Proc. Natl. Acad. Sci. USA
Vol. 88, pp. 7366–7370, August 1991
Biochemistry

Human immunodeficiency virus type 1 regulator of virion expression, rev, forms nucleoprotein filaments after binding to a purine-rich "bubble" located within the rev-responsive region of viral mRNAs

(RNA–protein interaction/RNA stem-loop structure/regulation of splicing/filament assembly)

SHAUN HEAPHY*, JOHN T. FINCH, MICHAEL J. GAIT, JONATHAN KARN, AND MOHINDER SINGH

Medical Research Council Laboratory of Molecular Biology, Hills Road, Cambridge CB2 2QH, United Kingdom

Communicated by Aaron Klug, May 30, 1991

ABSTRACT **The human immunodeficiency virus type 1 rev protein binds with high affinity (K_d < 1–3 nM) to a purine-rich "bubble" containing bulged GG and GUA residues on either side of a double-helical RNA stem-loop located toward the 5′ end of rev-response element RNA. High-affinity rev binding is maintained when the bubble is placed in heterologous stem-loop structures, but rev binding is reduced when either the bulged residues or flanking base pairs in the stem are altered. Rev binding to the purine-rich bubble nucleates assembly of long filamentous ribonucleoprotein structures containing polymers of rev bound to flanking RNA sequences. It is proposed that rev regulates human immunodeficiency virus RNA expression by selectively packaging viral transcripts carrying the rev-response element sequence into rod-like nucleoprotein complexes that block splicing of the packaged mRNAs.**

Transcription of human immunodeficiency virus type 1 (HIV-1) begins with the production of short fully spliced mRNAs encoding the regulatory proteins tat and rev. As the infection progresses, the combined activity of the *rev* gene product and a cis-acting RNA sequence called the rev-responsive element (RRE) allows the expression of the unspliced virion RNA and partially spliced mRNAs, including the mRNA for the *env* gene (1–3). It is still unknown whether rev blocks splicing directly (4) or whether it increases the rate of transport of unspliced mRNAs from the nucleus (3).

Recent experiments have shown that the interaction between rev and RRE RNA is due to direct binding (5–7). Here we demonstrate that rev binds with high affinity to a purine-rich "bubble" containing GG and GUA residues on either side of a double-stranded stem-loop structure present in RRE RNA. Additional rev molecules can then polymerize and form long filamentous ribonucleoprotein structures in association with the RNA sequences that flank the high-affinity binding site. These observations suggest that rev regulates HIV mRNA expression by packaging unspliced mRNA precursors carrying RRE sequences into protein coats, thus preventing access to the splicing machinery.

MATERIALS AND METHODS

Expression and Purification of Rev. Rev protein was expressed and purified as described (7) or further purified by chromatography on heparin-Sepharose. Rev was applied to heparin columns in a buffer containing 200 mM NaCl/50 mM Tris·HCl, pH 8.0/1 mM dithiothreitol/0.1 mM EDTA/0.1% Triton X-100 and eluted in buffer containing 2 M NaCl. After gel filtration on Superose 6 (prep grade) columns equilibrated with 200 mM NaCl/50 mM Tris·HCl, pH 8.0/1 mM dithiothreitol/0.1 mM EDTA, the rev protein appeared homogeneous on SDS/polyacrylamide gels and free of RNA contaminants. Rev concentrations were determined by amino acid analysis of the purified protein.

RNA-Binding Assays. DNA inserts containing RRE-related sequences were cloned between the *Eco*RI (5′) and *Hin*dIII (3′) sites of pGEM-1, either by cloning PCR products or by cloning hybridized pairs of synthetic oligonucleotides. RNA transcripts for binding experiments were prepared by transcription of *Hin*dIII-cut plasmids with T7 RNA polymerase and purified by gel electrophoresis (7). These transcripts carry a 5′ extension of 15 nucleotides contributed by the vector and an extra adenine residue at the 3′ end from the *Hin*dIII site. Filter-binding reactions were done essentially as described (7, 8) by using RNA, rev, and salt concentrations as indicated in the figure legends.

Filament Formation. Protein filaments were grown from solutions containing 20–100 μg of rev protein per ml in 20 mM Tris·HCl, pH 7.4/50 mM NaCl/1.0 mM dithiothreitol by slow warming from 4°C to 25°C for a period of several hours. Protein filaments are not formed with rev exposed to Triton X-100. Complexes between rev protein and RNA transcripts carrying an RRE sequence at the 5′ end were formed using RNA at 0.01–0.1 μg/ml, and rev protein at 22–110 μg/ml in 20 mM Tris·HCl, pH 7.4/50 mM NaCl/0.1 mM EDTA/1.0 mM dithiothreitol/RNasin at 20 units/ml at 30°C for 1 hr. Complex formation was monitored by gel-mobility shift assays (7), sucrose gradient centrifugation, and electron microscopy. Filaments were negatively stained with uranyl acetate and photographed at a magnification of ≈50,000.

RESULTS

RRE RNA Contains a High-Affinity Rev-Binding Site. In our previous paper we reported that as the concentration of rev increases, progressively larger complexes with RRE RNA are formed, whereas rev is unable to form stable complexes with anti-sense RRE and other RNA sequences (7). This experiment, which has recently been repeated by others (9), strongly suggested that rev binds initially to a high-affinity site on the RRE and that subsequently additional rev molecules occupy adjacent sites. We have now shown that these additional rev molecules bind to the RRE RNA with lower affinity. As shown in Fig. 1, the Scatchard plot for rev binding to RRE RNA is nonlinear, whereas a protein that forms

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: HIV-1, human immunodeficiency virus type 1; RRE, rev-response element.

*Present address: Department of Microbiology, University of Leicester, Leicester, LE1 9HN, United Kingdom.

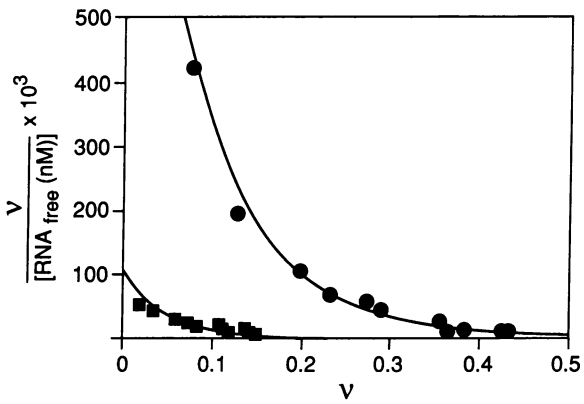

FIG. 1. Scatchard analysis of rev binding to a 225-nucleotide-long RRE RNA sequence. Binding reactions were done in buffers containing 50 mM Tris·HCl (pH 8.0), 1 μg of sonicated salmon sperm DNA, 0.45 μg of yeast tRNA, 20 units of RNasin (Promega), and either 200 mM (●) or 50 mM (■) KCl. Reactions at 200 mM KCl contained 9–10 nM rev and between 1 and 90 nM RRE RNA. Reactions at 50 mM NaCl contained 30–34 nM rev and between 1 and 90 nM RRE RNA. Data are plotted as the stoichiometry, ν (the ratio of the concentration of bound RNA to rev protein; abscissa), versus the ratio of ν to the free RNA concentration (ordinate).

one-to-one complexes with RNA, such as tat, produces a linear Scatchard plot (8).

We have estimated the K_d for high-affinity rev binding by a linear regression analysis of the high-affinity data. At 50 mM KCl ($\nu > 0.28$) there is a site to which rev binds with an apparent K_d of 2 ± 0.6 nM (50 mM KCl). At 200 mM KCl ($\nu > 0.10$) the K_d for high-affinity binding is 4 ± 1.0 nM. Both these values are consistent with previous estimates of a K_d of between 1.0 and 3.0 nM obtained from saturation-binding experiments (5, 7). However, it should be noted that estimates of K_d by any simple binding experiment that uses labeled RNA as a probe will include the contributions of both the high-affinity site and the adjacent lower-affinity sites (10).

The stoichiometry of rev binding to RRE RNA is highly dependent on ionic strength. At 50 mM KCl between six and eight rev monomers bind to the RRE RNA, whereas the stoichiometry of binding is ≈2:1 at 200 mM KCl (Fig. 1). In agreement with previous reports (11), we have found that rev elutes from gel filtration columns equilibrated with 200 mM NaCl with an apparent mass of 60 kDa (data not shown). These results suggest that rev exists in solution as a small oligomer, most likely a tetramer (11, 12), that can bind to RNA.

The Purine-Rich Bubble. A complicated stem-loop structure for RRE RNA has been proposed by Malim *et al.* (3) based on the RNA-folding programs of Maizel (13). However, the RNA-folding programs of Zuker (14) predict that a more stable structure could be formed by including the pairing of U^{33} and G^{34} with C^{62} and A^{63} on one stem and G^{65} with U^{85}, respectively, on an adjacent stem. As shown in Fig. 2, these base pairs create a purine-rich bubble.

The structure shown in Fig. 2 is more consistent with the nuclease-protection and chemical-probing data reported by Kjems *et al.* (9) than the model proposed by Malim *et al.* (3). For example, G^{59}, G^{64}, and G^{65} are strongly modified by kethoxal, whereas the residues G^{34}, G^{35}, and G^{36} are only weakly modified, and none of these residues is susceptible to cleavage by ribonuclease T1. In the structure proposed by Zuker (14), G^{34}, G^{35}, and G^{36} are stacked on one side of the bulge, whereas G^{59}, G^{64}, and G^{65} appear more accessible. In the original model, all these residues appeared in a large open loop. Furthermore, A^{32} and A^{86}, which are readily modified by diethylpyrocarbonate (9), now appear as bulged residues

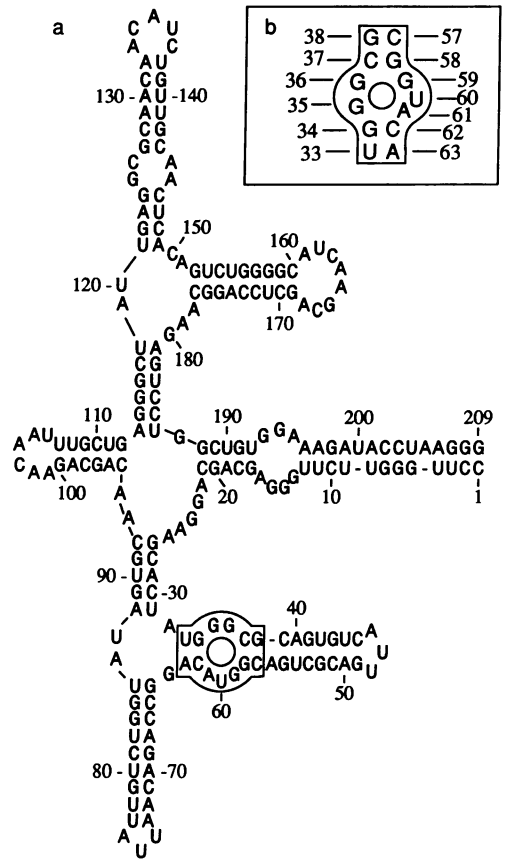

FIG. 2. (*a*) Secondary structure for the RRE region of HIV-1_{ARV-2} (residues 7786–8010) predicted by the RNA-folding programs of Zuker (14). Residue 1 of the RRE is the first nucleotide of the *Sty* I site used originally to define the location of the RRE sequence (3). This residue corresponds to residue 12 according to the numbering system of Kjems *et al.* (9). Our model differs from that of Malim *et al.* (3) because the pairings of U^{33}, G^{34}, and G^{65} with A^{63}, C^{62}, and U^{85}, respectively, allow formation of the purine-rich bubble. Base pairing between residues A^{113} and U^{118} and between A^{181} and U^{186} is allowed for the HIV-1_{ARV-2} sequence shown above, but this feature is absent in the HIV-1_{HXB2} sequence analyzed by Malim *et al.* (3). (*b*) Structure of the purine-rich bubble sequence.

in the structure, whereas in the original proposal these residues were base-paired.

Rev Binds to the Purine-Rich Bubble with High Affinity. Truncated RRE RNA transcripts containing the rev-binding site can bind to rev with a dissociation constant similar to that of full-length RRE (i.e., K_d = 1–3 nM). We have previously mapped the high-affinity rev-binding site to be between residues A^{26} and A^{96} by constructing a series of short RNA sequences spanning the RRE (7). Continuing this approach, we have now found that the shortest T7 transcript that can bind rev with a K_d of 1–3 nM includes RRE sequences beginning at U^{33} and ending at C^{66} (R19). This sequence is predicted to fold into the stable stem-loop containing the purine-rich bubble shown in Fig. 3*a*. The structure is stabilized by a 4-bp stem below the bubble, which contains CU residues derived from the T7 leader sequence.

Only the boxed sequences in the bubble region are required for rev binding. Deletion of the bulged A, at residue 56 (R22, Fig. 3*b*), or replacement of the entire upper stem sequence with the stable RNA hairpin-loop sequence CUUCGG [R38, Fig. 3*c*, (15)] produced transcripts that bound rev with K_d = 1–3 nM. Normal rev binding was also seen when the purine-rich bubble was inserted into an elongated RNA stem-loop structure (Fig. 3*d*, R33).

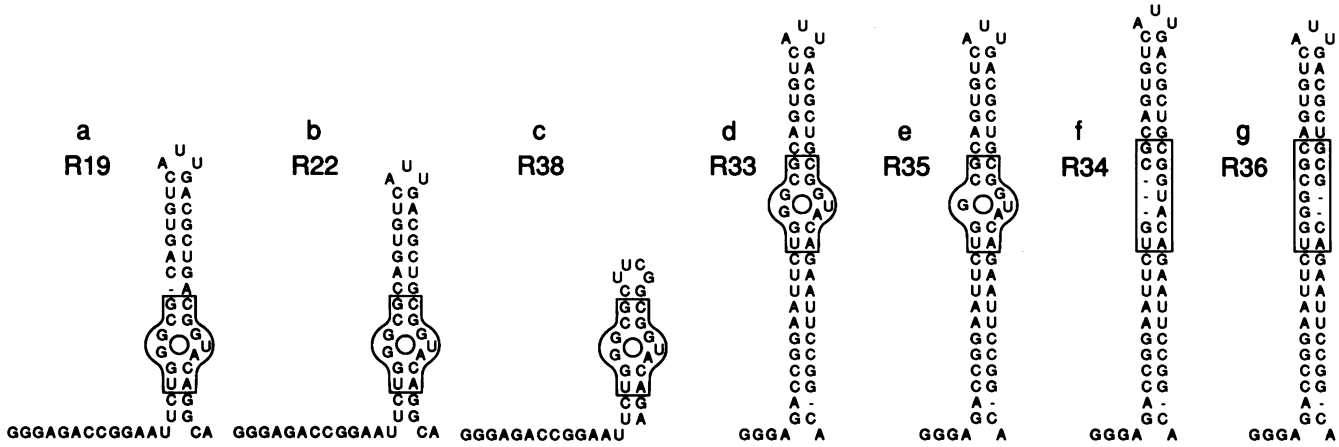

FIG. 3. Representative RNA stem-loop structures assayed for rev-binding. The sequences shown represent the full transcripts including residues derived from vector sequences. The optimal structures predicted by the method of Zuker (14) are shown, with the purine-rich bubble boxed. (*a*) Transcript R19, containing RRE sequences 33–66. (*b*) R22, residue A^{56} is deleted from R20. (*c*) R38, the stem-loop structure C^{39} to A^{56} is replaced by the stable loop sequence CUUCGG (15). (*d*) R33, the stem-loop formed by residues 33–63, with residue A^{56} deleted, was inserted on top of a stem containing 9 base pairs (bp). (*e*) R35, the purine-rich bubble is replaced by an altered sequence carrying a single bulged guanine residue on the 5′ side. (*f*) R34, the bulged residues G^{35} and G^{36} are deleted. (*g*) R36, the bulged residues G^{59}–A^{61} are deleted.

Sequence Requirements for Rev Binding. Because the R33 RNA is in a very stable predicted conformation ($\Delta G^{\circ} = -29.6$ kcal/mol), we were able to introduce deletions and substitutions within the purine-rich bubble region of R33 RNA without disrupting its overall structure (Fig. 3 *e–g*; Table 1).

The bulged residues on both sides of the purine-rich bubble are required for specific rev binding. Deletion of G^{59}, U^{60}, and A^{61} created a structure with a GG bulge on one side of the helix (R36, Fig. 3*g*) and resulted in loss of rev binding. Deletion of G^{35} and G^{36} from the other side of the helix (R34, Fig. 3*f*) or replacement of these residues with bulged adenine residues (R37, Table 1) also abolished specific rev binding. Nucleotide substitutions (Table 1) are tolerated at the U^{60} residue (R52) and at G^{36} (R50), but deletions or substitutions affecting the other bulged residues resulted in complete loss of specific rev binding. For example, replacement of G^{35} with adenine (R47) or A^{61} with guanine (R53) abolished rev binding. Deletion of G^{35} alone only reduced rev binding slightly (R35).

Table 1. Mutagenesis of the rev binding site

	Mutation	K_d, nM	RNA bound, % (10 nM rev)
A. Normal rev binding			
R7	wild type	3	50
R33	wild type	3	40
R57	G^{38}·C^{57} → A·U	3	40
B. Reduced rev binding			
R35	ΔG^{35}	4	35
R50	G^{36} → A	5	20
R52	U^{60} → C	5	18
R39	U^{33}·A^{63} → A·U	8	15
R54	U^{33}·A^{63} → C·G	6	17
R55	G^{34}·C^{62} → A·U	4	21
R42	G^{38}·C^{57} → C·G	8	18
C. Nonspecific rev binding			
R49	ΔG^{59}		2
R45	ΔU^{60}		6
R46	ΔA^{61}		1
R34	ΔG^{35}–G^{36}		3
R36	ΔG^{59}–A^{61}		4
R58	G^{59} → A		6
R53	A^{61} → G		5
R37	G^{34} → A; C^{35} → A; C^{36} → A; C^{62} → U		5
R47	G^{35} → A		6
R40	G^{34}·C^{62} → C·G		3
R56	C^{37}·G^{58} → U·A		6
R41	C^{37}·G^{58} → G·C		2

Filter binding assays contained 20 pg of uniformly labeled RNA probe (500 dpm per pg of RNA), 1 μg of salmon sperm DNA, 0.45 μg of yeast tRNA, and 40 units of RNasin (Promega) in 500 μl of buffer containing 43 mM Tris·HCl, pH 8.0, and 50 mM KCl. Mutations in the purine-rich bubble sequence (numbered as shown in Fig. 2) were introduced into the R33 stem-loop structure (Fig. 3*d*) by site-directed mutagenesis.

The 4 bp immediately adjacent to the bulged residues in the purine-rich bubble are also important for rev binding (Table 1). Replacement of C^{37}·G^{58} with a G·C bp (R41) or replacement of G^{34}·C^{62} with a C·G bp (R40) abolished specific rev binding. Alterations to the other base pairs in the bubble region also reduced rev binding significantly (Table 1). The only neutral mutation that we discovered was in R57, in which the G^{38}·C^{57} bp is replaced by an A·U bp.

The relative affinities of rev for the R33 transcript, mutants in the rev-binding site, and full-length RRE were also compared by competition experiments (Fig. 4). Unlabeled RRE RNA was an effective competitor and reduced rev binding to

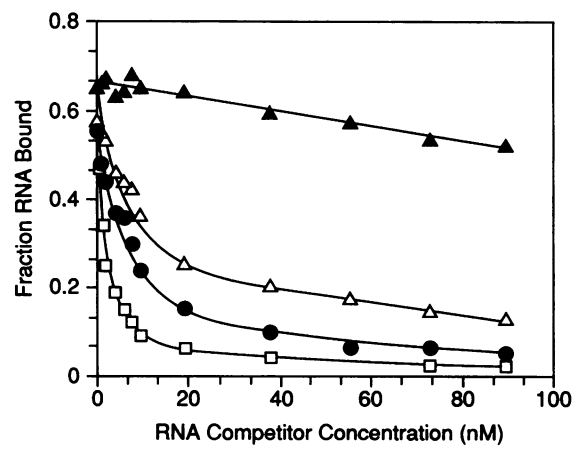

FIG. 4. Competition for rev binding to RRE RNA. Filter binding reactions contained 17 nM rev, 0.5 pM labeled RRE RNA (Fig. 2), and between 0 and 100 nM unlabeled competitor RNA. □, RRE RNA competitor; ●, R33 RNA competitor; ▲, R34 RNA competitor; and △, R35 RNA competitor.

the labeled RRE RNA by 50% of the initial value at a competitor concentration of 2 nM ($D_{1/2}$). The short R33 transcript was also an effective competitor and reduced rev binding with $D_{1/2}$ = 8 nM.

The mutations in the bubble either reduce or abolish specific rev binding. For example, R34, which carries a deletion of the bulged G residues G^{35} and G^{36}, does not bind rev with measurable affinity and did not compete efficiently for rev binding against the RRE ($D_{1/2}$ > 250 nM). R35, which has a bulge containing a single residue, and is typical of a mutation with reduced rev affinity had a K_d of 4 nM and showed intermediate competition behavior ($D_{1/2}$ = 16 nM).

Filament Formation by Rev. At concentrations >100 μg/ml rev polymerizes in low salt buffers (50 mM NaCl) and forms a gel (7). Electron micrographs show that these gels contain large filaments ≈14 nm wide and up to 1500 nm long (Fig. 5*a*). Filament formation is temperature dependent, and the longest filaments are grown by slowly increasing the temperature from 4°C to 25°C over a period of several hours, suggesting that filament formation is an entropically driven process dependent upon hydrophobic interactions between rev molecules. The structure of the rev-containing filaments is fairly regular, with units spaced at ≈4 nm. A band of negative stain runs down the middle of the filaments, suggesting that they are hollow tubes.

When an excess of rev is mixed with the 238-long RRE-7 transcript, short rod-like ribonucleoprotein complexes with a preferred filament length of 60 nm are formed (Fig. 5*b*). Filaments as long as 500–700 nm have been detected when a 2.4-kilobase (kb) transcript of the *env* gene is used as a template (Fig. 5*c*). The ratios of these two filament lengths suggest the RNA molecules are coated throughout their entire lengths. However, because the longer filaments are highly convoluted and tend to aggregate, a preferred length is not obvious, and we have not been able to determine accurately a packaging ratio.

The samples examined by electron microscopy were also analyzed by sucrose-gradient centrifugation and, for the 238 RRE RNA fragment, by nondenaturing gel electrophoresis (7). All the RNA transcripts were bound by rev and produced high-molecular-weight complexes that could be easily distinguished from free RNA and were protected from digestion by micrococcal nuclease (data not shown).

DISCUSSION

RNA Binding by Rev. The complex binding behavior of rev has led to some confusion as to whether rev recognizes a secondary structure or a specific sequence feature in the RRE RNA (9, 16, 17). The work reported here demonstrates that the RRE contains a purine-rich bubble that acts as the high-affinity rev-binding site. However, because of its ability to polymerize, rev is also able to bind RNA sequences adjacent to the high-affinity site. The binding of rev to these lower-affinity sites is responsible for the nonlinear Scatchard plots as well as for the formation of progressively larger complexes between rev and RRE RNA as rev concentrations are increased. When rev concentrations are sufficiently high, RNA is packaged into long ribonucleoprotein filaments, which can easily be detected by electron microscopy. Thus, rev binding to the high-affinity site within the RRE RNA may be considered to be the nucleation event for an assembly process during which RNA is packaged into filamentous coats. An analogous process occurs in the packaging of the RNA of tobacco mosaic virus and other RNA viruses by their coat proteins (18, 19).

Rev recognition of the bubble structure involves both the bulged nucleotides and the two adjacent base pairs on each side. All mutations known to abolish rev activity *in vivo* (20, 21) are expected to either delete or disrupt the bubble sequence. Residues within the bubble are highly conserved in different HIV-1 strains, except for U^{60}, which tolerates cytosine or guanine substitutions (13). Changes at residue 60 are not expected to impair RRE function significantly, because we have shown that the $U^{60} \rightarrow C$ substitution produces only a 2-fold reduction in rev binding.

The bubble sequence is highly resistant to nuclease cleavage as well as to modification by chemical reagents (9), suggesting that it forms a compact and rigid structure that locally distorts a double-stranded RNA helix. Details of the structure are still unknown, but the bubble could, perhaps, be stabilized by a non-Watson–Crick G·A bp (22) between G^{34} and A^{61}, as well as by stacking interactions.

Regulation of Gene Expression by Rev. RNAs carrying RRE sequences are efficiently packaged *in vitro* into rod-like filaments that can extend over many hundreds of nucleotides and coat the entire length of a template RNA molecule. Filament formation is facilitated by the presence of an RRE sequence. However, rev is also able to bind nonspecifically to RNA molecules with ≈20-fold lower affinity (5, 7). The nonspecific binding of rev allows RNA molecules that do not carry RRE sequences, such as tobacco mosaic virus RNA, to also be packaged into filaments *in vitro*, provided both the rev and RNA concentrations are sufficiently high (data not shown). The intracellular binding reaction is likely to involve a competition between rev and heterogenous ribonucleoprotein particle proteins (23), and this may restrict filament formation to the RRE-containing RNAs.

The RNA-binding properties of rev strongly suggests that it blocks splicing simply by packaging unspliced RNA transcripts containing the RRE sequence into inaccessible ribonucleoprotein complexes. Confirmation of our proposal will require the isolation of complexes containing rev and viral

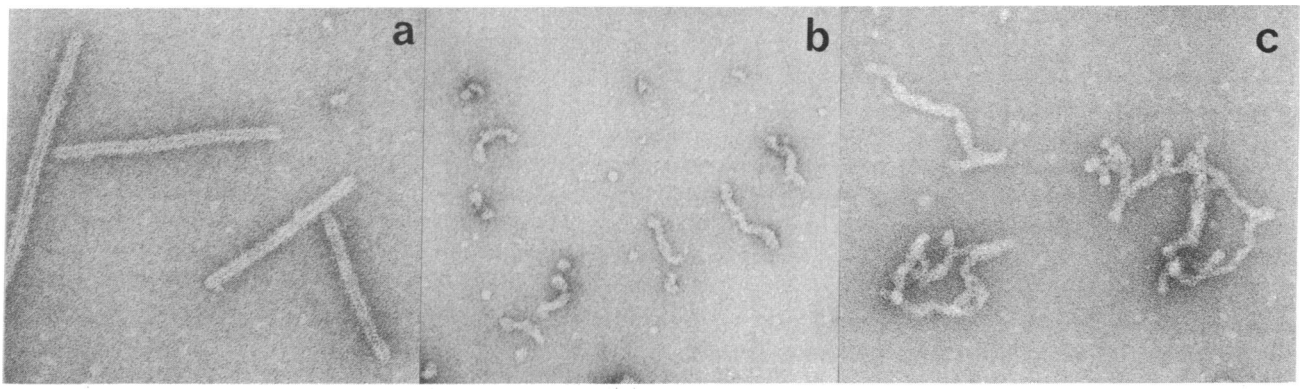

FIG. 5. Electron microscopy of filament formation by rev. (*a*) Rev protein filaments. (*b*) Complexes between rev protein and the 225-nucleotide-long RRE RNA transcript. (*c*) Complexes containing rev protein and a 2.4-kb transcript corresponding to env mRNA. Samples were negatively stained with uranyl acetate. (×120,000.)

mRNAs from infected cells. However, there is already indirect evidence in support of an RNA packaging model for rev activity. Rev is able to influence splicing when the RRE is placed either in an intron or in an unspliced exon and when the RRE is placed at various distances from splice sites (3, 4). In addition, rev is believed to disrupt splicing *in vitro* by blocking spliceosome formation (4). Finally, the *in vivo* activity of rev is believed to be highly concentration dependent because *rev*-minus viruses can only be rescued by high concentrations of transfected *rev*-expressing plasmids (24).

The packaging model also provides a simple kinetic explanation for the delayed appearance of the virion RNA relative to the 4.3-kb mRNAs, such as the env mRNA (1). Because the RRE sequence is only 535 nucleotides from the splice acceptor sequence for the second exons of the *tat* and *rev* genes, only a short rev filament would be needed to block splicing at this site and allow the production of the 4.3-kb mRNAs. Protection of the additional unused splice donor and acceptor sites located between 1.8 and 2.0 kb toward the 5′ end of the virion RNA would require either the formation of longer ribonucleoprotein filaments or the nucleation of filament formation by rev on secondary sites. In either case, these processes would be expected to be more efficient toward the end of an infectious cycle when intracellular rev protein concentrations might be expected to be maximal.

Although our proposal implies that the physical properties of rev can account for its biological activity, it is possible that cellular cofactor(s) are also required (25). Mouse cells infected by HIV have a *rev*-minus phenotype that can be reversed after fusion to human cells (25). However, in these experiments rev protein levels in the different cell lines were not measured, and it is possible that less rev was expressed in the mouse cells than in the human cells. By contrast, rev is functional in *Drosophila melanogaster* cells (26).

In conclusion we note that the assembly of rev protein on viral mRNAs carrying RRE sequences is a primary event in the HIV life-cycle and, therefore, constitutes an important target for therapeutic intervention. Small molecules that interfere with either rev binding to the bubble sequence or rev polymerization can be expected to show anti-HIV activity.

We thank Drs. P. J. G. Butler, C. Dingwall, A. Klug, and M. A. Skinner for helpful discussions; A. D. Lowe and S. M. Green for technical assistance, and C. Villa for photography. S.H. is a Medical Research Council Senior AIDS Research Fellow. J.K. is an Established Investigator of the American Heart Association.

1. Kim, S., Byrn, R., Groopman, J. & Baltimore, D. (1989) *J. Virol.* **63**, 3708–3713.
2. Rosen, C. A., Terwilliger, E., Dayton, A. I., Sodrowski, J. G. & Haseltine, W. A. (1988) *Proc. Natl. Acad. Sci. USA* **85**, 2071–2075.
3. Malim, M. H., Hauber, J., Le, S.-Y., Maizel, J. V. & Cullen, B. R. (1989) *Nature (London)* **338**, 254–257.
4. Chang, D. A. & Sharp, P. A. (1989) *Cell* **59**, 789–795.
5. Daly, T. J., Cook, K. S., Gary, G. S., Maione, T. E. & Rusche, J. R. (1989) *Nature (London)* **342**, 816–819.
6. Zapp, M. L. & Green, M. R. (1989) *Nature (London)* **342**, 714–716.
7. Heaphy, S., Dingwall, C., Ernberg, I., Gait, M. J., Green, S. M., Karn, J., Lowe, A. D., Singh, M. & Skinner, M. A. (1990) *Cell* **60**, 685–693.
8. Dingwall, C., Ernberg, I., Gait, M. J., Green, S. M., Heaphy, S., Karn, J., Lowe, A. D., Singh, M. & Skinner, M. A. (1990) *EMBO J.* **9**, 4145–4153.
9. Kjems, J., Brown, M., Chang, D. D. & Sharp, P. A. (1991) *Proc. Natl. Acad. Sci. USA* **88**, 683–687.
10. McGhee, J. D. & von Hippel, P. H. (1974) *J. Mol. Biol.* **86**, 469–489.
11. Nalin, C. M., Purcell, R. D., Antelman, D., Mueller, D., Tomchak, L., Wegrzynski, B., McCarney, E., Toome, V., Kramer, R. & Hsu, M.-C. (1990) *Proc. Natl. Acad. Sci. USA* **87**, 7593–7597.
12. Olsen, H. S., Cochrane, A. W., Dillon, P. J., Nalin, C. M. & Rosen, C. A. (1990) *Genes Dev.* **4**, 1357–1364.
13. Le, S.-Y., Malim, M. H., Cullen, B. R. & Maizel, J. V. (1990) *Nucleic Acids Res.* **18**, 1613–1623.
14. Zuker, M. (1989) *Science* **244**, 48–52.
15. Cheong, C., Varani, G. & Tinoco, I. J. (1990) *Nature (London)* **346**, 680–682.
16. Olsen, H. S., Nelbrock, P., Cohrane, A. W. & Rosen, C. A. (1990) *Science* **247**, 845–848.
17. Holland, S. M., Ahmad, N., Maitra, R. K., Wingfield, P. & Venkatesan, S. (1990) *J. Virol.* **64**, 5966–5975.
18. Klug, A. (1979) *Harvey Lect.* **74**, 141–172.
19. Turner, D. R., McGuigan, C. J. & Butler, P. J. G. (1989) *J. Mol. Biol.* **209**, 407–422.
20. Dayton, E. T., Powell, D. M. & Dayton, A. I. (1989) *Science* **246**, 1625–1629.
21. Malim, M. H., Tiley, L. S., McCarn, D. F., Rusche, J. R., Hauber, J. & Cullen, B. R. (1990) *Cell* **60**, 675–683.
22. SantaLucia, J. J., Kierzek, R. & Turner, D. H. (1990) *Biochemistry* **29**, 8813–8819.
23. Dreyfuss, G. (1986) *Annu. Rev. Cell Biol.* **2**, 459–498.
24. Pomerantz, R. J., Trono, D., Feinberg, M. B. & Baltimore, D. (1990) *Cell* **61**, 1271–1276.
25. Trono, D. & Baltimore, D. (1990) *EMBO J.* **9**, 4155–4160.
26. Ivey-Hoyle, M. & Rosenberg, M. (1990) *Mol. Cell. Biol.* **10**, 6152–6159.