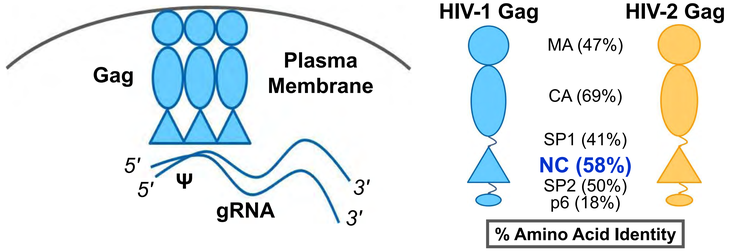
GAG DETERMINANTS OF SPECIFIC GENOME PACKAGING IN HIV-1 AND HIV-2

Jonathan M.O. Rawson[1], Olga A. Nikolaitchik[1], Xayathed Somoulay[1], Jennifer A. Yoo[1], Vinay K. Pathak[2], and Wei-Shau Hu[1]

Viral Recombination Section[1], Viral Mutation Section[2], HIV Dynamics and Replication Program, National Cancer Institute, Frederick, Maryland, USA

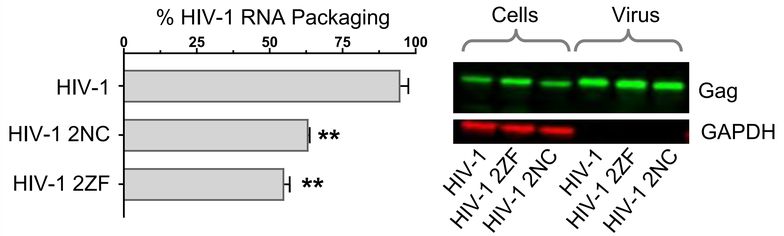
NATIONAL CANCER INSTITUTE 00167

BACKGROUND: HIV RNA PACKAGING



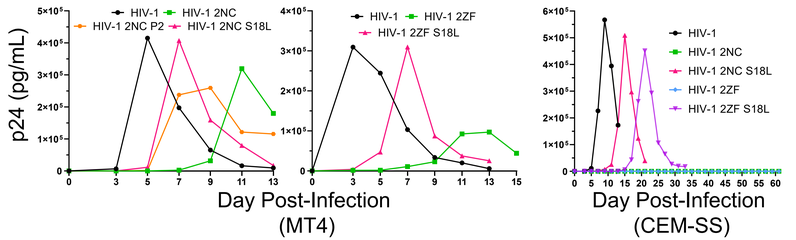
- The Gag polyprotein packages HIV genomic RNA (gRNA) by interacting with the RNA packaging signal (Ψ), located in the 5′ untranslated region.
- HIV-1 Gag packages HIV-2 RNA, but HIV-2 Gag does not package HIV-1 RNA (Kaye & Lever 1998, Dilley & Hu 2011).
- **Goal: To identify the Gag determinants of non-reciprocal cross-packaging in HIV-1 and HIV-2.**

RESULTS: CHIMERAS HAVE IMPAIRED HIV-1 RNA PACKAGING



- HIV-1 Gag chimeras with HIV-2 NC or ZF exhibited impaired packaging of HIV-1 RNA ($p < 0.01$, one-way ANOVA, n = 3).
- The chimeras did not affect Gag expression or particle release.
- **These results indicate that HIV-2 NC can recognize the HIV-1 packaging signal, but less efficiently than HIV-1 NC.**

RESULTS: CHIMERAS REPLICATE & ADAPT IN T CELLS



- Both chimeras replicated in MT4 T cells, but with delayed kinetics.
- HIV-1 2NC replicated faster when repassaged due to an S18L mutation in NC.
- The S18L mutation also enabled the chimeras to replicate in less permissive T cells (CEM-SS).

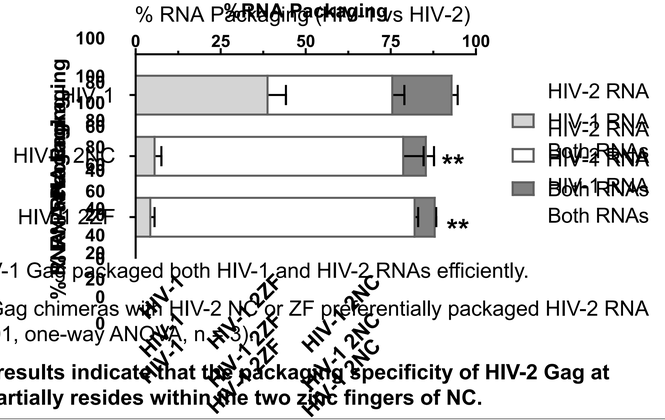
METHODS: HIV-1/HIV-2 GAG CHIMERAS

- HIV-1 Gag chimeras were constructed with HIV-2 nucleocapsid (NC) or just the two zinc fingers (ZF) of NC.



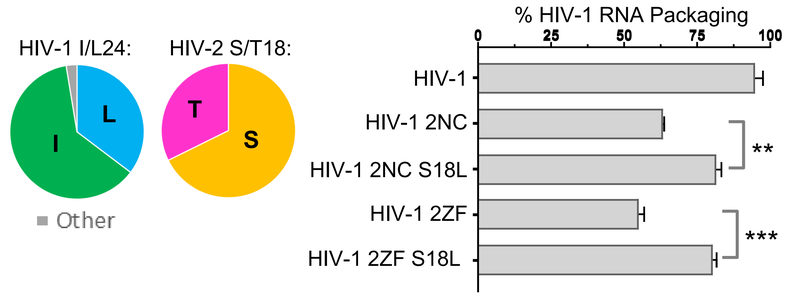
- HIV-1 and HIV-2 NC have 23 amino acid differences, but both have two CCHC-type ZFs and a number of basic residues.

RESULTS: CHIMERAS PREFERENTIALLY PACKAGE HIV-2 RNA



- WT HIV-1 Gag packaged both HIV-1 and HIV-2 RNAs efficiently.
- HIV-1 Gag chimeras with HIV-2 NC or ZF preferentially packaged HIV-2 RNA ($p < 0.01$, one-way ANOVA, n = 3).
- **These results indicate that the packaging specificity of HIV-2 Gag at least partially resides within the two zinc fingers of NC.**

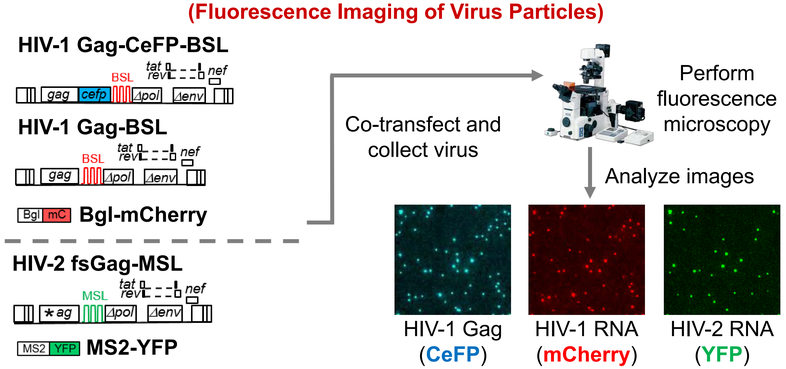
RESULTS: HIV-2 NC S18L MUTATION IMPROVES PACKAGING



- The S18L mutation occurred in the first zinc finger of HIV-2 NC and represents a switch from a typical HIV-2 to a typical HIV-1 residue at this position.
- The S18L mutation significantly improved packaging of HIV-1 RNA for both the HIV-1 2NC and HIV-1 2ZF chimeras.

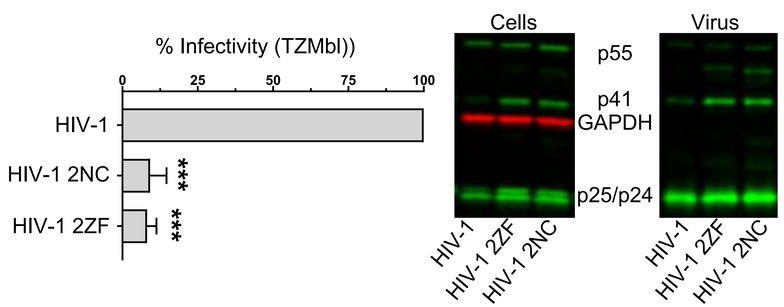
METHODS: SINGLE VIRION ANALYSIS

(Fluorescence Imaging of Virus Particles)



- Single virion analysis (SVA) was used to determine the RNA packaging efficiency of HIV-1 RNA alone or in competition with HIV-2 RNA.

RESULTS: CHIMERAS HAVE IMPAIRED INFECTIVITY



- HIV-1 Gag chimeras with HIV-2 NC or ZF exhibited low (~10%) but detectable infectivity in TZMbl cells ($p < 0.001$, one-way ANOVA, n = 3).
- The chimeras did not affect Gag expression or particle release, but did slightly impair Gag processing, as shown by higher levels of p41 in cells and virus.

CONCLUSIONS

- HIV-1 Gag chimeras with HIV-2 NC or ZF exhibit impaired packaging of HIV-1 RNA and altered packaging specificity, with a preference for HIV-2 RNA.
- These results indicate that the packaging specificity of HIV-2 Gag at least partially resides within the zinc fingers of NC.
- The S18L mutation within the first zinc finger of HIV-2 NC significantly improved chimera replication kinetics and HIV-1 RNA packaging efficiencies, indicating that this residue plays a key role in NC-RNA interactions.
- These studies may inform efforts to develop antivirals targeting RNA packaging and have implications for the possible emergence of HIV-1/HIV-2 recombinants in co-infected individuals.