Characterization of the Interaction of Staufen-1 with HIV-1 Rev and HERV-K(HML-2) Rec

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Abstract

BACKGROUND: The Rev protein of HIV-1 is an RNA transport factor that enhances nuclear export of intron-containing retroviral transcripts and appears to be critical for various aspects of subsequent RNA utilization pathways. The cellular protein Staufen-1 is in several ways functionally related to retroviral RNA transport proteins. It shuttles RNA out of the nucleus and has previously been shown to become incorporated into HIV-1 particles by direct binding to the genomic RNA and to interact with the Gag protein of HIV-1, promoting Gag oligomerization and RNA encapsidation. Moreover, we have recently demonstrated that it interacts with Rec and Gag proteins of HERV-K(HML-2).

METHODS: Using pull-down, coimmunipreciptation (CoIP) and flow cytometry-based FRET experiments along with deletion mutants we have mapped the interaction sites of Staufen-1 with Rev and Rec. The effect of Staufen-1 on nucleocytoplasmic export and translation was determined with reporter constructs in HEK 293T cells. Stress granules were induced with arsenite and the localization of the proteins in granules was investigated by immunofluorescence.

RESULTS: Having demonstrated a direct interaction between Staufen-1 and HIV-1 Rev we found the NLS of Rev to be the region required for binding. The RBD3 domain of Staufen-1 is the main mediator of the interaction with Rev. This is in contrast to the situation with Rec, where binding is mediated primarily through the RBD4 domain. While Staufen-1 strongly enhances nucleocytoplasmic export and/or translation of unspliced HERV-K(HML-2) transcripts in the presence of Rec, this appears not to be the case for unspliced HIV-1 transcripts in the presence of Rev. Moreover, we demonstrate that under cellular stress conditions, Rec and Rev associate in Staufen-1-positive stress granules. However, Staufen-1 appears to attract only Rec, but not Rev, into stress granules.

CONCLUSIONS: Although Staufen-1 interacts with both HIV-Rev and HERV-K(HML-2) Rec, only the nucleocytoplasmic transport and translation of HERV-K(HML-2) is significantly upregulated by Staufen-1. Our data also indicate that Rev and Rec are recruited into stress granules by different mechanisms.

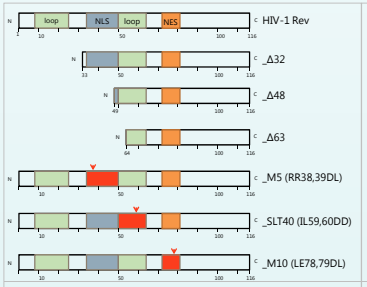


Fig. 1: HIV-1 Rev mutant constructs. Red arrows indicate amino acid substitutions.

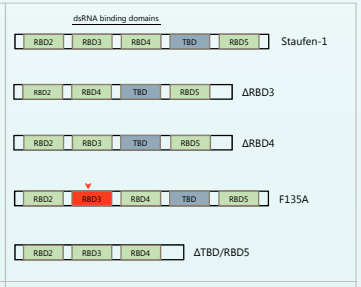


Fig. 2: Human Staufen-1 mutant constructs. The red arrow indicates the amino acid substitution.

Introduction

HIV Rev and HERV-K(HML-2) Rec are homologous regulatory proteins that facilitate nucleo-cytoplasmic export of incompletely spliced retroviral transcripts by binding to highly structured *cis*-acting stem-loop regions, called the Rev-responsive element (RRE) and Rec-responsive element (RcRE), respectively. We recently demonstrated that the human dsRNA-binding protein Staufen-1 interacts with Rec and associates with Rec in stress granules. It has also been demonstrated that Staufen-1 binds to the Gag proteins of HIV-1 and HERV-K. Nevertheless, the role played by Staufen-1 in the retroviral replication cycle remained largely unknown.

Methods

Pull-down experiments were done with recombinant His- and V5-tagged Staufen-1. For coimmunipreciptation, cells were transfected with pcDNA3.1-Rec-V5 or pcDNA3.1-Rev-V5 and pCMV-Staufen-1-FLAG. Rev or Rec were bound to V5-tagged agarose beads and analyzed by Western blot. Flow cytometry-based FRET (FACS-FRET) experiments were performed by transfection of cells with Rev-EYFP and ECFP-Staufen-1 fusion proteins (Banning *et al.*, PLoS ONE, 2010). Cell stress was induced by treatment with 0.5 mM sodium arsenite for 1 h. RNA-transport was measured using shuttle constructs (Wolff et al., PMC 2013 and Hanke et al. , J. Virol. 2013).

Results

Coimmunoprecipitation of overexpressed Staufen-1 (Fig. 3, A) and endogenous Staufen-1 (data not shown) with HIV-1 coRev-V5 (co=codon optimized) mutants revealed no interaction for all NLS-deficient coRev proteins. This could be confirmed in FACS-FRET experiments with Rev-EYFP and ECFP-Staufen-1, where a FRET signal could only be detected for NLS-functional Rev (Fig. 3, B). Prior to CoIP experiments, pull-downs with recombinant Staufen-1 and lysates from Rev-expressing cells yielded the same results (data not shown). Coprecipitation experiments using mutant Staufen-1 proteins demonstrated that neither the RBD3-deficient Staufen-1 protein (ΔRBD3) nor the F135A mutant interacted with coRev-V5 (Fig. 4, A).

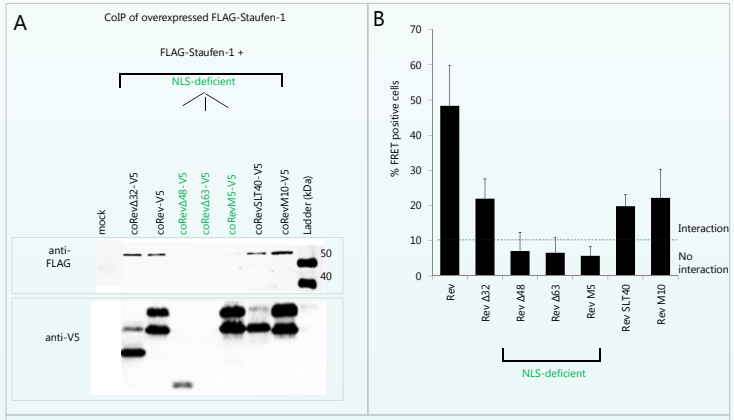


Fig. 3: (A) CoIP of overexpressed Staufen-1 using HIV-1 coRev-V5 bound to anti-V5 agarose. Loss of the NLS region prevents binding of Staufen-1 protein to Rev. (B) This could be confirmed by FACS-FRET, where a FRET signal could not be detected for NLS-deficient Rev mutants.

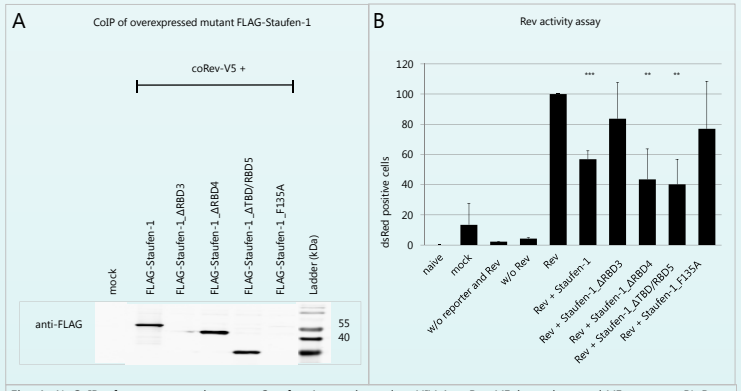


Fig. 4: A) CoIP of overexpressed mutant Staufen-1 proteins using HIV-1 coRev-V5 bound to anti-V5 agarose. B) Rev-dependent export of RRE-containing RNA is inhibited through Staufen-1 overexpression. Loss of a functional RBD3 region (ΔRBD3, F135A) obliterates the negative effect of Staufen-1.

Applying the Rev activity assay to coRev and Staufen-1, we found an inhibitory effect of Staufen-1 on coRev-dependent nucleocytoplasmic export of RRE-containing RNA. The inhibition was abrogated with the loss of a functional RBD3 domain in Staufen-1 (Fig. 4, B), which is in line with the CoIP experiments.

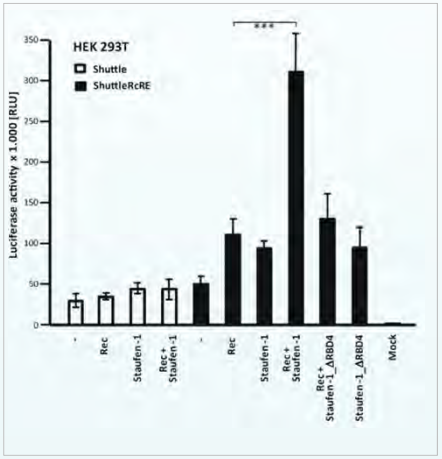


Fig. 5: Nucleocytoplasmic shuttle assay. Overexpression of Staufen-1 strongly enhances Rec-dependent export of RcRe-containing RNA, while deletion of RBD4 reverses the effect.

The situation with Rec was different, where the shuttle assay demonstrated an increased export of the vRNA by overexpression of Staufen-1. This was impaired by deletion of the RBD4 domain of Staufen-1 (Fig. 5).
Cellular Staufen-1 was previously shown to contribute to the transport of Rec and its viral HERV-K(HML-2) transcripts into stress granules (Hanke *et al.*, J Virol, 2013). Here we demonstrate that localization of Rec into stress granules in the presence of viral RcRE-containing RNA is promoted through Staufen-1 (Fig. 6). However, transport of Rev into stress granules does not seem to be influenced by Staufen-1 (Fig. 7).

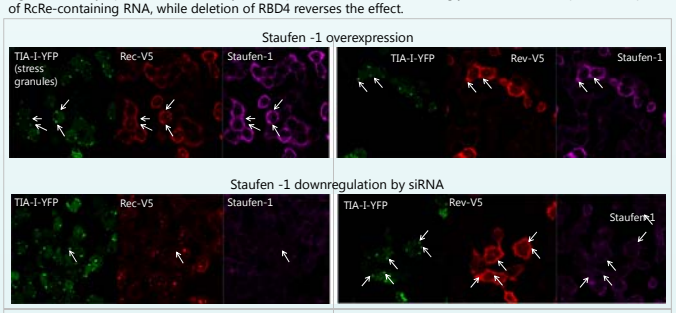


Fig. 6: Upper panel: Localization of Rec (red) into TIA-I-marked stress granules (green) upon Staufen-1 (violet) overexpression. Lower panel: Down-regulation of Staufen-1 expression by siRNA treatment results in a decrease of Rec protein in stress granules.

Fig. 7: Staufen-1 overexpression (upper panel). Lower panel: Downregulation of Staufen-1 through siRNA did not alter the amount of Rev protein (red) within TIA-I-marked (green) stress granules.

Conclusions

The functionally related proteins Rev and Rec are interaction partners of the human dsRNA-binding protein Staufen-1. We demonstrate here that the interaction of Staufen-1 with Rev is mediated through the NLS region of Rev and presumably the RBD3-domain of Staufen-1. The RBD4 domain appears to be mandatory for binding of Staufen-1 to Rec.
The interaction with Staufen-1 impacts RNA transport of HIV-1 and HERV-K(HML-2) differently. While Rev-dependent RNA export is reduced by Staufen-1, the activity of Rec is promoted. Staufen-1 is thought to participate in the transport of the viral protein Rec into stress granules. However, localization of Rev into stress granules does not appear to be mediated by Staufen-1.

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