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Differentiation-Dependent Human Immunodeficiency Virus Long Terminal Repeat Regulatory Elements Active in Human Teratocarcinoma Cells

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We have examined the transcriptional utilization of the human immunodeficiency virus type 1 (HIV-1) long terminal repeat (LTR) under differentiating conditions by using the embryonal carcinoma cell line NTERA-2. NTERA-2 cells undergo two distinct pathways of terminal differentiation, to a neuronal phenotype in response to retinoic acid and to a nonneuronal phenotype in response to hexamethylene bisacetamide. To identify LTR regulatory elements active in each cell type we used a set of HIV LTR linker substitution mutants, which contain mutations that progressively replace adjacent 18-bp segments across the U3 region and into the R region (between nucleotides −453 and +15 relative to the transcription start site). Although each differentiating cell type showed utilization of expected key elements (e.g., NF-κB, SP1, TATA) in the 3′ portion of the LTR (+1 to −112), the data indicated differentiation-dependent differences in the utilization of these elements. In addition, regions showing dramatic differentiation-dependent effects were detected in the 5′ portion of the LTR (−112 to −453), in positions where transcription control elements have not been described previously. The marked differences in the sets of LTR regulatory elements required by each cell type indicate that the LTR can function under a variety of differentiation conditions. Together with previous findings, the data suggest that the complexity of the HIV LTR for transcriptional control is much greater than was previously thought and that the LTR maintains elements which facilitate transcription in many cell types.

Human immunodeficiency virus (HIV) infects many cell types in addition to lymphocytes and macrophages. Infection of a variety of cell types may contribute to the diverse manifestations of HIV disease. In addition, it may mediate important aspects of HIV pathogenesis such as the initial infection that follows inoculation at a mucous membrane and the distinctive features that characterize infection of the developing fetus. HIV replication and gene expression depend upon regulatory sequences present in the HIV long terminal repeat (LTR). Although LTR sequences active in lymphoid cells have been well characterized (see reference 25 and references therein), those active in other cells have not. To examine the possibility that nonlymphoid cells use an alternate complement of regulatory sequences, we used a set of HIV LTR linker substitution mutants to identify LTR regulatory elements active in differentiating human embryonal carcinoma cells.

The embryonal carcinoma line NTERA-2 undergoes two distinct pathways of terminal differentiation. Retinoic acid (RA) induces extensive, and essentially complete, differentiation into a range of cell types (RA–NTERA-2), including cells resembling neurons (1, 2). Differentiation is accompanied by the expression of new marker antigens and various *HOX* genes (6, 21). Hexamethylene bisacetamide (HMBA) also induces differentiation of NTERA-2 cells. The HMBA-differentiated cells (HMBA–NTERA-2) are distinct from RA–NTERA-2; they do not include neuronlike cells, and they express neither the surface antigens nor the *HOX* genes induced by RA (3). Undifferentiated NTERA-2 cells do not support HIV replication, and the expression of HIV LTR reporter gene constructions is very low. In contrast, both RA– and HMBA–NTERA-2 support HIV growth and, when transfected with LTR reporter gene plasmids, show easily measurable levels of expression (9, 14).

Using the two differentiated NTERA-2 cell types, we have found that in addition to the requirement for known transcription control elements there are regions in the LTR which clearly show differentiation-dependent effects on transcriptional activity. These map not only to the 3′ portion of the LTR, where many transcription elements are known, but also to regions of the LTR which were previously thought to have little or no transcriptional function. Together with our previous data (25, 26), the present findings indicate that the structure of the HIV LTR, as it relates to transcription, is far more complex than was previously thought.

MATERIALS AND METHODS

Linker substitution mutants of the HIV LTR. The construction of the linker substitution mutants has been previously described (25). The mutants contain mutations that extend from the 5′ end of the LTR (−453 with respect to the transcription start site) to +15 beyond the start site. Each replaces 18 bp of wild-type (WT) sequence with an *Nde*I-*Xho*I-*Sal*I (NXS) polylinker (CATATGCTCGAGGTC GAC). The mutants are named *n*/*m* NXS, where *n* is the LTR nucleotide replaced by the 5′-end nucleotide of the polylinker and *m* is the nucleotide replaced by the 3′-end nucleotide of the polylinker (all numbering is with respect to the start site, +1).

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Cells and differentiation. NTERA-2 cl.D1 cells were seeded at 10^6 per 75-cm^2 flask in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal calf serum and either 10^{-5} M all-*trans*-RA or 3 mM HMBA (2, 3, 6). After growth at 37°C for 7 days, the cells were harvested with trypsin and reseeded at 10^6 per 3.5-cm tissue culture dish in DMEM–10% fetal calf serum, but without RA or HMBA. After incubation overnight, these cultures were transfected as described below.

Transfection and CAT analysis. Differentiated NTERA-2 cells were transfected with 1 μg of twice-banded CsCl gradient-purified wild-type (WT) or mutant LTR-chloramphenicol acetyltransferase (CAT) plasmid DNA and 10 μl of Lipofectin (Bethesda Research Laboratories), in 1 ml of serum-free DMEM. After 7 h, DMEM containing 10% fetal calf serum was added and the incubations were continued for an additional 48 h. To prepare the cells for the CAT assay we washed them twice in phosphate-buffered saline, lysed them with three freeze-thaw cycles, and assayed them for CAT activity by using the diffusion assay and scintillation counting (16). In each transfection experiment, samples were tested in triplicate. In all, three separate transfection experiments were done; each gave results similar to the ones reported in the text. The results and ranges are expressed relative to the activity of the WT LTR-CAT construction.

Factor-binding studies. Nuclear extracts were prepared as previously described (25) by using 10^9 NTERA-2 cells differentiated as described above. Labeled DNA fragments used as probes consisted of 113-bp fragments between −267 and −154 which were prepared by polymerase chain reaction (25) with the WT LTR plasmid and with mutant −255/−238 NXS or −219/−202 NXS as template (8). Two different binding and electrophoresis conditions were used (Tris-acetate and Tris-glycine; see specific conditions below). For each binding reaction 10^4 cpm of labeled fragment was incubated for 15 min on ice with 1 μg of nuclear extract protein in the appropriate binding buffer plus 200 ng of poly(dA-dT)-poly(dA-dT) as a nonspecific competitor. The binding reaction was analyzed by electrophoresis on a 4% polyacrylamide gel in the appropriate running buffer at 35 mA. Gels were dried and autoradiographed.

For the Tris-acetate system we used binding buffer (12% glycerol, 12 mM *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid [HEPES; pH 7.9], 60 mM KCl, 5 mM $MgCl_2$, 0.6 mM EDTA, 0.6 mM dithiothreitol) and running buffer (13.4 mM Tris [pH 7.8], 6.6 mM sodium acetate, 2 mM EDTA). For the Tris-glycine system we used binding buffer (10 mM Tris [pH 7.5], 50 mM NaCl, 1 mM EDTA, 5% glycerol, 1 mM dithiothreitol) and running buffer (50 mM Tris [pH 8.5], 380 mM glycine, 2 mM EDTA).

RESULTS AND DISCUSSION

To determine which HIV LTR regulatory sites were active in RA–NTERA-2 and HMBA–NTERA-2, we transfected the cells with a WT LTR-CAT plasmid (Fig. 1) and with a set of 26 linker substitution (LS) mutant LTR-CAT plasmids (Fig. 1) (25). The mutant LTR-CAT plasmids are named *n*/*m* NXS, where *n* and *m* are as defined in Materials and Methods. Figure 1 shows the exact locations of the NXS LS mutants with respect to known and suspected transcription elements.

After transfection as described in Materials and Methods, cells were assayed for CAT activity (Fig. 2; Table 1) and the activity of each mutant was normalized to that of the WT. Figure 2 shows a histogram analysis of the data comparing the activities in RA– and HMBA–NTERA-2 cells. The results are the average of triplicate transfections (two separate transfection experiments gave essentially identical results). In Fig. 1 the results are displayed graphically and compared with results previously obtained by using the same mutants in other cells (25, 26).

The data show that RA– and HMBA–NTERA-2 cells each use regulatory elements in the 3′ half of the LTR (−111 to +15) that are known to mediate expression in lymphocytes and fibroblasts (Fig. 1) (8, 11–13, 15, 18, 25, 26). However, the differentiated NTERA-2 cells also use potent regulatory elements within the 5′ half of the LTR that have not been previously described and do not function in lymphocytes and fibroblasts. Some of these regulatory elements exhibit markedly different functions in RA– and HMBA–NTERA-2 cells.

Known transcriptional control elements in the 3′ portion of the LTR show differentiation-dependent utilization. The best-characterized HIV LTR regulatory sequences lie in the region 3′ to −111 and include the two NF-κB, three Spl, TATA, and TAR elements, as well as a putative initiator element and binding sites for LBP-1/UBP-1 (8, 11–13, 15, 18, 24). Some mutations in this region had predictable effects that were essentially equivalent in the two cell types. For example, −39/−22 NXS, which eliminates TATA, dramatically decreased the activity in both cell types, and −57/−40 NXS, which affects the 3′-most Spl site, produced a moderate decrease in both types. Mutant −93/−76 NXS, which affects the 3′ NF-κB site, also severely decreased expression in both types. However, the data also suggested differentiation-dependent utilization of some of the well-characterized 3′-end regulatory sequences. Mutant −75/−58 NXS alters the two 5′-proximal Spl sites; it produced a more substantial decrease in expression in RA–NTERA-2 than in HMBA–NTERA-2. The mutant that destroys the 5′ NF-κB site, −111/−94 NXS, showed even more discordant effects in the two cell types. It decreased expression to 46% of WT levels in HMBA–NTERA-2, but slightly increased activity in RA–NTERA-2. This discordant behavior shown by the two NF-κB sites was previously noted in Jurkat-*tat* cells (Fig. 1) (25). Therefore, although the Spl and NF-κB elements make up an important part of the regulatory sequences of the LTR, their precise function changes in a cell type-specific fashion.

Significant differentiation-dependent elements map to the 5′ portion of the LTR. Mutations 5′ to −111 identified additional potent regulatory sites which are not used in lymphocytic cells. The activities of some of these sites varied markedly in a differentiation-dependent fashion. In Jurkat cells, mutations from −183 to −130 tended to reduce activity (Fig. 1) (25). Whereas one mutation in this region, −147/−130 NXS, decreased expression to 38% of WT levels in HMBA–NTERA-2, it produced slightly greater than WT activity in RA-NTERA-2.

Further 5′, mutants with mutations from −273 to −202 identified a zone of positive-control elements unique to differentiated NTERA-2 cells. In lymphocytic and fibroblastic cells these mutants showed close to WT activity (Fig. 1) (25, 26). Mutant −219/−202 NXS was particularly interesting, showing differentiation differences in the two cell types. The mutation decreased expression to 10% of WT levels in RA–NTERA-2 cells, but had no effect on activity in HMBA–NTERA-2 cells. Mutant −255/−238 NXS produced substantial decreases in levels of expression in both cell types. Mutant −273/−256 NXS decreased activity moderately in RA–NTERA-2, but substantially in HMBA–NTERA-2.

The linker-substitution mutants with mutations at the extreme 5′ end of the LTR, from −435 to −382, revealed

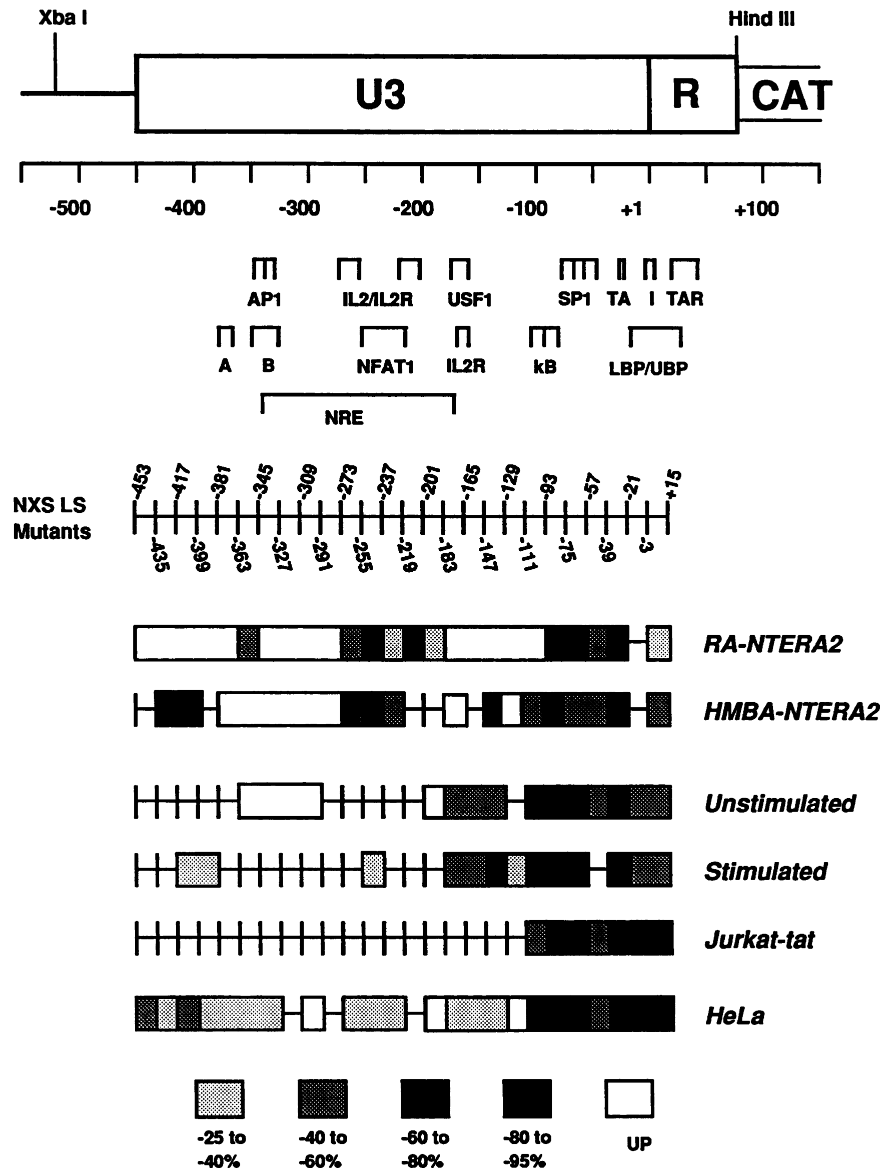

FIG. 1. Map of the HIV-1 LTR in the wild-type reporter plasmid pXLTR-CAT. The LTR insert is bracketed by the *Xba*I and *Hin*dIII sites used to construct the linker-scanning mutants (25) and is flanked at the 3′ end by the bacterial CAT gene. Under the map are a nucleotide scale (the transcription start site is +1) and a diagram indicating the locations of some of the characterized and potential regulatory sites noted in the LTR (4, 7, 8, 10–15, 17–20, 22, 24). Below the figure are shown the locations of the NXS linker-substitution mutations; the nucleotide number of the 5′ end of each mutant is indicated. The bottom part of the figure schematically diagrams the effects of the linker-substitution mutants in RA-treated and HMBA-treated NTERA-2 cells (see Fig. 2 and Table 1 for details) and compares the results obtained with NTERA-2 cells with those previously described for unstimulated, TPA-PHA-stimulated, and *tat*-expressing Jurkat cells as well as HeLa cells (25, 26). The mutations which reduced activity relative to the WT by 25 to 40%, 40 to 60%, 60 to 80%, and greater than 80% are indicated. In addition, mutations that caused a modest increase in activity relative to WT are noted (UP).

another prominent zone of previously undescribed regulatory sites. Again there were marked differentiation-dependent differences in the effects of these mutations between the two cell types. Mutants with mutations in this region all caused moderately increased CAT activity in RA–NTERA-2; however, in HMBA–NTERA-2 cells mutants −435/−418 NXS and −417/−400 NXS both caused substantially decreased activity. In contrast, mutant −399/−382 NXS, which caused the largest increase in activity in RA–NTERA-2 cells, caused essentially WT activity in HMBA–NTERA-2 cells.

Factor-binding studies. To determine whether LTR DNA-binding activities correlated with some of the newly found regulatory activities, we performed electrophoretic mobility shift assays (EMSAs) with RA– and HMBA–NTERA-2 nuclear extracts. We chose to examine the region containing mutations −255/−238 NXS and −219/−202 NXS for two reasons: (i) these mutations have significant effects in the RA– and HMBA–NTERA-2 cells but show little or no effect in lymphoid cells (Fig. 1 and 2), and (ii) although mutant −255/−238 NXS indicated an element with common functions in both cell types, mutant −219/−202 NXS showed a

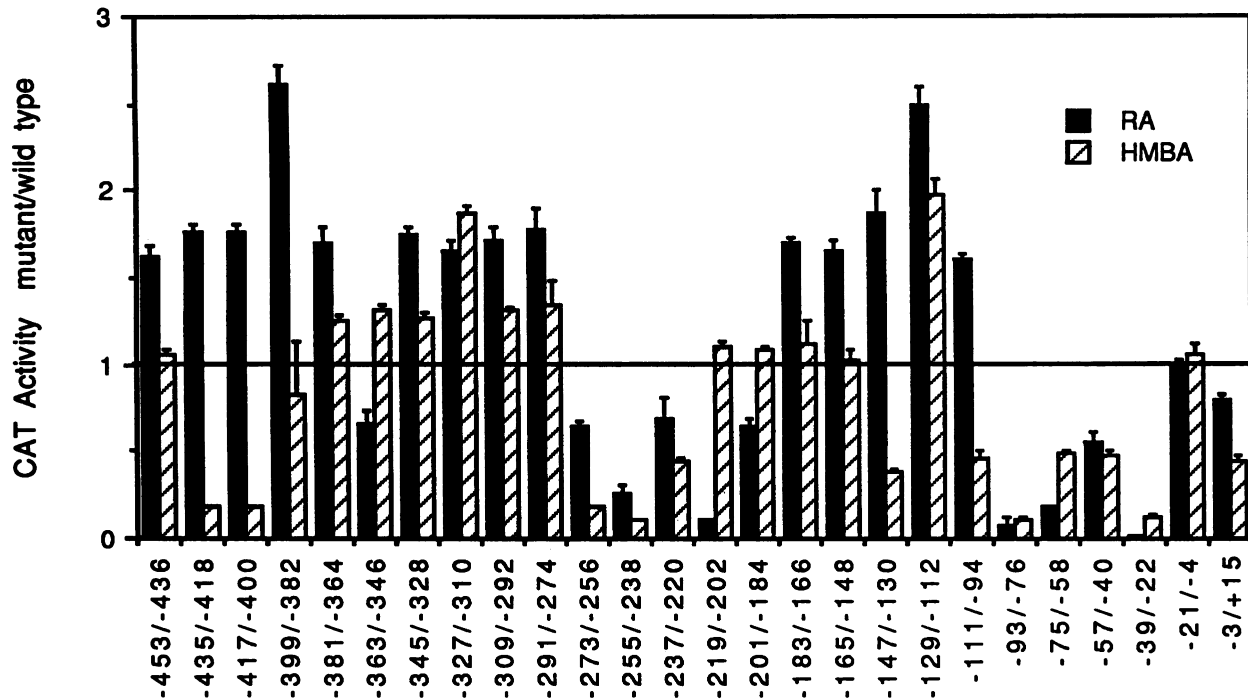

FIG. 2. Transient-transfection analysis of the linker-substitution mutants in RA- and HMBA-treated NTERA-2 cells. Wild-type pXLTR-CAT plasmid or NXS mutants were transfected into RA–NTERA-2 (solid bars) or HMBA–NTERA-2 (hatched bars) and assayed for CAT activity. The results are expressed relative to the activity of the WT plasmid and are the average of triplicate transfections; the error bars indicate the range of values. Two other experiments gave similar results.

marked differentiation-dependent difference, causing no effect in HMBA–NTERA-2 cells and a dramatic decrease in activity in RA–NTERA-2 cells.

EMSAs were performed by using two systems, a Tris-acetate low-ionic-strength system and a Tris-glycine high-ionic-strength system (5, 23). Labeled 113-bp probe fragments representing the nucleotides between −267 and −154 were prepared by the polymerase chain reaction with the WT LTR and with mutant −255/−238 NXS or −219/−202 NXS as template.

A WT probe and RA–NTERA-2 nuclear extracts yielded three prominent bands (Fig. 3A, lane 4) with the Tris-acetate system. The two slower-migrating bands represented specific binding events as judged by competition studies (data not shown). When the −255/−238 NXS mutant probe was used (Fig. 3A, lane 5), one of these bands (band 2) disappeared and a new, more highly shifted band (band 1) appeared. Band 1 was shown to be specific by competition analyses (data not shown). When HMBA–NTERA-2 extracts were used (lanes 7 to 9), the WT probe (lane 7) showed one specific binding event which comigrated with band 2 detected with RA–NTERA-2 extracts. As with the RA–NTERA-2 extracts, the −255/−238 NXS mutation eliminated band 2 when HMBA–NTERA-2 extracts were used (lane 8). Overall, the data suggest that band 2 represents a significant binding activity in the region from −255 to −238, which correlates with activation of HIV LTR transcription in both differentiated cell types.

As predicted from the above interpretation, the differentiation-dependent mutant probe, −219/−202 NXS, maintained normal band 2 formation with extracts from both cell types (Fig. 3A, lanes 6 and 9). For RA–NTERA-2 extracts, in which the −219/−202 mutation had a dramatic effect, the probe also showed a faint, more highly shifted band (lane 6) comigrating with band 1. Whether this band is the same as band 1 in lane 5 is unknown. It is also unknown whether this new band represents a binding event which causes the loss of transcriptional activity seen with the −219/−202 NXS mutation in RA–NTERA-2 cells.

The results with the −219/−202 NXS mutation did not show a definitive binding activity, which could account for the differentiation-specific loss of LTR transcriptional activity in RA–NTERA-2 cells. Therefore, additional EMSAs were performed by using the Tris-glycine system (Fig. 3B; see Materials and Methods). Under these conditions, a band seen with the WT probe clearly disappeared with the −219/−202 NXS probe (Fig. 3B, lane 6, band 1) and a new band appeared (band 2). In addition, the −255/−238 NXS mutation resulted in an apparent additional band migrating like band 2 (Fig. 3B, lane 5). It is intriguing that band 2 of Fig. 3B and band 1 of Fig. 3A appear with both mutations in each cell type; however, it is unknown whether they represent similar binding events.

Overall, the data from the two systems indicate that both the −255/−238 NXS and −219/−202 NXS mutations can affect specific binding activities which correlate with the associated loss of CAT activity in HMBA- and RA–NTERA-2. EMSAs were also performed to examine binding in the region from −435 to −400. Binding was observed; however, under the EMSA conditions tested thus far, the NXS mutations in this region had no definitive effect on the observable binding events (data not shown).

Conclusions. Transfections of the NXS LS mutants into differentiated NTERA-2 cells show that the HIV LTR contains several potent regulatory elements from −130 to −435 upstream of the start site, which are as important for LTR

TABLE 1. Relative CAT activities of LTR linker-scanning mutants after transfection into RA- or HMBA-treated NTERA-2 cells[a]

Mutant	Known or putative site in mutated region[b]	Relative CAT activity (mutant/wild type) (mean ± range)	
		RA-NTERA-2	HMBA-NTERA-2
−453/−436 NXS		1.63 ± 0.06	1.05 ± 0.03
−435/−418 NXS		1.76 ± 0.05	0.18 ± 0.01
−417/−400 NXS		1.76 ± 0.05	0.18 ± 0.18
−399/−382 NXS		2.62 ± 0.10	0.83 ± 0.30
−381/−364 NXS	Site A	1.70 ± 0.09	1.26 ± 0.03
−363/−346 NXS	AP-1	0.66 ± 0.07	1.31 ± 0.04
−345/−328 NXS	AP-1, site B, NRE	1.74 ± 0.05	1.27 ± 0.03
−327/−310 NXS	NRE	1.66 ± 0.06	1.87 ± 0.04
−309/−292 NXS	NRE	1.71 ± 0.08	1.31 ± 0.02
−291/−274 NXS	NRE, NFAT-1	1.77 ± 0.13	1.35 ± 0.14
−273/−256 NXS	NRE, NFAT-1, IL-2	0.64 ± 0.04	0.18 ± 0.01
−255/−238 NXS	NRE, IL-2	0.26 ± 0.04	0.10 ± 0.01
−237/−220 NXS	NRE, IL-2	0.69 ± 0.12	0.45 ± 0.01
−219/−202 NXS	NRE	0.10 ± 0.01	1.10 ± 0.04
−201/−184 NXS	NRE	0.65 ± 0.04	1.09 ± 0.01
−183/−166 NXS	NRE, IL-2Rα, negative regulatory factor, USF-1	1.70 ± 0.03	1.12 ± 0.13
−165/−148 NXS		1.66 ± 0.06	1.03 ± 0.06
−147/−130 NXS		1.86 ± 0.14	0.38 ± 0.02
−129/−112 NXS		2.50 ± 0.10	1.97 ± 0.09
−111/−94 NXS	NF-κB, HIVEN86A, EBP-1	1.60 ± 0.04	0.46 ± 0.04
−93/−76 NXS	NF-κB, HIVEN86A, EBP-1 (Sp1, first two bases)	0.08 ± 0.04	0.11 ± 0.01
−75/−58 NXS	Sp1	0.18 ± 0.01	0.49 ± 0.01
−57/−40 NXS	Sp1	0.55 ± 0.06	0.47 ± 0.03
−39/−22 NXS	TATA	0.01 ± 0.00	0.13 ± 0.01
−21/−4 NXS	LBP-1, UBP-1	1.00 ± 0.02	1.06 ± 0.05
−3/+15 NXS	LBP-1, UBP-1, (initiator)	0.79 ± 0.04	0.45 ± 0.03

[a] The data presented here are shown graphically in Fig. 1 and 2.
[b] References 4, 7, 8, 10–15, 17–20, 22, and 24.

activity in these cells as are the well-characterized elements such as NF-κB and Sp1. These 5′ elements are generally in regions which have not previously been associated with transcriptional activity in lymphocytic and fibroblastic cells. The activity of some of these regulatory sequences depends on differentiation since sequences that act as positive regulatory elements in one differentiated cell type may have no detectable activity in the other or may exhibit modest negative activity. Additionally, the data suggest differentiation-dependent utilization of some of the well-characterized 3′-end regulatory sequences. The discordant utilization of the NF-κB sites is of particular interest since a similar effect was noted in Jurkat cells which constitutively express *tat* (Fig. 1) (25).

Figure 1 shows a summary of activity profiles of the LTR as defined by the NXS LS mutations in a variety of cell types and under several growth conditions. These data are derived from the present and previous studies (25, 26). Besides the RA– and HMBA–NTERA-2 cells, the summary includes unstimulated, stimulated, and *tat*-expressing Jurkat cells (25) and HeLa cells (26). The extended regions which affect LTR activity, as well as the dramatic differences in effects of mutants between cells and conditions, clearly indicate that transcriptional control mediated by the HIV LTR is a more

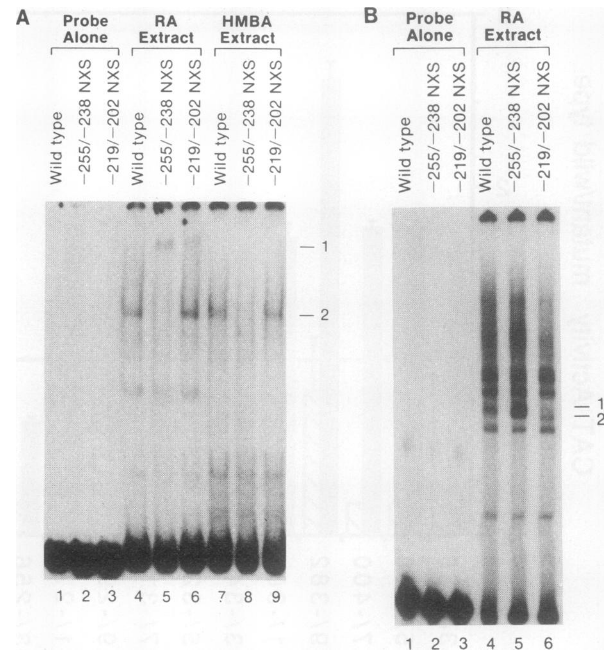

FIG. 3. DNA fragment mobility shift assays. A 113-bp fragment (−267 to −154) was prepared by polymerase chain reaction with WT, −255/−238 NXS, or −219/−202 NXS plasmids as template and labeled as described previously (25). For each reaction 1 μg of nuclear extract protein was incubated with 10^4 cpm of labeled fragment and 200 ng of poly(dA-dT)-poly(dA-dT) and then run in either a Tris-acetate buffer system (A) (5) or a high-ionic-strength Tris-glycine buffer system (B) (23). Two bands affected by the use of mutant probes in each panel are labeled 1 and 2. All affected bands resulted from specific binding events as judged by competition experiments (data not shown).

complex than was previously suspected. The LTR appears to include elements that can mediate transcription in many cell types and under a variety of growth and differentiation conditions. Therefore the ability of the virus to replicate in cells other than lymphocytes and monocytes may be important for virus survival and transmission. The existence of HIV LTR elements which affect transcription in cells other than lymphocytes suggests that the LTR may contribute to HIV tropism.

As is true for previously described LTR regulatory sequences active in lymphocytic cells, it is likely that factors which bind and mediate the activity of the differentiation-dependent elements also function during differentiation of normal, uninfected cells. In addition, the existence of differentiation-responsive LTR elements may help to explain some of the distinctive features observed when HIV infects a developing fetus.

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REFERENCES

1. **Andrews, P. W.** 1988. Human teratocarcinomas. Biochim. Biolphys. Acta **948:**2436–2444.
2. **Andrews, P. W.** 1984. Retinoic acid induces neuronal differentiation of a cloned human embryonal carcinoma cell line in vitro. Dev. Biol. **103:**285–293.
3. **Andrews, P. W., E. Nudelman, S. Hakomori, and B. A. Fenderson.** 1990. Different patterns of glycolipid antigens are expressed following differentiation of TERA-2 human embryonal carcinoma cells induced by retinoic acid, hexamethylene bisacetamide (HMBA) or bromodeoxyuridine (BUdR). Differentiation **43:**131–138.
4. **Bohnlein, E., J. W. Lowenthal, M. Siekevitz, D. W. Ballard, B. R. Franza, Jr., and W. C. Greene.** 1988. Same inducible nuclear proteins regulates mitogen activation of both the interleukin-2 receptor-alpha gene and type 1 HIV. Cell **53:**827–836.
5. **Carthew, R. W., L. A. Chodosh, and P. A. Sharp.** 1985. RNA polymerase II transcription factor binds to an upstream element in the adenovirus major late promoter. Cell **43:**439–448.
6. **Fenderson, B. A., P. A. Andrews, E. Nudelman, H. Clausen, and S.-I. Hakomori.** 1987. Glycolipid core structure switching from globo- to lacto- and ganglio- series during retinoic acid-induced differentiation of TERA-2-derived human embryonal carcinoma cells. Dev. Biol. **122:**21–34.
7. **Franza, B. R., Jr., F. J. Rauscher III, S. F. Josephs, and T. Curran.** 1988. Fos complex and fos-related antigens recognize sequence elements that contain AP-1 binding sites. Science **239:**1150–1153.
8. **Garcia, J. A., F. K. Wu, R. Mitsuyasu, and R. B. Gaynor.** 1987. Interactions of cellular proteins involved in the transcriptional regulation of the human immunodeficiency virus. EMBO J. **6:**3761–3770.
9. **Hirka, G., K. Prakash, H. Kawashima, S. A. Plotkin, P. W. Andrews, and E. Gonczol.** 1991. Differentiation of human embryonal carcinoma cells induces human immunodeficiency virus permissiveness which is stimulated by human cytomegalovirus coinfection. J. Virol. **65:**2732–2735.
10. **Jakobovits, A., D. H. Smith, E. B. Jakobovits, and D. J. Capon.** 1988. Discrete element 3′ of human immunodeficiency virus 1 (HIV-1) and HIV-2 mRNA initiation sites mediates transcription activation by an HIV *trans*-activator. Mol. Cell. Biol. **8:**2555–2561.
11. **Jones, K. A., J. T. Kadonaga, P. A. Luciw, and R. Tjian.** 1986. Activation of the AIDS retro virus promoter by the cellular transcription factor, Spl. Science **232:**755–759.
12. **Jones, K. A., P. A. Luciw, and N. Dychange.** 1988. Structural arrangements of transcription control domains within the 5′ untranslated leader regions of the HIV-1 and HIV-2 promoters. Genes Dev. **2:**1104–1114.
13. **Kaufman, J. D., G. Valandra, G. Roderiquez, G. Busher, C. Giri, and M. A. Norcross.** 1987. 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.
14. **Maio, J., and P. L. Brown.** 1988. Regulation of expression driven by human immunodeficiency virus type 1 and human T-cell leukemia virus type I long terminal repeats in pluripotential human embryonic cells. J. Virol. **62:**1398–1407.
15. **Nabel, G. S., and D. Baltimore.** 1987. An inducible transcription factor activates expression of human immunodeficiency virus in T cells. Nature (London) **326:**711–713.
16. **Neumann, J. R., C. A. Morency, and K. Russian.** 1987. A novel rapid assay for CAT gene expression. BioTechniques **5:**444–447.
17. **Orchard, K., N. Perkin, C. Chapman, J. Harris, V. Emery, G. Goodwin, D. Latchman, and M. Collins.** 1990. Novel T-cell protein which recognizes a palindromic sequence in the negative regulatory element of the human immunodeficiency virus long terminal repeat. J. Virol. **64:**3234–3239.
18. **Rosen, C. A., J. G. Sodroski, and W. A. Haseltine.** 1985. Location of cis-acting regulatory sequences in the human T cell lymphotropic virus type III (HTLV III) long terminal repeat. Cell **41:**813–823.
19. **Shaw, J. P., P. J. Utz, D. B. Durand, J. J. Toule, E. A. Emmel, and G. R. Crabtree.** 1988. Identification of a putative regulator of early T cell activation genes. Science **241:**202–203.
20. **Siekevitz, M., S. F. Josephs, M. Dukovich, N. Peffer, F. Wong-Staal, and W. C. Greene.** 1987. Activation of the HIV-1 LTR by T cell mitogens and the trans-activator protein of HTLV-I. Science **238:**1575–1578.
21. **Simeone, A., D. Acampora, L. Arcioni, P. W. Andrews, E. Bonicinelli, and F. Mavilio.** 1990. Sequential activation of HOX2 homeobox genes by retinoic acid in human embryonal carcinoma cells. Nature (London) **346:**763–766.
22. **Smith, M. R., and W. C. Greene.** 1989. The same 50-kDa cellular protein binds to the negative regulatory elements of the interleukin 2 receptor alpha-chain gene and the human immunodeficiency virus type 1 long terminal repeat. Proc. Natl. Acad. Sci. USA **86:**8526–8530.
23. **Staudt, L. M., H. Singh, R. Sen, T. Wirth, P. A. Sharp, and D. Baltimore.** 1986. Lymphoid-specific protein binding to the octamer motif of immunoglobulin genes. Nature (London) **323:**640–643.
24. **Wu, F. K., J. A. Garcia, D. Harrich, and R. B. Gaynor.** 1988. Purification of the human immunodeficiency virus type 1 enhancer and TAR binding proteins EBP-1 and UBP-1. EMBO J. **7:**2117–2129.
25. **Zeichner, S. L., J. Y. H. Kim, and J. C. Alwine.** 1991. Linker-scanning mutational analysis of the transcriptional activity of the human immunodeficiency virus type 1 long terminal repeat. J. Virol. **65:**2436–2444.
26. **Zeichner, S., J. Y. H. Kim, and J. C. Alwine.** 1991. Analysis of the human immunodeficiency virus long terminal repeat by in vitro transcription competition and linker scanning mutagenesis. Gene Expression **1:**15–27.