Identification of a Novel Cellular Cofactor for the Rev/Rex Class of Retroviral Regulatory Proteins

Hal P. Bogerd, Robert A. Fridell, Steven Madore,*
and Bryan R. Cullen
Howard Hughes Medical Institute
and Department of Genetics
Duke University Medical Center
Durham, North Carolina 27710

Summary

HIV-1 Rev is the prototype of a class of retroviral regulatory proteins that induce the sequence-specific nuclear export of target RNAs. This function requires the Rev activation domain, which is believed to bind an essential cellular cofactor. We report the identification of a novel human gene product that binds to not only the HIV-1 Rev activation domain in vitro and in vivo but also to functionally equivalent domains in other Rev and Rex proteins. The Rev/Rex activation domain-binding (Rab) protein occupies a binding site on HIV-1 Rev that precisely matches that predicted by genetic analysis. Rab binds the Rev activation domain when Rev is assembled onto its RNA target and can significantly enhance Rev activity when overexpressed. We conclude that Rab is the predicted activation domain-specific cofactor for the Rev/Rex class of RNA export factors.

Introduction

The human immunodeficiency virus type 1 (HIV-1) Rev protein is required for the nucleocytoplasmic transport, and hence translation, of a class of incompletely spliced HIV-1 mRNAs that encode the viral structural proteins (reviewed by Cullen, 1992). In the absence of Rev, these late viral RNAs remain sequestered in the nucleus until they are either spliced or degraded (Emerman et al., 1989; Felber et al., 1989; Malim et al., 1989b). Rev function is therefore essential for the production of progeny virions by HIV-1 infected cells (Feinberg et al., 1986; Sodroski et al., 1986).

Rev-mediated nuclear RNA export requires the direct interaction of Rev with a cis-acting, ~234 nt RNA stem-loop structure termed the Rev response element (RRE) (Daly et al., 1989; Malim et al., 1989b, 1990). It has been proposed that Rev first binds to a high affinity site within the RRE as a monomer (Bartel et al., 1991; Tiley et al., 1992b). Subsequently, additional Rev monomers assemble onto the RRE in an ordered process mediated by both protein–protein and protein–RNA interactions. This multimerization event is critical for Rev function via the RRE (Malim and Cullen, 1991; Iwai et al., 1992; McDonald et al., 1992).

*Present address: Department of Dermatology, University of Michigan Medical Center, Ann Arbor, Michigan 48109-0528.

Mutational analysis of the 116 amino acid Rev transactivator has identified two functional domains (Figure 1). A more amino-terminal basic domain acts as both a nuclear/nucleolar localization signal and as a sequence-specific RNA-binding domain (Malim et al., 1989a; Cochrane et al., 1990; Kjems et al., 1992). This domain is flanked by sequences that are essential for efficient Rev multimerization (Malim and Cullen, 1991). Toward the carboxyl terminus of Rev lies a short (~10 amino acid) leucine-rich sequence, termed the Rev activation domain, that is dispensable for RRE binding but nevertheless essential for Rev function (Figure 1) (Malim et al., 1989a, 1991; Venkatesh and Chinnadurai, 1990). At least two lines of evidence suggest that the activation domain represents a binding site for an essential cellular Rev cofactor. Most importantly, this short sequence element is the only part of Rev whose integrity is essential for Rev function via a heterologous RNA target site (McDonald et al., 1992). Thus, it has been demonstrated that a fusion protein consisting of Rev linked to the MS2 bacteriophage coat protein can induce the nuclear export of an RNA containing multiple copies of the MS2 coat protein RNA-binding site. Mutations known to block the activity of the Rev RNA-binding or multimerization sequence (or both) prevent the function of this fusion protein via the RRE yet fail to inhibit function via the MS2 RNA operator. In contrast, mutation of the Rev activation domain abrogates function via either RNA target (McDonald et al., 1992). These data therefore imply that the primary role of the Rev RNA-binding/multimerization domain is to facilitate the assembly of a Rev–RRE ribonucleoprotein complex that then recruits the appropriate cellular cofactor to the Rev activation motif.

A second line of evidence indicating that the activation domain is a Rev cofactor-binding domain derives from the observation that mutant Rev proteins containing an intact RNA-binding/multimerization domain, but lacking a functional activation domain, exhibit a potent dominant negative phenotype in vivo (Malim et al., 1989a, 1991; Venkatesh and Chinnadurai, 1990). It has been proposed that these Rev mutants participate with wild-type Rev in the formation of the Rev–RRE ribonucleoprotein complex but then block the function of the wild-type Rev by interfering with the cooperative recruitment of a cellular cofactor. The observation that Rev is functional in cells derived from a wide range of eukaryotes, including primates, mice, birds, frogs, fruit flies, and even yeast, implies that this unidentified cellular cofactor must have been evolutionarily conserved (Ivey-Hoyle and Rosenberg, 1990; Malim et al., 1991; Fischer et al., 1994; Stutz and Rosbash, 1994).

While HIV-1 Rev is the most intensely studied retroviral RNA transport factor, other members of the lentivirinae also encode Rev proteins, while the effectively unrelated T cell leukemia viruses, including human T cell leukemia virus type I (HTLV-I), encode an equivalent regulatory protein termed Rex (Cullen, 1992). For many of these other Rev and Rex proteins, it has proven possible to muta-

RNA Binding Domain   Activation Domain
1   118

75 – L P P L E R L T L D –84   HIV-1 REV
73 – I Q H L Q G L T I Q –82   HIV-2 REV
106 – M V G M E N L T L E –115   VMV REV
93 – L G A L A E L T L E –102   CAEV REV
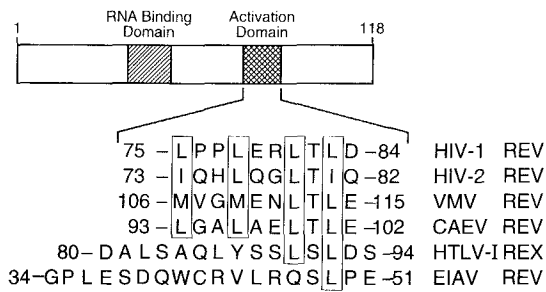80– D A L S A Q L Y S S L S L D S –94   HTLV-I REX
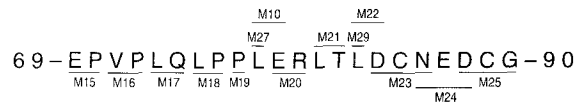34–G P L E S D Q W C R V L R Q S L P E –51   EIAV REV

Figure 1. Primary Sequence of the Minimal Activation Domains of Selected Rev and Rex Proteins

HIV-1 Rev contains an amino-terminal sequence that mediates RNA binding and Rev multimerization and a carboxy-terminal activation domain that is required for the recruitment of an essential cellular cofactor. The HIV-1 Rev activation domain is ~10 amino acids in length and includes four important leucine residues. Rev proteins encoded by other primate and ovine/caprine lentiviruses are similar to HIV-1 Rev in both their domain organization and their activation domain sequence. In contrast, while both EIAV Rev and HTLV-I Rex also contain defined domains that can functionally replace the activation domain of HIV-1 Rev, these display little sequence similarity.

tionally define short protein motifs that can functionally substitute for the activation domain of HIV-1 Rev (Hope et al., 1991; Tiley et al., 1991). In the case of the other primate lentiviruses, as well as the more divergent ovine/caprine lentiviruses visna–maedi virus (VMV) and caprine arthritis encephelitis virus, the Rev activation domain has been found to be similar in size and composition to that present in HIV-1 Rev (Malim et al., 1991; Tiley et al., 1991) (Figure 1). However, both the Rev protein encoded by the lentivirus equine infectious anemia virus (EIAV) and the Rex protein of HTLV-I shares no significant sequence homology with HIV-1 Rev and contains larger, divergent activation domains (Hope et al., 1991; Weichselbraun et al., 1992; Fridell et al., 1993; Mancuso et al., 1994). In the case of HTLV-I Rex, this ~15 amino acid sequence can be at least partially aligned with the relevant HIV-1 Rev sequence (Figure 1). In contrast, the ~18 amino acid activation domain of EIAV Rev displays no evident sequence similarity to the ~10 amino acid HIV-1 Rev activation motif, even though it can effectively substitute for the latter in mediating HIV-1 Rev function. Nevertheless, the functional equivalence of each of these protein sequences in vivo strongly suggests that they are each likely to interact with the same cellular cofactor. Here, we report the identification and initial characterization of this shared activation domain–specific Rev/Rex cofactor.

Results

Using the yeast two-hybrid protein interaction trap (Fields and Song, 1989; Fridell et al., 1995), we sought to identify a human cDNA that encoded a protein able to interact not only with HIV-1 Rev but also with HTLV-I Rex in an activation domain–dependent manner. The screened library consisted of the VP16 transcription activation domain fused to cDNA sequences derived from the human CEM T cell line. This screen led to the identification of three

                    M10   M22
                M27   M21 M29
69 – E P V P L Q L P P L E R L T L D C N E D C G – 90
    M15 M16 M17 M18 M19   M20   M23 ____ M25
                                              M24

Rev Mutant	Rev Activity	Rab Binding
WT	++	1.00
M10	–	<0.01
M15	++	0.57
M16	++	1.06
M17	++	0.25
M18	+	0.10
M19	++	1.79
M20	+	0.16
M21	–	<0.01
M22	–	<0.01
M23	++	3.63
M24	+	0.12
M25	++	1.87
M27	–	<0.01
M29	–	<0.01

Figure 2. The Ability of Rev Activation Domain Mutants to Function In Vivo Closely Correlates with Their Ability to Bind Rab

A set of scanning missense mutations across the HIV-1 Rev activation domain has been previously described (Malim et al., 1991). In brief, all two amino acid mutations (M10, M15–M18, and M20–M22) have aspartic acid and leucine substituted in place of the wild-type sequence. Single amino acid mutations feature a substituted aspartic acid (M19) or alanine (M27 and M29) residue, while three amino acid mutations contain the inserted sequence glutamic acid–aspartic acid–leucine (M23 and M25) or lysine–aspartic acid–leucine (M24). The in vivo biological activity of these Rev mutants has been reported (Malim et al., 1991) and is as follows: double plus, >50% of wild-type Rev function; plus, ≤50% of wild-type; and minus, negative phenotype. Each Rev mutant was expressed in the yeast indicator strain GGY1:: 171 as a GAL4 fusion protein, and the level of β-gal activity induced by coexpression of the VP16–Rab fusion was determined. This measure of the ability of each Rev mutant to bind Rab is expressed relative to the wild-type GAL4–Rev fusion protein, which is set at 1.0.

clones that encoded VP16 fusion proteins that specifically interacted with the GAL4–Rev bait protein and 10 clones expressing VP16 fusion proteins that specifically bound GAL4–Rex. None of the three Rev-specific clones were able to interact with GAL4–Rex, while only one of the 10 Rex-specific clones could bind to GAL4–Rev. Further experiments demonstrated that this interaction was entirely blocked by the introduction of point mutations into the activation domains of either Rev or Rex (data not shown). Based on this initial observation, we designated this human protein as the Rev/Rex activation domain–binding protein (Rab).

Rab Binds the Rev Activation Domain Specifically

To define more fully the protein sequence specificity of the interaction of Rev with Rab, we took advantage of a previously described (Malim et al., 1991) set of scanning missense mutations that precisely map the HIV-1 Rev activation domain (Figure 2). These mutations each affect from one to three amino acids either within or adjacent to the activation domain and give rise to wild-type, intermediate, or negative phenotypes when assayed for Rev function in mammalian cells. This complete set of Rev mutants was cloned into a yeast GAL4 fusion protein expression plasmid, and each mutant was individually tested for its ability to interact with the VP16–Rab fusion protein in the yeast cell nucleus, as assessed by the level of activation

```
  1  MAASAKRKQEEKHLKMLRDMTGLPHNRKCF
 31  DCDQRGPTYVNMTVGSFVCTSCSGSLRGLN
 61  PPHRVKSISMTTFTQQEIEFLQKHGNEVCK
 91  QIWLGLFDDRSSAIPDFRDPQKVKEFLQEK
121  YEKKRWYVPPEQAKVVASVHASISGSSASS
151  TSSTPEVKPLKSLLGDSAPTLHLNKGTPSQ
181  SPVVGRSQGQQQEKKQFDLLSDLGSDIFAA
211  PAPQSTATANFANFAHFNSHAAQNSANADF
241  ANFDAFGQSSGSSNFGGFPTASHSPFQPQT
271  TGGSAASVNANFAHFDNFPKSSSADFGTFN
301  TSQSHQTASAVSKVSTNKAGLQTADKYAAL
331  ANLDNIFSAGQGGDQGSGFGTTGKAPVGSV
361  VSVPSQSSASSDKYAALAELDSVFSSAATS
391  SNAYTSTSNASSNVFGTVPVVASAQTQPAS
421  SSVPAPFGATPSTNPFVAAAGPSVASSTNP
451  FQTNARGATAATFGTASMSMPTGFGTPAPY
481  SLPTSFSGSFQQPAFPAQAAFPQQTAFSQQ
511  PNGAGFAAFGQTKPVVTPFGQVAAAGVSSN
541  PFMTGAPTGQFPTGSSSTNPFL
```

Figure 3. Predicted Primary Sequence of the Human Rab Protein

The *rab* cDNA contains a single long ORF that is here translated from the first in-frame initiation codon. In vitro translation of this sequence gives rise to a protein that is the same size as the Rab protein detected in vivo (see below). Phenylalanine residues, including the dipeptide motif FG, as well as runs of serines are indicated.

A   **B**

C   **D**

Figure 4. Analysis of *rab* RNA and Protein Expression

(A) Northern blot analysis of poly(A)$^+$ RNA from the human cell lines CEM and HeLa. The relative mobility of marker RNA species is shown at the left of the blot while the extrapolated size of the hybridizing RNA species is given at right in kilobases.

(B) Western blot analysis of Rab protein expression. Proteins extracted from cells obtained from the indicated species were separated by 12% SDS–PAGE, transferred to nitrocellulose, and then subjected to Western blot analysis using an affinity-purified rabbit anti-Rab antiserum. The blot shown on the right was performed in the presence of soluble Rab protein to assess signal specificity.

(C) Immunofluorescence analysis of CV1 cells probed with the rabbit anti-Rab antiserum followed by rhodamine-conjugated goat anti-rabbit antiserum.

(D) Interaction of Rab with HIV-1 Rev in vitro. Equal levels of a radioactively labeled preparation of the ~60 kDa Rab protein were loaded onto columns containing GST, GST fused to HIV-1 Rev (GST–Rev), or GST fused to the M10 Rev activation domain mutant (GST–M10). After extensive washing, bound proteins were eluted from the columns and subjected to 12% SDS–PAGE. The Rab protein did not bind to the GST or GST–M10 columns but was specifically retained by the GST–Rev column. The relative mobility of marker proteins of the indicated size (in kilodaltons) is given at the left of (B) and (D).

of an integrated *lacZ* indicator gene. Each of these mutant GAL4–Rev fusion proteins was equivalently stable in yeast, as measured by Western blot analysis (data not shown).

As shown in Figure 2, five Rev mutants that entirely lack effector domain function, including two mutants (M27 and M29) that bear only single amino acid changes in Rev, all proved entirely unable to interact with the Rab fusion protein. In contrast, all of the Rev mutants previously shown (Malim et al., 1991) to exhibit substantially wild-type Rev activity in vivo also induced substantial levels of β-galactosidase (β-gal) activity, ranging from a minimum of one quarter to a maximum of approximately three times the level seen with wild-type Rev. Strikingly, the three Rev mutants (M18, M20, and M24) previously shown (Malim et al., 1991) to exhibit only partial (≤50%) Rev function in vivo also proved to be significantly attenuated in their ability to interact with Rab (≤16% of wild type). It is therefore apparent that there is a very close concordance between the ability of Rev activation domain mutants to function in the recruitment of the Rev cofactor in mammalian cells and the ability of these same Rev activation domain mutants to interact with the Rab protein in the yeast cell nucleus.

Sequence of the *rab* Gene Product

The *rab* cDNA clone contains a 2584 bp insert flanked 3′ by a stretch of A residues, consistent with priming at the mRNA poly(A) tail. Starting at the first in-frame methionine residue, this cDNA contains an open reading frame (ORF) of 562 amino acids that would be predicted to encode a protein of ~58 kDa (Figure 3). Computer analysis of available sequence databases failed to identify any proteins displaying significant homology to the predicted Rab ORF. Similarly, we were also unable to identify any nucleic
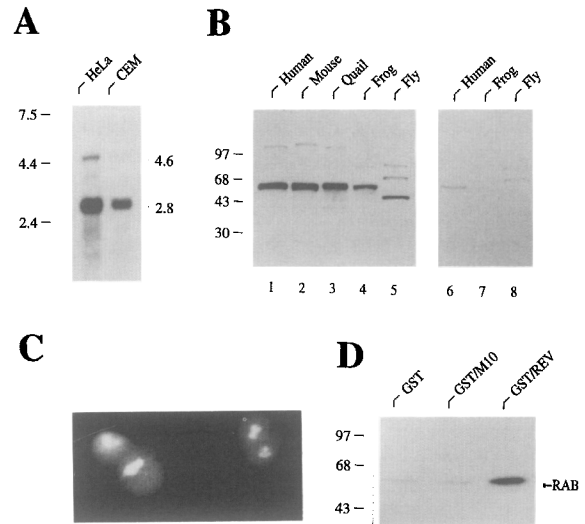
acid sequences that displayed significant homology to *rab* except for two short, unidentified expressed sequence tags. *rab* is therefore a novel human gene. An unusual aspect of the Rab protein sequence is the high concentration of phenylalanine residues, including 10 in the form of the dipeptide motif FG, as well as several runs of serine residues, found concentrated toward the carboxyl terminus of Rab. The potential significance of these is discussed below.

Analysis of *rab* mRNA and Protein Expression

The *rab* cDNA insert was used to probe a Northern blot of mRNA derived from the human T cell line CEM (the origin of the cDNA clone) and the human cell line HeLa. In both cases, a prominent band of ~2.8 kb was observed (Figure 4A). Both cell lines also expressed a hybridizing band of ~4.6 kb, although this was faint in the CEM cells. Similarly, Northern blot analysis of *rab* mRNA expression in a range of human tissues also identified a major 2.8 kb and a minor 4.6 kb *rab* transcript in all tissues examined

(data not shown). Based on this analysis, it therefore appeared that this cloned *rab* cDNA is close to the full length of the major species of *rab* mRNA expressed in human cell lines and tissues.

An interesting characteristic of both Rev and Rex is that these retroviral regulatory proteins are functional in a wide range of animal cells (Ivey-Hoyle and Rosenberg, 1990; Malim et al., 1991; Fischer et al., 1994). Therefore, it is predicted that the cellular cofactor for Rev and Rex should be conserved across species boundaries. To test this hypothesis, we performed a Western blot analysis on cellular protein extracts of mammalian, avian, amphibian, and invertebrate origin using an affinity-purified polyclonal rabbit anti-Rab antiserum (Figure 4B). In humans, this procedure identified a predominant band of ~60 kDa (Figure 4B, lane 1), and bands of identical mobility were also detected in cellular extracts of mouse, quail, and frog origin (lanes 2–4). In the fruit fly, a slightly smaller cross-reactive band was observed (Figure 4B, lane 5). These bands all represented authentic Rab-related proteins, in that reactivity could be specifically blocked by addition of a purified, soluble maltose-binding protein (MBP)–Rab fusion protein to the Western blot during the antibody-binding step (Figure 4B, lanes 6–8).

An indirect immunofluorescence analysis of the localization of endogenous Rab protein in the primate cell line CV1, using the Rab-specific rabbit antiserum, is shown in Figure 4C. This experiment revealed that Rab is concentrated in the nucleus and nucleolus of these cells in a pattern that is similar to that observed previously with HIV-1 Rev and HTLV-I Rex (Siomi et al., 1988; Felber et al., 1989). No specific signal was detected when a matched preimmune rabbit serum was used (data not shown). While Western blot analysis of nuclear and cytoplasmic protein fractions derived from HeLa cells further confirmed the nuclear concentration of Rab, this latter analysis also detected significant levels of cytoplasmic Rab protein (data not shown). It therefore appears that Rab is primarily, but not exclusively, nuclear in localization.

Although we have been unable to express the full-length Rab protein in a soluble form in bacteria, we are able to express limited amounts of soluble Rab protein in vitro by translation of *rab* mRNA in a rabbit reticulocyte lysate. Translation of the Rab ORF indicated in Figure 3 gave rise to a protein that migrated on polyacrylamide gels with the same relative molecular mass as the major Rab antibody–reactive species detected in human cells in vivo (Figure 4). Therefore, the Rab ORF shown in Figure 3 is full length.

We next used ^{35}S-labeled full-length Rab protein generated by in vitro translation to ask whether Rab would bind the Rev activation domain specifically in vitro (Figure 4D). For this purpose, we loaded equal levels of the ^{35}S-labeled Rab protein onto columns containing either glutathione S-transferase (GST) or fusion proteins consisting of GST linked to Rev (GST–Rev) or the M10 activation domain Rev mutant (GST–M10), conjugated to agarose beads. After extensive washing, remaining bound proteins were eluted and subjected to SDS–polyacrylamide gel electrophoresis (SDS–PAGE). As shown in Figure 4D, the labeled

Rab protein was not retained by the GST or GST–M10 columns but did bind to the GST–Rev column. These data therefore demonstrate that the specific interaction of Rab with the Rev activation domain can be recapitulated in vitro and also strongly suggest that this interaction is direct.

Rab Binds the Activation Domains of Diverse Rev and Rex Proteins In Vivo

We next wished to ask whether the interaction of Rab with Rev could be demonstrated in mammalian cells and whether other Rev and Rex proteins, particularly including the highly divergent EIAV Rev (see Figure 1), would also bind to Rab specifically. To address these issues, we performed a mammalian version of the two-hybrid analysis using transient transfection of the primate cell line COS (Figure 5). The pG5B-CAT indicator plasmid used in this analysis contains five GAL4 sites located 5' to a minimal promoter element directing expression of the chloramphenicol acetyl transferase (CAT) gene (Bogerd and Greene, 1993). In this assay, the identities of the bait-and-prey proteins were reversed such that the Rab protein was now expressed as a GAL4–Rab fusion while the full-length HIV-1, VMV, and EIAV Rev proteins and the HTLV-I Rex protein were each expressed as VP16 fusion proteins. For each Rev and Rex protein, plasmids bearing a deleterious
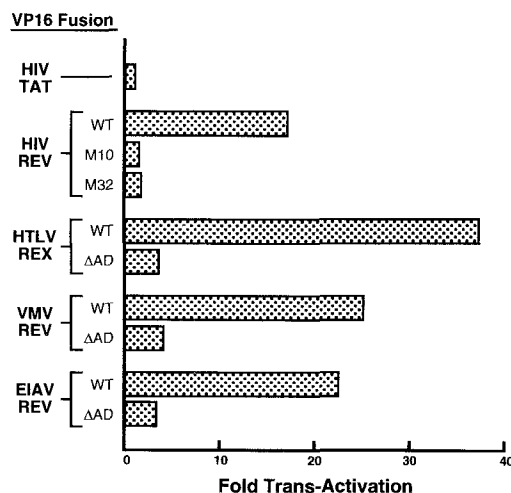

Figure 5. Rab Interacts with the Activation Domains of Multiple Rev and Rex Proteins in the Mammalian Nucleus

The Rab protein was expressed as a GAL4 fusion and the indicated retroviral regulatory proteins as VP16 fusions. The level of CAT enzyme activity detected in a COS cell culture transfected with pBC12-GAL4-Rab and the pG5B-CAT indicator plasmid was set at 1.0. Levels of CAT expression observed when COS cultures were additionally cotransfected with the indicated VP16 fusion protein expression plasmids are given as multiples of this value. VP16 fusion proteins containing the wild-type forms of HIV-1, VMV and EIAV Rev, or HTLV-I Rex protein were each able to assemble onto the GAL4–Rab protein bound to the pG5B-CAT-indicator plasmid, resulting in a 20- to 40-fold increase in CAT enzyme activity. In contrast, mutation of the activation domains present in each of these Rev or Rex proteins (indicated by M10 or M32 for HIV-1 Rev or ΔAD for the other proteins) effectively blocked this in vivo interaction. Data shown are representative of multiple independent transfection experiments that were each internally controlled by cotransfection of a β-gal expression plasmid. (See Experimental Procedures for description of the mutants used.)
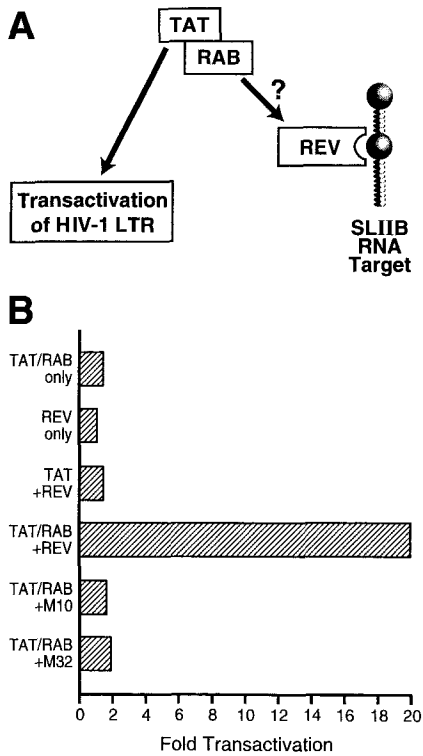
Figure 6. The Rab Protein Binds the Rev–RRE Ribonucleoprotein Complex In Vivo

(A) Schematic representation of an in vivo monohybrid assay for Rab binding to the Rev–RRE ribonucleoprotein complex. See text for discussion of the experimental strategy.

(B) Activation of the SLIIB–CAT indicator construct in HeLa cells upon cotransfection of the indicated expression plasmids. Only the combination of both the Tat–Rab fusion protein and the wild-type Rev protein results in activation of the HIV-1 LTR-linked CAT indicator gene. Introduction of the M10 or M32 mutation into the HIV-1 Rev activation domain blocks the in vivo interaction of Rab with the RNA-bound Rev protein.

missense mutation in the mutationally defined activation domain were also generated (Figure 5). All VP16 fusion proteins were expressed at comparable levels after transfection into COS cells (data not shown).

Transient transfection of COS cell cultures with the pG5B-CAT indicator plasmid, along with the pBC12-GAL4-Rab expression plasmid, gave only a low level of CAT enzyme activity. While this level was not significantly enhanced by coexpression of a Tat–VP16 fusion protein, a similar Rev–VP16 fusion protein produced a marked (~ 18-fold) induction in the level of CAT activity (Figure 5). In contrast, two Rev–VP16 fusion proteins bearing either the M10 or the M32 missense mutation of the Rev activation domain (Malim et al., 1991) each failed to enhance expression of the CAT indicator gene.

Similar evidence for an in vivo interaction with Rab was also obtained using VP16 fusion proteins containing either the HTLV-I Rex protein or the VMV Rev or more highly divergent EIAV Rev protein (Figure 5). In each case, introduction of a missense mutation (ΔAD) known to block activation domain function markedly inhibited the in vivo inter-

action with GAL4–Rab. We therefore conclude that Rab can interact with a range of highly divergent Rev and Rex proteins in the mammalian cell nucleus and that the integrity of the mutationally defined activation motifs is, in each case, critical to this interaction. It should be noted that the endogenously expressed Rab protein (see Figure 4) would be predicted to act as an efficient competitive inhibitor of the in vivo interaction between GAL4–Rab and these various Rev or Rex fusion proteins. It is therefore likely that the data presented in Figure 5 significantly underestimate the efficiency of this in vivo interaction.

Rab Binds to the Rev Activation Domain When Rev Is Bound to the RRE

An important predicted property of the cellular cofactor for HIV-1 Rev is that it should be able to interact efficiently with the Rev activation domain when Rev is assembled onto the RRE RNA target. To test whether Rab satisfied this prediction, we devised an in vivo genetic assay that uses the HIV-1 *tat* RNA sequence-specific transcriptional activator to provide an indirect measure of the assembly of a protein complex onto an RNA target sequence (Figure 6A). Normally, Tat activates gene expression from the HIV-1 long terminal repeat (LTR) after binding a *cis*-acting RNA target sequence, termed TAR, that forms the first 59 nt of all HIV-1 transcripts (Cullen, 1992). We have previously shown that if the TAR RNA stem–loop is replaced with the RRE-derived stem–loop IIB (SLIIB) minimal RNA target sequence for HIV-1 Rev, then *trans*-activation of the HIV-1 LTR can only be observed if Tat is fused to an HIV-1 Rev protein bearing an intact RNA-binding domain (Tiley et al., 1992a).

The assay delineated in Figure 6A takes this approach one step further. Here, the TAR element is again replaced by RRE SLIIB in an indicator construct in which the HIV-1 LTR is linked to CAT (pSLIIB-CAT). However, in this case, Rev is expressed in its wild-type form while Tat is expressed as a Rab fusion protein. Only if Rab can induce the efficient recruitment of this Tat–Rab fusion protein to the RRE-bound Rev protein will Tat be brought to the HIV-1 LTR promoter element and, hence, be able to activate HIV-1 LTR-driven CAT expression (Figure 6A).

Results obtained using this monohybrid assay on the homologous RRE RNA target are presented in Figure 6B. As can be readily seen, neither HIV-1 Rev alone nor the Tat–Rab fusion protein alone was able to induce a level of CAT expression that exceeded the basal level seen in HeLa cells transfected with the pSLIIB-CAT indicator construct alone. However, the simultaneous expression of both Rev and Tat–Rab in the nuclei of these human cells induced a readily detectable ~ 19-fold induction in CAT protein expression. Assembly of Rab onto the Rev–RRE ribonucleoprotein complex was, as predicted, fully dependent on the integrity of the Rev activation domain as shown by the lack of activity of the Rev M10 and M32 activation domain mutants in this assay (Figure 6B). Importantly, these two mutations have no effect on the ability of Rev to bind the RRE in vivo (Tiley et al., 1992a).
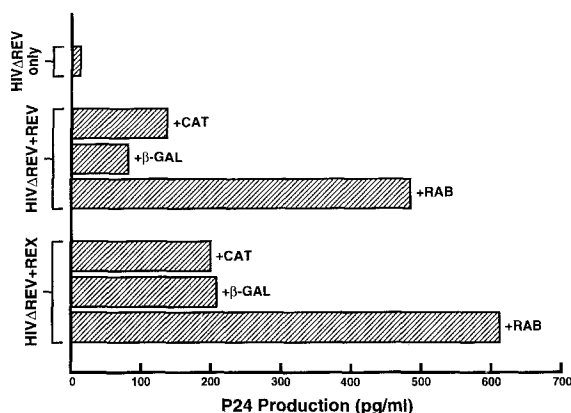
Figure 7. Overexpression of Rab Enhances HIV-1 Rev and HTLV-I Rex Function

COS cell cultures (35 mm) were transfected with 100 ng of the Rev-defective HIV-1 provirus expression plasmid pHIVΔRev, together with either 50 ng of the HIV-1 Rev expression plasmid pcRev or 100 ng of the HTLV-I Rex expression plasmid pcRex. In addition, each culture was transfected with 1 μg of a pBC12-CMV-based plasmid designed to express CAT, β-gal, or Rab and 20 ng of the indicator plasmid pBC12-CMV-SEAP. At 88 hr after transfection, the supernatant media were harvested and levels of secreted p24 capsid protein and SEAP determined. While Rab coexpression increased the Rev- or Rex-induced production of HIV-1 capsid protein by from 3- to 6-fold, it did not significantly affect the low basal expression of p24 (<20 pg/ml) seen in the absence of Rev and Rex (data not shown). Similarly, Rab overexpression also had no significant effect on the production of the SEAP internal control (setting mean SEAP activity at 1.0, the observed standard deviation was ± 0.27). Data are representative of three independent experiments.

Overexpression of Rab Can Enhance Rev and Rex Function In Vivo

The data presented in Figure 6 show that Rab can bind to the Rev activation domain when Rev is part of the Rev-RRE ribonucleoprotein complex. Therefore, Rab can effectively compete with the endogenous Rev cofactor for binding to the Rev activation domain. This finding suggested that an in vivo assay for Rev function performed under conditions in which the level of Rev expression was suboptimal should be able to uncover a phenotype for Rab even in the face of a significant endogenous level of Rab expression. In particular, we hypothesized that, if Rab is not the authentic Rev cofactor, then overexpression of Rab should competitively inhibit Rev function. In contrast, if Rab is the authentic Rev cofactor, then overexpression of Rab might promote the recruitment of Rab to the RRE RNA target by suboptimal levels of Rev protein, leading to an increase in Rev activity. Similarly, the HTLV-I Rex protein, which can also act via the HIV-1 RRE element (Rimsky et al., 1988), might also display enhanced activity in the presence of Rab if this protein is indeed the authentic Rev/Rex cofactor.

To test this hypothesis, we first introduced a premature termination codon into the rev gene present in a full-length HIV-1 proviral clone. We then transfected the resultant HIVΔRev provirus into COS cells along with different levels of the HIV-1 Rev expression plasmid pcRev or the HTLV-I Rex expression plasmid pcRex (Rimsky et al., 1988), and we determined the level of each of these plas-

mids that induced ~ 10% of the level of p24 capsid protein production that was seen with a saturating level of Rev. We next transfected COS cells with the HIVΔRev proviral clone, along with this limiting level of either pcRev or pcRex, together with an ~ 20-fold molar excess of a pBC12-CMV based plasmid (Malim et al., 1991) expressing the full-length Rab protein or expressing either CAT or β-gal as negative control proteins. Based on immunoprecipitation analysis, we estimate that cells transfected with the Rab expression plasmid express 5- to 10-fold higher levels of Rab than normal (data not shown). Transfection efficiency was monitored by cotransfecting a constant level of an expression plasmid encoding the secreted alkaline phosphatase (SEAP) indicator gene (Berger et al., 1988). After ~ 88 hr, the supernatant media were removed from the transfected cultures and levels of p24 capsid protein and SEAP activity determined. As shown in Figure 7, we consistently observed a 3- to 6-fold increase in the level of HIV-1 p24 capsid protein secreted by the culture overexpressing the Rab protein when compared with the cultures expressing either CAT or β-gal. In contrast, Rab overexpression had no significant effect on the level of supernatant SEAP activity. These data therefore strongly support the hypothesis that Rab is an important human cellular cofactor for HIV-1 Rev and HTLV-I Rex.

Discussion

The Rev transactivator encoded by HIV-1 is of considerable scientific interest for at least two reasons. First, Rev function is critical for the expression of HIV-1 structural proteins and, hence, for viral replication in vivo (Feinberg et al., 1986; Sodroski et al., 1986). Rev therefore represents an attractive target for chemotherapeutic intervention in HIV-1 induced disease. Second, HIV-1 Rev is the prototype of a novel class of retroviral regulatory proteins that act at the level of sequence specific nuclear RNA export (Cullen, 1992). Relatively little is known about how nucleocytoplasmic RNA transport is regulated in the eukaryotic cell, and a more complete understanding of the mechanism of action of Rev may, therefore, also lead to important insights into how this fundamental step in the expression of the eukaryotic genome is regulated. Importantly, recent data suggesting that human cells express an endogenous activity that can also induce the sequence-specific nuclear export of target mRNAs (Huang and Liang, 1993; Bray et al., 1994) also suggest that Rev-like regulatory proteins, rather than being restricted to complex retroviruses, might also play a critical role in mediating the expression of specific cellular genes.

Previous work on the HIV-1 Rev protein and on the functionally equivalent Rev and Rex proteins encoded by other complex retroviruses led to the mutational definition of activation domains that are believed to represent binding domains for a cellular cofactor (Figure 1). In the case of HIV-1 and other primate and ovine/caprine lentiviruses, this activation domain is a discrete (~ 10 amino acid) sequence containing four critically spaced leucine or similarly large hydrophobic amino acids (Figure 1) (Malim et al., 1991; Tiley et al., 1991). However, the activation do-

mains of the EIAV Rev protein and of the Rex protein of HTLV-I, while functionally equivalent to the sequence seen in HIV-1 Rev, are nevertheless distinct in terms of not only their larger size but also their primary sequence (Hope et al., 1991; Weichselbraun et al., 1992; Fridell et al., 1993; Mancuso et al., 1994). Together, this divergent set of Rev and Rex sequences are nevertheless predicted to bind to the same cellular cofactor and thus represent an invaluable tool for the experimental identification and validation of this factor.

In this paper, we report the identification and initial characterization of a novel human protein that specifically binds to each of these Rev and Rex activation domains in the mammalian cell nucleus (Figure 5). In fact, this cellular protein, which we term Rab, displays precisely the protein binding specificity predicted for the authentic cellular cofactor for HIV-1 Rev, as demonstrated by the close correlation between the biological activity and Rab binding ability of a large set of HIV-1 Rev activation domain missense mutants (Figure 2). We further show that Rab can specifically bind to the HIV-1 Rev activation domain in vitro (Figure 4D) and that Rab can efficiently assemble onto the activation domain when Rev is bound to its homologous RRE RNA target in vivo (Figure 6). Most importantly, we demonstrate that overexpression of the Rab protein can specifically enhance the ability of either HIV-1 Rev or HTLV-I Rex to activate HIV-1 structural protein expression in transfected cells in culture (Figure 7). Overall, these data provide clear evidence in support of the hypothesis that Rab is both the cellular target of the various Rev and Rex activation domains listed in Figure 1 and an important cofactor for HIV-1 Rev and HTLV-I Rex function in vivo.

Earlier efforts to identify cellular cofactors for HIV-1 Rev have led to the identification of two other candidate proteins. Luo et al. (1994) have demonstrated that the cellular protein p32, identified as a factor copurifying with the cellular splicing factor ASF/SF2, can bind the arginine-rich RNA-binding domain of HIV-1 Rev, and they have also argued that overexpression of p32 can enhance Rev function. It remains unclear whether the interaction of p32 with Rev is truly specific, in that p32 also binds at least equally well to the arginine-rich RNA-binding domain of HIV-1 Tat (Fridell et al., 1995). In any event, while p32 may well affect Rev function, it does not bind to the experimentally defined Rev activation domain and therefore cannot be the cellular factor that mediates activation domain function.

In contrast with p32, the other protein that has been proposed as a cellular cofactor for HIV-1 Rev, the eukaryotic translation initiation factor 5A (eIF-5A), has also been suggested to bind to the Rev activation domain, based on specific cross-linking of eIF-5A to an activation domain peptide in vitro (Ruhl et al., 1993). Data have also been presented suggesting that microinjection of the eIF-5A protein into Xenopus oocytes, which do not express detectable endogenous eIF-5A, is required to permit HIV-1 Rev function in this system (Ruhl et al., 1993). However, we have been unable to demonstrate any interaction between eIF-5A and HIV-1 Rev or HTLV-I Rex using the various genetic assays described in this paper (data not shown), while others have recently presented data arguing

that Xenopus oocytes are fully permissive for HIV-1 Rev function even in the absence of exogenously added eIF-5A (Fischer et al., 1994). The question of whether eIF-5A indeed plays a specific role in mediating HIV-1 Rev function therefore remains to be fully addressed.

While the data presented in this manuscript argue strongly for a critical role for Rab in mediating the function of retroviral Rev/Rex proteins, they shed relatively little light on its normal cellular function. Certainly, the remarkable evolutionary conservation of the Rab protein across widely divergent eukaryotic species (Figure 4B) is not only strongly suggestive of an important biological role but also consistent with the lack of species specificity observed for HIV-1 Rev function (Ivey-Hoyle and Rosenberg, 1990; Malim et al., 1991; Fischer et al., 1994). However, this result also makes it difficult to address the normal role of Rab by complementation in trans.

While the primary sequence of the predicted Rab protein does not show any significant homology to proteins of known function, Rab does contain a remarkably high 43 phenylalanine residues, including 10 copies of the FG amino acid pair, and 16 runs of two or more tandem serine residues (Figure 3). These protein sequence elements, which are concentrated in the carboxy-terminal half of Rab, are also found in several nucleoporin proteins, including particularly the human CAN or nup214 protein, which not only contains multiple copies of a highly degenerate FG based tetrapeptide repeat element but also numerous short serine runs (Kraemer et al., 1994). While the significance of these elements for nucleoporin function remains unclear, it is possible that they play a role in mediating nucleoporin interactions (Davis, 1995). However, an immunofluorescence analysis of the intracellular localization of Rab, while clearly demonstrating that Rab is concentrated in the cell nucleus, did not indicate a localization in the nuclear membrane (Figure 4C). While the subcellular localization of Rab does not, therefore, support the hypothesis that Rab is a true nucleoporin, the fact that Rab contains nucleoporin-like sequence elements does raise the intriguing possibility that Rab might interact with certain nucleoporins, in a manner analogous to the many established internucleoporin interactions (Davis, 1995), while mediating the nuclear export of target RNA species. Alternatively, it is also possible that Rev sequentially interacts with Rab and then with authentic nucleoporins, perhaps using the same activation domain protein-binding site, as part of an ordered pathway leading out of the nucleus to the cytoplasm. While both these scenarios must be viewed as highly speculative at present, they do provide models for the mechanism of action of the Rev/Rex class of RNA export factors that are not only interesting but also testable.

Experimental Procedures

Yeast Two-Hybrid Interaction Experiments

The yeast expression plasmid pGAL4-Rev encodes a fusion protein consisting of the GAL4 DNA-binding domain linked to the full-length HIV-1 Rev protein (Fridell et al., 1995). A series of 14 missense mutations, in or near the HIV-1 Rev activation domain, were generated in the pGAL4-Rev context by replacement of the wild-type rev cDNA sequence (XbaI–EcoRI) with polymerase chain reaction (PCR)-generated cDNA fragments bearing the previously described mutations

(Malim et al., 1991) listed in Figure 2. The plasmid pGAL4-Rex was constructed by substituting amino acids 2–189 of HTLV-I Rex in place of the Rev sequence present in pGAL4-Rev.

The Y190 yeast indicator strain, the preparation of the oligo(dT)-primed, pVP16-based CEM cDNA library, and the methodology used to screen for interacting proteins have been described previously (Harper et al., 1993; Fridell et al., 1995). Following rescue, library plasmids expressing relevant VP16 fusion proteins were rescreened by transformation into the yeast indicator strain GGY1::171 (Gill and Ptashne, 1987) together with the panel of GAL4 fusion protein expression plasmids described in Figure 2. After 3 days of selection on culture plates, double transformants were transferred to selective liquid medium. The next day, equal cell equivalents were analyzed for levels of β-gal expression (Fridell et al., 1995).

When appropriate, molecular clones were sequenced using the dideoxy chain termination method and the Sequenase version 2.0 sequencing kit (United States Biochemical). The *rab* cDNA insert was sequenced by the same method using both dGTP and dITP.

RNA and Protein Expression Analysis

Northern blot analysis of poly(A)⁺ RNA derived from the human cell lines CEM and HeLa was performed as previously described (Malim et al., 1990) using a random-primed, [α-^{32}P]dCTP-labeled probe prepared from an internal 853 bp BamHI–AccI *rab* cDNA fragment.

The pGEX-4T plasmid was used to express a fusion protein consisting of GST linked to amino acids 101–562 of the Rab ORF (Figure 3) in the BL21 (lon⁻) strain of Escherichia coli. Expression was induced by addition of 0.1 mM IPTG, and the resultant GST–Rab fusion protein was extracted, purified, and used to immunize rabbits. A second fusion protein, consisting of MBP linked to amino acids 101–326 of Rab, was expressed in E. coli using the pMAL-C2 expression plasmid, purified using a commercial kit (MBP Fusion and Purification System, New England Biolabs) and then coupled to cyanogen bromide–activated agarose beads (Pierce). The agarose-coupled MBP–Rab fusion protein was used to affinity purify Rab-specific antibodies from the serum of the GST–Rab-injected rabbits using buffers and procedures detailed in the Amino Link Immobilization kit (Pierce). The resultant Rab-specific rabbit antiserum was concentrated and dialyzed against phosphate-buffered saline (PBS) prior to use.

The tissue culture cell lines used to make protein extracts were HeLa (human), C127 (mouse), QC13 (quail), and Schneider 2 (Drosophila). Frog protein extracts were prepared from Xenopus oocytes. Cells were washed with PBS and then resuspended in 100 μl of PBS. After addition of 100 μl of 2× Laemmli's gel loading buffer containing 2-mercaptoethanol, the samples were sonicated, boiled, and centrifuged to remove debris. Soluble proteins were separated by 12% SDS–PAGE, transferred to a nitrocellulose filter, and then incubated with a 1:20,000 dilution of the affinity-purified rabbit Rab antiserum. After vigorous washing, bound antibodies were detected using a horseradish peroxidase–conjugated goat anti-rabbit antiserum and enhanced chemiluminescence (Amersham).

Immunofluorescence analysis was performed essentially as previously described (Malim et al., 1989a). CV1 cells were fixed and permeabilized prior to incubation with a 1:1000 dilution of the rabbit anti-Rab antiserum. After extensive washing, the fixed cells were incubated with a 1:100 dilution of an affinity-purified rhodamine-conjugated goat anti-rabbit antiserum (Boehringer Mannheim) and examined using a Zeiss Axioskop immunofluorescence microscope.

GST Affinity Chromatography

Both the GST–Rev and the GST–M10 fusion proteins contain the full-length Rev ORF, in the former case containing an intact activation domain and in the latter the defective M10 mutant form of the activation domain (Malim et al., 1989a). In addition, both Rev fusion proteins also contain the previously described M6 missense mutation of the Rev RNA-binding domain (Malim et al., 1989a). This mutation, which affects amino acids 41–44 of Rev, is located well outside the Rev activation domain (Figure 1). However, inclusion of this mutation markedly increases the yield of full-length Rev protein upon expression in E. coli (data not shown). Bacterially expressed GST, GST–Rev, and GST–M10 proteins were purified as previously described (Malim et al., 1991). Equivalent levels were then coupled to cyanogen bromide–activated agarose beads (Pierce) and used to prepare columns with

a 0.5 ml bed volume. A DNA fragment encoding the Rab ORF (amino acids 1–562; NcoI–XhoI) was cloned into the pGEM3ZF(+) expression plasmid, and ^{35}S-labeled Rab protein was prepared in a 200 μl rabbit reticulocyte lysate-coupled transcription–translation reaction (Promega) using T7 RNA polymerase and [^{35}S]methionine/[^{35}S]cysteine. The lysate was then diluted 1:5 with chromatography buffer (CB) (10 mM HEPES [pH 7.5], 0.1 M NaCl, 1 mM EDTA, 1 mM DTT, 10% glycerol, 2 mg/ml BSA, 0.5% NP-40, and 0.25 mM PMSF), and equal amounts were loaded onto the immobilized GST, GST–Rev, and GST–M10 affinity columns. These were then washed with 10 column volumes of CB before bound proteins were eluted with 4 column volumes of 0.1 M glycine (pH 2.8). Eluted proteins were concentrated and dialyzed against CB prior to analysis by 12% SDS–PAGE.

Mammalian Two-Hybrid Assay

All mammalian expression plasmids were constructed in the context of plasmid pBC12-CMV (Malim et al., 1991) with the exception of the pG5B-CAT indicator construct, which has been described previously (Bogerd and Greene, 1993). pBC12-GAL4-Rab expresses the GAL4 DNA-binding domain (amino acids 1–147) linked to the full-length *rab* cDNA sequence. pRev-VP16 and pRex-VP16 have been described previously (Bogerd and Greene, 1993) and express full-length HIV-1 Rev and HTLV-I Rex linked to the VP16 transcription activation domain. Variants of Rev (M10 and M32) and Rex (ΔAD) bearing defective activation domains were substituted into this same expression plasmid by excision of the wild-type *rev* gene by cleavage with SacI and BglII and replacement with a PCR-generated SacI–BclI DNA fragment encoding the appropriate Rev or Rex mutants. Similarly, PCR-generated SacI–BclI DNA fragments encoding wild-type and mutant (ΔAD) forms of VMV Rev and EIAV Rev were also substituted in place of HIV-1 Rev to generate the relevant VP16 fusion protein expression plasmids. All Rev and Rex activation domain mutants have been previously described and characterized (Hope et al., 1991; Malim et al., 1991; Tiley et al., 1991; Fridell et al., 1993). The introduced mutations are the following: HIV Rev M10, LG(78–79) to DL; HIV Rev M32, L78, L81, and L83 all to A; RexΔAD, LSLD(90–93) to GGGG; VMV RevΔAD, LE(114–115) to DL; EIAV RevΔAD, L49 and I54 to A.

COS cell cultures (100 mm) were transfected with 1 μg of pG5B-CAT, 500 ng of pBC12-GAL4-Rab, 5 μg of a VP16 fusion protein expression plasmid, and 500 ng of the internal control indicator plasmid pBC12-CMV-βgal using the DEAE–dextran procedure. The parental pBC12-CMV expression plasmid served as a negative control. At ~48 hr posttransfection, cell extracts were prepared and CAT and β-gal expression quantified (Fridell et al., 1995).

Mammalian Monohybrid Assay

The pSLIIB indicator construct and plasmids expressing wild-type (pcRev) and mutant (pM10 and pM32) forms of Rev have been described previously (Malim et al., 1991; Tiley et al., 1992a). The pcTat-Rab plasmid expresses a fusion protein consisting of the full-length Tat protein linked to the first amino acid of the Rab ORF indicated in Figure 3. HeLa cultures (35 mm) were transfected with 1 μg of the pSLIIB-CAT reporter plasmid, 0.5 μg each of the Tat–Rab fusion protein and Rev protein expression plasmid, and 1 μg of carrier DNA using the calcium phosphate procedure. The parental pBC12-CMV expression plasmid was used as a negative control. Cultures were harvested at ~48 hr after transfection and CAT activity quantified (Fridell et al., 1995).

HIV-1 Virus Rescue

HIV-1 provirus rescue assays were performed in COS cell cultures essentially as described by Malim et al. (1991). The Rev⁻ provirus expression plasmid pHIVΔRev and the pcRev, pcRex, pBC12-CMV-SEAP, pBC12-CMV-CAT, and pBC12-CMV-βgal expression plasmids have been described previously (Rimsky et al., 1988; Malim et al., 1991). The Rab expression plasmid pBC12-CMV-Rab contains the 562 amino acid Rab ORF indicated in Figure 3 (NcoI–XhoI) cloned into the expression plasmid pBC12-CMV. Transfections were performed as described in Figure 7. Secreted p24 levels were quantified using a commercial ELISA kit (DuPont), while SEAP activity was determined as described previously (Berger et al., 1988).

References

Bartel, D. P., Zapp, M. L., Green, M. R., and Szostak, J. W. (1991). HIV-1 Rev regulation involves recognition of non-Watson–Crick base pairs in viral RNA. Cell 67, 529–536.

Berger, J., Hauber, J., Hauber, R., Geiger, R., and Cullen, B. R. (1988). Secreted placental alkaline phosphatase: a powerful new quantitative indicator of gene expression in eukaryotic cells. Gene 66, 1–10.

Bogerd, H., and Greene, W. C. (1993). Dominant negative mutants of human T-cell leukemia virus type I Rex and human immunodeficiency virus type 1 Rev fail to multimerize in vivo. J. Virol. 67, 2496–2502.

Bray, M., Prasad, S., Dubay, J. W., Hunter, E., Jeang, K.-T., Rekosh, D., and Hammarskjöld, M.-L. (1994). A small element from the Mason–Pfizer monkey virus genome makes human immunodeficiency virus type 1 expression and replication Rev-independent. Proc. Natl. Acad. Sci. USA 91, 1256–1260.

Cochrane, A. W., Perkins, A., and Rosen, C. A. (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.

Cullen, B. R. (1992). Mechanism of action of regulatory proteins encoded by complex retroviruses. Microbiol. Rev. 56, 375–394.

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

Davis, L. I. (1995). The nuclear pore complex. Annu. Rev. Biochem. 64, 865–896.

Emerman, M., Vazeux, R., and Peden, K. (1989). The rev gene product of the human immunodeficiency virus affects envelope-specific RNA localization. Cell 57, 1155–1165.

Feinberg, M. B., Jarrett, R. F., Aldovini, A., Gallo, R. C., and Wong-Staal, F. (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., Hadzopoulou-Cladaras, M., Cladaras, C., Copeland, T., and Pavlakis, G. N. (1989). Rev protein of human immunodeficiency virus type 1 affects the stability and transport of the viral mRNA. Proc. Natl. Acad. Sci. USA 86, 1495–1499.

Fields, S., and Song, O.-K. (1989). A novel genetic system to detect protein–protein interactions. Nature 340, 245–246.

Fischer, U., Meyer, S., Teufel, M., Heckel, C., Luhrmann, R., and Rautmann, G. (1994). Evidence that HIV-1 Rev directly promotes the nuclear export of unspliced RNA. EMBO J. 13, 4105–4112.

Fridell, R. A., Partin, K. M., Carpenter, S., and Cullen, B. R. (1993). Identification of the activation domain of equine infectious anemia virus Rev. J. Virol. 67, 7317–7323.

Fridell, R. A., Harding, L. S., Bogerd, H. P., and Cullen, B. R. (1995). Identification of a novel human zinc finger protein that specifically interacts with the activation domain of lentiviral Tat proteins. Virology 209, 347–357.

Gill, G., and Ptashne, M. (1987). Mutants of GAL4 protein altered in an activation function. Cell 51, 121–126.

Harper, J. W., Adami, G. R., Wei, N., Keyomarsi, K., and Elledge, S. J. (1993). The p21 Cdk-interacting protein Cip1 is a potent inhibitor of G1 cyclin-dependent kinases. Cell 75, 805–816.

Hope, T. J., Bond, B. L., McDonald, D., Klein, N. P., and Parslow, T. G. (1991). Effector domains of human immunodeficiency virus type 1 Rev and human T-cell leukemia virus type I Rex are functionally interchangeable and share an essential peptide motif. J. Virol. 65, 6001–6007.

Huang, J., and Liang, T. J. (1993). A novel hepatitis B virus (HBV) genetic element with Rev response element–like properties that is essential for expression of HBV gene products. Mol. Cell. Biol. 13, 7476–7486.

Ivey-Hoyle, M., and Rosenberg, M. (1990). Rev-dependent expression of human immunodeficiency virus type 1 gp160 in Drosophila melanogaster cells. Mol. Cell. Biol. 10, 6152–6159.

Iwai, S., Pritchard, C., Mann, D. A., Karn, J., and Gait, M. J. (1992). Recognition of the high affinity binding site in rev-response element RNA by the human immunodeficiency virus type-1 rev protein. Nucl. Acids Res. 20, 6465–6472.

Kjems, J., Calnan, B. J., Frankel, A. D., and Sharp, P. A. (1992). Specific binding of a basic peptide from HIV-1 Rev. EMBO J. 11, 1119–1129.

Kraemer, D., Wozniak, R. W., Blobel, G., and Radu, A. 1994. The human CAN protein, a putative oncogene product associated with myeloid leukemogenesis, is a nuclear pore complex protein that faces the cytoplasm. Proc. Natl. Acad. Sci. USA 91, 1519–1523.

Luo, Y., Yu, H., and Peterlin, B. M. (1994). Cellular protein modulates effects of human immunodeficiency virus type 1 Rev. J. Virol. 68, 3850–3856.

Malim, M. H., and Cullen, B. R. (1991). HIV-1 structural gene expression requires the binding of multiple Rev monomers to the viral RRE: implications for HIV-1 latency. Cell 65, 241–248.

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

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

Malim, M. H., McCarn, D. F., Tiley, L. S., and Cullen, B. R. (1991). Mutational definition of the human immunodeficiency virus type 1 Rev activation domain. J. Virol. 65, 4248–4254.

Mancuso, V. A., Hope, T. J., Zhu, L., Derse, D., Phillips, T., and Parslow, T. G. (1994). Posttranscriptional effector domains in the Rev proteins of feline immunodeficiency virus and equine infectious anemia virus. J. Virol. 68, 1998–2001.

McDonald, D., Hope, T. J., and Parslow, T. G. (1992). Posttranscriptional regulation by the human immunodeficiency virus type 1 Rev and human T-cell leukemia virus type I Rex proteins through a heterologous RNA binding site. J. Virol. 66, 7232–7238.

Rimsky, L., Hauber, J., Dukovich, M., Malim, M. H., Langlois, A., Cullen, B. R., and Greene, W. C. (1988). Functional replacement of the HIV-1 Rev protein by the HTLV-1 Rex protein. Nature 335, 738–740.

Ruhl, M., Himmelspach, M., Bahr, G. M., Hammerschmid, F., Jaksche, H., Wolff, B., Aschauer, H., Farrington, G. K., Probst, H., Bevec, D., and Hauber, J. (1993). Eukaryotic initiation factor 5A is a cellular target of the human immunodeficiency virus type 1 Rev activation domain mediating trans-activation. J. Cell Biol. 123, 1309–1320.

Siomi, H., Shida, H., Hyun Nam, S., Nosaka, T., Maki, M., and Hatanaka, M. (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.

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

Stutz, F., and Rosbash, M. (1994). A functional interaction between Rev and yeast pre-mRNA is related to splicing complex formation. EMBO J. 13, 4096–4104.

Tiley, L. S., Malim, M. H., and Cullen, B. R. (1991). Conserved functional organization of the human immunodeficiency virus type 1 and visna virus Rev proteins. J. Virol. 65, 3877–3881.

Tiley, L. S., Madore, S. J., Malim, M. H., and Cullen, B. R. (1992a). The VP16 transcription activation domain is functional when targeted to a promoter-proximal RNA sequence. Genes Dev. 6, 2077–2087.

Tiley, L. S., Malim, M. H., Tewary, H. K., Stockley, P. G., and Cullen, B. R. (1992b). Identification of a high-affinity RNA-binding site for the human immunodeficiency virus type 1 Rev protein. Proc. Natl. Acad. Sci. USA 89, 758–762.

Venkatesh, L. K., and Chinnadurai, G. (1990). Mutants in a conserved region near the carboxy-terminus of HIV-1 Rev identify functionally important residues and exhibit a dominant negative phenotype. Virology 178, 327–330.

Weichselbraun, I., Farrington, G. K., Rusche, J. R., Bohnlein, E., and Hauber, J. (1992). Definition of the human immunodeficiency virus type 1 Rev and human T-cell leukemia virus type I Rex protein activation domain by functional exchange. J. Virol. 66, 2583–2587.

GenBank Accession Number

The accession number for the sequence reported in this paper is L42025.