JOURNAL OF VIROLOGY, Dec. 1991, p. 7051–7055
0022-538X/91/127051-05$02.00/0

Vol. 65, No. 12

Functional Mapping of the Human Immunodeficiency Virus Type 1 Rev RNA Binding Domain: New Insights into the Domain Structure of Rev and Rex

ERNST BÖHNLEIN, JOHANNES BERGER, AND JOACHIM HAUBER*

SANDOZ Research Institute, Brunner Strasse 59, 1235 Vienna, Austria

Received 15 July 1991/Accepted 28 August 1991

Expression of human immunodeficiency virus type 1 (HIV-1) structural proteins requires the direct interaction of the viral *trans*-activator protein Rev with its *cis*-acting RNA sequence (Rev-response element [RRE]). A stretch of 14 amino acid residues of the 116-amino-acid Rev protein is sufficient to impose nucleolar localization onto a heterologous protein. Our results demonstrated that these same amino acid residues confer Rev-specific RRE binding to the heterologous human T-cell leukemia virus type I Rex protein. In addition, our results indicated that amino acids distinct from the nuclear localization signal are important for Rex-specific RRE RNA binding.

The regulation of gene expression in human immunodeficiency virus type 1 (HIV-1) is critically dependent on the function of the viral *trans*-activator protein Rev (13, 36, 38, 40). Rev is a 19-kDa phosphoprotein (4, 19) which acts from the nucleolus (5, 26, 42) through the recognition of a highly structured target sequence, termed the Rev-response element (RRE), present in viral primary transcripts (9, 11, 17, 28, 35, 43). This Rev-RRE interaction results in the cytoplasmic accumulation of unspliced (*gag-pol*) or singly spliced (*env*) mRNA encoding the viral structural proteins (12, 15, 18). The exact molecular mechanism of HIV-1 Rev function still remains to be resolved (3, 15). However, for functional activity, the Rev *trans*-activator protein must comply with several criteria: (i) correct subcellular localization to the nucleolus (5, 26, 42), (ii) specific recognition of a substructure (stem-loop II) in the RRE sequence (8, 10, 20, 29, 33), and (iii) the presence of an activation domain for interaction with a cellular transport and/or splicing factor (26, 30, 41). Several studies have tried to attribute these functions to specific domains of the Rev protein (5, 22, 23, 26, 30, 32, 42). In particular, the idea that the Rev RNA binding domain may overlap with the Rev nuclear localization signal (NLS) was initially triggered by a study on bacteriophage antiterminator proteins (25). These proteins interact with RNA hairpins by a novel recognition sequence termed the arginine-rich motif. Notably, the Rev amino acid sequence contains a similar arginine-rich motif which has been shown to be responsible for the nucleolar accumulation of Rev (5, 21, 42). In this study, we directly demonstrated that the Rev NLS also confers RRE binding specificity to a heterologous protein.

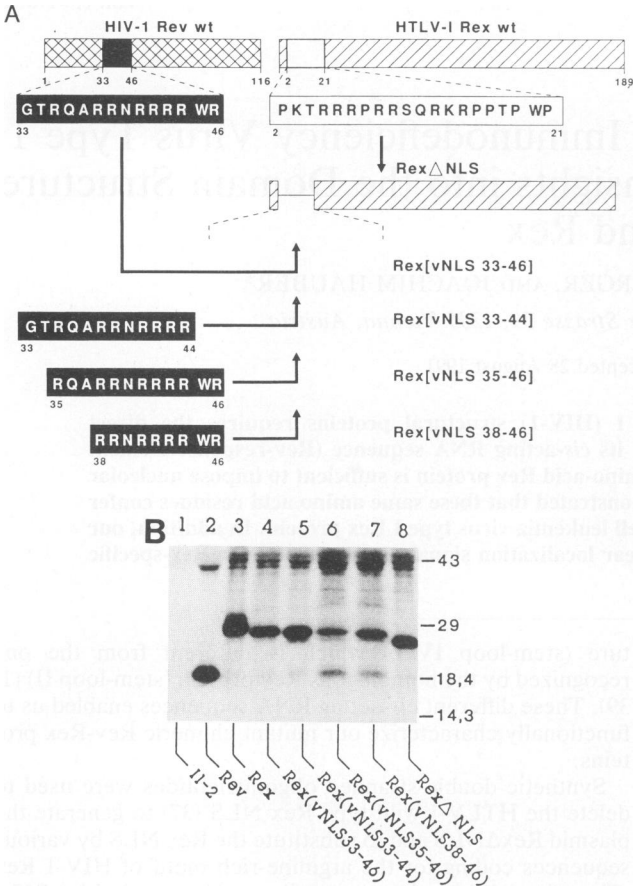
For these analyses, we exploited the fact that the related lentivirus human T-cell leukemia virus type I (HTLV-I) encodes a functionally equivalent *trans*-acting protein, Rex, which is able to act on the HIV-1 RRE sequence (14, 34). Recently, we have shown that substitution of the wild-type Rex NLS (Rex amino acid residues 2 to 21) by Rev amino acid residues 33 to 46 resulted in a hybrid protein which was phenotypically active on the full-length HIV-1 RRE sequence (21). Additional studies revealed that the 189-amino-acid wild-type Rex protein interacts with an RRE substructure (stem-loop IV/V) which is different from the one recognized by the homologous Rev protein (stem-loop II) (1, 39). These different *cis*-acting RNA sequences enabled us to functionally characterize our mutant chimeric Rev-Rex proteins.

Synthetic double-stranded oligonucleotides were used to delete the HTLV-I wild-type Rex NLS (37) to generate the plasmid RexΔNLS and to substitute the Rex NLS by various sequences coding for the arginine-rich motif of HIV-1 Rev (Fig. 1A). All introduced mutations were verified by DNA sequencing (Sequenase 2.0; U.S. Biochemicals). Following transfection of COS cells (6), the resulting mutant proteins were characterized by immunoprecipitation (2) and immunofluorescence analysis (2) (Fig. 1B and C) with either the Rev-specific polyclonal rabbit antiserum, Rev1/20 (7), or the Rex-specific polyclonal rabbit antiserum, Rex173/189 (2).

As expected, immunoprecipitation analysis showed that the wild-type HIV-1 Rev and the wild-type HTLV-I Rex proteins migrated at relative molecular masses of 19 and 27 kDa, respectively (Fig. 1B, lanes 2 and 3) (7, 24). Clearly, specific signals were also detectable for the Rev-Rex hybrid proteins (Fig. 1B, lanes 4 to 7) and the NLS-deficient protein encoded by RexΔNLS (Fig. 1B, lane 8). No signal was detectable in a control experiment expressing the human interleukin-2 gene, which indicates the specificity of the Rex antiserum used (Fig. 1B, lane 1).

Fluorescence immunocytochemistry of transfected COS7 cells revealed differences in the subcellular localization of the various mutant proteins. As demonstrated previously (21), the protein encoded by Rex[vNLS33-46] was directed to the correct nucleolar compartment by a stretch of 14 amino acid residues (Rev amino acid residues 33 to 46) derived from the nuclear localization domain of the HIV-1 Rev *trans*-activator protein (Fig. 1C, panel C versus panels A and B). Any partial deletion of the Rev NLS sequence abrogated the nucleolar localization of the hybrid proteins. The protein Rex[vNLS35-46], which lacks the first two amino acid residues of the Rev NLS, displayed clear nuclear staining but was excluded from nucleoli (Fig. 1C, panel E). Deletion of three additional Rev NLS amino acid residues (Rex[vNLS38-46]) abolished nuclear localization since this hybrid protein was detected in both the cytoplasmic and nuclear compartments (Fig. 1C, panel F). Similar results

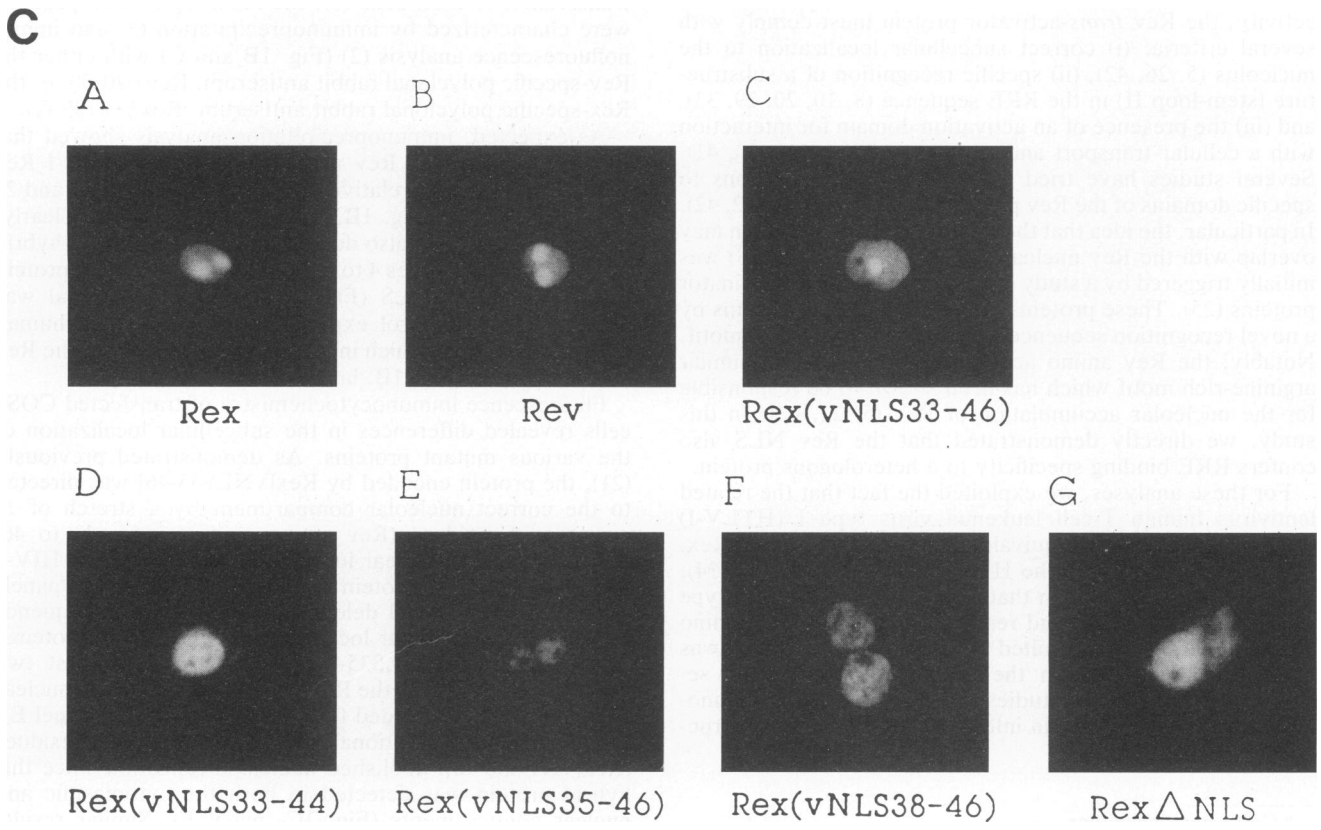
* Corresponding author.



were obtained with Rex[vNLS33-44] and the Rex protein encoded by RexΔNLS (Fig. 1C, panels D and G).

Our next experiments focused on the biological activity of the hybrid Rev-Rex molecules. To test their *trans*-activation capacity on the HIV-1 RRE, we used the Tat-shift assay (27). The expression of a genomic version of the HIV-1 *tat* gene results in the formation of the 86-amino-acid form of the Tat protein encoded by two exons. Because of the presence of a conserved intronic stop codon, the cotransfection of any functional HIV-1 *rev* or HTLV-I *rex* gene results in the appearance of the 72-amino-acid form of Tat protein encoded by the first *tat* exon (1). Substitution of the full-length RRE sequence in the original genomic *tat* gene by RRE

FIG. 1. Structure and expression of the molecular clones. (A) Using synthetic double-stranded oligonucleotides, the amino-terminal arginine-rich motif, which serves as the NLS (37) of HTLV-I Rex (hatched box), was deleted or substituted by the indicated sequences from the nuclear localization domain of the HIV-1 Rev *trans*-activator (crosshatched box). Rex[vNLS33-46] is identical to the previous published plasmid Rex[vNLS] (21). (B) Analysis of protein expression by immunoprecipitation experiments. All genes used in this study were expressed by the cytomegalovirus immediate-early promoter as described previously (2). After transfection of COS cell cultures with the indicated DNA, immunoprecipitation analysis was performed using anti-Rex (lanes 1 and 3 to 8) or anti-Rev (lane 2) antiserum (6). Precipitated proteins were resolved on discontinuous sodium dodecyl sulfate–13% polyacrylamide gels and visualized by autoradiography. Molecular mass standards (in kilodaltons) are shown on the right. IL-2, interleukin-2. (C) Subcellular localization of wild-type and mutant proteins by indirect immunofluorescence. COS7 cell cultures were transfected and subjected to immunofluorescence with anti-Rex (panels A and C to G) or anti-Rev (panel B) antiserum as described previously (2).



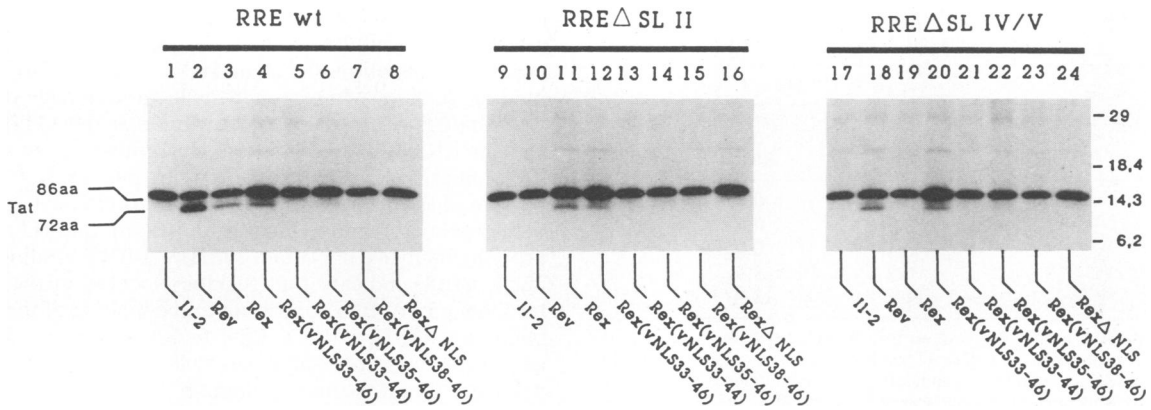


FIG. 2. Functional analysis of wild-type Rev, wild-type Rex, and mutant Rev-Rex proteins. COS cell cultures were cotransfected with the indicated DNA and subjected to Tat-specific immunoprecipitation analysis (2) with a murine monoclonal anti-Tat antibody (21). To assay the interaction with different RRE substructures, indicator plasmids harboring the wild-type RRE (RRE wt; lanes 1 to 8), an RRE stem-loop II deletion (RREΔSLII; lanes 9 to 16), and an RRE stem-loop IV/V deletion (RREΔSLIV/V; lanes 17 to 24) were used. Molecular mass standards (in kilodaltons) are indicated on the right. The 86-amino-acid (aa) and 72-amino-acid forms of Tat are indicated on the left. IL-2, interleukin-2.

deletion mutants provided an assay system to dissect the sites of Rev-RRE and Rex-RRE interaction (1). Therefore, we were able to test our various mutant proteins for their activity on the wild-type RRE sequence (RRE wt [27]), on a stem-loop II RRE deletion mutant (RREΔSLII [1]), and on a stem-loop IV/V RRE deletion mutant (RREΔSLIV/V [1]).

COS cell cultures were cotransfected as described previously (2). At 60 h posttransfection, the cells were metabolically radiolabeled with [^{35}S]cysteine and subjected to Tat-specific immunoprecipitation analysis (2) with a murine monoclonal anti-Tat antibody (21). The results clearly demonstrated that the HIV-1 Rev and the HTLV-I Rex *trans*-activator proteins induce the formation of the 72-amino-acid form of the Tat protein by recognition of different substructures on the HIV-1 RRE sequence (Fig. 2). In agreement with recent studies (1, 39), the Rev protein interacted with RRE stem-loop II while stem-loop IV/V was the target site for the Rex protein (Fig. 2, lanes 10 and 11 versus lanes 18 and 19). As expected, every protein which was unable to localize to the nucleolus (Fig. 1C, panels D to G) failed in this assay system (Fig. 2, lanes 5 to 8, 13 to 16, and 21 to 24), proving the necessity of correct nucleolar localization for the *trans*-acting phenotype. However, the Rev-Rex hybrid protein, Rex[vNLS33-46], which was shown to be active on the wild-type RRE (21) (Fig. 2, lane 4), displayed both RRE stem-loop II and RRE stem-loop IV/V binding specificity (Fig. 2, lanes 12 and 20). Obviously, the stretch of 14 amino acid residues derived from the Rev *trans*-activator protein (Rev amino acid residues 33 to 46) conferred the HIV-1 Rev RNA binding specificity to the HTLV-I Rex protein. Furthermore, these experiments present evidence that amino acid residues located outside of the HTLV-I Rex NLS contribute to the binding of Rex[vNLS33-46] to RREΔSLII.

The Rev *trans*-activator protein specifically recognizes radiolabeled RRE target RNA in in vitro binding assays (9). Our in vivo results demonstrated that HIV-1 amino acid residues 33 to 46 are able to confer Rev activity onto a truncated HTLV-I Rex protein. To directly address whether these amino acid residues represent the RNA binding domain, we performed filter binding experiments using HIV-1 Rev-derived peptides (Neosystem Laboratories). These studies indicated that Rev peptides which contain the Rev amino acid residues 33 to 46 in full (Rev aa22-52, Fig. 3) or in part (Rev aa34-46, Fig. 3) bound radiolabeled RRE sense RNA with significantly higher affinity than RRE antisense RNA (Table 1). Of note, recombinant Rev protein bound RRE RNA with three- to fivefold higher affinity than the peptides used in this study (data not shown). Rev peptides for amino acid residues 9 to 26 and 52 to 64 (Rev aa9-26 and

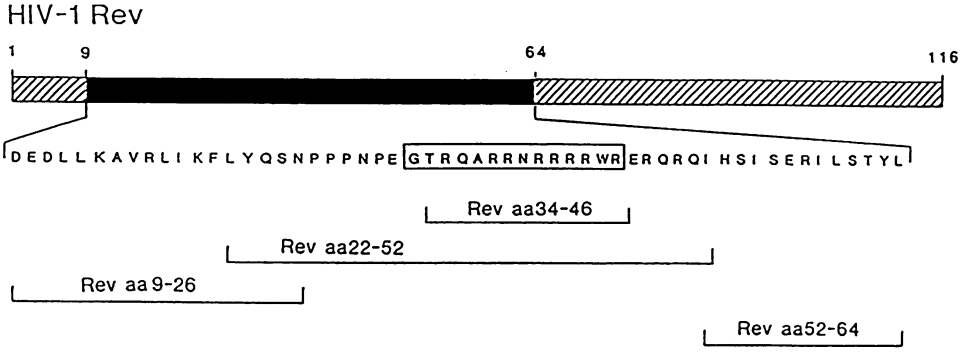


FIG. 3. Location of the Rev-derived peptides used in RRE-specific filter binding analysis. A partial amino acid sequence of the 116-amino-acid HIV-1 Rev protein is shown. Peptides used in the RRE binding assay (Table 1) are indicated by brackets. The REV RNA binding domain (Rev amino acid residues 33 to 46 [Rev aa33-46]), as determined by in vivo analysis, is boxed.

TABLE 1. RNA filter binding analysis with Rev-derived peptides[a]

Amt of peptide added (ng)	% of binding			
	Rev aa34-46		Rev aa22-52	
	RRE	as	RRE	as
0	0.0	0.0	0.0	0.0
80	1.8	0.9	0.4	0.0
100	4.6	0.7	0.9	0.2
150	9.1	0.7	2.4	0.2
250	21.0	1.7	12.3	0.8

[a] The assay was performed as described previously (9). In brief, radiolabeled HIV-1 sense (RRE) or antisense (as) RRE RNA was heated to 80°C for 3 min and cooled slowly to room temperature in a reaction mixture containing 0.1 μg of 5S RNA per ml from *Escherichia coli*, 50 μg of bovine serum albumin per ml, 10 U of RNasin (Promega), and phosphate-buffered saline (PBS). Various amounts of Rev peptides were added, and the reaction mixture was incubated for an additional 10 min. Finally, this incubation mixture was filtered carefully through cellulose-nitrate membranes and washed extensively with PBS. The radioactivity retained on the filters was determined and compared with total input activity. The given values indicate the percentage of binding of radiolabeled sense RRE or antisense RRE RNA to the various Rev-derived peptides. A value of zero (0.0) indicates background activity. Rev amino acids 9 to 26 and Rev amino acids 52 to 64 showed only background activity.

Rev aa52-64, Fig. 3), which do not include Rev amino acid residues 33 to 46, failed in this assay system (Table 1).

We characterized the RNA binding domain of the Rev *trans*-activator protein of HIV-1 using both in vivo and in vitro assay systems. In contrast to previous studies using mutational analysis which rendered the HIV-1 Rev protein nonfunctional, we generated chimeric Rev-Rex proteins which gained function, thereby directly demonstrating the biological activity of the transferred HIV-1 Rev amino acid sequences. The results of these experiments showed that a domain of Rev which serves as nuclear localization signal (21) also constitutes the Rev RNA binding domain. This stretch of basic amino acid residues is sufficient to confer Rev RNA binding specificity onto a mutant HTLV-I Rex protein (Fig. 2, lane 20). This binding activity, which is crucially important for Rev function (22, 23, 26, 32), was mapped to amino acid residues 33 to 46 in the 116-amino-acid Rev protein. In addition, immunofluorescence experiments revealed that this amino acid sequence could be functionally dissected with respect to nuclear and nucleolar localization. Deletion of two amino acid residues from the NLS motif (Rev amino acid residues 33 and 34) prevented the nucleolar accumulation of Rev (Fig. 1C, panel C versus panel E) which is absolutely required for Rev function (5, 26, 42). Therefore, the wild-type arginine-rich motif of the HIV-1 Rev protein serves at least two functions, nuclear localization and RNA binding.

Furthermore, our results also have implications for the HTLV-I Rex *trans*-activator protein. The Rex arginine-rich motif (Rex amino acid residues 1 to 19, Fig. 1A) which serves as the Rex nuclear localization signal (31, 37) has also been shown to be important for RNA binding (16). In contrast to wild-type Rev protein (Fig. 2, lane 10), the Rex[vNLS33-44] chimeric protein is functionally active on RREΔSLII (Fig. 2, lane 12). This result indicates that HTLV-I Rex amino acid residues located outside of the NLS functionally contribute to the binding of Rex to RRE stem-loop IV/V since the Rev RNA binding domain is by itself unable to recognize this target RNA sequence (1, 39). Thus, the functionally equivalent HIV-1 Rev and HTLV-I Rex *trans*-activators display a different domain organization with respect to subcellular localization and RNA recognition. The nuclear localization signal of HIV-1 Rev is also the RNA binding domain, while sequences located outside of the Rex nuclear localization signal contribute to the HTLV-I Rex-specific RRE RNA recognition. Currently, we are constructing chimeric Rev-Rex proteins with mutations in the Rex portion of the hybrid protein. These proteins will allow us to define relevant amino acid residues in the HTLV-I Rex protein which are important for HIV-1 RRE binding. Preliminary results indicate that residues located in the carboxy-terminal part of Rex contribute to the binding of the Rev-Rex chimeras to the HIV-1 Rev target sequence. However, additional mutational analyses will be required to precisely delineate this Rex binding domain.

We thank M. H. Malim for the RREΔSLII and RREΔSLIV/V plasmids, J. Rusche (Repligen) for recombinant Rev protein, J.-M. Seifert for oligonucleotide synthesis, and M. Ruhl for secretarial support.

REFERENCES

1. **Ahmed, Y. F., S. M. Hanly, M. H. Malim, B. R. Cullen, and W. C. Greene.** 1990. Structure-function analyses of the HTLV-I Rex and HIV-1 Rev RNA response elements: insights into the mechanism of Rex and Rev action. Genes Dev. **4**:1014–1022.
2. **Böhnlein, S., F. P. Pirker, L. Hofer, K. Zimmermann, H. Bachmayer, E. Böhnlein, and J. Hauber.** 1991. Transdominant repressors for human T-cell leukemia virus type I Rex and human immunodeficiency virus type 1 Rev function. J. Virol. **65**:81–88.
3. **Chang, D. D., and P. A. Sharp.** 1989. Regulation by HIV Rev depends upon recognition of splice sites. Cell **59**:789–795.
4. **Cochrane, A., R. Kramer, S. Ruben, J. Levine, and C. A. Rosen.** 1989. The human immunodeficiency virus *rev* protein is a nuclear phosphoprotein. Virology **171**:264–266.
5. **Cochrane, A. W., A. Perkins, and C. A. Rosen.** 1990. Identification of sequences important in the nucleolar localization of human immunodeficiency virus Rev: relevance of nucleolar localization to function. J. Virol. **64**:881–885.
6. **Cullen, B. R.** 1987. Use of eukaryotic expression in the functional analysis of cloned genes. Methods Enzymol. **152**:684–704.
7. **Cullen, B. R., J. Hauber, K. Campbell, J. G. Sodroski, W. A. Haseltine, and C. A. Rosen.** 1988. Subcellular localization of the human immunodeficiency virus *trans*-acting *art* gene product. J. Virol. **62**:2498–2501.
8. **Daefler, S., M. E. Klotman, and F. Wong-Staal.** 1990. Trans-acting Rev protein of the human immunodeficiency virus 1 interacts directly and specifically with its target RNA. Proc. Natl. Acad. Sci. USA **87**:4571–4575.
9. **Daly, T. J., K. S. Cook, G. S. Gray, T. E. Maione, and J. R. Rusche.** 1989. HIV-1 recombinant Rev protein binds specifically to the Rev response element *in vitro*. Nature (London) **342**:816–819.
10. **Dayton, E., D. Powell, and A. Dayton.** 1989. Functional analysis of CAR, the target sequence for the Rev protein of HIV-1. Science **246**:1625–1629.
11. **Dillon, P. J., P. Nelböck, A. Perkins, and C. A. Rosen.** 1990. Function of the human immunodeficiency virus types 1 and 2 Rev proteins is dependent on their ability to interact with a structured region present in *env* gene mRNA. J. Virol. **64**:4428–4437.
12. **Emerman, M., R. Vazeux, and K. Peden.** 1989. The rev gene product of the human immunodeficiency virus affects envelope-specific RNA localization. Cell **57**:1155–1165.
13. **Feinberg, M. B., R. F. Jarrett, A. Aldovini, R. C. Gallo, and F. Wong-Staal.** 1986. HTLV-III expression and production involve complex regulation at the levels of splicing and translation of viral RNA. Cell **46**:807–817.
14. **Felber, B. K., D. Derse, A. Athanassopoulos, M. Campbell, and G. N. Pavlakis.** 1989. Cross-activation of the Rex proteins of

HTLV-I and BLV and of the Rev protein of HIV-1 and nonreciprocal interactions with their RNA responsive element. New Biol. 1:318–330.
15. **Felber, B., M. Hadzopoulou-Cladaras, C. Cladaras, T. Copeland, and G. N. Pavlakis.** 1989. The rev protein of HIV-1 affects stability and transport of the viral mRNA. Proc. Natl. Acad. Sci. USA 86:1495–1499.
16. **Grassmann, R., S. Berchtold, C. Aepinus, C. Ballaun, E. Boehnlein, and B. Fleckenstein.** 1991. In vitro binding of human T-cell leukemia virus *rex* proteins to the *rex*-response element of viral transcripts. J. Virol. 65:3721–3727.
17. **Hadzopoulou-Cladaras, M., B. K. Felber, C. Cladaras, T. Copeland, and G. N. Pavlakis.** 1989. The *rev* (*trs/art*) protein of human immunodeficiency virus type 1 affects viral mRNA and protein expression via a *cis*-acting sequence in the *env* region. J. Virol. 63:1265–1274.
18. **Hammarskjöld, M. L., J. Heijmer, B. Hammarskjöld, I. Sangwan, L. Albert, and D. Rekosh.** 1989. Regulation of human immunodeficiency virus *env* gene expression by the *rev* gene product. J. Virol. 63:1959–1966.
19. **Hauber, J., M. Bouvier, M. H. Malim, and B. R. Cullen.** 1988. Phosphorylation of the *rev* gene product of human immunodeficiency virus type 1. J. Virol. 62:4801–4804.
20. **Heaphy, S., C. Dingwall, I. Ernberg, M. J. Gait, S. M. Green, J. Karn, A. D. Lowe, M. Singh, and M. D. Skinner.** 1990. HIV-1 regulator of virion expression (Rev) protein binds to an RNA stem-loop structure located within the Rev response element. Cell 60:685–693.
21. **Hofer, L., I. Weichselbraun, S. Quick, G. K. Farrington, E. Böhnlein, and J. Hauber.** 1991. Mutational analysis of the human T-cell leukemia virus type I *trans*-acting *rex* gene product. J. Virol. 65:3379–3383.
22. **Hope, T. J., X. Huang, D. McDonald, and T. G. Parslow.** 1990. Steroid-receptor fusion of the human immunodeficiency virus type 1 Rev transactivator: mapping cryptic functions of the arginine-rich motif. Proc. Natl. Acad. Sci. USA 87:7787–7791.
23. **Hope, T. J., D. McDonald, X. Huang, J. Low, and T. G. Parslow.** 1990. Mutational analysis of the human immunodeficiency virus type 1 Rev *trans*-activator: essential residues near the amino terminus. J. Virol. 64:5360–5366.
24. **Kiyokawa, T., M. Seiki, S. Iwashita, F. Shimizu, and M. Yoshida.** 1985. $p27^{x}$-III and $p21^{x}$-III, proteins encoded by the pX sequence of human T-cell leukemia virus type I. Proc. Natl. Acad. Sci. USA 82:8359–8363.
25. **Lazinski, D., E. Grzadielska, and A. Das.** 1989. Sequence-specific recognition of RNA hairpins by bacteriophage antiterminators requires a conserved arginine-rich motif. Cell 59:207–218.
26. **Malim, M. H., S. Böhnlein, J. Hauber, and B. R. Cullen.** 1989. Functional dissection of the HIV-1 Rev *trans*-activator—derivation of a *trans*-dominant repressor of Rev function. Cell 58:205–214.
27. **Malim, M. H., J. Hauber, R. Fenrick, and B. R. Cullen.** 1988. Immunodeficiency virus rev *trans*-activator modulates the activity of the viral regulatory genes. Nature (London) 335:181–183.
28. **Malim, M. H., J. Hauber, S.-Y. Le, J. V. Maizel, and B. R. Cullen.** 1989. The HIV-1 rev *trans*-activator acts through a structured target sequence to activate nuclear export of unspliced viral mRNA. Nature (London) 338:254–257.
29. **Malim, M. H., L. S. Tiley, D. F. McCarn, J. R. Rusche, J. Hauber, and B. R. Cullen.** 1990. HIV-1 structural gene expression requires binding of the Rev *trans*-activator to its RNA target sequence. Cell 60:675–683.
30. **Mermer, B., B. K. Felber, M. Campbell, and G. N. Pavlakis.** 1990. Identification of *trans*-dominant HIV-1 rev protein mutants by direct transfer of bacterially produced proteins into human cells. Nucleic Acids Res. 18:2037–2044.
31. **Nosaka, T., H. Siomi, Y. Adachi, M. Ishibashi, S. Kubota, M. Maki, and M. Hatanaka.** 1989. Nucleolar targeting signal of human T cell leukemia virus type I *rex*-encoded protein is essential for cytoplasmic accumulation of unspliced viral mRNA. Proc. Natl. Acad. Sci. USA 86:9798–9802.
32. **Olsen, H. S., A. W. Cochrane, P. J. Dillon, C. M. Nalin, and C. A. Rosen.** 1990. Interaction of the human immunodeficiency virus type 1 Rev protein with a structured region in env mRNA is dependent on multimer formation mediated through a basic stretch of amino acids. Genes Dev. 4:1357–1364.
33. **Olsen, H. S., P. Nelböck, A. W. Cochrane, and C. A. Rosen.** 1990. Secondary structure is the major determinant for interaction of HIV rev protein with RNA. Science 247:845–848.
34. **Rimsky, L. T., J. Hauber, M. Dukovich, M. H. Malim, A. Langlois, B. R. Cullen, and W. C. Greene.** 1988. Functional replacement of the HIV-1 Rev protein by the HTLV-I Rex protein. Nature (London) 335:738–740.
35. **Rosen, C. A., E. Terwilliger, A. Dayton, J. G. Sodroski, and W. A. Haseltine.** 1988. Intragenic cis-acting *art* gene responsive sequences of the human immunodeficiency virus. Proc. Natl. Acad. Sci. USA 85:2071–2075.
36. **Sadaie, M. R., T. Benter, and F. Wong-Staal.** 1988. Site-directed mutagenesis of two *trans*-regulatory genes (*tat*-III, *trs*) of HIV-1. Science 239:910–914.
37. **Siomi, H., H. Shida, S. H. Nam, T. Nosaka, M. Maki, and M. Hatanaka.** 1988. Sequence requirements for nucleolar localization of human T cell leukemia virus type I pX protein, which regulates viral RNA processing. Cell 55:197–209.
38. **Sodroski, J. G., W. C. Goh, C. Rosen, A. Dayton, E. Terwilliger, and W. A. Haseltine.** 1986. A second posttranscriptional transactivator gene required for HTLV-III replication. Nature (London) 321:412–417.
39. **Solomin, L., B. K. Felber, and G. N. Pavlakis.** 1990. Different sites of interaction for Rev, Tev, and Rex proteins within the Rev-responsive element of human immunodeficiency virus type 1. J. Virol. 64:6010–6017.
40. **Terwilliger, E., R. Burghoff, R. Sia, J. Sodroski, W. Haseltine, and C. Rosen.** 1988. The *art* gene product of human immunodeficiency virus is required for replication. J. Virol. 62:655–658.
41. **Trono, D., and D. Baltimore.** 1990. A human cell factor is essential for HIV-1 Rev action. EMBO J. 9:4155–4160.
42. **Venkatesh, L. K., S. Mohammed, and G. Chinnadurai.** 1990. Functional domains of the HIV-1 *rev* gene required for *trans*-regulation and subcellular localization. Virology 176:39–47.
43. **Zapp, M., and M. Green.** 1989. Sequence-specific RNA binding by the HIV-1 rev protein. Nature (London) 342:714–716.