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# Identification of a *cis*-Acting Element in Human Immunodeficiency Virus Type 2 (HIV-2) That Is Responsive to the HIV-1 *rev* and Human T-Cell Leukemia Virus Types I and II *rex* Proteins

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**A simian virus 40 late replacement vector encoding human immunodeficiency virus type 1 (HIV-1) gp120 (pGP120) was used to define a region within the HIV-2 genome that could work as a *rev*-responsive element (RRE). Our previous work showed that gp120 expression in this system required a functional RRE in *cis* and required the *rev* protein in *trans* (M.-L. Hammarskjöld, J. Heimer, B. Hammarskjöld, I. Sangwan, L. Albert, and D. Rekosh, J. Virol. 63:1959–1966, 1989). Using pGP120, we first mapped an RRE to a 1,042-base-pair (bp) *Sau*3a fragment in the *env* region of HIV-2. Both HIV-1 *rev* (*revI*) and HIV-2 *rev* (*rev2*) could work in conjunction with this fragment. Further mapping showed that a 272-bp subfragment within the 1,042-bp region was sufficient as an RRE. Surprisingly, the smaller fragment worked only with the *revI* protein and not with its homologous *rev2* protein. In addition, the *rev2* protein failed to function together with the RRE from HIV-1. We also utilized this system to examine the ability of the *rex* genes of human T-cell leukemia virus types I and II to functionally substitute for *rev*. These experiments showed that complementation by both the *rexI* and *rexII* proteins required the presence of an RRE. The *rex* proteins worked well in conjunction with either the HIV-1 or the HIV-2 RRE (the 1,042-bp as well as the 272-bp fragment).**

During the past decade, several human retroviruses have been identified. These can be divided into distinct subfamilies within the retrovirus family. One subgroup consists of the T-cell leukemia viruses, human T-cell leukemia virus type I (HTLV-I) and type II (28, 38, 56); the other consists of the viruses associated with acquired immunodeficiency syndrome, human immunodeficiency virus type 1 (HIV-1) and type 2 (3, 6, 20, 29, 39). DNA sequence analysis has shown that the genetic organization of both groups is quite similar to that of other retroviruses, in that the *gag*, *pol*, and *env* genes are arranged 5′ to 3′ in the genome RNA. In the proviral DNA these genes are flanked by two long terminal repeats (LTRs) (20, 40, 49, 51). However, in HTLV-I and -II, there are two additional genes called *tax* and *rex* which play a major role in the regulation of viral gene expression (5, 15, 25, 46, 48, 50). In HIV-1 and -2, seven additional genes have been identified (24, 36, 55). At least three of these genes, *tat*, *rev*, and *nef*, are involved in gene regulation (1, 2, 13, 16, 19, 53, 54).

The *tax* gene of the HTLVs and the *tat* gene of the HIVs seem to serve analogous functions. Both increase overall viral gene expression and both require a *cis*-acting sequence to be present in the viral LTR (2, 5, 34, 44, 46). Although the mechanisms by which transactivation occurs in the two groups of viruses are not understood in detail, a sequence required for *tax* activation appears to be 5′ of the start of transcription (4, 34, 43, 50, 52), while at least part of the sequence required for *tat* transactivation is 3′ of the start site and present within the viral RNA (26, 37, 44). Complementation between the two genes has not been reported. However, HTLV-I *tax* (*taxI*) has been shown to be able to functionally substitute for HTLV-II *tax* (*taxII*) and vice versa (34, 45). For the HIV group, it has been shown that HIV-1 *tat* (*tat1*) can transactivate the HIV-2 LTR but that HIV-2 *tat* (*tat2*) fails to efficiently activate the HIV-1 LTR (11, 20).

There also appears to be a functional parallel between the HIV-1 *rev* (*revI*) and HTLV-I *rex* (*rexI*) gene products. Both proteins are differential regulators of the viral structural protein genes, and similar mechanisms have been proposed for their modes of action. The products of both genes appear to promote the export of viral structural protein mRNAs from the nucleus and work only if certain sequences are present within the viral mRNAs (9, 12, 14, 21, 22, 25, 32, 33, 47, 48, 50). In HIV-1, this sequence has been called the *cis*-acting region (CAR) (9, 47) or *rev*-responsive element (RRE) (21, 22, 33) and has been mapped to a 242-base-pair (bp) fragment which also contains the coding information for the amino-terminal portion of gp41 (33). In HTLV-I, two *cis*-acting sequences have been mapped: a 5′ splice signal and an element within a 258-bp segment of the 3′ LTR (50).

In a recent study (42), *rexI* was shown to functionally substitute for *revI*. The study did not address whether *rexI* required the HIV-1 RRE to act. Since the *rexI* and *revI* proteins show little sequence homology and the RRE of HIV-1 also has no significant homology with any portion of the HTLV-I genome, the functional equivalence which was demonstrated was rather surprising. This issue has been discussed more fully in a recent review (8).

In the present study we examined the ability of *revI* and HIV-2 *rev* (*rev2*) to functionally complement each other and to identify an RRE in HIV-2. We also confirmed the observation that the *rexI* gene can complement a *revI* defect and showed that the same holds true for HTLV-II *rex* (*rexII*). In addition we showed that this complementation requires a HIV RRE to be present.

## MATERIALS AND METHODS

**Cells and transfections.** CMT3 COS cells (18) were transfected by using DEAE-dextran, as previously described (23).

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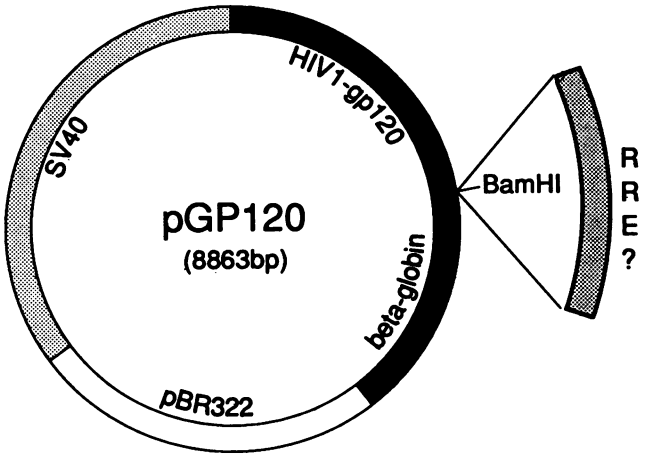


FIG. 1. RRE reporter plasmid pGP120. This construct was made from pSVSX1Δ4 (22) by partial cleavage with *Bam*HI followed by T4 polymerase repair and religation. This removed the *Bam*HI site at the pBR322-SV40 boundary, making the remaining *Bam*HI site unique. The plasmid is composed of sequences derived from HIV-1 (BH10 clone), rabbit β-globin, pBR322, and SV40. It does not express gp120 unless an RRE is inserted into the *Bam*HI site as shown and *rev* function is supplied in *trans*.

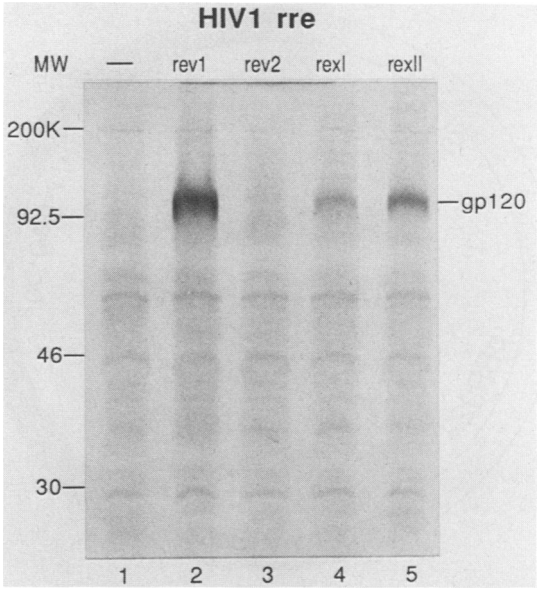


FIG. 2. Western blot analysis of proteins from cotransfections of pGP120-RRE1-r and plasmids which supply *rev* or *rex* function in *trans*. Proteins from transfected cells were separated on a sodium dodecyl sulfate–12% polyacrylamide gel. The blot was developed with a goat serum raised against a fragment of gp120 (p343) synthesized in *E. coli*. Each lane contains extracts from cells cotransfected with pGP120-RRE1-r and plasmids expressing the indicated gene. In lane 3, the plasmid used to express *rev2* was pREV2b. The construction of all plasmids is described in Materials and Methods. The positions of proteins used as molecular weight (MW) markers are indicated (in thousands [K]).

The cells were harvested 65 to 72 h posttransfection and prepared for Western blot (immunoblot) analysis.

**Western blot analysis.** The Western blotting was performed by using Immobilon P membranes (Millipore Corp.) as previously described (22, 23). The serum used for developing the blots was either human serum from an HIV-positive individual or a goat serum directed against gp120 (a kind gift from Tun Ho Lee). The latter serum was produced by using a fragment of gp120 (amino acids 343 to 512) made in *Escherichia coli*.

***rev*- and *rex*-expressing plasmids.** The plasmid used to express *rev1* was pRev1. It contains the *rev* protein-coding coding sequences from an HIV-1 cDNA clone (pCV1) under the control of the promoter-enhancer region from the simian cytomegalovirus IE94 gene (−650 to +30) (27). This plasmid was constructed by inserting a *Bsu*36I (*Mst*II) fragment from pCV1 (2) into the vector pCMV. In pCMV the simian virus 40 (SV40) sequences contained within pBABY (41) have been exchanged for the cytomegalovirus promoter region. The plasmids used to express *rev2* were pRev2a and pRev2b. pRev2a contains a *Dra*I-*Kpn*I fragment (nucleotides 6042 to 9019) from the HIV-$2_{SBL/ISY}$ clone (17) inserted into the T4 polymerase-repaired *Xho*I site of pBABY. pRev2b contains an *Hae*II-*Kpn*I fragment (nucleotides 5861 to 9019) from the same clone inserted into pBABY at the same site. The plasmids used to express the *rex* proteins were pBcRexI and pBcRexII (I. Chen, J. Rosenblatt, and J. Williams, manuscript in preparation). These plasmids were made by inserting HTLV-I and -II cDNA clones containing the *rex* open reading frame downstream of the cytomegalovirus immediate early promoter in the pBC12/CMV vector (provided by Bryan Cullen) (7). These constructs do not express *tax*.

**Computer analysis.** The programs utilized for homology searches and RNA structure prediction were contained within the package supplied by the University of Wisconsin Genetics Computer Group (10).

## RESULTS

In our earlier studies, we showed that an SV40 late replacement vector (pSVSX1) which efficiently expresses the *rev* and *env* genes of HIV-1 could be used to study *rev* function in transfected COS cells (22). *env* gene expression from this plasmid was clearly dependent on the presence of an intact *rev* gene and the RRE, since deletion of either totally abrogated the production of detectable envelope protein. Supplying *rev* in *trans* from a second vector could restore envelope expression, but only if the RRE was present.

We decided to exploit this system to determine if the *rex* and *rev* proteins from the other human retroviruses were capable of functionally substituting for *rev1* protein and also to identify the RRE in HIV-2. To do this we utilized the plasmid pGP120 (Fig. 1). This plasmid is a derivative of pSVSX1, the original vector which expressed *rev* and *env*. pGP120 cannot express *env*, since both the second coding exon of *rev* and the RRE are deleted. However, when a functional RRE is cloned into pGP120 in the correct orientation, gp120 should be expressed if *rev* function is supplied in *trans* from a second plasmid. (pGP120 is identical to pSVSX1Δ4 described by Hammarskjöld et al. [22], except that a second *Bam*HI site at the pBR322-SV40 boundary has been removed to make the remaining *Bam*HI site unique.)

Our initial experiment was designed to assess the abilities of *rev2*, *rexI*, and *rexII* to functionally substitute for *rev1* by using two derivatives of pGP120 which contained the HIV-1 RRE in either the right (pGP120-RRE1-r) or the wrong (pGP120-RRE1-w) orientation. In each case the RRE was provided by the insertion of a 854-bp *Bgl*II-*Bam*HI fragment of HIV-1 (nucleotides 7620 to 8474, BH10 clone, Hxb2cg numbering system [GenBank]). When COS cells were transfected with either of these plasmids alone, no detectable gp120 was produced (Fig. 2, lane 1, for pGP120-RRE1-r;

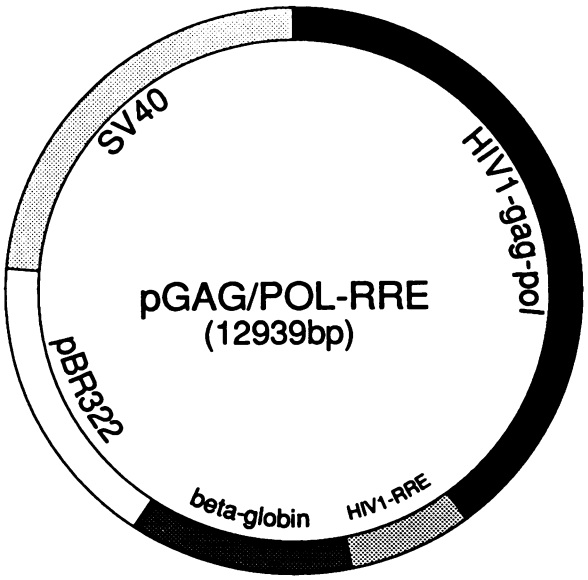


FIG. 3. Expression plasmid pGAGPOL-RRE. The plasmid is composed of sequences derived from HIV-1 and inserted into a previously described (41) shuttle vector (pBABY) containing rabbit β-globin, pBR322, and SV40. The HIV-1 sequences consist of a *Sac*I-*Sal*I fragment (nucleotides 679 to 5785), which contains the *gag*, *pol*, and *vif* genes, and a *Bgl*II-*Bam*HI fragment containing the RRE (nucleotides 7620 to 8474). Although these fragments were derived from the BH-10 clone, the HIV-1 nucleotide numbering corresponds to the reference genome (Hxb2cg) in GenBank.

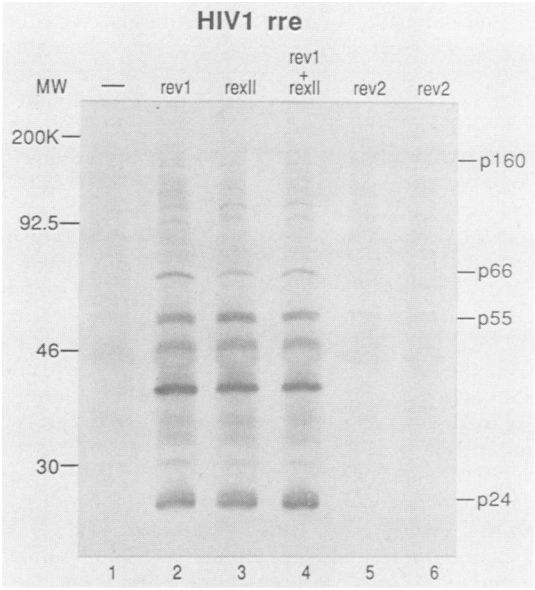


FIG. 4. Western blot analysis of proteins from cotransfections of pGAGPOL-RRE and plasmids which supply *rev* or *rex* function in *trans*. Proteins from transfected cells were separated on a sodium dodecyl sulfate–12% polyacrylamide gel. The blot was developed with serum from an HIV-positive individual. Each lane contains extracts from cells cotransfected with pGAGPOL-RRE and plasmids expressing the indicated gene(s). For *rev2*, two different plasmids were used. In lane 5, the plasmid expressing *rev2* was pREV2a; in lane 6, it was pREV2b (see Materials and Methods). The positions of proteins used as molecular weight (MW) markers are indicated (in thousands [K]).

data not shown for pGP120-RRE1-w). When cells were cotransfected with pGP120-RRE1-r and either a *rev1* (Fig. 2, lane 2), *rexI* (lane 4), or *rexII* (lane 5) protein-producing plasmid, gp120 production was observed. However, cotransfection of pGP120-RRE1-r with a plasmid that produced *rev2* protein failed to yield detectable gp120 (Fig. 2, lane 3). In addition, gp120 could not be detected in cells transfected with pGP120-RRE1-w and any of the *rev*- and *rex*-producing plasmids (data not shown). From this experiment we conclude that both *rexI* and *rexII* can functionally substitute for *rev1* in promoting *env* expression and that they require the presence of the *cis*-acting RRE in the correct orientation. In contrast, *rev2* fails to promote *env* expression in conjunction with the HIV-1 RRE.

In another experiment, we utilized a different SV40 late replacement vector construction (pGAGPOL-RRE; Fig. 3) to examine the ability of *rexII* and *rev2* to functionally substitute for *rev1*. In this plasmid, the region of HIV-1 encoding the *gag* and *pol* genes, and the 854-bp fragment containing the HIV-1 RRE, are cloned downstream of the SV40 late promoter. Rabbit beta-globin sequences positioned after the cloned HIV fragment provide a polyadenylation signal. Expression of *gag* and *gag-pol* gene products from this vector was expected to be *rev* dependent, since it has been shown that *gag* expression, with other vector systems, requires the RRE and *rev* (9, 21, 31). To examine the effects of *rev* and *rex* on expression, pGAGPOL-RRE was transfected into COS cells either alone or together with the *rev* and *rex* protein-producing plasmids. The cells were harvested 65 h posttransfection, and extracts were prepared

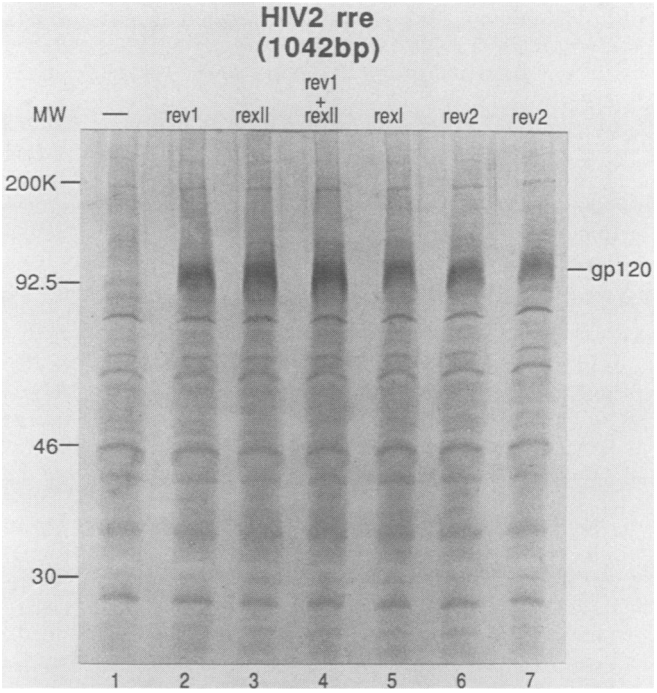


FIG. 5. Western blot analysis of proteins from cotransfections of pGP120-RRE2-1042 and plasmids which supply *rev* or *rex* function in *trans*. Proteins were separated and analyzed as described in the legend to Fig. 2. Each lane contains extracts from cells cotransfected with pGP120-RRE2-1042 and plasmids expressing the indicated gene(s). For *rev2*, two different plasmids were used. In lane 6, the plasmid expressing *rev2* was pREV2a; in lane 7, it was pREV2b (see Materials and Methods). The positions of proteins used as molecular weight (MW) markers are indicated (in thousands [K]).

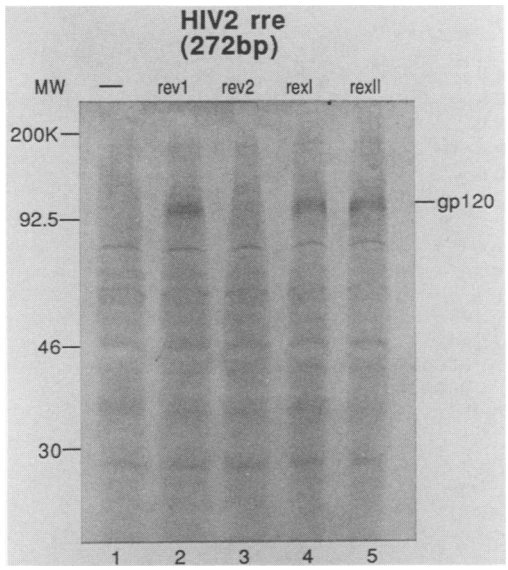


FIG. 6. Western blot analysis of proteins from cotransfections of pGP120-RRE2-272 and plasmids which supply *rev* or *rex* function in *trans*. Proteins were separated and analyzed as described in the legend to Fig. 2. Each lane contains extracts from cells cotransfected with pGP120-RRE2-272 and plasmids expressing the indicated gene. The positions of proteins used as molecular weight (MW) markers are indicated (in thousands [K]).

and examined on a Western blot with serum from an HIV-positive individual (Fig. 4). Little reactivity was observed in the lanes corresponding to the cell extracts from pGAGPOL-RRE transfected alone (Fig. 4, lane 1) or together with plasmids that supplied *rev2* protein (lanes 5 and 6). Strong bands, which corresponded in size to the known products of *gag* and *gag-pol*, were observed in the extracts from cells transfected with pGAGPOL-RRE and *rev1* (Fig. 4, lane 2), *rexII* (lane 3), or *rev1* and *rexII* (lane 4). From these data we conclude that *gag* and *gag-pol* expression from pGAGPOL-RRE was indeed *rev1* dependent and that *rexII* could supply *rev1* function. Interestingly, once again it appeared that *rev2* was unable to substitute for *rev1*. A more detailed description of the products produced from pGAGPOL-RRE will be presented elsewhere (A. J. Smith, M.-I. Cho, M.-L. Hammarskjöld, and D. Rekosh, submitted for publication).

We next addressed the question of whether a region of the HIV-2 genome could function as an RRE in our assay and substitute for the HIV-1 sequence utilized in the experiments described above. For this purpose, a 1,042-bp *Sau*3a fragment (nucleotides 7100 to 8142) from the HIV-$2_{SBL/ISY}$ clone (17) was inserted into the *Bam*HI site of pGP120 in the same orientation as the gp120 gene. The 659 bp at the 3′ end of this fragment are about 55% homologous to the 5′ portion of the 854-bp HIV-1 sequence previously shown to contain the RRE (22). The resulting plasmid, pGP120-RRE2-1042, was transfected into COS cells either alone or together with the various *rev* and *rex* protein-producing plasmids. The cells were analyzed for gp120 production 65 h posttransfection (Fig. 5). gp120 expression could not be detected when pGP120-RRE2-1042 was transfected alone (Fig. 5, lane 1). However, a strong gp120 band was observed when pGP120-RRE2-1042 was transfected together with the plasmids producing *rev1* (Fig. 5, lane 2), *rexII* (lane 3), *rev1* and *rexII* (lane 4), *rexI* (lane 5), or *rev2* (lanes 6 and 7) protein. Thus, we conclude that the cloned *Sau*3a fragment contained the HIV-2 RRE and that, in contrast to the results shown in Fig.

```
  1 CTAGGTTTTCTCACGACAGCAGGTGCTGCAATGGGCGCGCGGTCTCTGAC   ISY
    T-G---------G-A---------T--------------GC---C-----   ROD
    -----------G-A---------T----C---------GC---CT----   NIHZ

    --T--G--CT-GGGAG-------AAGCA-T--------AGCC--AA----   HXB2
    --T--G--CT-GGGAG-------AAGCA-T--------AGC---AA----   BH10
    C-T--G--CT-GGGAG-------AAGCA-G--------A-----AG----   ELI
    --T--G--CT-GGGAG-------AAGCA-G--------AGC---A--A--   MAL
    C-T--G--CT-GGGAG-------AAGCA-T--------AG-C--AA-A--   RF
    --T--G--CT-GGGAG-------AAGCA-G--------A-----AT----   Z2Z6


 51 GCTGTCGGCTCAGTCTCGGACTTTATTCCGTGGGATAGTGCAGCAACAGC   ISY
    CG-------C---------------C-GGCC-------------------   ROD
    ------A--------------------GGCC-------------------   NIHZ

    ----A---TA---G-CA-ACAA-----GTC---T-----------G---A   HXB2
    ----A---TA---G-CA-ACAA-----GTC---T-----------G---A   BH10
    ----A---TA---G-CA-ACAA---A-GTC---T--------A--G--AA   ELI
    ----A---TA---G-CA-ACAG---C-GTC---T--------A--G--AA   MAL
    ---AA---TA---G-CA-ACAC-----GTC---T--------A--G--AA   RF
    ----A---TA---G-CA-ACAG-----GTC---T--------A--G--AA   Z2Z6


101 AACAGCTGTTGGACGTGGTCAAGAGACAACAAGAAATGTTGCGACTGACC   ISY
    ------------------------------------C--------------   ROD
    --------------T-------------------------------------   NIHZ

    -CA-TT--C--AGG-CTA-TG--GCG-----GC-TC------A---C--A   HXB2
    -CA-TT--C--AGG-CTA-TG--GCG-----GC-TC------A---C--A   BH10
    -CA-TT--C--AGG-CTA-AG--GCG-----GC-TC------A---C--G   ELI
    -CA-TT--C--AGG-CTA-AG--GCG-----GC-TC------A---C--G   MAL
    -CA-TT--C--AGG-CTA-TG--GCG-----GC-TC------A---C--G   RF
    -CA-TT--C--AGG-CTA-AG--GCG-----GC-TC------A---C--G   Z2Z6


151 GTCTGGGGAACTAAAAACCTCCAGGCAAGAGTCACTGCTATTGAGAAGTA   ISY
    ----------G-------------------------------A--------   ROD
    -----------A-----T-----------------------A--------   NIHZ

    --------C-TC--GC-G-----A------A--CTA---G-G--A-GA--   HXB2
    --------C-TC--GC-G-----A------A--CTG---G-G--A-GA--   BH10
    --------C-T----C-G------------A--CTG---G-G--A-GA--   ELI
    --------C-T----C-G---------------CTG---G-G--A-GA--   MAL
    --------C-TC---C-G---------------CTG---G-G--A-GA--   RF
    --------C-T----C-G------------A--CTG---G-A----GA--   Z2Z6


201 CCTAGCAGACCAGGCGCGACTAAATTCATGGGGATGTGCGTTTAGACAAG   ISY
    ----CAG----------G-------------------------------   ROD
    ----AAG----------AG------------------------------   NIHZ

    ----AAG--T--ACA--TC---GGGATT-----T--CT-TGGA-A--TCA   HXB2
    ----AAG--T--ACA--TC---GGGATT-----T--CT-TGGA-A--TCA   BH10
    ----AAG--T--ACA--TC---GGAATT-----T--CT-TGGA-A---CA   ELI
    ----CAG--T--ACG--TC---GGAATG-----T--CT-TGGA-A---CA   MAL
    ----AGG--T--ACA--TC---GGAATT--------CT-TGGA-A--TCA   RF
    ----AAG--T--ACA--TC---GGAATT-----T--CT-TGGA-A--TCA   Z2Z6


251 TCTGCCACACTACTGTACCATG      ISY
    ---------------------      ROD
    ----------T----------      NIHZ

    -T---AC----G----G--T--     HXB2
    -T---AC----G----G--T--     BH10
    -T---AC-----A---G--C--     ELI
    -T---AC---ATT---G--T--     MAL
    -T---AC---------G--T--     RF
    -T---AC---------G--C--     Z2Z6
```

FIG. 7. Sequence comparison of the HIV-$2_{SBL/ISY}$ RRE with the corresponding regions of the genomes from other HIV-1 and HIV-2 isolates. The alignments are a composite of the individual comparisons made between the sequence of the *Sty*I fragment of HIV-$2_{SBL/ISY}$ and the sequence of each isolate, using the "Gap" program of the University of Wisconsin Genetics Computer Group (10). –, Nucleotide is the same as in the ISY isolate. HIV-2 clones are ISY, ROD, and NIHZ; all other clones are HIV-1.

2 and 4, *rev2*, as well as *rev1*, *rexI*, and *rexII*, promoted gp120 expression from this vector.

We next wanted to see if we could localize the HIV-2 RRE to a smaller region within the 1,042-bp *Sau*3a segment. The restriction enzyme *Sty*I cuts this sequence twice, generating four fragments which, 5′ to 3′, are 335, 239, 268, and 200 bp in size. A computer homology search of each of these fragments against the HIV-$2_{ROD}$ sequence or the HIV-$1_{HXB2}$ sequence revealed that the 268-bp fragment showed significantly higher homology to both sequences than did any of

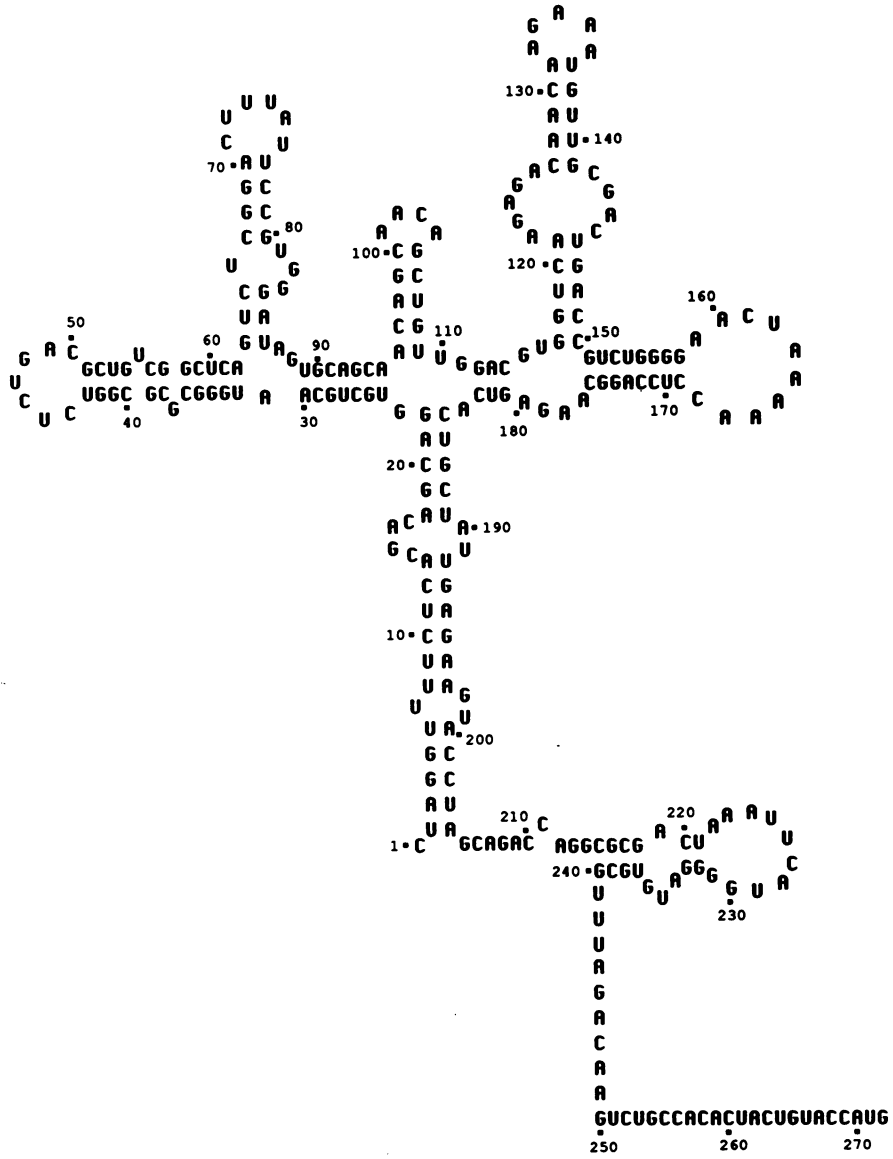

FIG. 8. Computer-predicted secondary structure for the HIV-$2_{SBL/ISY}$ RRE. The sequence was folded by using the "Fold" program of Zuker, as modified for the University of Wisconsin Genetics Computer Group (GCG) program package (10, 57). The diagram is adapted from the output of the GCG "Squiggles" program. The calculated free energy for the structure in the region of the molecule between nucleotides 1 and 240 is −74 kcal (ca. −310 kJ). The region of the molecule between nucleotides 241 and 272 has not been folded, since its predicted structure is not independent of context (see text).

the other fragments. It was 92% homologous to a region in HIV-$2_{ROD}$, compared with values of 87, 85, and 87% for the other fragments, and it was 60% homologous to a region in HIV-$1_{HXB2}$, compared with values of 47, 48, and 51%. This region also corresponded closely to a sequence which was previously identified as the HIV-1 minimal RRE (33). For these reasons it seemed probable that the 268-bp fragment would contain the HIV-2 RRE. To test this directly, the fragment was repaired with T4 DNA polymerase (generating a 272-bp fragment) and cloned into pGP120 to create the plasmid pGP120-RRE2-272, which was then assayed for its ability to produce gp120 in COS cells when cotransfected with plasmids that supplied either *rev* or *rex* protein in *trans* (Fig. 6). As expected, pGP120-RRE2-272 produced no detectable gp120 when transfected alone (Fig. 6, lane 1). However, high levels of gp120 were obtained in cotransfections with either *rev1* (Fig. 6, lane 2), *rexI* (lane 4), or *rexII* (lane 5), indicating that the 272-bp fragment could indeed function as an RRE. Surprisingly, no envelope protein was detected in cotransfections with the plasmid supplying *rev2* protein (Fig. 6, lane 3), indicating a failure of the *Sty*I fragment to be recognized as an RRE with its homologous *rev* protein. This experiment was repeated several times with the same negative result for *rev2* (data not shown).

In Fig. 7, the sequence of the 272-bp repaired *Sty*I fragment from HIV-$2_{SBL/ISY}$ is compared with the corresponding regions of the genomes of several of the other sequenced HIV-2 and HIV-1 isolates. The overall homology among the different HIV-2 sequences was about 92%, as was the overall homology among the HIV-1 isolates. The homology between HIV-1 and HIV-2 in this region was lower, about 60%. Analysis of the alignments revealed that there

were several stretches of sequence which remained totally conserved. It seems likely that at least some of these conserved regions will have functional significance. This hypothesis is strengthened by analysis of the computer-predicted secondary structure of the RNA in this region (Fig. 8) (10, 57). The predicted structure was complex and contained several different areas which were folded into stem-loops. The structure for the region between nucleotides 1 and 243 was the predicted most-stable folding, independent of whether the fragment was analyzed alone, as part of the pGP120-RRE2-272 plasmid, or within its natural context in the larger 1,042-bp *Sau*3a fragment (data not shown). A striking feature of this structure is that four of the totally conserved stretches formed base pairs with each other in the stems of two of the stem-loops (Fig. 8, nucleotides 127 to 142 and nucleotides 151 to 176). Confirmation of the predicted structure and the significance of the conserved stem-loops await further experimentation.

## DISCUSSION

In the experiments described in this article, a plasmid which contained HIV-1 *env* sequences but lacked a functional RRE was used to define a region within the HIV-2 genome that could work as an RRE. We showed that a 1,042-bp fragment of HIV-2 could act in conjunction with the HIV-1 *rev* protein as well as with the HIV-2 *rev* protein to promote *env* expression. However, when a 272-bp subfragment of this fragment was utilized, *rev2* could no longer enhance *env* expression, despite the fact that the smaller fragment still worked well with *rev1*. The *rev2* protein also failed to promote *env* expression when the HIV-1 RRE was used.

In general, the overall homology between the *rev1* and *rev2* proteins is only between 35 and 40%, while the homologies among the different *rev1* proteins and different *rev2* proteins are much higher, about 80 to 90%. In this regard, it should be noted that the HIV-$2_{SBL/ISY}$ *rev2* protein used in this study is predicted to be larger than the proteins from the two other sequenced HIV-2 isolates (ROD and NIHZ). Although it closely resembles the other *rev2* proteins in its first 100 amino acids, DNA sequencing predicts that the HIV-$2_{SBL/ISY}$ *rev2* protein is about 70 amino acids longer than the other *rev2* proteins, due to a lack of a stop codon in the second coding exon of *rev*. Since all three HIV-2 clones are infectious, determination of the roles, if any, that these additional sequences play in *rev* protein function awaits further experimentation.

The nonreciprocal complementation seen between *rev1* and *rev2* parallels that observed with *tat1* and *tat2*, in that *tat1* and *rev1* work with HIV-2 sequences, whereas the HIV-2 proteins do not work with the HIV-1 control elements (11, 20). Since this paper was submitted, similar results were reported for the HIV-$2_{ROD}$ *rev2* protein (30). These results and the fact that the *rev2* protein fails to work on the short HIV-2 RRE suggest that the regulatory proteins of HIV-2 may be more discriminating than those of HIV-1. How this relates to the capacity of these viruses to replicate and cause disease is unclear at this point.

The results of the experiments using the *rexI* and *rexII* proteins clearly showed that *rex* facilitation of HIV-1 *env* expression required the presence of an RRE in *cis*. It was surprising that these proteins were able to work in conjunction with both the HIV-1 and HIV-2 RREs, including the small 272-bp fragment, especially since *rev2* protein failed to work together with the same fragment or with the HIV-1 RRE. These results suggest similar functions and mechanisms of action for *rev* and *rex*. In previous work with this vector system, we showed that HIV-1 *env* mRNA accumulated in the nucleus in the absence of *rev*, allowing the conclusion that *rev* promotes the transport of *env* mRNA from the nucleus to the cytoplasm (22). The fact that we have now been able to obtain envelope protein expression with *rex* clearly indicates that *rex* also facilitates the nuclear export of HIV-1 *env* mRNA. Northern (RNA) blot analysis of RNA from transfected cells supports this conclusion (data not shown).

The role played by *rex* in promoting HIV-1 *env* expression is consistent with its postulated normal function, since recent studies using HTLV-I-infected cells and various expression vectors showed that *rex* promotes nuclear export or stabilization of structural mRNAs. The results showed that this required a specific sequence in the mRNA derived from the 3′ LTR, as well as a 5′ splice signal (50). A 3′ splice signal was not required. In contrast, it has been reported from several laboratories that *rev* function is independent of the presence of a splice donor (14, 33). However, recent results obtained in our laboratories suggest that HIV-1 *env* expression, in our SV40 vector system, requires the presence of the 5′ splice site upstream of *env*, in addition to the RRE (X. Lu, J. Heimer, D. Rekosh and M.-L. Hammarskjöld, manuscript in preparation), making the requirements for *rev* regulation very similar to those reported for *rex* regulation. Thus, viruses in two evolutionarily different retrovirus subgroups seem to exploit a common mechanism to obtain differential regulation of their genes.

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