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HIV Transcription is Induced with Cell Killing

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Abstract

Previous work has shown that HeLa cells stably transfected with an HIV-LTR-CAT construct are induced to express chloramphenicol acetyl transferase (CAT) following exposure to DNA-damaging agents such as ultraviolet radiation, γ rays, neutrons, and others. In this report, we demonstrate that this induction of HIV-LTR transcription occurs when stably transfected HeLa cells are exposed to agents which mediate cell killing, such as UV radiation, electroporation of sucrose buffer, prolonged heating, and low and high pH. Cells cultured following UV exposure demonstrated a peak in CAT expression that is evident in viable (but not necessarily cell division-competent) cells 24 h after exposure; this inductive response continued until at least 72 h after exposure. HIV-LTR induction was dose-dependent, and the amount of CAT transcription induced was correlated with the amount of cell killing that occurred in the culture. Other agents which caused no cell killing (such as heat-shock for up to 2 h, treatment with metronidazole, exposure to sunlight, vitamin C treatment, and others) had no effect on HIV-LTR induction. These results suggest that HIV transcription is induced as a consequence of the turn on of a cellular death or apoptotic pathway.

Valerie *et al.* (1) described the induction of transcription from the human immunodeficiency virus long terminal repeat (HIV-LTR) following exposure to DNA-damaging agents in HeLa cells stably transfected with a construct containing the chloramphenicol acetyl transferase (CAT) reporter gene driven by the HIV-LTR promoter. Since then, many reports have reproduced and expanded upon those findings (2-6), even demonstrating *in vivo* induction in transgenic mouse systems. Recent work from our laboratory has shown enhanced expression from the HIV promoter following exposure of cells to fission-spectrum neutrons at doses which also induce expression of apoptosis-associated genes (6). We set out in these experiments to determine whether this HIV-LTR induction was a consequence of DNA damage, as suggested by the initial reports, or whether it was associated with the onset of cell death in general (such as occurs during apoptosis or programmed cell death).

Figure 1 details the results of experiments examining the effects of electroporation of sucrose buffer on the induction of CAT expression driven by HIV-LTR. In these experiments, cells were exposed to differing electric voltage levels (0.1 or 0.3 kV) in phosphate-buffered sucrose. At the same time, each culture was monitored for the number of total viable cells when the total number of input cells for each experimental group was the same. These results demonstrated maximal induction of the LTR-CAT construct at voltages which resulted in maximal cell killing. It should be noted that equal numbers of viable cells only were used

for each CAT assay so as to avoid variation caused by the number of dead cells resulting from the treatment conditions. These and all other results are from a single experiment since cpm are not directly comparable from one experiment to the next; all results have been repeated twice after the original observation to confirm validity.

Next, the effects of UV exposure on expression of the CAT reporter gene were examined; Figure 2 shows results demonstrating induction of CAT following UV exposure. The response peaked at 24 h following exposure and was maintained at high levels even as late as 72 h following exposure; conditions were such that cell death was induced. Again, it should be noted that in these experiments, equal numbers of viable cells were used in determining the number of cells to be used in each CAT assay. The fact that this response remains high in those cultures in which cell death had been triggered, but not in sublethally treated cultures, further supports the concept that induction of HIV-LTR is associated with cell death rather than with DNA damage.

Figure 3 demonstrates the results of experiments examining UV effects on both colony formation (14-day assay) and CAT expression in the same cell cultures. Cellular colony formation was used as a measure of cell division/propagation capability. These experiments demonstrate a correlative relationship between HIV-LTR-mediated CAT expression and the lack of cell survival following UV exposure, i.e., cells that are not able to go on to

divide express higher levels of LTR-CAT than those that do go on to divide. Figure 4 similarly examines the effects of multiple UV doses on HIV-LTR-CAT induction. Exposures of 5 J/m² were not additive, and a dose between 5 and 20 J/m² was required for induction of HIV-LTR. These results suggest that HIV-LTR has a threshold UV dose for induction of CAT and that this threshold dose corresponds to a dose at which cell killing is first detectable in these cultures.

Table I describes the results of a series of many experiments in which various agents were tested under different conditions for their abilities to induce HIV-LTR-CAT expression. These experiments demonstrated that treatments which reduced cell viability also induced CAT expression. Treatments which induced HIV-LTR-mediated CAT expression included low pH (6.4), high pH (8.4), electroporation, UV exposure, and excess heat (700 W [microwave] for 10 s in a small volume of PBS). On the other hand, treatments which had no effect on viability and no effect on HIV-LTR-CAT induction included co-culture with metronidazole (a DNA-damage-inducing drug), vitamin C treatment, microwave exposure in an excess volume of medium to reduce overheating, exposure to electromagnetic fields, and heat-shock (10 min at 43 °C, 2 h at 39 °C).

These results suggest a potentially causative association between cell killing and the induction of HIV expression. Past work has shown the requirement for new protein synthesis for induction of HIV (7). We hypothesize that one pathway for

inducing cellular death (apoptosis or programmed cell death), which also requires new protein synthesis (8,9), causes induction of HIV expression. The fact that this response is not repressed within the first 72 h following UV exposure (Fig. 2) suggests that the HIV inductive response is not capable of being directly repressed by the dying cell. We propose a model whereby HIV lays dormant in cellular DNA until apoptosis or cell death is naturally induced; this activation of apoptosis then turns on HIV expression so that maximal viral transcription occurs in apoptotic cells. Much work has demonstrated a relationship between HIV and apoptosis (10-12), with most observations suggesting that HIV itself induces the apoptotic process. Our cell system does not produce active virus, so we cannot examine the possibility that HIV itself induces apoptosis, as shown by others (10-12). Our data suggest, however, that the cell death/apoptotic response induces HIV. This could explain why HIV is expressed *in vivo* most commonly in T cells which undergo natural developmental apoptosis in adults.

Finally, past work has suggested that UV-induced HIV transcription is mediated directly by DNA damage (13). Our model proposes that one mechanism by which HIV is induced is as a consequence of a cell death response; this response can be induced by a variety of agents, including those which damage DNA (UV, neutrons), those which denature proteins (excess heat; pH variations), and those which disrupt cell signaling (electroporation). The actual intracellular signal that induces

programmed cell death responses is not known but may provide important insights for studies of HIV gene regulation.

References and Notes

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Table I. Effects of Various Agents on HIV-LTR-CAT Induction

Agent	Fold HIV-LTR-CAT Induction*	No. Experiments
No treatment	1.0	>20
pH 6.7-8.1	0.7-1.0	3
pH 6.4	1.5-2.0*	2
pH 8.4	1.6-2.0*	3
Microwaves (700 W), 5-12 s, 25 ml PBS/pen/strep (temp. <43 °C)	0.8-1.1	4
Microwaves (700 W), 10 s, 10 ml PBS/pen/strep (temp. <43 °C)	2.2-2.5*	4
Electromagnetic radiation (60 Hz)	1.0-1.2	4
Ultraviolet radiation (≥ 25 J/m ²)	2.0-29.2*	>20
Ultraviolet radiation (<2.5 J/m ²)	1.0-1.3	>20
Vitamin C (0.1-1.0 mg/ml)	0.8-1.2	2
Metronidazole (6-12 μ g/ml)	0.8-1.3	2
Electroporation of buffered sucrose (0.1-0.3 kV)	3.7-36.1*	3
Heat shock (38-45 °C, 10 min; 39 °C, 2 h)	0.7-1.1	3
Fission-spectrum neutrons (48 cGy)	1.2-2.0*	3
Solar radiation (240 KJ/m ²)	1.0-1.3	3

*All measurements 12-24 h following exposure

*Significantly different from controls at $p < .01$

FIGURE LEGENDS

Figure 1. Equal numbers and concentrations of HeLa cells stably transfected with the HIV-LTR-CAT construct were exposed in triplicate to varying doses of electric current at the indicated voltages; electroporation was carried out in the presence of PBS. Cells were harvested 24 h following exposure and counted. A: Cell survival documents the numbers of viable cells remaining in the culture as determined by trypan blue dye exclusion at the time of cell harvest. The number atop each column indicates the percentage of viable cells remaining in the culture. B: Equal numbers of viable cells were counted and used in the CAT assays for the ^3H acetylation of chloramphenicol. Assays were performed as described previously (6).

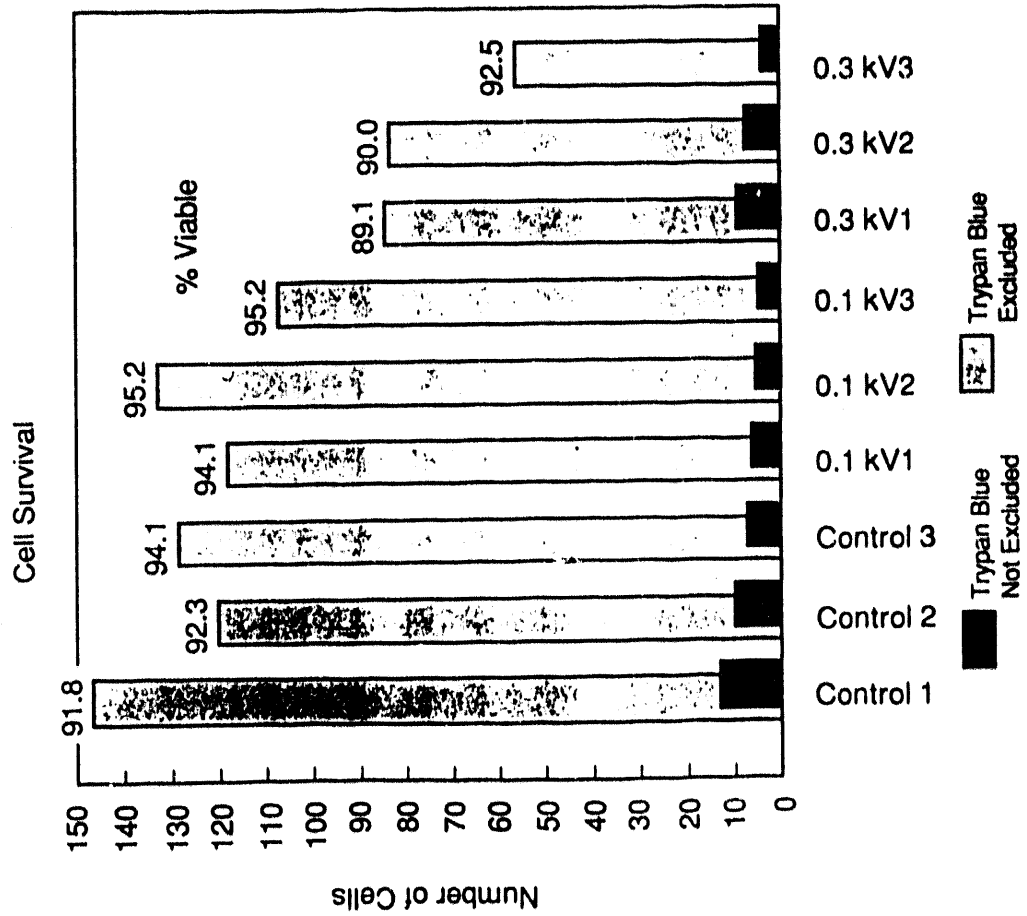
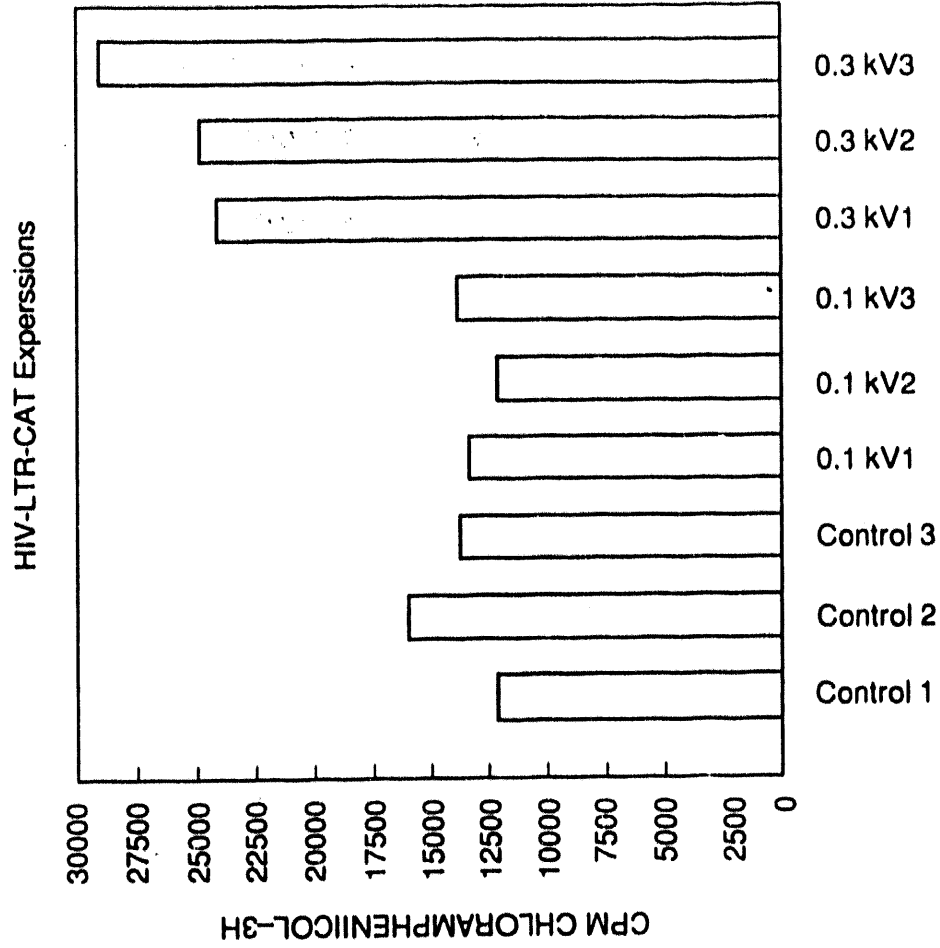
Figure 2. HeLa cells stably transfected with the HIV-LTR-CAT construct were exposed to different doses (as indicated) of UVC (254 nm) germicidal lamp. Cells were harvested 24-180 h following exposure. Equal numbers of viable cells were used in each CAT assay (6). At the high dose (25 J/m^2), there were no viable cells remaining for assay after 96 h post-exposure.

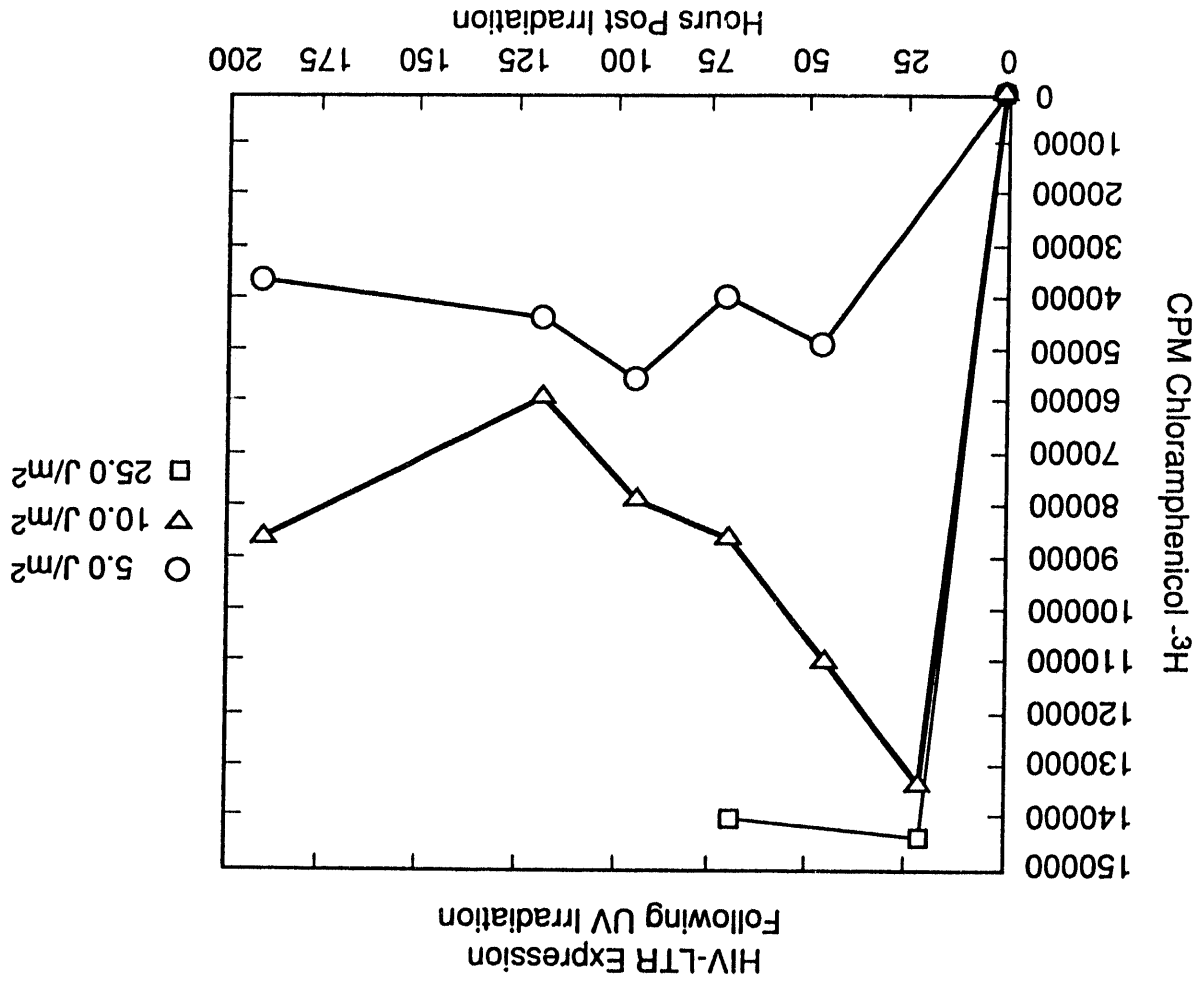
Figure 3. HeLa cells stably transfected with HIV-LTR-CAT construct were exposed to doses (J/m^2) as indicated of UVC (254 nm) germicidal lamp. From each culture, an equal number of cells for each treatment were used for 14-day colony assays for cell

survival determination (A; expressed as the number of colonies/number of cells plated \times 100) and equal numbers of viable cells were set up in CAT assays for expression studies (B; 6).

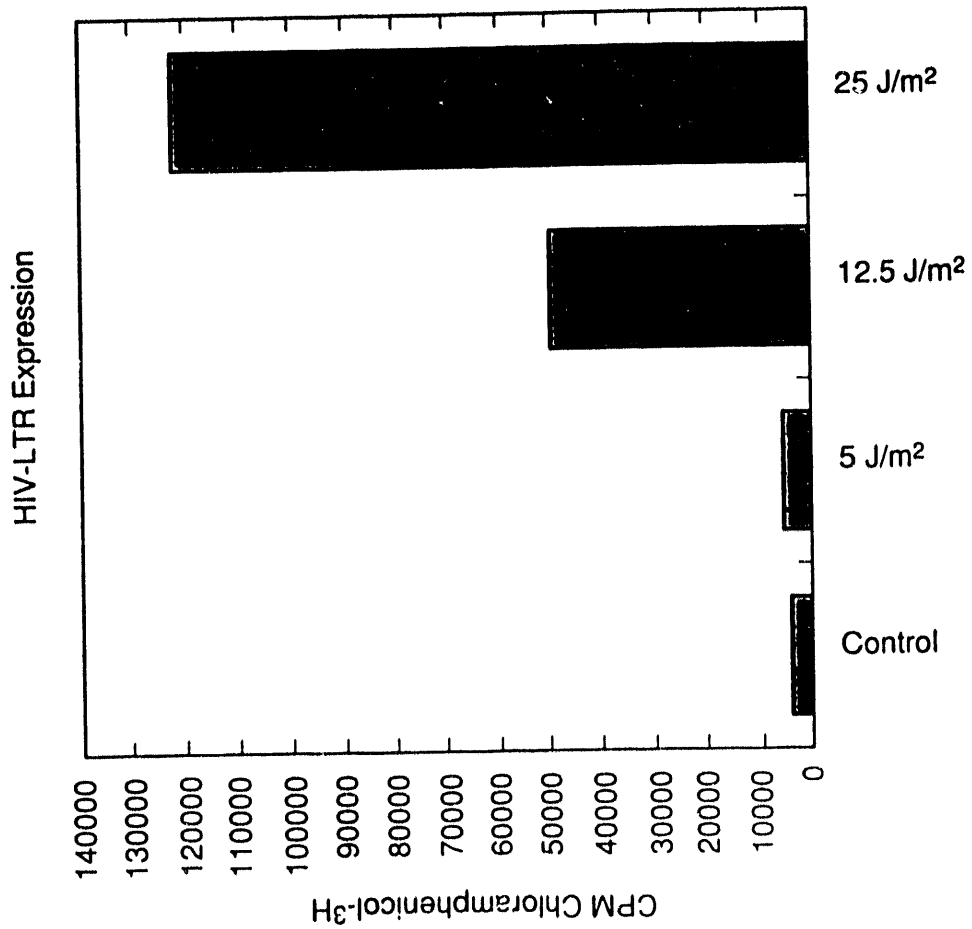
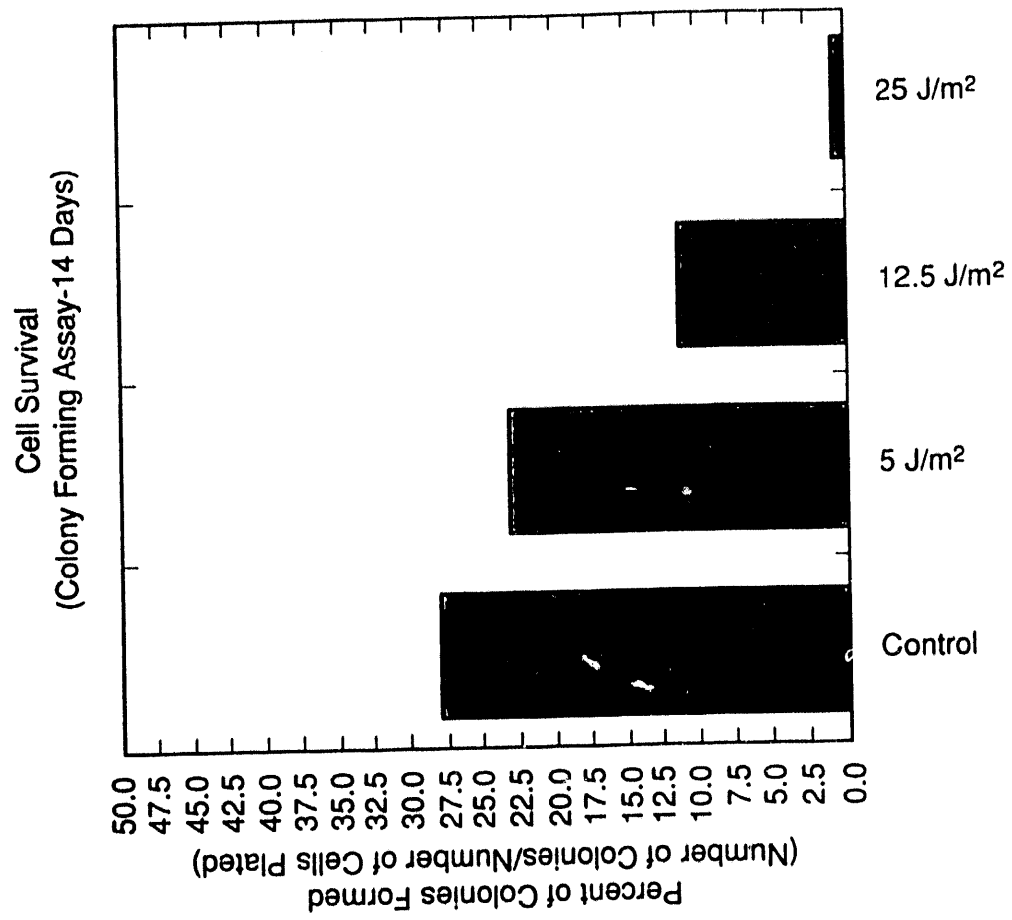
Figure 4. HeLa cells stably transfected with HIV-LTR-CAT were exposed at 24-h intervals to 0, 5 or 10 Jm^{-2} UVC (254 nm) germicidal lamp. Cells were harvested at 24 h and 48 h post-exposure. Equal numbers of viable cells were used for CAT assays as described (6).

Effects of Electroporation

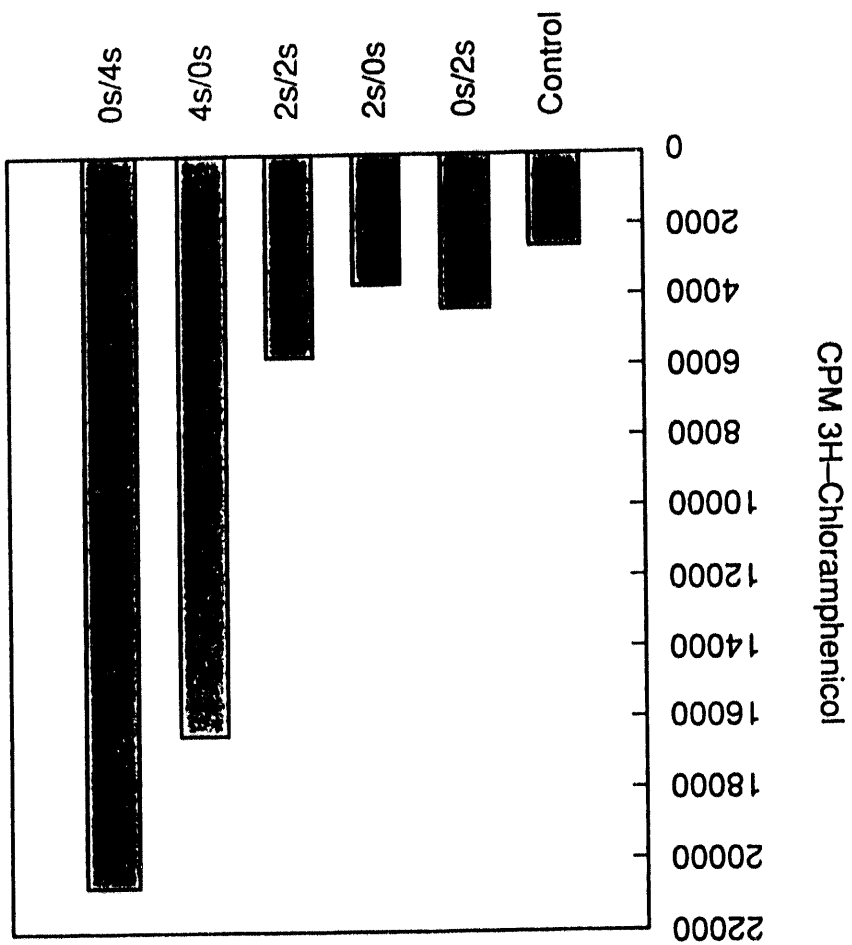




Effects of UV



Effects of Ultraviolet Radiation on HIV-LTR Expression



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