellular DNA Synthesis—We recently showed that ATM kinase inhibition, but not ATM protein disruption, blocks sister chromatid exchange following DNA damage (13). To determine whether ATM kinase inhibition also influences DNA synthesis, we labeled the DNA of IMR90 human lung fibroblasts expressing shRNA, which disrupts ATM, or of a GFP-expressing control line (Fig. 1C, left panel) with 13C-labeled thymidine for one cycle. Cells where then divided and treated with the ATM kinase inhibitor KU60019 or vehicle from 30 min prior to a pulse of 30 min with 3H-labeled thymidine to the end of the pulse. At the end of the pulse the cells were harvested, and the incorporation of 3H and 14C into high molecular weight material was then measured. The ratio of 3H over 14C was used as a measure for relative DNA synthesis. Treatment with ATM inhibitor more than halved thymidine incorporation during the observed time interval in IMR90 cells, whereas DNA synthesis was not affected in IMR90 with disrupted ATM (Fig. 1A). The observed inhibitory effect of KU60019 on DNA synthesis is therefore ATM-dependent. Similarly, we tested the H460 large cell lung cancer cells with wild-type or knockout levels of ATM (Fig. 1C, right panel). Again KU60019 inhibited DNA synthesis in an ATM-dependent manner (compare the first two lanes in the left and right panels in Fig. 1B). Irradiation of H460 with 5 Gy of ionizing radiation decreased DNA synthesis in an ATM-dependent manner (compare the first and third lanes in the left and right panels in Fig. 1B) as expected given the well established phenomenon of radioresistant DNA synthesis caused by ATM deficiency. Treatment with KU60019 decreased DNA synthesis even further in irradiated IMR90 with wild-type ATM levels. We next wanted to test whether a kinase-inactive ATM mutant showed a similar phenotype as observed with ATM inhibition. To do so we reconstituted ATM knockdown cells with wild-type or mutant ATM. The mutant ATM, ATM D2870A,N2875K, has been described previously (16) and lacks kinase activity. Fig. 1D shows part of the 3'-UTR of ATM mRNA. The underlined nucleotides are targeted by the shRNA vector used to make stable knockdown cell lines. A Bsu36I/XhoI double restriction of the expression vectors as well as sequencing was used to verify the lack of 3'-UTR in the wild-type and kinase activity-deficient ATM (Fig. 1E and under “Experimental Procedures”). An ATM vector lacking the 3'-UTR, but not an ATM vector containing the 3'-UTR, was able to reconstitute the ATM knockdown cell line (Fig. 1F). ATM knockdown cells expressing similar amounts of wild-type or kinase activity-deficient ATM (Fig. 1G) were used for DNA synthesis assays. Although wild-type ATM did not affect DNA synthesis, ATM lacking kinase activity strongly decreased DNA synthesis (Fig. 1H). Abolition of kinase by mutation and inhibition thus show the same phenomenon. The ATM inhibition-mediated stalling of DNA replication is associated with activation of Chk1 signaling as evidenced by phosphorylation of Chk1 at serine 317 (Fig. 1I) observed 1 h after ATM inhibition. Given that acute ATM inhibition, but not ATM disruption inhibited DNA synthesis, we speculated that ATM might interact with the replication machinery per se and interfere with DNA synthesis if kinase-inactivated.

ATM Interacts with PCNA in Vivo—PCNA plays a key role in DNA replication and DNA repair by forming a sliding homotrimeric ring around DNA that serves as a docking platform for the recruitment of various DNA-modifying enzymes to the replication fork. Such enzymes include nucleases, ligases, helicases, and most importantly DNA polymerases (14). By tethering these enzymes to the DNA template, PCNA increases the processivity and catalytic activity of the machineries involved in DNA synthesis.

Because of the unique ubiquitous role of PCNA in DNA synthesis processes and to study the role of ATM in DNA synthesis, we tested whether ATM binds to PCNA. Whole cell lysates of H460 cells were incubated with antibodies against PCNA and precipitated. Anti-rabbit immunoglobulins served as negative control. The immunoprecipitates with Protein A/G-agarose beads were tested for PCNA and ATM by immunoblotting. ATM co-precipitated with PCNA independently of cell irradiation or treatment with the ATM-specific kinase inhibitor KU60019 (Fig. 2A, compare lanes 1 to 4 and lanes 5 to 8). To further corroborate the physical interaction of ATM with PCNA in vivo, FLAG-tagged PCNA or HA-tagged ATM were expressed in U2OS cells and the tagged PCNA precipitated with M2 (anti-FLAG)-agarose. After repeated washes the eluates were tested for the presence of PCNA and ATM. Endogenous
ATM co-precipitated with PCNA, whereas no ATM was detected in the control cells transfected with HA-tagged ATM (Fig. 2B, compare lanes 1 and 2). Similarly, we expressed FLAG-tagged ATM in 293T cells (allowing better expression of ATM than U2OS) and found PCNA to co-precipitate with endogenous ATM (Fig. 2C). Lysate from untransfected cells served as control. To test whether the interaction of PCNA with ATM was mediated by DNA, we co-precipitated ATM and FLAG-tagged PCNA in the presence or absence of ethidium bromide or DNase. Neither treatment affected ATM co-precipitation with PCNA (Fig. 2D), indicating that DNA was neither bridging the interaction partners nor required for potential conformational changes associated with the interaction.
ATM Directly Interacts with PCNA in Vitro—ATM is a protein of ~350 kDa of 3056 amino acids. Despite its size, relatively few direct interaction partners have been identified to date. Examples of such binding partners include ATM itself (ATM forms a homodimer) (10), Tel2 (18), and the MRN complex (19). To establish whether PCNA binds ATM directly and to

**FIGURE 2. Direct interaction of ATM with PCNA.** A, in vivo interaction of ATM with PCNA. PCNA from H460 cells treated in the indicated way was precipitated with an antibody against PCNA. Bound material was washed and analyzed by immunoblotting. Mock precipitates from identically treated cells served as negative controls. B, exogenous PCNA interacts with endogenous ATM. U2OS cells were transfected with a vector for either HA-tagged ATM or FLAG-tagged PCNA. Inputs (4%) and anti-FLAG (M2-agarose) immunoprecipitates were tested for ATM and PCNA. C, exogenous ATM interacts with endogenous PCNA. HEK293T cells were transfected with a vector for FLAG-tagged PCNA or not. Inputs (4%) and anti-FLAG (M2-agarose) immunoprecipitates were tested for ATM and PCNA. D, the interaction between PCNA and ATM is not mediated by DNA. Cells were transfected with FLAG-tagged PCNA. The inputs and anti-FLAG immunoprecipitates from lysates in the absence or presence of ethidium bromide (EtBr) or DNase were tested for ATM. Lysates from untransfected cells served as negative control. E, verification and mapping of binary ATM-PCNA interactions. GST alone or GST-fused truncation mutants of ATM were bound to glutathione-Sepharose and incubated with in vitro translated, 35S-labeled PCNA. Beads were washed, and eluted proteins were analyzed by autoradiography after SDS-PAGE. The lower panel shows a scheme, and the upper right panel shows a Coomassie stain of GST-fused ATM fragments. The interaction site of the ATM with PCNA lies within the C-terminal regions F7 (2400–2700) and F8 (2680–3056) (upper left panel).
ATM Interaction with PCNA and DNA Synthesis

**Figure 3.** A and B, GST-fused truncation mutants of ATM were used to narrow down the interaction domain with PCNA. Interaction assays were done as described in Fig. 2E. ATM amino acids 2680–2700 (PBP) were sufficient to bind PCNA. C, scheme of GST-fused ATM truncations used. D, comparison of homologous regions in the PIKK family and their interaction with PCNA. GST-fused ATM PBP, but not the homologous peptides in other members of the PIKK family and their interaction with PCNA. GST-PCNA.

To further define the PCNA interaction sites for ATM PBP, we attempted to generate shorter PCNA truncations but found that several translated very poorly. Nevertheless, we were able to show with two reasonably expressing fragments that PCNA binding to ATM PBP does not bind to the PCNA interconnector loop (Fig. 4B, the interconnector loop spans PCNA amino acids 119–134), in agreement with the absence of a PIP consensus motif in the ATM PBP sequence.
ATM Interaction with PCNA and DNA Synthesis

ATM Stimulates PCNA-dependent DNA pol δ Activity—DNA pol δ is one of the three replicative polymerases and is believed to be the enzyme primarily responsible for lagging strand synthesis (27). Pol δ is also involved in DNA repair by filling the gap during mismatch repair and nucleotide excision repair and extending the invading strand in homologous recombination (28, 29). The activity of pol δ is strongly stimulated by PCNA and RFC, the clamp loader for PCNA (17) (Fig. 5A). To determine whether binding of ATM could influence PCNA-dependent DNA polymerase activity, we assayed DNA synthesis in vitro with recombinant proteins. In a primer extension assay containing pol δ and PCNA, DNA synthesis was stimulated by the addition of increasing amounts of an ATM-(2680–2700) peptide (compare lanes 1 to 3 in Fig. 5C). High concentrations of the same peptide inhibited pol δ in the same assay (lane 4, Fig. 5C). A quantitation of three independent experiments is shown in Fig. 5D.

Because the interaction of ATM PBP is not disrupted by 300 mM KCl (Fig. 3D), we speculate that the binding to PCNA is mediated by hydrophobic residues. We therefore hypothesized that mutating the ATM threonine 2682 and serine 2685 to the charged residue glutamic acid, which is also a phosphomimetic, would interfere with the binding of PBP to PCNA. We purified GST-ATM-(2680–2700) and GST-ATM-(2680–2700)-2682E,2685E (see Coomassie stain in Fig. 5B, lower panel) and tested the interaction of PCNA with these bead-immobilized proteins. The mutant ATM fragment bound PCNA 6–8 times weaker than wild-type (Fig. 5B, upper panel).

We then compared the ability of the wild-type and mutant ATM peptides, ATM-(2680–2700) and ATM-(2680–2700)-2682E,2685E, to stimulate pol δ in vitro. The wild-type ATM fragment stimulated DNA synthesis by pol δ to a greater extent than the mutant fragment compromised in PCNA binding (compare lanes 2 to 3 with lanes 5 to 6 in Fig. 5C). Similar results were observed when purified GST-ATM-(2680–2720) and GST-ATM-(2680–2720)-2682E,2685E were used in the DNA synthesis assay (data not shown). Unlike the wild-type ATM peptide, the PCNA-binding-impaired peptide did not inhibit pol δ at higher concentration (compare lanes 4 and 7 in Fig. 5C and see the quantitation in Fig. 5D).

Finally we tested the effect of wild-type and mutant ATM fragments on pol δ in the presence of both PCNA and RFC. Both wild-type and mutant ATM peptides inhibited DNA synthesis under these conditions, but the wild-type ATM peptide had a much stronger inhibitory effect than the mutant ATM peptide with the lower affinity for PCNA. In summary, these observations indicate that ATM does interact with PCNA and can influence PCNA activity in vitro. The interaction assays with PCNA deletion mutants suggest that ATM-(2680–2700) interacts with PCNA in the groove between two monomer domains (Fig. 4D). It could be that pol δ preferentially interacts with PCNA when one or more of three potential peptide binding sites on the trimer are unoccupied at low peptide concentration, whereas occupancy of the three binding sites at higher peptide concentration inhibits the interaction with pol δ. The maximum stimulatory effect on the peptide on DNA synthesis by pol δ and PCNA was 6- to 10-fold, whereas RFC stimulates the same reaction more than 100-fold as a consequence of active PCNA loading (Fig. 5D and data not shown).
FIGURE 5. A, DNA synthesis assays with DNA pol δ, PCNA, and RFC. A primer extension assay was used to test the effect of PCNA and RFC on DNA pol δ activity. A, mutant ATM-(2680–2720)-2682E,2685E peptide has a lower affinity for PCNA than wild-type ATM-(2680–2720). Interaction assays with wild-type and mutant ATM PBP show the lower affinity of the mutant with PCNA (upper panel shows a GST pulldown assay of in vitro translated PCNA; the lower panel shows a Coomassie stain of the baits.). C, DNA synthesis assays with DNA pol δ and PCNA with or without RFC were done in the presence or absence of wild-type or mutant ATM peptides. The PCNA-binding peptide of ATM-(2680–2700) (ATMwt) stimulates DNA synthesis several-fold in the absence of RFC and inhibits it in the presence of RFC. D, quantitation of DNA synthesis shown in C. ATM-(2680–2700) (ATMwt) shows a much stronger ability to stimulate PCNA-dependent DNA pol δ activity than ATM-(2680–2720)-2682E,2685E (ATMmut). This figure shows ratios of fully extended primers over total primers as determined by autoradiography. Error bars represent standard deviations of three independent experiments.
The inhibitory effect of the ATM peptide on RFC-stimulated DNA synthesis could be due to reduced loading of PCNA trimers bound by the peptide.

DISCUSSION

Following our initial observation that an ATM-specific inhibitor influences DNA synthesis in an ATM-dependent manner, we investigated whether ATM binds to PCNA, a docking platform for many factors involved in DNA replication and DNA repair. Surprisingly, we found that ATM and PCNA physically interact both in vivo and in vitro. We were able to define the ATM site sufficient for PCNA binding to a small region near the ATM kinase domain. This small peptide, ATM PBP, stimulates DNA pol δ in a PCNA-dependent manner in an in vitro assay.

ATM Binding to PCNA Does Not Involve a PIP Box—Unlike many other PCNA interacting proteins, the ATM fragment sufficient to bind PCNA does not have a motif that would fit the consensus of either a PIP box or a related KA box (described in Ref. 30). Consistent with the absence of a PIP box, this interaction is not mediated by the PCNA interconnector loop that plays an essential role in p21 (22), pol δ (p66), and FEN1 binding (23). ATM is a 350-kDa protein that forms a dimer in solution when in its inactive state (10). Future studies will determine whether ATM binds to PCNA as a monomer or as a dimer. Our interaction data with ATM fragments suggest that ATM dimerization is not needed for PCNA binding, and it is probable that monomerization is needed to expose the region close to the kinase domain for the interaction. Of note, theoretically trimeric PCNA contains three identical binding sites for a partner. Nevertheless, given ATM’s size it is unlikely that three ATM molecules could simultaneously bind the trimeric ring. Instead it is possible that ATM and the DNA polymerase share PCNA as a docking platform during DNA synthesis.

Regulation of PCNA-ATM Interaction—Recent studies have shown that PCNA activity is highly regulated by post-translational modification (31). In yeast, monoubiquitylation of PCNA at Lys-164 facilitates the recruitment of translesion polymerases that enable DNA damage bypass, whereas polyubiquitylation of the same residue dislodges translesion synthesis polymerases from PCNA enabling a template-switching mechanism in the error-free pathway of post-replication repair. At least in yeast, PCNA is also SUMOylated at Lys-164 and Lys-127 resulting in the inhibition of recombination via recruitment of Srs2 or inhibition of cohesion establishment via repulsion of Eco1 respectively (32, 33). Future studies will show whether the interaction of PCNA with ATM is also regulated by post-translational modification of PCNA or - as in the case of p21, RFC1 and Fen1 (31) by phosphorylation of its binding partner.

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