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X-Irradiation Activates the *Drosophila* 1731 Retrotransposon LTR and Stimulates Secretion of an Extracellular Factor That Induces the 1731-LTR Transcription in Nonirradiated Cells

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Using constructs expressing the reporter gene under the control of the entire or deleted long terminal repeats (LTRs) of 1731, a *Drosophila melanogaster* retrotransposable element, we show that 1731-LTR is activated by X-irradiation in a dose- and time-dependent manner, and that a sequence located in the U3 region of these LTRs is required. The *cis*-acting element conferring X-responsiveness shows similarities to kappaB (κB)-like binding sequence. In response to X-irradiation, S2 *Drosophila* cells produced an extracellular factor which activates the 1731-LTR in nonirradiated cells. This factor was detected both when transfected cells were cocultured with inducing cells and when a conditioned medium taken from irradiated cultures was added.

Key words: dorsal, retrotransposon 1731, X-irradiation.

1731, a *Drosophila melanogaster* retrotransposon belonging to the Ty1-copia group, whose complete nucleotidic sequence has been determined (*1*), is unidirectional and fully transcribed in a polyadenylated messenger RNA, and its transcripts decrease under ecdysterone control (*1*). 1731 displays a retrovirus-like structure with 5′ and 3′ long terminal repeats (LTRs) and coding sequences for the *gag* and *pol* domains (*1*) and resembles the Ty elements of yeast and Intracisternal A particle (IAP) genes of *Mus musculus* in their ability to transpose (*2*). Particles with associated reverse transcriptase activity (*3*) and containing polyadenylated RNA (unpublished results) accumulate in the cytoplasm. Some mobile genetic elements, *e.g.*, some of those present in yeast, *Drosophila* and muridae, are known to be transcriptionally activated by environmental stresses such as high temperature (*4, 5*), exposure to certain chemicals (*6-8*) and irradiation (*9-11*). X-irradiation was for a long time used to enhance random occurrence of genetic mutations. Especially in *Drosophila* species, many mutations were due to the mobilization of transposable elements (*12*). Investigations have been carried out to examine the effects of direct or indirect X-irradiation on the transcription from 1731-LTR.

MATERIALS AND METHODS

Plasmids—The constructs B9, D3, D64, and L2 that were used throughout these experiments contained the entire or partially deleted 5′ LTR of the D.m. 1731 retrotransposon (Fig. 1). Briefly, plasmid B9 contains the entire 5′ LTR (*5*). Subclone D64 has the last 76 nucleotides deleted from the 5′ LTR (*5*). The L2 construct has the last 76 and the first 28 nucleotides deleted from the LTR, together with an 8-bp internal sequence (*13*). In construct D3, both the first 58 and the last 76 nucleotides were removed from the LTR (*6*).

Cell Culture—D. melanogaster-Schneider 2 (S2) cell line (*14*) was grown in Schneider medium containing 5% decomplemented fetal calf serum in 78.5 cm^2 culture plates. Logarithmically growing cells were transfected using the DNA calcium phosphate procedure (*15*). Cells were replated 6 h before transfection and the medium was changed as cell adhesion was completed. For series of coculture experiments, two kinds of cell cultures were performed: 24 h after cell seeding, the culture medium was removed from half of the cultures and the cells were washed with prewarmed (28°C) PBS, then cells were transfected as indicated; the other cultures either did not receive any treatment or were X-irradiated at a dose of 100 cGy. Culture was continued for a further 6 h. Then 1×10^6 cells, irradiated or not, or the medium taken from these cultures were added. In each case, the volume of the medium was kept constant in relation to that of the initial culture. For some of the X-indirect experiments, 0.3 mM suramin was added (generous gift of Dr. J. Marvaldi). In some experiments, HT29 cells were cultivated in the presence of 100 μM pyrrolidine dithiocarbamate (PDTC) (Sigma Chemical, St. Louis, MO, USA) or 100 μM pyrrolidone, a cyclic molecule structurally related to PDTC but lacking a dithiocarboxy group. These compounds were added just before irradiation and the culture was continued for 6 h. For the indirect experiment, the conditioned medium containing PDTC or pyrrolidone was taken 6 h after X-irradiation and added to transfected cells; the culture was continued for 6 h.

X-Irradiation—The X-ray irradiation of cells was performed using a Saturne 20 (*CGR*, France) with an elec-

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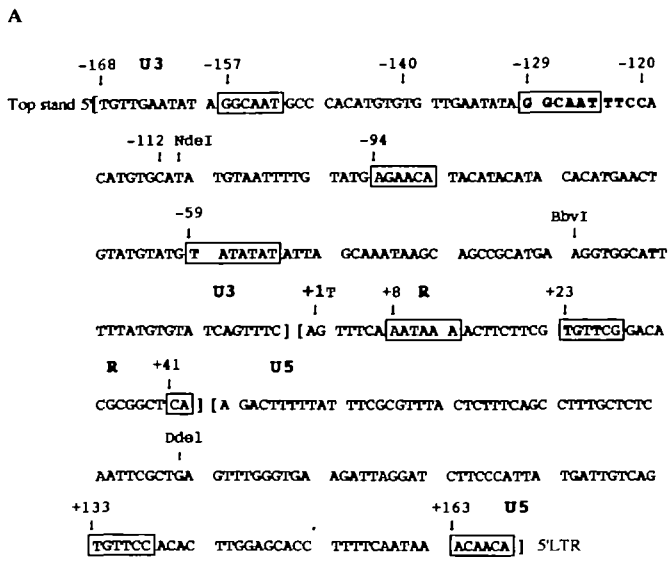

Fig. 1. **Nucleotide sequence of 1731 5′ LTR and schematic representation of subclones, adapted from Ref. 5.** (A) Nucleotide sequence of 1731 5′ LTR. Direct repeats are underlined. The putative control sequences are framed: positions −157 and −129, CAAT-boxes; position −59, TATA-box; position +8, polyadenylation signal; position +41, putative polyadenylation site; positions −94, +133, and +163, sequences similar to the hexanucleotide involved in the binding of the glucocorticoid receptor on MMTV LTRs; the putative κB-like binding sequence at position −129 to −120 is shown in bold letters. The +1T indicates the initiation of transcription. (B) Schematic representation of 1731 subclones bearing either an entire or a partially deleted 5′ LTR (solid bars); *Drosophila* genomic fragments of 729 or 63 bp upstream of the LTR are included respectively in B9 and D64 constructs (open bars).

tronic beam emitting at a fixed dose rate of 4 grays (Gy)/min as determined by dosimetry. The X-ray energy was 18 MeV. Before each irradiation, a dosimetric test was performed in water phantom. X-irradiation was carried out on cells in 5 ml of Schneider medium containing 5% fetal calf serum (v/v).

Chloramphenicol Acetyltransferase (CAT) Assays and Expression of Results—CAT assays were performed as previously described (*5*). Ten to 20 μg of protein was used for each assay. The enzymatic reaction was continued for 3 h. Protein concentrations were determined by use of the Pierce reactant. The percentage of the chloramphenicol converted into acetylated derivatives was calculated, and the basal rate (without treatment) for each construct was taken as unity for normalization: the effects of irradiation were expressed as the ratio of the percentage of chloramphenicol conversion in X-treated assays to that of the corresponding control without treatment. For the experiments with PDTC, CAT was quantified by immunoassay (CAT Elisa, Boehringer, Mannheim, Germany). All the experimental points are means from at least three experiments, and the standard error of the mean (SEM) was calculated.

RESULTS

Preliminary experiments were carried out to determine a dosing window from counts of S2 transfected cells surviving for 48 h after a single X-irradiation (Fig. 2). As repeatedly reported (*12*), *Drosophila* cells display less sensitivity towards radiation than mammalian cells. An irradiation dose as high as 100 cGy was required in order to kill less than 1% of S2 cells. Doses of 200 and 500 cGy resulted in respectively ~15 and ~50% of cell death.

The CAT activity displayed by S2 cells transfected by

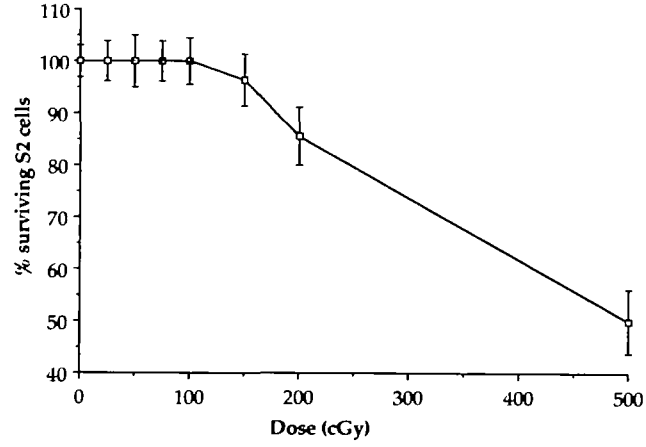

Fig. 2. **Effects of X-irradiation on the viability of S2 transfected cells.** Eight hours after transfection, the cells were exposed to various doses of X-irradiation at time 0, and culture was continued. Two days later, the cells were gently resuspended and their viability was checked using the Trypan blue exclusion method.

different plasmids bearing the entire or partially deleted 1731-LTR linked to the *cat* gene was measured either 6 or 15 h in the case of L2 subclone following X-irradiation and a dose-response curve was established (Fig. 3A). For the entire construct tested (B9), the relative CAT activity increased with increasing X-irradiation doses, reaching a maximum at 25-50 cGy, followed by a drop in activation, which returned to a basal level for an X-dose of around 500 cGy. For the B9 construct, a maximum activation compared to the control of as high as 12.5-fold was obtained.

To estimate the functional relevance of the X-responsiveness of the different LTR transcriptional signals, previously described LTR-deletion mutants (*5, 13*) were

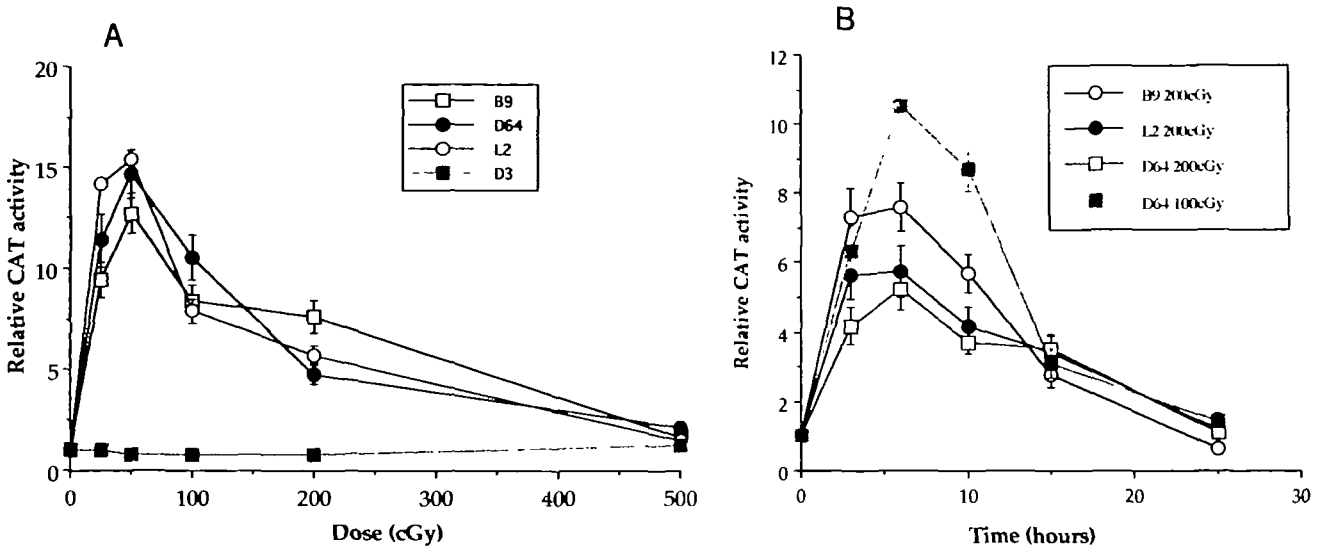

Fig. 3. **Effects of X-irradiation on promoter activity of the 5′ LTR/CAT subclones in S2 transfected cells.** (A) Dose response of CAT activity. Cells were exposed to X-rays and the CAT activity was measured 6 h later. Control plates not submitted to X-treatment were included, and the ratios of CAT activity exhibited between treated and untreated cells were calculated. (B) Response of CAT activity as a function of time after applying 200 cGy X-irradiation (and after 100 cGy X-irradiation in the case of D64 transfected cells). Corresponding control plates not submitted to X-treatment were included and the ratios of CAT activity exhibited between treated and untreated cells were calculated.

tested. L2 and D64 constructs contain a partially deleted 1731-LTR in which a "silencer" region is removed (see Fig. 1). These constructs were tested using the same protocol as described for B9. As a consequence of the deletion, an approximately 15-fold maximum increase occurred in D64 and L2 (6 h) transfected cells after exposure to a dose of 50 cGy. For the two deleted LTRs, an identical response to X-rays was obtained (Fig. 3A). More than 99% of the S2 cells survived an X-dose exposure inducing maximum activation (Figs. 2 and 3A).

The use of the construct D3, in which the putative enhancer sequence is deleted, allowed delimitation of the *cis*-acting sequence elements required for X-response (Fig. 1B). The major enhancer element located between positions −140 and −111 is necessary in order to confer X-responsiveness to the promoter. The use of construct D3 shows that a 5′ deletion to position −111 destroys reactivity; the putative κB-like binding sequence was located at positions −129 to −120 (*5*) (Fig. 3A).

Constructs containing an LTR placed in opposite orientation with respect to the CAT gene displayed neither basal nor X-stimulated activity (data not shown).

The kinetics of the response of CAT activity to X-irradiation is shown in Fig. 3B. B9 and L2 transfected cells were exposed to a dose of 200 cGy, and D64 transfected cells to a dose of 100 cGy. The transfected cells induced an increase of CAT activity after a delay of between 2 and 3 h; the maximum stimulation was reached 6 h later. The cell culture medium pretreated with X-rays, then added to the transfected cells, did not enhance CAT activity, suggesting that X-ray induced effects do not occur *via* the activation of a factor present in the culture medium.

As previously reported (*5*), a constant level of basal CAT activity was reached at around 20 h after transfection, and that level persisted up to 60 h (data not shown).

To confirm the specificity of the 1731 promoter response to X-irradiation, we tested constructs bearing irrelevant promoters from either mouse, such pCH 110, or *Drosophila* genome, such as pDr*lacZ* (generous gift of Dr. D. Bensaude, Paris), which contains the *lacZ* gene linked to the promoter of the *hsp 70* gene. When checked by transfection into S2 cells, these constructs were unresponsive to X-irradiation (data not shown).

1731	5′-GGC**AA**TTTCC-3′
dorsal consensus sequence	5′-GR**GAAAA**NCC-3′
NF-κB consensus sequence	5′-GGGRNNYYCC-3′
HIV-1 NF-κB sites	5′-GGG**ACTTT**CC-3′

Fig. 4. **Similarities between 1731, HIV-1, and the consensus sequence of dorsal and NF-κB.** Adapted from Ref. *5*. The similarities between dorsal consensus sequence and the other sequences are indicated in bold letters.

The 12- to 15-fold X-ray-directed activation of 1731-LTR was much higher than the reported activation rate of the LTR of Moloney murine sarcome provirus, for which a maximum 2-fold increase in the CAT activity occurred at a dose of 90 cGy (*16*).

To determine more precisely the mechanism underlying LTR activation and to examine the possible involvement of extracellular factors in that process, non-transfected cells were irradiated (100 cGy) and tested for their ability to stimulate CAT expression in nonirradiated transfected cells. These assays, subsequently referred to as indirect activation, were performed by adding either X-treated cells or a conditioned medium taken from an irradiated cell culture medium to wells containing 1731-LTR transfected cells.

During preliminary experiments, S2 cells transfected by D64 construct were exposed for various periods to condi-

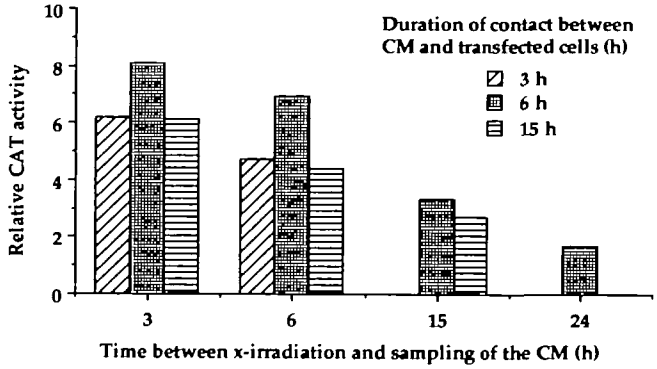

Fig. 5. **Effects of time on X-indirect activation of the D64 construct.** Medium from cultured cells was taken at various times after the (100 cGy) X-irradiation and added for various periods to D64 transfected cells. Culture times after X-irradiation and after the addition of conditioned medium (CM) are indicated in the figure. CAT assays were performed by enzymatic reaction as indicated under "MATERIALS AND METHODS."

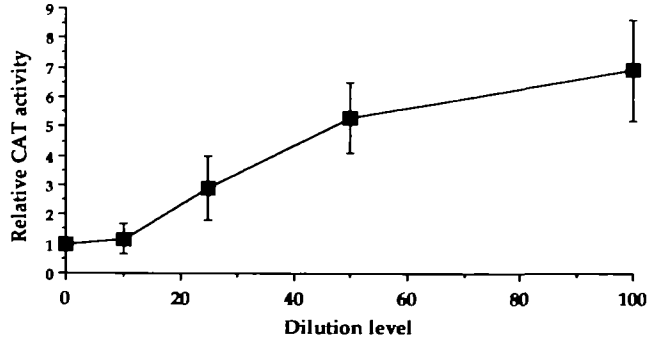

Fig. 6. **Effects of the dilution of the conditioned medium provided by (50 cGy) X-irradiated S2 cells on activation of the D64 construct transfected in S2 cells.** Medium from cultured cells was taken 6 h after X-irradiation, diluted or not with a fresh culture medium, and added to transfected cells. Culture was then continued for 6 h. CAT assay was performed by enzymatic reaction as indicated under "MATERIALS AND METHODS."

TABLE I. **Comparative effects of X-direct and -indirect activation of CAT activity and suramin-mediated inhibition of the CAT stimulation.** S2 cells transfected by various 1731 5′ LTR-derived subclones were given one of following 12 h later. A: A 50 cGy dose of X-rays (direct activation). B, C: X-treated (50 cGy) nontransfected cells were added (1×10^6 cells/well), together with (B) or without (C) suramin (0.3 mM). D, E: Conditioned medium from X-treated nontransfected cells, taken 6 h after irradiation, was added (0.5 ml/well), together with (D) or without (E) suramin (0.3 mM). In each experiment, cultures were continued for 6 h after treatment, then cells were recovered and CAT assays performed. The relative CAT activity was calculated by normalizing the percent conversion of chloramphenicol to its acetyl derivates, taking arbitrarily the results obtained for each construct without treatment as equal to 1.

	Fold induction of LTR-directed CAT activity				
	Direct X-irradiation of cells	Indirect X-irradiation		Conditioned medium from X-irradiated culture	
	A	B	C	D	E
		−suramin	+suramin	−suramin	+suramin
L2	15.4±1.3	9.6±2.4	4.9±1.3	5.4±1.1	2.6±0.5
D3	0.82±0.13	0.91±0.16	0.87±0.17	0.93±0.21	0.97±0.18
B9	12.7±1.0	7.4±1.3	N.D.	N.D.	N.D.
D64	14.7±1.2	6.9±1.7	N.D.	N.D.	N.D.

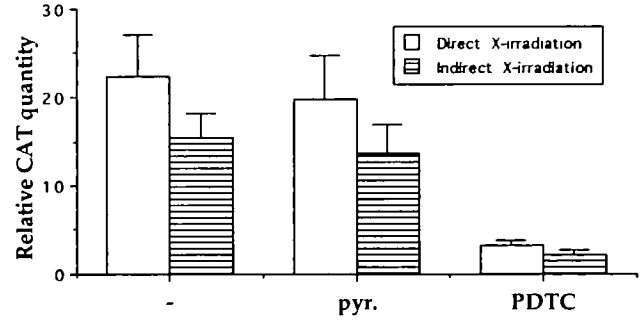

Fig. 7. **Effect of thiol compounds on activation of D64 construct by X-direct or -indirect 50 cGy irradiation.** HT29 cells were cultivated in the presence of 100 μM PDTC just before (50 cGy) X-irradiation, and the culture was continued for 6 h. For the indirect experiment, the conditioned medium containing PDTC or pyrrolidone was taken 6 h after X-irradiation and added to transfected cells, then culture was continued for 6 h. Pyrrolidone, a cyclic molecule structurally related to PDTC but lacking a dithiocarboxy group, was used as a negative control at 100 μM. The compounds added are indicated on the figure: −, no addition (control); pyr., 100 μM pyrrolidone; PDTC, 100 μM PDTC. CAT was quantified by immunoassay.

tioned medium taken at various times after X-irradiation. The maximum peak was obtained when the conditioned medium was taken 3 h after X-irradiation and the contact time between the conditioned medium and the responsive cells was 6 h (Fig. 5). When the conditioned medium was taken 6 h after X-irradiation and added to transfected cells for 6 h, activation was slightly lower; and these conditions were employed for all subsequent experiments. Activation declined to the basal level with medium taken 15 to 24 h after X-irradiation.

The level of stimulation induced by a conditioned medium taken from X-irradiated S2 cell culture was concentration-dependent up to 1/10 dilution (Fig. 6).

B9, D64, and L2 constructs displayed significant indirect activation, as shown in Table I. The highest activation was achieved by pairing X-treated cells with cells transfected by L2 subclone. For constructs L2, D64, and B9, indirect activation reached a lower level compared to direct activation (Table I, column A); but construct D3 displayed a similar responsiveness to both direct and indirect activation (Table I). In control experiments, addition of nonirradiated cells to responsive cells did not affect CAT activity (data not shown).

This observation suggests the involvement of an extracellular factor produced by cells in response to X-irradiations, and transducing a signal from one cell to another.

This hypothesis was investigated by two different approaches: first by supplementing transfected cells with a medium taken from an irradiated cell culture, and secondly by the use of the drug suramin, a strong polyanionic molecule which has been reported to trap growth factors and thus interfere with mitogenic signals and especially autocrine mechanisms in tumor cell cultures (*17, 18*).

Medium taken from X-treated cells 6 h after irradiation and added to transfected cells for 6 h before the CAT assay was still able to enhance CAT activity. However, this

activation was weak in comparison with that induced by the irradiated cells themselves (Table I, column D).

To test whether the X-indirect-induced LTR stimulation resulted from the cell release of a factor, which in turn interacted with a target on the cell surface, initiating a signal transduction pathway, the drug suramin was finally tested in X-indirect assays. When cocultures of X-treated cells together with responsive cells were performed in the presence of suramin, a 50% reduction of indirect activation ensued for construct L2 (Table I, column C). Under the same conditions, suramine did not alter or only weakly stimulated the basal *cat* expression (not shown). When included in a conditioned medium taken from an irradiated cell culture, suramin impaired but did not totally abolish the enhancement of CAT activity (Table I, column E).

As, in vertebrate cells, all nuclear factor-$\varkappa$B (NF-$\varkappa$B)-activating stimuli described to date are inhibited by radical-scavenging compounds (*19-21*), we tested whether this was also true of X-ray-mediated NF-$\varkappa$B/rel related activation in a *Drosophila* cell line (S2). Among several dithiocarbamate derivates tested, pyrrolidine dithiocarbamate (PDTC), as a potent NF-$\varkappa$B inhibitor, was best suited for tumor cell culture experiments (*19*). Consequently, we analyzed the *in vivo* effects of PDTC on S2 X-irradiated cells. S2 cells were preincubated in the presence of the antioxidant PDTC (100 μM) just prior to stimulation by X-rays (Fig. 7). In these experiments, another method of CAT dosage was used (immunoassay), which explains the differences in the X-direct or -indirect assays without treatment compared with the other experiments. Using this technique, the totality of the CAT protein (active or inactive form) was quantified, and compared to the previous experiments, where only the active enzymatic form was quantified. The values of the relative CAT quantity should indicate that after X-irradiation, the ratio of the inactive form to the active form increases. CAT assays confirmed that PDTC efficiently inhibited X-rays-direct or -indirect induced 1731-LTR activation in S2 cells (Fig. 7). In sharp contrast, 100 mM pyrrolidone, the end product of PDTC oxidation but lacking a dithiocarboxy group, had no detectable effect (Fig. 7).

DISCUSSION

In an attempt to determine the molecular steps involved in X-induced 1731-LTR transcription, using transient transfections of different 1731-LTR promoter-reporter gene constructs, we have shown that the X-induced expression of 1731 is regulated at the transcriptional level and is based on the activation of a protein closely related to the NF-$\varkappa$B/Rel family and its binding to the major enhancer element. The transcription factor NF-$\varkappa$B is a heterodimer of the p50 and p65 (rel A) subunits, each of which is related to the products of the proto-oncogene *c-rel* and the *Drosophila* morphonogen *dorsal* (*dl*) (reviewed in Refs. *22-24*). Dorsal is a transcriptional factor first described as a main regulator intervening in the process of morphogenetic dorso-ventral determination of the *Drosophila* embryo (*25, 26*). There is also a strong similarity between the consensus binding sequence of dorsal and NF-$\varkappa$B (Fig. 4). A new *Drosophila* Rel protein Dif (for dl-related immune factor), not functional during early embryogenesis, is expressed in the larval fat body (*27*), and Dif is the likeliest candidate for X-induced 1731-LTR activation. Since dorsal and Dif are normally localized in the cytoplasm in an inactive form, the X-induced signal transduction pathway involves a cytoplasm-located step (reviewed in Refs. *23* and *24*).

Other works have shown that the UV-induced transcription of the HIV-1 promoter is mediated through the tandem NF-$\varkappa$B tandem binding sequence located in the viral LTR (*28*), and it has been demonstrated that X-rays induce expression, nuclear localization and DNA binding activity of NF-$\varkappa$B (*29*).

A silencer region has been discovered in the 1731-LTR (*5*). When this sequence was deleted, 1731 LTR displayed an enhancement of its strength. This silencer region does not seem to be involved in the X-response: by using constructs containing these partially deleted LTRs, a similar rate of X-stimulated transcription was obtained. On the other hand, considering that the X-response is drastically reduced when a region containing the $\varkappa$B-like binding sequence is removed, we conclude that this sequence supported the up-regulation due to irradiation. As the stimulation was totally abolished, this suggests that the $\varkappa$B-like binding site could be the only operative pathway of signal transduction in the LTR sequence of the D3 subclone.

This present report shows that cells could be indirectly affected, probably through a process similar to those described for HIV-1 activation by UV irradiation (*28*), for which other authors have already demonstrated, in mammalian cells, a release into the extracellular space of a set of factors efficient in activation of the transcription of various genes in nonirradiated cells (*30*). Recently, secretion of growth factors subsequent to UV irradiation has been reported (*31*). UV-induced factor expression has been suggested in several mammalian cell types. The secreted factor was not tissue-specific (*28*).

X-indirect activation was indeed transmitted by conditioned medium taken from X-irradiated cells. This observation suggests the intervention of soluble factors released by the X-irradiated cells. Among the various cellular effects attributed to suramin, a role in trapping growth factors has been repeatedly reported (*17, 18, 32*). This antiparasitic drug (a heparin analogue) inhibits binding of various (mammalian) growth factors (*e.g.*, PDGF, bFGF, TGF-beta, EGF, IGF-I, IGF-II) to their receptors *in vitro* (reference in Ref. *32*). This polyanion has been reported to inhibit the activity of a cell-to-cell contact-induced extracellular factor (*31*). This effect is assumed to support phenotypic reversion of tumor cell line in culture (*33*), no doubt by removing autocrine factors released in the cell culture medium (*18*). The partial abolition, in the presence of this drug, of the X-ray-directed transactivation suggests that a growth factor-based mechanism may play a role in the transfer of the stimulation message from X-irradiated cells to responsive cells *via* the conditioned medium. The ability of X-irradiated cells to transfer activation argues against a simple direct action of short-lived free radicals emitted in response to irradiation. If a function for these chemical species is assumed, it is necessary to consider in addition a cascade of events in which some extracellular factors could play a role, as was suggested by our experiments with suramin. It was surprising to observe a considerable suramin-resistant fraction of indirect activation, but similar results were obtained with the D64 or B9 constructs

transfected in S2 cells (data not shown), or during X-indirect activation of HIV-1-LTR construct transfected in human melanoma-derived cell line (IGR39) or murine fibroblast BALB/c (D152) (data not published). During the experiment of phenotypic reversion of a human colonic carcinoma cell line (HT29) in culture, similar results were also observed (J. Fantini, unpublished results). Two explanations are possible: on the one hand, only some of the species of secreted "growth factor" are blocked in the presence of this drug; or, on the other hand, for a given extracellular factor, the trapping could be partial.

In vertebrates, although several independent results are consistent with a potential role for Red/ox process in the control of NF-$\varkappa$B, the exact mechanism involved has not yet been entirely characterized (*19-21, 34, 35*). In our experiments, under antioxidant oxidations, the X-direct or -indirect irradiation was found to be incapable of activating the 1731-LTR. This suggests that, like all other known NF-$\varkappa$B inducers, X-irradiation increases the production of reactive oxygen intermediates within the cell, which may act as second messengers leading to activation of the transcription factor. However, a number of hypotheses have arisen, as in this work, from the results of PDTC-based experiments, and the situation is far from clear, since PDTC is known to display at least two different chemical properties: in addition to its oxygen radical-scavenging effect itself, PDTC exhibits a chelating activity towards heavy metals, thus interfering with metal-dependent enzymes. Since some of these enzymes play a role in oxygen metabolism, the two activities could either synergize or, conversely, antagonize. This could explain some apparent discrepancies between reports using PDTC in different biological systems. These two mechanisms have been shown to be definitely involved in Jurkat T cells treated by a set of inducers, such as H_2O_2, PMA, or TNF-α (*19*).

It has long been known that different agents inducing the stress response, including some carcinogens, facilitate the induction of different retroviruses or retrotransposons (*5-11, 16, 36, 37*), and there is considerable evidence to support the view that transcriptional activation of LTRs is due to a stress response from carcinogens (*16*).

The significance of these results regarding the possible influence of the enhancement of transcription on the transposition rate of 1731 remains debatable. The relationship between the rate of, respectively, transcription, translation and transposition of transposable elements is still shrouded in uncertainty (*38, 39*).

The capacity of ionizing-radiation to activate 1731 adds one more hazard to the list of deleterious ionizing-induced effects. Given that all genomes contain retrotransposons, experiments using 1731 promoter are of general interest in that they support the view that biological stress induced by radiation could be indirectly responsible for mutations through the potential enhanced mobility of these genetic elements.

The evidence provided in this report and in other studies that 1731-LTR can be activated by various agents causing DNA damage, such as carcinogens (X-rays, PMA-PHA), and other types of stress, such as heat shock, indicates that this LTR might be a good reporter system for signals arising as a result of DNA damage.

Future studies should explore by gel shift assay the binding of a putative member of the NF-$\varkappa$B/Rel family on the $\varkappa$B-like sequence of the 1731-LTR, and try to characterize the nature of the protein(s) able to bind to this sequence.

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