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Characterization of HIV-1 REV protein: binding stoichiometry and minimal RNA substrate

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ABSTRACT

The HIV-1 REV protein binds to the stem II region of the REV-responsive element (RNA). Studies to further define the RNA sequence and structure specifically bound by REV protein identify a minimal RNA element of 40 nucleotides. Analysis of RNA fragments by gel retardation and filter binding suggest that a core element composed of one particular stem with flanking sequences capable of forming a second double stranded region is essential for specific recognition by REV protein. Stable REV-RNA complexes are formed in a stoichiometry of 1 REV: 1 RNA. The minimal RNA element binds 1 REV molecule while the stem II saturates at 3 REV molecules per RNA. These results establish that REV recognizes a primary binding site within the RRE and support the notion that the initial viral transcript binding event involves a monomeric REV protein.

INTRODUCTION

Lentiviruses encode proteins that regulate viral gene expression. All cytoplasmic viral RNAs are derived by alternative splicing from the same precursor RNA but appear in the cytoplasm at different times after infection. The HIV-1 rev gene encodes a protein which facilitates the transport of unspliced or incompletely spliced RNA into the cytoplasm (1−4). This function results in a switch from early to late gene expression and is essential to the virus replication cycle. Genetic and biochemical studies have indicated that REV binds to an RNA element (REV responsive element, RRE) which must be present for the 'late' RNA to be transported to the cytoplasm. Mutations in specific regions of REV (5, 6) or the cis acting RRE sequence (1, 7) prevent the expression of viral structural proteins.

To understand the mechanism for regulating gene expression by altering the cytoplasmic transport of specific RNA species requires a detailed understanding of REV protein and its cognate RNA. In the absence of REV incompletely spliced viral RNA is retained in the nucleus and interacts with components of the splicing machinery (8, 9). When present REV can facilitate the transport of incompletely spliced RNA to the cytoplasm but it is not understood whether this is accomplished by a blockade of splicing or through activation of a transport mechanism (1−4, 8, 9).

REV protein has been shown to bind *in vitro* with high affinity and specificity to RRE RNA (10−15). The RNA ligand, RRE, is thought to adopt a highly ordered stem-loop structure in which loss of the cis acting transport function can be mapped to a subregion termed stem II (14, 16−18). A competition experiment suggested that purified REV bound within the stem II region of the RRE (14) and recent studies provide evidence for REV binding to RNAs containing all or part of stem II and additional flanking sequences derived from the RRE (13, 15, 18). To refine models of how REV-RRE complex formation may facilitate RNA transport requires identifying a primary binding site(s) and understanding whether this site is recognized by a protein monomer or multimer. Studies described here define a 40 nucleotide subfragment of stem II RNA that is bound by REV specifically and with high affinity. We also demonstrate formation of REV-RNA complexes with a stoichiometry of 1:1.

MATERIALS AND METHODS

Purification and ^{125}I-Labeling of REV Protein

REV was expressed in *E. coli* as a nonfusion protein and was purified by ion exchange and gel filtration chromatography as described previously (10). Purified REV was labeled with [^{125}I] NaI using immobilized chloramine T reagent (Pierce) after pretreatment of the protein with methyl methanethiosulfonate to prevent oxidation of cysteine residues. ^{125}I-labeled REV was isolated from the reaction mixture by gel filtration and quantitated by BioRad protein determinations. The specific activity of the REV was 7×10^3 cpm/pmol; ^{125}I-labeled Rev binds RRE RNA with the same affinity and specificity as unlabeled REV (data not shown).

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Production and ^{32}P-labeling of RNA Fragments

RNAs were synthesized and labeled by incorporation of [α-^{32}P] UTP by transcription *in vitro*. The template for RRE was the Bluescript plasmid bearing a 280 base pair insert corresponding to nucleotides 7333–7612 of the RRE (10) and for RRE Δ41/105 was plasmid pΔ41/105 provided by B. Cullen (14). Templates for *in vitro* synthesis of stem II and the truncated fragments were prepared by PCR amplification from the Bluescript plasmid with the 280 base pair RRE insert. PCR amplifications were carried out under standard conditions using 100 pmoles of each primer and 0.5 pmoles DNA template (19). Each 'forward' PCR primer contained a 5′ extension of 17 nucleotides corresponding to the T7 promoter. The amplified DNAs were used directly as template for *in vitro* transcription by T7 RNA polymerase resulting in RNA containing no additional flanking sequences. *In vitro* transcription reactions contained 40 mM Tris-HCl (pH 8.0), 8 mM $MgCl_2$, 2 mM spermidine, 50 mM NaCl, 30 mM DTT, 400 μM each ATP, GTP and CTP, 200–400 nM PCR-derived DNA template or 2.5 μg of plasmid template, 50 U T7 RNA polymerase and 50 μCi (2.5 nmoles) [α-^{32}P] UTP in a volume of 25 μl. T7 RNA polymerase was purified from *E. coli* strain BL21 bearing plasmid AR 1219 essentially as described (20). After incubation, the reaction products were separated by electrophoresis through denaturing 8% polyacrylamide gels. Full-length RNA transcripts were located by autoradiography, collected by electroelution and concentrated by ethanol precipitation. The RNAs were resuspended in 100 μl 10 mM Tris-HCl (pH7.5), 1mM EDTA. The solutions were heated to 90°C and allowed to cool to 4°C over two hours. To produce large amounts of unlabeled RNA for competition experiments, the reactions were increased in scale 40-fold and the concentration of all nucleotide triphospates was increased to 1 mM. Following transcription, the products were gel purified as described above.

RNA fragments F1, F2 and F3 were chemically synthesized on a Cyclone DNA synthesizer (Milligen/Biosearch) using ribonucleoside phosphoramidites as described (21). After deprotection, the full length products were purified by electrophoresis on denaturing 12% polyacrylamide gels and collected by electroelution. 50 pmol of each RNA was labeled by reaction with 20 μCi [γ-^{32}P] ATP and T4 polynucleotide kinase (21). The labeled RNAs were repurified by electrophoresis as described above and concentrated from the buffer eluate of gel slices by chromatography on a C-18 Sep-Pak columns. RNA fragments were annealed by mixing 2 pmol of the unlabeled oligomers with 85,000 cpm (Cerenkov) ^{32}P-labeled RNA tracer in a total volume of 10 μl containing 50 mM Tris-HCl (pH7.5) and 0.5 mM EDTA. The solutions were heated to 50°C and allowed to cool to 4°C over 2 hours.

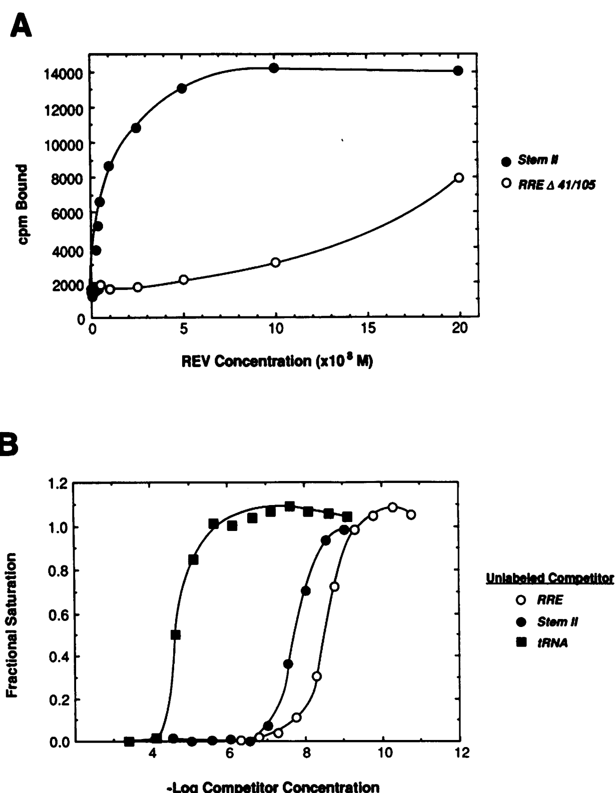

Figure 1: Rev binding to Stem II RNA and an RRE RNA lacking Stem II. (A) Nitrocellulose filter binding assays measuring direct binding of Rev to ^{32}P-labeled stem II RNA (●) and RRE Δ41/105 (○), a RRE RNA with an internal deletion of Stem II. (B) Competition of Rev binding to ^{32}P-labeled RRE RNA by unlabeled RRE RNA (○), Stem II RNA (●) and tRNA (■). Concentrations of competitor RNAs were determined from measurements of absorbance at 260 nm. Fractional saturation is calculated by dividing the cpm retained in the presence of competitor RNA by the cpm retained in the absence of competitor.

RNA Binding Assays

Nitrocellulose filter binding assays were performed by the method of Riggs *et al.*, (22) with minor modifications. Direct binding of REV to stem II or RRE-3 RNAs was measured using 10 fmol of ^{32}P-labelled RNA (10,000 cpm) and REV at concentrations from 0–200 nM in reactions of 500 μl containing phosphate buffered saline, 50 μg/ml BSA, 5 mM DTT, 10 μg tRNA and 5 units of RNasin (Promega). Following incubation at room temperature for 20 minutes, the mixtures were filtered at a rate of 15 ml/minute and radioactivity bound to the filters was measured by liquid scintillation counting.

Nitrocellulose filter binding assays designed to measure the ability of unlabeled RNAs to compete with RRE RNA for binding to REV were performed in 50 μl. The binding reactions using the conditions described above contained 10 nM REV, 10,000 cpm of ^{32}P-labeled RRE RNA (2–20 fmol) and unlabeled competitor RNAs at concentrations from 10^{-3} to 10^{-11} M. To reduce background due to the small reaction volume, the filters were washed once with 0.2 ml phosphate buffered saline.

Gel mobility shift assays were carried out by mixing 10,000 cpm of ^{32}P-labeled RNA (2–20 fmol) and REV at concentrations from 0–50 μM in reaction volumes of 10 μl containing phosphate buffered saline, 1 mM DTT, 10 U RNAsin and 10 μg tRNA. After incubation for 20–30 minutes on ice, the RNA-protein complexes were separated by electrophoresis at 4°C through nondenaturing polyacrylamide gels (29:1, acrylamide; bisacrylamide) run in Tris-borate buffer. Gels were dried and complexes were visualized by autoradiography. RNA fragments F1, F2, F3 and annealed combinations of these fragments were tested for REV binding by gel mobility shift assays containing 2.5 μl of the annealing reactions described above (0.5 pmol of each RNA, 22,000 cpm Cerenkov). Binding reactions were as described above except the amount of tRNA was decreased to 5 μg.

Stoichiometry of REV-RNA complexes

REV-RNA complexes were formed in binding reactions as described for the gel mobility shift assays except that the

concentration ^{32}P-labeled RNA was increased to 1 μM, ^{125}I-labeled REV was present at 3 to 6 μM depending on the experiment and tRNA was decreased to 1 μg. Stem II and F8 RNAs were adjusted to specific activities of 1×10^3 cpm/pmol and REV was iodinated at a specificity activity of 7×10^3 cpm/pmol. Complexes were resolved on 8% polyacrylamide gels cross-linked with DHEBA (N, N'-dihydroxyethylene-bis-acrylamide, Biorad), identified by autoradiography and excised from the wet gel. After solubilizing the gel slices with periodic acid, the radioactivity was measured by liquid scintillation counting. Corrections for quenching and spillover were made and the molar ratios of RNA and REV in each complex were calculated.

RESULTS

REV protein shows high affinity, specific binding to stem II of the HIV-1 RRE

Previous studies quantitatively measured the binding of HIV-1 REV protein to the REV Response Element (RRE), a 367 nucleotide RNA fragment (10). A competition experiment suggested that the REV protein binds specifically to a subregion of the RRE termed stem II (14). To investigate the specificity of REV binding to stem II, quantitative measurements of direct binding to portions of the RRE were performed. Figure 1A shows the protein dependent retention of radiolabeled RNA on filters from binding reactions performed in the presence of an unlabeled nonspecific RNA, tRNA. Stem II RNA was retained on filters by REV at low concentrations with saturation occurring at about 10 nM REV. The equilibrium dissociation constant (Kd) for REV-stem II binding measured in the absence of nonspecific RNA competitor (not shown) is 0.8 nM, a value somewhat higher than the Kd of 0.3 nM for REV-RRE interaction under the same experimental conditions (10). RRE Δ41/105, a full length RRE RNA (Figure 2, top) with stem II deleted, was poorly retained on the filters. Binding was only observed at high concentrations of REV reflecting nonspecific interactions. This result supports the suggestion based on genetic studies and a competition experiment (14) that RRE Δ41/105 is nonfunctional because REV is incapable of specifically binding this fragment.

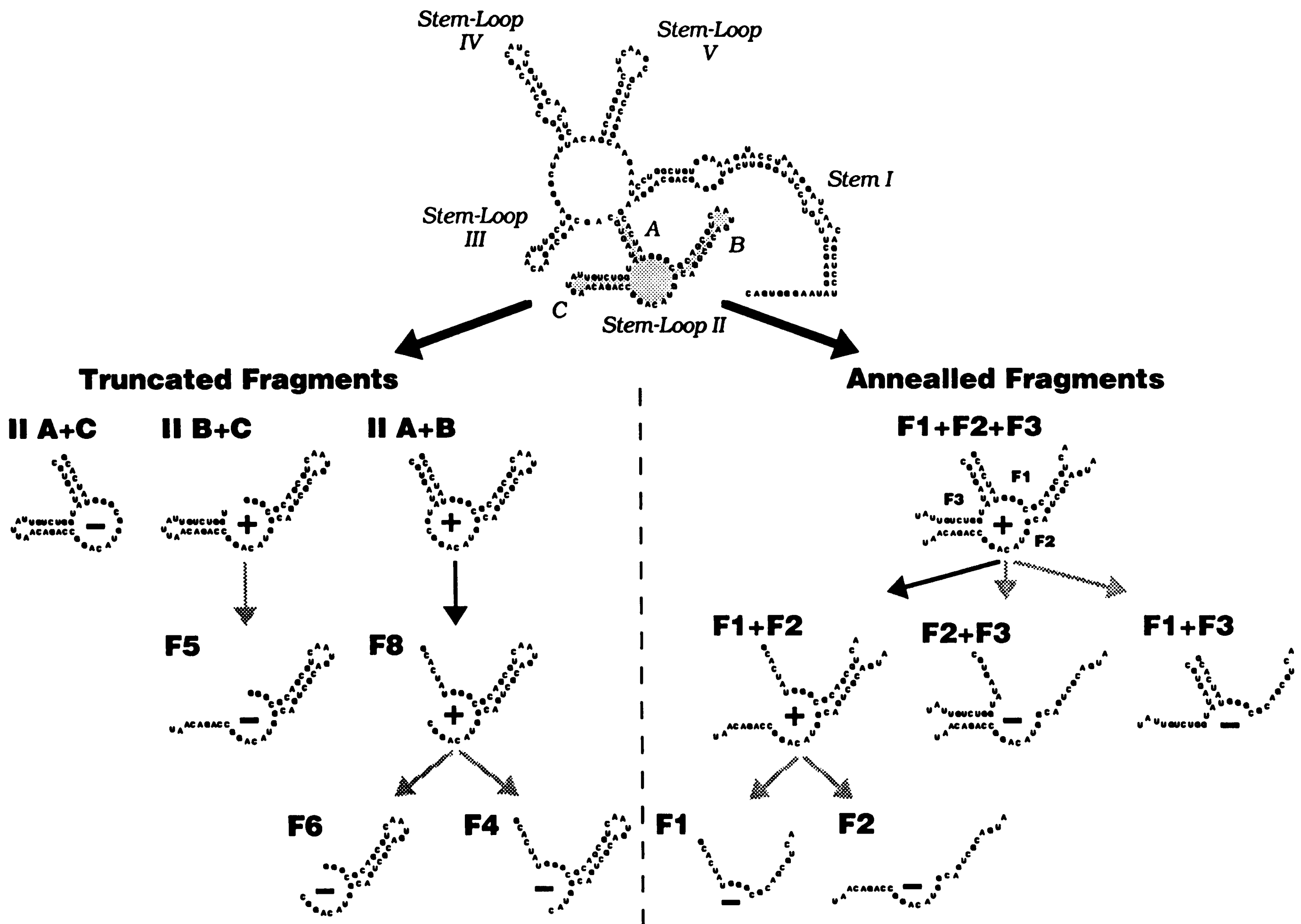

Figure 2: Schematic diagram of the RNAs tested for Rev binding. The top figure shows the predicted secondary structure for the minimal RRE defined by mutational analysis (1). The RRE RNA we have employed has 55 additional nucleotides at the 5' end and 68 additional nucleotides at the 3' end. Stem II is highlighted by shading. Individual deletions of stems A, B or C from stem II gave rise to the truncated fragments IIA+C, IIB+C and IIA+C. 3' deletions of the IIB + C and IIA + B produced RNAs F5 and F8, respectively. Further deletion of 5' and 3' sequences from F8 yielded F6 and F4. Stem II and all truncated RNA fragments were synthesized by *in vitro* transcription of PCR-derived templates. Synthetic RNA fragments F1, F2 and F3 were annealed to produce the structure designated F1+F2+F3. Pairwise annealing reactions are predicted to form the F1+F2, F2+F3 and F1+F3 structures. Plus signs in the structures denote binding by Rev in gel mobility shift assays while minus signs indicate little or no binding.

Another method to compare the relative binding affinities of protein to these RNA fragments is by competition assays performed at equilibrium. Figure 1B shows the results of REV binding to labeled RRE in the presence of various amounts of unlabeled RNA competitors. A nonspecific RNA, tRNA, is approximately 10^4-fold less effective as a competitor on a molar basis than unlabeled RRE fragment. The stem II RNA fragment is about five-fold less effective on a molar basis than RRE, but is bound by REV with approximately 2,000-fold higher affinity than tRNA.

Identification of a minimal RNA element

To further delineate a region of RNA required for specific binding by REV protein, smaller segments of the stem II RNA were constructed and tested for REV binding in gel mobility shift. The first set of truncated RNA fragments (Figure 2, left) are composed of two of the three double-stranded domains in stem II, A, B and C. Each truncated RNA capable of binding REV was made smaller until RNA fragments unable to react specifically with the REV protein were obtained. Figure 3A shows an audio-radiogram depicting the electrophoretic mobility of stem II RNA fragments with or without the addition of 1 μM REV protein. RNA fragments containing the entire stem II, IIA + B or IIB + C were capable of being recognized and bound by the REV protein as indicated by their shifted mobilities. IIA + C, however, was not recognized specifically by the REV protein. IIA + B was decreased in size by removing 9 nucleotides that form the 3′ side of stem II A. The resulting 40 nucleotide fragment, F8, was efficiently recognized and bound by REV. Further deletions of this RNA fragment by removing the other strand of stem IIA producing F6 or by shortening the 3′ end by deletion of four nucleotides yielding F4 resulted in RNA fragments not efficiently bound by REV. Likewise, removing one of the complimentary strands of stem IIC resulted in a RNA fragment (F5) that showed no REV-dependent mobility shift. These results localized the minimal element for RNA binding to the region consisting of the 5′ strand of stem IIA, three G residues, stem IIB and eight flanking nucleotides on the 3′ end.

Similar binding studies were performed with RNA fragments produced by solid phase chemical synthesis. Three fragments were designed so that they could anneal to form all three double stranded regions in stem II and pairwise annealing reactions would produce RNAs with subsets of the three stems (Figure 2, right). The annealed RNAs have scissions in the loops at the ends of

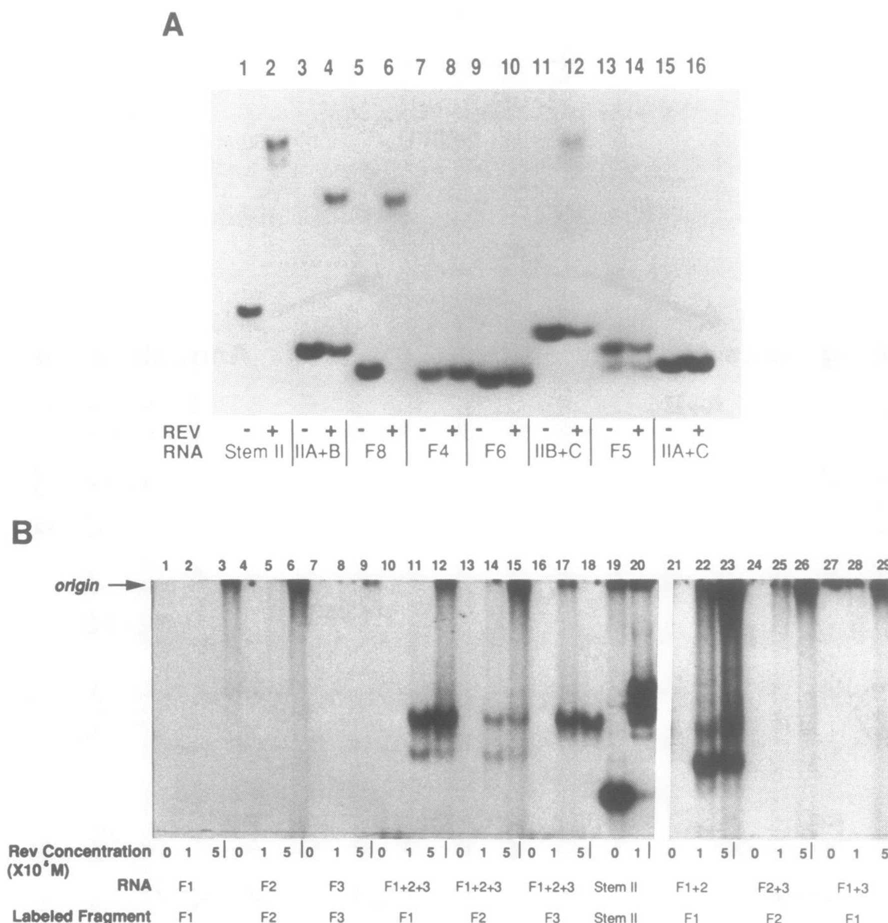

Figure 3: Gel mobility shift analysis of Rev binding to stem II subfragments and annealed RNA fragments. (A) Truncated RNAs were electrophoresed through an 8% polyacrylamide gel after incubation in the absence (−) or presence (+) of Rev at 1 μM. On overexposed autoradiograms, very low amounts of shifted F4 and F6 RNAs are detectable (not shown). (B) RNA fragments F1, F2, F3 and annealed combination of fragments were incubated in the absence or presence of 10 μM and 50 μM Rev. Complexes were separated by electrophoresis through 6% polyacrylamide gels. Unlabeled RNA fragments and the ^{32}P-labeled RNA included in the annealing mixtures, are indicated on the figure. Upon annealing, F1 and F2 RNAs form a species having slightly lower mobility than the individual fragments (not shown), and this species shifted further to a position of lower mobility in the presence of REV. F2 + F3 produce a lower mobility annealed complex (not shown) that fails to bind Rev. An annealed form of F1 + F3 was not detected but may have been masked by the free ^{32}P-labeled F1 RNA in the annealing mixture

stem IIB and stem IIC. As indicated on Figure 3B, reannealing all three RNA fragments to form F1 + F2 + F3 was monitored by labeling each one of the three fragments. Regardless of the RNA fragment used to introduce a labeled tracer, an annealed RNA species was bound by Rev and shifted to a position of lower mobility (lanes 11, 12, 14, 15, 17 and 18 versus lanes 10, 13 and 16). This result suggests that all three RNA fragments are present in the complex corresponding to the major band. The minor band evident in lanes 11, 12, 14 and 15 may result from a complex composed of REV and RNAs F1 and F2 but not F3 since this band is absent when RNA F3 is the labeled tracer (lanes 17 and 18). The species produced by reannealing of RNAs F1 and F2 (forming a species similar to F8) was bound by the REV protein (lanes 22 and 23), while annealing of the other fragment pairs (F2 + F3 or F1 + F3) did not result in a mobility shift by REV protein. None of the fragments bind REV individually.

Table 1. Relative Affinity of REV Protein for RRE, Stem II and Stem II Fragments.

Competitor RNAs	Concentration of Competitor RNA Required for 50% Decrease in RRE-REV Binding
RRE	3×10^{-9} M
F8	1×10^{-8} M
Stem II	2×10^{-8} M
IIA + B	3×10^{-7} M
F4	5×10^{-7} M
IIB + C	6×10^{-7} M
F6	2×10^{-6} M
F5	3×10^{-6} M
IIA + C	1×10^{-5} M
tRNA	2×10^{-5} M

Competition filter binding assays were performed using ^{32}P-labeled RRE, 10nM REV and varying concentrations of the unlabeled RNAs listed above. The concentration of unlabeled competitor required to decrease by 50% the REV dependent retention of RRE was determined from the midpoint of the competition curves.

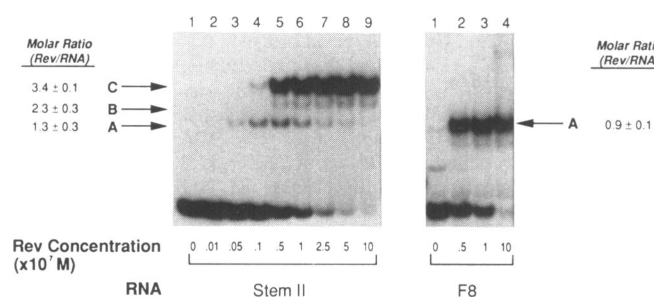

Figure 4: Rev concentration dependence of complexes formed with Stem II and RNA F8. Gel mobility shift assays were performed with Rev concentrations from 0–10 μM and ^{32}P-labeled RNA. Complexes were resolved as described in the legend to Figure 3(A) and R_f values calculated from the autoradiogram shown. Molar ratios of Rev to RNA in each complex were determined from parallel experiments (not shown) performed with ^{125}I-labeled Rev, ^{32}P-labeled RNAs and solubilizable polyacrylamide gels. Ratios of 1.3 ± 0.4, 2.3 ± 0.3 and 3.4 ± 0.1 for complexes A, B and C, respectively, are averages from four determinations. For the single complex formed on F8, the ratio of 0.9 ± 0.1 is based on eight samples. Single label experiments using the same protein and RNA concentrations were performed. The amount of Rev in a complex of ^{125}I-Rev and unlabeled F8 RNA was compared with the amount of RNA in a complex of ^{32}P-labeled F8 and unlabeled Rev gave a ratio of 1.1 ± 0.1, confirming the results from the double label experiments.

These results are consistent with the experiments on stem II subfragments which demonstrate the requirements of one portion of stem IIA, stem IIB and some flanking sequence to the 3′ side of stem IIB for binding by the REV protein. In addition, annealing of the fragments shows that an intact loop on stem IIB is not necessary for recognition by REV.

Truncated RNAs derived from stem II that are unable to bind Rev with sufficient affinity for ready detection by gel mobility shift assay may still bind Rev with some affinity greater than that for a nonspecific RNA. Our observation of shifted F4 and F6 RNAs on overexposed autoradiograms from the experiment shown in Figure 3A supports this suggestion. To address this possibility, the efficacies of RNA fragments as competitors of Rev binding to RRE RNA were measured by nitrocellulose filter binding assays. The molar concentrations of each RNA fragment required to decrease Rev binding to ^{32}P-labeled RRE RNA by 50% are reported in Table 1. The truncated RNAs can be grouped in three categories as follows: 1) F8 and stem II are very effective, competing at concentrations within ten fold higher that of RRE RNA, 2) IIA + B, IIB + C, F4, F5 and F6 are intermediate in efficacy requiring 100 to 1,000 times more RNA than RRE, and 3) IIA + C, the only fragment lacking Stem IIB, does not compete until added at a very high concentration comparable to that needed for competition by tRNA, a nonspecific RNA.

Stoichiometry of HIV-1 REV binding to RNA

Previous studies using filter retention to measure stoichiometry showed that approximately eight REV molecules bind to one RRE molecule (10). In agreement with the stoichiometry measurements, gel mobility shift assays revealed a number of low mobility REV-RRE complexes (10) in addition to the major complex which had the lowest mobility and presumably represented saturated RRE RNA. Gel mobility shift experiments with smaller RNA fragments produced simpler profiles. Figure 4 shows titrations of REV protein with stem II and F8 RNA fragments. The autoradiogram reveals three distinct complexes with stem II, denoted A, B, and C, having step-wise decreases in electrophoretic mobility. The 40 nucleotide fragment, F8, resulted in a single, predominant low mobility species in the gel. These gel patterns would be consistent with the stem II binding three REV molecules and the smaller RNA, F8, binding but one. Alternatively, each binding event and resulting complex may involve a multimeric form of Rev protein. In addition, stem II complexes A, B and C could all have the same stoichiometry but differ in their conformations. To distinguish among these possibilities the following experiment was done.

The ratio of protein to RNA was measured in complexes formed with ^{125}I-labeled REV and ^{32}P-labeled stem II or F8 RNAs. After electrophoresis the complexes were excised from the gel and the amount of each isotope was determined by liquid scintillation counting. The molar ratios of protein to RNA in each complex are indicated on Figure 4. The single REV-F8 complex contains equimolar amounts of protein and RNA. Similarly, the highest mobility stem II species, complex A, also has a molar ratio of one. Stem II complexes B and C are composed of REV and RNA in molar ratios of 2 and 3, respectively.

DISCUSSION

Studies presented here demonstrate high affinity binding of REV to stem II RNA and to subfragments derived from stem II. Truncated fragments created by deletion of an individual double

stranded domain bound REV when stem IIB was retained in the RNA. Further reduction in RNA size to a 40 nucleotide fragment produced F8, a fragment capable of binding REV with high affinity. This fragment represents a minimal RNA containing essential elements required for REV binding since further deletion of either seven nucleotides from the 5′ side or four nucleotides from the 3′ side eliminated REV binding in gel shift assays. In parallel experiments an RNA containing stem IIB produced by annealing two fragments also bound REV. The REV binding RNAs identified through these approaches, IIA + B, IIB + C, F8 and F1 + F2, share the following features: 1) stem IIB, 2) the trinucleotide GGG immediately 5′ of stem IIB and 3) either a second stem in the cases of IIA + B and IIB + C or flanking sequences capable of forming additional double stranded regions. In the case of F8, a secondary structure prediction (not shown) using the Zuker RNA Fold program (23) reveals the possibility of forming six noncontiguous base pairs in the sequences flanking stem IIB. Deletion of only 4 nucleotides from the 3′ end of F8 results in F4, an RNA incapable of binding REV and lacking four of the base pairs predicted to occur in the flanking sequences of F8. The fragment reannealing experiments demonstrate that the minimal RNA need not contain an intact loop at the base of stem IIB, an observation consistent with the nonessential role of this loop *in vivo* (14, 16, 18). Further studies are underway to precisely map points of contact between F8 RNA and REV. Mutational analysis of nucleotides found to interact with REV will define the contributions of specific sequences to REV binding *in vitro*.

Heaphy *et al.* (13) predicted that REV interacts with a 71 nucleotide fragment defined by the boundaries of overlapping 96 and 98 nucleotide REV-binding RNAs. F8 is fully contained within the 71 nucleotide fragment and it occurs at the 5′ end, a region proposed by the authors to contain essential elements for REV binding. Other studies of REV binding *in vitro* have examined the role of stem II in the context of the complete RRE (17) or in subfragments of the RRE (15, 18). Single or compensatory substitutions in stem II localized binding to stem B (17) or stem B and part of stem A (18). Our *in vitro* binding studies as well as those cited above correlate well with genetic studies (14, 17, 18) showing that stem II is essential for REV function *in vivo*. The minimal RNA element necessary for *in vivo* Rev function appears to contain stem II but requires additonal flanking sequence as well (24). Equilibrium binding constants and competition experiments reveal REV to have a slightly higher (5 fold) affinity for the complete RRE than for the stem II fragment. This difference may reflect added stability afforded to stem II RNA in the context of the entire RRE. Interestingly, F8, binds REV with an affinity comparable to that for intact stem II. High affinity binding to F8 indicates the presence of a primary binding site for REV within this 40 nucleotide fragment.

Stoichiometry measurements define the composition of REV-RNA complexes. Upon incubating F8 with REV the predominant complex consists of equimolar amounts of RNA molecule and protein. The three complexes formed with stem II RNA differ by one REV molecule per RNA with the lowest mobility complex having a protein to RNA ratio of 3. Additional studies with RRE (367 nts) gave a ratio of 8 in agreement with previous stoichiometry measurements (10). The simplest explanation of these results is that REV binds as a monomer to a primary site in F8 or stem II RNAs. It is likely that additional monomers of REV then bind to the longer RNA, stem II, giving rise to the slower mobility complexes. However, it is also possible that oligomers of REV may specifically bind to RNA. The stoichiometry experiments described here suggest that REV protein oligomerization is not a prerequisite for specific, high affinity binding to RRE.

The composition and mode for assembling REV-RNA complexes that are functional in RNA transport *in vivo* is unknown. Olsen *et al* (25) have recently presented studies correlating multimerization of REV with binding to RNA *in vitro* and function of the REV mutants *in vivo*. If multimerization of REV in solution reflects the ability of REV to polymerize on an RNA substrate *in vivo*, their findings support the notion that the biologically relevant REV-RNA species is composed of multiple REVs on RRE-containing RNA. Such functional complexes may be formed after initial binding of monomeric REV and RNA. Our studies delineating a minimal RNA element and demonstrating *in vitro* binding of REV as a monomer provide the basis for further characterization of the RNA site recognized by REV. These and subsequent studies may facilitate development of specific inhibitors of REV binding to RNA.

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