Molecular Mechanism of Rev/Rex-Dependent *Trans*-Activation of Viral Genes

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Rev of human immunodeficiency virus 1 (HIV-1) and Rex of human T-cell leukemia virus type I (HTLV-I) are post-transcriptional *trans*-activator proteins of viral gene expression. Rev and Rex are localized in the nucleolus and bind specifically to the Rev responsive element (RRE) and Rex responsive element (RxRE) sequences in viral RNAs. Furthermore, the interactions of Rev/Rex proteins with cellular cofactors are essential for Rev/Rex-dependent *trans*-activation *in vivo*. By means of affinity chromatography and biospecific interaction analysis, we identified 38-kDa nucleolar shuttle protein B23 and 18-kDa proteins (eukaryotic initiation factor 5A and prothymosin α) as the major proteins binding to the nucleolar localization signal/RNA-binding domain and leucine-motif/activation domain of Rev/Rex, respectively. Recently, nucleopolin-like proteins, Rab and Rip1p were also identified as the Rev/Rex activation domain-binding proteins. The functional relationship and molecular interaction between Rev/Rex, their binding proteins, and RRE/RxRE-containing viral RNAs are discussed.

Keywords: Retrovirus / HIV-1 / HTLV-I / Gene expression / Posttranscriptional regulation / Nucleolar localization signal / Shuttle protein

Rev of human immunodeficiency virus type 1 (HIV-1) and Rex of human T-cell leukemia virus type I (HTLV-I) (1) are novel post-transcriptional trans-activators. They are known to bind to their specific targets on viral unspliced (*gag-pol*) and partially spliced (*env*) mRNAs to enable the expression of viral structural proteins by accumulating these mRNAs to cytoplasm, hence they are critically required for viral transcription followed by replication (1-6). Besides having such a functional similarity, it has also been reported that both proteins are phosphorylated and are localized predominantely in the nucleolus when they are expressed in eukaryotic cells (2). As shown in Figure 1, they each possess two distinct functional domains (4,6). One is nucleolar targeting signal (NOS)/RNA-binding domain. The other is the leucine-rich motif/activation domain.

The NOS/RNA-binding domain features a very long basic amino-acid stretch which is capable of forming an amphipathic α-helix structure. The NOS/RNA-binding domain was shown to bind directly to the nucleolar protein B23 and the stem-bulge structure in the Rev/Rex responsive element (RRE/RxRE) of viral mRNAs (4). B23 has been shown

RESEARCH FACILITY OF NUCLEIC ACIDS

Scope of Research

With emphasis on regulatory mechanisms of gene expression in higher organisms, the research activity has been focused on analyses of signal structures at the regulatory regions of transcriptional initiation and of molecular mechanisms involved in post-transcriptional modification by the use of eukaryotic systems appropriate for analysis. As of December 1994, studies are concentrated on the molecular mechanism of RNA editing in mitochondria of kinetoplastids and of post-transcriptional trans-activation of human retroviral genes.

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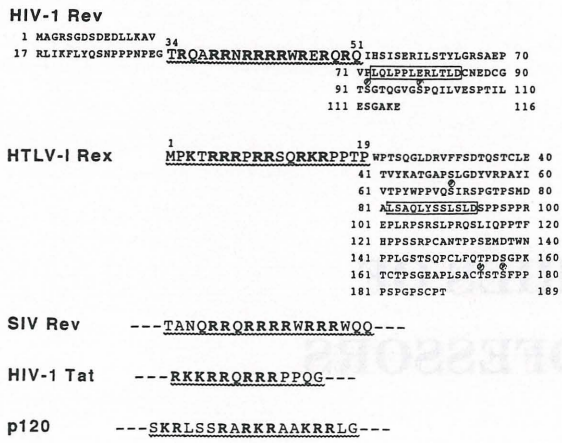

Figure 1. Molecular structure of HIV-1 Rev, HTLV-I Rex, and related proteins. The wavy underlines indicate a basic, arginine-rich core which is essential for both the nucleolar localization and sequence-specific binding to the RRE/RxRE RNAs. The open boxes indicate a leucine-rich motif/activation domain which promotes the cytoplasmic transport of RRE/RxRE RNAs by interaction with specific cofactors. The circled P means *in vivo* phosphorylation site.

to migrate between the nucleolus and cytoplasm, acting as a shuttle protein for the nucleocytoplasmic transport of ribosomal components across the nuclear membrane. Rev and Rex utilize this shuttle service to be imported into the nucleolus as the Rev-B23 and Rex-B23 complexes via the NOS/RNA-binding domain. The RRE/RxRE RNAs having higher affinities for Rev/Rex than B23 are interesting in view of *in vivo* function of these interactions. Our findings suggest that the nucleolar-imported complexes formed by Rev/Rex and B23 are specifically dissociated by RRE/RxRE RNAs. The Rev-RRE/Rex-RxRE complexes replaced via NOS/RNA-binding domain may then be ready for the nuclear export machinery (Figure 2).

Mutational analysis has revealed that the leucine-rich motif/activation domains are also important for the complete functions of Rev/Rex (3). Interestingly, peptide core motifs within these domains are functionally interchangable between Rev and Rex (1-4). These findings suggest that Rev and Rex should require common cellular cofactors involved in the cytoplasmic transport and/or accumulation of RRE/RxRE RNAs, which should interact through the activation domains. Recently, eukaryotic initiation factor 5A (eIF-5A), proththymosin α (ProTα) (6), nucleopolin-like proteins (Rab and Rip1p) were identified as the activation domain-binding proteins. These proteins are capable of binding to the activation domain when Rev and Rex assembled onto RRE/RxRE RNAs via NOS/RNA-binding domain and of significantly enhancing Rev/Rex-dependent *trans*-

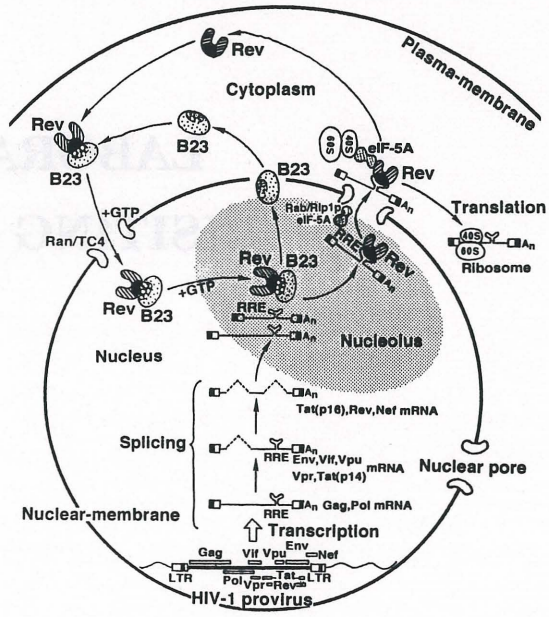

Figure 2. Possible mechanism of Rev-dependent *trans*-activation of HIV-1 genes.

activation of viral genes. It seems possible that the subsequent interaction of the Rev-RRE/Rex-RxRE complexes with eIF-5A, ProTα, Rab or Rip1p via the activation domain may induce the nuclear export of the complexes across the nuclear pores followed by the preferential accumulation and translation of RRE/RxRE RNAs (Figure 2).

References

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