AIM

Association for Information and Image Management

1100 Wayne Avenue, Suite 1100
Silver Spring, Maryland 20910

301/587-8202

Centimeter

Inches

MANUFACTURED TO AIM STANDARDS
BY APPLIED IMAGE, INC.

1 of 1

Modulation of Expression of Genes Encoding Nuclear Proteins Following Exposure to JANUS Neutrons or γ -Rays

Gayle E. Woloschak¹ and Chin-Mei Chang-Liu

**Argonne National Laboratory
Biological and Medical Research Division
9700 South Cass Avenue
Argonne, IL 60439-4833**

and

**¹Loyola University Medical Center
Department of Pathology
2140 South First Avenue
Maywood, IL 60153**

***Author to whom correspondence should be addressed**

The submitted manuscript has been authored by a contractor of the U. S. Government under contract No. W-31-109-ENG-38. Accordingly, the U. S. Government retains a nonexclusive, royalty-free license to publish or reproduce the published form of this contribution, or allow others to do so, for U. S. Government purposes.

DISCLAIMER

This report was prepared as an account of work sponsored by an agency of the United States Government. Neither the United States Government nor any agency thereof, nor any of their employees, makes any warranty, express or implied, or assumes any legal liability or responsibility for the accuracy, completeness, or usefulness of any information, apparatus, product, or process disclosed, or represents that its use would not infringe privately owned rights. Reference herein to any specific commercial product, process, or service by trade name, trademark, manufacturer, or otherwise does not necessarily constitute or imply its endorsement, recommendation, or favoring by the United States Government or any agency thereof. The views and opinions of authors expressed herein do not necessarily state or reflect those of the United States Government or any agency thereof.

MASTER

DISTRIBUTION OF THIS DOCUMENT IS UNLIMITED

Running Title: Radiation-Induced Modulation of Gene Expression

Key words: fission neutrons, ionizing radiation; nuclear proteins; oncogenes; transcriptional regulation

ABSTRACT

Previous work has shown that exposure of cells to ionizing radiations causes modulation of a variety of genes, including those encoding *c-fos*, interleukin-1, tumor necrosis factor, cytoskeletal elements, and many more. The experiments reported herein were designed to examine the effects of either JANUS neutron or γ -ray exposure on expression of genes encoding nucleus-associated proteins (H4-histone, *c-jun*, *c-myc*, *Rb*, and *p53*). Cycling Syrian hamster embryo cells were irradiated with varying doses and dose rates of either JANUS fission-spectrum neutrons or γ -rays; after incubation of the cell cultures for 1 h following radiation exposure, mRNA was harvested and analyzed by Northern blot. Results revealed induction of transcripts for *c-jun*, H4-histone, and (to a lesser extent) *Rb* following γ -ray but not following neutron exposure. Expression of *p53* and *c-myc* genes was unaffected by radiation exposure. Radiations at different doses and dose rates were compared for each of the genes studied.

INTRODUCTION

Past work by our group (1-4) and others (5-9) has shown the modulation of specific genes following exposure of cells to ionizing radiation and other DNA-damaging agents. Many classes of genes have been found to be modulated in response to ionizing radiation including those encoding cytoskeletal elements (2,3), cell growth arresting proteins (5), cytokines (1,6), and cellular oncogenes (7,8). The functions of this specific modulation of gene expression are currently being investigated by several groups; it has been suggested that gene modulation in response to radiation plays a role in cellular repair of DNA damage, cell survival, or cellular transformation (1-7).

Several groups have examined induction of nuclear proto-oncogenes following exposure to DNA-damaging agents. Work by Sherman *et al.* (1990) has recently identified *c-jun* as a proto-oncogene induced at early times following exposure of cells in culture to ionizing radiations. In addition, past work by Hollander and Fornace (8), as well as from our own group (4), has established that *c-fos* is induced following exposure to low linear energy transfer (LET) radiations such as γ -rays and X-rays. In this report, we present results of experiments aimed at examining modulation of expression of genes encoding *c-jun* and other nuclear proteins (such as *Rb*, *p53*, H4-histone, and *c-myc*) following exposure to either high-LET (JANUS fission-spectrum neutrons) or low-LET (γ -rays) radiations. This work confirmed induction of *c-jun* by low-LET (but not high-LET) radiation and established induction of genes encoding H4-histone and, to a

lesser extent, Rb protein by low- but not high-LET radiations. Accumulation of p53 and c-myc transcripts was unaffected following exposure to either high- or low-LET radiations. In addition, dose- and dose-rate-response effects were examined.

MATERIALS AND METHODS

Cells and Culture Conditions.

In all experiments, we examined modulation of gene expression by ionizing radiations in Syrian hamster embryo (SHE) fibroblasts, which are normal diploid cells that can be neoplastically transformed by low doses of ionizing radiations (10). All cell cultures were established in Dulbecco's modified Eagle's medium containing 20% fetal calf serum, 2 mM glutamine, penicillin (100 units/ml), and streptomycin (100 units/ml). Cells were grown to 75% confluence prior to irradiation to ensure that cells were cycling at the time of irradiation. Studies of preirradiated SHE cells grown under this protocol show them to be a mixed population of fibroblasts with more than 90% to 95% of the cell cycling. Cells were from passage 2 or 4. Preliminary studies showed no differences with regard to gene expression responses between these passages.

Radiation Treatment and DNA Analysis.

Cells plated in 100-mm Petri plates containing 10 ml of medium were irradiated with ^{60}Co γ -rays or fission-spectrum neutrons (0.85 MeV) from the JANUS reactor (11). All irradiations were performed at 37 °C on cycling cells; comparisons of doses of neutrons versus γ -rays were determined on the basis of survival data (i.e., 21 cGy of neutrons and 90 cGy

of γ -rays each cause 10% decreases in cell survival relative to nonirradiated cells, as measured by cloning efficiencies, and a significant frequency of morphological transformation, as determined in a 10-day colony assay) (10). At the time points used in these experiments, few if any transformed cells should be present in the cultures.

Our studies focused on establishing the presence of a molecular transcriptional response to ionizing radiation evident within the first hour after radiation exposure because it has already been well established that changes in DNA synthetic rates, cell volume, cell shape, and DNA repair capabilities all occur within this early time after cells have been exposed to radiation (12-14). We wished to determine whether consistent modulation of expression of specific genes occurred during this same time interval in response to ionizing radiation. Control cells were taken to the radiation chamber but not exposed to radiation. Plates of cells were then incubated at 37 °C for 1 h after completion of radiation exposure prior to harvest of the RNA. Clearly, at different dose-rates, exposure times vary.

Purification of RNA and Northern Blots.

The RNA was prepared by isolation in 6 M guanidine isothiocyanate, extraction with phenol, and precipitation from 3 M NaOAc, pH 6.0 (15-17). Poly(A)+ RNA was isolated by oligodeoxythymidylate-cellulose column chromatography until no unbound material absorbing at 254 nm was detected. The RNA was

stored as an ethanol precipitate at -20 °C. Routinely, we found that poly(A)+ RNA represented 5-10% of the total RNA fraction.

The RNA was separated by using formaldehyde-agarose gel electrophoresis, as described previously (18). Samples of poly(A)+ RNA (amounts in micrograms, as indicated in figure legends) were denatured in 50% formamide, 1.9 M formaldehyde, 0.2 M 3-[N-morpholino]propanesulfonic acid, 50 mM NaOAc, and 1 mM Na₂EDTA, pH 7.5, for 15 min at 55 °C and then separated on 1.2% agarose gels in 0.2 M 3-[N-morpholino]propanesulfonic acid, 50 mM NaOAc, 1 mM Na₂EDTA, and 2.2 M formaldehyde. The rRNA (*Escherichia coli* and mouse) and RNA ladder (BRL Laboratories, Bethesda, Maryland) markers were stained with ethidium bromide and photographed under UV light for use in sizing.

Northern transfers were performed as previously described (18). Blots were hybridized to ³²P nick-translated or oligo-labeled cDNA probes. Hybridization conditions were 50% deionized formamide, 0.75 M NaCl, 75 mM sodium citrate, 25-50 mM sodium phosphate (pH 6.5), 0.2% SDS, 0.2% bovine serum albumin, 0.2% ficoll, 0.2% polyvinylpyrrolidone, and sonicated denatured herring sperm DNA (50 µg/ml) 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 hybridized blots three times for 1 h each at 43 °C in 45 mM sodium citrate (pH 7.4), 0.45 M NaCl, 0.2% Ficoll, 0.2% polyvinylpyrrolidone, 0.2% bovine serum albumin, herring sperm DNA (50 µg/ml sonicated and denatured), 0.1% SDS, and then three

times for 1 h each in 1.5 mM sodium citrate (pH 7.4), 15 mM NaCl, herring sperm DNA (50 µg/ml sonicated and denatured), and 0.1% SDS. The blots were then dried and exposed to X-ray film at -20 °C.

In some experiments the same blot was washed and hybridized successively to several different probes. Each probe was eluted by washing for 24 h in distilled deionized water at 43 °C, and blots were checked (for total removal of the labeled probes) by 24-h exposure to X-ray film. Blots were washed three times in hybridization buffer before rehybridization to a different probe. All results reported here are representative of results from three independent experiments.

Although equal amounts (based on weight) of RNA were loaded into each well of a given gel, as determined by spectrophotometry, we found sufficient variation from one preparation to another to make poly(A)+ analysis essential. Systems for mRNA analysis measuring the molar concentration of RNA with 3' poly(A)+ tails (Molecular Genetics Resources, Tampa, Florida) were used for all poly(A)+ RNA preparations, and only RNA samples showing equimolar concentrations of poly(A)+ RNA were loaded onto the same gel. Equal amounts of mRNA on the blot were confirmed by hybridization to p53 or c-myc (see Tables 1 and 2).

Quantitation of blots was carried out using a Hirschman microdensitometer. For preparations expressing several transcripts (such as H4-histone), the amounts for all transcripts were added in each preparation (see Tables 1 and 2).

cDNA clones. We obtained cDNA clones from the following sources: *c-myc* from Dr. A. Balmain (Beatson Institute, Glasgow), H4-histone from Dr. G. Stein (Univ. Florida, Gainesville), *Rb* gene from Dr. Dryja (Harvard Medical School, Boston, MA), *c-jun* from Dr. W. W. Lamph (Salk Institute, LaJolla, CA), and *p53* from American Type Culture Collection (Bethesda, MD).

RESULTS

Experiments were performed to determine the effects of radiation dose, dose rate, and quality on expression of genes encoding nuclear proteins (*c-jun*, *Rb*, H4-histone, *p53*, and *c-myc*). Cycling SHE cells were exposed to varying doses (0 to 200 cGy) of γ -rays administered at either high (14-cGy/min) or low (1-cGy/min) dose rates. One hour after exposure, RNA was harvested from the cells and analyzed by Northern blot hybridization for expression of genes encoding nuclear proteins. Representative Northern blots are evident in Figure 1, and microdensitometric analyses of a series of Northern blots are shown in Table 1. From these results, it is apparent that transcripts specific for *c-jun* and H4-histone are induced within 1 h following exposure of SHE cells to γ -rays. This induction is greater at the high dose rate for *c-jun* and is slightly elevated at the low dose rate for H4-histone. *Rb* mRNA is slightly induced following γ -ray exposure, while expression of *p53* transcripts is unaffected. *c-myc* mRNA is depressed during the first h following γ -ray exposure; the absolute amount of repression could not be determined due to the almost undetectable *c-myc* expression evident in SHE cells following γ -ray exposure.

Results from similar experiments examining the effects of JANUS neutron exposure on expression of genes encoding nuclear proteins are depicted in Figure 2, with microdensitometric analyses presented in Table 2. These results revealed that neutron exposure failed to induce genes shown in Table 1 to be inducible with γ -rays (*c-jun*, H4-histone, and *Rb*). In fact, for

a few genes at specific doses, expression was actually found to be repressed following neutron exposure. Interestingly, while *c-myc* expression was repressed following γ -ray exposure, its expression was unaffected by neutrons. The only gene examined in this study for which slight induction following neutron exposure (when administered at a high dose rate) was evident was *p53*.

DISCUSSION

The experiments reported herein were designed to examine the effects of radiation exposure on expression of genes encoding nuclear proteins in SHE cells. Past work from our laboratory and that of other groups had already shown induction of *c-fos* mRNA (encoding a nuclear protein) following exposure to X-rays, γ rays, and other DNA damage-inducing agents (4,8). Sherman et al. have also recently shown the induction of *c-jun* mRNA accumulation following exposure of cells to γ -rays (7). The experiments reported herein examined expression of *c-jun*, *Rb*, *p53*, *c-myc*, and H4-histone following exposure of SHE cells either to high-LET (JANUS neutrons) or to low-LET (γ -rays) radiations. Our results confirm the work of Sherman et al. (7), documenting induction of *c-jun* following exposure to low-LET radiations, and similarly demonstrate a failure of high-LET radiations (neutrons) to induce *c-jun* in SHE cells. Similarly, past work from our group has shown that *c-fos* and protein kinase C (PKC) (2,4,19) are induced following exposure to low-LET radiations (such as x-rays and γ -rays), but not following exposure to JANUS neutrons

(3,8). Interestingly, many reports have linked *c-fos* and *c-jun* induction with PKC activation.

We also examined induction of a number of other nucleus-associated genes, including the cancer suppressor oncogenes, *p53* and *Rb*. This work revealed a slight but reproducible induction of *Rb* following exposure to γ -rays, but not following neutron exposure. Conversely, *p53* mRNA showed a trend toward induction following exposure to neutrons but not following γ -rays. While providing support for the differential cellular response to high- and low-LET radiations, these results do not provide a clear indication of possible function for the induction. Both *Rb* and *p53* have been implicated as playing a key role in the progression of cells in culture through the cell cycle (23,24). These experiments examined only effects on cycling cells (since radiation damage is most effective on cycling cells). We have previously shown that at least some transcriptional responses can be observed in cycling but not in growth-arrested (G_0) fibroblasts (19). Past work by several groups has shown that radiation exposure induces general transcriptional repression (1,2,3,25) and inhibition of cell cycle progression (13,25,26). Regulation of *Rb* and *p53* gene products in response to ionizing radiation may provide the cell with a mechanism whereby cell cycle progression can be slowed.

Similarly, genes of the histone and *c-myc* families are also known to be regulated throughout the cell cycle (27,28). Modulation of H4-histone or *c-myc* (or both) in response to γ -rays

may be a key feature in the cell's modulation of progression through the cell cycle following a damage-induced response.

All of the experiments presented herein provide further support to the hypothesis that high- and low-LET radiations induce different cellular responses to radiation-induced damage. Genes induced by low-LET γ -rays (such as *c-jun*, H4-histone, and, to a lesser extent, *Rb*) were unaffected following neutron exposure. The gene, *p53*, which was modestly induced following γ -ray exposure, was unaffected by neutron exposure; and *c-myc*, which was repressed following γ -ray exposure, was unaffected following neutron exposure. Taken together with previous work from our laboratory documenting similar differences in induction of PKC, *c-fos*, α -interferon, and others (1-4), this work suggests that the actual event (whether it be DNA damage, oxidative damage, protein denaturation, or some other intracellular event) which modulates the cellular response to ionizing radiations may be different for high- and low-LET radiations. In fact, a recent report from Gottlieb and Karin¹ has demonstrated that induction of *c-jun* and *c-fos* following exposure to DNA-damaging agents can be attributed to oxidative damage in the cell. Failure of neutrons to elicit this response would implicate some alternate pathway for gene modulation following neutron exposure. It is clear, though, that the same genes (and, consequently, the same

¹Gottlieb, R., and Karin, M. AP-1 as a mediator of the mammalian UV response (abstract). Read before the AACR Conference, Cellular Responses to Environmental DNA Damage, Banff, Alberta, December 2, 1991.

genetic regulatory elements) are affected in different ways following exposure of SHE cells to high-LET radiations relative to low-LET radiations. The mechanism or mechanisms for this remain unknown.

ACKNOWLEDGEMENTS

We thank the following people who provided us with cDNA clones: *c-jun* cDNA was obtained from Dr. W. Lamph (Salk Institute); *Rb* clone from Dr. Dryja (Harvard University); H4-histone clone from Drs. G. and J. Stein (University of Florida); *c-fos* and *c-myc* from American Type Culture Collection; and *p53* from Dr. A. Levine (State University of New York at Stony Brook).

We also thank Ms. K. Bexson for excellent secretarial expertise, Mr. G. Holmblad for his assistance in all irradiations, and Drs. F. Collart, C. Giometti, R. Drmanc, and M. Churchill 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.

REFERENCES

1. Woloschak 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.
2. Woloschak GE, Liu CM, Shearin-Jones P, Jones CA. Regulation of protein kinase C by ionizing radiation. Cancer Res 50:3363-3967, 1990.
3. Woloschak GE, Shearin-Jones P, Liu C-M, Jones CA.
Effects of ionizing radiation on expression of genes encoding cytoskeletal elements: kinetics and dose effects. Mol Carcinogen 3:374-378, 1990.
4. Woloschak GE, Chang-Liu CM. Differential modulation of specific gene expression following high- and low-LET radiations. Radiat Res 124:183-187, 1990.
5. Fornace AJ Jr, Alamo I Jr, Hollander CM. DNA damage-inducible transcripts in mammalian cells. Proc Natl Acad Sci USA 85:8800-8804, 1988.
6. 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.

7. Sherman ML, Datta R, Hallahan DE, Weichselbaum RR, Rufe DW. Ionizing radiation regulates expression of the *c-jun* proto-oncogene. Proc Natl Acad Sci USA 87:5663-5666, 1990.
8. Hollander CM, Fornace AJ Jr, Induction of *fos* RNA by DNA-damaging agents. Cancer Res 49:1687-1692, 1989.
9. Angel P, Rahmsdorf HJ, Pöting A, Lücke-Huhle C, Herrlich P. 12-*O*-tetradecanoylphorbol-13-acetate (TPA)-induced gene sequences in human primary diploid fibroblasts and their expression in SV40-transformed fibroblasts. J Cell Biochem 29:351-360, 1985.
10. Borek C, Hall EJ, Rossi HH. Malignant transformation in cultured hamster embryo cells produced by X-rays, 430-keV monoenergetic neutrons, and heavy ions. Cancer Res 38:2997-3005, 1978.
11. Williamson FA, Frigerio NA. Field mapping and depth dosimetry in the JANUS high flux irradiation room, a fast neutron facility for biological research. In: Burger G, Schraube H., Ebert HG, (eds), Proceedings of the First Symposium on Neutron Dosimetry in Biology and Medicine. Commission of the European Communities, Luxembourg, 1972,

p. 743.

12. Barendsen GW. Effects of radiation on the reproductive capacity and proliferation of cells in relation to carcinogenesis. In: Upton AC, Albert RE, Burns FJ, Shore RE (eds.), Radiation Carcinogenesis. Elsevier Publishers, New York, 1986, pp. 86-106.
13. Painter RB, Young BR. DNA synthesis in irradiated mammalian cells. J Cell Sci 6 suppl:207-214, 1987.
14. Johnson RT, Collins ARS, Squires S, Mullinger AM, Elliott GG, Dounces CS, Rasko I. DNA repair under stress. J Cell Sci 6 suppl:263-288, 1987.
15. Woloschak GE, Krco CJ. Regulation of k/ λ immunoglobulin light chain expression in normal murine lymphocytes. Mol Immunol 24:751-757, 1987.
16. Woloschak GE. Comparisons of heavy chain isotype expression in Peyer's patch and splenic B-cells. Mol Immunol 23:581-591, 1986.
17. Woloschak GE, Dewald G, Bahn RS, Kyle RA, Greipp PR, Ash RC. Amplification of RNA and DNA specific for erb B in

unbalanced 1;7 chromosomal translocation in myelodysplastic syndrome. J Cell Biochem 32:23-34, 1986.

18. Woloschak GE. Immunoglobulin gene expression in xid mice: defective expression of secreted and membrane α -heavy chain RNA. Mol Immunol 24:995-1004, 1987.
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. McDonnell SE, Kerr LD, Matrisian LM. Epidermal growth factor stimulation of stromelysin mRNA in rat fibroblasts requires induction of proto-oncogenes *c-fos* and *c-jun* and activation of protein kinase C. Mol Cell Biol 10:4284-4293, 1990.
21. Lamph WW, Wamsley P, Sassone-Corsi P, Verma IM. Induction of proto-oncogene Jun/AP-1 by serum and TPA. Nature 334:629-631, 1988.
22. Nakabeppa V, Ryder K, Nathans O. DNA binding activities of three murine Jun proteins: stimulation by Fos. Cell 55:907-915, 1988.

23. Howe JA, Mymryk JS, Egan C, Branton PE, Bayley ST.
Retinoblastoma growth suppressor and a 300-kDa protein
appear to regulate cellular DNA synthesis. Proc Nat Acad
Sci USA 87:5883-5887, 1990.
24. Diller L, Kassel J, Nelson CE, Gryka MA, Litwak G,
Gebhardt M, Bressac B, Dzturk M, Baker SJ, Vogelstein B,
Friend SH. p53 functions as a cell cycle control protein in
osteosarcomas. Mol Cell Biol 10:5772-5781, 1990.
25. Munson GP, Woloschak GE. Differential effect of ionizing
radiation on transcription in repair-deficient and
repair-proficient mice. Cancer Res 50:5045-5048, 1990.
26. Painter RB. Inhibition of mammalian DNA synthesis by
ionizing radiation. Int J Radiat Biol 49:771-781, 1986.
27. Greenberg ME, Ziff EB. Stimulation of 3T3 cells induces
transcription of the c-fos proto-oncogene. Nature,
311:433-438, 1984.
28. Carozzi N, Marashi F, Plumb M, Zimmerman S,
Simmerman A, Coles LS, Wells JRE, Stein G, Stein J.
Clustering of human I1 and core histone genes.
Science, 224:1115-1117, 1984.

Table 1. Relative Expression of Transcripts Encoding Nuclear Proteins Following γ -Ray Exposure: Cycling Cells^a

Dose of γ -Rays (cGy)	Dose Rate (cGy/ min)	Transcripts ^b				
		c-jun	Rb	H4- Histone	p53	c-myc
0	0	1.0 (.04)	1.0 (.01)	1.0 (.04)	1.0 (.11)	1.0 (.03)
6	1.0	1.6 (.04) ^c	0.9 (.08)	1.4 (.08)	1.1 (.09)	HND ^c
25	1.0	2.2 (.07) ^c	1.3 (.03)	1.4 (.03)	1.3 (.09)	HND
50	1.0	1.8 (.12) ^c	1.7 (.03) ^c	1.7 (.08) ^c	1.3 (.06)	HND
75	1.0	1.7 (.17) ^c	1.3 (.08)	2.0 (.07) ^c	1.4 (.02)	HND
25	14.0	1.5 (.11) ^c	0.9 (.09)	1.4 (.02)	1.1 (.02)	HND
50	14.0	0.8 (.21)	0.7 (.11)	1.6 (.04) ^c	1.3 (.07)	HND
75	14.0	1.1 (.12)	1.3 (.06)	1.1 (.05)	1.3 (.02)	HND
200	14.0	4.7 (.03) ^c	1.7 (.19) ^c	1.4 (.05)	0.9 (.11)	HND

^aCycling cells were irradiated with ⁶⁰Co γ -rays at the doses or dose rates indicated 1 h prior to RNA harvest.

^bRNA levels were determined by Northern blot hybridization and quantitated by microdensitometry. Amount of gene-specific mRNA in untreated cells was set at 1.0. All other RNAs were expressed relative to that. Standard deviations based on three independent determinations are in parentheses.

^cSignificantly different from control at P < 0.05.

^dHND, Hybridizations not detected.

Table 2. Relative Expression of Transcripts Encoding Nuclear Proteins Following Neutron Exposure: Cycling Cells^a

Dose of Neutrons (cGy)	Dose Rate (cGy/min)	Transcripts ^b				
		<i>c-jun</i>	<i>Rb</i>	H4-Histone	<i>p53</i>	<i>c-myc</i>
0	0	1.0 (.14)	1.0 (.05)	1.0 (.10)	1.0 (.01)	1.0 (.02)
6	0.5	0.7 (.16)	0.5 (.04) ^c	HND ^d	0.8 (.04)	1.1 (.04)
12	0.5	0.9 (.18)	0.7 (.05)	0.8 (.09)	0.7 (.02)	1.0 (.02)
24	0.5	0.9 (.13)	0.6 (.05) ^c	0.6 (.12)	0.4 (.11) ^c	1.3 (.01)
36	0.5	1.1 (.30)	1.0 (.10)	1.2 (.01)	1.1 (.07)	1.0 (.05)
12	12	0.8 (.05)	0.8 (.06)	0.4 (.03)	0.7 (.03)	1.2 (.05)
24	12	1.6 (.14) ^c	0.7 (.04)	0.6 (.01)	0.8 (.01)	0.9 (.05)
48	12	0.5 (.06) ^c	0.4 (.03) ^c	0.5 (.07)	0.8 (.12)	0.9 (.03)
96	12	1.2 (.10)	1.2 (.08)	1.3 (.01)	1.6 (.01) ^c	1.3 (.03)

^aCycling cells were irradiated with JANUS neutrons at the doses or dose rates indicated 1 h prior to RNA harvest

^bRNA levels were determined by Northern blot hybridization and quantitated by microdensitometry. Amount of gene-specific mRNA in untreated cells was set at 1.0. All other RNAs were expressed relative to that. Standard deviations are in parentheses.

^cSignificantly different from control at $P < 0.05$.

^dHND, Hybridizations not detected.

FIGURE LEGENDS

Figure 1. Northern blot examining expression of *c-jun* (A) and H4-histone (B) in untreated cells (0) or 1 h following exposure of SHE cells to varying doses (6, 25, 50, 75, or 200 cGy) of γ -rays administered at high (14-cGy/min) or low (1-cGy/min) dose rates.

Figure 2. Northern blot examining expression of *Rb* (A) and *p53* (B) in untreated cells (0) or 1 h following exposure of cells to varying doses, (6, 12, 24, 36, 48, or 96 cGy) of JANUS fission-spectrum neutrons administered at either high (12-Gy/min) or low (0.5-cGy/min) dose rates. Arrows in Figure 2A mark two *Rb* transcripts.

DATE

FILMED

6/15/94

END