The Activity of HIV-1 Rev/RRE Varies Greatly Between Isolates

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BACKGROUND

HIV replication is a highly regulated process. The binding of the viral Rev protein to the Rev Response Element (RRE) is essential for the nucleocytoplasmic export of mRNAs that retain introns and thus for packaging of genomic RNA.[1,2,3] Previous studies have shown that there is variation in both Rev and RRE sequence between patients and between viral quasispecies isolated from a single patient.[4] We previously assessed the functional activity of the Rev-RRE regulatory system in viruses isolated from five patients at two different time points in the course of infection. Variation of Rev/RRE function was observed between patients and within a patient as HIV disease progressed.[4]

The global HIV epidemic exhibits considerable genetic diversity and naturally occurring isolates can be categorized into groups and subtypes. Occasionally, viruses from different subgroups can recombine to create a fit hybrid which circulates in the population.[5] One such circulating recombinant form (CRF), known as CRF02_AG, has an RRE region contributed by parental subtype A and both exons of rev contributed by parental subtype G (Figure 1). In the present study, we investigate variations in Rev-RRE functional activity between HIV genotypes and the contributions of the Rev and the RRE to that variation.

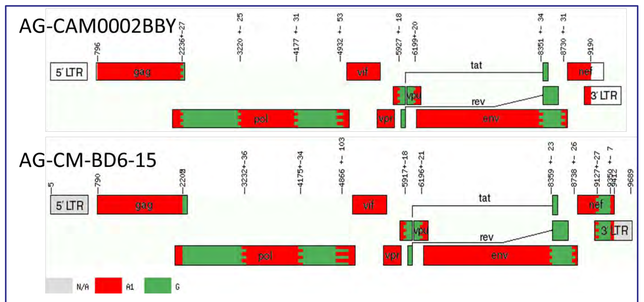


Figure 1. Genome map of two CRF02_AG recombinant HIV isolates. Image generated using jpHMM at GOBICS.[6]

METHODS

An assay was developed utilizing an HIV viral vector which relies on Rev-RRE for RNA export and packaging. The viral vector includes the HIV LTRs, an HIV packaging signal, and a hygromycin resistance cassette contained within an intron. Different RREs obtained from patient samples can be inserted into the vector. When the plasmid expressing the viral vector is transfected together with plasmids that supply Rev, envelope protein, GagPol, and Tat, virus-vector particles are produced. These virus-vector particles contain genomes that confer hygromycin resistance upon infection of target cells. Because the nuclear export of the hygromycin-resistance conferring viral vector RNA genome is dependent on the activity of the Rev-RRE system, the titer of infectious virus-vector produced in this way is a reflection of Rev-RRE function. To determine the titer, viral stocks obtained from the transient transfection system are serially diluted and used to infect target cells. Subsequent selection with hygromycin permits determination of viral titer by colony counting. Results for functional activity can be normalized to p24 production by the producer cells to account for variation in transfection efficiency. Assay results from different rounds can be compared by reference to the cognate pair activity of the laboratory strain NL4-3.

Eight HIV sequences were selected from the Los Alamos database representing two subtype A, two G and four CRF02_AG isolates.[7] Three of the CRF02_AG viruses were derived from Africa and one from Uzbekistan. Four subtype B patient isolates were also included as controls. Rev-RRE functional activity of the naturally occurring cognate pairs was determined as above. To evaluate the relative contributions of the Rev and RRE to cognate pair functional activity, artificial Rev-RRE pairs were created and assayed using one component from patient-derived virus and the other component from a reference. Both NL4-3 and one of the CRF02_AG viruses (6 Uzbek AG) were used as references for these artificial pairs. Artificial constructs were also created using both of the subtype G viruses for comparison with the respective cognate pairs. A linear regression was performed to evaluate the relationship between variation in cognate pair functional activity and variation in artificial pair activity using the patient-derived Rev or RRE component.

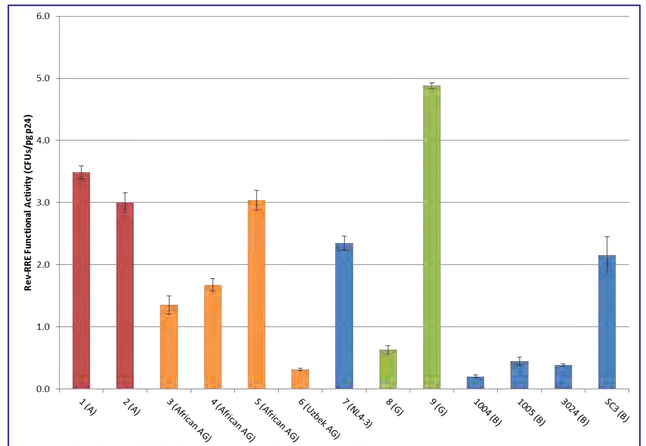


Figure 2. Rev-RRE Functional Activity of Selected Isolates. Functional activity measured by colony-forming units was normalized to p24 to account for transfection efficiency. Parentheses following virus number indicate subtype. NL4-3 is a subtype B laboratory strain.

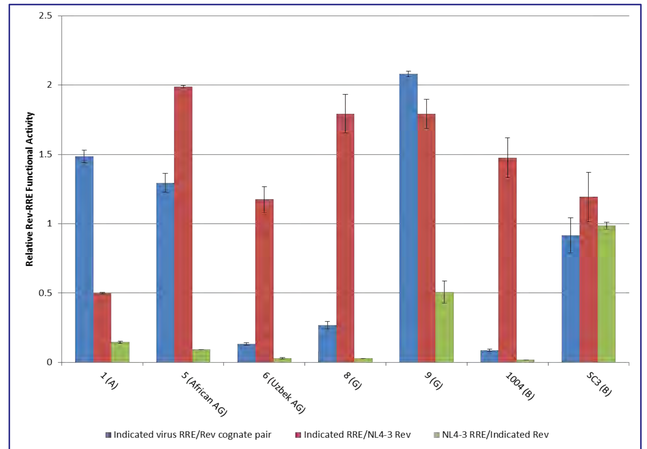


Figure 3. Relative functional activity of cognate Rev-RRE pairs compared with artificial pairs of patient-derived Rev or RRE with NL4-3 RRE or Rev. Functional activity is reported as multiples of NL4-3 cognate pair functional activity. Activity is normalized to p24 production to account for transfection efficiency. Parentheses following virus number indicate subtype.

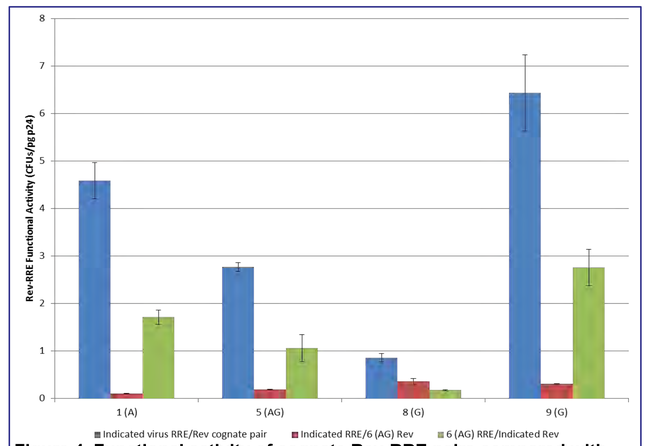


Figure 4. Functional activity of cognate Rev-RRE pairs compared with artificial pairs of patient-derived Rev or RRE with virus 6 (CRF02_AG) RRE or Rev. Functional activity is normalized to p24 production to account for transfection efficiency. Parentheses following virus number indicate subtype. As with other subtype AG virus, virus 6 (CRF02_AG) has an RRE sequence derived from parental subtype A and a Rev sequence derived from parental subtype G.

RESULTS

There was a 24-fold difference in functional activity between the most and least active naturally occurring Rev-RRE cognate pairs (Figure 2). There was no apparent clustering within subtypes. When selected Rev and RRE sequences were tested with NL4-3 RRE and Rev, the relative activity could not be predicted from the activity of the cognate pair (Figure 3). A similar result was found using the virus 6 (Uzbek AG) as a reference in place of NL4-3 (Figure 4). For the subtype G viruses, the cognate pair functional activity appears to track most closely with the Rev rather than the RRE component in the artificial pairs (Figure 5). In the linear regression analysis, there was low correlation of RRE activity but high correlation of Rev activity with the functional activity of the original cognate pair (Figure 6).

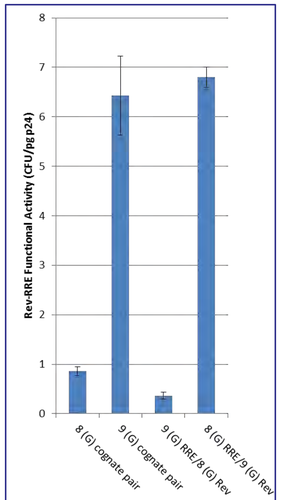


Figure 5. Functional activity of subtype G Rev-RRE cognate and artificial pairs. Functional activity is normalized to p24 production to account for transfection efficiency.

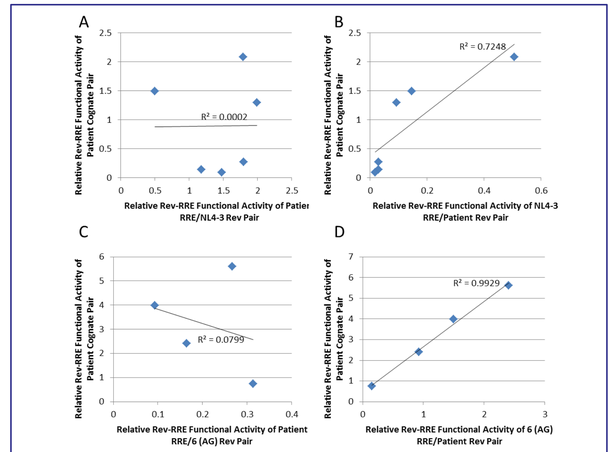


Figure 6. Linear Regression Analysis of Contributions of Rev and RRE to Cognate Pair Functional Activity Using Artificial Pairs. Figure A displays the relative Rev-RRE functional activity of the patient-derived cognate pair as a function of the relative functional activity of the patient RRE/NL4-3 Rev artificial pair. Figure B displays the relative Rev-RRE functional activity of the patient-derived cognate pair as a function of the relative functional activity of the NL4-3 RRE/patient Rev artificial pair. Figures C and D display the same relationships using the Rev and RRE from virus 6 (CRF02_AG), respectively, as a reference. Activity values are normalized to p24 and given as multiples of NL4-3 cognate pair activity. Virus SC3 was excluded from the analysis in A and B (see reference 3.) A linear regression for each figure was calculated and the relevant R2 value is displayed.

DISCUSSION

Rev/RRE functional activity varied dramatically between naturally occurring cognate pairs. This functional variation appeared to be primarily driven by differences in Rev. The clinical significance of this finding is still unclear, but our results demonstrate that there are clear differences in the Rev activity of circulating viruses. This may play a role in viral fitness and replication dynamics and be reflective of immune pressure. Identification of the most efficient Rev/RRE combination would also likely be of use in optimizing lentiviral vector systems for gene therapy.

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