HIV-1 Rev hijacks the host membrane trafficking protein PACS-1 to facilitate efficient viral protein-RNA complex localization during replication

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Introduction:

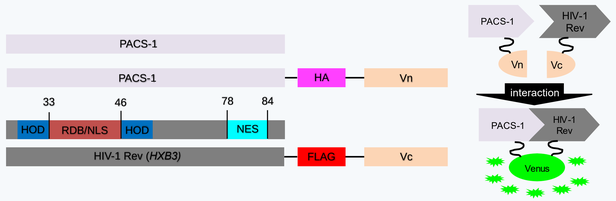
- •The HIV-1 Rev protein is required for translocating genomic HIV-1 RNA from the nucleus to the cytoplasm
- •PACS-1 is a novel host subcellular trafficking protein that interacts with Rev in vitro.
- •PACS-1 knockdown decreases HIV-1 Gag intracellular expression and its release into extracellular space.
- •Cytoplasmic Rev has been proposed to aid viral translation and assembly; however, the functional role of its interaction with PACS-1 remains elusive.

Objectives:

1. **Visualize the localization of the interaction between PACS-1 and HIV-1 Rev *in vitro*.**
2. **Determine how well-characterized HIV-1 Rev mutants affect the localization of the PACS-1:Rev complex.**
3. **Investigate the localization of HIV-1 Rev with HIV-1 nucleocapsid protein Gag during HIV-1 infection.**

Methods:

Bimolecular Fluorescent Complementation (BiFC)

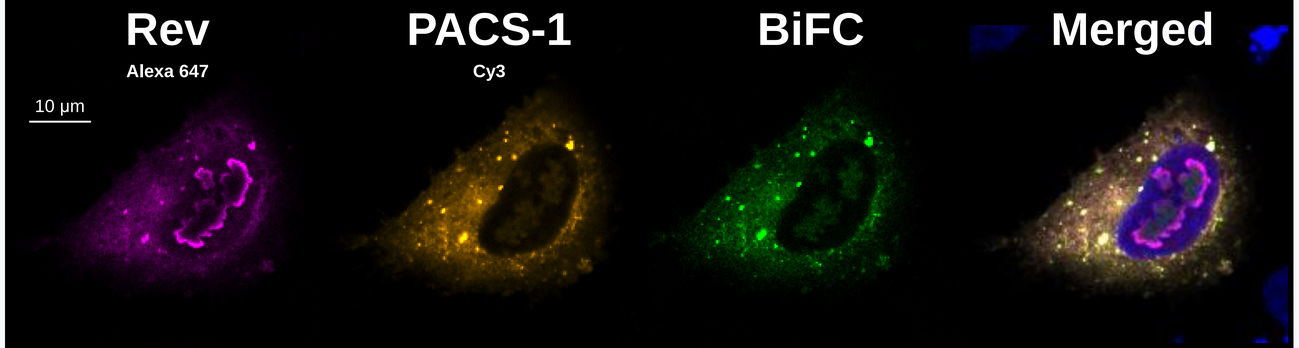


Confocal Microscopy

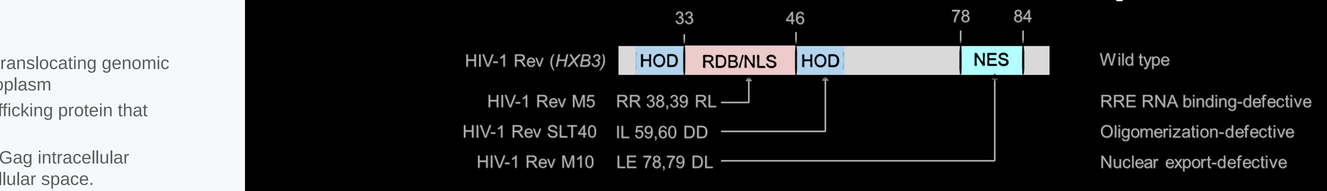
Co-localization of Fluorescent Signals

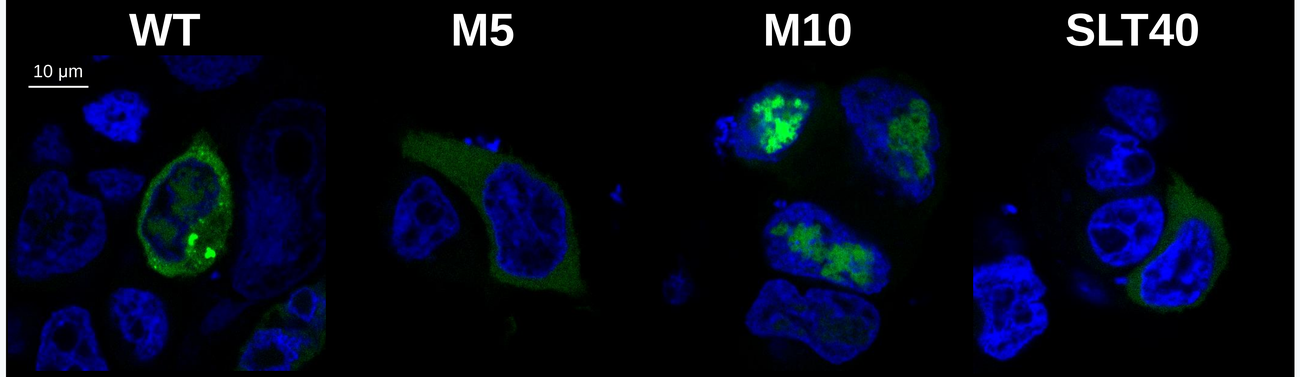
Pearson's Correlation Coefficient

The PACS-1 and Rev interaction can be visualized in cells.

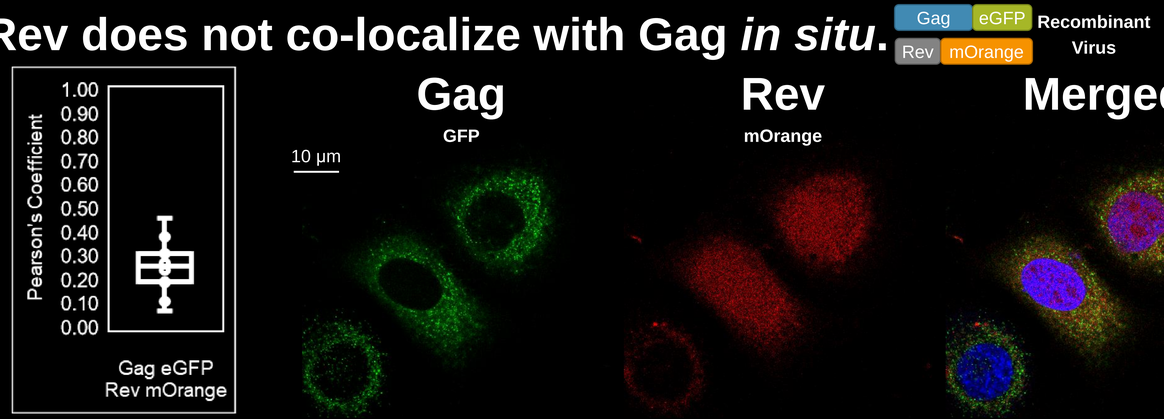


The localization of this interaction is dependent on Rev.





Rev does not co-localize with Gag *in situ*.



Results & Discussions:

We visualized the interaction between PACS-1 and HIV-1 Rev in transfected CD4+ HeLa cells (Top figure):

- The Venus fluorophore successfully reconstituted upon PACS-1:Rev protein-protein interaction.
- The PACS-1:Rev formed punctuates within the cytoplasm.
- The PACS-1:Rev formed globular regions within the nucleus surrounded by ring-like morphology of Rev proteins.
- The complex appeared to correspond more to PACS-1 localization.

We demonstrated that the localization of the PACS-1:Rev complex is dependent on Rev localization (Middle figure):

- BiFC fluorescent was reduced when Rev was restricted from the nucleus (M5) or oligomerizing (SLT40).
- The complex became trapped in the nucleus when Rev lost its nuclear export signal (M10).

The interaction between PACS-1 and HIV-1 Rev may initiate within a specific nuclear compartment such as the nucleolus or splicing speckles. The complex relies on Rev to export into the cytoplasm. Subsequently, the complex becomes incorporated into another subcellular structure in the cytoplasm that may represent endosomes or stress granules.

We elucidated that HIV-1 Rev did not have a strong colocalization with HIV-1 Gag during HIV-1 infection of CD4+ HeLa cells (Bottom figure):

- Pearson's coefficient ranged from ~0.10-0.40 with some fluctuation.

Rev may enhance HIV-1 assembly increasing structural protein expression and genome backpacking without directly interacting with Gag. However, it remains possible that Rev may serve as a molecular guide leading PACS-1 to associate with Gag downstream to facilitate viral assembly.

Conclusions:

Our work has provided insights in:

PACS-1 interacts with Rev in the nucleus and cytoplasm. This interaction is primarily cytosolic but appears to have a nuclear formation morphology and depends on Rev oligomerization.

Future questions to be answered:

- What are the molecular motifs responsible for the interaction? Are there any post-translational modifications that play a role? does phosphorylation play?
- How does the interaction influence HIV-1 RNA transport dynamic *in situ*?

Significances:

- The PACS-1:Rev complex is a novel anti-retroviral target.
- PACS-1 may be responsible for regulating RNA metabolism/.

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