Functional Analysis of Simian Immunodeficiency Virus SIV<sub>AGM</sub> Long Terminal Repeat

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Abstract. We have previously shown that long terminal repeats (LTRs) derived from various isolates of SIV<sub>AGM</sub> share a unique functional property. In the absence of viral Tat, all SIV<sub>AGM</sub> LTRs act as much more efficient promoters than any of the other LTRs derived from representative primate immunodeficiency viruses. In the presence of Tat, however, SIV<sub>AGM</sub> LTRs are activated relatively inefficiently. To map the elements that confer these features on the SIV<sub>AGM</sub> LTR, a number of deletion mutants were constructed, and their promoter activities were determined using a bacterial CAT gene as a marker. The results obtained indicated that various elements located in the U3 region may contribute to the high basal promoter activity and that no negative elements are present in the region. The Tat-responsive sequence TAR was localized to the R region as observed for the other LTRs. A mutant carrying a single nucleotide deletion in this region completely lost responsiveness to Tat protein.

Key words: SIV<sub>AGM</sub>, LTR, promoter, transcription, Tat, TAR

Numerous lentiviruses have been isolated from various species of primates, including human (1), and have been extensively studied biologically and biochemically. They are similar to one another in their complex genome organization and in their biological and functional properties (1). These primate immunodeficiency viruses are now subdivided into four major classes on the basis of sequence variability (2). The representative viruses are human immunodeficiency virus type 1 (HIV-1), HIV-2, simian immunodeficiency virus isolated from mandrill (SIV<sub>MND</sub>), and SIV from African green monkey (SIV<sub>AGM</sub>).

We have recently performed systemic and comparative genome analyses of these viruses (1). During the course of these studies, we have noticed that LTRs derived from SIV<sub>AGM</sub> are distinct in that they all are very strong transcriptional promoters in the absence of viral Tat protein and that they are poor responders to Tat proteins of various origins (3). In spite of this functional similarity of the SIV<sub>AGM</sub> LTRs, an extensive sequence heterogeneity exists among strains designated TYO-1, TYO-2, TYO-5, and TYO-7 (3). The U3 regions of the LTRs diverge, particularly due to deletions and/or insertions (3). Nonetheless, the signal elements in the LTRs, such as AP-1, NF-kB, and Sp1 binding sites, the TATA box, and the poly A signal, are fairly well conserved (3). Putative trans-acting responsive elements (TARs) in the R regions of the LTRs are also conserved (3). In this report we dissected functionally the SIV<sub>AGM</sub> TYO-7 LTR, the shortest of the four SIV<sub>AGM</sub> LTRs sequenced (3), by deletion analysis to gain insight into the molecular basis for its unique properties.
Materials and Methods

Cell Lines and DNA Transfection

A human colon carcinoma cell line, SW480 (4), and a CD4-positive human T-cell leukemia cell line, Molt4 clone 8 (M4-8) (5), were maintained in medium containing 10% fetal calf serum as previously described (6). For transfection of SW480 and M4-8 cells, the calcium-phosphate coprecipitation (7) and modified DEAF-dextran (8) methods were used, respectively.

CAT Assays

The chloramphenicol acetyltransferase (CAT) assay has been previously described (9). CAT levels were assayed in equivalent amounts of cell lysates prepared from transfected cells. CAT activity was quantitated by a bioimaging analyzer BAS2000 (Fuji Photo Film, Tokyo, Japan), as previously described (6).

DNA Constructs

The LTR-CAT construct, designated pSA7-CAT, bearing wild-type (WT) LTR of the TYO-7 strain of SIVAGM, has been previously described (3). The backbone promoterless plasmid for pSA7-CAT is pHdCAT, as previously described (3). All deletion mutants listed in Table 1 were constructed from pSA7-CAT. To introduce deletions into the restriction sites indicated later, standard recombinant DNA techniques were employed. T4 DNA polymerase and Bal31 were mainly used to generate deletions. To construct deletion mutants designated 4111, BD, XD, XB, X6, X8, AvD, HgD, K7, and K1 (see Table I for the location of deletion), the following restriction enzymes were used: HindIII in pHdCAT (3) for 4111, KpnI in pHdCAT (3) and BalI (nt 294) for BD, HindIII and XcmI (nt 258) for XD, XcmI for XB, XcmI for X6, XcmI for X8, KpnI and AvalII (nt 90) for AvD, KpnI and Hgal (nt 106) for HgD, KpnI for K7, and KpnI for K1. The structures of the mutants were determined by nucleotide sequencing. The expression vector of the SIVAGM Tat protein, designated pTata (previously referred to as ptata), has been previously described (3).

Results

Figure 1 shows the nucleotide sequence of SIVAGM TYO-7 LTR. This LTR is 450 bp long and is biologically active when monitored by the CAT assay system (3), consistent with the presence of several important signal elements (Fig. 1). To determine the contribution of these sequences in the LTR to its promoter activity, 10 deletion mutants were constructed. As indicated in Table 1, these mutants lacked sequences potentially important for promoter activity in the LTR. These deleted LTRs were placed in front of the CAT gene of pHdCAT (3), and transfection experiments were carried out. Two cell lines, SW480 (4) and M4-8 (5), were used for transfection. SW480 cells form monolayer and have been routinely and widely used for molecular characterization of HIV/SIV (1,3,4,6). M4-8 cells are CD4-positive lymphocytic cells and are highly susceptible to infection with various isolates of HIV/SIV (1,5).

Figure 2 shows the representative results obtained from transfection experiments. SW480 cells were transfected with various LTR-CAT constructs, and CAT activity in cells 48 hr post-transfection was monitored to determine the promoter activity of the LTR mutants. As clearly seen in Fig. 2A, deletions of the 5' portion of the LTR (mutants AvD, HgD, K7, and K1) reduced the basal promoter activity of the LTR. When sequences downstream of the TATA box were removed (4111, BD, XD, XB, X6, and X8), in