Published in final edited form as:

Curr HIV Res. 2020 ; 18(2): 85–98. doi:10.2174/1570162X18666200106112842.

Sequence and Functional Variation in the HIV-1 Rev Regulatory Axis

Patrick E. H. Jackson^{1,2}, Godfrey Dzhivhuho^{2,3}, David Rekosh^{2,3}, Marie-Louise Hammarskjöld^{2,3}

¹Division of Infectious Diseases and International Health, School of Medicine, University of Virginia, Charlottesville, Virginia, USA

²Myles H. Thaler Center for HIV and Human Retrovirus Research, University of Virginia, Charlottesville, Virginia, USA

³Department of Microbiology, Immunology, and Cancer Biology, School of Medicine, University of Virginia, Charlottesville, Virginia, USA

Abstract

Background.—To complete its replication cycle, HIV-1 requires the nucleocytoplasmic export of intron-containing viral mRNAs. This process is ordinarily restricted by the cell, but HIV overcomes the block by means of a viral protein, Rev, and an RNA secondary structure found in all unspliced and incompletely spliced viral mRNAs called the Rev Response Element (RRE). *In vivo* activity of the Rev-RRE axis requires Rev binding to the RRE, oligomerization of Rev to form a competent ribonucleoprotein complex, and recruitment of cellular factors including Crm1 and RanGTP in order to export the targeted transcript. Sequence variability is observed among primary isolates in both Rev and the RRE, and the activity of both can be modulated through relatively small sequence changes. Primary isolates show differences in Rev-RRE activity and a few studies have found a correlation between lower Rev-RRE activity and slower progression of clinical disease. Lower Rev-RRE activity has also been associated with evasion of cytotoxic T lymphocyte mediated killing.

Conclusions.—The HIV-1 Rev-RRE regulatory axis is an understudied mechanism by which viral adaptation to diverse immune milieus may take place. There is evidence that this adaptation plays a role in HIV pathogenesis, particularly in immune evasion and latency, but further studies with larger sample sizes are warranted.

Keywords

HIV Rev; HIV Rev Response Element; HIV sequence variation; HIV latency; RNA splicing; post-transcriptional gene regulation

CONFLICT OF INTEREST

The authors declare no conflicts of interest. P. E. H. J. was supported by grant K08AI136671 from the National Institutes of Health. Salary support for G.D. and D.R. was provided by the Myles H. Thaler Research Fund and Professorship at the University of Virginia. Salary support for M.-L. H. was provided by the Charles H. Ross Jr. Professorship at the University of Virginia. Research on HIV Rev in the laboratories of D.R. and M.-L. H. was supported by grants CA206275 and AI134208 from the National Institutes of Health.

1. INTRODUCTION

HIV-1 utilizes alternative mRNA splicing in order to produce multiple viral proteins from a relatively small genome. The primary HIV mRNA transcript is about 9 kb and contains multiple splice sites that generate over 30 mRNAs with nine primary open reading frames [1], the products of which can be further cleaved for a total of about fifteen viral proteins [2]. HIV mRNA transcripts can be divided into three classes: unspliced, partially spliced, and completely spliced. Thus many of the expressed mRNAs retain introns [3–6]. All three classes are required for the production of the structural and regulatory proteins that are essential for the completion of viral replication.

In general, mammalian cells permit fully spliced mRNAs to be exported from the nucleus to the cytoplasm while restricting export of intron-containing mRNAs [7–10]. Typically, unspliced and incompletely spliced transcripts from cellular genes are retained and eventually degraded in the nucleus [11]. However, some such transcripts contain *cis*-acting RNA secondary structures called constitutive transport elements (CTEs) that link the intron-containing RNA directly to the Nxf1 pathway to permit nucleocytoplasmic export [12–15]. CTEs are also present in some cellular genes [16–19], as well as in so-called “simpler” retroviruses [13, 20–23]. However, like all other “complex” retroviruses, HIV overcomes the host restriction on intron retention by means of both a viral protein and a *cis*-acting stem-loop structure present in all incompletely spliced and unspliced viral mRNAs [24–27].

The expression of HIV-1 proteins is biphasic. First, the “early” completely spliced viral mRNA species are exported via the same pathway used by most host mRNAs [28] to produce three viral proteins, one of which is the viral regulatory protein Rev [4–6, 29]. After translation, Rev shuttles back into the nucleus. To mediate the nucleocytoplasmic export of “late” intron-containing mRNAs, Rev next binds to and multimerizes on an RNA stem-loop structure found on all such mRNAs known as the Rev Response Element (RRE) [24–27, 30–37]. The resulting ribonucleoprotein (RNP) complex recruits key host factors including Crm1 (Chromosome maintenance factor 1) and RanGTP [38–45], which enables nucleocytoplasmic export. The mRNAs exported through the Crm1 pathway include the unspliced mRNA required for the synthesis of the viral proteins Gag and Gag-Pol (which is also the viral genome), as well as the singly spliced mRNAs for Vif, Vpr, Vpu, and Env [2, 10, 27, 30, 43, 46–49].

Many reviews have been written about the structural biology of the prototypical Rev-RRE interaction and the interaction of the RNP with host factors [50–54]. After a brief discussion of the Rev protein and the RRE, this review will focus on a description of variability in the Rev-RRE axis and the potential implications of that variability for pathogenesis. Major points include the genesis of sequence variation in Rev and the RRE, the mechanisms by which such sequence variation gives rise to differences in functional activity, and evidence of the importance of Rev-RRE activity levels in infection.

2. THE REV-RRE REGULATORY AXIS

2.1 The Rev protein

The prototypical Rev is a 116 amino acid protein that contains distinct functional domains including an arginine rich motif (ARM), a bipartite oligomerization domain (OD), and a nuclear export signal (NES), separated by linker regions (L) (Figure 1) [55]. The N-terminal and C-terminal regions appear to be disordered. The primary site of interaction with the RRE is the ARM. This domain forms an alpha helix that docks to the complementary groove in the high affinity binding site within RRE stem loop IIb [34, 56]. The ARM also contains a nuclear localization signal (NLS) necessary for the protein to be imported from the cytoplasm to the nucleus and nucleolus [34, 43, 57, 58]. The ODs facilitate Rev-Rev dimer and oligomer formation [59]. Each Rev monomer has two alpha-helical regions that form a hairpin structure that is capable of forming complexes with other Rev proteins [50]. The NES is a leucine-rich domain and is necessary for recruitment of Crm1 and other host proteins to facilitate export of the RNP from the nucleus to the cytoplasm [30, 60]. In older literature, the NES was often referred to as the activation domain.

2.2 The RRE

The HIV-1 RRE is a highly structured RNA element located within the *env* coding region of HIV that in some isolates adopts different secondary structures (Figure 2) [36]. The minimal functional RRE is about 250 nucleotides in length, while the complete RRE is about 351 nucleotides [61]. Because of its location, the RRE is present in all unspliced and partially spliced viral mRNAs. Extensive base pairing within the element creates a complex stem-loop structure containing a long, imperfect double helix (domain I) [62], a branched domain (domain II) with high affinity for Rev [58, 63, 64], and two or three additional domains that contribute to RRE function [65]. Recent work suggests that *in vivo*, the RRE forms a three dimensional “A” shaped structure that permits Rev interaction with multiple nucleotide regions [50, 66].

2.3 Rev-RRE binding

The structural biology aspects of Rev-RRE interactions have been extensively explored [37, 47, 67–74] [75]. Using the ARM, Rev initially binds to the high affinity site located at the base of stem-loop IIB. Through further cooperative binding, Rev then multimerizes by interacting with additional Rev molecules through its ODs and with the RNA through different faces of the ARM. This leads to a complex structure that has not been well characterized. The number of Revs that constitute the oligomer have been variously estimated from as few as four [50] to as many as thirteen [32, 47, 61, 76–80]. This RNP complex then recruits cellular proteins through the NESs present on the assembled Revs [81].

2.4 Cellular factors required for export

Chromosome maintenance protein 1 (Crm1; or exportin-1, Xpo1) is a major nuclear export factor that mediates nuclear export of hundreds of distinct mammalian proteins by recognizing NES short sequence motifs [39, 41, 42, 82]. However, recent studies suggest

that the NESs on the multimeric Rev-RRE complex interact differently with Crm1 than do the NESs on proteins that are transported without bound RNA [53, 83, 84]. Crm1 is also involved in the export of some cellular mRNAs, including those whose export is mediated by eIF4E [85, 86] and HuR/ELAV proteins [87–89]. Daugherty and colleagues show that the Rev-RRE RNP configuration only allows one or two Crm1-RanGTP complexes to form and that the Rev-RRE RNP complex is assembled in such a way that RanGTP can also bind. Once bound, the Rev-RRE-Crm1-RanGTP complex exports through the nuclear pore. Eventually Rev is released and can shuttle back into the nucleus for further rounds of nuclear export [28, 30, 39].

3. REV AND RRE SEQUENCE VARIABILITY

HIV infection is characterized by rapid sequence diversification, and mutations occur in all regions of the genome, including those encoding Rev and the RRE [90, 91]. Mutations arise through multiple simultaneous mechanisms, including errors in reverse transcription [92] and probably also as a consequence of RNA editing. Rates of mutation are not constant throughout the viral genome, and the *env* coding region (which includes the RRE and overlies *rev* exon 2) displays an increased rate of mutation relative to *gag*, *pol*, *vif*, and *nef* likely due to the action of the APOBEC3 family of cytidine deaminases [93]. The cellular protein ADAR1 also causes increased editing of *rev*, *env*, and the RRE, presumably as a consequence of the presence of double-stranded RNA secondary structure in these regions [94].

In primary isolates, *rev* sequence diversity has been reported as being low during acute infection, with a tendency towards increased variability over the course of disease progression [95]. Similarly, during vertical transmission there is low variability between maternal and child quasispecies, and functional regions of Rev are conserved in this context [96]. Immune pressure is one source of sequence variation and the accumulation of CTL escape mutations within Rev has been described during infection *in vivo* [97, 98].

Notably, the *rev* reading frame is overlapped in its entirety by the coding frames for Tat and Env. Despite this potential conservational pressure on the *rev* coding space, in fact HIV does not display a difference in mutation rates between any overlapped and non-overlapped regions of its genome [99]. The coding regions for functional domains of Tat are segregated from those of Rev, such that mutations that alter the function of one protein are well tolerated by the other [99]. While similar segregation has not been demonstrated between *rev* and *env*, *rev* has been shown to diversify *in vivo* at a rate independent of *env* suggesting independent evolution [100].

Sequence variation is also observed within the RRE among primary isolates, though again there is potential conservation pressure due to the overlying *env* gene. Nucleotide changes in the region tend to conserve the predicted RRE secondary structure, with mutations being more frequent in unpaired loop regions or occurring in stem regions in combinations which would not be expected to disrupt pairing. Synonymous changes in the RRE also predominate over nonsynonymous changes, suggesting that selection may occur at the level of the RNA secondary structure while conserving *env* [101]. Conservation of RRE primary sequences

directly involved in Rev binding and of secondary structures implicated in functional activity has also been observed during vertical transmission [102].

4. MECHANISMS OF REV-RRE FUNCTIONAL ACTIVITY VARIATION

Efficient nucleocytoplasmic export of intron-containing viral mRNAs *in vivo* requires several steps, including formation of a functional RRE three dimensional structure, assembly of the Rev-RRE RNP complex, and recruitment of cellular factors. Each of these levels of interaction affords an opportunity for Rev-RRE activity as a whole to be modified through changes in the RRE, Rev, or both.

4.1 RRE functional variation

The interaction of Rev and the RRE is both sequence-specific and structure-specific [103]. A number of *in vitro* selection studies have demonstrated that RRE sequences can be readily mutated with both increased and decreased Rev-binding affinity relative to a wild type sequence [104]. Even more strikingly, minimal changes in the RRE can also dramatically alter preferential affinity for Rev versus other RRE-binding aptamers [105–110].

RRE activity is the product of an ensemble of excited and ground-state conformations that exist in dynamic equilibrium in the cell and may have quite different Rev-binding affinities [111]. Co-existing alternate conformations may yield strikingly different activities as measured by both Rev-dependent protein production and vector packaging assays [63]. Mutations within the RRE need not result in large scale changes of the lowest energy secondary structure to affect activity but may simply alter the relative abundance of alternative conformers.

RNA modifications within the RRE, including methylation of adenosines at the N6 position (m⁶A), also appear to contribute to the level of RRE functional activity [112]. In this study, methylation of adenosines within the stem loop IIb region of the RRE enhanced Rev binding and RNA nuclear export. It remains unclear whether this difference in activity is due to m⁶A induced changes to the RNA secondary structure or whether there is direct Rev interaction with the methyl group.

RRE functional evolution employing some of these mechanisms of activity modulation has been observed during the course of natural infection. In one patient infected with virus that showed increasing Rev-RRE activity over the course of six years, the RRE acquired four single nucleotide mutations, which resulted in a gradual increase in functional activity over time [113, 114]. Mutations associated with increased activity resulted in both large scale changes in RRE secondary structure, as well as increased entropy in the Rev binding site.

Changes in the RRE to preserve function in the face of extrinsic pressure has also been observed. Rev M10 contains a mutation within the NES that abolishes Rev function in a *trans*-dominant fashion. In the presence of a sufficient amount of Rev M10, the replication of otherwise competent HIV expressing wild type Rev is inhibited [115]. Cellular therapy with Rev M10 has been explored as a therapeutic strategy for HIV infection though it has not entered clinical practice [116, 117]. In one study, when the replication-competent laboratory

HIV strain NL4–3 was passaged in cells constitutively expressing Rev M10, escape mutants spontaneously arose that had nucleotide changes in the RRE [118]. It was determined that two G to A changes that did not cause amino acid changes in the overlapping *env* reading frame were sufficient to confer partial resistance to Rev M10 and permit viral replication. These mutations resulted in large scale changes in the secondary structure of the RRE, but they did not alter the stoichiometry or RNA location of Rev-RRE binding [119]. The precise mechanism of resistance remains unclear.

Compensatory RRE mutations to rescue activity have also been seen in the context of the development of HIV resistance to peptide fusion inhibitors. Peptide fusion inhibitors are compounds related to the c-terminal or n-terminal heptad repeats of gp41 that inhibit formation of the fusion-active conformation of the viral protein [120]. When NL4–3 was passaged in the presence of the fusion inhibitor C34, resistance mutations spontaneously arose in the n-terminus region of gp41, which is encoded in a region that overlays the RRE. Amino acid changes that individually strongly increased C34 resistance but compromised replication tended to be combined with nonsynonymous mutations in the RRE that only slightly increased resistance but rescued replication capacity. The secondary mutations rescued replication in part by restoring the native RRE structure that was disrupted by primary resistance mutations [121]. Primary resistance mutations also changed the coding sequence of gp41 in viruses passaged in the presence of another peptide fusion inhibitor, enfuvirtide (T20), and these were similarly accompanied by synonymous nucleotide changes within the RRE that stabilize stem loop III [122].

4.2 Rev function variation

Modulation of the activity of the Rev-RRE system can also be accomplished through changes in the Rev protein. While more constrained from a functional standpoint than other regions of the Rev sequence, even the OD, ARM, and NES display substantial sequence variability [55]. Deletions in these functional domains can obliterate Rev activity, but subtler modifications can both increase and decrease function.

Rev function *in vivo* involves the formation of a homo-oligomer on the RRE scaffold. The Rev oligomer displays increased affinity for the RRE over the monomer, and oligomerization increases the export and translation of RRE-containing mRNAs. Rev monomers in the complex interact with different regions of the RRE using alternative points of contact within the ARM [62]. The ability of Rev to bind the RRE is robust to mutations within the ARM in part due to the capacity for Rev to adopt these alternative recognition strategies [123]. Despite this robustness, some minor changes in the ARM can alter relative binding affinity for the RRE and a related aptamer, suggesting extensive capacity for tuning Rev activity through changes in this region.

Within the OD, naturally occurring mutations have also been demonstrated to alter function as measured by reporter gene expression [124]. While some mutations in the OD can cause defects in Rev-RRE binding and Rev oligomerization, these effects do not closely track with changes in Rev activity as measured by reporter gene expression, suggesting a multiplicity of mechanisms for functional modulation.

Regions outside of the three known functional domains also impact Rev activity. For example, the intrinsically disordered C-terminal domain plays a role in the regulation of oligomerization and may prevent the formation of nonfunctional aggregates [125]. Rev nuclear localization has also been demonstrated to be affected by mutations outside of the ARM [126]. Mutants can be generated that show high activity and low nuclear localization and *vice versa*, suggesting that nuclear localization efficiency is a poor correlate of activity.

5. REV-RRE ACTIVITY VARIATION IN VIVO

5.1 Observations of Rev-RRE activity modulation

The combination of sequence variability and the multitude of mechanisms by which sequence changes in the Rev-RRE system alter activity give rise to primary isolates with functional variation in this axis due to differences in both Rev and the RRE.

Primary isolates have been described with naturally occurring changes in the Rev NES that result in attenuated Rev activity, likely due to reduced efficiency of interaction between the Rev and cellular export factors [127]. Another set of primary isolates from different subjects demonstrated variation in Rev-RRE activity up to 24-fold in an assay measuring RNA nuclear export and packaging, and differences in Rev-RRE activity tracked with differences in Rev rather than the RRE [128].

A separate study investigating Rev-RRE activity of HIV quasispecies in subjects at two different time points during infection found that activity tended to be similar among viruses in the same subject at the same time point, but that activity differed both between subjects and within the same subject at different times [114]. For this set of subjects, differences in RRE activity drove functional variation and significant differences in activity were observed with only four single nucleotide changes.

Rev-RRE changes that confer stable activity in the context of drug escape mutations also occur *in vivo*. An analysis of HIV sequences from enfuvirtide-treated patients showed resistance mutations in the portion of the gp41 region overlapping the RRE. However, a compensatory mutation in the Rev oligomerization domain that didn't overlap gp41 was also observed [129]. The presence of the Rev mutation was associated with increased HIV viral load and lower CD4 count, suggesting that Rev changes can provide a mechanism of restoring Rev-RRE activity even in the presence of a suboptimal RRE structure. A separate analysis of RRE sequences taken from subjects who had failed therapy with enfuvirtide identified several mutations that were predicted to cause structural changes *in silico*. However, the functional activity of the different RREs in terms of Rev-RRE binding, RNA export, and reporter protein production were similar to or higher than a comparable wild type RRE [130]. The authors suggested that this data demonstrated that RRE functional plasticity was sufficient to compensate for extrinsically induced RRE sequence changes, even when these changes would appear to alter the secondary structure.

5.2 Rev-RRE activity variation in clinical disease

Very few studies have attempted to correlate Rev-RRE activity in primary isolates with the clinical status of subjects. The conclusions that can be made from these studies are limited

by several factors including differences in clinical terminology, changes in the standard of care for HIV infected patients, and, above all, the small numbers of subjects.

One notable set of such studies came out of the Sydney Blood Bank cohort. This cohort was established to study the clinical outcomes for one blood donor and eight epidemiologically linked blood recipients who acquired subtype B HIV infection between 1981 and 1984 [131]. The donor's HIV sequence was notable for a defective *nef* and members of the cohort had variable clinical courses with a striking prevalence of slowly progressive or nonprogressing disease [132]. HIV sequence analysis of four members of the cohort who had not progressed to symptomatic infection after more than ten years revealed that all had *rev* genes with a predicted C-terminal extension of at least three amino acids [133]. A subsequent functional analysis of Revs taken from a partially overlapping subset of the cohort attempted to correlate Rev activity with clinical status as a slow progressor (i.e. the subject required initiation of ART during study follow up) or long-term nonprogressor (i.e. never initiated on ART) [134]. Two of four tested Revs showed decreased Rev-RRE binding relative to the laboratory strain NL4-3 and decreased reporter gene production, though the magnitudes of these two measures of activity did not correlate well. Thus there was no clear association in this set of Revs between Rev function and status as a long-term nonprogressor or a slow progressor, though this is perhaps unsurprising given the tentative nature of the clinical distinction. All four Revs used in the functional analysis also exhibited C-terminal extensions relative to the consensus subtype B sequence.

The C-terminal extension of Revs in the Sydney Blood Bank cohort is particularly interesting in light of an observation by Papathanasopoulos and colleagues who reported on two perinatally infected sibling subjects (ages 9.6 and 11) classified as "slow progressors" due to their lack of symptoms at the time of sampling [135]. Both subjects were infected with subtype C viruses that on sequencing were found to have similar extensions in the predicted Rev protein, though other HIV open reading frames were preserved. While the consensus subtype C Rev is 16 amino acids shorter than subtype B Rev [136], Rev in these two subjects exhibited a length that was three amino acids longer than the subtype B consensus. Unfortunately, functional studies of the Rev-RRE axis were not performed for these subjects.

Rev sequences from one additional non-term nonprogressor were evaluated by Iversen and coworkers [137]. The subject was so classified based on his maintenance of a CD4 count greater than 250 cells per mm³ for up to ten years after infection. Chimeric viruses created using *env* (and overlying the RRE, *tat*, and *rev*) taken from primary isolates replicated poorly in peripheral blood mononuclear cells (PBMCs). Sequencing revealed a mutation within the Rev NES relative to the consensus B sequence, L78I. Viruses carrying this mutation were able to replicate in a cell line expressing HTLV-1 Rex suggesting that Rex was able to complement the defective Rev and rescue replication. Chimeric viruses carrying the consensus residue L78 could also replicate, albeit poorly, in PBMCs further confirming the importance of the Rev NES mutation. The authors suggested that the attenuated *rev* allele could have contributed to the lack of disease progression in the subjects.

A study conducted in a Thai cohort that was followed longitudinally compared RRE activity between subjects divided into fast or slow progressors based on the rate of decline in CD4 count [138]. An arbitrary cutoff of 75 CD4 cells per mm³ per year was used to divide the groups. RRE sequences were generated for subjects at two time points separated by about 5 years and activity was measured by reporter gene expression. After excluding subjects with known host factors that affect disease progression, RRE activity at the early time point did not correlate with the rate of disease progression. However, higher RRE activity at the later time point was correlated with having a faster CD4 count decline.

Additional studies have been conducted based on the distinction between “symptomatic” and “asymptomatic” subjects with HIV infection. RRE activity was assessed in one such set of subjects, also from Thailand [139]. Two symptomatic and eight asymptomatic subjects were selected, but no additional clinical data were reported. Four RREs were sequenced from each subject and functional activity was determined by reporter gene expression. The authors reported a trend towards higher RRE activity in symptomatic versus asymptomatic subjects but no clear correlation with disease status. Interestingly, a 2.3-fold difference in cytoplasmic unspliced RNA and a 25.7-fold difference in reporter gene levels were found between the highest- and lowest-activity RREs, and variability in RRE activity was seen both within and between patients.

In a final study, the activity of Rev was determined for primary isolates taken from three patients with asymptomatic HIV and three patients with AIDS. Chimeras created with Revs taken from AIDS subjects had an increase in the relative production of Gag compared with the asymptomatic subjects, but there was no consistent difference in the production of a reporter gene in the *nef* position [140]. This result is consistent with increased Rev activity in the AIDS subjects compared with the asymptomatic subjects. Rev activity was also measured from two example viruses in this set by measuring RNA export, and consistent with this interpretation a Rev derived from the AIDS subject yielded a greater proportion of unspliced to spliced viral transcripts in the cytoplasm compared with a Rev from an asymptomatic subject.

The results of these limited clinical studies are summarized in Table 1.

5.3 Equine infectious anemia virus Rev-RRE activity variation

While the correlation between HIV Rev-RRE activity variation and clinical disease state is difficult to evaluate based on the currently available evidence, the data are more convincing in the case of the equine infectious anemia virus (EIAV). Like HIV, EIAV is a complex retrovirus that utilizes both a *trans*-acting viral protein, Rev, and a *cis*-acting RNA secondary structure, the RRE, to accomplish the nucleocytoplasmic export of intron-containing viral mRNAs via the Crm1 pathway. EIAV Rev and RRE are functionally homologous to HIV Rev and RRE, though both the protein and the RNA element are distinct in sequence and structure between the viruses [141]. As with HIV Rev, EIAV Rev activity can be significantly modulated by single amino acid changes, both within and without defined functional domains [142]. Work by Susan Carpenter and colleagues on EIAV Rev sequence and functional evolution during infection provide insights into the role of this regulatory

system in clinical disease in horses, and this work provides an analogy to Rev-RRE variation in HIV [143].

In experimental infection of a pony with EIAV, sequence diversification of Rev was observed resulting in the presence of multiple circulating quasispecies at different time points. Two clades of quasispecies with distinct phylogenetic structures were observed to coexist and to vary in prevalence over time. Moreover, Rev activity, as measured by reporter gene expression and chimeric virus replication, differed between the clades, with higher-activity Revs being most prevalent during febrile periods of illness and lower-activity Revs predominating during acute and afebrile periods [144, 145]. In one pony with an aggressive disease course, the more active Rev population predominated at all time points [145]. Analysis of individual quasispecies revealed that single amino acid changes occurring mostly outside of known functional domains of Rev were sufficient to cause significant alterations in activity, including three of four substitutions occurring in a nonessential region of the protein [146]. Interestingly, evolution of the RRE was not observed in these studies. These experiments are highly suggestive that tuning of EIAV Rev-RRE activity through changes in Rev plays a role in the pathogenesis of this disease. A similar scenario could well occur in HIV.

6. REV-RRE ACTIVITY IN HIV PATHOGENESIS

The role of Rev-RRE regulatory axis activity variation in HIV pathogenesis is unclear, but it may provide a viral mechanism of adaptation to differing fitness landscapes encountered over the course of infection. In the natural history of HIV infection, the virus is likely to encounter several different immune environments. The virus must navigate the transmission bottleneck to establish infection [147], maintain infection in early disease when the host immune system is relatively intact, and then continue to replicate during late disease when host immune defenses are severely compromised. Additionally, the virus may encounter body compartments, including the reproductive tract and the central nervous system, where immune pressures may differ from blood plasma or lymphoid tissues [148]. In these different environments, the replicative strategy that leads to optimal fitness may differ. Viruses with lower rates of replication and decreased production of immunogenic structural proteins may be more fit in environments with high immune surveillance, while viruses with a high rate of replication and antigen production may be more fit when immune control is less efficient.

While viral replication is dependent on several viral regulatory systems, including the Tat-TAR axis, the Rev-RRE axis is the only mechanism by which HIV can modulate the relative production of antigenic structural proteins like Gag versus the immune modulatory protein Nef. Experimental evidence demonstrates that cells infected with virus that has low Rev activity are better able to avoid killing by anti-Gag-specific cytotoxic T lymphocytes (CTLs) [140]. Thus, in patients and environments with high immune surveillance, a virus pursuing a strategy of CTL-avoidance through a less active Rev-RRE system may prove more fit than a virus with high Rev-RRE activity. In contrast, late in infection when the immune system is deteriorating, viruses with high Rev-RRE activity could serve to promote increased HIV replication.

The Rev-RRE system could also be involved in the maintenance of HIV latency. Absent or low level Rev-RRE activity would be a route by which HIV could maintain a situation in which viral replication and structural protein production is suppressed. Efficient export of unspliced mRNA transcripts and production of p24 is not linearly related to the amount of Rev present in the cell, but requires a critical threshold [149]. Viruses with low Rev-RRE activity may be better able to suppress structural protein expression during latency by effectively raising this threshold. This phenomenon has been demonstrated in a cell line containing a latent provirus that expresses very low levels of cytoplasmic unspliced viral mRNA. Superinfection of the cell line with intact HIV resulted in increased unspliced mRNA export and p24 production even in the absence of an increase in overall transcription of the latent genome [150]. This suggested that low Rev activity was essential for latency maintenance of the provirus

While the mechanism by which HIV establishes latency continues to be explored, one model is that latency is the result of a stochastic cell-driven process [151]. If this model is correct, then viruses which persist for longer in the cell would be more likely to become part of the latent reservoir. In fact, decreased Rev-RRE activity does appear to correlate with cell survival in HIV infected subjects. In a study of Rev M10 as an intracellular therapeutic, peripheral blood leukocytes were collected from patients with HIV, transfected with either a Rev M10 producing or control plasmid, and then reinfused [116]. Rev M10 cells that subsequently became infected with HIV would be expected to attenuate or suppress the Rev-RRE axis of the infecting virus. The survival *in vivo* of Rev M10 expressing leukocytes was greatly increased relative to controls. Prolonged survival could result from improved CTL evasion or from decreasing cellular toxicity by moderating the rate of replication.

7. DISCUSSION

The Rev-RRE regulatory axis in primary isolates displays significant genetic, structural, and functional variability. The Rev-RRE interaction is complex and structurally plastic. As such, it affords multiple opportunities for modulation and tuning. Changes in Rev or the RRE are sufficient to alter the level of Rev-RRE activity, and either element can compensate for induced changes in the other. Naturally occurring viruses display considerable diversity in levels of Rev-RRE activity and it would be remarkable if this did not result in different levels of fitness in specific immune environments. Indeed, the finding that circulating quasispecies within single subjects at single time points have clustered levels of Rev-RRE activity suggests the presence of evolutionary pressure on this system [114]. However, due to the limited number of investigations to date, any correlation between Rev-RRE activity level and clinical disease in HIV remains speculative.

If Rev-RRE activity modulation does permit viral adaptation to differing immune environments, then understanding this system could provide key insights into clinically relevant aspects of HIV infection. The phenomenon of the transmission bottleneck is well described, but the viral phenotype that is being selected during the events of transmission remains incompletely characterized. The level of Rev-RRE activity could be one factor by which a virus is adapted to the unique immune environment of the genital tract. If so, then

disruption of that system through pharmacologic means could provide another modality of infection prophylaxis.

As an essential system for viral replication, the Rev-RRE axis is conceptually a promising target for drug therapy. While a number of interventions have been proposed, ranging from small molecules to Rev and RRE aptamers, they have yet to enter clinical practice. Despite the great success of current antiretroviral therapy, there still are patients with extensive drug resistance. Thus additional modalities of antiretroviral therapy, especially to new viral targets, are very desirable. Any drug development targeting the Rev-RRE system will need to deal with the structural variability and plasticity intrinsic to both elements in order to avoid the development of resistance.

Even more pressing than improvements in HIV treatment are advances in the area of HIV cure. One of the most promising strategies to overcome HIV latency and potentially achieve a sterilizing cure is the so-called “kick and kill” approach [152]. If latently infected cells can be stimulated to produce viral proteins, then the latent reservoir could be cleared by immune or extrinsic mechanisms. A latent reservoir enriched with proviruses with attenuated Rev-RRE systems, however, would likely be especially challenging to reactivate. One potential strategy in that circumstance would be to combine existing latency reversing agents with agents to modify splicing patterns [153].

Despite more than 30 years of investigation, the functional role of the Rev-RRE regulatory axis in clinical HIV disease remains poorly defined. Advances in structural biology have illuminated the complex interaction of Rev, the RRE, and cellular factors, but the role the system plays in pathogenesis is less well understood. As the field looks towards ending the HIV pandemic, it is worth revisiting this often overlooked axis in search of further insights that may point the way to improved interventions for people living with HIV.

REFERENCES

1. Gallo R, Wong-Staal F, Montagnier L, Haseltine WA, Yoshida M. HIV/HTLV gene nomenclature. *Nature*. 1988;333(6173):504.
2. Frankel AD, Young JA. HIV-1: fifteen proteins and an RNA. *Annu Rev Biochem*. 1998;67:1–25. [PubMed: 9759480]
3. Hammariskjold ML. Regulation of retroviral RNA export. *Semin Cell Dev Biol*. 1997;8(1):83–90. [PubMed: 15001110]
4. Emery A, Zhou S, Pollom E, Swanstrom R. Characterizing HIV-1 Splicing by Using Next-Generation Sequencing. *J Virol*. 2017;91(6).
5. Purcell DF, Martin MA. Alternative splicing of human immunodeficiency virus type 1 mRNA modulates viral protein expression, replication, and infectivity. *J Virol*. 1993;67(11):6365–78. [PubMed: 8411338]
6. Schwartz S, Felber BK, Benko DM, Fenyo EM, Pavlakis GN. Cloning and functional analysis of multiply spliced mRNA species of human immunodeficiency virus type 1. *J Virol*. 1990;64(6):2519–29. [PubMed: 2335812]
7. Legrain P, Seraphin B, Rosbash M. Early commitment of yeast pre-mRNA to the spliceosome pathway. *Molecular And Cellular Biology*. 1988;8(9):3755–60. [PubMed: 3065622]
8. Reed R, Hurt E. A conserved mRNA export machinery coupled to pre-mRNA splicing. *Cell*. 2002;108(4):523–31. [PubMed: 11909523]

9. Erkmann JA, Kutay U. Nuclear export of mRNA: from the site of transcription to the cytoplasm. *Exp Cell Res*. 2004;296(1):12–20. [PubMed: 15120988]
10. Rekosh D, Hammarskjold ML. Intron retention in viruses and cellular genes: Detention, border controls and passports. *Wiley Interdiscip Rev RNA*. 2018;9(3):e1470. [PubMed: 29508942]
11. Liu H, Luo M, Wen JK. mRNA stability in the nucleus. *J Zhejiang Univ Sci B*. 2014;15(5):444–54. [PubMed: 24793762]
12. Terribilini M, Lee JH, Yan C, Jernigan RL, Carpenter S, Honavar V, et al. Identifying interaction sites in “recalcitrant” proteins: predicted protein and RNA binding sites in rev proteins of HIV-1 and EIAV agree with experimental data. *Pac Symp Biocomput*. 2006:415–26. [PubMed: 17094257]
13. LeBlanc JJ, Uddowla S, Abraham B, Clatterbuck S, Beemon KL. Tap and Dbp5, but not Gag, are involved in DR-mediated nuclear export of unspliced Rous sarcoma virus RNA. *Virology*. 2007;363(2):376–86. [PubMed: 17328934]
14. Bray M, Prasad S, Dubay JW, Hunter E, Jeang KT, Rekosh D, et al. 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 U S A*. 1994;91(4):1256–60. [PubMed: 8108397]
15. Gruter P, Tabernero C, von KC, Schmitt C, Saavedra C, Bachi A, et al. TAP, the human homolog of Mex67p, mediates CTE-dependent RNA export from the nucleus. *Mol Cell*. 1998;1(5):649–59. [PubMed: 9660949]
16. Li Y, Bor YC, Fitzgerald MP, Lee KS, Rekosh D, Hammarskjold ML. An NXF1 mRNA with a retained intron is expressed in hippocampal and neocortical neurons and is translated into a protein that functions as an Nxf1 cofactor. *Molecular biology of the cell*. 2016;27(24):3903–12. [PubMed: 27708137]
17. Li Y, Bor YC, Misawa Y, Xue Y, Rekosh D, Hammarskjold ML. An intron with a constitutive transport element is retained in a Tap messenger RNA. *Nature*. 2006;443(7108):234–7. [PubMed: 16971948]
18. Wang B, Rekosh D, Hammarskjold ML. Evolutionary conservation of a molecular machinery for export and expression of mRNAs with retained introns. *Rna*. 2015;21(3):426–37. [PubMed: 25605961]
19. Bor Y, Swartz J, Morrison A, Rekosh D, Lodomery M, Hammarskjold ML. The Wilms’ tumor 1 (WT1) gene (+KTS isoform) functions with a CTE to enhance translation from an unspliced RNA with a retained intron. *Genes Dev*. 2006;20(12):1597–608. [PubMed: 16738405]
20. Zolotukhin AS, Valentin A, Pavlakis GN, Felber BK. Continuous propagation of RRE(–) and Rev(–)RRE(–) human immunodeficiency virus type 1 molecular clones containing a cis-acting element of simian retrovirus type 1 in human peripheral blood lymphocytes. *J Virol*. 1994;68(12):7944–52. [PubMed: 7966585]
21. Ogert RA, Beemon KL, editors. Identification of a constitutive transport element in the 3’ untranslated region of the Rous Sarcoma virus RNA Retroviruses; 1995; Cold Spring Harbor: Cold Springs Harbor Laboratory.
22. Pessel-Vivares L, Ferrer M, Laine S, Mougél M. MLV requires Tap/NXF1-dependent pathway to export its unspliced RNA to the cytoplasm and to express both spliced and unspliced RNAs. *Retrovirology*. 2014;11:21. [PubMed: 24597485]
23. Sakuma T, Davila JI, Malcolm JA, Kocher JP, Tonne JM, Ikeda Y. Murine leukemia virus uses NXF1 for nuclear export of spliced and unspliced viral transcripts. *J Virol*. 2014;88(8):4069–82. [PubMed: 24478440]
24. Sodroski J, Goh WC, Rosen C, Dayton A, Terwilliger E, Haseltine W. A second post-transcriptional trans-activator gene required for HTLV-III replication. *Nature*. 1986;321(6068):412. [PubMed: 3012355]
25. Malim MH, Hauber J, Le SY, Maizel JV, Cullen BR. The HIV-1 rev trans-activator acts through a structured target sequence to activate nuclear export of unspliced viral mRNA. *Nature*. 1989;338(6212):254–7. [PubMed: 2784194]
26. Hadzopoulou-Cladaras M, Felber BK, Cladaras C, Athanassopoulos A, Tse A, Pavlakis GN. The rev (trs/art) protein of human immunodeficiency virus type 1 affects viral mRNA and protein

- expression via a cis-acting sequence in the env region. *J Virol.* 1989;63(3):1265–74. [PubMed: 2783738]
27. Hammariskjold ML, Heimer J, Hammariskjold B, Sangwan I, Albert L, Rekosh D. Regulation of human immunodeficiency virus env expression by the rev gene product. *J Virol.* 1989;63(5):1959–66. [PubMed: 2704072]
 28. Cullen BR. Nuclear mRNA export: insights from virology. *Trends Biochem Sci.* 2003;28(8):419–24. [PubMed: 12932730]
 29. Sadaie MR, Benter T, Wong-Staal F. Site-directed mutagenesis of two trans-regulatory genes (tat-III, trs) of HIV-1. *Science.* 1988;239(4842):910–3. [PubMed: 3277284]
 30. Pollard VW, Malim MH. The HIV-1 Rev protein. *Annu Rev Microbiol.* 1998;52:491–532. [PubMed: 9891806]
 31. Hope TJ. The ins and outs of HIV Rev. *Arch Biochem Biophys.* 1999;365(2):186–91. [PubMed: 10328811]
 32. Daly TJ, Cook KS, Gray GS, Maione TE, Rusche JR. Specific binding of hiv-1 recombinant Rev protein to the Rev-responsive element in vitro. *Nature.* 1989;342:816–9. [PubMed: 2481237]
 33. Daeffer S, Klotman ME, Wong-Staal F. Trans-activating rev protein of the human immunodeficiency virus 1 interacts directly and specifically with its target RNA. *Proc Natl Acad Sci U S A.* 1990;87(12):4571–5. [PubMed: 2191294]
 34. Heaphy S, Dingwall C, Ernberg I, Gait MJ, Green SM, Karn J, et al. HIV-1 regulator of virion expression (Rev) protein binds to an RNA stem-loop structure located within the Rev response element region. *Cell.* 1990;60(4):685–93. [PubMed: 1689218]
 35. Holland SM, Ahmad N, Maitra RK, Wingfield P, Venkatesan S. Human immunodeficiency virus rev protein recognizes a target sequence in rev-responsive element RNA within the context of RNA secondary structure. *J Virol.* 1990;64(12):5966–75. [PubMed: 2243382]
 36. Fernandes J, Jayaraman B, Frankel A. The HIV-1 Rev response element: an RNA scaffold that directs the cooperative assembly of a homo-oligomeric ribonucleoprotein complex. *RNA Biol.* 2012;9(1):6–11. [PubMed: 22258145]
 37. Fernandes JD, Booth DS, Frankel AD. A structurally plastic ribonucleoprotein complex mediates post-transcriptional gene regulation in HIV-1. *Wiley Interdiscip Rev RNA.* 2016.
 38. Henderson BR, Percipalle P. Interactions between HIV Rev and nuclear import and export factors: the Rev nuclear localisation signal mediates specific binding to human importin-beta. *J Mol Biol.* 1997;274(5):693–707. [PubMed: 9405152]
 39. Fornerod M, Ohno M, Yoshida M, Mattaj JW. CRM1 is an export receptor for leucine-rich nuclear export signals. *Cell.* 1997;90(6):1051–60. [PubMed: 9323133]
 40. Neville M, Stutz F, Lee L, Davis LI, Rosbash M. The importin-beta family member Crm1p bridges the interaction between Rev and the nuclear pore complex during nuclear export. *Curr Biol.* 1997;7(10):767–75. [PubMed: 9368759]
 41. Ossareh-Nazari B, Bachelier F, Dargemont C. Evidence for a role of CRM1 in signal-mediated nuclear protein export. *Science.* 1997;278(5335):141–4. [PubMed: 9311922]
 42. Fukuda M, Asano S, Nakamura T, Adachi M, Yoshida M, Yanagida M, et al. CRM1 is responsible for intracellular transport mediated by the nuclear export signal. *Nature.* 1997;390(6657):308–11. [PubMed: 9384386]
 43. Karn J, Stoltzfus CM. Transcriptional and posttranscriptional regulation of HIV-1 gene expression. *Cold Spring Harb Perspect Med.* 2012;2(2):a006916. [PubMed: 22355797]
 44. Daelemans D, Costes SV, Lockett S, Pavlakis GN. Kinetic and molecular analysis of nuclear export factor CRM1 association with its cargo in vivo. *Molecular and cellular biology.* 2005;25(2):728–39. [PubMed: 15632073]
 45. Askjaer P, Jensen TH, Nilsson J, Englmeier L, Kjems J. The specificity of the CRM1-Rev nuclear export signal interaction is mediated by RanGTP. *J Biol Chem.* 1998;273(50):33414–22. [PubMed: 9837918]
 46. Dlamini Z, Hull R. Can the HIV-1 splicing machinery be targeted for drug discovery? *HIV AIDS (Auckl).* 2017;9:63–75. [PubMed: 28331370]
 47. Rausch JW, Le Grice SF. HIV Rev Assembly on the Rev Response Element (RRE): A Structural Perspective. *Viruses.* 2015;7(6):3053–75. [PubMed: 26075509]

48. Malim MH, Tiley LS, McCarn DF, Rusche JR, Hauber J, Cullen BR. HIV-1 structural gene expression requires binding of the Rev trans-activator to its RNA target sequence. *Cell*. 1990;60(4):675–83. [PubMed: 2406030]
49. Smith AJ, Cho MI, Hammariskjöld ML, Rekosh D. Human immunodeficiency virus type 1 Pr55gag and Pr160gag-pol expressed from a simian virus 40 late replacement vector are efficiently processed and assembled into viruslike particles. *J Virol*. 1990;64(6):2743–50. [PubMed: 1692347]
50. Watts NR, Eren E, Zhuang X, Wang YX, Steven AC, Wingfield PT. A new HIV-1 Rev structure optimizes interaction with target RNA (RRE) for nuclear export. *J Struct Biol*. 2018;203(2):102–8. [PubMed: 29605570]
51. Cochrane A HIV-1 Rev function and RNA nuclear-cytoplasmic export. *Methods Mol Biol*. 2014;1087:103–14. [PubMed: 24158817]
52. Swenarchuk L, Harakidas P, Cochrane A. Regulated expression of HIV-1 Rev function in mammalian cell lines. *Can J Microbiol*. 1999;45(6):480–90. [PubMed: 10453476]
53. Booth DS, Cheng Y, Frankel AD. The export receptor Crm1 forms a dimer to promote nuclear export of HIV RNA. *Elife*. 2014;3.
54. Yue Y, Coskun AK, Jawanda N, Auer J, Sutton RE. Differential interaction between human and murine Crm1 and lentiviral Rev proteins. *Virology*. 2018;513:1–10. [PubMed: 29028476]
55. Jayaraman B, Fernandes JD, Yang S, Smith C, Frankel AD. Highly Mutable Linker Regions Regulate HIV-1 Rev Function and Stability. *Sci Rep*. 2019;9(1):5139. [PubMed: 30914719]
56. Kjems J, Frankel AD, Sharp PA. Specific regulation of mRNA splicing in vitro by a peptide from HIV-1 Rev. *Cell*. 1991;67(1):169–78. [PubMed: 1913815]
57. Hope TJ. The ins and outs of HIV Rev. *Archives of biochemistry and biophysics*. 1999;365(2):186–91. [PubMed: 10328811]
58. Battiste JL, Mao H, Rao NS, Tan R, Muhandiram DR, Kay LE, et al. Alpha helix-RNA major groove recognition in an HIV-1 rev peptide-RRE RNA complex. *Science*. 1996;273(5281):1547–51. [PubMed: 8703216]
59. Jain C, Belasco JG. Structural model for the cooperative assembly of HIV-1 Rev multimers on the RRE as deduced from analysis of assembly-defective mutants. *Mol Cell*. 2001;7(3):603–14. [PubMed: 11463385]
60. Fischer U, Huber J, Boelens WC, Mattaj IW, Luhrmann R. The HIV-1 Rev activation domain is a nuclear export signal that accesses an export pathway used by specific cellular RNAs. *Cell*. 1995;82(3):475–83. [PubMed: 7543368]
61. Mann DA, Mikaelian I, Zimmel RW, Green SM, Lowe AD, Kimura T, et al. A molecular rheostat. Co-operative rev binding to stem I of the rev-response element modulates human immunodeficiency virus type-1 late gene expression. *J Mol Biol*. 1994;241(2):193–207. [PubMed: 8057359]
62. Daugherty MD, D’Orso I, Frankel AD. A solution to limited genomic capacity: using adaptable binding surfaces to assemble the functional HIV Rev oligomer on RNA. *Mol Cell*. 2008;31(6):824–34. [PubMed: 18922466]
63. Sherpa C, Rausch JW, Le Grice SF, Hammariskjöld ML, Rekosh D. The HIV-1 Rev response element (RRE) adopts alternative conformations that promote different rates of virus replication. *Nucleic acids research*. 2015;43(9):4676–86. [PubMed: 25855816]
64. Lusvarghi S, Sztuba-Solinska J, Purzycka KJ, Pauly GT, Rausch JW, Grice SF. The HIV-2 Rev-response element: determining secondary structure and defining folding intermediates. *Nucleic acids research*. 2013;41(13):6637–49. [PubMed: 23640333]
65. O’Carroll IP, Thappeta Y, Fan L, Ramirez-Valdez EA, Smith S, Wang YX, et al. Contributions of Individual Domains to Function of the HIV-1 Rev Response Element. *J Virol*. 2017;91(21).
66. Fang X, Wang J, O’Carroll IP, Mitchell M, Zuo X, Wang Y, et al. An unusual topological structure of the HIV-1 Rev response element. *Cell*. 2013;155(3):594–605. [PubMed: 24243017]
67. Jayaraman B, Crosby DC, Homer C, Ribeiro I, Mavor D, Frankel AD. RNA-directed remodeling of the HIV-1 protein Rev orchestrates assembly of the Rev–Rev response element complex. *Elife*. 2015;3:e04120.

68. DiMattia MA, Watts NR, Cheng N, Huang R, Heymann JB, Grimes JM, et al. The Structure of HIV-1 Rev Filaments Suggests a Bilateral Model for Rev-RRE Assembly. *Structure*. 2016;24(7):1068–80. [PubMed: 27265851]
69. Jayaraman B, Mavor D, Gross JD, Frankel AD. Thermodynamics of Rev-RNA interactions in HIV-1 Rev-RRE assembly. *Biochemistry*. 2015;54(42):6545–54. [PubMed: 26422686]
70. Tanamura S, Terakado H, Harada K. Cooperative dimerization of a stably folded protein directed by a flexible RNA in the assembly of the HIV Rev dimer-RRE stem II complex. *J Mol Recognit*. 2016;29(5):199–209. [PubMed: 26620599]
71. Mann DA, Mikaelian I, Zimmel RW, Green SM, Lowe AD, Kimura T, et al. A molecular rheostat. Co-operative rev binding to stem I of the rev-response element modulates human immunodeficiency virus type-1 late gene expression. *J Mol Biol*. 1994;241(2):193–207. [PubMed: 8057359]
72. Malim MH, Cullen BR. HIV-1 structural gene expression requires the binding of multiple Rev monomers to the viral RRE: implications for HIV-1 latency. *Cell*. 1991;65(2):241–8. [PubMed: 2015625]
73. Heaphy S, Finch JT, Gait MJ, Karn J, Singh M. Human immunodeficiency virus type 1 regulator of virion expression, rev, forms nucleoprotein filaments after binding to a purine-rich “bubble” located within the rev-responsive region of viral mRNAs. *Proc Natl Acad Sci U S A*. 1991;88(16):7366–70. [PubMed: 1871141]
74. Iwai S, Pritchard C, Mann DA, Karn J, Gait MJ. Recognition of the high affinity binding site in rev-response element RNA by the human immunodeficiency virus type-1 rev protein. *Nucleic acids research*. 1992;20(24):6465–72. [PubMed: 1282702]
75. Hammariskjold MH, Rekosh D. A long-awaited structure is rev-ealed. *Viruses*. 2011;3(5):484–92. [PubMed: 21941623]
76. Daugherty MD, Booth DS, Jayaraman B, Cheng Y, Frankel AD. HIV Rev response element (RRE) directs assembly of the Rev homooligomer into discrete asymmetric complexes. *Proc Natl Acad Sci U S A*. 2010;107(28):12481–6. [PubMed: 20616058]
77. Kjems J, Brown M, Chang DD, Sharp PA. Structural analysis of the interaction between the human immunodeficiency virus Rev protein and the Rev response element. *Proc Natl Acad Sci U S A*. 1991;88(3):683–7. [PubMed: 1992459]
78. Bai Y, Tambe A, Zhou K, Doudna JA. RNA-guided assembly of Rev-RRE nuclear export complexes. *Elife*. 2014;3:e03656. [PubMed: 25163983]
79. Pond SJ, Ridgeway WK, Robertson R, Wang J, Millar DP. HIV-1 Rev protein assembles on viral RNA one molecule at a time. *Proc Natl Acad Sci U S A*. 2009;106(5):1404–8. [PubMed: 19164515]
80. Zapp ML, Hope TJ, Parslow TG, Green MR. Oligomerization and RNA binding domains of the type 1 human immunodeficiency virus Rev protein: a dual function for an arginine- rich binding motif. *Proc Natl Acad Sci U S A*. 1991;88(17):7734–48. [PubMed: 1715576]
81. Booth DS, Cheng Y, Frankel AD. The export receptor Crm1 forms a dimer to promote nuclear export of HIV RNA. *Elife*. 2014;3:e04121. [PubMed: 25486595]
82. Stade K, Ford CS, Guthrie C, Weis K. Exportin 1 (Crm1p) is an essential nuclear export factor. *Cell*. 1997;90(6):1041–50. [PubMed: 9323132]
83. Sherer NM, Swanson CM, Hue S, Roberts RG, Bergeron JR, Malim MH. Evolution of a species-specific determinant within human CRM1 that regulates the post-transcriptional phases of HIV-1 replication. *PLoS Pathog*. 2011;7(11):e1002395. [PubMed: 22114565]
84. Daugherty MD, Liu B, Frankel AD. Structural basis for cooperative RNA binding and export complex assembly by HIV Rev. *Nat Struct Mol Biol*. 2010;17(11):1337–42. [PubMed: 20953181]
85. Culjkovic B, Topisirovic I, Skrabanek L, Ruiz-Gutierrez M, Borden KL. eIF4E is a central node of an RNA regulon that governs cellular proliferation. *J Cell Biol*. 2006;175(3):415–26. [PubMed: 17074885]
86. Siddiqui N, Borden KL. mRNA export and cancer. *Wiley Interdiscip Rev RNA*. 2012;3(1):13–25. [PubMed: 21796793]

87. Fries B, Heukeshoven J, Hauber I, Gruttner C, Stocking C, Kehlenbach RH, et al. Analysis of nucleocytoplasmic trafficking of the HuR ligand APRIL and its influence on CD83 expression. *J Biol Chem.* 2007;282(7):4504–15. [PubMed: 17178712]
88. Kimura T, Hashimoto I, Nagase T, Fujisawa J. CRM1-dependent, but not ARE-mediated, nuclear export of IFN- α 1 mRNA. *J Cell Sci.* 2004;117(Pt 11):2259–70. [PubMed: 15126627]
89. Schutz S, Chemnitz J, Spillner C, Frohme M, Hauber J, Kehlenbach RH. Stimulated expression of mRNAs in activated T cells depends on a functional CRM1 nuclear export pathway. *J Mol Biol.* 2006;358(4):997–1009. [PubMed: 16580684]
90. Daniels RS, Smith MH, Fisher AG. Molecular characterization of biologically diverse envelope variants of human immunodeficiency virus type 1 derived from an individual. *J Virol.* 1991;65(10):5574–8. [PubMed: 1895406]
91. Li G, Piampongsant S, Faria NR, Voet A, Pineda-Pena AC, Khouri R, et al. An integrated map of HIV genome-wide variation from a population perspective. *Retrovirology.* 2015;12:18. [PubMed: 25808207]
92. Mansky LM, Temin HM. Lower in vivo mutation rate of human immunodeficiency virus type 1 than that predicted from the fidelity of purified reverse transcriptase. *J Virol.* 1995;69(8):5087–94. [PubMed: 7541846]
93. Cuevas JM, Geller R, Garijo R, Lopez-Aldeguer J, Sanjuan R. Extremely High Mutation Rate of HIV-1 In Vivo. *PLoS Biol.* 2015;13(9):e1002251. [PubMed: 26375597]
94. Biswas N, Wang T, Ding M, Tumne A, Chen Y, Wang Q, et al. ADAR1 is a novel multi targeted anti-HIV-1 cellular protein. *Virology.* 2012;422(2):265–77. [PubMed: 22104209]
95. Rossenkhon R, Novitsky V, Sebunya TK, Musonda R, Gashe BA, Essex M. Viral diversity and diversification of major non-structural genes vif, vpr, vpu, tat exon 1 and rev exon 1 during primary HIV-1 subtype C infection. *PLoS One.* 2012;7(5):e35491. [PubMed: 22590503]
96. Ramakrishnan R, Hussain M, Holzer A, Mehta R, Sundaravaradan V, Ahmad N. Evaluations of HIV type 1 rev gene diversity and functional domains following perinatal transmission. *AIDS Res Hum Retroviruses.* 2005;21(12):1035–45. [PubMed: 16379607]
97. Guillon C, Stankovic K, Ataman-Onal Y, Biron F, Verrier B. Evidence for CTL-mediated selection of Tat and Rev mutants after the onset of the asymptomatic period during HIV type 1 infection. *AIDS Res Hum Retroviruses.* 2006;22(12):1283–92. [PubMed: 17209772]
98. Salazar-Gonzalez JF, Bailes E, Pham KT, Salazar MG, Guffey MB, Keele BF, et al. Deciphering human immunodeficiency virus type 1 transmission and early envelope diversification by single-genome amplification and sequencing. *J Virol.* 2008;82(8):3952–70. [PubMed: 18256145]
99. Fernandes JD, Faust TB, Strauli NB, Smith C, Crosby DC, Nakamura RL, et al. Functional Segregation of Overlapping Genes in HIV. *Cell.* 2016;167(7):1762–73 e12. [PubMed: 27984726]
100. Martins LP, Chenciner N, Asjo B, Meyerhans A, Wain-Hobson S. Independent fluctuation of human immunodeficiency virus type 1 rev and gp41 quasispecies in vivo. *J Virol.* 1991;65(8):4502–7. [PubMed: 2072461]
101. Phuphuakrat A, Auewarakul P. Heterogeneity of HIV-1 Rev response element. *AIDS Res Hum Retroviruses.* 2003;19(7):569–74. [PubMed: 12908934]
102. Ramakrishnan R, Ahmad N. Derivation of primary sequences and secondary structures of rev responsive element from HIV-1 infected mothers and infants following vertical transmission. *Virology.* 2007;359(1):201–11. [PubMed: 17045321]
103. Dayton ET, Konings DA, Powell DM, Shapiro BA, Butini L, Maizel JV, et al. Extensive sequence-specific information throughout the CAR/RRE, the target sequence of the human immunodeficiency virus type 1 Rev protein. *J Virol.* 1992;66(2):1139–51. [PubMed: 1731093]
104. Werstuck G, Zapp ML, Green MR. A non-canonical base pair within the human immunodeficiency virus rev-responsive element is involved in both rev and small molecule recognition. *Chem Biol.* 1996;3(2):129–37. [PubMed: 8807838]
105. Aoyama S, Sugaya M, Kobayashi C, Masuda K, Maeda T, Sakamoto T, et al. An isostructural G-G to A-A substitution within the HIV RRE RNA switches the specificity towards arginine-rich peptides. *Nucleic Acids Symp Ser (Oxf).* 2009(53):271–2.

106. Sugaya M, Nishimura F, Katoh A, Harada K. Tailoring the peptide-binding specificity of an RNA by combinations of specificity-altering mutations. *Nucleosides Nucleotides Nucleic Acids*. 2008;27(5):534–45. [PubMed: 18569791]
107. Iwazaki T, Li X, Harada K. Evolvability of the mode of peptide binding by an RNA. *Rna*. 2005;11(9):1364–73. [PubMed: 16043495]
108. Harada K, Sugaya M, Nishimura F, Katoh A. Manipulation of the peptide-binding specificity of an RNA in a rational manner by combinations of specificity-altering mutations. *Nucleic Acids Symp Ser (Oxf)*. 2008(52):13–4.
109. Abdallah EY, Smith CA. Diverse mutants of HIV RRE IIB recognize wild-type Rev ARM or Rev ARM R35G-N40V. *J Mol Recognit*. 2015;28(12):710–21. [PubMed: 26130028]
110. Harada K, Iwazaki T, Li X, Yuda A, Kobayashi K. The variability of the peptide-binding specificity of RNA. *Nucleic Acids Res Suppl*. 2003(3):201–2. [PubMed: 14510450]
111. Chu CC, Plangger R, Kreutz C, Al-Hashimi HM. Dynamic ensemble of HIV-1 RRE stem IIB reveals non-native conformations that disrupt the Rev-binding site. *Nucleic acids research*. 2019;47(13):7105–17. [PubMed: 31199872]
112. Lichinchi G, Gao S, Saletore Y, Gonzalez GM, Bansal V, Wang Y, et al. Dynamics of the human and viral m(6)A RNA methylomes during HIV-1 infection of T cells. *Nat Microbiol*. 2016;1:16011. [PubMed: 27572442]
113. Sherpa C, Jackson PEH, Gray LR, Anastos K, Le Grice SFJ, Hammarskjöld ML, et al. Evolution of the HIV-1 Rev Response Element during Natural Infection Reveals Nucleotide Changes That Correlate with Altered Structure and Increased Activity over Time. *J Virol*. 2019;93(11).
114. Sloan EA, Kearney MF, Gray LR, Anastos K, Daar ES, Margolick J, et al. Limited nucleotide changes in the Rev response element (RRE) during HIV-1 infection alter overall Rev-RRE activity and Rev multimerization. *J Virol*. 2013;87(20):11173–86. [PubMed: 23926352]
115. Malim MH, Bohnlein S, Hauber J, Cullen BR. Functional dissection of the HIV-1 Rev trans-activator--derivation of a trans-dominant repressor of Rev function. *Cell*. 1989;58(1):205–14. [PubMed: 2752419]
116. Woffendin C, Ranga U, Yang Z, Xu L, Nabel GJ. Expression of a protective gene-prolongs survival of T cells in human immunodeficiency virus-infected patients. *Proc Natl Acad Sci U S A*. 1996;93(7):2889–94. [PubMed: 8610137]
117. Ranga U, Woffendin C, Verma S, Xu L, June CH, Bishop DK, et al. Enhanced T cell engraftment after retroviral delivery of an antiviral gene in HIV-infected individuals. *Proc Natl Acad Sci U S A*. 1998;95(3):1201–6. [PubMed: 9448309]
118. Hamm TE, Rekosh D, Hammarskjöld ML. Selection and characterization of human immunodeficiency virus type 1 mutants that are resistant to inhibition by the transdominant negative RevM10 protein. *J Virol*. 1999;73(7):5741–7. [PubMed: 10364325]
119. Legiewicz M, Badorrek CS, Turner KB, Fabris D, Hamm TE, Rekosh D, et al. Resistance to RevM10 inhibition reflects a conformational switch in the HIV-1 Rev response element. *Proc Natl Acad Sci U S A*. 2008;105(38):14365–70. [PubMed: 18776047]
120. Jing S, Zhao Q, Debnath AK. Peptide and non-peptide HIV fusion inhibitors. *Current pharmaceutical design*. 2002;8(8):563–80. [PubMed: 11945159]
121. Nameki D, Kodama E, Ikeuchi M, Mabuchi N, Otaka A, Tamamura H, et al. Mutations conferring resistance to human immunodeficiency virus type 1 fusion inhibitors are restricted by gp41 and Rev-responsive element functions. *J Virol*. 2005;79(2):764–70. [PubMed: 15613304]
122. Ueno M, Kodama EN, Shimura K, Sakurai Y, Kajiura K, Sakagami Y, et al. Synonymous mutations in stem-loop III of Rev responsive elements enhance HIV-1 replication impaired by primary mutations for resistance to enfuvirtide. *Antiviral Res*. 2009;82(1):67–72. [PubMed: 19428597]
123. Possik EJ, Bou Sleiman MS, Ghattas IR, Smith CA. Randomized codon mutagenesis reveals that the HIV Rev arginine-rich motif is robust to substitutions and that double substitution of two critical residues alters specificity. *J Mol Recognit*. 2013;26(6):286–96. [PubMed: 23595810]
124. Edgcomb SP, Aschrafi A, Kompfner E, Williamson JR, Gerace L, Hennig M. Protein structure and oligomerization are important for the formation of export-competent HIV-1 Rev-RRE complexes. *Protein Sci*. 2008;17(3):420–30. [PubMed: 18218716]

125. Faust O, Bigman L, Friedler A. A role of disordered domains in regulating protein oligomerization and stability. *Chem Commun (Camb)*. 2014;50(74):10797–800. [PubMed: 25054624]
126. Wolff H, Hadian K, Ziegler M, Weierich C, Kramer-Hammerle S, Kleinschmidt A, et al. Analysis of the influence of subcellular localization of the HIV Rev protein on Rev-dependent gene expression by multi-fluorescence live-cell imaging. *Exp Cell Res*. 2006;312(4):443–56. [PubMed: 16368434]
127. Hua J, Caffrey JJ, Cullen BR. Functional consequences of natural sequence variation in the activation domain of HIV-1 Rev. *Virology*. 1996;222(2):423–9. [PubMed: 8806526]
128. Jackson PE, Tebit DM, Rekosh D, Hammarskjöld ML. Rev-RRE Functional Activity Differs Substantially Among Primary HIV-1 Isolates. *AIDS Res Hum Retroviruses*. 2016;32(9):923–34. [PubMed: 27147495]
129. Svicher V, Alteri C, D'Arrigo R, Lagana A, Trignetti M, Lo Caputo S, et al. Treatment with the fusion inhibitor enfuvirtide influences the appearance of mutations in the human immunodeficiency virus type 1 regulatory protein rev. *Antimicrob Agents Chemother*. 2009;53(7):2816–23. [PubMed: 19124665]
130. Cunyat F, Beerens N, Garcia E, Clotet B, Kijms J, Cabrera C. Functional analyses reveal extensive RRE plasticity in primary HIV-1 sequences selected under selective pressure. *PLoS One*. 2014;9(8):e106299. [PubMed: 25170621]
131. Larmont J, Tindall B, Evans L, Cunningham A, Cunningham P, Wells J, et al. Long-term symptomless HIV-1 infection in recipients of blood products from a single donor. *Lancet*. 1992;340(8824):863–7. [PubMed: 1357294]
132. Zaunders J, Dyer WB, Churchill M. The Sydney Blood Bank Cohort: implications for viral fitness as a cause of elite control. *Curr Opin HIV AIDS*. 2011;6(3):151–6. [PubMed: 21378562]
133. Oelrichs R, Tsykin A, Rhodes D, Solomon A, Ellett A, McPhee D, et al. Genomic sequence of HIV type 1 from four members of the Sydney Blood Bank Cohort of long-term nonprogressors. *AIDS Res Hum Retroviruses*. 1998;14(9):811–4. [PubMed: 9643382]
134. Churchill MJ, Chiavaroli L, Wesselingh SL, Gorry PR. Persistence of attenuated HIV-1 rev alleles in an epidemiologically linked cohort of long-term survivors infected with nef-deleted virus. *Retrovirology*. 2007;4:43. [PubMed: 17601342]
135. Papathanasopoulos MA, Patience T, Meyers TM, McCutchan FE, Morris L. Full-length genome characterization of HIV type 1 subtype C isolates from two slow-progressing perinatally infected siblings in South Africa. *AIDS Res Hum Retroviruses*. 2003;19(11):1033–7. [PubMed: 14686322]
136. Rodenburg CM, Li Y, Trask SA, Chen Y, Decker J, Robertson DL, et al. Near full-length clones and reference sequences for subtype C isolates of HIV type 1 from three different continents. *AIDS Res Hum Retroviruses*. 2001;17(2):161–8. [PubMed: 11177395]
137. Iversen AK, Shpaer EG, Rodrigo AG, Hirsch MS, Walker BD, Sheppard HW, et al. Persistence of attenuated rev genes in a human immunodeficiency virus type 1-infected asymptomatic individual. *J Virol*. 1995;69(9):5743–53. [PubMed: 7637019]
138. Phuphuakrat A, Paris RM, Nittayaphan S, Louisirirochanakul S, Auewarakul P. Functional variation of HIV-1 Rev Response Element in a longitudinally studied cohort. *J Med Virol*. 2005;75(3):367–73. [PubMed: 15648073]
139. Phuphuakrat A, Auewarakul P. Functional variability of Rev response element in HIV-1 primary isolates. *Virus Genes*. 2005;30(1):23–9. [PubMed: 15744559]
140. Bobbitt KR, Addo MM, Altfield M, Filzen T, Onafuwa AA, Walker BD, et al. Rev activity determines sensitivity of HIV-1-infected primary T cells to CTL killing. *Immunity*. 2003;18(2):289–99. [PubMed: 12594955]
141. Carpenter S, Dobbs D. Molecular and biological characterization of equine infectious anemia virus Rev. *Curr HIV Res*. 2010;8(1):87–93. [PubMed: 20210784]
142. Ihm Y, Sparks WO, Lee JH, Cao H, Carpenter S, Wang CZ, et al. Structural model of the Rev regulatory protein from equine infectious anemia virus. *PLoS One*. 2009;4(1):e4178. [PubMed: 19137065]

143. Carpenter S, Chen WC, Dorman KS. Rev variation during persistent lentivirus infection. *Viruses*. 2011;3(1):1–11. [PubMed: 21994723]
144. Belshan M, Baccam P, Oaks JL, Sponseller BA, Murphy SC, Cornette J, et al. Genetic and biological variation in equine infectious anemia virus Rev correlates with variable stages of clinical disease in an experimentally infected pony. *Virology*. 2001;279(1):185–200. [PubMed: 11145901]
145. Baccam P, Thompson RJ, Li Y, Sparks WO, Belshan M, Dorman KS, et al. Subpopulations of equine infectious anemia virus Rev coexist in vivo and differ in phenotype. *J Virol*. 2003;77(22):12122–31. [PubMed: 14581549]
146. Sparks WO, Dorman KS, Liu S, Carpenter S. Naturally arising point mutations in non-essential domains of equine infectious anemia virus Rev alter Rev-dependent nuclear-export activity. *J Gen Virol*. 2008;89(Pt 4):1043–8. [PubMed: 18343848]
147. Carlson JM, Schaefer M, Monaco DC, Batorsky R, Claiborne DT, Prince J, et al. HIV transmission. Selection bias at the heterosexual HIV-1 transmission bottleneck. *Science*. 2014;345(6193):1254031. [PubMed: 25013080]
148. Blackard JT. HIV compartmentalization: a review on a clinically important phenomenon. *Current HIV research*. 2012;10(2):133–42. [PubMed: 22329519]
149. Pomerantz RJ, Seshamma T, Trono D. Efficient replication of human immunodeficiency virus type 1 requires a threshold level of Rev: potential implications for latency. *J Virol*. 1992;66(3):1809–13. [PubMed: 1738210]
150. Winslow BJ, Pomerantz RJ, Bagasra O, Trono D. HIV-1 latency due to the site of proviral integration. *Virology*. 1993;196(2):849–54. [PubMed: 8372452]
151. Siliciano RF, Greene WC. HIV latency. *Cold Spring Harb Perspect Med*. 2011;1(1):a007096. [PubMed: 22229121]
152. Margolis DM, Hazuda DJ. Combined approaches for HIV cure. *Curr Opin HIV AIDS*. 2013;8(3):230–5. [PubMed: 23446138]
153. Bates DO, Morris JC, Oltean S, Donaldson LF. Pharmacology of Modulators of Alternative Splicing. *Pharmacol Rev*. 2017;69(1):63–79. [PubMed: 28034912]

Figure 1.

Schematic representation of Rev domain organization. Numbers correspond to amino acid position. N-term = N-terminal region, OD = oligomerization domain, L = linker, ARM = arginine rich motif, NES = nuclear export signal, C-term = C-terminal region. Adapted from [55] and used under a Creative Commons Attribution 4.0 International License (<http://creativecommons.org/licenses/by/4.0/>).

Figure 2.

Two secondary structures of the "long" 351-nt NL4-3 RRE. A. The five stem-loop RRE structure. B. The four stem-loop structure. The region corresponding to the "short" 234-nt RRE and the primary and secondary Rev binding sites are indicated on both structures.

Figure is modified from [63], originally published in Nucleic Acids Research from Oxford University Press.

Table 1.

Summary of studies correlating a clinical parameter of HIV infection *in vivo* with a Rev-RRE functional activity measurement.

Clinical status	Number of subjects	Rev-RRE activity finding	Notes	Reference
Long-term nonprogressor versus slow progressor	2 slow progressors, 2 long term nonprogressors	Rev activity was low in 2/4 subjects but this was not correlated with clinical status.	Subjects all infected with virus characterized by deletion in <i>nef</i> LTR.	[134] ^a
Long-term nonprogressor	1	Subject's <i>rev</i> allele attenuates viral replication in culture.	One of four clones from the subject contained a premature stop codon in <i>nef</i>	[137] ^a
Fast versus slow rate of CD4 count decline	16 in described analysis	Higher RRE activity in later disease is associated with faster rate of CD4 count decline. RRE activity at an early time point was not associated with progression.	Subjects with known host alleles associated with disease progression were excluded.	[138] ^a
Symptomatic versus asymptomatic HIV infection	2 symptomatic, 8 asymptomatic	Trend towards higher RRE activity in symptomatic patients as measured by reporter gene production.	Significant variation was observed between primary isolates within subjects and no clear correlation between RRE activity and disease state was observed.	[139] ^a
AIDS versus asymptomatic HIV infection	6	Rev activity is higher for AIDS vs asymptomatic subjects as measured by the relative production of Gag and a substitute early gene.	One Rev from each group was also used in an activity assay assessing RNA export, giving a consistent result.	[140] ^a

^aRefer to the numbered reference in the text.