Virology 385 (2009) 93–104

Contents lists available at ScienceDirect

Virology

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

Mapping of determinants involved in the stimulation of HIV-1 expression by Sam68

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ARTICLE INFO

Article history:
Received 23 July 2008
Returned to author for revision
15 August 2008
Accepted 27 October 2008
Available online 16 December 2008

Keywords:
Sam68
HIV-1
Rev
Polyadenylation

ABSTRACT

Control of HIV-1 RNA processing is central to the replication of the virus. Previously, we demonstrated that the cellular protein Sam68 enhances HIV-1 structural protein expression and RNA 3′ end processing. In this report, we show that Sam68 interacts with unspliced HIV-1 RNA and that other members of the STAR/GSG protein family also promote viral RNA 3′ end processing. We define a portion of the GSG domain (Sam 97-255) as sufficient for enhancement of Rev-dependent expression. In contrast to Sam68, Sam 97-255 increases unspliced RNA processing only in the presence of Rev in 293T cells. In a different cell line, Sam 97-255 enhances HIV-1 gene expression without enhancing RNA 3′ end processing, suggesting that stimulation of 3′ end processing is not required for enhancement of HIV-1 gene expression. Overall, these results indicate that Sam68 and the mutants described affect the composition of the viral RNP to enhance viral protein synthesis.

Introduction

In HIV-1, all viral proteins must be expressed from a relatively small genome. To accomplish this feat, HIV-1 undergoes a number of alternative splicing events to produce over 30 RNAs from a single transcript (Cochrane et al., 2006; Stoltzfus and Madsen, 2006). The resulting mRNAs are grouped by size: the unspliced 9 kb encoding Gag and GagPol; the singly spliced 4 kb encoding Env, Vif, Vpr, or Vpu; and the multiply spliced 2 kb encoding Tat, Rev or Nef. Early during infection, the unspliced and singly spliced RNAs are restricted to the nucleus while the multiply spliced RNAs are exported to the cytoplasm. Once sufficient amounts of Rev are produced, it mediates the Crm1-dependent export of the incompletely spliced viral RNAs (Hope, 1999; Pollard and Malim, 1998).

Several host proteins have been shown to affect unspliced HIV-1 RNA export and expression (Fang et al., 2004; Fritz et al., 1995; Li et al., 2002a, 2002b; Luo et al., 1994; Modem et al., 2005; Pongoski et al., 2002; Sanchez-Velas et al., 2004; Yedavalli et al., 2004). One of these, Sam68 (Src-*a*ssociated during *m*itosis of 68kD), has been implicated in the regulation of Rev-dependent export (Li et al., 2002a; Modem et al., 2005) and cytoplasmic utilization of unspliced viral RNAs (Coyle et al., 2003; McLaren et al., 2004). Sam68 contains a KH RNA binding motif embedded within a larger region of homology termed the GSG (GRP33, Sam68, Gld1) domain (Lukong and Richard, 2003). Along with RNA binding (Chen et al., 1997; Chen and Richard, 1998; Lin et al., 1997), the GSG domain is required for multimerization of Sam68 with itself and other members of the STAR (*s*ignal *t*ransduction and *a*ctivation of *R*NA) protein family (Chen et al., 1997; Chen and Richard, 1998; Di Fruscio et al., 1999; Wu et al., 1999; Zorn and Krieg, 1997). RG-rich regions required for non-specific RNA binding (Chen et al., 1997) and proline-rich signaling motifs are found elsewhere in the protein. We have previously shown that overexpression of Sam68 enhances unspliced HIV-1 3′ end processing in the presence and absence of Rev (McLaren et al., 2004). However, in our hands, Sam68 did not greatly enhance export of the unspliced viral RNA (McLaren et al., 2004).

To better understand the properties that contribute to the enhancement of HIV-1 RNA processing and gene expression by Sam68, we examined the factors, both *cis* and *trans*, required for Sam68′s ability to modulate viral RNA processing and translation. In this report, we demonstrate that endogenous Sam68 is associated with viral RNA and that other members of the STAR protein family share the ability to promote HIV-1 RNA 3′ end processing. We subsequently show that a region encompassing the GSG domain (Sam 97-255) is sufficient to enhance HIV-1 structural gene expression in two cell lines. Interestingly, in 293T cells, Sam 97-255 and the RNA binding mutant Sam G178D enhance 3′ end cleavage of unspliced *env* RNA but only in the presence of Rev. In contrast, in 293 cells, these Sam68 mutants had no effect on viral RNA processing even though they enhanced HIV-1 gene expression. These results suggest that enhanced 3′ end cleavage of HIV-1 RNAs does not necessarily correlate with effects on HIV-1 protein synthesis. From this work, it appears that Rev, bound to the RRE, is able to recruit factors in addition to Crm1 to modulate viral RNA processing. In terms of Sam 97-255 and Sam G178D, these mutants likely impact viral RNA processing at multiple levels, both in the nucleus and cytoplasm, to promote HIV-1 gene expression.

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0042-6822/$ – see front matter © 2008 Elsevier Inc. All rights reserved.
doi:10.1016/j.virol.2008.10.049

Results

Endogenous Sam68 preferentially binds unspliced HIV-1 RNA

The ability of Sam68 to promote 3′ end processing of incompletely spliced HIV-1 RNA might be accomplished by either direct interaction with the affected RNA or modulation of host factor activity and/or expression. The former hypothesis is supported by the observation that mutational inactivation of the RNA binding activity of Sam68 (the mutant Sam G178D) results in loss of the capacity to enhance unspliced viral RNA 3′ end processing (McLaren et al., 2004). The Sam G178D mutant retains the capacity to multimerize with itself and wild type Sam68 (Chen et al., 1997). In a further attempt to discriminate between these hypotheses, we tested which RNA species endogenous Sam68 preferentially binds to (unspliced or spliced pgTat RNA). To this end, 293 cells were transfected with the HIV-1 *env* expression plasmid, pgTat, (Fig. 1) and harvested 48 h post-transfection. Lysates were diluted, precleared and incubated with antibody against Sam68 or rabbit IgG (rIgG). After immunoprecipitation, western blotting was carried out and showed that the majority of endogenous Sam68 was

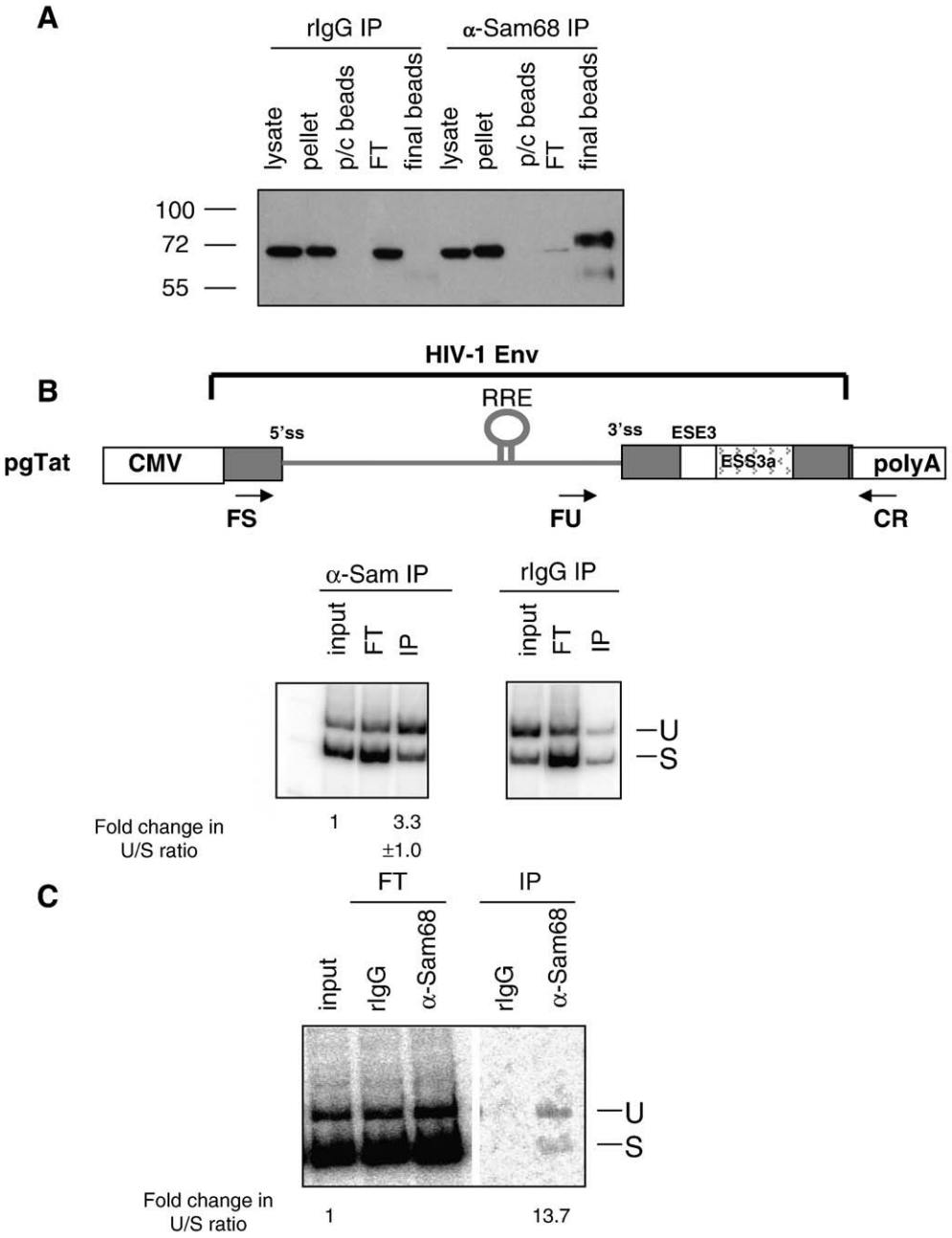

Fig. 1. Endogenous Sam68 preferentially interacts with unspliced HIV-1 RNA. 293 cells were transfected with the HIV-1 *env* expression plasmid pgTat and RNPs harvested 48 h later as detailed in "Materials and methods". RNPs were precipitated with anti-Sam68 antibody or nonspecific rabbit IgG (rIgG) and immune complexes captured with protein G sepharose beads. (A) Fractions of the reactions were reserved, run on 10% SDS-PAGE and blotted with anti-Sam68 antibody to analyze the efficiency of immunoprecipitation. Fractions tested were: lysate (cleared cell lysate), pellet (cell lysate pellet), p/c beads (preclear beads), FT (flowthrough), final beads (immunoprecipitate beads, IP). (B, C) RNA immunoprecipitations were carried out as described above and beads washed with (B) NT2 (50 mM Tris-HCl pH 7.5, 150 mM NaCl, 1 mM MgCl2, 0.05% NP40, 100 U/ml RNase OUT (Invitrogen), 2 mM DTT, 1X Complete mini EDTA-free protease tablet (Roche)) or (C) a more stringent buffer (75 mM NaCl, 0.25% deoxycholate, 0.5% Triton X-100, 0.05% SDS, 1 M urea, 100 U/ml RNase OUT (Invitrogen), 2 mM DTT, 1X Complete mini EDTA-free protease tablet (Roche)). RNA was harvested from lysate, FT and IP fractions and RT-PCR performed to amplify spliced (S) and unspliced (U) pgTat cDNAs. Shown at the top is a schematic of pgTat indicating the positions of the primers (FS, FU, CR) used in the RT-PCR analysis.