Exploring the Requirements for RNA Packaging Through a Non-Viral RNA-Binding Protein

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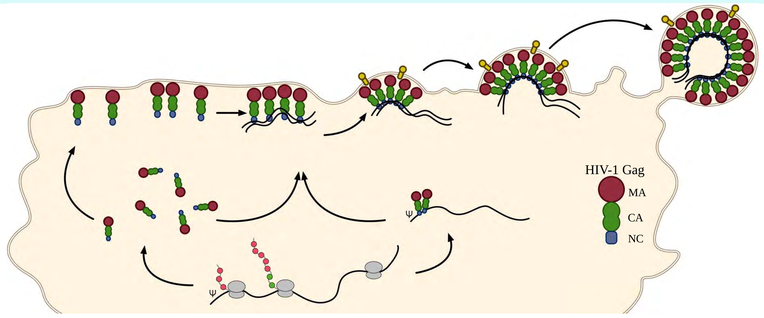
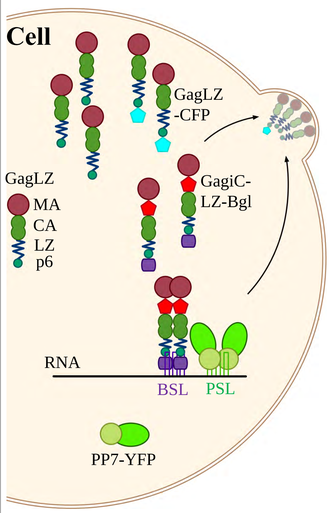
Introduction & Background



- During HIV viral assembly, two copies of RNA are packaged into virions
- Nucleocapsid (NC) interacts with a specific packaging signal (Psi) on the viral RNA to facilitate efficient packaging
- In the absence of RNA containing Psi, cellular RNAs are recruited to facilitate Gag VLP formation (Muriaux & Rein. **2001,** PNAS)
- VLPs can be made *in vitro* with virtually any single stranded RNA (Campbell & Rein. **1998,** JVI)
- Gag binds HIV-1 RNA with only a slight preference over cellular mRNA in the cytoplasm (Kutluay et al . **2014,** Cell**)**
- Most HIV-1 Particles have HIV-1 RNA (Chen et al. **2009**, PNAS)

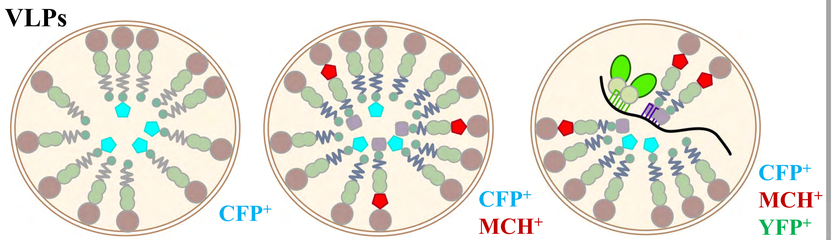
Hypothesis & Approach

We hypothesized that other regions of Gag contribute to RNA packaging and assembly. We designed an experimental system to examine the influence of individual Gag domains on RNA packaging in the absence of NC.

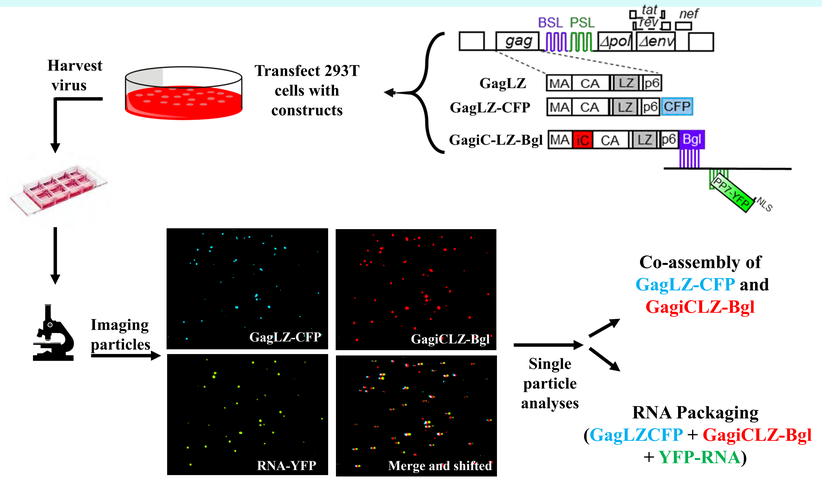


Approach

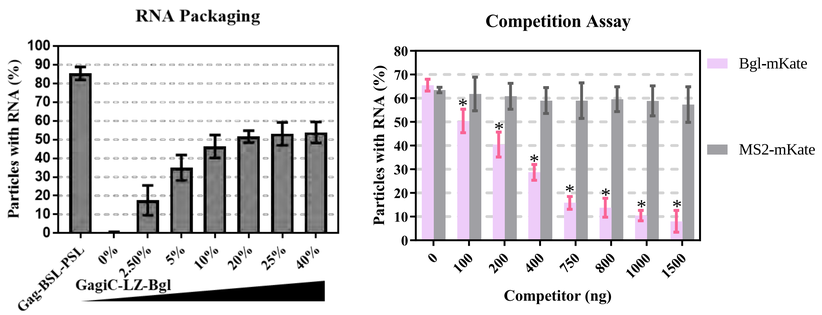
- To bypass assembly defects caused by deletion of NC, a LZ motif was inserted in place of NC
- A combination of GagLZ and GagLZ-CFP were used to make and label morphologically WT VLPs
- An internally mCherry labeled GagLZ construct (GagLZ-iC) expressing a bacterial RNA binding protein, BglG, was used to package RNA independently of NC
- All Gag plasmids contained BglG and bacterial phage PP7 coat protein specific stem loops (BSL and PSL) for packaging through BglG and detection by a PP7-YFP fusion protein respectively
- Systematic deletions of the GagiC construct were used to examine the impact of individual domains of Gag on RNA packaging



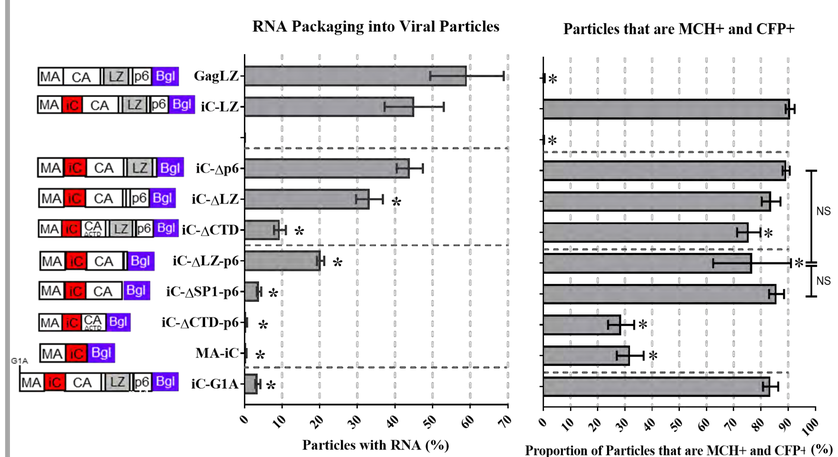
Experimental System – Single Virion Analysis



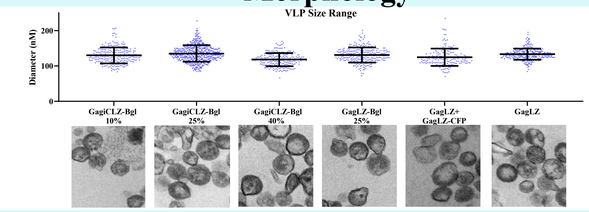
GagiCLZ-Bgl is Able to Package RNA Specifically Through Interactions Between Bgl:BSL



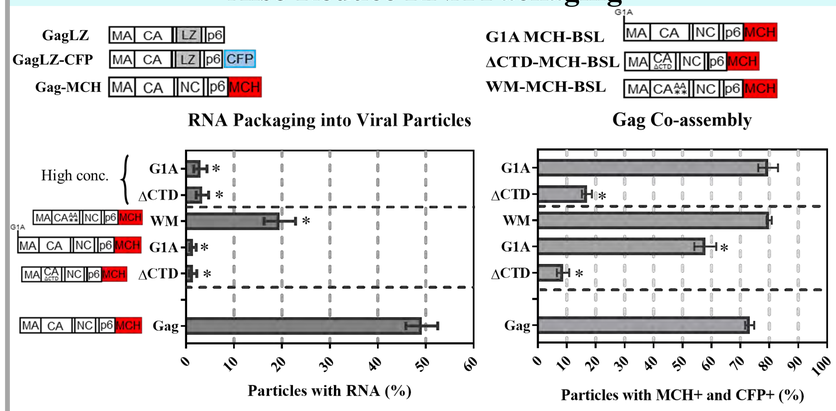
Deletion of Domains Involved in Gag Oligomerization and Membrane Anchoring Result in Severe Packaging Defects



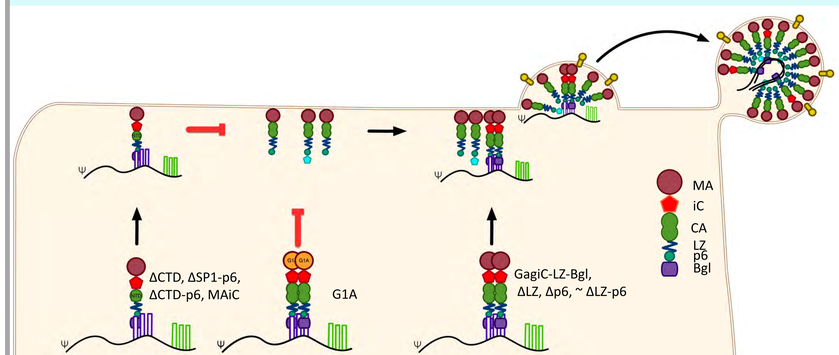
GagiCLZ-Bgl Does Not Significantly Effect VLP Morphology



G1A and Oligomerization Mutations in Full-Length Gag Also Reduce RNA Packaging



Summary



- binds BSL
- **unable** to oligomerize on RNA
- can anchor to the PM
- **not** able to be packaged into particles

- binds BSL
- able to oligomerize on RNA
- GagLZ bound RNA **cannot** anchor into the PM
- RNA is **not** packaged into particles

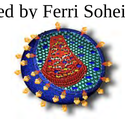
- binds BSL
- able to oligomerize on RNA
- anchors the RNA to the PM
- packaged into particles

In addition to RNA binding, Gag domains important for Gag oligomerization and membrane targeting are important for RNA genome packaging.

Acknowledgments

We thank Dr. Eric O. Freed for many productive discussions and experimental suggestions.

Electron microscopy of VLPs was performed by Ferri Soheilian at the Frederick National laboratory for Cancer Research (FNLCR).



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