Virology 462-463 (2014) 126–134

Contents lists available at ScienceDirect

Virology

journal homepage: www.elsevier.com/locate/yviro

RRE-dependent HIV-1 Env RNA effects on Gag protein expression, assembly and release

Claudia S. López [a,*], Rachel Sloan [a], Isabel Cylinder [a], Susan L. Kozak [b], David Kabat [b], Eric Barklis [a,*]

[a] Departments of Molecular Microbiology and Immunology, Oregon Health & Sciences University, 3181 SW Sam Jackson Park Road, Portland, OR 97239, United States
[b] Biochemistry and Molecular Biology, Oregon Health & Sciences University, 3181 SW Sam Jackson Park Road, Portland, OR 97239, United States

ARTICLE INFO

Article history:
Received 27 March 2014
Returned to author for revisions
16 April 2014
Accepted 17 May 2014

Keywords:
HIV-1
Env
Gag
RNA export
RRE

ABSTRACT

The HIV-1 Gag proteins are translated from the full-length HIV-1 viral RNA (vRNA), whereas the envelope (Env) protein is translated from incompletely spliced Env mRNAs. Nuclear export of vRNAs and Env mRNAs is mediated by the Rev accessory protein which binds to the rev-responsive element (RRE) present on these RNAs. Evidence has shown there is a direct or indirect interaction between the Gag protein, and the cytoplasmic tail (CT) of the Env protein. Our current work shows that *env* gene expression impacts HIV-1 Gag expression and function in two ways. At the protein level, full-length Env expression altered Gag protein expression, while Env CT-deletion proteins did not. At the RNA level, RRE-containing Env mRNA expression reduced Gag expression, processing, and virus particle release from cells. Our results support models in which Gag is influenced by the Env CT, and Env mRNAs compete with vRNAs for nuclear export.

Introduction

The HIV-1 envelope (Env) protein is encoded by the 3′ end of the HIV-1 viral RNA (vRNA) genome in a region that overlaps the Vpu open reading frame (ORF), and the Tat and Rev ORFs (Adachi et al., 1986; Li et al., 1992). Also encoded in this region is the rev responsive element (RRE) that binds to Rev (Adachi et al., 1986; Li et al., 1992). Ordinarily, the nuclear export signal (NES) of Rev binds to a complex of the cellular proteins Crm1(exportin-1, XPO-1) and Ran-GTP to facilitate the nuclear export of the HIV-1 vRNA that encodes the Gag and GagPol proteins, as well as other incompletely spliced HIV-1 RNAs (Fischer et al., 1994, 1995, 1999; Henderson and Percipalle, 1997; Yi et al., 2002; Swanson et al., 2004, 2010; Perales et al., 2005; Cullen, 2005; Groom et al., 2009; Sherer et al., 2011; Elinav et al., 2012). It is noteworthy that this exit pathway differs from that of most spliced mRNAs, which employ cellular Tap and Nxt proteins to mediate nuclear exit (Cullen, 2005). Interestingly, the Mason-Pfizer monkey virus (MPMV), a D-type retrovirus, does not encode a Rev protein, but uses a cis-active constitutive transport element (CTE) which binds Tap (NFX1) to foster the nuclear export of incompletely spliced MPMV RNAs (Pasquinelli et al., 1997; Wodrich et al., 2000; Swanson et al., 2004; Cullen, 2005).

The HIV-1 Env protein is translated from incompletely spliced 4 kb viral mRNAs to yield a glycosylated 160 kDa polyprotein (gp160) that is routed to the plasma membranes (PM) of infected cells via the vesicular transport pathway (Sodroski et al., 1986; Checkley et al., 2011). During its transit, gp160 is cleaved by furin-like proteases to yield the Env surface (SU, gp120) and transmembrane (TM, gp41) proteins, which remain associated, and assemble into trimer units (Welman et al., 2007; Checkley et al., 2011). At the PM, Env proteins may be endocytosed, by virtue of internalization motifs on the TM cytoplasmic tail (CT; Lodge et al., 1994, 1997; Egan et al., 1996; Boge et al., 1998; Courageot et al., 1999; Chan and Chen, 2006). Alternatively, Env proteins may be incorporated into assembling virus particles. Evidence suggests that assembly of wild type (WT) Env proteins into virus particles is dependent on an interaction between the Env CT and the matrix (MA) domain of the precursor Gag (PrGag) protein, but it is unclear whether this interaction is direct or indirect (Gabuzda et al., 1992; Wang et al., 1993; Yu et al., 1993; Freed and Martin, 1995a, 1995b, 1996; Mammano et al., 1995; Murakami and Freed, 2000a, 2000b; Wyma et al., 2000; Santos da Silva et al., 2013). For HIV-1 Env proteins that carry CT deletions (ΔCT), the scenerio is different. Such ΔCT proteins do not require an interaction with MA for virion incorporation (Gabuzda et al., 1992; Wang et al., 1993; Yu et al., 1993; Freed and Martin, 1995a, 1995b, 1996; Mammano et al., 1995). These observations support a model in which the long HIV-1 Env CT interferes with free diffusion into assembly sites, making it so that ΔCT proteins are passively incorporated into virions, whereas

* Corresponding authors. Tel.: +1 503 494 8098.
E-mail addresses: lopezcl@ohsu.edu (C.S. López), barklis@ohsu.edu (E. Barklis).

http://dx.doi.org/10.1016/j.virol.2014.05.019
0042-6822/

WT proteins require the CT–MA interaction (Gabuzda et al., 1992; Wang et al., 1993; Yu et al., 1993; Freed and Martin, 1995a, 1995b, 1996; Mammano et al., 1995; Murakami and Freed, 2000a, 2000b; Wyma et al., 2000; Muranyi et al., 2013; Santos da Silva et al., 2013).

During our studies on HIV-1 assembly, we observed that expression of HIV-1 Env proteins and RNAs exerted variable effects on Gag protein expression, assembly, and release. In particular, we observed a protein-mediated effect, in which WT Env protein expression reduced Gag expression and virus particle release, while ΔCT Env protein expression did not. A second effect was exerted at the RNA level. Specifically, we observed that expression of Env-encoding RNAs reduced Gag expression, processing, and release, irrespective of whether the Env protein itself was expressed. The RNA effect mapped to the RRE, and Gag proteins that were translated from RNAs that used the Tap-mediated nuclear exit pathway were unaffected. Overall our results support a model for a CT–MA interaction, and demonstrate the possibility of inhibiting HIV-1 replication via competition for RNA nuclear export.

Results

Effects of env on HIV-1 Gag

To examine interactions between the HIV-1 structural Gag proteins and the membrane-associated Env proteins, we initially utilized Gag expression vectors HIV-Luc and psPAX2 (Fig. 1; Connor et al., 1995; Zufferey et al., 1997). The former vector employs HIV-1 (Fig. 1) control elements, and therefore expression is controlled via the promoter in the long terminal repeat (LTR), and the products of the viral *tat* and *rev* genes. It is noteworthy that the *env* gene in this vector is non-functional due to both a frameshift and an insertion of the firefly luciferase (*luc*) gene (De Wet et al., 1986). In contrast to HIV-Luc, with psPAX2, the Gag and GagPol proteins are transcribed from a spliced mRNA expressed from a cytomegalovirus/chicken β actin (CAG) promoter, and most of the *env* gene is deleted (Zufferey et al., 1997). To complement Gag expression vectors, the HIV-1 Env protein was expressed from SIVIIIEnv (Sullivan et al., 1995) and SIVIIIΔCT, which respectively

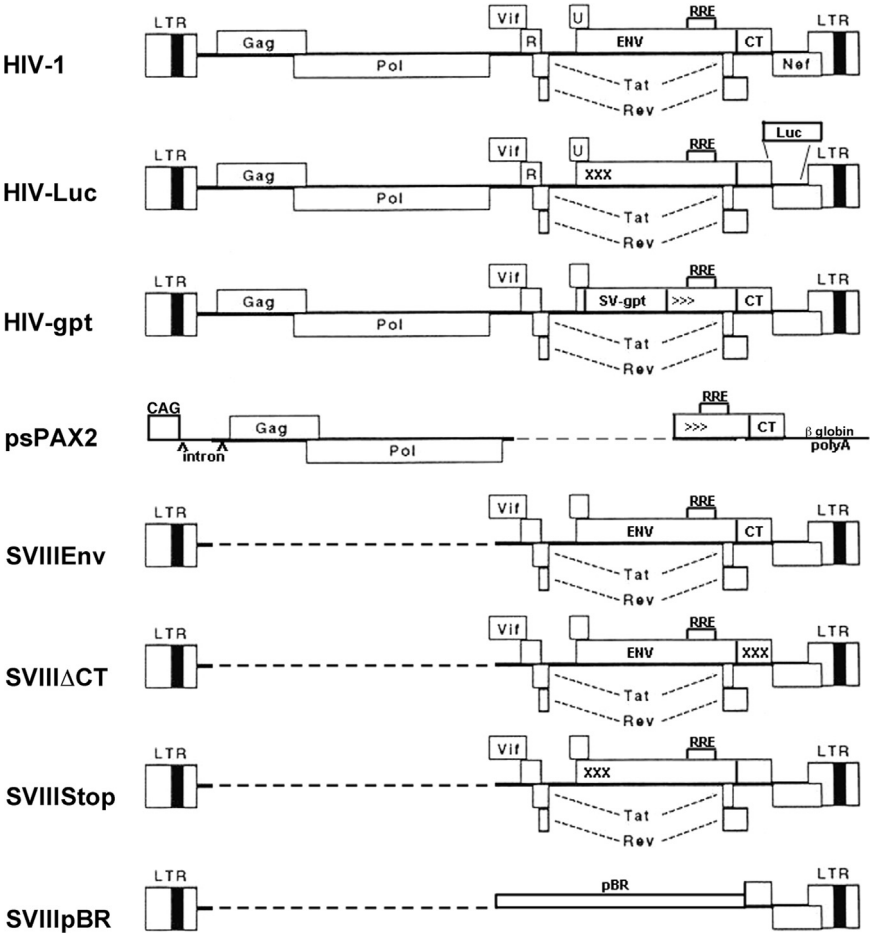

Fig. 1. Recombinant DNA constructs. HIV-1: shown is a map of the HIV-1 strain NL4-3 provirus with long terminal repeat (LTR) units, and the Gag, Pol Vif, Vpr (R), Vpu (U), Env, Nef, Tat and Rev open reading frames as indicated. Also depicted is the coding region for the Env cytoplasmic tail (CT), and the Rev responsive element (RRE). **HIV-Luc:** the HIV-Luc provirus is based on HIV-1 NL4-3, expresses Gag, Pol, Vif, Vpr, Vpu, Tat and Rev open reading frames, and carries the RRE element. It encodes the firefly luciferase gene (Luc) between NL4-3 nt 8201 of *env* and nt 8443 of *nef*. It is *env*- due to a frameshift at the 5′ end of *env* (nt 5950). **HIV-gpt:** the HIV-gpt provirus is based on the HIV-1 proviral clone HXB2, which is *vpr*-, *vpu*- and *nef*-. The HIV-gpt provirus bears a 1.2 kb deletion of the *env* gene (nt 6402–7620), which was replaced by a 1.1 kb fragment from pSV2gpt, encoding an SV40 promoter and origin of replication and the *E. coli* xanthine–guanine phosphoribosyl transferase (*gpt*) gene. **psPAX2:** psPAX2 expresses codon-optimized *gag*, and *pol* genes from spliced mRNAs driven by the a recombinant CMV enhancer/chicken β actin (CAG) promoter and employing the rabbit β globin polyadenylation signal (polyA). It carries the RRE and a fragment of the *env* gene, but does not express the Env protein. **SVIIIEnv:** this HXB2 proviral derivative carries the HIV-1 5′LTR, and encodes HXB2 sequences from an artificial SalI site at nt 5496 through the 3′ HXB2 LTR. The vector encodes *vif*, *tat*, *rev* and *env*, and carries an RRE. **SVIIIΔCT:** SVIIIΔCT is based on SVIIIEnv, but harbors a deletion of the Env CT, by virtue of two inserted termination codons. **SVIIIStop:** SVIIIStop is based on SVIIIEnv, but encodes a termination codon that truncates the Env protein after 63 codons. **SVIIIpBR:** the SVIIIpBR plasmid was created by replacing the HXB2 nt 5496–8470 in SVIIIEnv with a fragment from the bacterial plasmid pBR322. It does not carry the RRE and it expresses no HIV-1 genes.