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Research Communication

E2F1 Transcription is Induced by Genotoxic Stress Through ATM/ATR Activation

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Summary

E2F1, a member of the E2F family of transcription factors, plays a critical role in controlling both cell cycle progression and apoptotic cell death in response to DNA damage and oncogene activation. Following genotoxic stresses, E2F1 protein is stabilized by phosphorylation and acetylation driven to its accumulation. The aim of the present work was to examine whether the increase in E2F1 protein levels observed after DNA damage is only a reflection of an increase in E2F1 protein stability or is also the consequence of enhanced transcription of the E2F1 gene. The data presented here demonstrates that UV light and other genotoxics induce the transcription of E2F1 gene in an ATM/ATR dependent manner, which results in increasing E2F1 mRNA and protein levels. After genotoxic stress, transcription of cyclin E, an E2F1 target gene, was significantly induced. This induction was the result of two well-differentiated effects, one of them dependent on *de novo* protein synthesis and the other on the protein stabilization. Our results strongly support a transcriptional effect of DNA damaging agents on E2F1 expression. The results presented herein uncover a new mechanism involving E2F1 in response to genotoxic stress. © 2009 IUBMB

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Keywords E2F1; DNA damage response; UV irradiation; cyclin E; transcriptional regulation; ATM; ATR; phosphorylation; protein stability; genotoxic stress.

INTRODUCTION

E2F, a family of eight heterodimeric transcription factors, is central to the coordination of cellular proliferation by controlling the expression of both the genes required for cell cycle progression, particularly DNA synthesis, and the genes involved in apoptosis. E2F is regulated in a cell cycle-dependent manner, mainly through its temporal association with pocket protein family members, the prototype member being retinoblastoma tumour suppressor protein (*1, 2*). By integrating the control of retinoblastoma phosphorylation by cyclin-dependent kinases with the transcriptional activity of E2F target genes, E2F plays a pivotal role in the regulation of cell cycle progression. Different E2F family members have been found to play contrasting roles in different contexts, functioning as both activators and repressors of transcription (*3, 4*).

The DNA damage response involves multiple checkpoint pathways that are highly conserved and become activated upon genotoxic stress. Checkpoint activation triggers a cascade of events that ultimately lead to cell cycle arrest or apoptosis. In metazoans, the integrity of these pathways is critical to protect cells from agents that cause uncontrolled cellular proliferation and tumorigenesis (*5*). Key components of this response are the ataxia-telangectasia mutated (ATM) and the ATM and RAD3-related (ATR) kinases that typically function as sensors of DNA damage. The checkpoint kinases Chk1 and Chk2 act as effectors through phosphorylation of strategic substrates such as p53, Mdm2 and the cell cycle-regulating phosphatases Cdc25A and Cdc25C, among others (*6, 7*).

Beside its well-established function as a proliferative factor, E2F1, the first cloned member of this family, has been involved in the apoptotic cell death in response to DNA damage and oncogene activation, suggesting a role for E2F1 in checkpoint control and providing a plausible explanation for its tumour suppressor activity (*8, 9*).

Distinct modalities of E2F1 regulation might be responsible for opposite outcomes of its activation, cell cycle progression or apoptosis. In response to genotoxic stress, ATM/ATR kinases phosphorylate E2F1 at S31, and it has been suggested that this phosphorylation event might increase E2F1 half-life by inhibiting the binding and degradation through Skp2 (*10*). In addition,

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Wang et al. have provided evidences that 14-3-3τ, a member of the 14-3-3 family proteins, binds to S31-phosphorylated E2F1 and inhibits the ubiquitination of E2F1 during DNA damage (*11*). Moreover, E2F1 increased stability following DNA damage could be due to the reduced ubiquitination resulting from S364 phosphorylation by Chk2, possibly by disruption of the Mdm2/E2F1 interaction (*12*). These phosphorylations driven stabilization are required for the expression and induction of several E2F1-apoptotic target genes such as p73, Apaf-1, and caspases, during DNA damage (*11*). Recently, Liu et al. showed that E2F1 is regulated by a retinoblastoma protein-independent mechanism, in which TopBP1 (DNA topoisomerase IIβ binding protein I) recruits Brg1/Brm, a central subunit of SWI/SNF chromatin-remodeling complex, to inhibit E2F1 transcriptional activity. This regulation would be critical for the control of E2F1-dependent apoptosis during S phase and DNA damage (*13*).

In addition to phosphorylation, acetylation has also recently been recognized to play a role in the stabilization of the E2F1 protein following DNA damage. Acetylation, which occurs at three specific lysines adjacent to its DNA-binding domain, is mediated by the P/CAF and p300/CBP acetyltransferases, although only the first seems to be responsible for E2F1 stabilization. This modification specifically influences the DNA binding activity and transactivation potential of E2F1. Moreover, following strong DNA damage, E2F1 acetylation has been reported to affect its target promoter selectivity, favouring transcription of proapoptotic genes, such as p73 (*14, 15*).

These observations provide a molecular basis for the participation of E2F1 in the DNA damage response and point at protein stabilization as the main mechanism involved. Even though exploring the genomic-wide effects of DNA damage revealed the induction of a large number of genes involved in DNA repair and apoptosis, increased expression of E2F1, as a result of transcriptional activation, has been less considered as a potential mechanism of genotoxic action (*16*).

The aim of the present work was to examine whether the increase in E2F1 protein levels observed after DNA damage is only a reflection of an increase in E2F1 protein stability or is also the consequence of enhanced transcription of the E2F1 gene. The data presented here demonstrates that UV light and other genotoxics induce the transcription of E2F1 gene in an ATM/ATR dependent manner, which results in increasing E2F1 mRNA and protein levels. After genotoxic stress, transcription of cyclin E, an E2F1 target gene was significantly induced. This induction was the result of two well-differentiated effects, one of them dependent on *de novo* protein synthesis and the other on the protein stabilization. Our results strongly support a transcriptional effect of DNA damaging agents on E2F1 expression.

EXPERIMENTAL PROCEDURES

Cell Culture

The human fibroblast cell line WI38 was grown in minimum essential medium (Invitrogen) supplemented with 10% fetal bovine serum (FBS), 100 U/mL penicillin, 100 mg/mL streptomycin, 30 mg/mL gentamicin, 100 mM nonessential aminoacids, 2 mM glutamine (Invitrogen), and 1 mM sodium pyruvate at 37°C in a humidified 5% CO2 atmosphere. The hamster fibroblast BHK-21 was maintained in Dulbecco's modified Eagle medium supplemented as WI38 cells. Transfections were performed with Lipofectamine 2000 reagent (Invitrogen) following the manufacturer's protocol.

For establishment of stable clones, the vector containing expressing inducible E2F1 in fusion with the estrogen receptor ligand binding domain was used (kindly provided by B. Amati, Milan, Italy). For cell stable selectable resistance gene a puroBABE vector carrying a puromycin gene was used. Twenty-four hours after transfection, cells were replated at low density to allow the isolation of single colonies. The clonal cell lines derived from the transfectants were maintained in selective medium containing 1.5 μg/mL puromycin (Sigma). In stable transformants BHK/ER-E2F-1, derivative of hamster fibroblast BHK-21, E2F1 activity was induced by 300 nM 4-hydroxy tamoxifen (Sigma) for 5 h.

The inhibitors used in this work (cycloheximide, MG132, caffeine, SB218078, DBH (dopamine β-hydroxylase inhibitor) and Chk2 Inhibitor) have been previously used and controlled in our laboratory regarding their inhibitory activity.

UV Irradiation

Exponentially growing cells were trypsinized and seeded in 35-mm plates at 50–60% confluence. Twenty-four hours after plating, cells were irradiated in open dishes with UV 40 J/m^2, 254 nm (range 240–280 nm) at room temperature from a Philips ultraviolet lamp (TUV15WG15T8) calibrated to deliver 2.5 J/m^2 sec. Following exposure, the medium was replaced and cells were incubated in a 5% CO_2 humidified incubator at 37°C along times indicated in each case. For each experiment, control cells were treated identically, except for UV light exposure.

Oligodeoxynucleotides

Single strand oligodeoxynucleotides (ODN) were synthesized with phosphodiester linkage by Bio-Synthesis (Lewisville, TX) and diluted in H_2O to form a 500 μM stock. The sequences of circular dumbbell double-stranded decoy ODN are as follows (*17*) (consensus sequence is underlined):

E2Fdecoy wild type: 5′-ATGCGCGAAACGCGTTTTCGCG TTTCGCGCATAGTTTTCT-3′

E2Fdecoy mutated: 5′-ATAATCTAAACGCGTTTTCGCGTTT AGATTATAGTTTTCT-3′.

ODN were annealed and 1 unit T_4 DNA ligase (Invitrogen) was added to the mixture, followed by incubation for 24 h at 16°C to generate covalently ligated circular dumbbell decoy ODN molecules. E2F decoy ODN comprises two loops and one containing an E2F consensus sequence. Cells were transfected with E2Fwild type or E2Fmutated decoy ODN (100 nM) as described earlier.

E2F1 antisense ODN (5′-CCCGAGCAGGGCCTCCAGCGC-3′) was designed to block synthesis of this protein.

Western Blot Analysis

Preparation of cell lysates electrophoresis and transfer to a nitrocellulose membrane was carried out as previously described (*18*). The membrane was immunoblotted with mouse monoclonal antibodies anti-human E2F1 (Santa Cruz). β-Actin antibody (Santa Cruz) was used to ensure equal protein content. The antibodies were detected using horseradish peroxidase-linked goat antimouse IgG (Santa Cruz) and visualized by the ECL detection system (Amersham-Pharmacia) and a Bio-Imaging Analyzer Fujifilm LAS-1000.

RNA Isolation and Northern Blot Analysis

Total cellular RNA was isolated from cultured cells as described previously (*19*). The yield and purity of RNA samples were assessed by absorbance at 260 nm and the ratio of absorbance at 260 and 280 nm, respectively. For Northern blot analysis, 20 μg of total RNA was denaturated, electrophoresed in 1% glyoxal-agarose gels, and transferred to nylon membranes (Hybond N, Amersham Biosciences). The membranes were sequentially hybridized with ^{32}P-labeled probes to human E2F1, cyclin E and β-tubulin. The ODN and cDNA was purified and 5′-end labeled using [γ-^{32}P] ATP and T_4 polynucleotide kinase. Hybridization was carried out overnight at 68°C in the same prehybridization solution by adding the ^{32}P-labeled oligodeoxynucleotide (3×10^5 cpm/cm^2) as previously described (*20*). Membranes were stripped, prehybridized, hybridized, and washed in standard conditions as described before (*18*). The membranes were then exposed and scanned onto a Bio-Imaging Analyzer Fujifilm BAS-1800II and quantified using ImageJ software.

Nuclear Run-On Transcription Assay

Exponentially growing BHK-21 cells were plated in 10 cm dishes (8×10^6/dish). After 24 h, fibroblasts were irradiated with 40 J/m^2 UV and incubated at 37°C for 8 h. To perform the nuclear run-on transcription, isolated nuclei were incubated in 50 μL reaction buffer (10 mM Tris-HCl, pH 8.4, 1.5 mM MgCl2, 140 mM NaCl, 1 mM each of ATP, GTP, CTP and 0.34 μM, [^{32}P-α] UTP-labeled purchased from New England Nuclear, 10 mM phosphocreatine and 100 μg/mL phosphocreatine kinase) for 20 min at 30°C and radiolabeled RNA was extracted from each sample. All samples had very close total radioactivity and were hybridized to nylon membranes (GeneScreen Plus, Perkin Elmer) that were previously slot-blotted with E2F1 linearized plasmid probe and vector containing a cDNA of cyclin D1 (10 pmoles) or ODN (10 pmoles) including, p21 (5′TCTGTCATGCTGGTCTGCCGCCG3′), CDK4 (5′CCT CCTCCATTGGGGACTCTCACAC3′) and β-tubulin was also blotted as control. After washing, the membranes were scanned directly onto a Bio-Imaging Analyzer Fujifilm BAS-1800II and quantified using ImageJ software.

RESULTS AND DISCUSSION

E2F1 Transcription is Induced in Response to DNA Damage

We first examined whether E2F protein accumulates in response to UV irradiation in human fibroblast cell line WI38. We found that there was a clear induction of E2F1 protein at 8 h after 40 J/m^2 UV treatment. Moreover, this protein induction seems to be dependent on ATM/ATR activity since it was greatly diminished in cells treated with caffeine, a phosphoinositide 3-kinase related kinase (PIKK) inhibitor. This result is consistent with several reports describing the accumulation of E2F1 protein in various other cell lines in response DNA damage caused by a variety of agents including ionizing and UV irradiation and chemotherapeutic drugs (*10*).

Even though these effects are strongly supported by evidence, an additional effect of DNA damaging agents on E2F1 transcription can not be discarded. To assess the possibility that the induction of E2F1 was also attributable to transcriptional regulation, we examined its RNA levels at various postirradiation time points. We observed that E2F1 mRNA was significantly induced at 4 h after 40 J/m^2 UV treatment. These results suggest that UV induced the expression of E2F1 protein not only by increasing its half-life, but also by regulating some pretranslational step. The increase observed in E2F1 mRNA level after treatment with a genotoxic agent could have different causes. One could be changes in the transcriptional rate level. To study the effect of UV on the transcription initiation rate of E2F1, we performed a run-on assay. Cells were irradiated with 40 J/m^2 and processed 8 h later. E2F1 transcription showed a 2-fold increase in damaged cells compared to the control cells. As a control, we also checked the expression of some genes known to be involved in cell cycle or DNA repair, whose transcription was induced or repressed in response to UV light as reported. Thus, p21 was induced 3-fold by UV, whereas CDK4 and β-tubulin remained unaltered. Conversely, cyclin D1 showed a decrease in its initiation rate after UV irradiation. *In silico* analysis of the 5′-regulatory region of E2F1 revealed the presence of high homologous binding sites for: p53 and NF-kB. Several studies described participation of these factors in the cellular DNA damage response (*21, 22*). In addition, E2F1 has been reported to upregulate its own promoter (*23*). Therefore, it stands to reason that one or more of the aforementioned factors could be involved in E2F1 transcriptional induction. As a whole, our results demonstrate that E2F1 is transcriptionally induced by UV irradiation.

Causal Relationship Between Enhanced E2F1 and Cyclin E Transcriptional Expression in UV-Treated Cells

With the purpose of analyzing the physiological significance of this DNA damage-dependent increase in E2F1 protein, we studied the transcriptional expression of an E2F1 target gene in UV-irradiated cells. We selected cyclin E since it is a well known E2F responsive gene, containing various functional bind-

ing sites for the transcriptional factor in its regulatory region. As shown in Fig. 1B, expression of cyclin E mRNA was augmented in cells irradiated with 40 J/m^2 UV. These results are consistent with those reported previously (*24*). However, our results do not agree with those reported by Liu et al. In this article, the authors show that, following genotoxic stress, TopBP1 represses E2F1 activity by recruiting Brg1/Brm to interact with E2F1 on E2F1-responsive promoters, including cyclin E (*13*). We do not know the reason for this divergence of the effect of E2F1 on the expression of cyclin E, although the fact that Liu et al. used genotoxics, such as neocarzinostatin and adryamicin, that produce double stranded DNA damage, while we used UV, could be a reason to explain the observed differences.

To examine the connection between the accumulation of E2F in response to UV irradiation and cyclin E expression, we performed experiments using an E2F decoy oligodeoxynucleotide. The results depicted in Fig. 2A show that induction of cyclin E in response to UV light was blocked in cells previously transfected with E2F decoy. Conversely, the cyclin E induction was not modified when a mutated E2F decoy was used.

To assess whether the UV-mediated induction of cyclin E is specifically mediated by E2F1, we carried out similar experiments using an E2F1 antisense oligodeoxynucleotide. Interestingly, diminished E2F1 levels, caused by preincubation with E2F1 antisense, suppress the UV-mediated induction of cyclin E (Fig. 2B).

To further strengthen this association, we performed experiments in hamster fibroblast cell line BHK-21 stably transfected with an estrogen receptor-E2F1 fusion vector, in which E2F1 can be activated with tamoxifen. Northern blot assays show that, as it was observed in response to UV irradiation, E2F1 activation caused an increase in cyclin E mRNA (Fig. 2C). As expected, transfection with E2F decoy significantly blocked the cyclin E induced expression while the mutated E2F decoy did not affect it. Interestingly, E2F decoy also diminished the E2F1 expression as expected for the autoregulatory mechanisms for E2F1 transcription (*23*). Together these results demonstrate a causal relationship between enhanced E2F1 and cyclin E expression in UV-treated cells.

E2F1 Protein Accumulation in Response to UV Light is the Result of Both Transcriptional and Pretranslational Effects

Our results suggest that the E2F1 accumulation observed following UV irradiation is the consequence of two separate mechanisms, one operating at a posttranslational level that modulates the E2F1 stability and the other increasing the transcriptional expression of the E2F1 gene. If this hypothesis is correct, the transcriptional activity of E2F1 on a target gene, like cyclin E, will reflect a sum of both UV-mediated effects.

To test this hypothesis, we performed a set of experiments designed to dissect the mechanisms related to E2F1-mediated induction of cyclin E in cells irradiated with UV light. To do this, we used MG132, a proteasome inhibitor, to simulate the

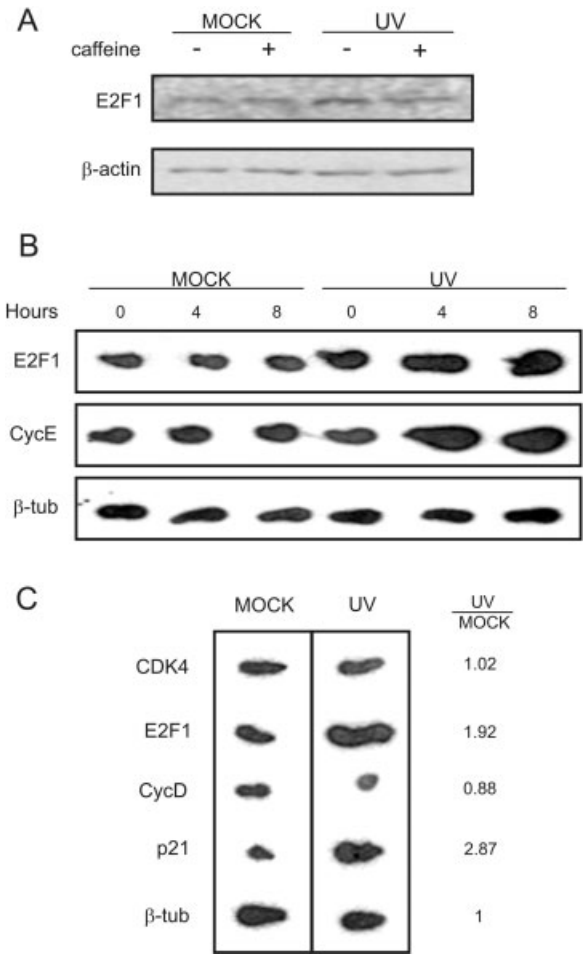

Figure 1. E2F1 transcription is induced by UV irradiation. (A) WI38 fibroblasts were treated with 5 mM caffeine and, after 1 h, cells were irradiated with 40 J/m^2 UV light and cell lysates prepared 8 h following irradiation. Equal amounts of proteins from cell lysates (100 μg) were analyzed by western blot with anti-E2F1 antibody. Anti-β-actin antibody was used as a protein loading control. (B) BHK-21 fibroblasts were irradiated with 40 J/m^2 UV light and harvested at the indicated times following irradiation. Total RNA (20 μg) was extracted from cells and subjected to Northern blot analysis using the ^{32}P-labeled probes indicated in the left margin. (C) BHK-21 fibroblasts were irradiated with 40 J/m^2 UV light and harvested 8 h after following irradiation. Denaturated DNA fragments containing sequences of E2F1, p21, CDK4, cyclin D1, and β-tubulin were slot blotted onto a nylon membrane and hybridized with [^{32}P-α] UTP-labeled nascent RNA prepared from BHK-21 cells. Transcription rates were normalized to each β-tubulin signal. Mean values of the transcription initiation rate for irradiated versus nonirradiated cells are indicated to the right. In (A–C) each figure shows a representative photograph of three independent experiments with similar results. CycE, cyclin E; β-tub, β-tubulin; CycD, cyclin D.

UV-mediated stabilization effect on E2F1, and cycloheximide, a potent inhibitor of protein synthesis, to block the synthesis of newly transcribed E2F1. The reasoning behind this experiment was that if UV light only stabilizes E2F1, the induction of cyclin E expression in UV treated cells would be quantitatively similar to that observed in MG132 incubated cells. On the contrary if, in addition to E2F1 stabilization, UV induces its gene expression, the cyclin E expression would be greater in irradiated than in MG132-treated cells. Experiments, whose results are depicted in Fig. 3, strongly support the latter. The induction of cyclin E mRNA in response to UV irradiation was significantly greater than that observed in the presence of 40 μM MG132, a concentration that almost completely inhibits proteasome activity (*25*), as evaluated by the ratio UV/MG132 = 1.60, at 4 h and 1.94 at 8 h.

Next, we evaluated the effect of cycloheximide on cyclin E expression in cells treated or not with UV light. If UV exerts its action on E2F stability solely, addition of cycloheximide would not modify cyclin E expression. Conversely, if E2F1 transcriptional induction is a functional UV effect, the added inhibitor is expected to impair cyclin E expression. Again, our results sustain a bifunctional action of UV on E2F1, since the presence of cycloheximide partially blocked the genotoxic-mediated induction of cyclin E expression, as indicated by the ratio UV/UV + cycloheximide = 1.64 at 4 h and 1.41 at 8 h. It is worth noting

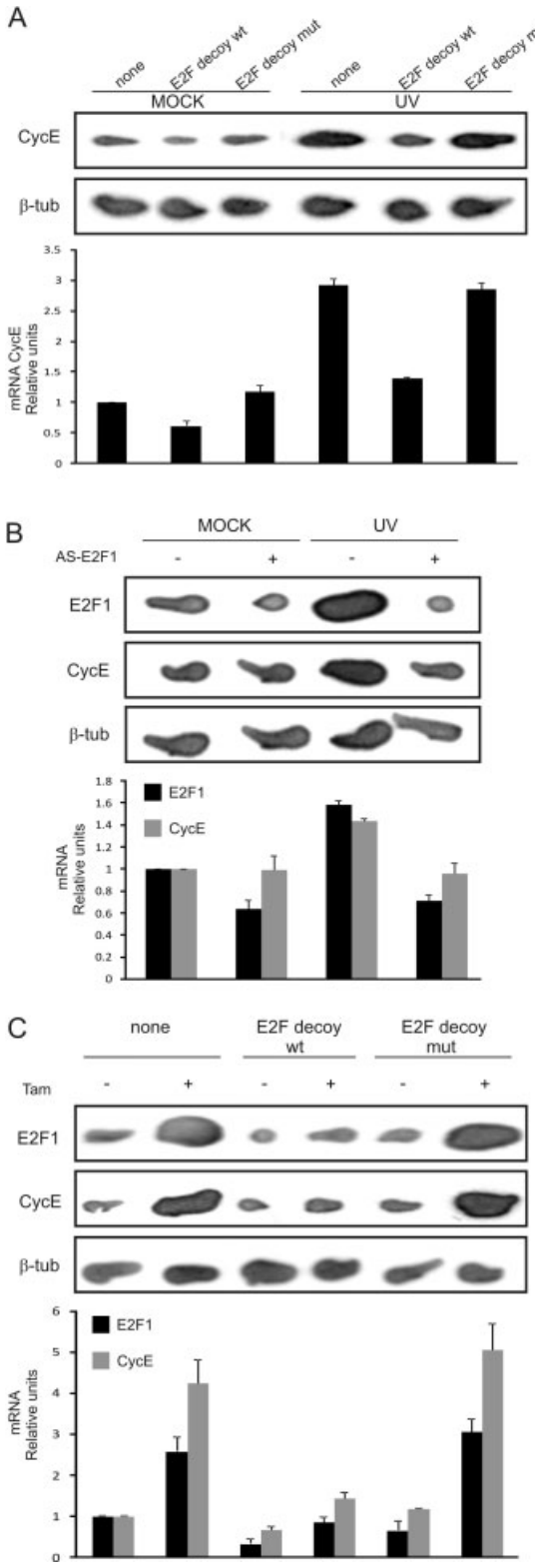

Figure 2. E2F1 mediates induction of cyclin E in response to UV irradiation. (A) BHK-21 cells were nontreated or transfected with an oligodeoxynucleotide E2F decoy (100 nM) or an E2F decoy mutated version (100 nM) and irradiated with 40 J/m^2 and harvested 8 h following irradiation. Total RNA (20 μg) was extracted from cells and subjected to Northern blot analysis using the ^{32}P-labeled probes indicated in the left margin. (B) BHK-21 fibroblasts were transfected with an E2F1 antisense oligodeoxynucleotide (2 μM). After 24 h, cells were irradiated with 40 J/m^2 UV light and harvested 8 h after irradiation. Total RNA (20 μg) was extracted from cells and subjected to Northern blot analysis using the ^{32}P-labeled probes indicated in the left margin. (C) BHK-21 fibroblasts stably transfected with an estrogen-E2F1 fusion vector in which E2F1 can be activated with tamoxifen (BHK/ER-E2F1) transfected with an oligodeoxynucleotide E2F decoy (100 nM) or an E2F decoy mutated version (100 nM). After 24 h, cells were incubated with 300 nM tamoxifen during 5 h. Total RNA (20 μg) was extracted from cells and subjected to Northern blot analysis using the ^{32}P-labeled probes indicated in the left margin. In (A–C) each figure shows a representative autoradiograph of three independent experiments with similar results. Cyclin E and E2F1 expression were normalized to β-tubulin signal. Results are expressed as relative cyclin E and E2F1 mRNA with respect to non transfected and nonirradiated or nontreated sample, which was set to 1. Low panel shows quantification of northern blot. Bars represent mean ± s.d. of three experiments. Cyc E, cyclin E; β-tub, β-tubulin; Tam, tamoxifen, AS-E2F1, E2F1 antisense oligodeoxynucleotide.

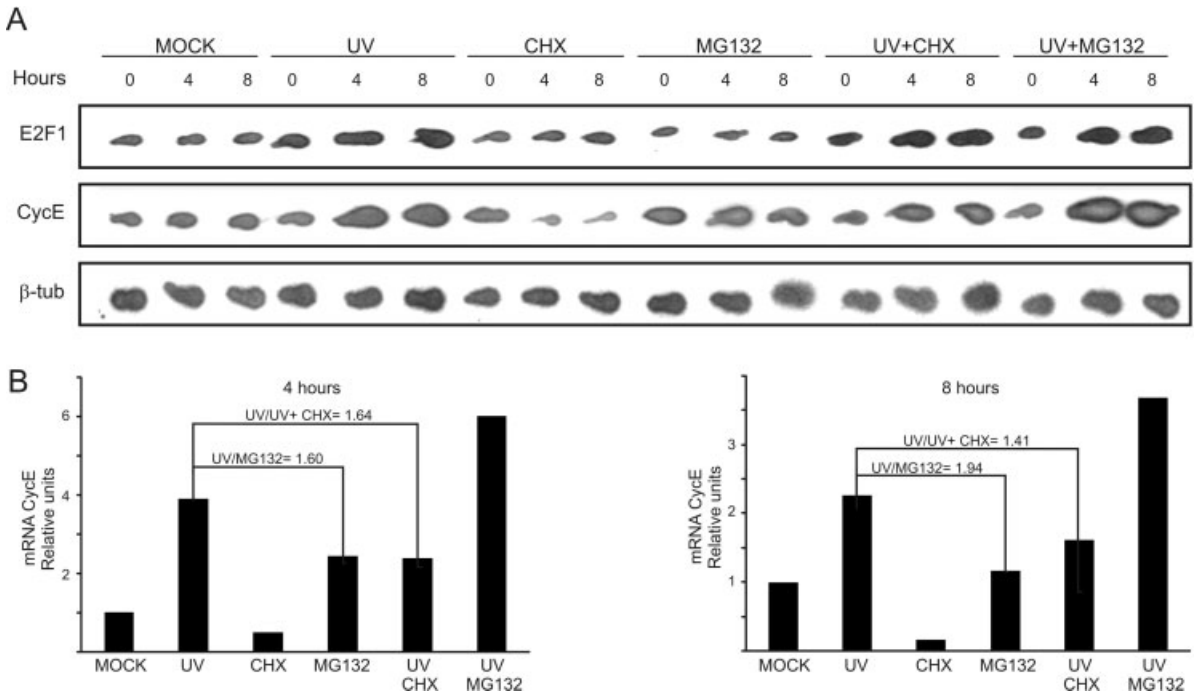

Figure 3. UV-promoted cyclin E highest induction requires E2F1 protein synthesis. (A) BHK-21 cells were irradiated with 40 J/m^2 UV or not after incubation with 10 μM cycloheximide or 40 μM MG132 for 1 h, and harvested at the indicated times following irradiation. Total RNA (20 μg) was extracted from cells and subjected to Northern blot analysis using a ^{32}P-labeled probes specific for cyclin E, E2F1, and β-tubulin mRNA. Figure shows a representative autoradiograph of two independent experiments with similar results. (B) Quantification of the Northern blot depicted in (A). Cyclin E expression was normalized to β-tubulin signal. Results are expressed as relative cyclin E expression with respect to nontreated samples, which was set to 1. Values in the graphic represent fold increases between the indicated treatments. Cyc E, cyclin E; β-tub, β-tubulin; CHX, cycloheximide.

that no changes were detected in E2F1 mRNA levels under cycloheximide or MG132 treatments.

Transcriptional Induction of E2F1 in Response to Genotoxic Stress Requires ATM/ATR Kinases

ATM/ATR activation is a very early event in the DNA damage response activating cell cycle checkpoints, DNA repair, stress response genes and apoptosis, through transduction cascades to a series of downstream effector molecules. As aforementioned, E2F1 stabilization, following UV irradiation, is a consequence, at least in part, of ATM and Chk2 phosphorylation (*26*). To explore the role of ATM/ATR in the UV-mediated induction of E2F1 mRNA, we performed northern blot assays in cells treated with a UV fluence of 40 J/m^2 in presence of caffeine. As shown in Fig. 4A, transcription of E2F1 was almost abolished in the presence of the PIKK inhibitor. Next, we asked whether the downstream effectors Chk1 or Chk2 are involved in E2F1 mRNA induction. To do this, cells were treated with SB218078 or DBH (dopamine β-hydroxylase inhibitor), a Chk1 inhibitor, or Chk2 inhibitor and UV irradiated. None of the inhibitors tested significantly reduced the induced E2F1 mRNA suggesting that neither Chk1 nor Chk2 activities are necessary.

To assess whether the DNA damage-mediated induction of E2F1 transcription is a common event rather than a genotoxic type-specific observation, we determined E2F1 expression in response to different DNA damaging agents such cis-platin and β-amyloid peptide. Cis-platin is a widely used chemotherapeutic agent that produces noncoding DNA adducts that interfere with replication and transcription. β-amyloid peptide is a component of the extracellular senile plaques which are present in patients with Alzheimer's disease, and is believed to be neurotoxic. Interestingly, E2F1 transcription was induced in cells challenged with 20 μM cis-platin or 10 μM β-amyloid. Consistent with the results obtained with UV light treated cells, induction of E2F1 mRNA in response to cis-platin or β-amyloid was dependent on ATM/ATR but not on Chk1 or Chk2 activities.

In summary, we have demonstrated that, in response to UV irradiation, E2F1 is transcriptionally induced in mammalian fibroblasts. This E2F1 transcriptional regulation seems not to be restricted to UV light, since other genotoxics, such cis-platin and peptide β-amyloid, cause a similar E2F1 upregulation. Finally, we conclude that the induction of E2F1 mRNA in response to DNA damaging agents involves ATM/ATR activation. The results presented herein uncover a new mechanism involving E2F1 in response to genotoxic stress.

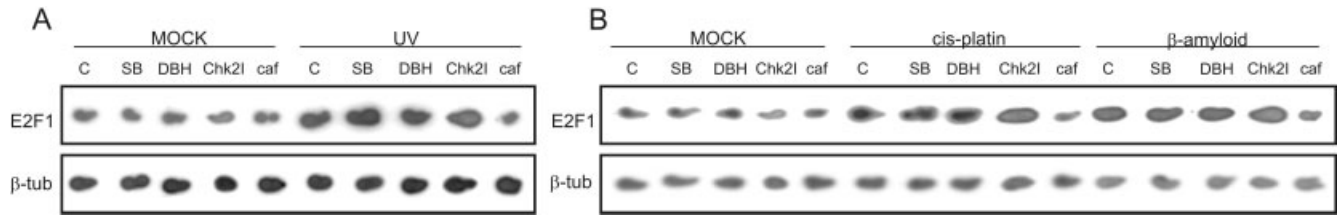

Figure 4. E2F1 upregulation in response to different genotoxics is dependent of ATM/ATR activation. (A) WI38 fibroblasts were irradiated with 40 J/m^2 after incubation with 3 μM DHB or 15 nM SB218078 or 20 nM Chk2 inhibitor or 5 mM caffeine for 1 h, and harvested at the indicated times following irradiation. (B) WI38 fibroblasts were treated with 20 μM cis-platin or 10 μM peptide β-amyloid after incubation with 3 μM DHB (dopamine β-hidroxylase inhibitor), or 15 nM SB218078 or 20 nM Chk2 inhibitor or 5 mM caffeine for 1 h, and harvested at the indicated times following genotoxic treatment. In (A and B) total RNA (20 μg) was extracted from cells and subjected to Northern blot analysis using a ^{32}P-labeled probe specific for E2F1 and reprobed for β-tubulin mRNA. Each figure shows a representative autoradiograph of two independent experiments with similar results. β-tub, β-tubulin; SB, SB218078; Chk2I, Chk2 inhibitor; caf, caffeine.

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