For submission to *Environmental Health Perspectives*

ANL/CMB/CP--91543
CONF-961150--1

Radiation-Induced Gene Responses

Gayle E. Woloschak[1], Tatjana Paunesku,
Pocahontas Shearin-Jones,[2] and Jeremy Oryhon[3]

Center for Mechanistic Biology and Biotechnology
Argonne National Laboratory
9700 South Cass Avenue
Argonne, Illinois 60439-4833

---

[1]Correspondence Author: Center for Mechanistic Biology and Biotechnology, Argonne National Laboratory, 9700 South Cass Avenue, Argonne, Illinois 60439-4833. Phone: (630) 252-3312; Fax: (630) 252-3387; E-mail: woloschak@anl.gov.

[2]Current address: Halifax Community College, P. O. Drawer 809, Weldon, North Carolina 27890

[3]Current address: University of Illinois, URH 276 Scott Hall, 202 E. Peabody Drive, Champaign, Illinois 61820

DISCLAIMER

Running Title: Radiation-induced gene responses

Key Words: radiation-induced genes; radiation; transcriptional regulation; transcription factors; DNA-damaging agents

Abbreviations: LDH — lactate dehydrogenase
UV — ultraviolet
E-UV — element regulated by UV
dd-RT-PCR — differential display-reverse transcriptase-polymerase chain reaction

ACKNOWLEDGEMENTS

The authors would like to thank Ms. Felicia King for outstanding secretarial assistance and Drs. David Grdina and Frank Collart for critical review of the manuscript.

This work was supported by the U.S. Department of Energy, Office of Health and Environmental Research, under Contract No. W-31-109-ENG-38 and NCI Grant #CA73042. This publication was also made possible by grant number ES07141-02 from the National Institute of Environmental Health Sciences (NIEHS), NIH. Its contents are solely the responsibility of the authors and do not necessarily represent the official views of the NIEHS, NIH.

ABSTRACT

In the process of identifying genes that are differentially regulated in cells exposed to ultraviolet (UV) radiation, we identified a transcript that was repressed following the exposure of cells to a combination on UV radiation and salicylate (a known inhibitor of NF-κB). Sequencing of this band determined that it had identity to lactate dehydrogenase, and Northern blots confirmed the initial expression pattern. Analysis of the sequence of the LDH 5′ region established the presence of NF-κB, Sp1, and two Ap-2 elements; two partial AP-1, one partial CRE, and two halves of E-UV elements were also found. Electromobility shift assays were then performed for the AP-1, NF-κB, and E-UV elements. These experiments revealed that (1) binding to NF-κB was induced by UV, but this was repressed with salicylic acid; (2) UV did not affect AP-1 binding, but salicylic acid inhibited it alone or following UV exposure; and (3) E-UV binding was repressed by UV, and salicylic acid had little effect. Since the binding of no single element correlated with the expression pattern of LDH-β, it is likely that multiple elements govern UV/salicylate-mediated expression.

INTRODUCTION

During the past decade, many studies have identified genes induced in response to DNA-damaging agents such as UV and ionizing radiation (1–29). The collective contribution of these studies has led to the implication that several different transcription or regulatory elements play key roles in the immediate early response, including p53, AP-1, NF-κB, and others (30–43), and to the identification of nuclear and nonnuclear events as playing essential roles in the actual induction process (15,16,44–49). For some of these transcription factors, target genes in the transcription factor regulon have been identified (16,50–54); for example, NF-κB and AP-1 activation contributes to the induction of HIV-LTR following UV exposure (15,16,35,55–61). In addition, AP-1 and NF-κB sites have been found in a large number of UV- and ionizing-radiation-induced genes (7,42,62–65). Nevertheless, the precise pathway following transcription factor activation by UV or ionizing radiation (or both) has not yet been mapped.

MATERIALS AND METHODS

Differential display - reverse - transcriptase polymerase chain reaction (dd-RT-PCR)

The differential display of eukaryotic messenger RNA (mRNA) by means of reverse transcription coupled with the polymerase chain reaction (dd-RT-PCR) is a technique developed by Liang and Pardee (66) in order to separate and, eventually, to clone individual mRNAs differentially expressed in mRNA preparations from similar cells. In our laboratory, however, we developed an improved dd-RT-PCR approach (26) that at the same time allows one to ignore polyT contamination and ensures that contamination with products of random priming by 5′ primers will not be detected on the sequencing gel. Briefly, we are using $(T)_{12}XY$ end-labeled primer for the PCR under conditions similar to the original, except for the use of higher concentrations of dNTPs.

Purification and sequencing of bands from dd-RT-PCR

Differentially expressed cDNAS were extracted from the dried sequencing gel for reamplification. Bands of interest were located and released from the dried gel by cutting through the film. Pieces of dried gel carrying the band of interest were soaked along with the 3MM paper (used as backing) in 100 µl of H_2O for 10 min at room temperature and then were boiled for 15 min. After a 2-min spin in the microcentrifuge, the supernatant was transferred to a clean microcentrifuge tube and mixed with 0.10 volume of 3 M sodium acetate, 0.05 volume

of glycogen (10 mg/ml stock), and 4 volumes of ethanol. The mixture was placed at –80 °C for 30 min and centrifuged for 10 min at +4 °C. The pellet was dissolved in 10 µl of distilled H_2O and stored at –20 °C. The band was reamplified twice.

Sequencing was done with the PRIZM dyedeoxy sequencing kit according to the manufacturer's instructions. Sequencing electrophoresis was carried out by first dissolving the dried sample in 4 µl of a 5:1 mixture of deionized formamide and 50 mM EDTA (pH 8.0). Immediately before loading, the sample was heated to 90 °C and then run on a standard sequencing gel prepared for use with a DNA sequencer (Applied Biosystems 373A). Gene sequences were compared to those available in the GenBank using the BLAST search program (available at BLAST@NCBI.NLM.NIH.GOV).

EMSA binding

Electromobility shift assays (EMSA) were performed by using the consensus- containing oligonucleotides that were labeled with $\gamma[^{32}P]$ATP in a T4 polynucleotide kinase reaction. For a nonspecific competitor (negative control), we used an irrelevant recognition sequence (Sp1). All binding conditions for proteins (1 µg) were similar to those described by Schreiber *et al.* (67). The reactions were done in the presence of sonicated salmon sperm DNA as a nonspecific binding inhibitor and with 0.8–5 µg of unlabeled crude nuclear protein extract. The assays were set up to use lysate from equal amounts of protein for each experiment. The free oligonucleotides were resolved from protein-DNA complexes by Tris-borate polyacrylamide gel

electrophoresis (70). The DNA bands were resolved by autoradiography on a PhosphorImager.

RNA analyses

For all genes, we verified expression patterns by Northern blots. RNA was routinely purified in our laboratory by isolation in guanidine isothiocyanate, extraction from phenol, and precipitation from 3 M sodium acetate, pH 6.0, (22–24). RNA was stored as an ethanol precipitate at –20 °C.

For Northern blot analysis, RNA was separated by using formaldehyde-agarose gel electrophoresis as described previously (22–24). Northern transfers were performed as described (22–24). The mass probe was an end-labeled 18S rRNA 30-nt-long probe; the LDH probe was labeled from dd-RT-PCR amplified with $\alpha[^{32}P]$dCTP as the sole source of dCTP. Hybridization conditions were 50% deionized formamide, 0.75 M NaCl, 75 mM sodium citrate, 25–50 mM sodium phosphate (pH 6.5), 0.2% sodium dodecyl sulfate (SDS), 0.2% bovine serum albumin, 0.2% Ficoll, 0.2% polyvinylpyrrolidone, and 50 μg/mL sonicated denatured herring sperm DNA at 43 °C. Prior to hybridization, all labeled probes were heat-denatured at 90 °C for 5 min. After hybridization, nonspecific binding was reduced by washing the blot three times for 1 h each at 65 °C in 45 mM sodium citrate (pH 7.4), 0.45 M NaCl, 0.2% Ficoll, 0.2% polyvinylpyrrolidone, 0.2% bovine serum albumin, 50 μg/mL herring sperm DNA (sonicated, denatured), and 0.1% SDS, followed by three more washings for 1 h each at 65 °C in 1.5 mM sodium citrate (pH 7.4), 15 mM NaCl, 50 μg/mL herring sperm DNA (sonicated, denatured), and

0.1% SDS. The blot was then dried and exposed (to x-ray film at –70 °C with intensifying screens) on the PhosphorImager.

RESULTS AND DISCUSSION

Previous studies by several groups have shown that NF-κB binding is inhibited in HeLa cells by the addition of salicylate to the medium (71,72). HeLa cells (which lack functional *p53*) were either untreated or were exposed to UV, UV plus salicylate (UV/sa), salicylate (sa), *cis*-Pt, *cis*-Pt plus salicylate (*cis*Pt/sa), vinblastine (vin), vinblastine plus salicylate (vin/sa), UV plus indomethacin (UV/indo), or *cis*Pt plus indomethacin (*cis*Pt/indo). Concentrations and exposures were as previously published by our group in studies demonstrating that salicylate inhibits UV- and *cis*Pt-mediated HIV-LTR transcription (72). Bands were selected by dd-RT-PCR using primers and sequencing protocols previously published by our group (26).

The sequence of one dd-RT-PCR band (T102) is presented in Table 1. It was found to have identity with the human lactate dehydrogenase β-chain (LDH-β) 3′ region. Northern blots of the LDH-β probe hybridized to RNAs derived from control cells and from cells exposed to UV or *cis*-Pt, with or without salicylic acid, revealed that LDH-β mRNA was not affected by any exposures except when UV and salicylate or cisplatin and salicylate were combined; these treatments caused a marked reduction in LDH-β mRNA accumulation (Table 2).

The 5′ flanking region of the LDH-β gene was analyzed using sequence information available from GenBank. A summary of these results is presented in Figure 1, showing the presence of complete sites for NF-κB, Sp1, and AP-2 elements as well as partial sites for AP-1 and CRE elements. In addition, an element recently defined in our laboratory called E-UV (73) was also found to be present, but with several base pair changes relative to the consensus

sequence. This element has been found to be split in several different genes and appears to be split in LDH-β as well.

Gel shift analyses of the AP-1, NF-κB, and E-UV sequences were performed using extracts of cells exposed to UV, salicylic acid, UV/salicylic acid, etc. An example of one such gel shift is presented in Figure 2, in which E-UV (Fds = full-length, double stranded) and AP-1 elements were examined. The results (Table 2) revealed that no single element showed precisely the same binding pattern as the expression pattern of LDH-β mRNA. Therefore it is likely that several of these elements (and perhaps some working in concert) govern regulation of LDH-β following UV/salicylate exposure. It has been documented in the literature that salicylate inhibits NF-κB binding to its element. The experiments in Figure 2 demonstrate that AP-1 binding is also inhibited by prior exposure of cells to salicylate. This suggests that UV-induced responses that are affected by salicylate may involve either the NF-κB or AP-1 pathways.

REFERENCES

1. Anderson A, Woloschak GE. Cellular proto-oncogene expression following exposure of mice γ-rays. Radiat Res 130:340–344 (1992).
2. Boothman DA, Wang M, Lee SW. Induction of tissue-type plasminogen activator by ionizing radiation in human malignant melanoma cells. Cancer Res 51:5587–5595 (1991).
3. Fornace Jr AJ, Alamo IJ, Hollander CM. DNA damage-inducible transcripts in mammalian cells. Proc Natl Acad Sci USA 85:8800–8804 (1988).
4. Fornace Jr AJ, Fargnoli J, Papathanasiou M, Holbrook NJ, Hollander CM, Nebert DW, Luethy JD. Mammalian genes coordinately regulated by growth arrest signals and DNA-damaging agents. Mol Cell Biol 9:4196–4203 (1989).
5. Fornace Jr AJ. Mammalian genes induced by radiation: activation of genes associated with growth control. Annu Rev Genet 26:507–526 (1992).
6. Herrlich P, Ponta H, Rahmsdorf HJ. DNA damage-induced gene expression: signal transduction and relation to growth factor signaling. Rev Physiol Biochem Pharmacol 119:187–216 (1992).
7. Hallahan DE, Spriggs DR, Beckett MA, Kufe DW, Weichselbaum RR. Increased tumor necrosis factor α mRNA after cellular exposure to ionizing radiation. Proc Natl Acad Sci USA 86:10104–10107 (1989).
8. Libertin CR, Panozzo J, Groh KR, Chang-Liu C-M, Schreck S, Woloschak GE. Effects of gamma rays, ultraviolet radiation, sunlight, microwaves and electromagnetic fields on gene expression mediated by human immunodeficiency virus promoter. Radiat Res 140:91–96 (1994).

9. Martin M, Cefaix J-L, Pinton P, Crechet F, Daburton F. Temporal modulation of TGR-β1 and β-actin gene expression in pig skin and muscular fibrosis after ionizing radiation. Radiat Res 134:63–70 (1993).

10. Munson G, Woloschak GE. Differential effect of ionizing radiation on transcription in repair-deficient and repair-proficient mice. Cancer Res 50:5045–5048 (1990).

11. Panozzo J, Bertoncini D, Miller D, Libertin CR, Woloschak GE. Modulation of expression of virus-like elements following exposure of mice to high- and low-LET radiations. Carcinogenesis (Lond.) 12:801–804 (1991).

12. Peak JG, Woloschak GE, Peak MJ, Enhanced expression of protein kinase C gene caused by solar radiation. Photochem Photobiol 53:395–397 (1991).

13. Ramsamooj P, Kasid U, Dritschilo A. Differential expression of proteins in radioresistant and radiosensitive human squamous carcinoma cells. J Natl Cancer Inst 84:622–628 (1992).

14. Ronai ZA, Okin E, Weinstein IB. Ultraviolet light induces expression of oncogenes in rat fibroblasts and human keratinocyte cells. Oncogene 2:201–204 (1988).

15. Stein B, Kramer M, Rahmsdorf HJ, Ponta H, Herrlich P. UV induced transcription from the HIV-1 LTR and UV-induced secretion of an extracellular factor that induces HIV-1 transcription in non-irradiated cells. J Virol 63:4540–4544 (1989).

16. Stein B, Rahmsdorf HJ, Steffen A, Liftin M, Herrlich P. UV-induced DNA damage is an intermediate step in UV-induced expression of human immunodeficiency virus type I, collagenase, c-fos, and metallothionein. Mol Cell Biol 9:5169–5181 (1989).

17. Valerie K, Delers A, Bruck C, Thiriart C, Rosenberg H, Debouck C, Rosenberg M. Activation of human immunodeficiency virus type I by DNA damage in human cells. Nature 333:78–81 (1988).

18. Woloschak GE, Chang-Liu C-M. Differential modulation of specific gene expression following high- and low-LET radiations. Radiat Res 124:183–187 (1990).

19. Woloschak GE, Chang-Liu C-M. Expression of cytoskeletal elements in proliferating cells following radiation exposure. Int J Radiat Biol 59:1173–1183 (1991).

20. Woloschak GE, Chang-Liu C-M. Effects of low-dose radiation on gene expression in Syrian hamster embryo cells: comparison of JANUS neutrons and gamma rays. In Proceedings of the International Conference on Low Dose Irradiation and Biological Defense Mechanisms (T Sugahara, L A Sagan and T Aoyama, Eds.) pp. 239–242. Kyoto, Japan, 1992.

21. Woloschak GE, Chang-Liu C-M. Modulation of expression of genes encoding nuclear proteins following exposure to JANUS neutrons or γ-rays. Cancer Lett 97:169–175 (1995).

22. Woloschak GE, Chang-Liu C-M, Shearin-Jones P. Regulation of protein kinase C by ionizing radiation. Cancer Res 50:3963–3967 (1990).

23. Wolsochak GE, Liu C-M, Jones PS, Jones CA. Modulation of gene expression in Syrian hamster embryo cells following ionizing radiation. Cancer Res 50:339–344 (1990).

24. Woloschak GE, Shearin-Jones P, Chang-Liu C-M. Effects of ionizing radiation on expression of genes encoding cytoskeletal elements: kinetics and dose effects. Mol Carcinog 3:374–378 (1990).

25. Woloschak GE, Chang-Liu C-M, Panozzo J, Libertin CR. Low doses of neutrons induce changes in gene expression. Radiat Res 138:S56–S59 (1994).

26. Woloschak GE, Paunesku T, Chang-Liu C-M, Grdina DJ. Expression of thymidine kinase messenger RNA and a related transcript is modulated by radioprotector WR1065. Cancer Res 55:4788–4792 (1995).

27. Woloschak GE, Felcher P, Chang-Liu C-M. Combined effects of ionizing radiation and cycloheximide on gene expression. Mol Carcinog 13:44–49 (1995).

28. Woloschak GE, Felcher P, Chang-Liu C-M. Expression of cytoskeletal and matrix genes following exposure to ionizing radiation: dose-rate effects and protein synthesis requirements. Cancer Lett 92:135–141 (1995).

29. Sakakeeny MA, Harrington M, Leif J, Merrill W, Pratt D, Romanik E, McKenna M, Fitzgerald TJ, Greenberger JS. Effects of gamma-irradiation on the M-CSF-promoter linked to a chloramphenicol acetyl transferase reporter gene expressed in a clonal murine bone marrow stromal cell line. Stem Cells 12:87–94 (1994).

30. Hallahan DE, Sukhatmen VP, Sherman ML, Virudachalam S, Kufe DW, Weichselbaum RR. Protein kinase C mediates X-ray inducibility of nuclear signal transducers EGR1 and JUN. Proc Natl Acad Sci USA 88:2156–2160 (1991).

31. Kastan MB, Onyekwere O, Sidransky D, Vogelstein B, Craig RW. Participation of p53 protein in the cellular response to DNA damage. Cancer Res 51:6304–6311 (1991).

32. Nelson WG, Kastan MB. DNA strand breaks: the DNA template alterations that trigger p53-dependent DNA damage response pathways. Mol Cell Biol 14:1815–1823 (1994).

33. Sun X, Shimizu H, Yamamoto K. Identification of a novel p53 promoter element in genotoxic stress-inducible p53 gene expression. Mol Cell Biol 8:4489–4496 (1995).

34. Brach MA, Hass R, Sherman ML, Gunji H, Weichselbaum R, Kufe D. Ionizing radiation induces expression of binding activity of the nuclear factor κB. J Clin Invest 88:691–695 (1991).

35. Angel P, Imagawa M, Chiu R, Stein B. Imbra RJ, Rahmsdorf JJ, Jonat C, Herrlich P, Karin M. Phorbol ester-inducible genes contain a common *cis* element recognized by a TPA-modulated *trans*-acting factor. Cell 49:729–739 (1987).

36. Andalabi A, Lia F, Imes S, Fogelman AM, Lusis AJ. Oxidized lipoproteins influence gene expression by causing oxidative stress and activating the transcription factor of NF-κB. Biochem Soc Trans 21:651–655 (1993).

37. Kharbanda S, Ren R, Pandey P, Shafman TD, Feller SM, Weichselbaum RR, Kufe DW. Activation of the c-Abl tyrosine kinase in the stress response to DNA-damaging agents. Nature 376:785–788 (1995).

38. Datta R, Rubin E, Sukhatme V, Qureshi S, Hallahan D, Weichselbaum RR, Kufe DW. Ionizing radiation activates transcription of the EGR1 gene via CArG elements. Proc Natl Acad Sci USA 89:10149–10153 (1992).

39. Datta R, Taneja N, Sukhatme V, Qureshi SA, Weichselbaum R, Kufe DW. Reactive oxygen intermediates target CC(A/T)GGG sequences to mediate activation of the early growth response 1 transcription factor gene by ionizing radiation. Proc Natl Acad Sci USA 90:2419–2422 (1993).

40. Mohan N, Meltz ML. Induction of nuclear factor κB after low-dose ionizing radiation involves a reactive oxygen intermediate signaling pathway. Radiat Res 140:97–104 (1994).

41. Prasad AV, Mohan N, Chandrasekar B, Meltz ML. Activation of nuclear factor κB in human lymphoblastoid cells by low-dose ionizing radiation. Radiat Res 138:367–372 (1994).

42. Sahijdak WM, Yang C-R, Zuckerman JS, Meyers M, Boothman DA. Alterations in transcription factor binding in radioresistant human melanoma cells after ionizing radiation. Radiat Res 137:47-51 (1994).

43. McKenna W, Iliakis G, Weiss MC, Bernhard EJ, Muschel RJ. Increased G2 delay in radiation-resistant cells obtained by transformation of primary rat embryo cells with oncogenes H-*ras* and v-*myc*. Radiat Res 125:283–287 (1991).

44. Uckun FM, Tuel-Ahlgren LM, Song CW, Waddick K, Myers DE. Kirihara J, Ledbetter JA, Schieven GL. Ionizing radiation stimulates unidentified tyrosine-specific protein kinases in human B-lymphocyte precursors, triggering apoptosis and clonogenic cell death. Proc Natl Acad Sci USA 89:9005–9009 (1992).

45. Uckun FM, Schieven GL, Tuel-Ahlgren LM, Dibirdik I, Myers DE, Ledbetter JA, Song CW. Tyrosine phosphorylation is a mandatory proximal step in radiation-induced activation of the protein kinase C signaling pathway in human B-lymphocyte precursors. Proc Natl Acad Sci USA 90:252–256 (1993).

46. Simon MM, Aragane Y, Schwarz A, Luger TA, Schwarz T. UVB light induces nuclear factor κB (NFκB) activity independently from chromosomal DNA damage in cell-free cytosolic extracts. J Invest Dermatol 102:422–427 (1994).

47. Devary Y, Rosette C, DiDonato JA, Karin M. NFκB activation by ultraviolet light not dependent on an nuclear signal. Science 261:1442–1445 (1993).

48. Hayashi T, Ueno Y, Okamoto T. Oxidoreductive regulation of NF-κB. Involvement of a cellular reducing catalyst thioredoxin. J Biol Chem 268:11380–11388 (1993).

49. Koong AC, Chen EY, Giaccia AJ, Hypozia causes the activation of nuclear factor κB through the phosphorylation of IκBα on tyrosine residues. Cancer Res 54:1425–1430 (1994).

50. Brach MA, Gruss HJ, Kaisho T, Asano Y, Hirano T, Herrmann F. Ionizing radiation induces expression of interleukin 6 by human fibroblasts involving activation of nuclear factor κB. J Biol Chem 268:8466–8472 (1993).

51. Dominquez I, Sanz L, Arenzana-Seisdedos F, Diaz-Meco MT. Inhibition of protein kinase C zeta subspecies blocks the activation of an NF-κB-like activity in *Xenopus laevis* oocytes. Mol Cell Biol 13:1290–1295 (1993).

52. Engstrom Y, Kadalayil L, Sun S-C, Samakovlis C, Hultmark D, *et al.* κB-like motifs regulate the induction of immune genes in *Drosophila.* J Mol Biol 232:327–333 (1993).

53. Finco TS, Baldwin AS. κB site-dependent induction of gene expression by diverse inducers of nuclear factor κB requires RAF-1. J Biol Chem 24:17676–17679 (1993).

54. Kunsch C, Rosen CA. NF-κB subunit-specific regulation of the interleukin-8 promoter. Mol Cell Biol 13:6137–6146 (1993).

55. Zmudzka B, Beer JZ. Yearly review: activation of human immunodeficiency virus by UV radiation. Photochem Photobiol 52:1153–1162 (1990).

56. Schreck R, Rieber P, Baeuerle PA. Reactive oxygen intermediates as apparently widely used messengers in the activation of the NF-κB transcription factor and HIV-1. EMBO J 10:2247–2258 (1991).

57. Schreck S, Panozzo J, Milton J, Libertin CR, Woloschak GE. The effects of multiple UV exposure on HIV-LTR expression. Photochem Photobiol 61:378–382 (1995).

58. Biswas DK, Dezube BJ, Ahlers CM, Pardee AB. Pentoxifylline inhibits HIV-1 LTR-driven gene expression by blocking NFκB action. J AIDS 6:778–786 (1993).

59. Kretzchmar M, Meisterenst M, Scheidereit C, Li G, Roeder RG. Transcriptional regulation of the HIV-1 promoter by NF-κB *in vitro*. Genes Dev 6:761–774 (1992).

60. Perkins ND, Edwards NL, Duckett CS, Agranoff AB. A cooperative interaction between NF-κB and Sp1 is required for HIV-1 enhancer activation. EMBO J 12:3551–3558 (1993).

61. Schmid RM, Perkins ND, Duckett CS, Andrews PC, Nabel GJ. Cloning of an NF-κB subunit which stimulates HIV transcription in synergy with p65. Nature 352:733–736 (1991).

62. Hiscott J, Marios J, Garoufalis J, D'Addario M. Characterization of a functional NF-κB site in the human interleukin 1 beta promoter: evidence for a positive autoregulatory loop. Mol Cell Biol 13:6231–6240 (1993).

63. Messer G. Weiss EH, Baeuerle PA. Tumor necrosis factor beta (TNF-β) induces binding of the NF-κB transcription factor to a high-affinity κB element in the TNF-β promoter. Cytokine 2:389–397 (1990).

64. Lacoste J, D'Addario M, Roulston A, Wainberg MA, Hiscott J. Cell-specific differences in activation of NF-κB regulatory elements of human immunodeficiency virus and β interferon promoters by tumor necrosis factor. J Virol 64:4726–4734 (1990).

65. Singh SP, Lavin MF. DNA-binding protein activated by gamma radiation in human cells. Mol Cell Biol 10:5279–5285 (1989).

66. Liang P, Pardee AB. Differential display of eukaryotic messenger RNA by means of the polymerase chain reaction. Science 257:969–971 (1992).

67. Schreiber E, Mathias P, Muleer MM, Schaffner W. Rapid detection of octamer binding proteins with "mini-extracts" prepared from a small number of cells. Nucleic Acids Res 17:6419 (1989).

68. Davis LG, Dibner MD, Battey JF. In: Basic Methods in Molecular Biology, New York:Elsevier Science Publishers, Inc., 1986;233–284.

69. Lederer JA, Liou JS, Kim S, Rice N, and Lichtman AH, Regulation of NF-κB activation in T helper and T helper 2 cells. J Immunol 156:56–63 (1996).

70. Jones KA, Yamamoto KR, Tjian R. Two distinct transcription factors bind to the HSV thymidine kinase promoter *in vitro*. Cell 42:559–572 (1985).

71. Ghosh S, Kopp K. Reply to Frantz and O'Neill. Science 270:2018–2019 (1995).

72. Woloschak GE, Panozzo J, Schreck S, Libertin CR. Salicylic acid inhibits ultraviolet- and *cis*-Platinum-induced human immunodeficiency virus expression. Cancer Res 55:1696–1700 (1996).

73. Woloschak GE, Paunesku T. Mechanisms of radiation-induced gene responses. Radiat Res In press.

FIGURE LEGENDS

Figure 1. Diagram of LDH-β 5′ flanking region with sites for complete elements (NF-κB, Sp1, Ap2) and partial elements (AP-1, CRE, E-UV) indicated.

Figure 2. Gel shifts were performed as described by Schreiber *et al.* (67) and Lederer *et al.* (69). For each experiment, HeLa cell extracts and consensus elements were purchased from Promega Biotech. The + and - strand oligonucleotides of the E-UV elements, annealed to create full-length double-stranded (Fds) sequence, were synthesized on a "gene synthesizer" (Applied Biosystems) according to the manufacturer's conditions. Reactions were performed in the presence of sonicated salmon sperm DNA, nonspecific binding inhibitor, and with 0.8 μg of HeLa unlabeled crude nuclear protein extract, or 5 μg HeLa nuclear extract purchased from Promega Biotech (comm. extract). Free oligonucleotides were resolved from protein-DNA complexes by Tris-borate polyacrylamide gel electrophoresis. The dried gel was exposed on the PhosphorImager screen.

Table 1. Sequence Comparison: Band T102 vs. Human LDH-B

```
T102     200 TCACACNACAATAGTTAATTTTATTTGTTCAAGAGCTCAGATTGCAAGCATTAAACCAAG
             ||||||  ||||||||||||||||||||||||||||||||||||||||||||||||||||
huLDH-B    6 TCACACTACAATAGTTAATTTTATTTGTTCAAGAGCTCAGATTGCAAGCATTAAACCAAG

           140 CATAGGCTTTGATTCTGTGAGCCCCAATTCACATATTGNAGNAGATCNAAGCNANCTGTG
               |||||||||||||||||||||||| |||||||||||| || |||||| |||| | ||||||
            66 CATAGGCTTTGATTCTGTGAGCCCAAATTCACATATTGAAGAAGATCAAAGCAAACTGTG

            86 ATCCATGTACATGGNTGAAAACTAAAGGCTCGAGTTAATCACATTGTAGTTTTTAAATTT
               |||||||||||||| ||||||||||||||||||||||||||||||||||||||||||||
           126 ATCCATGTACATGGATGAAAACTAAAGGCTCGAGTTAATCACATTGTAGTTTTTAAATTT

            20 CTACANCCTAGANCTCACTA  1
               |||||  ||||||  |||||||
           186 CTACAGCCTAGAGCTCACTA  205
```

T102 vs. huLDH-B
95 % identity

Table 2. Summary of Effects of Various Treatments on LDH-β mRNA and Transcription Factor Binding

	Control	UV	sal	UV/sal	*Cis*	*Cis*/sal	vin
LDH-β mRNA	0	0	0	↓	0	↓	0
AP-1 binding	0	0	↓	↓	0	↓	ND
NF-κB binding	0	↑	0	↓	↑	↓	↑
E-UV binding	0	↓	0	0	↓	0	ND

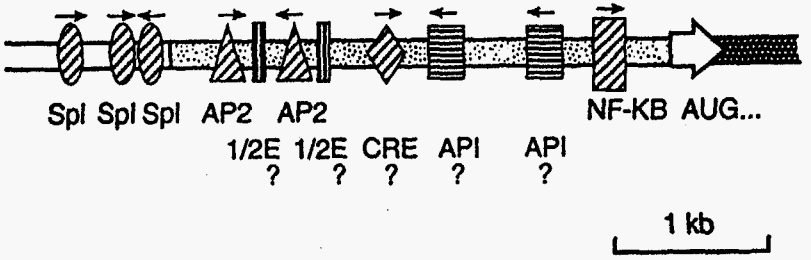
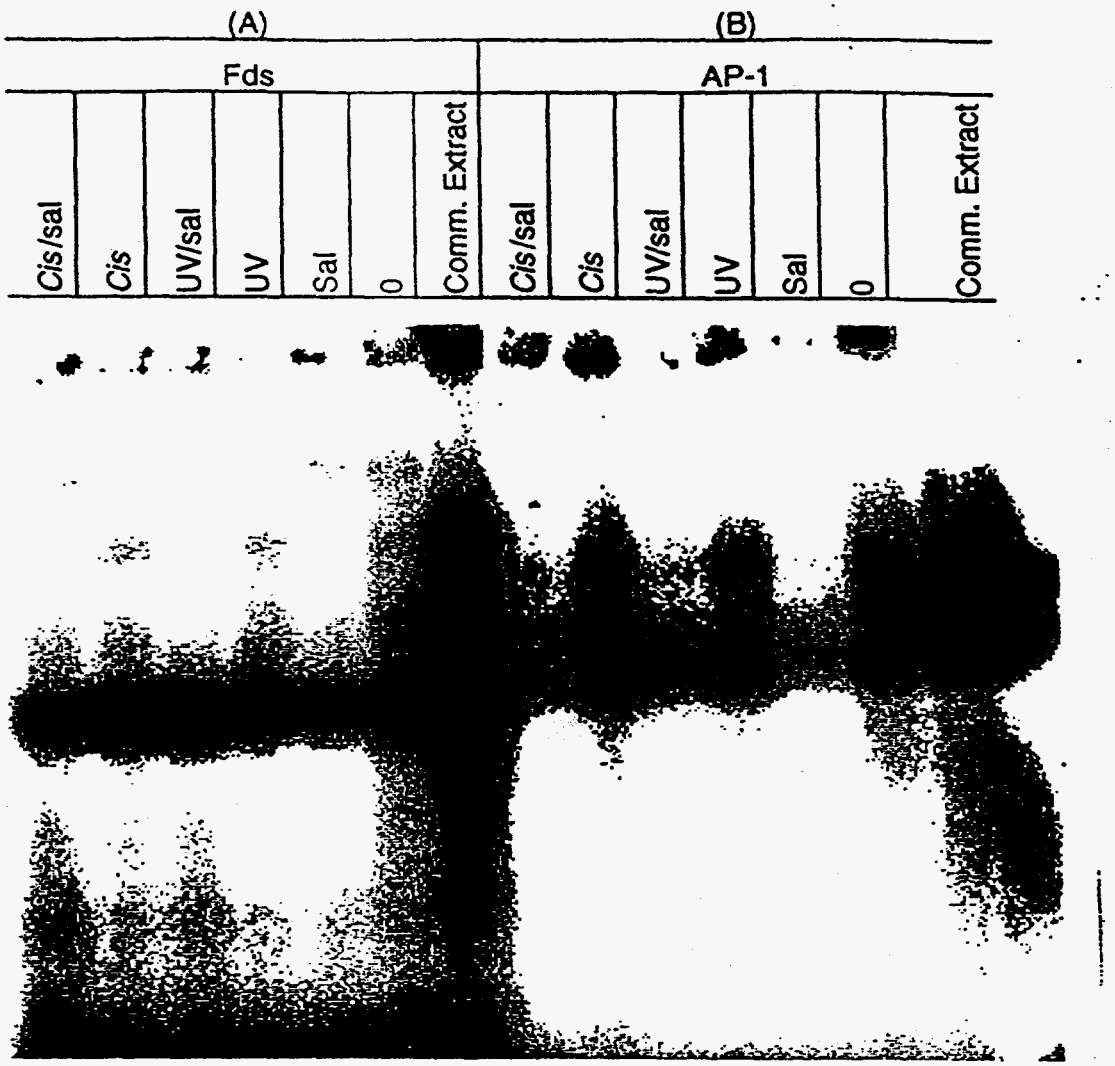


Fig. 1



Gel Shift Assay of HeLa cells nuclear extract after cells were treated with , UV (16 joule/m^2/sec), and *cis* platinum *(cis)* 50 μM/ml with or without salicylic acid (5 mg/ml). Labeled Fds (double stranded C1 oligonucleotide) and AP-1, a transcriptional element were reacted with nuclear proteins in a gel shift assay. Protein concentrations were standardized so each sample had 175 mg/ml. Sample volume was 5 μl (final concentration, 0.8 mg).

Fig. 2