RETROVIRUS-SPECIFIC DIFFERENCES IN PROTEIN-NUCLEIC ACID INTERACTIONS: IMPLICATIONS FOR GENOMIC RNA PACKAGING

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Retroviral RNA encapsidation involves a recognition event between genomic RNA packaging signals and one or more domains in Gag. In HIV-1, the nucleocapsid (NC) protein domain of Gag is known to be involved in genomic RNA packaging, and displays nucleic acid binding, aggregation, and chaperone functions. In contrast, HTLV-1 NC, a member of the deltaretrovirus genus, displays very weak nucleic acid binding affinity and very little chaperone or aggregation activity. It has also been demonstrated that mutation of conserved charged residues in the BLV matrix (MA) domain affects virus replication and viral RNA packaging efficiencies *in vivo*. Based on these observations, we hypothesized that the MA domain of Gag may generally contribute to nucleic acid binding and genome encapsidation for the deltaretroviruses. To gain further insight into the nucleic acid binding properties of MA, we examined the interaction between HTLV-2 and HIV-1 MA proteins and various nucleic acid constructs *in vitro*. Mutagenesis studies were designed to probe the role of conserved charged amino acid residues of MA in aggregating and binding nucleic acids. Fluorescence anisotropy measurements were performed to measure nucleic acid binding affinity. In general, HTLV-2 MA displays substantially higher binding affinity to nucleic acids and better chaperone and aggregation activities than either HIV-1 MA or HTLV-2 NC. The simultaneous mutation of two basic residues (R47A/K51A) in HTLV-2 MA α-helix II, results in a binding defect to non-specific ssDNA relative to wild-type, whereas single point mutations have more modest effects on binding. HTLV-2 MA binds with higher affinity and apparent specificity to SL2 RNA derived from the putative packaging signal of HTLV-2. Furthermore, an HIV-1 MA triple mutant, E40R/E42L/N47K, designed to mimic HTLV-2 MA α-helix II, dramatically improves binding affinity and chaperone activity of the HIV-1 MA protein *in vitro* and restores RNA packaging to a ΔNC HIV-1 variant *in vivo*. Taken together, these results are consistent with a role for HTLV-2 MA in deltaretrovirus RNA packaging.

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