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

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Interaction of the human immunodeficiency virus type 1 (HIV-1) Rev protein with a structured region within env mRNA (termed RRE) mediates the export of virus structural mRNAs from the nucleus to the cytoplasm. We show that the region encompassing the basic stretch of amino acids is essential for the ability of Rev to bind to RRE RNA and function in vivo. By use of a functional truncated Rev protein in conjunction with authentic Rev, effects on gel mobilities of the Rev–RRE RNA complex attributable to multimerization of Rev protein were observed. Rev proteins, unable to multimerize, failed to bind RRE RNA. Identification of Rev mutants capable of forming multimers, but unable to bind RRE RNA, suggests that the multimerization and RNA-binding domains can be distinguished and that multimerization is likely a prerequisite for formation of the RRE RNA-binding site. A mutant Rev protein, shown previously to function as a trans-dominant inhibitor of Rev function, bound to RRE RNA as a multimer to a similar extent as wild-type Rev. This observation is consistent with the hypothesis that regulation of HIV gene expression by Rev involves the interaction with cellular factors and that the trans-dominant Rev is probably defective in this function.

[Key Words: HIV-1; Rev protein; RRE RNA]

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The Rev protein is one of several regulatory proteins encoded by the human immunodeficiency virus type 1 (HIV-1) [Feinberg et al. 1986; Sodroski et al. 1986; Terrwilliger et al. 1988]. Expression of Rev is required for the export of HIV structural mRNAs from the nucleus to the cytoplasm [Emerman et al. 1989; Felber et al. 1989; Hammarskjöld et al. 1989; Malim et al. 1989b]. Recent studies demonstrate that the Rev proteins encoded by both HIV-1 and HIV-2 interact with a segment of RNA present within their respective env gene messages [Daly et al. 1989; Zapp and Green 1989; Cochrane et al. 1990a; P.J. Dillon, P. Nelbock, A. Perkins, and C.A. Rosen, in prep.]. Mutational analysis of the Rev-responsive elements [initially referred to as CAR; Dayton et al. 1988], termed RRE [Malim et al. 1989b], indicates that RNA secondary structures present within this region are the major determinant for Rev interaction and function [Heaphy et al. 1990; Malim et al. 1990; Olsen et al. 1990; P.J. Dillon, P. Nelbock, A. Perkins, and C.A. Rosen, in prep.]. Disruption of this region, in either intact proviral DNA or Rev-dependent heterologous gene expression systems, results in loss of Rev response [Rosen et al. 1988; Dayton et al. 1989; Hadzopoulou-Cladaras et al. 1989; Malim et al. 1989b]. Thus, direct interaction of Rev with this RNA, either alone or in conjunction with cellular factors, is likely to mediate nuclear export of the viral structural mRNA.

The Rev protein is a 20-kD highly charged, phosphorylated [Hauber et al. 1988; Cochrane et al. 1989c] nuclear protein [Cullen et al. 1988; Felber et al. 1989; Perkins et al. 1989] that accumulates in the nucleolus [Malim et al. 1989a; Cochrane et al. 1990b]. Mutational analysis of Rev has identified the presence of distinct nuclear [Malim et al. 1989a; Perkins et al. 1989] and nucleolar targeting signals [Cochrane et al. 1990b]. The nucleolar targeting signal consists of a stretch of basic amino acids similar to those present in other nuclear targeting signals [Kalderon et al. 1984; Burglin and Der Roberts 1987]. Although the importance of nucleolar localization remains to be determined, Rev mutants failing to
localize in either the nucleus or nucleolus are nonfunctional [Cochrane et al. 1990b]. In contrast, elimination of phosphorylation does not appear to have any significant effect on Rev function [Cochrane et al. 1989b; Malim et al. 1989a].

To identify the amino acids of Rev essential to its interaction with RRE RNA, the ability of mutated Rev proteins to bind RRE RNA in vitro and function in vivo was examined. Our results demonstrate that the region encompassing the basic stretch of amino acids confers the ability of Rev to interact with RRE RNA and to multimerize. A Rev mutant, shown previously to be a trans-dominant inhibitor of Rev function, was also found to bind to the RRE RNA as a multimer to a similar extent as authentic Rev. This observation is consistent with the hypothesis that Rev functions through interaction with cellular factors and suggests that the trans-dominant Rev protein lacks this ability.

Results

Whereas previous studies have delineated several functional domains within Rev, the mutational analysis described here was undertaken to identify the domain required for interaction with the RRE RNA. Site-directed mutations [Fig. 1; Table 1] were constructed by oligonucleotide mutagenesis [Kunkel 1985]. For expression and subsequent purification purposes, mutations were made within a modified synthetic Rev cDNA that contains a 5’ sequence encoding a stretch of histidine residues and codons most frequently used in Escherichia coli. We have shown previously that function of this modified Rev protein (H6 Rev) containing the histidine residues is comparable to that of authentic unmodified Rev [Cochrane et al. 1989a]. The localization and type of mutations made are shown in Table 1.

We and others have shown recently that purified Rev protein derived from E. coli forms a specific interaction with RRE RNA [Daly et al. 1989; Zapp and Green 1989; Cochrane et al. 1990a; Heaphy et al. 1990; Malim et al. 1990]. Because of possible difficulties associated with purification of each of the 14 mutations described, the feasibility of using Rev within E. coli lysates in the binding assays was examined. Rev expression was induced in mid-logarithmic growth as described [see Materials and methods]. Cells were harvested, resuspended in buffer, and sonicated. Following a brief centrifugation to remove cellular debris, an aliquot of the cleared lysate was used directly in the binding assay. As shown in Figure 2, the Rev protein obtained from the cleared lysate bound to the RRE containing RNA. Binding was indistinguishable from that obtained with purified protein. To assess the specificity of binding, the lysate was incubated with RRE RNAs, HSL-7 and HSL-5, mutations shown previously to be unable to interact with purified Rev [Olsen et al. 1990]. No interaction was detected between Rev-containing lysate and these mutated RREs. Furthermore, no binding of RRE RNA was observed with lysates obtained from E. coli not expressing Rev. These results demonstrate that binding of the modified Rev protein obtained from cleared E. coli lysates is comparable to that observed with the unmodified, purified Rev protein.

Identification of the Rev RNA-binding domain

Each of the mutations shown in Figure 1 and Table 1 was made within a Rev insert present in the pRC-25 bacterial expression vector [Crowl et al. 1985]. Cleared E. coli lysates prepared from exponentially growing cultures were prepared as described above. Western blot analysis with an anti-Rev antiserum raised against a synthetic peptide corresponding to amino acids 1–20 [residues not affected by the mutagenesis] showed comparable levels of Rev protein in each lysate [data not shown]. One microliter of cleared lysate was incubated with in vitro-transcribed RRE RNA transcripts, and binding was measured in a qualitative gel mobility-shift ribonuclease T1 protection assay [Cochrane et al. 1990a]. As shown in Figure 3, many of the Rev mutants failed to interact with the RRE RNA. Mutations that destroyed the ability of Rev to bind centered near or within the basic stretch of amino acids.

Function of Rev is dependent on its ability to interact with RRE RNA

The ability of the mutated Rev proteins to complement gene expression in Rev-dependent heterologous gene expression was examined. The Rev cDNA inserts harboring the individual mutations were cloned downstream of the SV40 promoter present in the eukaryotic expression vector pSVEX. The pSV–Rev expression vectors, together with plasmid pSVAR, were cotransfected into COS cells, and chloramphenicol acetyltransferase (CAT) assays were performed 48 hr post-transfection. Plasmid pSVAR [Cullen et al. 1988] contains the HIV env sequences that mediate nuclear retention of RNA in the absence of Rev; as such, CAT gene expression is dependent on expression of Rev [Cullen et al. 1988; Rosen et al. 1988]. Results obtained from the CAT assays were scored as plus or minus and are presented in...
Table 1. Functional characterization of Rev protein mutants

| Mutation and location | Amino acid changes | RRE RNA binding | Rev–Rev association | Trans-activation |
|-----------------------|-------------------|-----------------|---------------------|-----------------|
| R 38-39               | Arg-Arg to His-Glu|                 | +                   |                 |
| RΔ48-51               | ΔArg-Gln-Arg-Gln  |                 | +                   |                 |
|                      |                   |                 |                     |                 |
| RA45-51               | ΔTrp-Arg-Glu-Arg-Gln|                   | +                   |                 |
|                      |                   |                 |                     |                 |
| R 85                  | Cys to Phe        | +               | +                   | -               |
| R 75                  | Leu to Arg        | +               | +                   | TD              |
| RΔ70-72               | ΔPro-Val-Pro      |                 | +                   |                 |
| RΔ59-61               | ΔIle-Leu-Gly      |                 | +                   | -               |
| RΔ55-57               | ΔIle-Ser-Glu      |                 | +                   | -               |
| RΔ80-82               | ΔArg-Leu-Thr      |                 | +                   | TD              |
| RΔ63-65               | ΔTyr-Leu-Gly      |                 | +                   | -               |
| RΔ32-34               | ΔGlu-Gly-Thr      |                 | +                   | -               |
| RΔ28-31               | ΔPro-Pro-Asn-Pro  |                 | -                   | -               |
| RevAC-4               | Leu78-Glu79 to Asp-Leu|                   | +                   | ND              |
| Rev-916               | Ser92 to stop     |                 |                     | TD              |

Functional characterization of Rev protein mutants. Naming of Rev mutants is indicative of the region altered. Numbering of amino acids is as shown in Fig. 1. Amino acid deletions are designated with Δ. RNA binding to the RRE was analyzed by using a gel-shift RNase protection assay (Fig. 3) and scored as + or – for binding. Mutant Rev proteins were assayed for multimer formation in the absence of RNA, as shown in Fig. 5, and scored as positive (+), negative (–), or not determined (ND). Mutant Rev proteins were analyzed for function in a Rev-dependent transient gene expression system (Rosen et al. 1988) and scored as positive (+), negative (–), or transdominant suppression (TD).

Table 1. As anticipated, each of the Rev proteins that failed to interact with RRE RNA in vitro was nonfunctional in vivo. The lack of function was not a reflection of low levels of expression because Rev-immunoreactive products were detected in each of the transfections [data not shown]. Mutants R75 and RΔ80-82, which were nonfunctional, were also transdominant suppressors of Rev function (see below).

Rev binds to RRE RNA as a multimer

Many of the well-characterized protein–DNA and protein–RNA associations occur through the formation of protein complexes. To examine whether Rev interacts with RRE RNA as a monomer or multimeric structure, a truncated Rev protein lacking the 25 carboxy-terminal amino acids was used in the binding assay. We have shown previously that this truncated Rev, designated here as Rev-916, retains full function in vivo [referred to previously as RevSTP, Cochrane et al. 1989b]. As shown in Figure 4A, Rev-916 bound with RRE RNA and had a faster migration within the gel. If Rev–RRE interaction...
Figure 4. Multimer formation between truncated Rev protein and authentic or mutant Rev. Cleared lysates from E. coli expressing authentic or mutant Rev were prepared by resuspension of cells from a 25-ml culture in 1 ml of sonication buffer. Cleared lysates were then prepared as described in Materials and methods. (A) Lysate (1 μl) containing full-length Rev, or the carboxy-terminal truncation Rev-916 was incubated with RRE RNA. (B–E) The amount shown (in microliters) of Rev-916 lysate was combined with the indicated amount of cleared lysate from a culture expressing one of the mutant Rev proteins. Analysis of Rev–RRE RNA interactions was as described in Materials and methods.

occurs through complex formation, the mixing of full-length and truncated Rev in the binding reactions should produce intermediate size complexes. As shown in Figure 4B, intermediate complexes were formed when the truncated Rev-916 was combined with full-length protein. Lack of a single intermediate-size band between the two Rev species suggests that the Rev complex may be composed of more than two subunits.

Having established that Rev interacts with RRE RNA through multimer formation, the ability of the mutant Rev proteins to form multimers was examined. Each of those mutants, which failed to interact with the RRE RNA, was unable to form multimers with the truncated Rev-916 protein in the gel mobility-shift assay described above (Fig. 4). In contrast, multimer formation was observed with all of the mutants in which the RRE RNA interaction was unaffected.

Multimer formation occurs in the absence of RRE RNA

Because involvement of the basic stretch of amino acids in multimer formation was unexpected, we examined whether Rev could multimerize in the absence of RRE RNA. Purified Rev protein was biotinylated and incubated with 35S-labeled Rev protein produced in an in vitro reticulocyte translation system. Streptavidin-coupled agarose beads were added to the mixture, and complexes were collected by centrifugation. The pelleted material was analyzed by SDS-PAGE. As shown in Figure 5, labeled full-length and truncated Rev-916 was coprecipitated with the biotinylated Rev, despite the absence of RRE RNA. In contrast, no labeled material was coprecipitated from translation lysates programmed with RNA corresponding to Rev proteins RA55-57 and RA28-31, indicating that the association observed is specific. However, mutants R38-39, RA48-51, RA59-61, RA63-65, and RA32-34, each of which were unable to bind RRE RNA, were coprecipitated with biotinylated Rev. Thus, the inability to detect multimerization of these mutants by use of the RNA-binding assay suggests that the mutations have disrupted residues important for RNA binding as opposed to multimerization. These observations indicate that multimerization is mediated by protein–protein interactions, and the formation of the RNA-binding site is dependent on multimerization.

A trans-dominant suppressor of Rev function binds to RRE RNA as a multimer

It was shown previously that mutation of amino acids 78 and 79 produces a Rev protein that is a trans-dominant repressor of Rev function (Malim et al. 1989a). To examine the basis for the repressive effects exerted by the trans-dominant Rev (designated here as RevAC-4), we first tested its ability to repress Rev mediated regulation of CAT gene expression directed by the pSVAR plasmid. The results shown in Figure 6 indicate that RevAC-4 was indeed a trans-dominant repressor of Rev function in our assay, in accord with previous findings
Figure 5. Rev complex formation occurs in the absence of RRE RNA. In vitro reticulocyte translation lysates were programmed with RNA encoding authentic Rev or Rev mutants. Biotinylated Rev was added to the translation lysate, and ^35S-labeled Rev was coprecipitated with the biotinylated Rev by the addition of streptavidin-coupled agarose beads. Coprecipitated products were analyzed by 15% SDS-PAGE and visualized by autoradiography. Biotinylated Rev was incubated with translation lysates expressing the following: (lanes 2 and 6) authentic Rev; (lanes 3 and 7) Rev-916; (lane 4) RA55-57; (lane 5) RA28-31.

To elucidate the basis for the trans-dominant effects of RevAC-4, its ability to interact with RRE RNA was examined. As shown in Figure 7, RevAC-4, present in cleared E. coli lysates, formed a stable interaction with the RRE RNA, comparable to that observed with authentic Rev protein. Upon mixing RevAC-4 with the truncated Rev-916, an intermediate Rev–RRE complex was observed, indicating that the trans-dominant suppression is not attributable to failure to interact with RRE RNA or to form multimeric complexes. Similar findings were obtained by use of purified RevAC-4 (not shown) or Rev proteins R75 and RΔ80-82 obtained from E. coli lysates (Fig. 7).

Discussion

We examined the requirements for interaction of Rev protein with RRE RNA. Our major findings are that the region encompassing a highly charged basic stretch of amino acids is required for interaction with the RRE RNA, as well as the ability of Rev to form multimers and that trans-dominant suppressors of Rev function interact with RRE RNA.

Previous studies have concentrated on the RRE sequence elements required for interaction with Rev (Cochrane et al. 1990a; Heaphy et al. 1990; Malim et al. 1990; Olsen et al. 1990). The studies reported here examined the basis for this interaction at the protein level. Earlier studies have identified those domains within Rev required for phosphorylation (Cochrane et al. 1989b; Malim et al. 1989a) and nuclear [Malim et al. 1989b; Perkins et al. 1989] and nucleolar localization [Cochrane et al. 1990b]. Deletion analysis of Rev has also shown that the 8 amino-terminal [Malim et al. 1989a] and 25 carboxy-terminal amino acids (Cochrane et al. 1990b) can be deleted without impairing Rev function. Therefore, the internal 83 amino acids appear to be sufficient for full Rev function. Our results show that the RNA-binding domain centers in and around the group of positively charged amino acids. The presence of highly charged residues is characteristic of numerous well-characterized DNA- and RNA-binding domains (Gentz et al. 1989b; Landshulz et al. 1988; Turner and Tjian 1989).

The lack of RRE RNA binding obtained with mutations in the basic region could reflect either destruction of the RNA-binding site or disruption of a domain required for protein complex formation, which is essential to the interaction of Rev with the RRE RNA. To examine the latter possibility, binding reactions were done in the presence of full-length and truncated Rev protein. Our results, showing formation of intermediate-size Rev–RRE RNA complexes, indicate that Rev probably interacts with the RRE RNA as a protein multimer. Because a range of intermediate-size complexes, rather
than a single discrete species, was observed, it may be that the Rev protein complex consists of more than two subunits. Upon examination of the capacity of the mutants to form multimers, we observed that each of those mutations failing to interact with the RRE RNA was also unable to form multimers with functional Rev protein in the Rev protein–RRE RNA-binding assay. However, by use of biotinylated Rev protein to examine association between Rev proteins in the absence of RNA, all but two (mutants ΔA55-57 and ΔA28-31) of these RRE RNA nonbinders could be coprecipitated. These findings suggest that the residues important for multimerization can be distinguished from those required for RNA binding. Furthermore, the inability of the gel mobility-shift assay to detect heteromultimers of authentic Rev and the nonbinding mutants strongly suggests that multimer formation is required for the formation of the RNA-binding site. Thus, our current hypothesis is that the RNA-binding domain is formed following multimerization and lies very close, if not coincident, with the residues specifying multimerization. This concept is not unique to the Rev–RRE interaction described here. As an example, nucleocapsid assembly of tobacco mosaic virus is mediated through a capsid protein dimer that forms the RNA recognition site (Butler 1984). Formation of protein recognition sites on DNA can also proceed through a similar mechanism. For example, some DNA-binding proteins (i.e., Fos and Jun) form protein dimers through a so-called leucine zipper, which then creates a DNA-binding site that lies adjacent to the dimerization domain (Gentz et al. 1989a; Landschulz et al. 1988).

The mechanism for Rev-mediated export of viral structural mRNAs from the nucleus to cytoplasm is not understood. Whether this process is controlled by Rev alone or through interaction with cellular factors is also unclear. Recently, a Rev mutant was identified that functions as a trans-dominant suppressor of Rev function (Malim et al. 1989a). To understand the basis for trans-dominant suppression and to obtain insight into Rev function, we created the same mutation in prokaryotic and eukaryotic Rev expression vectors. Expression of this Rev protein, designated here as RevAC-4, resulted in trans-dominant suppression of Rev function in our assay system in accord with published findings (Malim et al. 1989a). We also found that mutations R75 and RA80-82, which are in the same vicinity as the RevAC-4 mutation, functioned as trans-dominant suppressors of Rev function. The RevAC-4 obtained from E. coli lysates or purified to homogeneity (not shown) bound to RRE RNA to a similar extent as that observed with wild-type protein. Moreover, the RevAC-4 also bound as a multimer. Similar results were obtained with mutants R75 and RA80-82. These data indicate that a simple interaction of Rev with the RRE is insufficient for Rev function. The data also suggest that at least two steps are required for Rev function: association with RRE RNA, followed by interaction of cellular factors with the Rev–RRE complex to mediate the export of viral structural mRNAs from the nucleus to the cytoplasm. Thus, the trans-dominant Rev suppressors probably lack the capacity to interact with these cellular factor(s) and, in turn, suppress Rev function by competing for wild-type Rev binding on the RRE RNA.

It has been shown that the Rev protein encoded by the human T-cell leukemia virus 1 (HTLV-I) functions through an element in the HTLV-I long terminal repeat (LTR) to mediate export of HTLV-I structural mRNAs from the nucleus to the cytoplasm (Hanly et al. 1989; Itoh et al. 1989). Moreover, Rex expression can complement a Rev-deficient HIV-1 provirus (Rimsky et al. 1988), and mutant Rex proteins, which are trans-dominant suppressors of Rex function, are also trans-dominant suppressors of Rev function (Rimsky et al. 1989). Therefore, it is likely that the same factor(s) required for export of the HIV-1 and HTLV-I structural mRNAs interacts with the Rev and Rex proteins. This probably holds true for the evolutionarily conserved HIV-2 and simian immunodeficiency virus (SIV) viruses that encode Rev proteins with similar function (Malim et al. 1989c; P.J. Dillon, P. Nelbock, A. Perkins, and C.A. Rosen, in press). Identification of cellular factors that interact with Rev and Rex should provide further understandings.
standing of the underlying mechanisms for Rev- and
Rev-mediated regulation of human retrovirus gene ex-
pression.

Materials and methods

REV–RRE binding assay

The gel mobility-shift RNA protection assay was carried out as
described previously (Cochrane et al. 1990a). Briefly, crude E.
coli extract containing Rev protein or purified Rev protein
was incubated in binding buffer [5 mM HEPES (pH 7.0), 25 mM
KCl, 2 mM MgCl2, 3.8% glycerol] with 10 μg of tRNA as a nonspe-
cific competitor. After a 5-min incubation, in vitro-synthesized
RNA (~100,000 cpm) containing the RRE was added, and the
mixture was incubated for an additional 15 min at 30°C. The
samples were then digested with 7 units of RNase T1 before
electrophoresis on a nondenaturing polyacrylamide gel [4 or
6.5%]. For the experiments with Rev antibodies, binding reac-
tions were preincubated with antibodies for 15 min prior to ad-
dition of the RRE RNA probe.

Recombinant DNA procedures

Site-directed mutagenesis was performed as described (Kunkel
1985) by use of a Bio-Rad mutagenic kit (Bio-Rad). The muta-
tions were introduced into the Bluescript vector (Stratagene)
harboring a synthetic Rev gene altered to give more efficient
codon usage in prokaryotes [Cochrane et al. 1989a]. The syn-
thetic Rev gene used contains a stretch of histidine residues at
the amino terminus, facilitating purification of biological by
active protein [Cochrane et al. 1989a]. The modified Rev genes
were introduced into the pRC-25 vector under control of the
heat-inducible ApL promoter [Crowl et al. 1985]. Function of
the mutated Rev proteins in vivo was assessed by a previously
described heterologous gene expression assay [Rosen et al.
1988].

The amino acid sequence and the construction of Rev mu-
tants AC-4 [Malim et al. 1989a] and 916 (Cochrane et al. 1989b)
have been described previously. The RevAC-4 mutant is mu-
tated at residues 78 and 79 and has been shown to function as a
trans-dominant suppressor of Rev function [Malim et al.
1989a]. The Rev-916 mutant is a truncated Rev protein lacking
25 carboxy-terminal amino acids.

Preparation of Rev protein from E. coli extracts

Expression of Rev from the pRC-25 vectors was induced from
mid-logarithmic cultures by shifting the temperature from 30
to 42°C. Cells were recovered by centrifugation, the pellet was
resuspended in lysis buffer [50 mM Tris (pH 8.0), 1 mM EDTA, 5
mM DTT, 0.5 mM phenylmethylsulfonyl fluoride, or alterna-
tively, with the addition of 0.8 M KCl and 1% NP-40] and soni-
cated for ~1 min. Cell debris was removed by centrifugation,
and the cleared lysate was used directly in the binding reaction.
The presence of Rev protein was confirmed by Western blot
analysis with an antibody raised against Rev amino acids 1–20
(Perkins et al. 1989).

Rev complex formation

In vitro translation of the Rev-encoding RNA was performed
according to the manufacturer’s suggestions [Promega]. The in
vitro-translated protein was incubated in binding buffer in the
presence of 10 μg of nonspecific competitor tRNA. Biotinylated
Rev protein was added (~50–100 ng), and the mixture was in-
cubated at 30°C. After 10 min, the Rev protein complexes were
precipitated by addition of streptavidin–agarose [BRL, Gaith-
ersburg, MD], followed by centrifugation. Pellets were washed
three times in binding buffer, resuspended in gel loading buffer,
and analyzed on a 15% SDS gel. Rev protein was biotinylated
with biotin-N-hydroxysuccinimide-ester (BRL).

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References

Burglin, T.R. and E.M. DerRoberts. 1987. The nuclear migra-
tion signal of Xenopus laevis nucleoplasmin. EMBO J. 6:
2617–2625.

Burt, P.J.G. 1984. The current picture of the structure and as-
sembly of tobacco mosaic virus. J. Gen. Virol. 65: 253–279.

Cochrane, A.W., C.H. Chen, and C.A. Rosen 1990a. Specific in-
teraction of the HIV rev transactivator protein with a struc-
tured region in the env mRNA. Proc. Natl. Acad. Sci.
87: 1198–1201.

Cochrane, A.W., A. Perkins, and C.A. Rosen. 1990b. Identifica-
tion of sequences important in the nucleolar localization of
human immunodeficiency virus rev: Relevance of nucleolar
localization to function. J. Virol. 64: 881–885.

Cochrane, A.W., C.H. Chen, R. Kramer, L. Tomchak, and C.A.
Rosen. 1989a. Purification of biologically active human immu-
no deficiency virus rev protein from E. coli. Virology
173: 335–337.

Cochrane, A.W., E. Golub, D. Volsky, S. Ruben, and C.A.
Rosen. 1989b. Functional significance of phosphorylation to
the human immunodeficiency virus rev protein. J. Virol.
63: 4436–4440.

Cochrane, A.W., R. Kramer, S. Ruben, J. Levine, and C.A.
Rosen. 1989c. The human immunodeficiency virus rev pro-
tein is a nuclear phosphoprotein. Virology 171: 264–266.

Crowl, R., C. Seamans, C. Lomedico, and S. McAndrew. 1985.
Versatile expression vectors for high-level synthesis of
cloned gene products in Escherichia coli. Gene 38: 31–38.

Cullen, B. R., J. Hauber, K. Campbell, J.G. Sodroski, W. Hasel-
tine, and C.A. Rosen. 1988 Subcellular localization of the
human immunodeficiency virus trans-acting rev gene
product. J. Virol. 62: 2498–2501.

Daly, T., K. Cook, G. Gray, T. Maione, and J. Rusche. 1989.
Specific binding of HIV-1 recombinant rev protein to the
rev-responsive element in vitro. Nature 342: 816–819.

Dayton, A.I., E.F. Terwilliger, J. Potz, M. Kowalski, J.G. So-
droski, and W.A. Haseltine. 1988. Cis-acting sequences re-
sponsive to the rev gene product of the human immunodefi-
cency virus. J. AIDS 1: 441–452.

Dayton, E., D. Powell, and A. Dayton. 1989. Functional anal-
ysis of CAR, the target sequence for the rev protein of
HIV-1. Science 246: 1625–1629.

Dillon, P.J., P. Nelbock, A. Perkins, and C.A. Rosen. 1990.
Function of the human immunodeficiency virus types 1 and
2 Rev proteins is dependent upon their ability to interact
with a structural region present in the env gene mRNA. J.
Virol. [in press].
Emerman, M., R. Vazquez, and K. Peden. 1989. The rev gene product of the human immunodeficiency virus affects envelope-specific RNA localization. Cell 57: 1155–1165.

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.

Felber, B.K., M. Hadzopoulo-Cladaras, C. Cladaras, T. Copeyland, and G.N. Pavlakis. 1989. The rev protein of HIV-1 affects the stability and transport of the viral mRNA. Proc. Natl. Acad. Sci. 86: 1495–1499.

Gentz, R., C.H. Chen, and C.A. Rosen. 1989a. Bioassay for trans-activation using purified human immunodeficiency virus tat-encoded protein: Trans-activation requires mRNA synthesis. Proc. Natl. Acad. Sci. 86: 821–824.

Gentz, R., F.J. Rauscher, C. Abate, and T. Curran. 1989b. Parallel association of Fos and Jun leucine zippers juxtaposes DNA binding domains. Science 243: 1695–1699.

Gorman, C.M., L.F. Moffat, and B. Howard. 1982. Recombinant genomes which express chloramphenicol acetyl transferase in mammalian cells. Mol. Cell. Biol. 2: 1044–1051.

Hadzopoulo-Cladaras, M., B.K. Felber, C. Cladaras, A.A. Anthanassopoulos, A. Tse, and G.N. Pavlakis. 1989. The rev (trs/art) protein of the 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.

Hammarskjöld, M.L., J. Heijmer, B. Hammarskjöld, I. Sangwan, L. Albert, and D. Rekosh. 1989. Regulation of human immunodeficiency virus env expression by the rev gene product. J. Virol. 63: 1959–1966.

Hanly, S.M., L.T. Rimsky, M.H. Malim, J.J. Kim, J. Hauber, M. DucDodon, S.-Y. Le, J.V. Maizel, B.R. Cullen, and W.C. Greene. 1989. Comparative analysis of the HTLV-I Rex and HIV-1 rev trans-regulatory proteins and their RNA response elements. Genes Dev. 3: 1534–1544.

Hauber, J., M. Bouvier, M. Malim, and B. Cullen. 1988. Phosphorylation of the rev gene product of the human immunodeficiency virus type 1. J. Virol. 62: 4801–4804.

Heaphy, S., C. Dingwall, I. Ernberg, M.J. Gait, S.M. Green, J. Karn, A.D. Lowe, M. Singh, and M.A. 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.

Itoh, M., J. Inoue, H. Toyoshima, T. Akizawa, M. Higashi, and M. Yoshida. 1989. HTLV-I Rex and HIV-1 Rev act through similar mechanisms to relieve suppression of unspliced RNA expression. Oncogene 4: 1275–1279.

Kalderon, D., B.L. Roberts, W.D. Richardson, and A.E. Smith. 1984. A short amino acid sequence able to specify nuclear localization. Cell 31: 813–823.

Kunkel, T.A. 1985. Rapid and efficient site-specific mutagenesis without phenotypic selection. Proc. Natl. Acad. Sci. 82: 488–492.

Landschulz, W.H., P.F. Johnson, and S.L. McKnight. 1988. The leucine zipper: A hypothetical structural common to a new class of DNA-binding proteins. Science 240: 1759–1764.

Malim, M.H., S. Bohnlein, J. Hauber, and B.R. Cullen. 1989a. Functional dissection of the HIV-1 rev trans-activator-derivation of a trans-dominant repressor of rev function. Cell 58: 205–214.

Malim, M.H., J. Hauber, S.Y. Le, J.V. Maizel, and B.R. Cullen. 1989b. The HIV-1 rev transactivator acts through a structured target sequence to activate nuclear export of unspliced viral mRNA. Nature 338: 254–257.

Malim, M.H., S. Bohnlein, R. Fenrick, S.-Y. Le, J.V. Maizel, and B.R. Cullen. 1989c. Functional comparison of the Rev transactivators encoded by different primate species immunodeficiency virus species. Proc. Natl. Acad. Sci. 86: 8222–8226.

Malim, M.H., L.S. Tiley, D.F. McCorm, J.R. Rusche, J. Hauber, and B.R. Cullen. 1990. HIV-1 structural gene expression requires binding of the Rev transactivator to its RNA target sequence. Cell 60: 675–683.

Olsen, H.S., P. Nelbock, 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.

Perkins, A., A. Cochrane, S. Ruben, C. Rosen. 1989. Structural and functional characterization of the human immunodeficiency virus rev protein. J. AIDS 2: 256–263.

Rimsky, L.M.D. Dodon, E.P. Dixon, and W.C. Greene. 1989. Trans-dominant inactivation of HTLV-I and HIV-1 gene expression by mutation of the HTLV-I Rex transactivator. Nature 341: 453–456.

Rimsky, L., 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 335: 738–740.

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. 85: 2071–2075.

Sodroski, J., W.C. Goh, C. Rosen, A. Dayton, E. Terwilliger, and W.A. Haseltine. 1986. A second post-transcriptional transactivator gene required for the HTLV-III replication. Nature 321: 412–417.

Terwilliger, E., R. Burghoff, R. Sia, J. Sodroski, W. Haseltine, and C. Rosen. 1988. The art gene product of the human immunodeficiency virus is required for replication. J. Virol. 62: 655–658.

Turner, R. and R. Tjian. 1989. Leucine repeats and an adjacent DNA-binding domain mediate the formation of functional c-fos and c-jun heterodimers. Science 243: 1689–1694.

Zapp, M. and M. Green. 1989. Sequence-specific RNA binding by the HIV-1 rev protein. Nature 342: 714–716.
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.

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