

# Response of Human Immunodeficiency Virus Long Terminal Repeat to Growth Factors and Hormones

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**Abstract.** We have employed a recombinant plasmid, pBHIV1, carrying the long terminal repeat (LTR) of the human immunodeficiency virus-1 (HIV-1) linked to the reporter chloramphenicol acetyl transferase (cat) gene and to the aminoglycoside phosphotransferase (aph) gene as a selectable marker. We have introduced pBHIV1 in rat 208F and human MRCSV40TGR fibroblasts and obtained stable geneticin resistant RFBHIV1-1 and SVTGHIV1-1 transfectant cells respectively. Both RFBHIV1-1 and SVTGHIV1-1 cells express CAT activity from the HIV LTR promoter. The response to insulin, epidermal growth factor, hydrocortisone and dexamethasone was studied on the LTR regulated CAT activity in both cell lines. It was found that, at optimal concentrations, insulin, epidermal growth factor and hydrocortisone regulate positively the expression of CAT from the HIV LTR in rat RFBHIV1-1 but not in human SVTGHIV1-1 cells. On the other hand dexamethasone at  $10^{-5}M$  stimulated CAT activity in both types of cells.

Human immunodeficiency viruses are the cause of acquired immune deficiency syndrome (AIDS) (1,2). These viruses infect and destroy the T4 lymphocytes and establish chronic infections (3). Understanding the regulation of HIV gene expression is of paramount importance in order to prevent and eventually cure AIDS. The HIV LTR is a complex modular structure of protein binding sites through which cellular factors can regulate gene expression. Stimuli which are known to affect HIV LTR activity include several mitogens i.e. phytohemagglutinin (4, 5), phorbol esters (6, 8), ionomycin (4) and gene products i.e. *tat* (9, 10), or *ras* p21 (11).

The demonstration that several hormones enhance the

production of HIV by mononuclear leukocytes infected *in vitro* and that hydrocortisone in particular facilitates the isolation of virus from peripheral blood mononuclear cells established in cell culture from AIDS patients (12) prompted us to examine further whether hormones and growth factors act through the virus LTR sequences. We find that both types of regulators can augment gene expression from the HIV LTR sequences in an *in vitro* system.

## Materials and Methods

**Recombinant plasmids and cell lines.** Plasmid pBHIV1 carrying a 728 bp XhoI-HindIII DNA fragment containing the HIV-1 LTR sequences was constructed by inserting a 1.9 Kb BamHI fragment carrying the *aph* gene into the single BamHI site of plasmid pBC12 | HIV | CAT (13). Plasmid pBC12 | HIV | CAT was obtained from B.R. Cullen (13).

The spontaneously immortalized rat 208F and the SV40 immortalized human MRCSV40TGR fibroblasts were used as recipients to obtain the RFBHIV1-1 and SVTGHIV1-1 stable geneticin resistant transfectant cell lines with plasmid pBHIV1 (14). DNA transfections were carried out using the calcium phosphate technique (15) as modified (16).

**Treatment of cells and CAT assays.** Cells were plated at  $1.5 \times 10^6 / 75\text{cm}^2$  flask in Ham's SF12 medium containing 10% FCS at 37° C. 24h later the medium was replaced with Ham's SF12 medium containing 0.5% FCS and left for another 24h at 37°C. Then the medium was changed with Ham's SF12 containing 0.5% FCS and the various concentrations of growth factor or hormone. Cells were harvested 24h later and tested for CAT activity as previously described. (17).

## Results

**Effects of insulin and epidermal growth factor (EGF) on the HIV LTR.** It has been shown previously that the HIV LTR contains the primary *cis*-acting regulatory sequences of the virus. It was therefore of interest to examine the effect of the growth factors and hormones on the transcriptional regulation of HIV LTR. Stable transfectants expressing the CAT gene driven by the HIV LTR were obtained. The effect of insulin on the LTR was tested as described in Materials and Methods. Autoradiographs of representative chromatograms from CAT assays are shown in Figure 1 for rat cells and in Figure 2 for human fibroblasts. The corresponding histograms representing the increase in CAT activity are shown in

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Key Words: HIV, AIDS, growth factors, hormones.

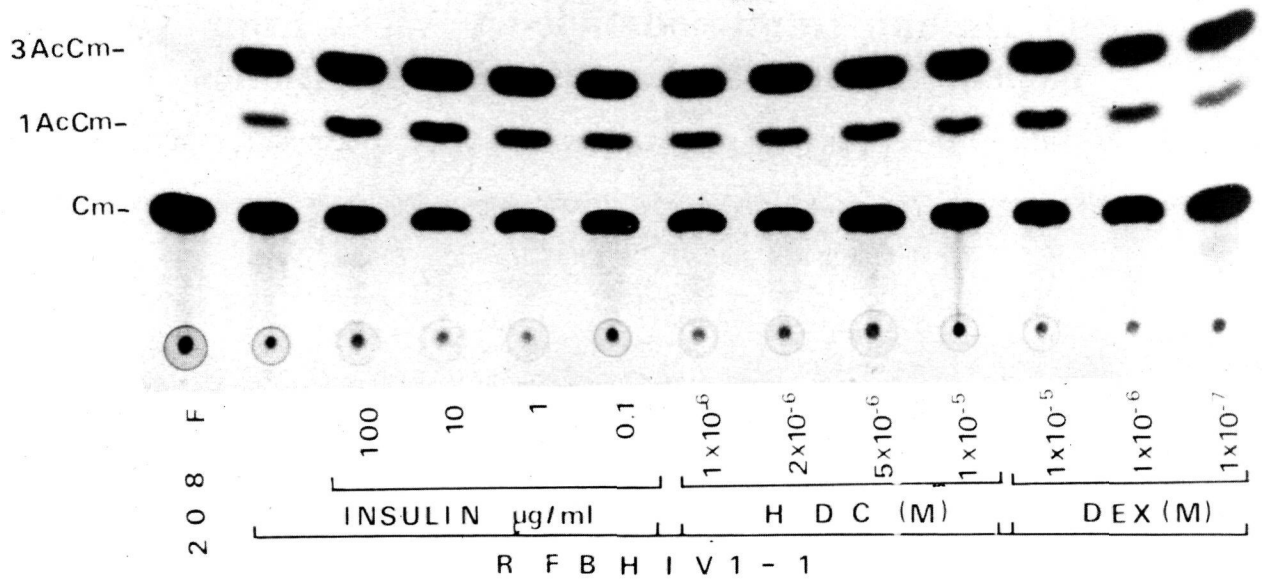


Figure 1. Chromatogram of representative CAT assays with extracts from recipient and transfectant RFBHIV1-1 cells with and without treatment with insulin, HDC or DEX.

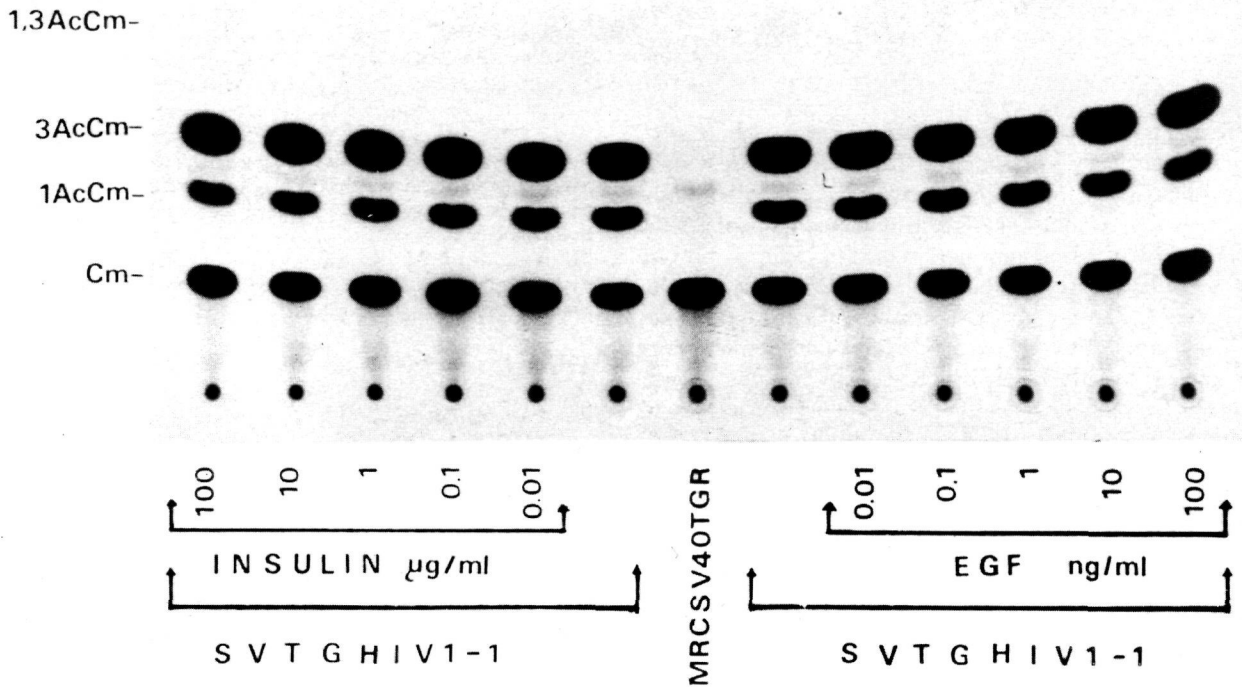


Figure 2. Chromatogram of representative CAT assays with extracts from recipient and SVTGHIV1-1 cells with and without treatment with insulin or EGF.

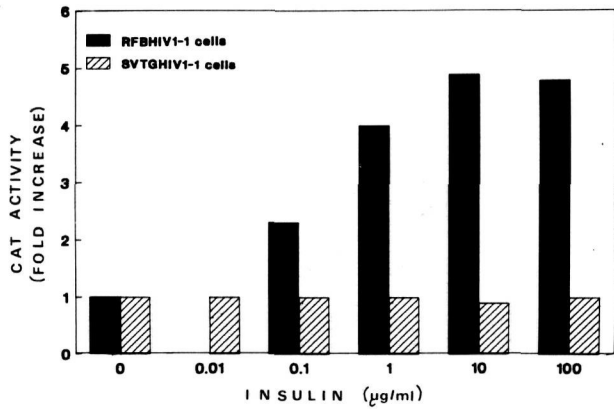


Figure 3. Induction of CAT activity by insulin. RFBHIV1-1 and SVTGHIV1-1 cells were plated at  $1.5 \times 10^6/75 \text{ cm}^2$  flask in Ham's SF12 medium containing 10% FCS at 37° C. 24 h later the medium was replaced with Ham's SF12 medium containing 0.5% FCS and left for another 24 h at 37° C. Then the medium was changed with Ham's SF12 containing 1% FCS and the various concentrations of insulin. Cells were harvested 24h later and tested for CAT activity as described in Materials and Methods. Relative values of CAT activity in RFBHIV1-1 and SVTGHIV1-1 cells were 13 and 29 pmole acetylated chloramphenicol /µg protein per hour incubation, respectively. Average from three experiments is given. Standard deviation was less than 10% of average values.

Figure 3. Whereas at 1-100µg insulin/ml, a 4 to 5-fold stimulation of CAT activity was found in rat cells, in human cells insulin did not alter CAT activity from HIV LTR.

The effect of EGF on HIV LTR was also tested. Similarly, CAT assays are shown in Figure 4 for the rat and Figure 2 for human fibroblasts. The corresponding histograms representing the increase in CAT activity are shown in Figure 5. Whereas at 0.1-100 ng/ml EGF a 2.3 to 3.8-fold increase in CAT activity was found in rat cells, EGF had no effect on the human cells.

The effect of hydrocortisone (HDC) and dexamethasone (DEX) on the HIV LTR. The effect of HDC was tested at concentrations varying from  $1 \times 10^{-5}$  to  $1 \times 10^{-6}$  M. Representative chromatograms from CAT assays are shown in Figure 1 and Figure 6 for rat and human cells respectively. The corresponding histograms representing the increase in CAT activity are shown in Figure 7. Whereas at  $1 \times 10^{-6}$  to  $1 \times 10^{-5}$ M HDC a 1.9 to 2.8-fold increase in CAT activity was found in rat cells, in human cells HDC has no effect.

The effect of DEX was also tested. At  $10^{-5}$  M DEX a 3.6-fold increase in CAT activity was observed in rat (Figure 1 and 8) and a 2.4-fold increase in human (Figures 6 and 7) cells, respectively.

### Discussion

Several regulatory elements have been described on the HIV-1 LTR. Among them are included a trans-acting respon-

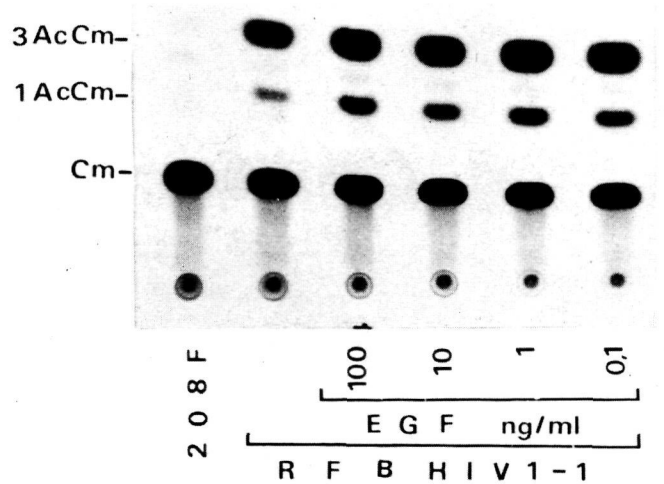


Figure 4. Chromatogram of representative CAT assays with extracts from recipient and transfectant RFBHIV1-1 cells with and without treatment with EGF.

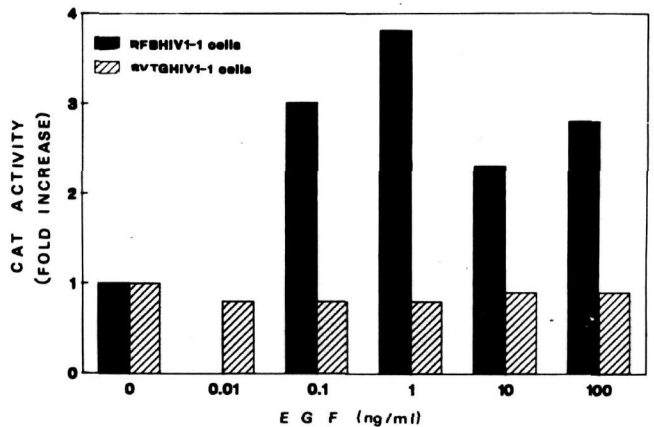


Figure 5. Induction of CAT activity by EGF. CAT values were obtained as described in Figure 3.

sive element (9, 10), an enhancer region including binding sites for NF-κB and Sp1 transcription factors (18, 19), a phorbol ester inducible element (5) and a negative regulatory element (10).

Glucocorticosteroids are known to have a wide range of effects including the modulation of expression of some cellular genes (20-24).

Previous studies have suggested a role for corticosteroids and possible gonadal steroids in the modulation of virus expression and/or release and have suggested that the capacity of these and other compounds to induce virus replication

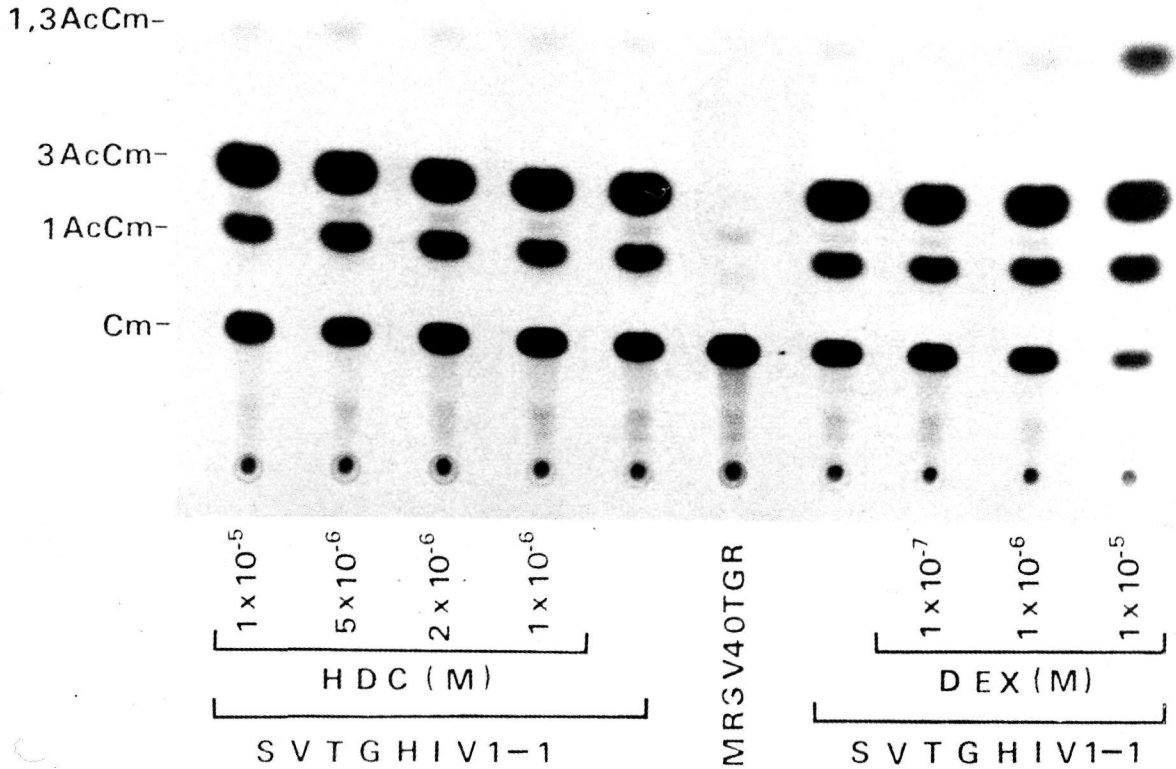


Figure 6. Chromatogram of representative CAT assay with extracts from recipient and SVTGHIV1-1 cells with and without treatment with HDC or DEX.

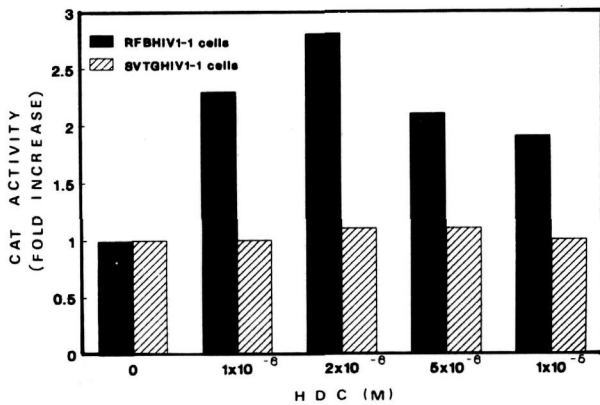


Figure 7. Induction of CAT activity by HDC. CAT values were obtained as described in Figure 3.

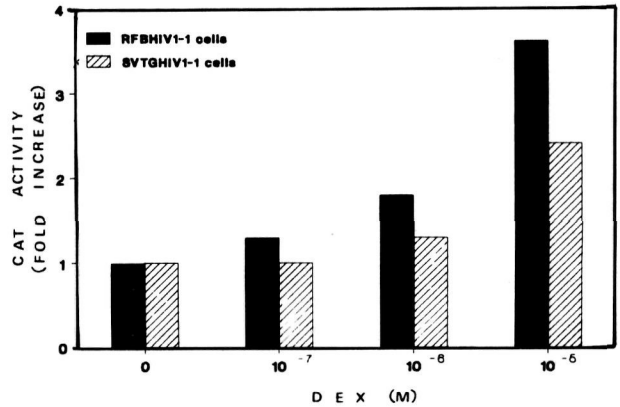


Figure 8. Induction of CAT activity by DEX. CAT values were obtained as described in Figure 3.

should be considered prior to their possible clinical use (12).

In the present study we have investigated the effect of growth factors, i.e. insulin and EGF, and hormones, i.e. HDC and DEX, on the transcriptional activity of HIV LTR. We found that the response was cell-type dependent. Although in rat fibroblasts the HIV LTR responded strongly

to all the above factors, in human fibroblasts the LTR responded only to DEX.

The mechanism of action of these various factors on the HIV LTR is yet unknown. However, it may be postulated that they operate through the modification of DNA binding proteins which interact with the HIV LTR.

References

- 1 Barre-Sinoussi F, Chermann J, Rey R, Nugey M, Chamaret S, Gruest J, Dautet C, Axler-Blin C, Vezinet-Brun F, Rouzioux C, Rosenbaum W and Montagnier L: Isolation of a T-lymphotropic retrovirus from a patient at risk for acquired immunodeficiency syndrome. *Science* 220: 868-871, 1983.
- 2 Gallo R, Salahuddin S, Papovic M, Shearer G, Kaplan M, Haynes D, Falker T, Redfield R, Oleske J, Safai B, White G, Fester P and Markham T: Frequent detection and isolation of cytopathic retroviruses (HTLV-III) from patients with AIDS and at risk for AIDS. *Science* 224: 500-503, 1984.
- 3 Fauci AS: The human immunodeficiency virus: Infectivity and mechanisms of pathogenesis. *Science* 239: 617-622, 1988.
- 4 Siekevitz M, Josephs SF, Dukovich M, Peffer N, Wong-Staal F and Greene WC: Activation of the HIV-1. *Science* 238: 1575-1578, 1987.
- 5 Bohnlein E, Lowenthal JW, Siekevitz M, Ballard DW, Franza BR and Greene WC: The same inducible nuclear proteins regulates activation of both the interleukin-2 receptor-alpha gene and type 1 HIV. *Cell* 53: 827-836, 1988.
- 6 Kaufman JD, Valandra G, Roderiquez G, Bushar G, Giri C and Norcross MA: Phorbol ester enhances human immunodeficiency virus-promoted gene expression and acts on a repeated 10-base-pair functional enhancer element. *Mol Cell Biol* 7: 3759-3766, 1987.
- 7 Nabel G and Baltimore D: An inducible transcription factor activates expression of human immunodeficiency virus in T cells. *Nature* 326: 711-713, 1987.
- 8 Harada S, Koyanagi Y, Nakashima H, Kobayashi N and Yamamoto N: Tumor promoter, TPA, enhances replication of HTLV-III/LAV. *Virology* 154: 249-258, 1986.
- 9 Tong-Starksen SE, Luciw PA and Peterlin BM: Human immunodeficiency virus long terminal repeat responds to T-cell activation signals. *Proc Natl Acad Sci USA* 84: 6845-6849, 1987.
- 10 Rosen CA, Sodroski JG and Haseltine WA: The location of cis-acting regulatory sequences in the human T cell lymphotropic virus type III (HTLV-III/LV) long terminal repeat. *Cell* 41: 813-823, 1985.
- 11 Spandidos DA, Yiagnisis M, Pintzas A: Human immunodeficiency virus long terminal repeat responds to transformation by the mutant T24 H-ras I oncogene and it contains multiple AP-1 binding TPA-inducible consensus sequence elements. *Anticancer Res* 9: 383-386, 1989.
- 12 Marham PD, Salahuddin SZ, Veren K, Orndorff S and Gallo RC: Hydrocortisone and some other hormones enhance the expression of HTLV-III. *Int J Cancer* 37: 67-72, 1986.
- 13 Bergel J, Hauber R, Geiger R and Cullen BR: Secreted placental alkaline phosphatase: a powerful new quantitative indicator of gene expression in eucaryotic cells. *Gene* 66: 1-10, 1988.
- 14 Spandidos DA, Zoumpourlis V, Kotsinas A, Maurer HR and Patsilinnacos P: Transcriptional activation of the human immunodeficiency virus long terminal repeat sequences by cis-platin. *Genetic Anal Techn Appl*: in press.
- 15 Graham FL and van der Eb AJ: A new technique for the assay of infectivity of human adenovirus 5 DNA. *Virology* 52: 456-461, 1973.
- 16 Spandidos DA and Wilkie NM: Expression of exogenous DNA in mammalian cells. In: *In vitro* transcription and translation- a practical approach (Hames B.D. and Higgins, S. J. eds) Oxford, IRL Press, pp1-48, 1984.
- 17 Spandidos DA and Riggio M: Promoter and enhancer like activity at the 5' - end of normal and T24 Ha-ras1 genes. *FEBS Lett* 203: 169-174, 1986.
- 18 Muesing MA, Smith DH and Capon DJ: Regulation of mRNA accumulation by a human immunodeficiency virus trans-activator protein. *Cell* 48: 691-701, 1987.
- 19 Jones KA, Kadonaga JJ, Luciw PA and Tjian R: Activation of AIDS retrovirus promoter by the cellular transcription factor Sp1. *Science* 232: 775-758, 1986.
- 20 Arya SK, Wong-Staal F and Gallo RC: Dexamethasone - mediated inhibition of human T cell growth factor and  $\gamma$ -interferon messenger RNA. *J Immunol* 133: 273-276, 1981.
- 21 Belanger L, Frain , Gingras MC, Bartkowiak J and Sala-Trepas JM: Glucocorticosteroid suppression of  $\alpha$ -fetoprotein synthesis in developing rat liver. Evidence for selective gene repression at the transcriptional level *Biochemistry* 20: 6665-6672, 1981.
- 22 Firzlf JM and Diggelman H: Dexamethasone increases the number of RNA polymerase II molecular transcribing integrated mouse mammary tumor virus DNA and flanking mouse sequences. *Mol Cell Biol* 4: 1057-106, 1984.
- 23 Grossman CJ: Interactions between the gonadal steroids and the immune system. *Science* 227: 257-261, 1985.
- 24 Hagar LJ and Palmer RD: Transcriptional regulation of mouse liver metallothionein-I gene by glucocorticoids. *Nature* 291: 340-342, 1981.

Received July 20, 1990  
Accepted August 3, 1990