c-jun and Egr-1 Participate in DNA Synthesis and Cell Survival in Response to Ionizing Radiation Exposure

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Exposure of mammalian cells to ionizing radiation results in the induction of the immediate early genes, c-jun and Egr-1, which encode transcription factors implicated in cell growth as well as the cellular response to oxidative stress. We studied the role of these immediate early genes in cell cycle kinetics and cell survival following x-irradiation of clones containing inducible dominant negatives to c-jun and Egr-1. The dominant negative constructs to c-jun (Δ9) and Egr-1 (WT/Egr) prevented x-ray induction of transcription through the AP-1 and Egr binding sites, respectively. Twenty percent of confluent, serum-deprived SQ20B human tumor cells, normal fibroblasts, and fibroblasts from patients with ataxia telangiectasia entered S phase within 5 h of irradiation. Clones containing inducible Δ9 and WT/Egr dominant negative constructs demonstrated attenuation of the percentage of cells exiting G1 phase and reduced survival following irradiation. These data indicate that the dominant negatives to the stress-inducible immediate early genes Egr-1 and c-jun prevent the onset of S phase and reduce the survival of human cells exposed to ionizing radiation.

Although DNA damage-induced cell cycle delay has been the focus of recent reports (1–5), certain aspects of the cellular response to ionizing radiation resemble a growth-like response. For example, the kinetics of tumor and normal tissue cell repopulation is accelerated after irradiation (6–8). Cell survival and proliferation following x-irradiation are in part regulated by growth factors and cytokines that are induced by ionizing radiation (9–12). Furthermore, a recent report demonstrated that when irradiated, cells beyond the G1 restriction point enter S phase (3). To study the proliferative response to x-irradiation, we irradiated serum-deprived cells and determined whether x-irradiation initiates DNA synthesis in a subpopulation of G1 phase cells. Following a transient G1 delay, a reduction in the percentage of cells in G1 was evident within 2 h after irradiation. This reduction was temporally related to c-jun and Egr-1 expression.

Egr-1 and c-jun are immediate early genes encoding transcription factors that are implicated in the response of cells to a variety of stressful stimuli (13–17). Induction of these genes is associated with cell growth as well as the cellular response to oxidative stress (12, 18, 19; reviewed in Refs. 20 and 21). The transcriptional regulation of genes in response to ionizing radiation results in the expression of many genes, including Egr-1 and c-jun (14–15, 22). The role of these genes in the cellular response to x-rays may be the recruitment of quiescent cells into the cell cycle and subsequent repopulation of tissues exposed to oxidative stress (23). In particular, c-jun is a protooncogene encoding the transcription factor J un, a component of the AP-1 protein complex which regulates the transcription of a number of genes, including growth factors and cytokines (reviewed in Ref. 13). We therefore studied the role of Egr-1 and c-jun in the radiation response by using dominant negative constructs to Egr-1 and c-jun. The c-jun dominant negative (Δ9) was created by replacing the transcriptional activation domain of c-jun with the transcriptional inhibitory domain of Lex A, while maintaining the DNA binding region (24). The mutant J un lacks an activation domain and blocks stimulation of transcription by several oncoproteins, including Ras and v-Src, as well as by phorbol esters. We constructed the Egr-1 dominant negative by replacing the transcriptional regulatory domain with that of a negative transcriptional regulator of WT-1 (25). The repression function was mapped to the glutamine- and proline-rich NH2 terminus of WT-1; fusion of this domain to the zinc finger region of Egr-1 converted Egr-1 into a transcriptional repressor. This construct (WT/Egr) binds to the Egr-1 binding site (EBS)1 to block the transcriptional activation of downstream genes regulated by EBS cis-acting elements. We found that WT/Egr and Δ9 prevented x-ray induction of EBS-CAT and AP1-CAT reporter constructs and abated G1/S transition after x-irradiation.

Ataxia telangiectasia (AT) is an autosomal recessive disorder characterized by progressive ataxia, immunodeficiency, a predisposition to the development of cancer, and marked radiosensitivity (reviewed in Ref. 26). Cells isolated from patients with some AT complementary groups demonstrate neither inhibition of DNA synthesis nor G1 delay following exposure to ionizing radiation (27). We found that radiation-mediated c-jun and Egr-1 expression was prolonged in AT fibroblasts. This unopposed immediate early gene expression was associated with a prolonged exit from G1 into S phase after irradiation. These data indicate that persistent DNA damage in x-irradiated AT fibroblasts may provide a signal for unabated immediate early gene induction and subsequent exit from G1. We found that the c-jun and Egr-1 dominant negatives also pre-

1 The abbreviations used are: EBS, Egr-1 binding site; AT, ataxia telangiectasia; Gy, gray; FACS, fluorescence-activated cell sorter; CAT, chloramphenicol acetyltransferase.
vented irradiated AT fibroblasts from exiting G1. These data demonstrate phenotypes for the x-ray-inducible early genes c-jun and Egr-1.

**EXPERIMENTAL PROCEDURES**

**Cell Cultures**—Human epithelial tumor (SQ-20B) cells were grown from human laryngeal carcinoma (28). IMR90 fibroblasts and AT5BI cells were obtained from ATCC. Cell lines were maintained in medium consisting of 72.5% Dulbecco's modified Eagle's medium and 22.5% Ham's nutrient mixture F-12 (Life Technologies, Inc.), 10% fetal bovine serum (Renhautin), 100 units/ml of penicillin, and 100 μg/ml of streptomycin. Human epithelial tumor (SQ-20B) cells (28) were grown to confluence for 4 days and serum-deprived for 3 days at 37°C. The medium was removed, and the cultures were washed with PBS and then irradiated with 10 Gy (GE Maxitron x-ray generator, dose rate, 1 Gy/min). Cells were incubated for 1 h prior to irradiation, and fresh serum-free medium was added to both experimental and control plates prior to irradiation. Experiments were performed three to four times, and the mean and standard error of the mean was determined.

**RNA Analysis**—Cells were grown to confluence, serum-deprived for 24 h, and exposed to 10 Gy (GE Maxitron x-ray generator) as described previously (29). RNA was extracted by use of the single step guanidinium thiocyanate-phenol/chloroform method (30) following irradiation and was treated under otherwise identical conditions. RNA from irradiated cells were size-fractionated by 1% formaldehyde-agarose electrophoresis. Ethidium bromide staining of the RNA demonstrated equal loading of each lane. RNA gels were then transferred to a nylon membrane (Hybond N+, DuPont NEN). Northern blotting experiments were supported by the recent report that cell populations exiting S phase following x-irradiation required, in addition to the proposed signals for G1/S transition, that the cells exit G1 (31).

**Cell Cycle Analysis**—SQ20B, IMR90, and AT5BI cells were x-irradiated with 5 Gy from an x-ray generator (GE Maxitron at 2 Gy/min) at a dose rate of 1 Gy/min. Plates were returned to the incubator for time intervals ranging from 0 to 8 h following irradiation. SQ20B human epithelial tumor cells and AT5BI fibroblast lines containing dominant negatives were grown to confluence for 4 days and serum-deprived for 1 day and again treated with 5 Gy (GE Maxitron at 2 Gy/min). During induction of the dominant negatives, ZnSO4 (100 μM) was added for 5 h prior to x-irradiation. Fresh serum-free medium was added to both experimental and control plates prior to irradiation. At the indicated times, cells were trypsinized, washed in phosphate-buffered saline, and fixed in ice-cold 70% ethanol. Cells were then stained with propidium iodide. Flow cytometry and FACS analysis (FACScanner, Becton Dickinson) were used to quantify the distribution of DNA fluorescence and intensity. The proportion of cells in G1, S, and G2/M phases was calculated with the Cell Fit program (Becton Dickinson). DNA content was presented as DNA histograms, and the percentage of cells in G1, S, and G2/M was calculated for each of the time intervals following irradiation. Experiments were performed three to four times, and the mean and standard error of the mean was determined.

**Dominant Negative Constructs and Cloning**—Inducible dominant negative constructs were created by blunt end ligation of the metallothionein promoter upstream of the transcriptional start site of WT/Egr and Δ9. SQ20B human tumor cells and AT5BI fibroblasts were cotransfected with the Egr-1 dominant negative on the metallothionein-inducible promoter and the EBS linked to a CAT reporter. Transfectants were irradiated 18 h later. Fibroblasts were cotransfected with the c-jun dominant negative, Δ9, on the metallothionein-inducible promoter and the AP-1 binding element linked to a CAT reporter. Transfectants were also irradiated 18 h later. CAT enzyme activity was quantified as described previously (16).

For creation of stably integrated transfectants, inducible dominant negative constructs were transfected into SQ20B, IMR90, and AT5BI cells with Lipofectin reagent (Life Technologies, Inc.). Stably integrated transfectants were selected by growth in G418 (800 μg/ml) and trypsinization of individual colonies by use of cloning cylinders. IMR90 colonies stopped growing after several divisions and could not be cloned. Similarly, SQ20B colonies containing Δ9 discontinued growth and could not be cloned. Stable and AT5BI dominant negative-containing clones were maintained in G418 (800 μg/ml). We determined the inhibition of x-ray induction of the EBS-CAT or 3×TRE-CAT constructs when ZnSO4 was added. The clone demonstrating the greatest inhibition was then maintained in G418 and used for FACS and survival analysis.

The Qualification of X-ray-mediated Promoter/Reporter Constructs—SQ20B and AT5BI clones containing the dominant negatives were transfected with promoter reporter constructs. The promoter which binds Egr-1 into the chloramphenicol transfer gene (EBS-CAT) or the AP-1 binding agent into the chloramphenicol transfer gene (3×TRE-CAT) was transfected into clones with the Lipofectin reagent (Life Technologies, Inc.). The Lipofectin reagent was removed after 6 h, and cultures were maintained overnight before stimulation with 12-O-tetradecanoylphorbol-13-acetate or x-irradiation (10 Gy) with or without ZnSO4. Thirty hours following radiation, CAT was assayed by use of freeze-thawing as we have described previously (16). The Δ9 and WT/ Egr containing clones that demonstrated the greatest inhibition of x-ray induction were then maintained in G418. These clones were used for FACS and survival analysis.

**Radiation Survival Analysis**—Exponentially growing cells were trypsinized, counted, and plated onto 100 plates. Plating efficiencies were determined by colony forming assay as described previously (33). Cells were irradiated with a GE Maxitron x-ray generator (dose rate, 1 Gy/min). Plates were stained and colonies were counted 14 days following irradiation. During the induction of the dominant negatives, ZnSO4 (100 μM) was added for 5 h prior to irradiation, and fresh serum-free medium was added to both experimental and control plates prior to irradiation. Experiments were performed three to four times, and the mean and standard error of the mean was determined.

**RESULTS**

**Cell Cycle Kinetics**—To study the proliferative response following x-irradiation, we exposed irradiated serum-deprived cells and determined whether x-irradiation initiates DNA synthesis in G1 phase cells. We found that 83% of confluent normal human fibroblasts accumulated in G2/G1 when the cells were nutritionally deprived. After irradiation, fibroblasts were fixed and stained at 1-h intervals, and the percentages of cells in G1, S, and G2/M phases were determined by FACS analysis. A reduction in the percentage of cells in G1 was evident within 24 h after irradiation, following a transient delay of the irradiated fibroblasts in G1 (Fig. 1A). The maximal percentage of cells exiting G1 was observed 3 h after irradiation, reaching 18% of all cells (22% of cells in G2/G1). To determine whether transformed human tumor cells exhibited a similar response, we irradiated confluent serum-deprived SQ-20B cells. Of these noncontact inhibited cells, 55% were in G1/G2 prior to irradiation (Fig. 1B). These cells also began to exit G1 and enter S phase within 2 h of irradiation in the absence of serum. A maximum of 15% of all cells (27% of G1 cells) exited G1 4 h after irradiation.

We next examined AT fibroblasts that are known to be deficient in G1 delay. As with non-AT cells, 15% of AT fibroblasts (AT5BI) exited G1 and accumulated in S phase within 5 h, but exit from G1 continued for 9 h, with a total of a 22% reduction in the percentage of cells in G1 (Fig. 1C). These findings are supported by the recent report that cells beyond the G1 restriction point enter S phase following x-irradiation (31).

**Immediate Early Gene Expression**—We studied whether cell entry into S phase following x-irradiation is temporally associated with expression of c-jun and Egr-1. The time course for radiation-mediated c-jun and Egr-1 expression is shown in Fig. 1. In quiescent serum deprived cells, transient induction of c-jun and Egr-1 after exposure to ionizing radiation was indeed temporally associated with exit from G1, and subsequent entry into S phase. Expression of c-jun gene increased 8-fold at 30 min, whereas Egr-1 expression increased 9-fold. Increased expression of both Egr-1 and c-jun persisted for 9 h following irradiation of the AT cells, in contrast to normal fibroblasts and tumor cell lines in which immediate early gene induction was transient (Fig. 1) The prolonged immediate early gene expression following irradiation was associated with prolongation of the G2/S transition. These data indicate that ionizing radiation-induced exit from G1 and entry into S phase are temporally associated with immediate early gene induction.
in a manner analogous to their expression following stimulation with mitogens.

Dominant Negative Mutants of c-jun and Egr-1 Block X-ray-mediated Transcription of AP-1 and Egr Binding Sites—To study the role of radiation-mediated Egr-1 expression in cell cycle regulation and survival, we utilized the dominant negative to Egr-1 (WT/Egr) (25). We first cotransfected the plasmid containing WT/Egr with the Egr binding site linked to the CAT reporter construct (EBS-CAT) to determine whether WT/Egr prevented radiation-mediated transcriptional activation through Egr-1 (Fig. 2A). Both plasmids (pMT-WT/Egr and pEBS-CAT) were added. A 10-fold increase in CAT expression resulted from irradiation of AT5BI cells transfected with EBS-CAT. The 10-fold increased expression of EBS-CAT was attenuated to 2-fold when WT/Egr was cotransfected with EBS-CAT. Similarly, a 3-fold increase in CAT expression was observed after irradiation of SQ20B cells transfected with EBS-CAT (Fig. 2A). This induction was abolished by cotransfection

![Fig. 1. DNA quantification following irradiation. Confluent, serum-deprived cells were irradiated and DNA was stained with propidium iodide. The DNA quantity of cells was quantified by FACS analysis, and the percentages of cells in G1 and S were calculated by means of the Cell Fit program (Becton Dickinson). RNA was extracted at the indicated times following irradiation. Northern blots were hybridized to 32P-labeled c-jun and Egr-1 DNA probes followed by autoradiography and quantification by densitometry. Data presented show the mean and S.E. of three to four Northern blots. A, c-jun and Egr-1 gene expression and percentage of irradiated SQ-20B cells and G1 in S phase. B, c-jun and Egr-1 gene expression and the percentage of IMR-90 fibroblasts in G1 in S phase. C, c-jun and Egr-1 gene expression and AT5BI fibroblasts in G1 and S phase.](http://www.jbc.org/)

![Fig. 2A.](http://www.jbc.org/)

![Fig. 2B.](http://www.jbc.org/)

![Fig. 2C.](http://www.jbc.org/)
A  SQ-20B  
| fold induction EBS-CAT | 1 | 2 | 3 | 4 |
|------------------------|---|---|---|---|
| wild type              |   |   |   |   |
| control                |   |   |   |   |
| x-ray                  |   |   |   |   |
| WT/Egr transfected     |   |   |   |   |
| control                |   |   |   |   |
| x-ray                  |   |   |   |   |

B  AT5BI  
| fold induction EBS-CAT | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
|------------------------|---|---|---|---|---|---|---|---|---|---|
| wild type              |   |   |   |   |   |   |   |   |   |   |
| control                |   |   |   |   |   |   |   |   |   |   |
| x-ray                  |   |   |   |   |   |   |   |   |   |   |
| WT/Egr transfected     |   |   |   |   |   |   |   |   |   |   |
| control                |   |   |   |   |   |   |   |   |   |   |
| x-ray                  |   |   |   |   |   |   |   |   |   |   |

C  AT5BI  
| fold induction AP1-CAT | 1 | 2 | 3 | 4 |
|-------------------------|---|---|---|---|
| wild type               |   |   |   |   |
| control                 |   |   |   |   |
| x-ray                   |   |   |   |   |
| A9 transfected          |   |   |   |   |
| control                 |   |   |   |   |
| x-ray                   |   |   |   |   |

Fig. 2. Dominant negative mutants to c-jun and Egr-1 prevent x-ray induction through AP-1 and Egr binding sites. Cells were cotransfected with the Egr-1 dominant negative (WT/Egr) and the Egr-1 binding site (EBS) linked to a CAT reporter. Transfectants were irradiated 18 h later. Shown are CAT enzyme levels calculated as the percent conversion of [14C]chloramphenicol to the acetylated forms. AT1VB fibroblasts were cotransfected with the c-jun dominant negative (A9), and the AP-1 binding element linked to a CAT reporter (3×TRE-CAT). Transfectants were irradiated 18 h later. Shown are the means and S.E. from three experiments. A, SQ20B cells transfected with WT/Egr dominant negative. B, AT5BI transfected with WT/Egr dominant negative. C, AT5BI fibroblasts transfected with A9.

with WT/Egr, Cotransfection of control plasmid (pUC) with reporter-reporter construct (EBS-CAT) did not affect x-ray mediated induction of transcription.

To study the role of c-jun induction in the onset of S phase following irradiation, we utilized the transacting dominant negative to c-jun (A9) in which the DNA binding region is maintained, and the transcriptional activation domain is replaced with the transcriptional inhibitory domain of Lex A (24). We first cotransfected the A9 expression vector with the AP-1-CAT reporter construct (3×TRE) to determine whether A9 prevented radiation-mediated AP-1 activation. Both plasmids (pMT-D9 and p3×TRE-CAT) were added to Lipofectin solution and incubated. A reduction in the radiation inducibility of AP-1 by A9 was observed (Fig. 2C). A 3-fold increase in CAT expression resulted from irradiation of AT5BI cells transfected with 3×TRE-CAT. This increased expression was attenuated when A9 was cotransfected with 3×TRE-CAT.

Dominant Negative Mutants Blocked X-ray-mediated Exit from G1—We studied the biological effects of the WT/Egr dominant negative construct on the radiation response in SQ-20B and AT5BI cells. Stably transfected clones containing WT/Egr under the transcriptional regulation of the metallothionein promoter were selected by use of the neomycin resistance gene and colony formation in the presence of G418. Clones transfected with WT/Egr demonstrated attenuated transcriptional activation of the transfected EBS-CAT reporter following irradiation. The SQ20B clone containing WT/Egr was irradiated and stained with propidium iodide, and the DNA content was quantified in the population of cells by FACS analysis. The WT/Egr clone demonstrated no increase in the percentage of cells entering S phase in response to x-irradiation (Fig. 3A). The AT fibroblast clone containing WT/Egr was also irradiated, and the cell cycle distribution was analyzed by flow cytometry. This clone demonstrated no significant decrease in the percentage of cells in G1, and no increase in the percentage of cells in S phase in response to irradiation (Fig. 3B). These data indicate that transcriptional regulation through the Egr binding site in part controls exit from G1 and entry into S phase following irradiation of human cells.

Stably transfected clones containing the A9 dominant negative under the transcriptional control of the metallothionein promoter were selected for expression of the neomycin resistance gene. Colonies grown in the presence of G418 demonstrated no 3×TRE induction by x-irradiation. AT clones containing A9 demonstrated attenuated exit from G1 when pretreated with ZnSO4 for 4 h prior to irradiation. Clones treated in the absence of ZnSO4 demonstrated a 15% reduction in the percentage of cells in G1 within 9 h after irradiation (Fig. 3C). Exit from G1 after x-irradiation of nontransfected cells was not affected by ZnSO4. A concomitant increase in the percentage of cells in S phase occurred during this time interval.

The Effects of c-jun and Egr-1 Dominant Negatives on Survival of Irradiated Cells—To study the effects of Egr-1 dominant negative constructs on cell killing by ionizing radiation, we irradiated clones containing WT/Egr with or without ZnSO4 added prior to irradiation. The survival of SQ20B clones containing WT/Egr was reduced slightly when these were treated with ZnSO4 as compared with nontransfected wild-type SQ-20B cells. We corrected for this reduced plating efficiency during analysis of radiation killing. The survival rate of the SQ20B clone containing WT/Egr was significantly less than that of nontransfected wild-type SQ-20B cells (Fig. 4). Radiation survival analysis was performed by means of the colony-forming assay. Fig. 4 shows that, after irradiation with 11 Gy, the surviving fraction of wild-type SQ20B cells was 0.0005, as compared with 0.0005 in WT/Egr containing clones with ZnSO4 (p < 0.01). We also utilized the AT fibroblast clones containing the WT/Egr dominant negative construct to verify the effects of WT/Egr on radiation cell killing. The plating efficiency for AT-WT/Egr clones was 25% and did not differ from that of wild-type AT5BI fibroblasts or clones treated with ZnSO4. Fig. 4 shows that, following 5 Gy (a dose equitoxic to 11 Gy in SQ20B cells), the surviving fraction of AT5BI WT/Egr clones without ZnSO4 was 0.0004 as compared with 0.0001 in WT/Egr-containing clones treated with ZnSO4 (p < 0.05). Thus, attenuation of radiation-mediated transcriptional activation through the Egr binding site is associated with reduced cell survival following irradiation.

To study the effects of c-jun dominant negative constructs on
cell killing by radiation, we irradiated the clone containing D9 with or without ZnSO4 added prior to the irradiation. The plating efficiency for the AT-D9 clone was 25% and did not differ from that for wild-type AT5BI fibroblasts or clones treated with ZnSO4. Radiation survival analysis was performed with the colony-forming assay. Fig. 4 shows that, following treatment with 5 Gy, the surviving fraction of wild-type AT5BI cells was 0.0006 and was 0.0002 in D9-containing clones treated with ZnSO4 (p < 0.05). Thus, attenuation of transcriptional activation through AP-1 is associated with reduced cell survival following x-irradiation.

DISCUSSION
Our objective in this study was to determine the role of the immediate early genes c-jun and Egr-1 in the response of human cells to ionizing radiation. We found that radiation-mediated expression of c-jun and Egr-1 preceded the departure of cells from G1. To study the requirement for these genes during the exit from late G1 into S phase, we utilized inducible dominant negatives to c-jun and Egr-1. Basal expression of these constructs was observed as diminished transcriptional activation in the absence of ZnSO4. Each of the clones that grew in the presence of G418 was tested for inhibition of x-ray induction of the promoter-reporter constructs (EBS-CAT and 3TRE-CAT) in the presence of zinc sulfate. These results indicated the extent to which the dominant negatives are inducible in these clones. Each clone that demonstrated optimal induction of dominant negative activity was maintained in G418. FACS analysis and radiation survival analysis were performed only on these clones, demonstrating optimal dominant negative induction by ZnSO4. We found that dominant negatives to both Egr-1 and c-jun attenuated the G1 exit of irradiation cells. These cells were serum-deprived and confluent, and therefore a large percentage of them were already in G1. Following a transient G1 delay, irradiated mammalian cells typically demonstrate an accumulation in S phase (45) (reviewed in Ref. 46). This has been referred to as an S phase arrest which is followed by a more pronounced G2 delay. It is important to note that the percentage of cells in G2 did not vary throughout the 8 h of observation in these experiments. Taken together, these data indicate that transcriptional regulation through AP-1 and the Egr binding site in part regulates exit from G1 following irradiation. These findings do not exclude the possibility that these dominant negatives act through mechanisms other than blocking transcriptional activation. Jun and Egr-1 may have metabolic functions that have not been determined. One possible example of this is the demonstration that c-jun participates in DNA replication (37, 38). Furthermore, the transcriptional activation domain of j un is required for both transcriptional activation and DNA synthesis (47). Regardless of its mechanism of action, data presented herein...
indicate that Jun is induced by ionizing radiation and is required for transition from G1 to S phase.

We found a 1-h delay in G1 before cells enter S phase as shown by previous studies (34, 46). These data are supported by the recent finding that cells beyond the G1 restriction point enter S phase after X-irradiation (3). Cell cycle-regulated genes such as thymidine kinase, dihydrofolate reductase, DNA polymerase-α, and PCNA are all induced during late G1 to early S phase (48). In addition, the retinoblastoma susceptibility gene (Rb), as well as genes encoding the transcription factors E2F1, c-Myc, and J un, have been shown to be induced in late G1. Their respective gene products are presumably involved in the regulation of subsequently expressed genes required for DNA synthesis. We have found that 20% of cells begin to synthesize DNA following a brief delay of 1–2 h after irradiation. The transient G1 delay observed in this study is consistent with that found in previous studies (49). This brief delay that precedes the onset of DNA synthesis may be due to the prerequisite formation of protein complexes such as E2F-pRb or E2F combined with other cell cycle-regulating elements such as cyclin-E, cdk2, p107, and p130 (50).

We found that the rapid induction of c-jun and Egr-1, and the subsequent onset of DNA replication, were associated with the survival response of mammalian cells to ionizing radiation. Although there is a correlation between exit from G1 and cell survival in the present study, a causal relationship has not been established. One of the difficulties in further establishing the impact of dominant negative constructs to c-jun and Egr-1 on radiation survival is that these constructs induce cell killing. We found that the c-jun dominant negative construct (Δ9) prevented colony formation in both IMR-90 fibroblasts and SQ-20B cells whereas, WT/Egr prevented colony formation in IMR-90 fibroblasts only. It is not known why SQ-20B cells will grow following transfection with WT/Egr but do not grow when Δ9 is expressed at low basal levels. Although these dominant negative constructs are regulated by the metallocodonin promoter, we found that there is low basal expression. Even in the absence of ZnSor, these constructs reduce radiation induction through their respective DNA binding sites. Thus, colony formation will not occur in unstimulated cells.

This study also presents new findings on the transcriptional regulation of the c-jun protooncogene in irradiated AT fibroblasts. Cells isolated from patients with some AT complementary groups demonstrate negative inhibition of DNA synthesis or G1 delay following exposure to ionizing radiation (27). DNA damage-induced cell cycle arrest is proposed to be a primary means to protect against replication of a damaged DNA template. Genetic instability occurs in a number of disorders related to abnormalities in p53, including Li Fraumeni syndrome and AT. In this regard, one potential biological consequence of the lack of a G1 check point in AT cells is the replication of a damaged DNA template which may be associated with the propagation of genetic errors (51, 52).

We examined c-jun expression in irradiated cells, because c-jun expression is associated with cellular proliferation. Evidence that c-jun plays a role in G1/S transition is that c-jun mRNA levels peak prior to the exit of cells from G0/G1 (34–35). Furthermore, microinjection of excess AP-1 binding sequences (36) or J un antibodies into proliferating cells prevents the onset of DNA replication (37). Moreover, J un is a regulator of DNA replication (38) and may be required for regulation of genes that control progression through G1, such as cyclin D1 (39). We have shown here that blocking the effects of J un in irradiated cells prevents the G1/S transition and enhances cell killing by X-rays. However, the extreme radiosensitivity of AT cells, despite prolonged immediate early gene induction and entrance into S phase, suggests that induction of Egr-1 and c-jun is not sufficient to rescue these cells from radiation-induced death, but is a consequence of persistent damage. Taken together with the findings that the premitotic checkpoint and cell viability are dependent upon the onset of S phase of the cell cycle in lower eukaryotes (40), the role of x-ray-mediated c-jun expression may be in the initiation of DNA replication following genotoxic stress.

One recently proposed function of the stress-activated protein kinases has been to activate c-jun induction following exposure to oxidative and other types of stress (18–19, 41–43). We have previously shown that x-ray-mediated microtubule-associated protein and pp90 (44) kinase activation regulates induction of c-jun and Egr-1 (44). We suggest that microtubule-associated protein, pp90 (45) and (MAPK/extracellular signal-regulated kinase) kinase kinase-stress-activated protein kinase kinases may mediate the G1 to S phase transition reported here. As in lower eukaryotes, mammalian cells are likely to require independent, parallel, but potentially interactive signaling pathways which allow for maximum adaptability to stress (19, 41). It is likely that the microtubule-associated protein, pp90 (46) and (MAPK/extracellular signal-regulated kinase) kinase kinase-stress-activated protein kinase signaling pathways are activated by ionizing radiation and may act independently or interact by as yet unknown mechanisms to control cell cycle progression and survival. Egr-1 and c-jun regulation of the onset of S phase following X-irradiation may be due to direct interactions with genes that govern cell cycle regulation as well as transcriptional regulation of downstream genes that enhance cell survival.

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