Conditional regulatory elements of human immunodeficiency virus type 2 long terminal repeat

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Mutational analysis of the human immunodeficiency virus type 2 (HIV-2) long terminal repeat (LTR) revealed a novel cis-acting positive and a negative regulatory element in the U3 region, located upstream of the enhancer–promoter region. These elements acted in a cell type-specific manner, being most active in human lymphocytic CEM cells, more active in Jurkat cells than in human monocytic U937 cells and least active in epithelioid HeLa cells. The down-modulatory effect of the negative regulatory element was abolished by HIV-2 Tat, suggesting the involvement of upstream DNA elements in optimal Tat-mediated trans-activation. The sequence elements that respond to T cell activation signals were also located in the upstream U3 region. Notably, the magnitude of the effect of the upstream regulatory elements depended on the basal activity of the LTR, which was also cell type-dependent. This emphasizes the importance of the cell-specific transcriptional factors and other effectors in regulating HIV gene expression. These observations may be relevant to the cell type-specific restriction of virus replication in vivo.

Introduction

Human immunodeficiency virus type 2 (HIV-2) differs from HIV-1 in several aspects of its structure and biological properties. HIV-1 is aetiologically associated with AIDS and is spread worldwide. HIV-2, which is more closely related to simian immunodeficiency virus than to HIV-1, is presently restricted in distribution to regions of West Africa (Guyader et al., 1987; Evans et al., 1988; Kong et al., 1988; Kumar et al., 1990). Although some isolates of HIV-2 are associated with AIDS, others may be far less pathogenic and HIV-2 as a group may be less pathogenic than HIV-1 (Dufoort et al., 1988; Marlink et al., 1988; Poulsen et al., 1989). A study of the regulation of the replication of this virus may contribute to the understanding of HIV pathogenesis. The replication capacity and virulence of HIV is governed, at least in part, by the coordinate expression of trans-acting regulatory genes and interaction of their gene products with cis-acting regulatory elements. HIV gene expression is in addition modulated by host factors, some of which affect HIV gene expression by interacting with the cis-acting regulatory elements.

Many of the regulatory elements of HIV are located in the long terminal repeat (LTR). Some of these cis-acting elements (such as promoters and enhancers) are common to other genes but others are unique to HIV. Among the latter is an element called TAR (tat response), located downstream of the transcriptional initiation site (Rosen et al., 1985; Arya et al., 1987; Hauber & Cullen, 1988). Important among the non-viral factors that influence HIV gene expression are the immune or T cell activation signals (Zagury et al., 1986; Fauci, 1988). The elements that respond to these signals are also located in the LTR, upstream of the transcriptional initiation site (Nabel & Baltimore, 1987; Tong-Starksen et al., 1987, 1990; Arya, 1990). Although both HIV-1 and HIV-2 contain similar elements, they differ in their details. For example, whereas the TAR element of HIV-1 consists of one stem–loop structure, the TAR element of HIV-2 is composed of at least two stem–loop structures (Arya, 1988; Arya & Gallo, 1988; Fenrick et al., 1989). Functionally, the HIV-1 TAR element is able to respond to HIV-1 and HIV-2 Tat equally well, however the HIV-2 TAR element responds to HIV-2 Tat somewhat more efficiently than to HIV-1 Tat (Emerman et al., 1987; Arya, 1988; Arya & Gallo, 1988; Fenrick et al., 1989). Furthermore, the T cell activation response element of HIV-1 is a single direct repeat of two subelements consisting of the NFκB sites; the response element of HIV-2 is more complex (Nabel & Baltimore, 1987; Arya, 1990; Markovitz et al., 1990). Substitution mutation analysis of the HIV-2 LTR has revealed it to be composed of at least four cis-acting subelements which act in synergy; the NFκB site found in both the HIV-1 and HIV-2 LTRs is only one of these (Markovitz et al., 1992; Leiden et al., 1992). We report here that the HIV-2 LTR contains other novel elements that function in a
cell type-dependent manner. These elements are located upstream of the enhancer–promoter region and their activity is modulated by HIV-2 Tat. We also present a deletion mutation analysis of the HIV-2 LTR delineating the T cell activation response elements.

Methods

Molecular clones. The wild-type LTR clone of HIV-2(ST) (clone SLTR) was subcloned from a full-length biologically active proviral clone pSXb-1 (JSB-27) (Kumar et al., 1990; Arya & Sadaie, 1993). It contained nucleotide −554 to +427, composed of U3, R, U5 and some sequences downstream of the U5 region. The wild-type LTR clone of HIV-2(ROD) was constructed from a mutant clone (RLTR). The mutant RLTR clone was subcloned from a biologically active HIV-2(ROD) proviral clone that had a deletion spanning nucleotides −184 to −556 at the 5’ end of U3 region, but was otherwise similar to the clone SLTR. The deletion in the RLTR clone was repaired with the contained nucleotide −554 to +427, composed of U3, R, U5 and some 2(ROD) proviral clone that had a deletion spanning nucleotides −184 to −556 at the 5’ end of U3 region, but was otherwise similar to the clone SLTR. The deletion in the RLTR clone was repaired with the appropriate fragment from an HIV-2(ROD) cDNA clone (Emerson et al., 1987). The mutant LTR clones were created by standard mutagenesis procedures which sometimes involved the use of synthetic DNAs or linkers and/or PCR amplification of specific regions. The authenticity of the clones was checked by determining the DNA sequence of the relevant regions. The LTRs were placed upstream of the chloramphenicol acetyltransferase (CAT) gene in the proper orientation. The HIV-2(ST) tat clone was obtained by cDNA cloning as previously described (Arya et al., 1985; Arya & Sethi, 1990; Arya, 1991), and recloned to place it downstream of the cytomegalovirus early gene promoter (pCM-TAT; Arya, 1993).

DNA-mediated transfection and CAT assays. Suspension culture cells (human lymphocytic CEM, Jurkat and monocytic U937 cells) were transfected by the DEAE-dextran protocol and monolayers of human epithelial HeLa cells were transfected by the calcium phosphate procedure as previously described (Arya et al., 1985; Arya, 1993). Between 8 x 10^6 and 10 x 10^6 lymphocytic or monocytic cells or about 1 x 10^6 HeLa cells were transfected with 4 μg of LTR-CAT DNA. About 44 to 48 h post-transfection the cells were processed to obtain 200 μl of cytoplasmic extract. Aliquots (10 to 100 μl) were incubated for various times and the induction of CAT gene expression was measured. To ensure that the assays measuring the CAT gene expression were in the linear range of CAT enzyme activity, it was often necessary to alter the amount of extract or time of incubation or both. This was particularly the case when different cell types and activation protocols were used.

Tat-mediated trans-activation and T cell activation. In order to evaluate the trans-activation of the HIV-2 LTR by HIV-2 Tat, the cells were cotransfected with the LTR-CAT DNA (4 μg) and tat DNA (pCM-TAT; 1 μg) as described above. For T cell activation the cells were transfected with the LTR-CAT DNA and 20 to 24 h later the culture was treated with phorbol 12-myristate 13-acetate (PMA; 10 ng/ml) for an additional 20 to 24 h.

Results

Basal expression and Tat-mediated trans-activation

The LTRs from two isolates of HIV-2 were used in this study. One, SLTR, was derived from the non-fusogenic and non-cytopathic HIV-2(ST) and the other, RLTR(R), was from the fusogenic and cytopathic HIV-2(ROD).

These two LTRs are about 90% identical at the DNA sequence level. Fig. 1 shows the sequence comparison of the U3 region of the two LTRs upstream of the transcriptional initiation site. There is a limited and scattered sequence divergence between the two LTRs consisting of nucleotide substitutions and a few additions or deletions. The R region (downstream of the transcriptional initiation site) of these LTRs is even less divergent. These two LTRs differed in their ability to direct LTR-dependent CAT gene expression when transfected into human T lymphocytic CEM cells. The results showed that the HIV-2(TAGOD) LTR was roughly twice as active as the HIV-2(ST) LTR (Fig. 2). However, in the presence of Tat provided in trans, the two LTRs produced nearly equivalent levels of CAT gene expression.

To delineate the regulatory elements located upstream of the transcriptional initiation site of the HIV-2(ST) and HIV-2(ROD) LTRs, mutation analysis of these LTRs...
Fig. 2. Basal expression and Tat-mediated trans-activation of the HIV-2(ST) and HIV-2(ROD) LTRs in human T lymphocytic CEM cells. The data represent the results of two to four or more independent transfections.

HIV-2 (ST) LTR

<table>
<thead>
<tr>
<th>SLTR</th>
<th>Relative gene expression</th>
<th>Basal</th>
<th>+TAT</th>
<th>Fold increase</th>
</tr>
</thead>
<tbody>
<tr>
<td>SLTR</td>
<td>1.0</td>
<td>3152 ± 883</td>
<td>3152 ± 883</td>
<td></td>
</tr>
<tr>
<td>SLTR4</td>
<td>2.2 ± 0.6</td>
<td>2900 ± 340</td>
<td>1367 ± 253</td>
<td></td>
</tr>
<tr>
<td>SLTR3</td>
<td>2.5 ± 1.0</td>
<td>4690 ± 1964</td>
<td>1082 ± 712</td>
<td></td>
</tr>
<tr>
<td>SLTR2</td>
<td>1.0 ± 0.5</td>
<td>2110 ± 198</td>
<td>3220 ± 600</td>
<td></td>
</tr>
<tr>
<td>SLTR1</td>
<td>6.4 ± 1.9</td>
<td>4818 ± 987</td>
<td>940 ± 414</td>
<td></td>
</tr>
<tr>
<td>SLTR0</td>
<td>0.3 ± 0.1</td>
<td>1712 ± 462</td>
<td>5362 ± 1651</td>
<td></td>
</tr>
<tr>
<td>SLTR(R1)</td>
<td>15.6 ± 2.0</td>
<td>5255 ± 1390</td>
<td>270 ± 22</td>
<td></td>
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</table>

HIV-2 (ROD) LTR

<table>
<thead>
<tr>
<th>RLTR(R)</th>
<th>Relative gene expression</th>
<th>Basal</th>
<th>+TAT</th>
<th>Fold increase</th>
</tr>
</thead>
<tbody>
<tr>
<td>RLTR4</td>
<td>2.2 ± 0.7</td>
<td>4426 ± 1013</td>
<td>2453 ± 660</td>
<td></td>
</tr>
<tr>
<td>RLTR3</td>
<td>3.2 ± 1.0</td>
<td>3186 ± 664</td>
<td>1670 ± 347</td>
<td></td>
</tr>
<tr>
<td>RLTR2</td>
<td>2.8 ± 0.6</td>
<td>2890 ± 270</td>
<td>1092 ± 337</td>
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<tr>
<td>RLTR1</td>
<td>15.1 ± 3.6</td>
<td>3203 ± 200</td>
<td>1310 ± 326</td>
<td></td>
</tr>
<tr>
<td>RLTR5</td>
<td>14.2 ± 4.7</td>
<td>6058 ± 1704</td>
<td>483 ± 86</td>
<td></td>
</tr>
<tr>
<td>RLTR6</td>
<td>0.80 ± 0.17</td>
<td>3003 ± 1346</td>
<td>3965 ± 1006</td>
<td></td>
</tr>
<tr>
<td>RLTR7</td>
<td>0.78 ± 0.10</td>
<td>402 ± 100</td>
<td>514 ± 60</td>
<td></td>
</tr>
<tr>
<td>RLTR8</td>
<td>1.15 ± 0.10</td>
<td>2408 ± 330</td>
<td>2126 ± 456</td>
<td></td>
</tr>
<tr>
<td>RLTR(S1)</td>
<td>8.1 ± 1.8</td>
<td>4004 ± 364</td>
<td>512 ± 70</td>
<td></td>
</tr>
<tr>
<td>RLTR(S)</td>
<td>1.4 ± 0.2</td>
<td>2960 ± 925</td>
<td>2732 ± 224</td>
<td></td>
</tr>
</tbody>
</table>

Fig. 3. Analysis of the negative regulatory element of the HIV-2 LTR located in the U3 region. WT, Wild-type.
was undertaken. The transfection of the wild-type and mutant clones into CEM cells showed that a deletion of more than one-half of the U3 region of the LTRs (nt -233 to -556) had a modest (two-fold or less) effect on their activity (Fig. 2). However, further deletion in this region [nt -183 to -556 for the HIV-2(ST) LTR (clone SLTR1) and nt -184 to -556 for the HIV-2(ROD) LTR (clone RLTR)] increased the basal level of activity six- to eightfold relative to the wild-type clones in CEM cells. Deletions further downstream abolished the enhancement of basal activity. For example, the basal level of activity of the mutant clone RLTR8 (deletion of nt -132 to -556) was not significantly higher than that of the wild-type clone. Similarly, the basal activity of the mutant clone RLTR6 (deletion of nt -142 to -147 in addition to the nt -181 upstream sequence) was not higher than that of the wild-type clone. The results with chimeric clones of the HIV-2(ST) and HIV-2(ROD) LTRs substantiated the specific sequence-dependent up-modulation of the LTR-directed gene expression. When the sequence segment between nucleotides -556 to -181 of the HIV-2(ST) LTR was substituted with the corresponding segment from the HIV-2(ROD) LTR [clone SLTR(R1)], the activity of the chimeric clone was about 15-fold higher compared with the parental wild-type clone (Fig. 2). The reciprocal chimeric clone in which a similar segment of the HIV-2(ROD) LTR was exchanged with that of the HIV-2(ST) clone was about eightfold more active than the wild-type clone.

The effect of these regulatory elements in CEM cells in the presence of Tat was quite different from that in its absence. Tat, provided in trans, activated the expression directed by the wild-type and mutant clones to different degrees. For example, the expression directed by the wild-type HIV-2(ROD) LTR was trans-activated 2000- to 3000-fold whereas the expression directed by the mutant clones lacking the sequence elements upstream of nucleotide -183 (clone RLTR) or upstream of nucleotide -147 (clone RLTR5) were trans-activated only 400- to 500-fold (Fig. 1). Similarly, the trans-activation of the chimeric clones lacking the upstream sequence elements [clones SLTR(R1) and RLTR(S1)] was about five- to tenfold lower than that of the parental wild-type clones. In contrast, a chimeric clone containing the upstream sequences [clone RLTR(S)] was trans-activated just as effectively as the wild-type clones (Fig. 2). In general, the Tat-mediated trans-activation of the clones with a lower level of basal activity was more pronounced than that of the clones with a higher level of basal activity, such that the total level of expression by the majority of the clones in the presence of Tat appeared to be roughly equivalent. The mutant clone RLTR7 was anomalous in this respect; it had a lower basal level of activity as well as a lower degree of trans-activation compared to other clones (e.g. wild-type). It is noteworthy that the mutant clone SLTRO lacking sequence elements upstream of nucleotide -81 was trans-activated by Tat provided in trans.

Further definition of the upstream regulatory elements

The results presented in Fig. 2 suggested that the sequence elements located upstream of nucleotide -182/-183 in the U3 region had a down-modulatory effect on LTR-directed gene expression, with the more important elements being located between nucleotides -181/-183 and -232. Therefore, this segment of the U3 region was more intensively investigated by creating more deletion mutants with a set of staggered deletions of about 20 nucleotides each. The transfection of these additional mutant clones into CEM cells showed that nucleotides -188 to -232 had a marginal effect only on basal level LTR-directed gene expression (Fig. 3). The deletion of sequence elements between nucleotides -183 and -188 had a clear up-modulatory effect on the basal level expression. As with other clones, there was no marked difference in the expression directed by these mutant LTR clones in the presence of Tat supplied in trans (Fig. 3).

T cell activation

To define the sequence elements that may be responsible for activating gene expression in response to T cell activation signals, the wild-type and selected mutant HIV-2(ROD) LTR clones were transfected into CEM cells and the transfected cells were treated with PHA-P plus PMA. The gene expression directed by the wild-type LTR was activated about 30-fold in response to T cell activation in these cells (Fig. 4). There was no measurable difference in the response of the wild-type clone and a set of clones with deletions between nucleotides -556 and -188. This set contained a number of staggered deletion mutants. These results suggested that the segment of HIV-2 LTR between nucleotides -188 and -556 lacks elements that respond either positively or negatively to T cell activation signals. There was no indication of the existence of the compensatory elements in this region. In contrast, deletion of the region downstream of nucleotide -188 resulted in a clear decrease in the ability of the LTR to respond to T cell activation signals. The response of the mutant clone with a deletion between nucleotide -188 and -183 (clone RLTR) was about one-third of that of the wild-type clone. Additional deletions downstream of nucleotide -183 further reduced the response ability of the LTR. Independent deletions in the region between nucleotides -183 and -112 further reduced the response ability to about one-sixth to one-tenth of that of the wild-type LTR (Fig. 4).
Novel HIV-2 LTR regulatory elements

Table 1. Cell type-dependent expression of the HIV-2 LTR regulatory elements*

<table>
<thead>
<tr>
<th>HIV-2 clone</th>
<th>CEM</th>
<th>Jurkat</th>
<th>U937</th>
<th>HeLa</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Chloramphenicol acetylation (%)</td>
<td>Fold increase</td>
<td>Chloramphenicol acetylation (%)</td>
<td>Fold increase</td>
</tr>
<tr>
<td>Wild-type</td>
<td>0.4±0.1</td>
<td>34.9±0.3</td>
<td>33.72±438</td>
<td>3.2±0.1</td>
</tr>
<tr>
<td>RLTR</td>
<td>47.2</td>
<td>466</td>
<td>668±0.4</td>
<td>68.2±17.3</td>
</tr>
<tr>
<td>RLTR5</td>
<td>46.7±15</td>
<td>49.4±0.4</td>
<td>427±40</td>
<td>68.2±127</td>
</tr>
<tr>
<td>RLTR6</td>
<td>0.3±0.2</td>
<td>27.2±3.2</td>
<td>4410±1100</td>
<td>666±1.6</td>
</tr>
<tr>
<td>RLTR7</td>
<td>0.3</td>
<td>3.8</td>
<td>517</td>
<td>1.7±0.2</td>
</tr>
<tr>
<td>RLTR8</td>
<td>0.5</td>
<td>33.9</td>
<td>29.48</td>
<td>-</td>
</tr>
</tbody>
</table>

* The values in this table correspond to the following incubation conditions of the extract. CEM and Jurkat cells: basal, 100 lal for 4 h; +TAT, 20 lal for 30 min. U937 cells: basal, 50 lal for 4 h; +TAT, 5 lal for 10 min. HeLa cells: basal, 100 lal for 4 h; +TAT, 10 lal for 10 min.

Cell type-dependent expression

The LTR clones were also tested in cell types other than CEM. Table 1 shows the results obtained with lymphocytic Jurkat, monocytic U937 and epithelioid HeLa cells. Table 1 also contains the data for CEM cells that were obtained in direct comparative experiments. The upstream regulatory elements noted above for CEM cells appeared to have only a modest effect in Jurkat cells and this effect was nullified by Tat provided in trans. The basal level activity of the mutant clone RLTR or RLTR5 was about 10-fold higher than the wild-type clone in CEM cells in these experiments, but was only twofold higher in Jurkat cells. Conversely, the Tat-mediated trans-activation of these mutant clones was about six- to eightfold lower than that of the wild-type clone in CEM cells; it was not noticeably different in Jurkat cells. The upstream regulatory elements had no significant effect in U937 and HeLa cells. The expression directed by the clones RLTR or RLTR5 did not differ appreciably from that directed by the wild-type clone in these cells in the absence or presence of Tat (Table 1). The reasons for the four- to fivefold lower activity of clones RLTR6 and RLTR7 in HeLa cells in the absence of Tat remain to be determined.

It is curious that the negative and positive regulatory elements were more effective under conditions of low rather than high levels of basal activity. The basal level conversion of chloramphenicol to acetylated chloramphenicol by the wild-type HIV-2(ROD) LTR if the equivalent of 100 lal of extract was incubated for 4 h was 0.4 (-0.1)% in CEM cells, 3.2 (+0.1)% in Jurkat cells and 67.4 (+23.2)% in U937 cells. Although not strictly comparable because of differences in transfection protocol, it was also much higher in HeLa cells. In a sense, even in CEM cells these elements were not strongly effective in the presence of Tat where levels of expression were enhanced.
Discussion

The two LTRs used in this study were derived from different strains of HIV-2 that differ in their phenotypes. HIV-2(ST) was obtained from a healthy individual at risk of AIDS and is non-fusogenic and non-cytopathic in vitro (Kong et al., 1988; Kumar et al., 1990). HIV-2(ROD), obtained from a patient with AIDS, is in contrast both fusogenic and cytopathic in vitro (Guyader et al., 1987). The divergent phenotype of the two viruses is reproduced in studies with molecular clones derived from the two viruses (Hoxie et al., 1991; Arya & Sadaie, 1993). The level of gene expression directed by these two LTRs in T lymphocytic cells in culture was also different; the HIV-2(ROD) LTR was apparently twice as active as the HIV-2(ST) LTR (Fig. 2). The two LTRs are about 90% identical at the DNA sequence level and the limited divergence largely consists of scattered nucleotide substitutions and a few additions and deletions (Fig. 1). It is unclear at present whether this limited and diffuse divergence can account for the difference in the Tat-independent basal level gene expression directed by the two LTRs. The reciprocal chimeric clones SLTR(R1), containing nucleotides -107 to -181 from HIV-2(ROD) LTR in the HIV-2(ST) LTR background, and clone RLTR(S1) with similar transfer of HIV-2(ST) LTR onto the HIV-2(ROD) LTR background, also differed from each other by about a factor of two in their basal level expression. This suggests that at least part of the reason for the difference between the two LTRs may reside in this sequence segment. Any connection between the difference in the activities of these two LTRs and the profound differences in the biological properties of their parental viruses (Arya & Sadaie, 1993) remains to be established.

The mutational analysis of the LTRs revealed that they contained both a positive and a negative regulatory element in the U3 region. Comparison of the clones SLTR2 and SLTR1 for HIV-2(ST) LTR and RLTR2 and RLTR for HIV-2(ROD) LTR (Fig. 2) suggests that the major part of the positive regulatory element was located between nucleotides -232/-231 and -183/-182, as deletion of this sequence element resulted in six- to eightfold enhancement of the basal level activities of the LTRs. These observations were supported by the results obtained with chimeric LTR clones where positive regulatory elements of HIV-2(ST) and HIV-2(ROD) have been exchanged [clones SLTR(R1) and RLTR(S), Fig. 2]. Further analysis of the sequence elements between nucleotides -232 and -183 using HIV-2(ROD) LTR as a prototype (Fig. 3) suggested that the dominant part of the negative regulatory element was located between nucleotides -188 and -183 and was composed of a stretch of five purine nucleotides (AGGAA). The positive regulatory element was apparently located downstream of nucleotide -147. Comparison of the clones RLTR, RLTR5 and RLTR6 (Fig. 2) suggests that the most important of the positive elements was located between nucleotides -142 and -147(TCAGG).

Interestingly, the magnitude of the Tat-mediated trans-activation of the wild-type and mutant LTR clones depended on their basal level of expression. In general, the magnitude of trans-activation was greater for clones with a lower level of basal activity than the clones with a higher level of basal activity. Since the lower basal activity could be largely due to the influence of the negative regulatory element, one function of Tat may be to abolish the down-modulatory effect of the negative regulatory element allowing the positive regulatory element to function. The net result would be that in the presence of Tat the clones containing the negative regulatory element will behave similarly to the clones lacking this element, as appears to be the case (Fig. 2 and 3). Alternatively, the upstream region where the negative regulatory element was located may be required for full trans-activation mediated by Tat, suggesting that specific upstream DNA elements may be important for Tat-mediated trans-activation. This would be in addition to the downstream TAR element. A third possibility is that the LTR is already maximally trans-activated by Tat such that the deletion of the upstream element increases the basal level expression without a corresponding increase in the Tat-mediated trans-activation. Notably, the gene expression directed by the mutant clone SLTRO lacking the NFκB site was also trans-activated by Tat, suggesting that the NFκB enhancer element itself was not essential for Tat-mediated trans-activation. However, the composite enhancer–promoter region, including Sp1 binding site(s) and TATA box, apparently contributed to the optimal Tat-responsiveness of the HIV-1 LTR (Berkhout et al., 1990; Berkhout & Jeang, 1992; Olsen & Rosen, 1992) as well as to that of the HIV-2 LTR (Arya & Gallo, 1988).

Tat is thought to activate transcription both by increasing the rate of transcriptional initiation and by enhancing the efficiency of transcript elongation (Laspia et al., 1989). It was recently reported for the HIV-1 LTR that two different types of transcriptional complexes are formed, processive and non-processive (Lu et al., 1993). The processive complexes are assembled at the enhancer–promoter region without requiring a TATA box whereas non-processive complexes required the presence of the TATA box. HIV-1 Tat appears to trans-activate the non-processive complexes only, without significantly affecting the processive complexes, suggesting that HIV-1 Tat affects a critical step in the formation of elongation-competent transcriptional com-
plexes. It has also been reported that under conditions of low basal level transcription, HIV-1 Tat functions primarily to increase the rate of initiation of transcription from the HIV-1 LTR, presumably by promoting the formation of more efficient transcription initiation complexes (Kessler & Mathews, 1991). On the other hand, when the basal level of transcription is high, Tat primarily functions to increase the efficiency of transcript elongation by facilitating the formation of more stable transcriptional complexes (Kessler & Mathews, 1991). It is conceivable that the negative regulatory element noted above hinders the formation of efficient transcriptional initiation complexes in CEM cells this being overcome by Tat, such that in CEM cells Tat functions to increase both transcriptional initiation as well as transcript elongation. In other cell types, such as U937, transcriptional initiation complexes of sufficient efficiency might be formed such that Tat functions primarily to enhance transcript elongation in these cells. It is also possible that under conditions of maximal Tat-mediated trans-activation, the upstream element influences transcript initiation independent of Tat and Tat promotes transcript elongation.

While these studies were in progress, a substitution mutational analysis of the HIV-2 LTR delineating sequence elements responding to T cell activation was presented (Markovitz et al., 1992). Reportedly, the HIV-2 LTR contains three distinct cis-acting sequence elements, in addition to the NFκB site, that respond to T cell activation signals. Two of these sites are composed of purine-rich sequences called purine-rich boxes (PUBs) 1 and 2 and the third site was termed pets. These sites are reportedly the targets of an ets oncogene-related transcriptional factor, Elf-1 (Leiden et al., 1992). These interactions are thought to be required for the activation of HIV-2 LTR-directed gene expression in response to T cell activation signals, particularly those mediated through the T cell receptor. Our studies did not specifically target purine boxes but our analysis of the HIV-2 LTR T cell activation response elements based on the deletion mutants was consistent with results obtained with substitution mutation analysis noted above. For example, the observation that mutant clone RLTR5 responded about eightfold less well than the wild-type clone to T cell activation signals may be because it lacks the PUB2 site (Fig. 3). Similarly, it is likely that the mutant clones RLTR6 and RLTR8 were poor in responding to T cell activation signals because they lack the pets and PUB1 sites, the clone RLTR8 in addition lacking PUB2 site (Fig. 3). The diminished response of the mutant clone RLTR is probably related to the fact that this clone has a part of the ‘enhancer’ element deleted. We have previously reported that the HIV-2 LTR contains an imperfect direct repeat in the U3 region that can function as an ‘enhancer’ element and participates in the T cell activation response (Arya, 1990).

Significantly, the regulatory elements delineated here apparently function in a cell type-dependent manner, even among cell types of similar lineage. For example, these elements were more effective in human T lymphocytic CEM cells than in Jurkat cells. This is important because it implies that owing to these regulatory elements subsets of cells within a particular lineage may differ from each other in their ability to support HIV-2 replication. The effectiveness of these regulatory elements appeared to be related to the basal level of the LTR-directed gene expression, being more effective in cells with lower basal level expression. This may be important in initiating a state of restricted virus gene expression in certain target cells. This state would be maintained until sufficient Tat protein is accumulated to overcome the effect of the negative regulatory element. Notably, tat gene expression will also be under the control of the regulatory elements. It was thought until recently that virus replication occurs in three phases during the course of infection and development of the disease. After the initial burst of virus replication, the infected cells enter a phase of attenuated virus expression (clinical latency) which is followed by a high level of virus replication at the later stages. It now appears that the ‘latent’ phase of virus infection may not occur (Embretson et al., 1993; Pantaleo et al., 1993) and that the virus continues to replicate to high levels in lymph nodes and other lymphoid tissues throughout the course of infection. However, there are T cells in peripheral blood that are infected with the virus but do not produce infectious virus particles. There may be infected cells with only restricted virus gene expression, a situation similar to that described above for lymphocytic CEM cells in culture. This culture system may serve as a model for the study of cellular factors that regulate virus expression.

We thank Dr Robert C. Gallo for continued support and our colleagues for their comments on the manuscript. The HIV-2(ROD) clone was kindly provided by R. Desrosier through B. Hahn. The original HIV-2(ROD) LTR cDNA clone was created by M. Emerman and was kindly provided by D. Markowitz. The contributions of Ms Jennifer Mohr, a student at Duke University, were made possible by a summer award by the Student Research Training Program of the National Cancer Institute.

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(Received 7 March 1994; Accepted 15 April 1994)