INVESTIGATING THE ROLE OF DIMERIZATION AND CAP SEQUESTRATION IN SELECTIVE PACKAGING

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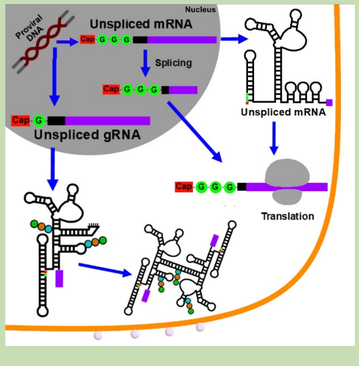
ABSTRACT

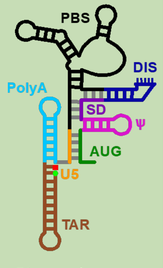
The HIV-1 lifecycle requires its RNA to serve dual purpose: as a messenger RNA and as genomic RNA. To dictate the function of the RNA, HIV-1 utilizes heterogenous transcription start sites, producing RNAs beginning with either 1 (Cap1G) or 3 (Cap3G) guanosines. The number of guanosines dictates the structure of the untranslated region of the genome, the 5′-Leader—a region essential to the regulation of translation, splicing, and packaging. Cap3G RNAs are monomeric, hiding Gag binding sites and exposing the 5′-cap, enabling eIF4E binding which allows it to serve as a messenger RNA. Cap1G, on the contrary, adopts a dimeric structure where polyA is stacked with TAR, allowing the dimerization initiation site (DIS) to be available for dimerization, while simultaneously sequestering the 5′-cap. Cap1G's structure will ensure high affinity binding of Gag to allow HIV-1 packaging. Previous literature suggests the stability of polyA is the primary determinant of the selective packaging HIV-1 RNA. Based on competitive packaging assays between wild type HIV and various mutants designed to either disrupt or stabilize the polyA, they hypothesize that polyA stability dictates selective packaging. However, our work has yielded alternative results, where it was deduced that stable polyA mediates cap sequestration—a process we deem essential to efficient HIV-1 packaging. Our project aims to conclude whether cap sequestration is a dominant regulator for packaging. We plan to further explore these mutants from a series of EMSAs with eIF4E, a cap binding protein, in order to evaluate the exposure of the cap to see if these results dictate their packaging tendencies. We also plan to use NMR to visualize the exact effects of the mutations on structure. Successful completion of our work will help identify the dominant features of selective packaging of the HIV-1 genome.

BACKGROUND

- HIV-1 uses a heterogenous transcription start site to produce RNAs beginning with 1 (Cap1G) or 3 (Cap3G) guanosines
- Cap1G is typically used in packaging
- Cap3G is typically used in translation

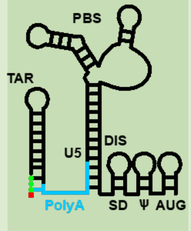


Cap1G



- 5′-cap is sequestered due to TAR-polyA stacking
- Poly-A hairpin is intact
- Exposed DIS allows for dimerization

Cap3G



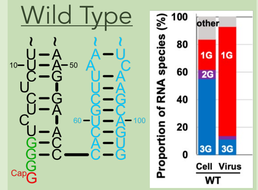
- 5′-cap is exposed
- PolyA is unstructured
- Sequestered DIS prevents dimerization

PRIOR LITERATURE

Prior literature has hypothesized that the stability of the polyA region is the primary regulator of selective packaging

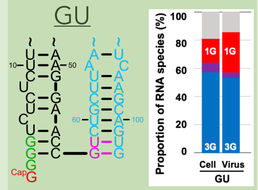
They concluded this by running 5′RACE experiments on various mutants designed to either stabilize or disrupt the polyA structure

Wild Type



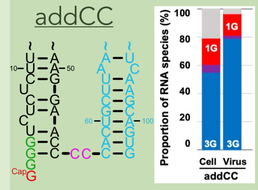
- Wild type
- 1G is selectively packaged

GU

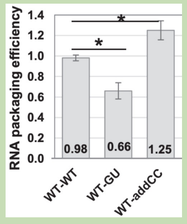


- Destabilized polyA
- 1G and 3G both are inefficiently packaged

addCC



- Stabilized polyA
- 3G is packaged

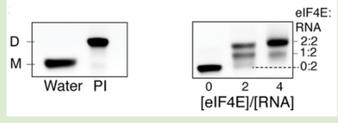


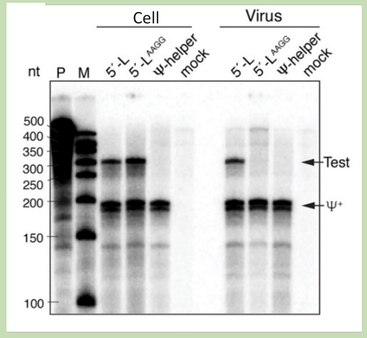
- To further test their hypothesis, they ran a packaging efficiency assay which showed that the addCC mutant packaged more efficiently than both the wild type and the GU mutant
- This suggests that polyA stability matters

OUR THEORY

Dr. Pengfei Ding, a previous postdoc from our lab, conducted research which seems to contradict this prior literature by suggesting that cap sequestration is the dominant regulator of selective packaging

To come to this conclusion, he created a mutant that both dimerizes and binds eIF4E (a cap binding protein)





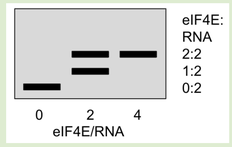
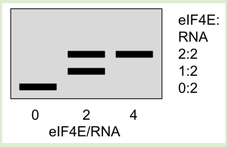
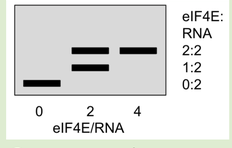
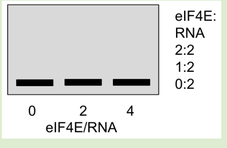
He used this mutant to conduct a gel study where he found that this mutant was expressed in cells but was not found in the virus, which proves that even though it has a stable polyA, it is not selectively packaged

FUTURE DIRECTIONS

Our goal is to observe whether cap sequestration is a dominant regulator for packaging by understanding the cap structure of mutants from previous literature

1. Recreate the GU and addCC mutants with a cap1G and cap3G
2. Observe whether each construct binds eIF4E
3. Use NMR to visualize the effects of mutations on structure

PREDICTIONS

	Cap1G	Cap3G
GU	 • Cap exposed • eIF4E can bind	 • Cap exposed • eIF4E can bind
addCC	 • Cap exposed • eIF4E can bind • With the CC added, polyA structure will not change	 • Cap sequestered • eIF4E cannot bind • The two CC base pair with the GG, allowing it to stack

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- Ding, Pengfei, et al. "5′-Cap sequestration is an essential determinant of HIV-1 genome packaging." Proceedings of the National Academy of Sciences 118.37 (2021): e2112475118

FUNDING

This research was funded by the NIAID U54150470 grant and the Howard Hughes Medical Institute at UMBC