Ataxia-telangiectasia-mutated (ATM) Is a T-antigen Kinase That Controls SV40 Viral Replication in Vivo*

Yuling Shi†, Gerald E. Dodson‡, Sophie Shaikh‡, Kathleen Rundell§, and Randal S. Tibbetts†

From the †Department of Pharmacology, University of Wisconsin Medical School, Madison, Wisconsin 53706 and the ‡Department of Microbiology-Immunology, Northwestern University, Chicago, Illinois 60611

Received for publication, September 30, 2005 Published, JBC Papers in Press, October 11, 2005, DOI 10.1074/jbc.C500400200

The eukaryotic DNA damage response represents a series of highly integrated and tightly regulated pathways that coordinate DNA repair, cell cycle, and homeostatic responses to abnormal DNA structures arising endogenously or following exposure to extrinsic genotoxic stimuli. Central to the DNA damage response are a pair of structurally and functionally related protein kinases, designated ATM† (ataxia-telangiectasia-mutated) and ATR (ATM-Rad3-related) belonging to the phosphoinositide 3-kinase-related kinase (PIKK) gene superfamily. ATM and ATR share a conserved carboxy-terminal catalytic domain and display highly overlapping substrate specificities in vitro (1–3). Substrates for ATM and ATR include the p53 and BRCA1 tumor suppressors among many other proteins involved in cell cycle checkpoint activation, DNA repair, and transcriptional regulation (1, 3). Mutations in ATM cause the cancer susceptibility-neurodegeneration syndrome, ataxia-telangiectasia (1, 4). ATM-deficient cells are grossly defective in the ionizing radiation (IR)-induced G1/S, intra-S phase, and G2/M checkpoints and are profoundly sensitive to IR and other agents that induce DNA double-strand breaks (DSBs) (1). Although structurally and functionally related to ATM, the major functions of ATR pertain to its roles in DNA replication (5). ATR prevents premature firing of DNA replication forks (6–10). Although null mutations in ATR are lethal, hypomorphic splicing mutations that reduce ATR protein levels are associated with a rare congenital condition known as Seckel’s syndrome (11–13).

The catalytic activity of ATM is rapidly up-regulated in response to IR and other DSB-inducing agents. Catalytic activation involves the trans-autophosphorylation of inactive, dimeric ATM on Ser-1981, followed by dissociation into active monomers (14). The trimeric complex of MRE11, RAD50, and NBS1 (MRN) facilitates ATM activation and ATM-dependent substrate phosphorylation through recruitment of ATM to DSBs and/or orientation of the ATM catalytic domain (15–18). Recent studies suggest that the recruitment of ATM is mediated by the carboxyl terminus of NBS1 (19, 20). Although ATR isolated from DNA-damaged cells does not show enhanced kinase activity, its recruitment to regions of stalled DNA replication is regulated through binding to the ATR-interacting protein (ATRIP) and replication protein A (RPA) (19, 21). Among many key substrates for ATR and ATM are the checkpoint effector kinases, CHK1 and CHK2, which are phosphorylated by ATM and ATR in response to DSBs and DNA replication stress, respectively (6, 22–24). CHK1 and CHK2 promote checkpoint arrest through phosphorylation and inactivation of CDC25 family phosphatases (25–28).

Virus infection can also elicit ATM-dependent DNA damage responses in mammalian cells. The E1b55K/E4orf6 proteins of adenovirus mediate the degradation of MRE11 and suppression of ATM activation, suggesting that subversion of the DNA damage response is an important aspect of the adenoviral replication cycle (29). More recently, it was shown that ATM is activated in response to infections with HIV or herpes simplex virus (HSV) (30–32). Remarkably, inhibition of ATM greatly reduces the magnitude of HIV and HSV infections in vitro, suggesting that the activity of ATM is central to the replication cycle of these viruses (30, 31). Thus, under some circumstances, viruses have co-opted endogenous checkpoint regulators to ensure their own efficient replication.

SV40 is a circular double-stranded DNA tumor virus that modulates cellular DNA damage checkpoints at several levels. The SV40 large tumor antigen (LTag) is an essential viral replicator that recruits host DNA replication factors to the viral origin and functions as the replicative SV40 DNA helicase. LTag is also a well characterized oncoprotein that mediates the degradation of MRE11 and suppression of ATM activation, suggesting that subversion of the DNA damage response is an important aspect of the adenoviral replication cycle (29). More recently, it was shown that ATM is activated in response to infections with HIV or herpes simplex virus (HSV) (30–32). Remarkably, inhibition of ATM greatly reduces the magnitude of HIV and HSV infections in vitro, suggesting that the activity of ATM is central to the replication cycle of these viruses (30, 31). Thus, under some circumstances, viruses have co-opted endogenous checkpoint regulators to ensure their own efficient replication.

SV40 is a circular double-stranded DNA tumor virus that modulates cellular DNA damage checkpoints at several levels. The SV40 large tumor antigen (LTag) is an essential viral replicator that recruits host DNA replication factors to the viral origin and functions as the replicative SV40 DNA helicase. LTag is also a well characterized oncoprotein that...
disrupts G1/S checkpoint control through binding and inactivation of pRB and p53 (33). LTag also disrupts S phase checkpoint control, causing uninterrupted rounds of cellular DNA synthesis (endoreduplication) through binding and inactivation of NBS1 (34). SV40 replication is itself regulated by host checkpoint control pathways in response to DNA damage. Extracts prepared from human cells exposed to IR, camptothecin, or alkalytating agents display reduced replication of SV40 ori-containing plasmids in vitro (35–37). The inhibition of SV40 replication following DNA damage has been ascribed to the inactivation and down-regulation of RPA, a single-stranded DNA-binding protein that is essential for SV40 replication in vitro (38–40). The phosphorylation of LTag may also contribute to the suppression of SV40 replication. Ser-120 and Ser-123 are inhibitory phosphorylation sites that must be dephosphorylated by PP2A for full activation of LTag replication potential in vitro (41–44). DNA-dependent protein kinase (DNA-PK) and casein kinase I phosphorylate LTag on Ser-120 in vitro and are thus implicated as negative regulators of SV40 replication (39, 43, 45). However, phosphorylation of Ser-120 in DNA-damaged cells has not been documented and the importance of DNA-PK for LTag phosphorylation in cells in vitro is unclear.

Our laboratory recently identified an ATM phosphorylation site in the cyclic AMP response element-binding protein (CREB) (46). In that study, we generated a phosho-specific antibody that recognizes the Ser-121 phosphorylation site of CREB, which is phosphorylated by ATM in cells in vitro following IR treatment. Here, we show that the phosho-CREB-121 antibody cross-reacts with LTag in extracts of irradiated mammalian cells. We demonstrate that ATM phosphorylates LTag on Ser-120 in vivo and that ATM-mediated phosphorylation of LTag regulates SV40 DNA replication. Our findings reveal a regulatory interaction between ATM and LTag that is required for optimal replication of SV40 in primate cells.

**EXPERIMENTAL PROCEDURES**

**Cell Culture and Antisera**—African green monkey CV1 cells and SV40 immortalized ATM+/+ and ATM−/− mouse embryo fibroblasts (MEFs) were maintained in DMEM containing 5% FCS. The ATM-deficient MEFs were kindly provided by Dr. John Petrini (Sloan Kettering Cancer Research Center). Human BJ fibroblasts and TERT-immortalized JM fibroblasts (47) were maintained in DMEM containing 10% FCS. Antibodies used in this study include: α-ATM (Santa Cruz Biotechnology), α-ATM-pS1981 (R&D Systems), α-CREB-pS121 (Novus Biologicals), α-CREB and α-γH2AX (Upstate Biotechnology), α-Chk2-pT68 and α-pS3-pS15 (Cell Signaling), α-RPA32 (Calbiochem), α-β-tubulin (Upstate Biotechnology), and α-ATM (GeneTex). γH2AX immunostaining was carried out as described (48).

**Plasmid Constructs and Transfections**—The p129 LTag expression plasmid was kindly provided by Dr. Janet Mertz (University of Wisconsin). Site-directed mutagenesis of this plasmid was carried out using the QuickChange method (Stratagene). 5 μg of plasmid DNA encoding wild-type LTag or the individual LTag phosphorylation site mutants were stably transfected into subconfluent, 10-cm dishes of BJ fibroblasts using FuGENE 6 transfection reagent (Roche Applied Science) according to the manufacturer’s instruction. Clonal outgrowth of the LTag-expressing cells occurred over a 3–5-week period. For ATM RNAi experiments we targeted the following ATM nucleotide sequence: (5′-AAUGAAGUCCAUUGCUAAUCA-3′). 60-mm dishes of subconfluent CV1 cells were transfected with 3 μg of annealed siRNA duplex (Dharmacon) using the calcium phosphate procedure as described previously (38). Cells were analyzed 48 h after transfection. The GST-T1 plasmid encoding LTag amino acids 5–172 was kindly provided by Dr. James Alewine (University of Pennsylvania). Recombinant GST-T1 was purified from Escherichia coli inclusion bodies by denaturation/renaturation. Briefly, a 50 ml culture of E. coli (strain BL-21) was induced with 0.1 mM isopropyl β-D-thiogalactopyranoside at 37 °C for 6 h. The cells were collected by centrifugation and ruptured by sonication in PBS containing protease inhibitors (0.2 mM phenylmethylsulfonyl fluoride, 10 μg/ml leupeptin, 10 μg/ml Aprotinin, and 5 μg/ml pepstatin A). Following addition of Triton X-100 (0.2%), the lysate was centrifuged at 20,000 × g for 20 min, and the pellet was resuspended in PBS containing 10% glycerol and protease inhibitors. Following centrifugation, the supernatant was dialyzed 1:2 in PBS and then sequentially dialyzed into PBS containing 10% glycerol and 2, 1, 0.5, and 0 μl urea. The dialyzed was clarified by centrifugation and stored at −80 °C until use.

**Protein Analysis and Kinase Assays**—Cell extraction and immunoblot procedures were performed as described (49), with the exception that protein extracts from SV40-infected CV1 cells were prepared by heating for 10 min in 2× SDS sample buffer (125 mM Tris–HCl, pH 6.8, 4% SDS, 20% glycerol, 10% β-mercaptoethanol, 0.05% bromphenol blue). ATM kinase assays were performed as described (49). Extracts were immunoprecipitated with 1 μg of control IgG or α-ATM (Ab-3, Calbiochem) at 4 °C for 2 h. The immunoprecipitates were washed and incubated with 2 μg of substrate per reaction. Large scale CREB-pS121 immunoprecipitations were performed as follows: 15 μg of CREB-pS121 antibody was incubated with 10 μg of whole cell extract prepared from irradiated (20 Gy) or unirradiated ATM+/+ or ATM−/− MEFs, together with 30 μg of protein A-Sepharose (Amersham Biosciences). The immunoprecipitations were resolved by SDS-PAGE and the gels stained with colloidal Coomassie Blue (Novex). Excised bands were sequenced using mass spectrometry performed by ProtTech Inc. (Pittsburgh, PA).

**Virus Procedures**—To produce SV40 virions in CV1 cells, a plasmid that contains the entire SV40 genome cloned via EcoRI sites into the kanamycin-resistant plasmid, pMK, was used (47). To attempt virus production from the LTag8120A plasmid, BstXI/BamHI fragments (SV40 nucleotides 4759 and 2533, respectively) were used to replace the WT sequences in pMK/SV40. EcoRI was used to release the SV40 genome from pMK; DNA was recircularized and then transfected into CV1 cells using DEAE-dextran. Virus-containing cell supernatants were maintained at −70 °C prior to use. SV40 was used to infect subconfluent 60 mm dishes of CV1 cells (m.o.i. = 3 or 10, as specified) in 0.4 ml of serum-free medium for 90 min at room temperature, on a rocking platform. Two milliliters of fresh medium were added and the cells incubated at 37 °C for the indicated lengths of time. SV40 DNA was purified from CV1 cells using QiAprep spin columns (Qiagen) as described (50). 0.2 to 0.1 α(v)/μCi of each DNA sample was digested with EcoRI prior to analysis by agarose gel electrophoresis. DNA bands were stained with ethidium bromide and quantified by densitometry where indicated. Thymidine incorporation assay was performed by pulse labeling triplicate cultures of SV40-infected cells with 10 μCi/ml [3H]thymidine (methyl-H-3) (ICN) for 12 h. Triplicate DNA samples were prepared, digested with EcoRI for 1 h, and resolved by agarose gel electrophoresis. Ethidium bromide-stained bands corresponding to the SV40 minichromosome were excised and solubilized in 500 μl of QX1 buffer (Qiagen). Incorporated [3H]signal was counted for 2 min in 15 ml of scintillation fluid in a Packard 1600CA fluid scintillation analyzer. Error bars are standard deviations from triplicate samples. Real-time PCR analysis of SV40 DNA levels was performed in an Applied Biosystems Prism Instrument, using the SYBR Green method based on protocols provided by the manufacturer. The primers used, SV40For3/ SV40Rev (SV40 4476–4453 and 4372–4399) were described previously (51).
ATM Phosphorylates Large T-antigen

RESULTS

Phospho-CREB Antibodies Identify SV40 LTag as a Candidate ATM Substrate—In our previous study, we generated a phospho-specific antibody that recognizes the Ser-121 phosphorylation site of CREB (α-CREB-pS121) (46). Immunoblotting experiments revealed that the α-CREB-pS121 antibody also recognized proteins with estimated molecular masses of 90 kDa (p90) and 100 kDa (p100) in extracts of irradiated MEFs (Fig. 1A). The p90 and p100 species were not observed in extracts prepared from irradiated ATM−/− MEFs, suggesting that they represent ATM-dependent phosphorylation products (Fig. 1A).

Large scale immunoprecipitation with α-CREB-pS121 was used to purify p90 and p100 from extracts of irradiated MEFs. The p90 species was highly abundant in immunoprecipitates prepared from irradiated, ATM−/− MEFs, whereas lower levels of p90 were observed in IPs prepared from unirradiated cells or ATM−/− MEFs (Fig. 1B). The band corresponding to p90 was sequenced by mass spectrometry (Fig. 1B), and a total of 11 derived peptide sequences yielded an unambiguous match to SV40 LTag (Fig. 1C). This result was consistent with the fact that the MEFs used for the immunopurification procedures had been immortalized by stable transfection with LTag.

To confirm that α-CREB-pS121 recognized LTag, we used the antibody to probe α-LTag immunoprecipitates prepared from control or irradiated ATM−/− and ATM−/+ MEFs. This experiment confirmed that α-CREB-pS121 showed strong reactivity with LTag prepared from irradiated ATM−/+ cells, although some residual reactivity was observed in the absence of ATM (Fig. 2A). From these findings we conclude that ATM phosphorylates LTag in vitro on one or more residues that are recognized by the α-CREB-pS121 antibody and that other kinases may also contribute to the phosphorylation of this site.

ATM Phosphorylates LTag on Ser-120 in Vivo and in Cellulo—Having identified LTag as a putative ATM substrate, we wished to identify which phosphorylation site(s) were recognized by the α-CREB-pS121 antibody. LTag contains six S/T-Q motifs that are candidate sites for phosphorylation by ATM and ATR. Three of the candidate phosphorylation sites, located at Ser-120, Ser-639, and Ser-665, occur in the sequence Asp-Ser-Glu (DSQ), which is identical to the Ser-121 phosphorylation site of CREB in cellulo (Fig. 2). To test whether ATM phosphorylates LTag on Ser-120, a glutathione S-transferase-LTag fusion protein (GST-T1) spanning amino acids 5–172 of LTag, was tested as a substrate in ATM immune complex kinase assays (49). SDS-PAGE and autoradiography revealed that ATM specifically phosphorylated the GST-T1 fragment over background levels obtained using a control rabbit serum (Fig. 2C). Of note, the phosphorylation of GST-T1 was weak in comparison to the phosphorylation of a GST-p53 fusion protein, indicating that this LTag amino-terminal fragment is a suboptimal substrate for ATM. Phosphorylation of GST-T1 by ATM was also observed using recombinant wild-type HA-ATM, but not kinase-dead HA-ATM, prepared from transiently transfected HEK 293T cells (Fig. 2D). These results suggest that ATM directly phosphorylates the amino-terminal region of LTag in vivo.

To determine which Ser or Thr residue in LTag conferred reactivity with α-CREB-pS121, we generated a panel of BJ fibroblast cell lines that stably express wild-type LTag or LTag mutants bearing Ala substitutions at each candidate ATM phosphorylation site. The stable cell lines were exposed to 20-Gy IR or mock irradiated, and cell extracts were analyzed by immunoblotting with α-CREB-pS121. A single Ser-120→Ala mutant abolished reactivity with the antibody, whereas the other Ala substitutions had little or no effect (Fig. 2E). This result corroborates the in vitro phosphorylation data and strongly implies that ATM phosphorylates LTag on Ser-120 in cellulo in response to DNA damage. For the remainder of this report we refer to the α-CREB-pS121 antibody as α-LTag-pS120.
ATM Phosphorylates Large T-antigen

FIGURE 3. ATM activation and LTag phosphorylation during lytic SV40 infection. A, the time course of LTag phosphorylation and ATM activation following infection of CV1 cells with SV40. CV1 cells were infected with SV40 (m.o.i. = 10) for the indicated lengths of time. Cell extracts were then analyzed by immunoblotting with α-LTag-pS120 and α-ATM-pS1981. The membrane was then reprobed with α-LTag and α-ATM antibodies. B, ATM activation and LTag phosphorylation correlate with peak SV40 DNA synthesis. CV1 cells were infected with SV40 (m.o.i. = 3) for 4–60 h and cell extracts immunoblotted with α-LTag-pS120 and α-ATM-pS1981 antibodies before being reprobed with α-LTag and α-ATM antibodies, respectively. The corresponding levels of SV40 minichromosome DNA are shown in the bottom panel.

LTag Is Phosphorylated by ATM during Lytic SV40 Infection—The above results showed that ATM phosphorylates LTag in stably transfected rodent cell lines in response to DNA damage. However, we wished to explore the ATM-LTag functional relationship in the more physiologic context of a lytic SV40 infection. Specifically, we sought to determine whether SV40 infection activated ATM kinase activity toward LTag. African green monkey CV1 cells, a permissive host for SV40, were infected with SV40 (m.o.i. = 10), and the levels of ATM autophosphorylation on Ser-1981 and LTag phosphorylation on Ser-120 were measured over a period of 39 h by immunoblotting. ATM autophosphorylation was strongly induced by 15-h post-infection (HPI) and remained at a nearly constant elevated level throughout the 39-h time course of the experiment (Fig. 3A). The phosphorylation of LTag on Ser-120 was also induced by SV40 infection and closely paralleled ATM activation. Ser-120 phosphorylation was detected by 15–19 HPI, which correlated with the accumulation of total LTag (Fig. 3A). This suggests that nascently synthesized LTag is phosphorylated on Ser-120 prior to the initiation of viral replication. A more extensive time course analysis revealed that ATM activation was not observed before 12 HPI and that both ATM autophosphorylation and LTag phosphorylation declined at late time points (48–60 h) after infection, when cells began to exhibit a viral cytopathic effect (CPE) (Fig. 3B). ATM activation and LTag phosphorylation closely paralleled the accumulation of full-replicated viral genomes, suggesting that both processes are linked to the viral DNA replication cycle (Fig. 3B).

SV40 Infection Induces the Phosphorylation of Endogenous ATM Substrates—Given that ATM underwent autophosphorylation following SV40 infection, we explored whether ATM-dependent signaling events were also induced during infection. Whole cell lysates from CV1 cells were prepared at various times after infection with SV40 and immunoblotted with a panel of phospho-specific antibodies detecting ATM phosphorylation sites in p53 (Ser-15), CREB (Ser-121), and CHK2 (Thr-68). The phosphorylation status of RPA32 was also monitored by electrophoretic mobility shift. The phosphorylation of p53, CREB, and RPA mirrored that of LTag in SV40-infected cells (Fig. 4A). CHK2 was also phosphorylated in CV1 cells, although with slightly delayed kinetics. Together, these findings suggest that SV40 infection activates ATM and induces ATM-mediated signaling events.

The phosphorylation of histone H2AX on Ser-139 is a highly sensitive marker for ATM activation and DSB induction in mammalian cells (52). The phosphorylated form of H2AX, designated γH2AX, rapidly accumulates in nuclear foci corresponding to sites of DNA damage. We therefore compared the γH2AX immunostaining pattern in CV1 cells exposed to IR or infected with SV40 for 24 or 38 h. Immunostaining with a γH2AX-specific mAb revealed a faint, diffuse staining pattern in untreated CV1 cells. As expected, IR exposure resulted in an intense, highly focal γH2AX immunostaining pattern (Fig. 4B). SV40-infected CV1 cells also exhibited intense γH2AX staining; however, the pattern of staining was qualitatively different from that of γ-irradiated cells. At 24 HPI SV40-infected CV1 cells exhibited intense nucleoplasmic staining with numerous γH2AX foci. The number and intensity of these cells were less than what was observed in cells exposed to 10-Gy IR (Fig. 4B). By 38 HPI the SV40-infected cells typically exhibited between 4 and 10 large, brightly staining foci in addition to strong nucleoplasmic staining. Interestingly, the nucleoplasmic staining was concentrated in perinucleolar regions. These findings provide additional support that SV40 activates ATM and suggest that SV40 infection may cause host cell DNA damage.

ATM Is Required for Optimal SV40 Replication in CV1 Cells—To determine whether infection-associated LTag phosphorylation was ATM-dependent, we transfected CV1 cells with an ATM-specific siRNA prior to infection with SV40 and measured LTag phosphorylation at 24 HPI. The phosphorylation of LTag on Ser-120 was strongly suppressed by prior transfection of an ATM-specific siRNA, indicating that ATM contributes to the phosphorylation of Ser-120 during infection (Fig. 5A). The reduced phosphorylation of LTag in ATM knockdown cells may be carried out by residual ATM or other cellular kinases, such as ATR or DNA-PK.
Previous studies have shown that Ala substitutions at Ser-120 drastically reduce SV40 virus replication in cultured monkey cells (53). This suggests that the phosphorylation of LTag Ser-120 by ATM may be an essential event in the viral replication cycle. Before testing this hypothesis, we first confirmed the replication defect of an SV40 mutant virus in our hands. Indeed, an SV40 plasmid expressing the LTag120A mutant failed to replicate or induce a CPE following its transfection into CV1 cells (Fig. 5B). Next, we tested whether prior transfection of ATM siRNA affected the steady-state levels of the SV40 minichromosome in SV40-infected CV1 cells. CV1 cells were transfected with control or ATM siRNA-transfected cells and levels of SV40 minichromosome DNA assessed by agarose gel electrophoresis and ethidium bromide staining. inhibition of ATM suppresses nascent SV40 DNA synthesis. CV1 cells were transfected with ATM siRNA 48 h prior to being infected with SV40 for 12 h. The cells were pulsed with [3H]thymidine and the amount of [3H]thymidine incorporation measured 12 h later as described under "Experimental Procedures."  

**DISCUSSION**

The activation of LTag replication potential requires a coordinated series of phosphorylation/dephosphorylation events leading to the assembly of double hexameric LTag complexes at the bipartite SV40 ori (54, 55). Ser-120 and Ser-123 are implicated as inhibitory sites that must be dephosphorylated by PP2A to fully activate LTag replication potential in vitro (41, 43, 55). Kinases previously implicated in Ser-120 phosphorylation include DNA-PK, an S/T-Q-directed kinase belonging to the PIKK superfamily, and CK1 (56). The phosphorylation of LTag by CK1 on Ser-120 and Ser-123 was shown to inhibit origin unwinding and DNA replication activity in vitro (43), and DNA-PK has a similar inhibitory effect when added to SV40 in vitro replication reactions (39). However, the relevance of DNA-PK or CK1 to LTag phosphorylation in vivo is uncertain, and until now, the regulation of Ser-120 phosphorylation in response to SV40 infection or specific stimuli has not been explored. Through the use of ATM-deficient cell lines and RNAi we have provided strong evidence that ATM is the major Ser-120 kinase that is activated by IR and during lytic SV40 infection. Our results do not rule out potential contributions of DNA-PK, CK1, and ATR. The α-LTag pS120 antibody can be used to test the roles of these kinases in LTag phosphorylation in response to diverse stimuli.

Our findings suggest that ATM-mediated phosphorylation of LTag on Ser-120 is required for optimal SV40 replication in cellulo. ATM activation and LTag phosphorylation were maximal during the period of peak viral DNA synthesis 24–48 HPI and were down-regulated later in the SV40 infection cycle when CV1 cells began to exhibit CPE (Fig. 3B). These findings are compatible with the notion that phosphorylation of Ser-120 is required for replication. Consistent with this idea, down-regulation of ATM through RNAi inhibited SV40 replication (Fig. 5D). The most straightforward explanation for the SV40 replication defect observed in ATM-deficient cells is that inhibition of Ser-120 phosphorylation compromises the replication potential of LTag. However, it is conceivable that ATM also modulates LTag function through phosphorylation of a carboxyl-terminal cluster of Ser-Gln residues, which are known to be phosphorylated in intact cells (57, 58). Because the replication defect of ATM siRNA-transfected cells was not complete, it is also possible that other Ser-120 kinases contribute to SV40 replication in cellulo.

The Ser-120 residue presents an intriguing regulation paradox; on the one hand, it is required for SV40 replication in vivo (53), yet, as noted above, Ser-120 is strongly implicated as an inhibitor of SV40 DNA replication in vitro. The known functions of ATM in chromosomal DNA replication may shed light on the apparently contradictory roles of Ser-120 in the viral replication cycle. In vertebrates, ATM and ATR function to prevent premature replication origin firing during the normal cell cycle and in response to genotoxic stimuli down-regulation of phase promoting kinases CDC7-DBF4 and CDK2 (7). It is possible that ATM serves a similar function in the context of SV40 viral DNA replication. We speculate that the phosphorylation of Ser-120 regulates the timing of SV40 replication by preventing premature activation of LTag helicase activity at the ori. Alternatively, the phosphorylation of LTag by ATM may terminate individual SV40 minichromosome replication cycles by liberating LTag from the DNA template, thus, allowing LTag to initiate additional replication cycles.

SV40 DNA replication in CV1 cells occurs despite robust ATM activation and initiation of host cell checkpoint responses, including the phosphorylation of CREB, RPA, CHK2, p53 (Fig. 4A), H2AX, and CHK1 (59). How SV40 replicates in the face of hostile checkpoint conditions is unclear. However, a recent report showed that LTag binds to and partially inactivates the replication checkpoint functions of NBS1 (34). Inactivation of NBS1 is responsible for the endoreduplication of host DNA observed in SV40-infected cells, and loss of NBS1 causes hyper-replication of SV40 DNA (34). Thus, partial inactivation of NBS1 may...
be required to counter the potential inhibitory effects of ATM activation on SV40 replication.

Activation of ATM has recently been reported during cellular infection with HIV and HSV and therefore appears to represent a general response to viral infection (30–32). In the HIV system, activation of ATM requires the viral integrase, and the essential function of ATM appears to be mitigation of integrase-induced cytotoxicity (30). Because ATM autophosphorylation is closely correlated with the onset of SV40 DNA synthesis, it is possible that one or more viral replication intermediates initiate ATM activation. Alternatively, the activation of ATM may reflect extensive host cell chromosome damage. Consistent with this latter possibility, SV40-infected cells exhibited dramatic γH2AX nuclear foci (Fig. 4B). Further experiments should define the mechanism of ATM activation by SV40 and elucidate how ATM regulates the replication and transformation potential of LTAg.

Acknowledgment—We thank Dr. Ellen Fanning (Vanderbilt University) for helpful discussions.

REFERENCES

1. Shih, Y. (2003) Nat. Rev. Cancer 3, 155–168
2. Kim, S. T., Lim, D. S., Canman, C. E. & Kastan, M. B. (1999) J. Biol. Chem. 274, 37538–37543
3. Abraham, R. T. (2001) Trends Biochem. Sci. 26, 353–360
4. Becker-Catania, S. G. & Gatti, R. A. (2001) J. Biol. Chem. 276, 34517–34523
5. Casper, A. M., Nghiem, P., Arlt, M. F. & Glover, T. W. (2002) Proc. Natl. Acad. Sci. U.S.A. 99, 10952–10957
6. Grasser, F. A., Mann, K. & Walter, G. (1987) Cell 50, 499–506
7. Xu, X., Avni, D., Chiba, T., Yan, F., Zhao, Q., Lin, Y., Heng, H. & Livingston, D. (2004) Genes Dev. 18, 1305–1316
8. Ali, S. H. & DeCaprio, J. A. (2001) Semin. Cancer Biol. 11, 15–23
9. Wu, X., Ave, D., Chiba, T., Yan, F., Zhao, Q., Lin, Y., Heng, H. & Livingston, D. (2004) Genes Dev. 18, 1305–1316
10. Nghiem, P., Park, P. K., Kim, Y., Vaziri, C. & Schreiber, S. L. (2001) Proc. Natl. Acad. Sci. U.S.A. 98, 9092–9097
11. Brown, E. J. & Kelly, T. J. (1991) J. Virol. 65, 2098–2110
12. Brown, A. L., Lee, C. H., Schwarz, J. K., Mitiku, N., Piwnica-Worms, H. & Chung, J. H. (1999) Proc. Natl. Acad. Sci. U.S.A. 96, 3745–3750
13. Sanchez, Y., Wong, C., Thoma, R. S., Riehman, R., Wu, Z., Piwnica-Worms, H. & Elledge, S. J. (1997) Science 277, 1497–1501
14. Mailand, N., Falck, J., Lukas, C., Syljuasen, R. G., Weicker, M., Bartek, J. & Lukas, J. (2000) Science 288, 1425–1429
15. Ali, S. H. & DeCaprio, J. A. (2001) Semin. Cancer Biol. 11, 15–23
16. Bakkenist, C. J. & Kastan, M. B. (2003) EMBO J. 22, 5621–5627
17. Shiloh, Y. (2003) Trends Biochem. Sci. 28, 155–168
18. Uziel, T., Lerenthal, Y., Moyal, L., Andegeko, Y., Mittelman, L. & Shiloh, Y. (2003) J. Cell Biol. 162, 169–175
19. Carson, C. T., Schwartz, R. A., Stracker, T. H., Lilley, C. E., Lee, D. V. & Weitzman, M. D. (2005) Proc. Natl. Acad. Sci. U.S.A. 102, 5844–5849
20. Liu, Q., Guentoko, S., Cui, X. S., Matsuoaka, S., Cortez, D., Tamai, K., Luo, G., Carattini-Rivera, S., DeMayo, F., Bradley, A., Donehower, L. A. & Elledge, S. J. (2000) Genes Dev. 14, 1448–1459
21. Brown, A. L., Lee, C. H., Schwarz, J. K., Mitiku, N., Piwnica-Worms, H. & Chung, J. H. (1999) Proc. Natl. Acad. Sci. U.S.A. 96, 3745–3750
22. Sanchez, Y., Wong, C., Thoma, R. S., Riehman, R., Wu, Z., Piwnica-Worms, H. & Elledge, S. J. (1997) Science 277, 1497–1501
23. Mailand, N., Falck, J., Lukas, C., Syljuasen, R. G., Weicker, M., Bartek, J. & Lukas, J. (2000) Science 288, 1425–1429
24. Busino, L., Donzelli, M., Chiesa, M., Guadavaccaro, D., Ganoth, D., Dorrello, N. V., Hershko, A., Pagano, M. & Draetta, G. F. (2003) Nature 426, 87–91
25. Falck, J., Mailand, N., Syljuasen, R. G., Bartek, J. & Lukas, J. (2001) Nature 410, 842–847