27. P. Sung, S. Prakash, L. Prakash, *Proc. Natl. Acad. Sci. U.S.A.* **87**, 2695 (1990).
28. V. Bailly, J. Lamb, P. Sung, S. Prakash, L. Prakash, *Genes Dev.* **8**, 811 (1994).
29. A. J. Rattray and L. S. Symington, *Genetics* **138**, 587 (1994).
30. R. C. Von Borstel, K. T. Cain, C. M. Steinberg, *ibid.* **69**, 17 (1971).
31. V. I. Korogodin *et al.*, *Yeast* **7**, 105 (1991).
32. E. Balbinder *et al.*, *Genetics* **66**, 31 (1970).
33. F. Palombo, E. Kohfeldt, A. Calcagnile, P. Nehls, E. Dogliotti, *J. Mol. Biol.* **223**, 587 (1992).
34. S. Gangloff, M. R. Lieber, R. Rothstein, *Experientia* **50**, 261 (1994).
35. B. D. Davis, *Proc. Natl. Acad. Sci. U.S.A.* **86**, 5005 (1989).
36. C. Melamed, Y. Nevo, M. Kupiec, *Mol. Cell. Biol.* **12**, 1613 (1992).
37. L. K. Derr, J. N. Strathern, D. J. Garfinkel, *Cell* **67**, 355 (1991).
38. L. K. Derr and J. N. Strathern, *Nature* **361**, 170 (1993).
39. J. N. Strathern, B. Shafer, C. B. McGill, *Genetics* in press.
40. M. S. Fox, J. P. Radicella, K. Yamamoto, *Experientia* **50**, 253 (1994).
41. P. C. Hanawalt, *Science* **266**, 1957 (1994).
42. R. Rothstein, *Methods Enzymol.* **194**, 281 (1991).
43. U. N. Fleig, R. D. Pridmore, P. Philippsen, *Gene* **46**, 237 (1986).
44. D. E. Lea and C. A. Coulson, *J. Genet.* **49** 264 (1949).
45. Plasmid pSR247, a pGEM2 derivative containing the *pGAL-lys2ΔBgl* allele and the yeast *URA3* gene, was constructed as follows. The unique Bgl II restriction site near the 5′ end of the *LYS2* gene on plasmid pDP6 (*43*) was filled-in with Klenow, adding 4 base pairs to the coding sequence and creating a Cla I site (plasmid pSR108). A 3-kb Nco I fragment containing the distal two-thirds of the *lys2ΔBgl* allele was deleted from pSR108 to give plasmid pSR227. A 1.5-kb Eco RV–Hind III fragment from pSR227 was subcloned into Hinc II-Hind III–digested pGEM2, yielding a plasmid (pSR232) containing a promoterless 5′ portion of the *lys2ΔBgl* allele. A 1-kb fragment derived from chromosomal sequences upstream of the *LYS2* locus (from plasmid pDCH6) (*43*) was subcloned into Eco RI–digested pSR232 yielding plasmid pSR233. A Pst I fragment containing the *GAL1-10* promoter region {the Bam HI–Eco RI promoter fragment of pBM150 [M. Johnston, R. W. Davis, *Mol. Cell. Biol.* **4**, 1440 (1984)] with added Pst I linkers} was then inserted at the unique Pst I site adjacent to the promoterless *lys2ΔBgl* fragment of pSR233 to give plasmid pSR239; the *lys2* sequences are transcribed from the *GAL10* promoter. Finally, a 1.2-kb *URA3* Hind III fragment was inserted at the unique Hind III site of pSR239 to give plasmid pSR247.
46. The *pGAL-lys2ΔBgl* allele was introduced by a two-step replacement procedure (*42*) in which pSR247 was targeted to integrate at the *LYS2* locus by digestion with Nru I. After selection of transformants on uracil-deficient medium, Ura$^-$ segregants were selected on 5-FOA and screened for a Lys$^-$ phenotype. The structure of the presumptive chromosomal *pGAL-lys2ΔBgl* allele was confirmed by Southern (DNA) blot analysis. Plasmid pSR244 contains a *gal80::HIS3* disruption allele and was constructed by replacing a Bgl II fragment within the *GAL80* coding sequence [T. E. Torchia, R. W. Hamilton, C. L. Cano, J. E. Hopper, *Mol. Cell. Biol.* **4**, 1521 (1984)] of plasmid pSR243 (3.1-kb *GAL80* Hind III fragment in pGEM3) with a 1.7-kb *HIS3* Bam HI fragment. One-step disruption (*42*) of *GAL80* was accomplished by transforming yeast with Nco I–Sma I–digested pSR244 and selecting His$^+$ transformants. The presence of the *gal80::HIS3* disruption in the resulting strain (SJR282) was confirmed by sensitivity of cells to 2-deoxygalactose and by Southern blot analysis.
47. F. Sherman, *Methods Enzymol.* **194**, 3 (1991).
48. S. E. Luria and M. Delbruck, *Genetics* **28**, 491 (1943).
49. All gene disruptions were done with a *hisG-URA3-hisG* cassette [E. Alani, L. Cao, N. Kleckner, *Genetics* **116**, 541 (1987)]. The *rev3Δ::hisG-URA3-hisG* allele was contained on plasmid pPM292 (obtained from L. Prakash), the *rad6Δ::hisG-URA3-hisG* allele on plasmid pR671 [J. F. Watkins, P. Sung, S. Prakash, L. Prakash, *Genes Dev.* **7**, 250 (1993)], the *rad18Δ::hisG-URA3-hisG* allele on plasmid pJJ239 [J. F. Watkins, P. Sung, S. Prakash, L. Prakash, *Genes Dev.* **7**, 250 (1993)], and the *rad52::hisG- URA3-hisG* allele on plasmid pSR136 [D. F. Steele, M. E. Morris, S. Jinks-Robertson, *Genetics* **127**, 53 (1991)]. SJR297 and SJR298 were transformed with appropriately digested plasmids and Ura$^+$ transformants were selected. Disruptions were confirmed by PCR or Southern blot analysis.
50. We thank P. Hanawalt and C. Lawrence for helpful comments on the manuscript and L. Prakash for plasmids. Supported by NIH grant GM38464 to S.J.R.

24 October 1994; accepted 11 April 1995

Correction of Radiation Sensitivity in Ataxia Telangiectasia Cells by a Truncated IκB-α

Mira Jung, Yin Zhang, Sung Lee, Anatoly Dritschilo*

Cells from patients with ataxia telangiectasia (AT) are hypersensitive to ionizing radiation and are defective in the regulation of DNA synthesis. A complementary DNA that corrects the radiation sensitivity and DNA synthesis defects in fibroblasts from an AT group D patient was isolated by expression cloning and shown to encode a truncated form of IκB-α, an inhibitor of the nuclear factor kappa B (NF-κB) transcriptional activator. The parental AT fibroblasts expressed large amounts of the IκB-α transcript and showed constitutive activation of NF-κB. The AT fibroblasts transfected with the truncated IκB-α expressed normal amounts of the IκB-α transcript and showed regulated activation of NF-κB. These results suggest that aberrant regulation of NF-κB and IκB-α contribute to the cellular defect in AT.

Ataxia telangiectasia (AT) is a human autosomal recessive disease characterized by neurological, immunological, and radiobiological deficiencies. Four genetic complementation groups and two variants have been identified by heterokaryon analysis (*1*). Cells from patients with AT are hypersensitive to ionizing radiation and show aberrant regulation of DNA synthesis (*2*). To identify genes that contribute to the radiation sensitivity of AT cells, we used an Epstein-Barr virus (EBV)–based expression vector to screen a complementary DNA (cDNA) library for the ability to restore normal radiosensitivity to AT cells. When this vector (pCNCNot) is transfected into cells that produce the EBV nuclear antigen–1 (EBNA-1), the plasmids are maintained episomally and can be retrieved by Hirt DNA extraction methods (*3*, *4*).

SV40-immortalized fibroblasts from an AT group D patient (AT5BIVA) were first transfected with the p266CH2 plasmid (which carries the EBNA-1 gene), and a clonally derived cell line (ATCL2) was established to serve as the recipient for gene transfer experiments (*4*). A cDNA library from SQ-20B cells, a human squamous carcinoma cell line previously characterized as radiation-resistant, was constructed in the pCNCNot vector (*4*, *5*). We reasoned that these cells would express adequate quantities of the mRNAs associated with the radioresistant phenotype.

After cDNA transfection and drug selection, cells were pooled, expanded, and exposed to radiation selection with 8 gray (Gy) of ionizing radiation. Cells that survived the first radiation exposure were expanded and re-exposed. Several surviving colonies were then subcloned and cultured for further studies. Clonally derived cell lines ATCL2-8 and ATCL2-11 were isolated from independent, but procedurally similar transfection experiments.

Radiation sensitivity is defined by the steepness of the terminal slope of the survival curve when cells are exposed to graded doses of ionizing radiation. As determined from target theory analysis, the slope is proportional to $1/D_0$, where D_0 is the dose required to reduce cell survival to 37% (*6*). Values for D_0 range from 1.2 to 1.4 Gy for normal human fibroblasts and from 1.1 to 1.9 Gy for SV40-immortalized fibroblasts (*7*). The ATCL2-8 and ATCL2-11 cells were less sensitive to radiation (D_0 = 1.7 and 1.6 Gy, respectively) than were parental AT5BIVA cells (D_0 = 0.7 Gy) (Fig. 1).

Episomal DNAs were obtained by Hirt extractions, revealing seven candidate cDNAs in ATCL2-11 and one in ATCL2-8. The cDNAs ranged in size from 0.2 to 2 kb, and sequence analysis revealed that a 1-kb cDNA was common to both cell lines. The candidate 1-kb cDNA was then retransfected into ATCL2 cells, yielding

M. Jung, Departments of Radiation Medicine and Microbiology and Immunology, Georgetown University School of Medicine, 3800 Reservoir Road, NW, Washington, DC 20007, USA.
Y. Zhang, S. Lee, A. Dritschilo, Department of Radiation Medicine, Georgetown University School of Medicine, 3800 Reservoir Road, NW, Washington, DC 20007, USA.
*To whom correspondence should be addressed.

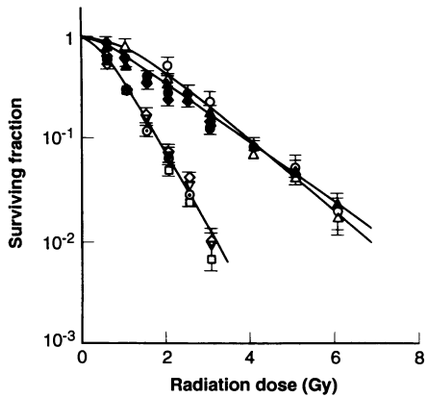

Fig. 1. Correction of radiation sensitivity in AT cells. Clonogenic survival of cells was determined after their exposure to graded doses of ionizing radiation from a clinical Theratron 80 Cobalt 60 machine. Data were fit with a single-hit multitarget model (*6*) to determine values for D_0 and n, the extrapolation number (*21*). Complemented AT cells (ATCL2-11), closed circles and triangles; D_0 = 1.6 Gy, n = 1.0. Normal human fibroblasts (NC-1), open circles and triangles; D_0 = 1.2, n = 2.5. Remaining open symbols show a composite curve for parental AT cells AT5BIVA. Experimental points were determined in triplicate; error bars represent ±SEM. Each symbol represents the results of an independent experiment.

transfectants with normal radiation sensitivity (ATCL2-JD1; D_0 = 1.3 Gy).

Failure to arrest DNA synthesis in response to irradiation is another characteristic of AT cells and is the basis for the genetic complementation groups of AT. We therefore studied DNA synthesis in the parental and transfected AT cells after exposing them to ionizing radiation. Like normal fibroblasts, ATCL2-JD1 cells showed an inhibition of DNA synthesis, whereas the parental AT5BIVA cells showed no inhibition (Fig. 2).

The candidate 1-kb cDNA encodes a truncated form of IκB-α (ΔIκB-α), a protein that inhibits the transcription factor NF-κB (*8*). NF-κB is a member of the Rel family of proteins; it binds to specific DNA sequences (κB sites) and functions as a transcriptional activator in the nucleus (*9*). IκB-α forms a complex with NF-κB that is maintained in the cytoplasm. When NF-κB is activated (for example, in response to cytokines, cellular stress, and reactive oxygen intermediates), IκB-α becomes phosphorylated and undergoes proteolysis (*9*). The unbound NF-κB then translocates to the nucleus, where it activates transcription.

To determine if ΔIκB-α was functionally replacing a mutant, endogenous IκB-α in the AT5BIVA cells, we investigated the endogenous and transfected genes by single-strand conformational polymorphism (SSCP) assays and sequence analysis. Polymerase chain reaction (PCR) primers were based on the published IκB-α sequence (*10*), and sequencing was confirmed by duplicate automated and manual techniques (*11*). No point mutations were found in either of the IκB-α sequences (*8*). However, the transfected 1-kb cDNA encoded a truncated form of IκB-α (ΔIκB-α) that was missing 45 amino acids at the NH_2-terminus. Analysis of IκB-α in SQ-20B cells revealed only full-length species, suggesting that the truncation occurred during construction of the cDNA library.

Because IκB-α plays a central role in the regulation of NF-κB, we investigated the expression of IκB-α in AT and control cells by Northern (RNA) blot analyses. The AT5BIVA cells contained larger amounts of the 1.6-kb IκB-α transcript than did the transfected AT cells or control fibroblasts (Fig. 3A). AT cells from complementation groups A and C also expressed elevated amounts of the IκB-α transcript (Fig. 3B), although transfection of group A fibroblasts

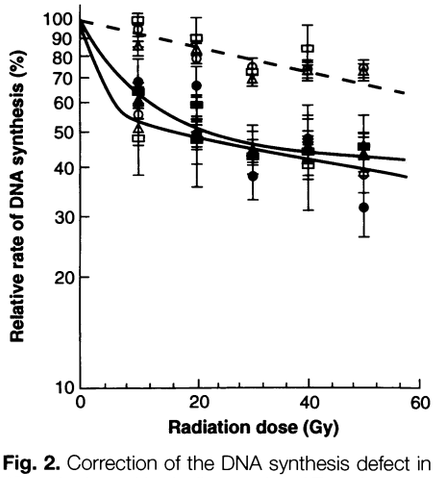

Fig. 2. Correction of the DNA synthesis defect in AT cells. Logarithmically growing AT and control cells (10^4 MRC5VI fibroblasts) were incubated at 37°C for 48 hours in minimal essential medium containing [^{14}C]thymidine (0.02 μCi/ml) and exposed to the indicated doses of radiation. The cells were then pulse-labeled with [^{3}H]thymidine (5 μCi/ml) for 90 min and subsequently lysed and acid-precipitated. Relative incorporation of [^{3}H]thymidine was determined as the ratio of ^{3}H disintegrations per minute to ^{14}C disintegrations per minute. Curves for AT5BIVA data (broken line), ATCL2-JD1 (closed symbols, solid line), and MRC5V1 (open symbols, solid line) were fit by inspection. Data were determined in triplicate, and each symbol represents the results of an independent experiment. Error bars represent ±SD.

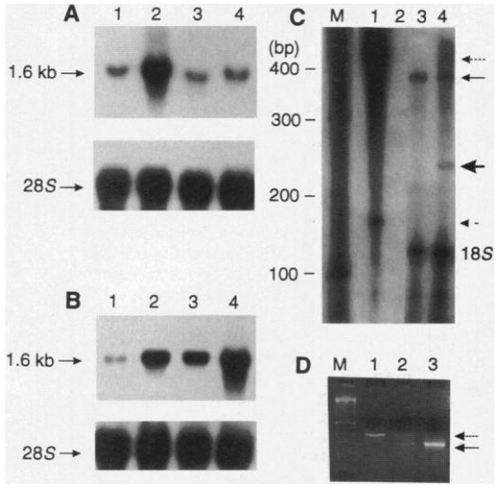

Fig. 3. Expression of IκB-α mRNA in AT cells. Total RNA was extracted with an RNAzol B kit (Tel-Test, Friendswood, Texas) and subjected to electrophoresis on a 1.2% formamide-agarose gel. Hybridizations were performed with an IκB-α–specific probe prepared by digestion of the plasmid containing an IκB-α partial cDNA [350 base pairs (bp)] and labeled by random priming. The membrane was washed and rehybridized with a probe for 28S ribosomal RNA (Clontech Lab, Palo Alto, California). (**A**) Northern analysis was performed on the total RNA (20 μg) from the following cell lines: MRC5CV1 (lane 1), AT5BIVA (lane 2), ATCL2-11 (lane 3), and ATCL2-8 (lane 4). (**B**) Comparison of IκB-α mRNA expression in other complementation groups (A and C) of AT fibroblasts: MRC5CV1 (lane 1), AT3BIVA (lane 2), AT4BIVA (lane 3), and AT5BIVA (lane 4). (**C**) A ribonuclease protection assay was performed with a 387-nucleotide cDNA fragment of IκB-α in PCRII (Invitrogen). The plasmid was linearized with Xba I and transcribed with Sp6 polymerase to yield a 480-nucleotide antisense transcript. Ten micrograms of total RNA was hybridized with [α-^{32}P]uridine triphosphate–labeled antisense RNA probes (8×10^4 counts per minute) of IκB-α and 18S ribosomal RNA (control) for 16 hours at 45°C. After hybridization of yeast RNA (lane 2), AT5BIVA RNA (lane 3), and ATCL2-11 RNA (lane 4) to the probes, the samples were digested with 0.5 unit of ribonuclease A and 20 units of RNase T_1 for 30 min at 37°C. Lane 1 was hybridized with yeast RNA without ribonuclease treatment. The samples were subjected to electrophoresis in 6% polyacrylamide gels containing 8 M urea and exposed at −20°C to Kodak XAR-5 film. The protected IκB-α and ΔIκB-α fragments are marked with small and large solid arrowheads, respectively. Unprotected fragments are indicated with broken arrows. "M" represents the end-labeled molecular size marker. (**D**) RT-PCR analysis was performed for determination of IκB-α and ΔIκB-α expression in AT5BIVA (lane 1), ATCL2-11 (lane 2), and ATCL2-JD1 (lane 3) cells with specific primers for the 5′ end of the endogenous and exogenous sequences and the common 3′ end. Samples were subsequently subjected to electrophoresis on a 2% agarose gel containing ethidium bromide (20 μg/ml). The expected molecular sizes are indicated at the right with broken and solid arrows for IκB-α (385 bp) and ΔIκB-α (280 bp) PCR products, respectively. "M" represents the 100-bp ladder molecular size marker.

with ΔIκB-α did not correct their radiosensitivity (*12*).

We confirmed that both IκB-α and ΔIκB-α transcripts were expressed in transfected AT cells by reverse transcription–PCR analysis (Fig. 3D). A quantitative ribonuclease protection assay revealed that the transfected cells contained ~10 times more of the endogenous IκB-α transcript than the exogenous ΔIκB-α transcript (Fig. 3C).

We also analyzed IκB-α and ΔIκB-α in AT cells at the protein level with an antibody to the NH_2-terminus of IκB-α. Immunoblot analysis revealed a single band, reflecting the endogenous protein, in both parental and transfected AT cells (Fig. 4A, lanes 1 and 2). An antibody to the COOH-terminus yielded a single band in parental AT cells, but two bands in transfected cells, confirming the presence of the truncated ΔIκB-α protein (lanes 3 and 4). The second band was identified as ΔIκB-α by immunoblot analysis and by its comigration with the ΔIκB-α in vitro translation product (*13*).

Activated NF-κB has been reported to regulate the expression of IκB-α through an autoregulatory feedback loop (*14*). We therefore studied NF-κB activity in the AT cells (measured as the binding of NF-κB to its κB recognition site) by a gel shift assay (Fig. 4B). The parental AT5BIVA cells showed constitutive activation of NF-κB, whereas ATCL2-11 cells showed regulated activation of NF-κB. Furthermore, although the level of NF-κB–DNA binding should increase in irradiated cells (*15*), no such enhancement was observed in AT5BIVA cells. In contrast, the transfected ATCL2-11 cells did show an increase of protein binding to κB sequences after irradiation. Taken together, these data provide evidence that ΔIκB-α restored regulated activation of NF-κB in AT cells.

The mechanism by which ΔIκB-α corrects the aberrant regulation of NF-κB in AT5BIVA cells remains unclear but may relate to the deletion of IκB-α Ser^{32} and Ser^{36}, which appear to be critical residues for signal-induced phosphorylation and degradation of IκB-α (*16*). However, regulation of IκB-α amounts is complex and also involves the PEST-like sequence (where P is Pro; E, Glu; S, Ser; and T, Thr) at the COOH-terminus of IκB-α (*9*), which is retained in our truncated IκB-α protein. Regardless of the mechanism, the functional correction of radiation hypersensitivity by ΔIκB-α suggests that aberrant NF-κB regulation may contribute to the cellular defect in AT. Furthermore, the involvement of NF-κB in immunoglobulin gene regulation, and the roles of other members of this gene family (*REL*, *BCL3*) in lymphomagenesis, are consistent with several nonradiological aspects of the AT phenotype (*17*). Finally, recent reports have noted that NF-κB is constitutively expressed at high levels in neurons, suggesting a possible link to central nervous system symptoms in AT (*18*).

IκB-α is unlikely to be the primary defect in AT group D, as there are no mutations in the coding sequence of the IκB-α gene in the AT5BIVA cells. Furthermore, the gene has been localized to chromosome 14 (*19*), whereas genetic linkage analysis has mapped the putative AT gene to chromosome 11q22-23 (*20*). We hypothesize that the NF-κB–IκB complex contributes to the AT phenotype but acts downstream of the gene representing the primary defect.

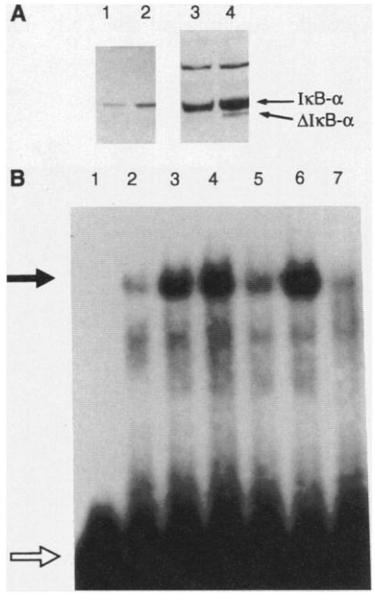

Fig. 4. (**A**) Detection of exogenous ΔIκB-α in ATCL2-11 cells. Equal concentrations (10 μg) of whole-cell extracts were loaded in each lane and immunoblotted. AT5BIVA cells (lanes 1 and 3) and ATCL2-11 cells (lanes 2 and 4) were probed with rabbit polyclonal antibodies. Antibodies (Santa Cruz Biotech, Santa Cruz, California) directed against the human IκB-α NH_2-terminus (amino acids 6 to 20) (lanes 1 and 2) and against the COOH-terminus (amino acids 297 to 317) (lanes 3 and 4) were used in these protein immunoblots. The endogenous IκB-α migrates slower than ΔIκB-α. (**B**) Expression of NF-κB in AT cells was measured by a gel-shift assay with a κB probe corresponding to the binding motif present in the human κ immunoglobulin enhancer. Cell extracts were prepared as described (*22*). Nuclear extracts (10 μg) were incubated with a κB probe end-labeled with [γ-^{32}P]adenosine triphosphate (20,000 counts per minute) and 3 μg of poly(dI:dC) in 10 mM tris-HCl (pH 7.5), 50 mM NaCl, 1 mM dithiothreitol, 1 mM EDTA, and 6% glycerol. DNA-protein complexes were fractionated on 4% native acrylamide gels in 25 mM tris-HCl, (pH 8.3), 190 mM glycine, and 1 mM EDTA. Lane 1, no cell extract; lane 2, HeLa cells; lane 3, AT5BIVA cells; lane 4, AT5BIVA cells, 3 hours after exposure to 6 Gy; lane 5, ATCL2-11 cells; lane 6, ATCL2-11 cells, 3 hours after exposure to 6 Gy; and lane 7, competition with unlabeled κB probe (20-fold excess) with AT5BIVA cell extract. The position of the NF-κB–DNA complex is indicated by the solid arrow. The position of the free probe is indicated by the open arrow.

REFERENCES AND NOTES

1. N. G. J. Jaspers, R. A. Gatti, C. Baan, P. C. M. L. Linssen, D. Bootsma, *Cytogenet. Cell Genet.* **49**, 259 (1988).
2. A. M. R. Taylor *et al.*, *Nature* **258**, 427 (1975); R. B. Painter and B. R. Young, *Proc. Natl. Acad. Sci. U.S.A.* **77**, 7315 (1980).
3. J. Yates, N. Warren, D. Reisman, B. Sudgen, *Proc. Natl. Acad. Sci. U.S.A.* **81**, 3806 (1984); S. Lupton and A. J. Levine, *Mol. Cell. Biol.* **5**, 2533 (1985); B. Hirt, *J. Mol. Biol.* **26**, 265 (1967).
4. Z. Salehi *et al.*, in *Neoplastic Transformation in Human Cell Culture*, J. S. Rhim and A. Dritschilo, Eds. (Humana Press, Totowa, NJ, 1991), pp. 377–386. The pCNCNot vector contains the cytomegalovirus immediate-early gene promoter and EBV *ori*P sequences.
5. R. R. Weichselbaum, M. A. Beckett, J. L. Schwartz, *Int. J. Radiat. Oncol. Biol. Phys.* **15**, 575 (1988).
6. D. E. Lea, *Actions of Radiations on Living Cells* (Cambridge Univ. Press, London, 1962), pp. 74–75.
7. C. F. Arlett, M. H. L. Green, A. Priestly, S. A. Harcourt, L. V. Mayne, *Int. J. Radiat. Biol.* **54**, 911 (1988).
8. S. Haskill *et al.*, *Cell* **65**, 1281 (1991).
9. M. J. Lenardo and D. Baltimore, *ibid.* **58**, 227 (1989); I. M. Adcock, O. Kwon, P. J. Barnes, *Biochem. Biophys. Res. Commun.* **199**, 1518 (1994); P. A. Baeuerle and D. Baltimore, *Science* **242**, 540 (1988); S. Miyamoto, M. Maki, M. J. Schmitt, M. Hatanaka, I. M. Verma, *Proc. Natl. Acad. Sci. U.S.A.* **91**, 12740 (1994); Y. Lin, K. Brown, U. Siebenlist, *ibid.* **92**, 552 (1995).
10. The GenBank accession number for the sequence is M69043 (*8*).
11. M. Jung, A. Dritschilo, U. Kasid, *PCR Methods Appl.* **1**, 171 (1992); R. Hopgood, M. Kevin, S. Gill, P. Gill, *BioTechniques* **13**, 82 (1992).
12. After transfection with the plasmid DNA from ATCL2-11 that encoded ΔIκB-α, stably transfected AT3BIVA cells were pooled and used for survival experiments with the method described in Fig. 1. Values for D_0 and *n* were 0.8 Gy and 1.0, respectively.
13. ΔIκB-α was translated in vitro and electrophoresed on a 10% SDS-polyacrylamide gel in parallel with the whole-cell extracts of AT5BIVA and ATCL2-11. In vitro transcription-translation (Ambion, Austin, TX) was performed with the PCNCNot vector, which contains an Sp6 polymerase promoter at the 5′ end of the cDNA insert and an Eco RI restriction site at the 3′ end.
14. S. C. Sun *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **91**, 1346 (1994); K. Brown, S. Park, T. Kanno, G. Franzoso, U. Siebenlist, *ibid.* **90**, 2532 (1993).
15. M. A. Brach *et al.*, *J. Clin. Invest.* **88**, 691 (1991).
16. K. Brown, S. Gerstberger, L. Carlson, G. Franzoso, U. Siebenlist, *Science* **267**, 1485 (1995).
17. H. Ohno, G. Takimoto, T. W. McKeithan, *Cell* **60**, 991 (1990); A. Neri *et al.*, *ibid.* **67**, 1075 (1991); E. Boder, in *Ataxia Telangiectasia: Genetics, Neuropathology, and Immunology of a Degenerative Disease of Childhood*, R. A. Gatti and M. Swift, Eds. (Liss, New York, 1985), pp. 1–61.
18. C. Kaltschmidt, B. Kaltschmidt, H. Neumann, H. Wekerle, P. A. Baeuerle, *Mol. Cell. Biol.* **14**, 3981 (1994).
19. M. M. LeBeau *et al.*, *Genomics* **14**, 529 (1992).
20. R. Gatti *et al.*, *Nature* **336**, 577 (1988); M. S. Meyn, J. M. Lu-Kuo, L. B. K. Herzing, *Am. J. Hum. Genet.* **53**, 1206 (1993).
21. N. Albright, *Radiat. Res.* **112**, 331 (1987).
22. J. D. Dignam, R. M. Lebowitz, R. G. Roeder, *Nucleic Acids Res.* **11**, 1475 (1983).

23. We thank F. G. Kern and Z. Salehi for construction of the cDNA library; E. Sorbello, E. Tuturea, Y. Perelmutor, and J. Siddiqui for technical assistance; E. North for manuscript preparation; and I. Verma, R. R. Weichselbaum, and R. Narayanan for helpful discussions. Supported in part by NIH grants R29 CA63023 (to M.J.) and CA45408 (to A.D.).

25 October 1994; accepted 2 May 1995