Structural and Functional Analysis of the Visna Virus Rev-Response Element

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The distantly related lentiviruses human immunodeficiency virus type 1 (HIV-1) and visna virus each encode a posttranscriptional regulatory protein, termed Rev, that is critical for expression of the viral structural proteins. We genetically mapped the *cis*-acting target sequence for visna virus Rev, the visna virus Rev-response element or RRE-V, to a complex 176-nucleotide RNA stem-loop structure that coincides with sequences encoding the N terminus of the transmembrane component of envelope. The computer-predicted structure of the RRE-V was validated by *in vitro* analysis of structure-specific RNase cleavage patterns. The visna virus Rev protein was shown to interact specifically with the genetically defined RRE-V *in vitro* but was unable to bind the HIV-1 RRE. Similarly, HIV-1 Rev was also unable to bind the RRE-V specifically. We therefore conclude that the HIV-1 and visna virus Rev proteins, while functionally analogous, nevertheless display distinct RNA sequence specificities. These findings provide a biochemical explanation for the observation that these two viral regulatory proteins are functional only in the homologous viral system.

Lentiviruses are a family of complex retroviruses that induce chronic, degenerative diseases in the infected host (7, 10). Phylogenetic analysis suggests that lentiviruses can be broadly divided into two subgroups (27, 34). These are the relatively cohesive primate immunodeficiency viruses and a somewhat more diverse group of nonprimate lentiviruses that primarily infect ungulates (27, 34). The prototype of the nonprimate lentiviruses is visna virus, first described as the etiologic agent of a chronic degenerative syndrome observed in domestic sheep (10). Visna virus and other nonprimate lentiviruses such as caprine arthritis-encephalitis virus and equine infectious anemia virus continue to be economically significant livestock pathogens. However, the most significant lentivirus is clearly human immunodeficiency virus type 1 (HIV-1), the etiologic agent of AIDS and the prototype of the primate lentivirus subgroup (4, 7).

Although HIV-1 and visna virus display little primary sequence homology (32), they do share a complex pattern of viral gene expression that is facilitated by the action of virally encoded regulatory proteins (4, 6, 8, 12, 15, 25, 31, 35, 39). In particular, both HIV-1 and visna virus have been shown to encode a nuclear protein, termed Rev, that is required for the cytoplasmic expression of the unspliced and singly spliced mRNAs that encode the viral structural proteins (8, 9, 15, 22, 31, 35, 39). In the case of HIV-1, this action requires the direct binding of multiple Rev protein molecules to a highly structured viral RNA target site, the Rev-response element or RRE (5, 11, 21, 24, 28, 40). We have previously provided genetic evidence for the existence of an RRE in visna virus (35). Here, we precisely map the visna virus RRE (RRE-V) to a 176-nucleotide (nt) computer-predicted RNA stem-loop structure located in the viral envelope gene. We show that the RRE-V indeed forms the predicted RNA secondary structure and demonstrate that the RRE-V can serve as a highly specific binding site for visna virus Rev protein (Rev-V).

MATERIALS AND METHODS

Plasmid constructs. The eukaryotic expression plasmids pBC12/CMV, pcL, pL/Rev, pRev/L, pcRev, pgTat; the prokaryotic expression plasmid pGST-Rev; and the HIV-1 RRE RNA expression construct pGEM/RRE have been described previously (21, 22, 24, 35, 36). The coding regions of Rev-V and of the L/Rev and Rev/L chimeric proteins were isolated by using the polymerase chain reaction (26). The primers used introduced a unique *Nco*I site coincident with the first AUG of Rev/L or the second AUG of Rev-V and L/Rev and also introduced *Eco*RI sites downstream of each translation termination codon. These sequences were substituted into the glutathione-S-transferase (GST) fusion protein expression vector pGST-Rev (21) in place of the HIV-1 Rev gene after cleavage with *Nco*I and *Eco*RI. The resultant pGST-Rev-V, pGST-L/Rev, and pGST-Rev/L plasmids are predicted to encode each of these Rev proteins fused to the C terminus of GST.

Visna virus RNA segments are named according to their location within the visna virus proviral genomic sequence reported by Sonigo et al. (32). pSK⁺/RRE-V(7970-8262) contains the *Pst*I (7970)-to-*Dra*I (8262) fragment of the visna virus *env* gene cloned between the *Pst*I and *Eco*RV sites of pBluescript SK⁺ (Stratagene). pGEM/RRE-V(7923-8124) and pGEM/RRE-V(8001-8202) were constructed by cloning polymerase chain reaction-generated DNA fragments from the indicated regions of the visna virus *env* gene between the *Eco*RI and *Hind*III sites of pGEM-3Zf(+) (Promega). These same DNA fragments were also cloned between the *Eco*RI and *Hind*III sites of pBluescript SK⁺ and subsequently excised as *Bam*HI-to-*Hind*III DNA fragments, thus acquiring a *Bam*HI site from the pBluescript polylinker. They were then inserted between the *Bgl*II and *Hind*III sites of pgTAT to produce pgTAT/RRE-V(7923-8124) and pgTAT/RRE-V(8001-8202).

Purification of GST fusion proteins. HIV-1 Rev, Rev-V, L/Rev, and Rev/L were expressed in *Escherichia coli* as GST fusion proteins as previously described (21, 30). After adsorption of the cell lysate to glutathione-Sepharose affinity columns, the recombinant proteins were either eluted as

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intact fusion proteins by displacement with excess glutathione or released by proteolytic digestion with thrombin. The GST-Rev-V fusion protein possesses a unique thrombin cleavage site at the junction between GST and Rev-V. The column matrix was incubated with 0.1 U of thrombin (Boehringer Mannheim) in 10 mM Tris (pH 8.2)–150 mM NaCl–2.5 mM CaCl₂ for 30 min at room temperature. The eluted proteins were concentrated, and their diluents were changed by three rounds of centrifugal ultrafiltration in Centricon 10 microconcentrators (Amicon). Polyacrylamide gel and Western blot (immunoblot) analyses suggested that these recombinant protein preparations were between 25 and 50% pure (data not shown). The proteins were stored in 10% glycerol–10 mM HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid) (pH 7.5)–50 mM NaCl–10 mM KCl–0.5 mM EGTA [ethylene glycol-bis(β -aminoethyl ether)-*N,N,N',N'*-tetraacetic acid]–2 mM dithiothreitol at -70°C .

In vitro transcription and binding assays. RNA transcripts were synthesized by using standard methodology and materials from a commercially available kit (Promega). The combinations of template plasmid, site of linearization, and RNA polymerase used for the various transcripts were as follows: pGEM/RRE-V(7923-8124), *Hind*III, T7; pGEM/RRE-V(8001-8202), *Hind*III, T7; pSK⁺/RRE-V(7970-8262), *Hind*III, T3; pGEM/RRE, *Xba*I, T7. The 7970 to 8091 RNA probe was generated from pSK⁺/RRE-V(7920-8262) by T3 transcription after being linearized at an *Nco*I site located at 8091 within the inserted visna virus sequence. Transcripts were radioactively labeled either by incorporation of [α -³²P]UTP during the transcription reaction or by labeling at the 5' or 3' ends as previously described (37).

Gel shift assays were done as previously described (21, 24, 37). Standard binding reactions (10 μ l) contained 10⁵ cpm ($\sim$ 1 ng) of RNA probe in 10 mM HEPES (pH 7.5)–0.5 mM EGTA–2 mM MgCl₂–10% glycerol–25 mM NaCl–150 mM KCl–1 mM dithiothreitol–7.5 mg of bovine serum albumin per ml–175 U of RNAGuard (Pharmacia) per ml–20 μ g of yeast tRNA per ml. Purified REV proteins and additional RNA competitors were added as indicated in the text.

Structure probing and footprinting. Reaction volumes of 100 μ l identical in composition to those described for gel shift assays, except for the omission of RNAGuard, were subjected to limited digestion with either RNase T₂ (GIBCO-BRL) (20 U/ml) or RNase V1 (Pharmacia) (0.04 U/ml) for 10 min at 37°C. The reactions were terminated by phenol-chloroform extraction and then ethanol precipitation. The digestion products were resolved on a 6% polyacrylamide sequencing gel containing 8 M urea. Identification of specific cleavage products was facilitated by comparison with RNA sequencing ladders generated by using base-specific RNases supplied in a commercially available kit (Pharmacia). The influence of Rev-V protein on the structure-specific cleavages was examined by preincubating the test RNA with 100 μ g of purified Rev-V protein per ml for 10 min on ice prior to the RNase digestion step.

RESULTS

The HIV-1 RRE coincides with a 234-nt RNA secondary structure that also encodes the N-terminal portion of the transmembrane protein component of the viral envelope protein (22). Similarly, the sequences that encode the N terminus of the visna virus transmembrane protein are also predicted to form a highly significant RNA folding region (29, 35). We have previously demonstrated that this sequence, extending from 7923 to 8262 within the visna virus

FIG. 1. Identification of the RRE-V. COS cell cultures were transfected (2) with the indicator construction pgTAT (22) containing the HIV-1 RRE (lanes 1 to 3) or with derivatives in which the HIV-1 RRE had been replaced with the visna virus sequence 7923 to 8124 (lanes 4 and 5) or 8001 to 8202 (lanes 6 and 7). Cultures were cotransfected with the HIV-1 Rev expression vector pcRev (lane 2), the Rev-V expression vector pcL (lanes 3, 5, and 7), or the negative control vector pBC12/CMV (NEG) (lanes 1, 4, 6, and 8). At 72 h after transfection, cultures were labeled with [³⁵S]cysteine as previously described (2) and subjected to immunoprecipitation with a 1:140 dilution of a rabbit polyclonal antiserum specific for the HIV-1 Tat protein (2, 22). Precipitated proteins were resolved on a discontinuous sodium dodecyl sulfate-14% polyacrylamide gel and visualized by autoradiography. Rev activity is indicated by the appearance of a truncated, $\sim$ 14-kDa form of the HIV-1 Tat protein. Numbers on right show sizes in kilodaltons.

genome, indeed contains a biologically active RRE (35). However, computer analysis indicates that this region can be folded to give two highly stable, but mutually exclusive, RNA secondary structures (data not shown). The first of these is a 176-nt sequence extending from 7933 to 8108 within the visna virus genome, while the second potential RNA structure is 202 nt in length and extends from 8001 to 8202 (29, 35).

To distinguish which of these potential RNA structures forms the actual RRE-V, we used a previously described assay for Rev function based on the expression vector pgTat (22, 23). This construct contains both exons of the HIV-1 Tat protein separated by an intron that includes the HIV-1 RRE. In the absence of HIV-1 Rev, the pgTat construct expresses exclusively a spliced cytoplasmic Tat mRNA that encodes a 16-kDa form of Tat visualizable by immunoprecipitation (Fig. 1, lane 1). In the presence of HIV-1 Rev, an unspliced cytoplasmic Tat mRNA encoding a truncated 14-kDa form of Tat is also expressed (Fig. 1, lane 2). As previously shown (36), Rev-V is not active in this HIV-1 RRE-based assay (Fig. 1, lane 3). However, if the HIV-1 RRE is substituted with the RRE-V, then it is predicted that Rev-V should be able to rescue the expression of the unspliced mRNA encoding the 14-kDa form of Tat. A derivative of pgTat containing the visna virus sequence 7923 to 8124, but not a construct containing the 8001 to 8202 sequence, was indeed highly responsive to Rev-V (Fig. 1, lanes 4 to 7). We therefore conclude that the RRE-V is contained between 7923 and 8124 in the visna virus genome.

Recombinant HIV-1 Rev protein can specifically bind to, and multimerize on, the HIV-1 RRE in vitro (5, 11, 17, 21, 24, 28, 40). It has previously been demonstrated that HIV-1

FIG. 2. RRE-V specifically binds multiple Rev-V protein molecules in vitro. A constant level of a uniformly labeled 202-nt probe, derived from 7923 to 8124 in the visna virus genome, was incubated (24) with increasing levels of the recombinant GST-Rev-V protein, as indicated. Binding of probe by the Rev-V fusion protein was detected as slower migration through a native polyacrylamide gel. At least two distinct protein-RNA complexes are visualized by this procedure. The GST protein itself was unable to bind the RRE-V probe (data not shown).

Rev expressed as a fusion protein attached to the C terminus of GST retains the same in vitro RNA-binding specificity as nonfusion Rev (21, 37). Purified recombinant GST-Rev-V protein was therefore examined for its ability to bind the genetically defined RRE-V 7923 to 8124 sequence. As shown in Fig. 2, the GST-Rev-V protein indeed bound a radiolabeled RNA probe derived from this region. As first reported for HIV-1 Rev (21), increasing levels of GST-Rev-V resulted in the formation of first one and then multiple specific protein-RNA complexes that could be separated by electrophoresis through a nondenaturing polyacrylamide gel. To further demonstrate the specificity of this protein-RNA interaction, we asked whether binding of GST-Rev-V to the radiolabeled 7923 to 8124 RNA probe could be blocked by an excess of selected unlabeled RNAs. As shown in Fig. 3,

FIG. 3. Competition analysis of the visna virus Rev-RRE interaction. The indicated visna virus RNA sequences, as well as the entire HIV-1 RRE, were assessed for their ability to competitively inhibit binding of the labeled 7923 to 8124 RRE-V probe by the GST-Rev-V protein. A constant level (160 ng) of each unlabeled competitor RNA was preincubated with 400 ng of GST-Rev-V before addition of ~1 ng of the labeled 7923 to 8124 RRE-V probe. The effect of this preincubation on the level of protein-RNA complex formation was then determined by gel retardation analysis. No Competitor indicates standard reaction conditions, which include 200 ng of *E. coli* tRNA.

FIG. 4. Sequence specificity of the HIV-1 Rev and Rev-V proteins. The L/Rev protein consists of the N-terminal domain of Rev-V fused to the C-terminal activation domain of HIV-1 Rev (36). Similarly, the Rev/L protein contains the N-terminal domain of HIV-1 Rev fused to the C-terminal domain of Rev-V (36). Each of these two Rev chimeras was expressed as a GST fusion protein, and their ability to bind the RRE-V (lanes 1 to 5) or the HIV-1 RRE (lanes 6 to 10) was compared with that of the parental visna virus (GST-Rev-V) and HIV-1 [GST-Rev(HIV)] proteins. Only proteins containing the N-terminal basic domain of Rev-V bound the RRE-V (lanes 2 and 3), while proteins containing the HIV-1 Rev basic domain specifically bound only the HIV-1 RRE (lanes 9 and 10). This pattern therefore recapitulates the target specificity of these Rev proteins previously determined in vivo (36).

RNA segments derived from visna virus coordinates 7923 to 8124 (i.e., the probe sequence), 7970 to 8262, and, minimally, 7970 to 8091 all efficiently competed for Rev-V binding. In contrast, tRNA as well as RNAs derived from visna virus coordinates 8001 to 8202 (i.e., containing the alternate RNA structure) or containing the full-length HIV-1 RRE were unable to compete effectively for Rev-V binding (Fig. 3). The conclusion that the specific binding site(s) for Rev-V on the RRE-V is located between 7970 and 8091 in the visna virus genome was further supported by the direct demonstration of Rev-V binding to a radioactively labeled 7970 to 8091 probe (data not shown).

The HIV-1 Rev protein can be divided into at least two distinct functional domains (13, 20, 21, 28, 38, 40). Sequences adjacent to the protein N terminus that include, but extend beyond, a highly basic sequence are critical both for binding to the HIV-1 RRE and for Rev multimerization (18, 20, 21, 28, 40). A second essential domain, located adjacent to the C terminus of HIV-1 Rev, is not involved in Rev binding or multimerization in vitro yet is essential for Rev function in vivo (13, 20, 21, 23, 28, 40). It has therefore been proposed that this leucine-rich sequence forms the Rev activation domain (20, 23). Previously, we have shown that chimeric proteins consisting of the N-terminal region of HIV-1 Rev and the C-terminal region of Rev-V (Rev/L), or vice versa (L/Rev), were fully active on the RRE cognate for the protein N terminus (36). This result suggested that HIV-1 Rev and Rev-V, despite very limited sequence homology, were likely to share the same domain organization. However, these chimeric proteins, like the parental HIV-1 Rev and Rev-V, were completely inactive on the noncognate RRE (35, 36). To address the molecular basis for the sequence specificity of HIV-1 Rev, Rev-V, and the chimeric

FIG. 5. Secondary structure of the RRE-V. The figure shows the entire 202-nt 7923 to 8124 sequence shown by both genetic and biochemical criteria to contain the RRE-V (Fig. 1 to 3). Position 1 equals coordinate 7923 in the visna virus provirus sequence reported by Sonigo et al. (32). The start of the visna virus transmembrane protein (TMP) coincides with position 2 (7924). The computer-predicted RRE-V stem-loop structure extends from 11 (7923) to 186 (8108) within this larger sequence. The minimal sequence shown to bind Rev-V in vitro extends from 7970 (48) to 8091 (169) (Fig. 3) and is indicated by a darker line. Specific cleavage sites for the single-strand-specific RNase T_2 and the double-strand-specific RNase V1 were determined as described in the legend to Fig. 6. The data presented here represent a summary of cleavage data derived from three independent experiments.

Rev/L and L/Rev proteins, we purified each protein as a GST-Rev fusion and determined whether they could specifically bind to the HIV-1 RRE or RRE-V. As shown in Fig. 4, both proteins active on the RRE-V bound the RRE-V efficiently but failed to bind the HIV-1 RRE (Fig. 4, lanes 2, 3, 7, and 8). In contrast, the two proteins active on the HIV-1 RRE bound the HIV-1 RRE efficiently but failed to interact with the RRE-V (Fig. 4, lanes 4, 5, 9, and 10). We therefore conclude that the ability of these Rev proteins to function in only one of these two viral systems is due to their distinct RNA sequence specificities. These are, in turn, determined by the origin of the basic domain of each protein.

The data presented in Fig. 1 to 4 support the hypothesis that the RRE-V coincides with a computer-predicted 176-nt RNA stem-loop structure located between 7933 and 8108 in the viral genome (Fig. 5) (29, 35). As previously shown for the somewhat longer HIV-1 RRE (17, 22), this structure is predicted to consist of a long presentation stem surmounted by a set of shorter stem-loop structures that, in the HIV-1 RRE, contain primary sequence information critical for HIV-1 Rev binding (1, 11, 17, 24, 37, 40). To assess the validity of the RNA secondary structure visualized in Fig. 5, we radioactively labeled either the 5' or the 3' end of the 202-nt 7923 to 8124 RNA probe that contains the entire predicted RRE-V secondary structure (Fig. 5). These molecules were then subjected to limited digestion with the single-strand-specific RNase T_2 or the double-strand-specific RNase V1. Sites of cleavage were identified after electro-

phoresis through a denaturing polyacrylamide gel by reference to sequence-specific RNase cleavage ladders run in parallel (Fig. 6). These data, which are compiled in Fig. 5, strongly support the validity of the predicted RRE-V secondary structure.

As a first step toward localizing the sites of Rev-V binding on the RRE-V, we next asked whether the binding of Rev-V to the RRE-V would interfere with any of these specific RNase cleavage events (Fig. 6, lanes 9 to 12 and 21 to 24). Nucleotides protected by Rev-V binding are indicated in Fig. 6 and are compiled in Fig. 5. The protected nucleotides were found to be concentrated in several clusters near the apex of the RRE-V RNA structure. This pattern is therefore similar to that reported for RNase protection of the HIV-1 RRE by Rev (17). The hypothesis that one or more of the shorter RNA stem-loops predicted for the RRE-V are critical for Rev-V binding is further supported by the observation that the short 7970 (48) to 8091 (169) RRE-V sequence, which does not contain the RRE-V presentation stem, is both necessary and sufficient for specific Rev-V binding in vitro (Fig. 3).

DISCUSSION

Although both HIV-1 and visna virus are members of the lentivirus family, they have diverged considerably during evolution and now retain only limited primary sequence identity (32). In addition, visna virus appears to have a

FIG. 6. RNase cleavage and protection patterns of the RRE-V. The 202-nt 7923 to 8124 RRE-V probe shown in Fig. 5 was synthesized in vitro by using T7 RNA polymerase (24). Subsequently, this RNA probe was uniquely end labeled at the 5' end, using [γ - ^{32} P]ATP and T4 polynucleotide kinase (lanes 1 to 12), or at the 3' end, using [^{32} P]pCP and T4 RNA ligase (lanes 13 to 24) (37). Aliquots of each RNA were incubated on ice in the presence (+) or absence (-) of Rev-V protein (lanes 8, 10, 12, 20, 22, and 24 and lanes 7, 9, 11, 19, 21, and 23, respectively). The samples were then subjected to limited digestion with either single-strand-specific RNase T₂ (lanes 9, 10, 21, and 22) or double-strand-specific RNase V1 (lanes 11, 12, 23, and 24). Lanes 6, 7, 19, and 20 correspond to the digestion controls in which no RNase was added. Alkaline hydrolysis (AH) lanes as well as lanes showing cleavage patterns induced by sequence-specific RNases (lanes 1 to 5 and 13 to 17) were run in parallel to facilitate identification of cleavage sites. RRE-V nucleotides that were specifically protected from RNase cleavage by bound Rev are indicated. The RNase cleavage and protection data derived from this and other experiments are compiled in Fig. 5.

significantly less complex genomic organization than HIV-1 and is not believed to encode equivalents of the HIV-1 *vpr*, *vpu*, and *nef* genes (4, 29, 32). Furthermore, while both viruses encode transcriptional activators of their homologous long terminal repeat promoters, these are functionally distinct. HIV-1 Tat is a highly potent *trans* activator that is believed to primarily function by stimulating the elongation

of nascent proviral transcripts (reviewed in reference 3). Remarkably, the target sequence for Tat has been shown to be an RNA stem-loop structure. In contrast, the visna virus S gene product only modestly enhances visna virus transcription and appears to do so by directly or indirectly interacting with AP-1 and AP-4 DNA target sites that are located in the U3 region of the viral long terminal repeat (12).

Despite the considerable differences that exist between these two prototypic lentiviruses, genetic and biochemical analyses of the Rev-V *trans* activator reveal that this protein is remarkably similar in both function and mechanism of action to the Rev *trans* activator of HIV-1. Previously, we had demonstrated that Rev-V, like HIV-1 Rev, activates the cytoplasmic expression of the incompletely spliced mRNAs that encode the viral structural proteins, including Gag and Env (35). Subsequently, we reported that Rev-V and HIV-1 Rev display the same functional organization, marked particularly by an N-terminal basic domain that determines sequence specificity and by a conserved C-terminal leucine-rich motif that acts as the Rev activation domain (36). Here, we extend our previous genetic data for the existence of RRE-V by precisely mapping the RRE-V to a 176-nt RNA stem-loop structure that coincides with sequences encoding the N terminus of the transmembrane protein component of the viral envelope. This location is therefore identical to that observed for the HIV-1 RRE (22). The secondary structure adopted by the RRE-V was validated by analysis of *in vitro* RNase cleavage patterns and appears generally similar to that adopted by the somewhat larger (234-nt) HIV-1 RRE (22). In particular, both RREs consist of a helical region of 30 to 40 bp surmounted by three or four shorter RNA stem-loops. In the case of HIV-1, these apical stem-loop structures have been shown to contain primary sequence information that is critical for HIV-1 Rev binding (1, 11, 17, 24, 37, 40). Similarly, we demonstrated that Rev-V can also specifically bind to, and multimerize on, the RRE-V. *In vitro* RNase protection data, as well as a preliminary mutational analysis of the RRE-V, indicate that the primary target for Rev-V binding is also likely to be located within one or more of the shorter stem-loops observed in the RRE-V.

The remarkably similar properties of the Rev proteins of HIV-1 and visna virus are particularly surprising given the completely different mechanisms of action reported for the HIV-1 Tat and visna virus S transcription factors (3, 12). This evolutionary conservation suggests that Rev function is dependent on the specific interaction of Rev with a highly conserved cellular factor(s) involved in the splicing and/or transport of eukaryotic mRNA (9, 13, 18, 20, 23, 28, 36, 40). This hypothesis is supported by the observation that HIV-1 Rev is functional in a wide variety of species, including cells of avian and insect origin (14, 23). The extensive similarity of the Rev proteins of the prototypic lentiviruses HIV-1 and visna virus reported here may also imply that the Rev *trans* activators recently reported to exist in several other nonprimate lentiviruses (16, 33) are likely to retain many of these same functional characteristics.

Although it is evident that HIV-1 Rev and Rev-V function by essentially identical mechanisms, it is also clear that Rev-V is unable to functionally substitute for the HIV-1 Rev protein and vice versa. Here, we demonstrate that this results from the inability of Rev-V to bind the HIV-1 RRE. HIV-1 Rev has recently been shown to specifically recognize the primary sequence in and adjacent to a 5-nt RNA bulge observed in the HIV-1 RRE (1, 11, 37). As this structured sequence is not conserved in the RRE-V, it is not surprising that HIV-1 Rev is also unable to bind to, and function through, this visna virus element. It will clearly be of interest to determine the precise sequence requirements for Rev-V binding to determine whether any aspects of the sequence or structure of the HIV-1 Rev primary binding site have been conserved during the evolutionary divergence of these two distinct viruses. Such comparative analyses may eventually lead to an understanding of the mechanisms that underlie the

sequence specificity of these novel arginine-rich RNA-binding proteins (19, 20) and might also prove useful in the development of specific antagonists of this critical viral regulatory step.

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