Solar UVA, but not UVC, reaches the earth’s surface and therefore is an important etiological factor for the induction of human skin cancer. ATM kinase is an important regulator of cell survival and cell cycle checkpoints. Here, we observe that UVA, unlike UVC, triggers ATM kinase activity, and the activation may occur through reactive oxygen species produced after irradiation of cells with UVA. We also show that ATM activation is involved in the apoptotic response to UVA but not UVC. Furthermore, we provide evidence that ATM-dependent p53 and c-Jun N-terminal kinase (JNK) pathways are linked to UVA-induced apoptosis. On the other hand, UVC-induced apoptosis occurs through ATR-dependent p53 phosphorylation as well as the JNK pathway. Therefore, these results suggest that ATM, like p53, is involved in the UVA-induced apoptosis to suppress carcinogenesis.

The solar UV light that reaches the earth’s surface is divided into ultraviolet A (UVA) (320–400 nm) and ultraviolet B (UVB) (290–320 nm) (1, 2). UVB is absorbed mainly by the earth’s ozone layer and can also be blocked efficiently by protective strategies such as sunscreens (2). Ultraviolet C (UVC) (200–290 nm) is completely absorbed by the ozone layer and, therefore, is unlikely to have a major pathophysiological effect, although most of the reported UV-stimulated biological effects result from experiments with UVC (1–3). On the other hand, UVA constitutes over 90% of solar UV at ground level and can penetrate the skin. Both in vitro and in vivo experiments clearly confirmed that UVA, like UVB and UVC, is a mutagen and carcinogen (1, 2). These observations, therefore, demonstrate that UVA is an important contributor to UV-induced carcinogenesis.

Carcinogenesis appears to arise as a consequence of a combination of disturbances in signal transduction pathways that control cell cycle checkpoints, cell survival, arrest, and apoptosis (2–4). Such disturbed signaling may result from mutation of key regulatory genes. For example, in many cancers, the tumor suppressor gene p53 has become dysfunctional or is completely lost and thus fails to repress carcinogenesis (5). Atm (ataxia telangiectasia mutated) is also shown to function as a tumor suppressor gene, and its mutation leads to ataxia telangiectasia (AT), a multisystemic genetic disorder with an extensive combination of somatic and physiological defects and high incidence of cancer (6–8). Moreover, an accumulation of evidence, indicating that acquired resistance toward apoptosis is a hallmark of most and perhaps all types of cancer (4), suggests that apoptotic signaling pathways in cancer-prone AT cells may be disturbed by mutation of Atm. The gene product, ATM, has indeed been shown to be a serine/threonine protein kinase with a carboxyl-terminal domain significantly similar to the catalytic subunit of phosphatidylinositol 3-kinase and to play a vital role in multiple signal transduction pathways influencing cell cycle checkpoint controls (7, 9–11). However, the role that ATM kinase may play in p53-dependent or -independent apoptosis pathways is less understood.

ATM was shown to be activated in the cellular response to ionizing radiation (IR), but not to UVC exposure (10–13), and the activated ATM was recruited to double strand breaks (DSBs) for repair (14). These findings are frequently cited as evidence that UV-induced signal transduction does not require ATM activation. However, of the solar UV, UVA, and perhaps to a lesser extent UVB, but not UVC, are responsible for human skin diseases including cancer, and thus UVA-induced signal transduction should be more relevant to human skin carcinogenesis. Whether ATM kinase is activated and involved in the apoptotic response to UV is as yet unknown. In this report, we provide evidence that ATM is activated by UVA, but not UVC, and is involved in the cellular decision to trigger p53- and c-Jun N-terminal kinase (JNK)-dependent apoptotic pathways in response to UVA.

MATERIALS AND METHODS

Cell Lines, Cell Culture, and UV Sources—Mouse wild-type (p53+/+) or Jnk−/−) and knockout (p53−/− or Jnk1−/−, or Jnk2−/−) embryonic fibroblasts (15, 16) were grown in Dulbecco’s modified Eagle’s medium (DMEM) (Invitrogen) containing 10% fetal bovine serum (FBS; from Gemini Bio-Products, Inc., Calabasas, CA), 2 mm l-glutamine, 100 units/ml penicillin, and 100 μg/ml streptomycin in a humidified atmosphere of 5% CO₂ at 37 °C. Human cell lines stably expressing a wild-type or kinase-defective mutant of ATM or ATR (ATM) and Rad3-related were used in this study because of the small amounts of ATM or ATR in normal mammalian cells (12, 17). The AT cells transfected with an empty mammalian expression vector pEBST7 (Atm) or a construct, pEBST7-Y25, containing recombinant full-length ATM (Atm) (18) were maintained in the above described 10% FBS-DMEM supplemented with 100 μg/ml of hygromycin B (Sigma) for selection. Human fibroblast GM847 lines stably expressing wild-type full-length (ATRwt) or a kinase-inactive allele (ATRkd) of ATR (17) were cultured in 10% FBS-DMEM containing 400 μg/ml G418 (Gemin Bio-Products) for selection. The cell lines were treated with UVA or UVC irradiation, and nonirradiated cell samples were used as negative controls. For the detailed description of UVA or UV sources, see our previous reports (15, 16, 19).

DNA Fragmentation Ladder Assay—The experimental cells (2.5 × 10⁶ to 3 × 10⁶) were seeded in 150-mm dishes and cultured for 12–24 h. The embryonic fibroblasts described above were not starved, but Atm−/−, N-terminal kinases; IR, ionizing radiation; DSB, double strand break; DMEM, Dulbecco’s modified Eagle’s medium; FBS, fetal bovine serum; IP, immunoprecipitation; GST, glutathione S-transferase.
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ATm−, ATRwt, and ATRkd cells were starved for 4–8 h in 1% FBS-DMEM. The cells were harvested 14–18 h after irradiation with UVA or UVC at the indicated doses. Subsequent DNA fragmentation laddering assays were performed according to previously described methods (19, 20).

**Western Blot Analysis**—Equal numbers of experimental cells (1 × 10⁶ to 5 × 10⁶) were cultured for 12–24 h in 100-mm dishes. After 70–80% confluence was reached, the cells were starved for 24–48 h in serum-free DMEM. At the indicated times after irradiation, the cells were harvested and washed once with ice-cold phosphate-buffered saline. Then the cells were disrupted in 200 μl of radioimmuno precipitation buffer (50 mM Tris-HCl (pH 7.5), 150 mM NaCl, 1 mM EDTA, 1 mM EGTA, 1% (v/v) Triton X-100, 2.5 mM sodium pyrophosphate, 1 mM β-glycerol phosphate, 1 mM Na₃VO₄, 10 μg/ml leupeptin, 10 μg/ml aprotinin, and 1 mM phenylmethylsulfonyl fluoride). The clarified supernatant fractions containing equal amounts of protein were subjected to separation of activated caspase-3 activity. Then samples were subjected to separation by 5% (w/v) SDS, 30% (v/v) glycerol, 150 mM dithiothreitol, and 0.3% (w/v) bromphenol blue. Then samples were subjected to separation by 5% (for ATM) or 8% (for JNKs and β-actin) SDS-PAGE followed by Western blot analysis according to the reported methods (16, 19). For detection of ATM expression, 150 μg of protein was loaded in each lane for 5% SDS-PAGE. The ATM polyclonal antibody (Ab-3) was from Oncogene, Inc. (Cambridge, MA). Antibodies against phospho-JNKs (Thr183/Tyr185), nonphosphorylated JNKs, or β-actin were from Cell Signaling, Inc. (Beverly, MA). For analysis of caspase-3 activation, the cells were starved in 1% FBS-DMEM and then harvested at the indicated times following irradiation. Subsequently, activated caspase-3 was separated on a 10–20% gradient PAGE gel (Bio-Rad) followed by Western blotting with an antibody against cleaved caspase-3 (D175) (Cell Signaling).

**Immunoprecipitation Assay for p53**—Following culture for 12–24 h, the experimental cells were starved for 24 h in 1% FBS-DMEM. The cells were harvested at the indicated times following irradiation and lysed in 250 μl of IP buffer (20 mM Tris (pH 7.5), 150 mM NaCl, 1 mM EDTA, 1 mM EGTA, 1% (v/v) Triton X-100, 2.5 mM sodium pyrophosphate, 1 mM β-glycerol phosphate, 1 mM Na₃VO₄, 10 μg/ml leupeptin, 10 μg/ml aprotinin, and 1 mM phenylmethylsulfonyl fluoride). The clarified supernatant fractions containing equal amounts of protein were subjected to immunoprecipitation following by Western blot analysis according to the described methods (16, 21). The immune complex beads were washed twice with IP buffer and twice with phosphate-buffered saline. A mouse monoclonal antibody against p53 (Ab-1) (Oncogene) was used for immunoprecipitation of p53 protein, and then rabbit polyclonal antibodies against phospho-p53 (Ser15 or Ser20) (Cell Signaling) were used for Western blotting.

**Kinase Activity Assays for ATM or ATR**—In vitro kinase activity assays for ATM or ATR were performed according to the reported methods (22) with some modification. Briefly, before irradiation as described above, the experimental cells were or were not preincubated for 60 min with ascorbic acid, catalase, N-acetyl cysteine, or t-erothoidealine (from Sigma). Then the cells were harvested at the indicated times after irradiation. The cells were disrupted in IP buffer (above) supplemented with 4 mM EDTA, 50 mM NaCl, 0.2% (v/v) dodecyl β-maltoside (Sigma), and 1 mM phenylmethylsulfonyl fluoride. The clarified supernatant fractions containing equal amounts of protein were subjected to preimmunoprecipitation for 2 h with normal IgG (Upstate Biotechnology, Lake Placid, NY) and protein A/G plus-agarose beads (Santa Cruz Biotechnology, Inc., Santa Cruz, CA) and subsequent immunoprecipitation overnight with an antibody to ATM (Ab-3) or ATR (Ab-2) (Oncogene). The beads containing immune complexes of ATM or ATR were washed twice with the above-modified IP buffer and twice with kinase buffer A (50 mM Hepes (pH 7.5), 150 mM NaCl, 4 mM MgCl₂, 6 mM MgCl₂, 10% (v/v) glycerol, 1 mM diethiothreitol, and 100 μM Na₃VO₄ (prepared fresh)). The immune complex of ATM was incubated for 15 min at 30 °C with 1 μg of PHAS-1 (phosphorylated heat- and acid-stable protein regulated by insulin; Stratagene, Los Angeles, CA) in 30 μl of kinase buffer A containing 20 μM unlabeled ATP and 10 μCi of [γ-³²P]ATP. The reaction was stopped by the addition of 3× SDS sample buffer and then separated by 15% SDS-PAGE. After autoradiography, radiolabeled phosphate blots were quantified using the ImageQuant software (Molecular Dynamics, Inc., Sunnyvale, CA). After incubation of the ATM complexes with full-length p53 fusion protein (residues 1–393) conjugated with glutathione S-transferase (GST-p53) (Santa Cruz) in kinase buffer A containing 200 μM ATP, the reaction was subjected to separation by 8% SDS-PAGE followed by Western blot analysis with phospho-p53 (Ser⁰⁵⁵) antibody.

**RESULTS**

**ATM-dependent Apoptosis Induced by UVA but Not UVC**—DNA ladder is believed to be a significant marker of apoptosis, reflecting cleavage of internucleosomal DNA strands by the nuclease (known as caspase-activated DNase) to generate the multiple DNA fragments (23, 24). The fragmentation laddering assays can thus be used to evaluate whether apoptosis is induced by UVA or UVC. Failure to induce apoptosis (Fig. 1A) was observed in UVA-irradiated AT cells transfected with an empty vector pEBS7 (Atm−). Conversely, UVA-induced apoptosis was restored (Fig. 1A) after ectopic expression of recombinant full-length ATM in AT cells stably transfected with a construct pEBS7-YZ5 (Atm−). On the other hand, no difference in UVC-induced apoptosis was observed in either Atm− or Atm− cell lines (Fig. 1B). Caspase-3 has been shown to be one

Fig. 1. Requirement of ATM for induction of apoptosis by UVA but not UVC. AT cells were transfected with an empty vector pEBS7 (Atm−) or a construct pEBS7-YZ5 encoding wild-type ATM kinase (Atm+). The cells were grown in 10% FBS-DMEM and then starved for 8 h by replacing growth medium with 1% FBS-DMEM before irradiation. A, Atm− and Atm+ cells were harvested 18 h after irradiation with UVA at 20, 40, or 80 kJ/m². B, Atm− and Atm+ cells were harvested 14 h following irradiation with UVA at 20, 40, 60, or 120 J/m². DNA fragmentation laddering was visualized by 1.8% agarose gel electrophoresis. C, after UVA or UVC irradiation, total cell lysates containing equal amounts of protein were subjected to separation of activated caspase-3 on 10–20% gradient PAGE gel followed by Western blotting with an antibody to cleaved caspase-3. These data are representative of at least three similar independent experiments.

**JNK Activity Assay**—Activity assays for JNKs were carried out according to the protocol recommended by Cell Signaling. In brief, immunoprecipitates with the beads conjugated previously with c-Jun fusion protein (Cell Signaling) were incubated for 30 min at 30 °C in kinase buffer B (50 mM Tris-HCl (pH 7.5), 10 mM MgCl₂, 1 mM EGTA, 1 mM diethiothreitol, and 0.01% (v/v) Brij 35) containing 0.5 μM ATP. Then these reactive products were separated by 10% SDS-PAGE followed by Western blotting with a phospho-c-Jun (Ser⁶³) antibody.
of the central executioners of apoptosis, being responsible for the proteolytic cleavage of many key proteins (24). Western blotting results using a cleaved caspase-3 antibody showed that caspase-3 was activated in Atm\(^+\) cells, but only very weakly in Atm\(^-\) cells following UVA irradiation (Fig. 1C, upper panel). Similar activation of caspase-3 by UVC occurred in both Atm\(^+\) and Atm\(^-\) cell lines (Fig. 1C, lower panel). Overall, these results suggest that ATM appears to be required for induction of apoptosis by UVA but not UVC.

**UVA, Unlike UVC, Activates ATM Kinase**—Previous reports (10–13) showed that IR, but not UVC, activates ATM kinase, but whether UVA activates ATM kinase is as yet unknown. Here, Western blot analysis using an ATM antibody showed stable expression of ATM in Atm\(^+\) cells, but no significant change of ATM expression after irradiation with UVA or UVC (Fig. 2A, upper panel). In contrast, weaker signals for ATM were observed in Atm\(^-\) cells (Fig. 2A), suggesting possible expression of an unstable protein product of mutated Atm (8, 18). Additionally, no change in \(\beta\)-actin expression was found in the two cell lines (Fig. 2A, lower panel). The identification of ATM Is Required in UVA-induced Signaling and Apoptosis

**Fig. 2. Activation of ATM kinase by UVA but not UVC.** A, Atm\(^+\) and Atm\(^-\) cells were starved for 24 h in serum-free DMEM and then were or were not irradiated with UVA (80 J/m\(^2\)) or UVC (60 J/m\(^2\)). The cells were harvested 2 h after irradiation. The cell lysates were separated by 5% (upper panel) or 8% (lower panel) SDS-PAGE followed by Western blotting with an ATM (Ab-3) or \(\beta\)-actin antibody. B, after starvation for 12 h in 0.5% FBS-DMEM, the cells were harvested 15 min following irradiation with or without UVA (80 J/m\(^2\)) or UVC (60 J/m\(^2\)). Then the cell lysates were subjected to immunoprecipitation with an ATM antibody followed by determination of ATM kinase activity toward PHAS-1 a substrate. C, the cells were starved and irradiated as described for B and then were harvested at the indicated times. Subsequently, ATM kinase activity was assayed in the immunoprecipitates. These results are repeated in at least four independent experiments.

**Fig. 3. Inhibition of UVA-induced ATM kinase activity by ROS scavengers.** A, starved Atm\(^+\) cells were or were not preirradiated for 60 min with 0.5 mM ascorbic acid (AA), 300 units ml\(^{-1}\) catalase (CAT), 15 mM N-acetylcysteine (NAC), or 0.5 mM \(\alpha\)-ergothioneine (LET) and then were or were not treated with UVA (80 J/m\(^2\)). B, hydrogen peroxide (H\(_2\)O\(_2\)) stimulation of cells was used as an internal positive control. The cells were harvested 15 min after UVA irradiation (A) or 90 min after H\(_2\)O\(_2\) treatment (B), and then ATM immunoprecipitation and determination of kinase activity were performed as described for Fig. 2. The data are representative of two similar independent experiments.
Phosphorylation of p53 at Ser 15 and Ser20 in an amino-
UVA exposure of cells to UVA irradiation. (Fig. 3A-

Atm activity was almost undetectable in ATRwt cells, but a poorer induction
of ATM-dependent p53 phosphorylation may be ATM-independent
and UVA-induced apoptosis. (Fig. 4A). Additionally, exposure of cells to H2O2 also activated
ATM kinase, and the activation was blocked by preincubation
with catalase or L-ergothioneine (Fig. 3B). Furthermore, our data showed that UVA- or UVC-induced
apoptosis was significantly blocked in p53−/− cells compared with corresponding induction in p53+/+ cells (Fig. 4B). Taken together with data in Fig. 1, these results indicate that UVA-induced apoptosis may be mediated by activation of an ATM-dependent p53 pathway, but UVC-induced apoptosis appears to occur through an ATM-independent p53 pathway.

**JNKs Link ATM to the Apoptotic Response Induced by UVA**—Our previous report suggested that a sphingomyelinase-dependent and -independent signaling pathway might be involved in JNKs-mediated apoptosis in the UVA response (19). Here, phosphorylation (Fig. 5A) and activation (Fig. 5C) of JNKs was stimulated strongly in Atm−/− cells exposed to UVA irradiation, but the stimulation of JNKs by UVA was defective in Atm1−/− cells (Fig. 5, A and C). On the other hand, a strong phosphorylation of JNKs induced by UVC was not different in either Atm+ or Atm− cell lines (Fig. 5B). Additionally, no change of the basal JNK levels was observed in the experimented cells (Fig. 5, A and B), suggesting that expression of JNKs was unaffected by deficiency of ATM. Therefore, the results indicate that ATM kinase activation is required for stimulation of JNKs by UVA, but not UVC. Interestingly, UVA- or UVC-induced apoptosis was inhibited partially in Jnk−/− cells, but was attenuated completely in Jnk−/− cells, compared with corresponding controls in Jnk+/+ cells (Fig. 5D). Together with the results in Fig. 1, these data suggest that an ATM-mediated JNKs pathway may be required for apoptosis induced by UVA, but UVC-induced apoptosis appears to occur through an ATM-independent JNK pathway.

**UVC Induces ATR Signaling to p53/JNKs-mediated Apoptosis**—The above-mentioned data (Figs. 1B, 4B, and 5D) appear to suggest that UVC-induced apoptosis may require ATM-independent signaling through p53 or JNKs. ATR, being functionally and structurally similar to ATM (11), was shown to be involved in the regulation of cell survival and cell cycle checkpoints (17, 28), but a role of ATR in activation of the apoptotic response pathways is as yet unknown. To clarify the role, therefore, we used human fibroblast lines stably expressing wild-type full-length (ATRwt) or a kinase-inactive allele (ATRkd) of ATR. Both ATRwt and ATRkd cell lines were previously established and shown to have wild-type active or dominant negative effects on ATR kinase activity, respectively (17, 28). The full-length p53 protein conjugated with glutathione S-transferase (GST-p53) was used as a substrate in kinase activity assays for studying ATR activity. Results showed a rapidly stimulated increase of ATR kinase activity toward GST-p53 in UVC-irradiated ATRwt cells, but the ATR activity was significantly inhibited in ATRkd cells (Fig. 6A, lower panel). In addition, a strong phosphorylation of Ser15 in endogenous p53 was detected in ATR immune complexes isolated from UVC-irradiated ATRwt cells, but a poorer phosphorylation was observed in the complex from ATRkd cells exposed to UVC (Fig. 6A, lower panel). Conversely, neither the ATR kinase activity nor the endogenous p53 phosphorylation was detected in the complexes immunoprecipitated from UVC-stimulated ATRwt or ATRkd cells (Fig. 6A, upper panel). These data suggest that UVC, but not UVA, activates ATR kinase and that the activated ATR may co-precipitate with the p53 protein. Furthermore, UVC-induced apoptosis was significantly inhibited in ATRkd cells compared with ATRwt cells (Fig. 6B). Additionally, no typical apoptosis was observed in either ATRwt or ATRkd cells exposed to UVA (data not shown). Together with Fig. 4B and previous reports (17, 28), these data indicate a requirement of ATR-dependent p53 signaling activation for induction of apoptosis by UVC, but not UVA.

However, UVC-induced apoptosis was not completely atten-
knockout (Jnk1<sup>−/−</sup> or Jnk2<sup>−/−</sup>) embryonic fibroblasts were irradiated with UVA (80 kJ/m<sup>2</sup>) or UVC (60 J/m<sup>2</sup>). Then the cell lysates were subjected to DNA fragmentation laddering assays. P, phosphorylated; np, nonphosphorylated. The data are representative of at least three independent experiments.

**DISCUSSION**

Mutation of the Atm gene is known to cause AT disorder with pleiotropic biological markers including high cancer risk, hypersensitivity to DNA-damaging agents, cell cycle checkpoint alterations, increased chromosomal breakage, and telomere end fusion (7, 9–11), indicating that a role of ATM in multiple response pathways is probably altered or even lost in AT cells. ATM kinase was shown to be activated in the cellular responses to DSBs (14, 29) and such breaks induced by IR (12, 13, 30), radiomimetic drugs (12), or arsenite (31) but not to UVB/UVA or base-damaging agents (11, 13, 17, 28, 30), suggesting that ATM may act specifically in response to DSBs. However, broken DNA is not required for in vitro activation of ATM kinase (30), suggesting that besides DSBs, other mechanisms may be involved in ATM activation. In addition, various abnormalities other than hypersensitivity to IR in AT have raised the possibility of a role for ATM in other cellular responses. Indeed, recent reports showed activation of ATM kinase by oxidative stresses including H<sub>2</sub>O<sub>2</sub> (25) and CdCl<sub>2</sub> (32, 33) and even by insulin (34). Here, evidence is provided that UVA particularly induces rapid activation of ATM kinase, but ATM activation was not observed within at least 2 h following UVC. This observation supports the idea that the photobiological effects of UVA (e.g. free radical or reactive oxygen species production and other damage by UVA-absorbing proteins and lipids) are different from those induced by UVC (e.g. base damage by UVC-absorbing DNA) (1–3, 35). Indeed, the damage induced by UVA has been shown to occur largely via reactive oxygen intermediates or radicals generated on endogenous UVA-absorbing non-DNA chromophores (called photosensitizers), because of the low absorption of DNA (1–3). Here, our data showed that ATM kinase was activated by oxidative stresses including UVA and H<sub>2</sub>O<sub>2</sub> suggesting that ATM kinase is an oxidative stress sensor.

The high predisposition of AT cells to progress toward malignant transformation suggests possible resistance of AT cells to apoptosis. The resistance appears to result from defective apoptotic responses in ATM-deficient cells (5, 36–38). However, whether ATM mediates apoptosis is not fully understood, because contradictory observations have been reported. Some reports showed either no effect or an inhibitory effect of ATM kinase on the apoptotic responses to IR (39) or oxidative stress (40); conversely, other reports indicated a requirement of ATM kinase for induction of apoptosis (5, 36–38, 41).

**Fig. 5.** A connection between ATM-mediated JNK phosphorylation and UVA-induced apoptosis. A, inhibition of UVA-induced phosphorylation of JNKs by ATM deficiency. Atm<sup>−/−</sup> and Atm<sup>−/−</sup> cells were starved for 36 h in serum-free FBS-DMEM and then harvested at the indicated times following irradiation with UVA (80 kJ/m<sup>2</sup>). Total cell lysates were subjected to Western blotting with antibodies against phosphorylated or nonphosphorylated JNKs. B, no difference in phosphorylation of JNKs induced by UVC between ATM-expressing and -deficient cells. C, inhibition of UVA-stimulated JNK activity by deficiency of ATM. Atm<sup>−/−</sup> and Atm<sup>−/−</sup> cells were treated as described for Fig. 4A and then harvested 15 or 30 min after UVA (80 kJ/m<sup>2</sup>). The cell lysates were subjected to immunoprecipitation with c-Jun fusion protein beads followed by JNK activity assay and subsequent Western blot analysis with a phospho-c-Jun (Ser<sup>63</sup>) antibody. D, suppression of UV-induced apoptosis by deficiency of JNKs. Mouse wild-type (Jnk<sup>−/−</sup>) and uated in p53<sup>−/−</sup> or ATRkd cells (Figs. 4B and 6B), indicating that besides ATR-mediated p53 signaling, other pathways may also be involved in this process. For example, protein kinase C was previously reported to activate a JNK-mediated apoptotic pathway in the UVC response (20). Interestingly, phosphorylation of JNKs corresponding with activation of JNKs was observed to be significantly inhibited in UVC-irradiated ATRkd cells, in comparison with correspondingly treated ATRwt cells (Fig. 6C, lower panel). On the other hand, UVA-induced phosphorylation of JNKs was not affected in ATRkd cells compared with that in ATRwt cells (Fig. 6C, upper panel). Additionally, no change of basal levels of JNKs was observed in these experimental cells (Fig. 6C). Together with data in Figs. 5D and 6A, these results suggest that activation of ATR kinase may be involved in JNK mediation of apoptotic responses induced by UVC but not by UVA.
assumption is supported by our demonstration that ATM kinase activation may be involved in UVA-induced apoptosis. The discrepancies among these reported apoptotic responses, although not fully understood, may be associated with cell types, even cellular states (e.g. proliferative or quiescent) or extracellular microenvironments of the same target, as well as with the kind and intensity of the apoptotic stimuli.

A role of ATM in mediating the p53- and JNK-activated apoptotic pathways is not well clarified, although p53 (36–39) and JNKs (19, 41–43) have been shown to be involved in activation of the apoptotic response pathways. Previous experiments with IR showed p53 phosphorylation of Ser\textsuperscript{15} by ATM directly (10–14, 29) and of Ser\textsuperscript{20} by ATM-dependent Chk2 indirectly (11, 44, 45). However, a link of the ATM-mediated apoptotic response to UVA is as yet unknown. Here, we present evidence that ATM-dependent p53 activation is required for apoptosis.
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UVA-induced apoptosis, inasmuch as a defective UVA-stimulated phosphorylation of p53 at Ser15 and Ser20 in ATM-deficient cells was restored in ATM-expressing cells, and the apoptotic response to UVA was significantly inhibited in ATM- or p53-deficient cells. Interestingly, another link between ATM and UVA-induced apoptosis was here found to be through an ATM-dependent JNK response pathway. This indication is supported by our experiments showing inhibition of UVA-induced apoptosis and JNK activation by deficiency of ATM or JNKS. In fact, phosphorylation of c-Jun was shown to activate apoptosis and JNK activation by deficiency of ATM or p53-deficient cells. Interestingly, another link between ATM and Andria Hansen for secretarial assistance.

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FIG. 7. A proposed model for involvement of ATM or ATR in activation of the apoptotic pathway. UVA or UVC activates ATM or ATR, respectively. In the UVA response, p53 and JNKS link ATM to activation of apoptotic pathways. ATR is linked by p53 and JNKS to the cellular apoptotic response induced by UVC. Sphingomyelinase or protein kinase C signaling to JNKS is also suggested to mediate activation of apoptosis by UVA or UVC, respectively. The arrows indicate direct (thick arrow) or indirect activation (thin arrow).
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