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HIV-1 Rev can specifically interact with MMTV RNA and upregulate gene expression

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Abstract

We present evidence that the HIV-1 Rev protein can heterologously regulate expression of the simple beta retrovirus mouse mammary tumour virus (MMTV). Up to 10-fold upregulation was seen in a functional assay system when specific MMTV sequences were substituted for the HIV-1 Rev responsive element (RRE). RNA gel shift analysis showed that purified recombinant Rev could specifically bind to MMTV unique region 3 prime (U3) RNA and that these sequences could compete for wild-type Rev-RRE binding approximately 20-fold more efficiently than a non-specific competitor RNA. Using a combination of in silico and deletion mutation analyses, it was not possible to define any single specific secondary structure responsive to Rev, suggesting that a structure or combination of structures that only form in the context of the complete U3 transcript is/are required to interact with Rev. Taken together, these results suggest that HIV-1 Rev can directly bind to MMTV RNA as well as mediate upregulation of MMTV gene expression.

Keywords: Protein–RNA interaction; mRNA nuclear export; Luciferase

1. Introduction

Regulator of expression of the virion (Rev) is responsible for the nucleo-cytoplasmic shuttling of unspliced and singly spliced viral RNAs required for the expression of structural proteins and genomic RNA in certain members of the retroviridae. For human immunodeficiency virus type 1 (HIV-1), Rev achieves this by binding in a highly specific manner to an RNA secondary structure found in the envelope gene (*env*) intron, called the Rev responsive element (RRE) (Zapp and Green, 1989; Tiley et al., 1992). By binding to RRE, Rev counteracts multiple instability elements throughout the HIV genome in order to ensure the expression of RNAs that would otherwise be processed by the cellular splicing machinery. Subsequent interactions with a number of host proteins including eukaryotic initiation factor 5A (eIF-5A) (Bevec et al., 1996), chromosome region maintenance 1 (CRM1) (Askjaer et al., 1998), Src-associated in mitosis 68 kDa (Sam68) (Li et al., 2002a), the human nucleoporin-like protein hRIP/Rab (Bogerd et al., 1995; Fritz et al., 1995) and several other nuclear pore complex proteins (nups) such as nup153 (Ullman et al., 1999), nup98 and nup214 (Zolotukhin and Felber, 1999) have been shown to be necessary for Rev-mediated nuclear

Abbreviations: BSA, bovine serum albumin; DNase, desoxyribonuclease; bp, base pairs; *cat*, gene encoding chloramphenicol acetyl transferase; DTT, dithiothreitol; HIV, human immunodefiency virus; IPTG, isopropyl-beta-D-thiogaloactpyranoside; kDa, kilo Daltons; LTR, long terminal repeat; MCS, multiple cloning site; nt, nucleotide; ORF, open reading frame; PAGE, polyacrylamide gel electrophoresis; wt, wild-type.

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export. This route of viral mRNA export from the nucleus is known as the CRM1-dependent pathway.

It is not fully understood how all alpha, beta and gamma retroviruses achieve trafficking of genomic length and singly spliced mRNA out of the nucleus. However, the constitutive transport elements (CTE) of beta retroviruses such as Mason–Pfizer monkey virus (MPMV) (Bray et al., 1994), simian retrovirus type 1 (SRV-1) and type 2 (SRV-2) (Zolotukhin et al., 1994) have been well described as *cis*-acting RNA elements capable of acting independently of viral regulatory factors like Rev (Tabernero et al., 1996; Guan et al., 2001). CTE-mediated nuclear export of mRNAs can functionally replace the Rev/RRE control mechanism (Rizvi et al., 1996; Tabernero et al., 1996) and works by directly sequestering the host nuclear export receptor TAP/NXF1, a member of a family of highly conserved nuclear export factors (Gruter et al., 1998; Herold et al., 2000).

Previously, HIV-1 Rev was thought to be limited in its use of unrelated lentiviral RREs and RRE like elements, i.e., it could only cross-activate with HIV-2 and SIV (Felber and Pavlakis, 1993). More recently, however, Rev has been shown to *trans*-activate the Rec/cORF-responsive element (RcRE), an RRE functional homologue found in the 3′LTR of the human endogenous retrovirus, HERV-K (Magin-Lachmann et al., 2001). Interestingly, the RcRE is not related to the RRE either at the primary sequence or structure level and in contrast forms a complex, folded RNA structure within its responsive element. HERV-K is related at a nucleotide and amino acid level to beta retroviruses, especially mouse mammary tumour virus (MMTV) (Callahan et al., 1982; Westley and May, 1984). Little is, however, known about mechanisms that promote MMTV mRNA nuclear export.

During investigation of these mechanisms in MMTV, we tested whether the HIV-1 Rev protein could promote expression of transcripts carrying the 3′LTR region of the endogenous/exogenous provirus Mtv-2 and indeed could show that Rev can facilitate the expression of transcripts carrying MMTV sequences and directly bind to RNA from the U3 region. Although the biological significance of this is still unclear, these findings strongly suggest that a similar Rev-like factor may be encoded by MMTV.

2. Materials and methods

2.1. Plasmid constructs

As a parental construct for all luciferase reporter plasmids, pRSVenv was used. pRSVenv is based on the cloning vector pBR322 (bp 2469–4351, GenBank J01749) and contains the promoter region of the Rous Sarcoma Virus (RSV) Schmidt-Ruppin A strain 5′LTR (bp 2668–3189, GenBank L29199) followed by the MMTV *env* gene and 3′LTR. This MMTV *env*/LTR region was derived from the MMTV genomic clone, pGR102, and ligated into the RSV vector using *Hin*dIII/*Bss*HII restriction sites to create pRSVenv. pGR102 has been previously described (Salmons et al., 1985). To create pLucenv, the luciferase gene was removed from pXP1 (commercially available, ATCC 37576) using *Hin*dIII and *Dra*I and inserted into pRSVenv in between the RSV promoter and the MMTV *env* gene using *Hin*dIII and *Msc*I. Long-template PCR was performed on pLucenv to remove a strong splice donor which existed in a non-coding region between the luciferase and the MMTV *env* gene in order to confer full responsiveness of luciferase activity to Rev. The sense primer env4674 (GCGACTAGTACGAGGCTATGCTTGTGTT) and the antisense primer env4269c (CGCACTAGTATCGCTGAATACAGTTACAT) both contain the *Spe*I restriction sites for religation of the PCR fragment to create pLucenv.dsd. Any remaining splice sites were predicted to be sub-optimal in silico and all luciferase reporter constructs generated from pLucenv.dsd contain this modification. pLucHenv was generated by removing the MMTV *env* gene from pLucenv.dsd with *Bgl*II and *Spe*I and replacing it with the *Bbs*I/*Bsr*FI HIV *env* gene fragment from the HIV *env* expression construct pRCMV-Henv. pRCMV-Henv was derived from pBH10R3 (Roy et al., 1989). To create pLHenv.pA, the *Nco*I/*Xmn*I MMTV LTR fragment was removed from pLucHenv and swapped for the *Nco*I/*Pvu*II flanked Simian Virus 40 polyadenylation sequence (SV40 pA), from pRCMV-Henv. For pLucHenv and pLHenv.pA, the HIV-1 *env* and *vpu* open reading frames are present downstream of the luciferase gene but not the major splice acceptor for *env*. The complete wild-type HIV-1 RRE sequence is therefore present within the *env* gene in pLucHenv and pLHenv.pA. The MMTV LTR construct, pRLucO, was made by removing MMTV *env* gene from pRSVenv with *Hin*dIII and *Ava*I, and replacing it with the *Hin*dIII/*Dra*I luciferase gene from pXP1. The natural splice acceptor for ORF is not present in this construct. The RSV driven luciferase expression control plasmid, pRLS, was derived from pLHenv.pA by removing the HIV *env* gene with *Nco*I/*Spe*I and religating. Further control plasmids pRSVLuc, pActinLuc and pHIVLuc have been previously described (Wintersperger et al., 1995). The HIV-1 Rev expression construct, pCMVsRevBF, was derived from the plasmid pCMVsrev (a kind gift from Barbara Felber); the cloning of which has been previously described (Schwartz et al., 1990). For production of HIV-1 RRE RNA, the RRE sequence was generated by PCR using pRCMV-Henv as template (sense primer with *Hin*dIII restriction site, GATCGCAAGCTTCAGTGGGAATAGGAGCTTTG; antisense primer with *Bam*HI, GATCGCGGATCCAGGAGCTGTTGATCCTTTAG). The *Bam*HI/*Hin*dIII RRE PCR fragment was inserted into the multiple cloning site of the T7 in vitro transcription vector, pTZ19R in an orientation-dependent manner. pTZ19R is a commercially available in vitro transcription vector (Fermentas) and has been previously described (Mead et al., 1986). For the expression of MMTV U3 RNA, several constructs were generated based on the pTZ19R vector by