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Isolation of x-ray-inducible transcripts from radioresistant human melanoma cells

(differential hybridization/cDNA cloning/ionizing radiation/gene expression)

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ABSTRACT **Twelve x-ray-induced transcripts (xips), differentially expressed 8- to 230-fold in x-irradiated versus unirradiated radioresistant human melanoma (U1-Mel) cells, were isolated as cDNA clones (xip1 through xip12) after four rounds of differential hybridization. Northern analyses revealed rare, medium, and abundant xips, ranging in size from 1.2 to 10 kb. All transcripts were transiently expressed and induced by low, but not by high (>600 cGy), doses of radiation. Three transcripts (xip4, -7, and -12) were induced only by ionizing radiation, and many (i.e., xip1, -2, -3, -5, -6, -8, -9, -10, and -11) were also induced by UV irradiation or phorbol 12-myristate 13-acetate. Heat shock did not induce any of the xips, but it decreased basal levels of xip4, -7, -11, and -12. Three xip cDNA clones were identified as encoding thymidine kinase, DT diaphorase, and tissue-type plasminogen activator. The remaining nine cDNA clones showed little homology to known genes. Three clones contained regions homologous to c-*fes*/*fps* protooncogene, recombination activating gene 1, or the human angiogenesis factor gene. X-ray-inducible genes may function in damaged cells to regulate DNA repair, apoptosis, mutagenesis, and carcinogenesis.**

Several known mammalian genes are modulated by ionizing radiation. These include tissue-type plasminogen activator (t-PA) (1), oncogenes [e.g., c-*jun*, interleukin-1, and *egr-1* (2–4)], and genes regulating cell growth [e.g., cyclins A and B (5, 6), proliferating cell nuclear antigen (PCNA) (7), basic fibroblast growth factor (8), and p53 (9)]. Much uncertainty exists as to the number of genes induced and their function(s) within the cell, since many changes in genes occurred only after supralethal radiation doses. Some gene changes may result from aberrant transcription, with no new protein synthesized (10).

Boothman *et al.* (11) demonstrated the induction and repression of several proteins in radioresistant, confluence-arrested, human malignant melanoma (U1-Mel) cells in response to ionizing radiation. Induced or repressed proteins were present at low levels and were not affected by other cytotoxic agents (11). Unfortunately, cDNA expression cloning of their corresponding transcripts has been difficult.

In this study, we used differential hybridization to clone transcripts which were induced after ionizing radiation. Our objective was to define the spectrum of genes induced, especially those which occur in radioresistant human cells after radiation. The expression of one transcript, that for t-PA, has been reported (1, 12).

MATERIALS AND METHODS

Cells and Culture Conditions. U1-Mel cells and normal nonfetal human fibroblasts (GM2936B) were obtained, maintained, and grown to confluence arrest (1, 11). The number of labeled nuclei for each cell was lowered from 30–50% on day 2 of growth to ≤5.2% ± 0.1% (mean ± SEM) at confluence. Cells were treated with ionizing radiation, UV radiation, phorbol 12-myristate 13-acetate (PMA), or heat shock, with or without actinomycin D or cycloheximide, and survival was determined from colony-forming ability (1, 11, 12).

RNA Isolation and Northern Blot Analyses. Total RNA was purified, electrophoretically separated, and transferred to nylon-based Zeta-Probe membranes (Bio-Rad) by capillary transfer (1, 13–15). RNA dot-blot analyses were performed as described (1, 13–15). Transcript levels were quantified and normalized to loading variations (1, 14) by using 36B4 cDNA (16) and β_2-microglobulin cDNA (1).

cDNA Library Production, Differential Hybridization, and Cross-Hybridization Analyses. X-ray-inducible clones were isolated by using a modified differential hybridization procedure. Total RNA was extracted from x-irradiated (600 cGy) or unirradiated U1-Mel cells and poly(A)$^+$ RNA was batch selected (13). Five micrograms of poly(A)$^+$ RNA extracted from x-irradiated U1-Mel cells was used to construct a cDNA library in Lambda Zap II (Stratagene). The primary library was plated onto XL1-Blue *Escherichia coli* at 10,000 plaque-forming units per 150-mm^2 Petri dish. Duplicate plaque lifts were hybridized with ^{32}P-labeled cDNA probes generated from 10 μg of poly(A)$^+$ RNA from unirradiated or x-irradiated U1-Mel cells by using reverse transcriptase from Moloney murine leukemia virus (13); a 10-fold excess of unirradiated probe was used. Filters were then washed at high stringency (1, 13, 15). Sixty differentially expressed plaques (i.e., xip clones) were purified by three additional differential hybridizations and rescued by automatic excision, which converted them to pBluescript plasmids (17). Duplicate clones were eliminated by cross-hybridization (17). Twelve independent cDNA clones (i.e., xip1–xip12) containing the largest inserts were purified. xip inserts were amplified by PCR (13) or excised by *Kpn* I–*Sma* I double digestion to generate xip cDNA probes (1, 12).

DNA Sequence Analyses. Insert sizes of isolated xip cDNA clones were xip1, 0.9 kbp; xip2, 1.5 kbp; xip3, 1.8 kbp; xip4, 2.2 kbp; xip5, 1.0 kbp; xip6, 3.2 kbp; xip7, 1.7 kbp; xip8, 1.2 kbp; xip9, 2.1 kbp; xip10, 4.2 kbp; xip11, 1.2 kbp; and xip12, 2.5 kbp. pBluescript xip cDNA templates were sequenced (18)‖ and data were compared to known sequences in GenBank (Release 72, January 1993) using MacVector DNA analysis software (IBI).

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Abbreviations: PMA, phorbol 12-myristate 13-acetate; t-PA, tissue-type plasminogen activator; xips, x-ray-inducible gene transcripts.

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‖The sequences discussed in this paper have been deposited in the GenBank data base (accession nos. L19362–L19370).

RESULTS

Isolation and Purification of X-Ray-Inducible Transcripts (i.e., xips). Inserts from the 12 cDNA clones, which were induced 3 h after irradiation from U1-Mel cells, were labeled and used as probes in Northern analyses (Fig. 1) of RNA from x-irradiated and unirradiated U1-Mel cells. Purified, differentially expressed xip transcripts were as follows: xip1, 5.0 kb; xip2, 8.0 kb; xip3, 4.0 kb; xip4, 2.5 kb; xip5, 8.0 kb; xip6, 3.2 kb; xip7, 7.5 kb; xip8, 7.0 kb; xip9, 6.5 kb; xip10, 3.5 kb; xip11, 1.2 kb; and xip12, 9.0 kb (Fig. 1 *B–M*, respectively). Transcript sizes were estimated by using 2.37 kb for 18S rRNA and 6.33 kb for 28S rRNA. The x-ray-inducibility of these transcripts at 4 h was 11-, 110-, 38-, 47-, 29-, 78-, 5-, 6-, 80-, 35-, 6-, and 4-fold for xip cDNA clones 1–12, respectively. xips reached peak levels at various times after radiation (see Table 1). The inducible expression of xips was not affected by post-x-ray treatment of U1-Mel cells with cycloheximide at 10 μg/ml for 12 h (1, 11); there was no evidence for superinduction of xips or that mRNA induction required protein synthesis (data not shown). A block in, or slowing of, rRNA processing was noted after radiation (Fig. 1*A*). We did not observe blockage of mRNA processing in more than 30 known gene transcripts (data not shown).

Temporal and Dose–Responsive Expression of xip mRNAs. The duration of xip expression after a single dose of ionizing radiation in two types of confluence-arrested human cells was studied. U1-Mel cells were exposed to 450 cGy and normal GM2936B fibroblasts were exposed to 290 cGy [equitoxic doses giving 10% survival (11)]. RNA was extracted at various times. xip induction levels in these cells varied from 3-fold for xip11 to >230-fold for xip2 (Fig. 2, Table 1). All xips were transiently induced, although their peak levels varied greatly (Fig. 2). Except for an early xip6 (i.e., t-PA) peak, all xips were inhibited by actinomycin D (5 μg/ml) (data not shown), and all xips demonstrated only one peak over time. Analysis of xip6 (i.e., t-PA) revealed two induction peaks; an early peak (at 10 min to 1 h) due to mRNA stabilization, and a larger peak (at 12–14 h) due to new transcription (Fig. 2*B*; ref. 1). Times of peak xip induction in U1-Mel and GM2936B cells are summarized in Table 1 and ranged from 1 h to 14 h. Many transcripts (i.e., xip2, -3, -4, -5, -9, -10, and -11) were expressed within 4 h in both U1-Mel and GM2936B cells but did not necessarily appear at maximal levels at this time. All

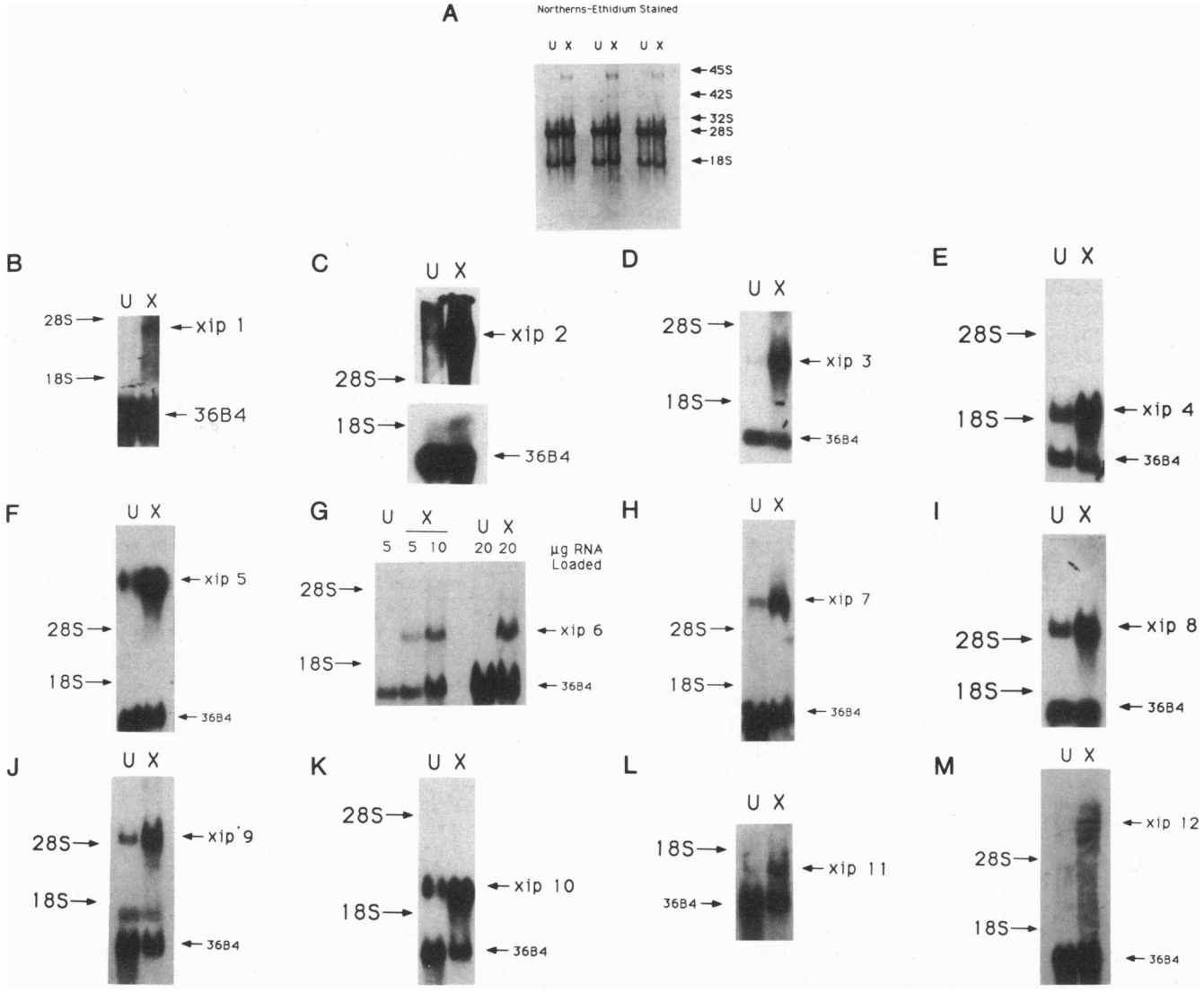

FIG. 1. Confirmation of xips by Northern analyses. RNA from unirradiated (U) or x-irradiated (X) U1-Mel cells was purified 4 h after irradiation, and Northern transfers were performed, photographed (*A*), and hybridized with ^{32}P-labeled xip cDNA inserts and with a *Pst* I 36B4 cDNA insert (0.7 kb) as an internal loading control. Filters were exposed to autoradiographic film for 6–12 h (xip2 and xip6, for 30 min to 1 h). *B–M* are Northern blots of xip1 through -12, respectively. In *G*, numbers near the origin show the amount (in μg) of RNA loaded.

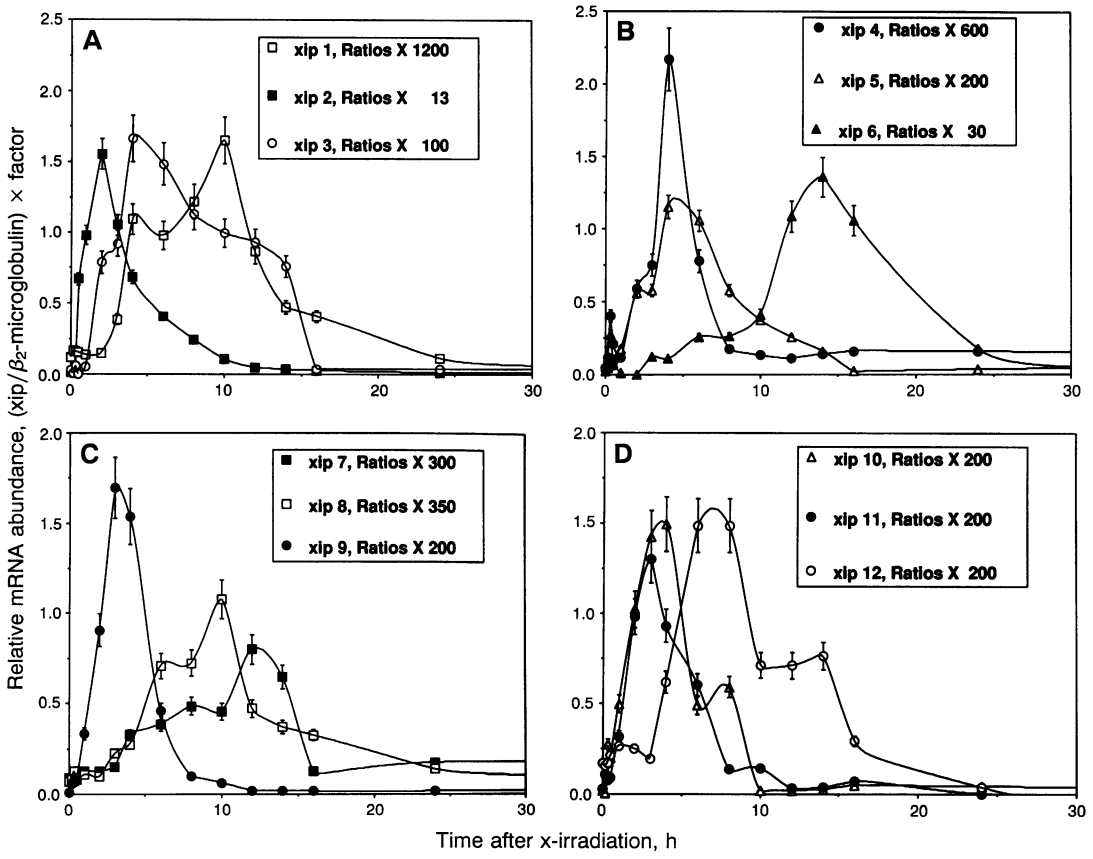

FIG. 2. Temporal expression of xips. U1-Mel cells were x-irradiated (450 cGy) and RNA was extracted at various times. xip levels were quantified via RNA dot-blot analyses. Relative mRNA abundances were calculated by dividing xip levels by β_2-microglobulin mRNA levels (1) for each time point. To graph relative mRNA abundances on a similar scale, ratios were multiplied by an arbitrary factor. The higher the factor, the less abundant the xip transcript. Factors are given in the *Insets*.

xips were induced in greater amounts in U1-Mel than in normal GM2936B cells (Table 1).

Confluence-arrested U1-Mel cells were also exposed to various doses of ionizing radiation and RNA was extracted at peak expression times for each xip (in Fig. 2 and Table 1). xip expression was then monitored by dot-blot analyses (Fig. 3). Transcripts hybridizing to xip1–8, -11, and -12 were maximally induced by 500 cGy. xip9 and xip10 were maximally induced by 100 cGy and 400 cGy, respectively (Fig. 3*A*). Many xips were induced by as little as 10 cGy (xip1, -2, -4, -5, -6, -9, -10, and -12). Other xips (xip3, -7, -8, and -11) were not induced in U1-Mel cells with doses of less than 200 cGy. All xips were down-regulated with doses exceeding 600 cGy (Fig. 3).

Table 1. Summary of x-ray-induced clones and corresponding transcripts (xips)

cDNA clone	Length,* kb	Inducibility,† -fold		mRNA induction peak,† h		Relative abundance‡
		U1-Mel	GM2936B	U1-Mel	GM2936B	
xip1	5.0	50	13	10	12	Low
xip2	8.0	230	5	2	4	High
xip3	4.0	38	3	4	2–4	Medium
xip4	2.5	47	3	4	4	Low
xip5	8.0	30	4	4–6	4	Low
xip6	3.2	150	5	14	4–6	High
xip7	7.5	11	3	12	4–12	Low
xip8	7.0	18	3	10	12	Low
xip9	6.5	83	2	4	3	Low
xip10	3.5	35	4	4	4	Low
xip11	1.2	8	2	3	1–2	Low
xip12	9.0	9	3	6–8	4–6	Low

*As determined in Northern blots.

†Confluence-arrested human cells were treated with or without ionizing radiation (450 cGy for U1-Mel, 290 cGy for GM2936B) and xip levels were quantitated at various times by dot-blot analyses from data presented in Fig. 2 or from GM2936B data, which were not presented. Induction levels (i.e., -fold) at optimal expression times (Fig. 2) were obtained from RNA dot-blot analyses. xip induction ratios were obtained by comparing xip expression levels in RNA from x-irradiated vs. unirradiated cells at times when transcripts reached maximal levels. Induction ratios, at 4-h time points, were also determined by densitometric readings of Northern blots (Fig. 1), and these levels are listed in the text.

‡Subjective measure of relative xip transcript abundance, using t-PA as an abundant transcript and xip1 or xip11 (i.e., thymidine kinase) as relatively rare transcripts in unirradiated or x-irradiated confluence-arrested U1-Mel cells.

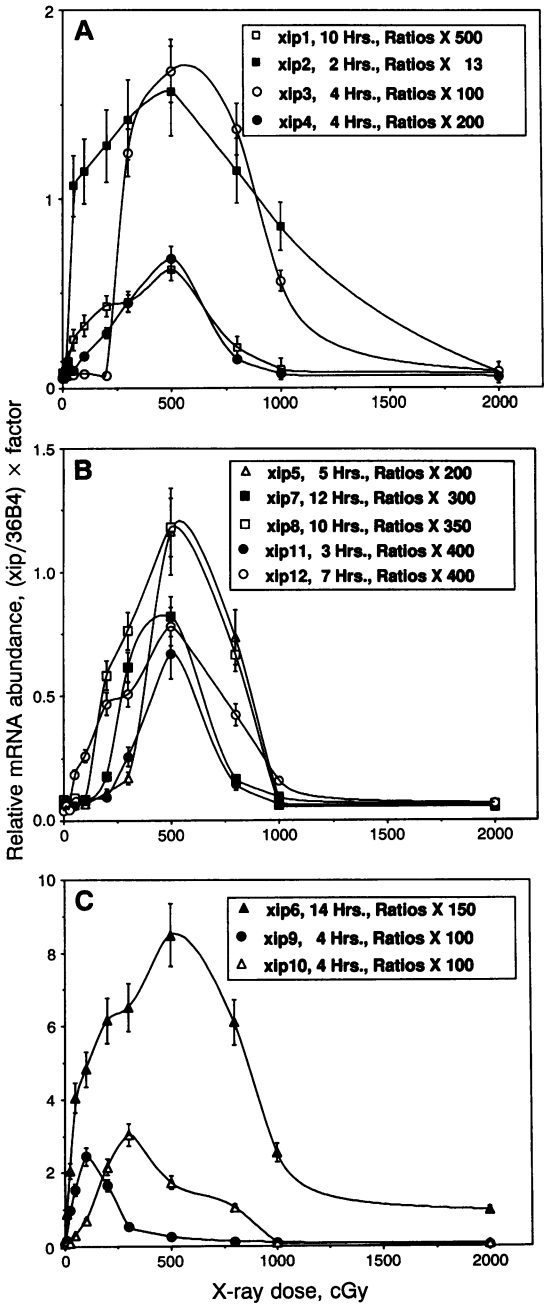

FIG. 3. Dose–response expression of xips. U1-Mel cells were exposed to various doses of ionizing radiation and RNA was extracted at optimal xip expression times (in Fig. 2). RNA was purified and analyzed by dot-blotting, and relative mRNA abundances were calculated as described for Fig. 2.

Induction of xips by Other Agents. Confluence-arrested U1-Mel cells were exposed to: (*i*) ionizing radiation of 200, 500, and 700 cGy; (*ii*) UV irradiation at 1, 10, and 30 J/m^2; (*iii*) PMA at 1, 10, and 100 nM; or (*iv*) heat shock at 42°C or 44°C for 1 h. RNA was then extracted at various times and examined by dot-blot analyses for xip induction (Table 2). Ionizing radiation supplied the greatest stimulus for xip synthesis; only t-PA (xip6) transcripts were induced to greater levels by UV irradiation and PMA. xip4, -7, and -12 were induced only by ionizing radiation. xip1, -2, -10, and -11 were greatly induced by ionizing radiation and only slightly increased by UV irradiation. xip3, -5, and -6 were induced by all cytotoxic treatments except for heat shock. xip1, -2, -9, -10, and -11 were induced by ionizing radiation and UV radiation but not by PMA or heat shock. Only xip8 was induced by ionizing radiation and PMA, but not by UV radiation. Heat shock did not induce any of the xips; instead it decreased basal levels of xip4, -7, -11, and -12. β_2-Microglobulin or 36B4 level remained constant.

Table 2. Induction of xips by other cytotoxic agents

xip	mRNA induction level after treatment*			
	Ionizing radiation (200–700 cGy)	Heat shock (44 or 42°C, 1 h)	PMA (1–100 nM)	UV radiation (1–30 J/m^2)
xip1	+++	0	0	+ (23%)
xip2	++++	0	0	+ (33%)
xip3	+++	0	++	+++
xip4	+++	−	0	0
xip5	+++	0	++	++
xip6	+++	0	++++	++++
xip7	++	−	0	0
xip8	+++	0	++	0
xip9	+++	0	0	++
xip10	+++	0	0	+ (21%)
xip11	+	−	0	+ (77%)
xip12	++	−	0	0

*Confluence-arrested U1-Mel cells were treated with various cytotoxic agents as described in the text. Other cells were not treated (i.e., untreated control). RNA from treated and untreated cells was harvested at 0, 4, 12, 24, and 48 h. Induction levels were quantitated (1) and grouped as follows: −, decreased; 0, no induction noted; +, increased by 10–99% (precise quantitation tabulated in some cases); ++, increased 2- to 9-fold; +++, increased 10- to 100-fold; ++++, increased greater than 100-fold. Induction levels were corrected by quantitation of variations in 36B4 expression.

Sequences and Identities of xips. Three xip transcripts were known: xip3, DT diaphorase [NAD(P)H:menadione oxidoreductase]; xip6, t-PA; and xip11, thymidine kinase. Temporal and dose-responsive induction of these transcripts was confirmed with authentic cDNA probes. Enzymatic analyses also confirmed their inductions (1). Other cDNA clones were not homologous to known genes, but they demonstrated homology to specific coding regions of certain genes: xip1, 67% identity with the 5′ end, exon 1 nucleotides 2825–3001, of ethanol-inducible cytochrome P450 (19); xip5, 47% identity with the 3′ end, exon 5 nucleotides 6495–6692, of human growth hormone (GH-1 and GH-2) and chorionic somatomammotropin (CS-1) (20); xip7, 62% identity with recombination activating gene 1 (*RAG-1*) mRNA and protein, amino acids 113–244 (21); xip9, 67% identity with the 3′ end, exon 4, of c-*fes/fps* protooncogene (22); and xip12, 37% identity with the 3′ end, nucleotides 1697–2425, of human angiogenesis factor mRNA (23). The remaining xip cDNA clones (i.e., xip2, -4, -8, and -10) showed very little (<10%) identity with known genes in GenBank.

DISCUSSION

Transcripts induced after ionizing radiation may regulate several biological responses in mammalian cells (e.g., DNA repair and apoptosis). To identify the spectrum of x-ray-inducible genes, we isolated several transcripts induced within 3 h after ionizing radiation. We chose confluence-arrested human cells to better understand survival recovery and to avoid cell cycle redistribution effects, which result in new transcripts due to cell cycle delays and are not necessarily x-ray-inducible, after x-irradiation. Only a few induced transcripts were observed after ionizing radiation, considering that more than 6 × 10^6 individual recombinant plaques were screened. Peak x-ray induction levels of these transcripts in U1-Mel cells varied greatly (from 4- to 230-fold) and appeared from 1 to 14 h. Similar responses were noted in normal GM2936B fibroblasts (Table 1), although xip levels

were 1/3 to 1/10. Since U1-Mel cells recover from x-ray damage 4- to 5-fold better than GM2936B cells (11), future investigations should examine differences in xips between normal and transformed cells (12) and the potential role of xips in DNA repair.

Although ionizing radiation provided the greatest stimulus, some xips were induced by other cytotoxic agents. Certain subsets of inducible transcripts were modulated by specific stresses caused by some cytotoxic agents but not by others (Table 2). Thus, cytotoxic agents induced both unique subsets of genes, but also general response transcripts (e.g., t-PA; Table 2). Heat shock did not stimulate any of the xips, but it decreased the unirradiated levels of four transcripts (Table 2). Down-regulation of xips by heat may play a role in hyperthermia (24).

The syntheses of unique transcript subsets at certain radiation doses, and at controlled times, may regulate intracellular signals that stimulate certain processes (e.g., DNA repair) but not others (e.g., apoptosis). We recently reported an adaptive survival response (ASR) in U1-Mel cells, where cells developed a 4-fold increase in radioresistance after stepwise priming exposures of 5 cGy/day for 4 days (6). ASRs were not observed above or below certain doses (6, 25). Similarly, xips were not synthesized simultaneously (Fig. 2) after radiation, and x-ray doses drastically affected which transcripts were produced and to which level they accumulated (Fig. 3). At doses exceeding an optimal level (200–600 cGy), xip induction was prevented (Fig. 3). Likewise, ASRs are observed at certain optimal x-ray doses (6, 25). Since xips appear to be transiently induced (Fig. 2, Table 1), multiple low doses of ionizing radiation may be required to build up gene transcripts and proteins to "poise" cells for greater DNA repair.

On the basis of xip gene identities and homologies, we can speculate about their function(s). xip clones can be divided into four functional groups: (*i*) regulatory factors of cell growth, DNA metabolism, and recombination [xip11, thymidine kinase; xip5, a clone homologous to human growth hormone genes; xip9, a clone homologous to c-*fps/fes*; and xip7, a clone homologous to *RAG-1*; (*ii*) metastasis (xip6, t-PA; and xip12, a clone homologous to the human angiogenesis factor gene); (*iii*) cellular defense mechanisms (xip1, a clone homologous to a cytochrome P450 gene; and xip3, DT diaphorase); and (*iv*) transcripts with unknown functions (xip2, -4, -8, and -10). After ionizing radiation, human cells respond with a controlled induction of a variety of mRNAs. Although some transcripts are produced to elicit and regulate functions within the cell to aid survival, it is unclear how these gene products interact to produce a unified response. Since U1-Mel cells demonstrated a 5-fold increase in survival in 5 h after ionizing radiation (1, 11), we are interested in the possible involvement of xip7, a gene with homology to *RAG-1*, in DNA repair. Alterations in *RAG-1* expression have been correlated with *scid* mouse hypersensitivity to x rays (22, 26).

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1. Boothman, D. A., Wang, M. & Lee, S. W. (1991) *Cancer Res.* **51,** 5587–5595.
2. Sherman, M. L., Datta, R., Hallahan, D. E., Weichselbaum, R. R. & Kufe, D. W. (1990) *Proc. Natl. Acad. Sci. USA* **87,** 5663–5666.
3. Hallahan, D. E., Sukhatme, Y., Sherman, M., Virudachalam, S., Kufe, D. W. & Weichselbaum, R. R. (1991) *Proc. Natl. Acad. Sci. USA* **88,** 2156–2160.
4. Woloschak, G. E., Chang-Liu, C.-M., Jones, P. S. & Jones, C. A. (1990) *Cancer Res.* **50,** 339–344.
5. Muschel, R. J., Zhang, H. B., Iliakis, G. & McKenna, W. G. (1991) *Cancer Res.* **51,** 5113–5117.
6. Meyers, M., Schea, R., Seabury, H., Petrowski, A., McLaughlin, W. P., Lee, I. & Boothman, D. A. (1992) in *Low Dose Irradiation and Biological Defense Mechanisms*, eds. Sugahara, T., Sagan, L. A. & Aoyama, T. (Elsevier, Amsterdam), pp. 263–266.
7. Lambert, M. & Borek, C. (1988) *J. Natl. Cancer Inst.* **80,** 1492–1497.
8. Haimovitz-Friedman, A., Vlodavsky, I., Chaudhuri, A., Witte, L. & Fuks, Z. (1991) *Cancer Res.* **51,** 2552–2558.
9. Kaston, M. B., Onyekwere, O., Sidransky, D., Vogelstein, B. & Craig, R. (1991) *Cancer Res.* **51,** 6304–6311.
10. Fornace, A. J., Jr., & Mitchell, J. B. (1986) *Nucleic Acids Res.* **14,** 5793–5811.
11. Boothman, D. A., Bouvard, I. & Hughes, E. N. (1989) *Cancer Res.* **49,** 2871–2878.
12. Fukunaga, N., Burrows, H. L., Meyers, M., Schea, R. A. & Boothman, D. A. (1992) *Int. J. Radiat. Oncol. Biol. Phys.* **24,** 949–957.
13. Maniatis, T., Fritsch, E. F. & Sambrook, J. (1982) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Lab. Press, Plainview, NY).
14. Hollander, M. C. & Fornace, A. J., Jr. (1990) *BioTechniques* **9,** 170–174.
15. Lee, S. W., Tomasetto, C. & Sager, R. (1991) *Proc. Natl. Acad. Sci. USA* **88,** 2825–2829.
16. Masiakowski, P., Breathnach, R., Bloch, J. H., Gannon, F., Krust, A. & Chambon, P. (1982) *Nucleic Acids Res.* **10,** 7895–7903.
17. Short, J. M., Fernandez, J. M., Sorge, J. A. & Huse, W. D. (1988) *Nucleic Acids Res.* **16,** 7583–7600.
18. Sanger, F., Nicklen, S. & Coulson, A. R. (1977) *Proc. Natl. Acad. Sci. USA* **74,** 5463–5467.
19. Umeno, M., McBride, O. W., Gelboin, H. V. & Gonzalez, F. J. (1988) *Biochemistry* **27,** 9006–9013.
20. Chen, E. Y., Liao, Y. C., Smith, D. H., Barrera-Saldana, H. A., Gelinas, R. E. & Seebury, P. H. (1989) *Genomics* **4,** 479–497.
21. Oettinger, M. A., Schatz, D. G., Gorka, C. & Baltimore, D. (1990) *Science* **248,** 1517–1523.
22. Roebroek, A. J., Schalken, J. A., Verbeek, J. S., Van den Ouweland, A. M., Onnekink, C., Bloemers, H. D. & Van de Ven, W. S. (1985) *EMBO J.* **4,** 2897–2903.
23. Kurachi, K., Davie, E. W., Strydom, D. J., Riordan, J. F. & Vallee, B. L. (1985) *Biochemistry* **24,** 5494–5499.
24. Bicher, H. I., Hetzel, F. W. & Sandhu, T. S. (1982) *Adv. Exp. Med. Biol.* **157,** 87–97.
25. Shadley, J. D., Afzal, V. & Wolff, S. (1987) *Radiat. Res.* **111,** 511–517.
26. Biedermann, K. A., Sun, J. R., Giaccia, A. J., Tosto, L. M. & Brown, J. M. (1991) *Proc. Natl. Acad. Sci. USA* **88,** 1394–1397.