CRM1 and the Viral RNA Packaging Element Promote Plasma Membrane Localization of the Rous Sarcoma Virus Gag Protein

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The mechanism by which Rous sarcoma virus (RSV) packages its genome represents a novel paradigm for retroviruses. The RSV Gag protein transiently enters the nucleus, binds to the psi viral packaging sequence, and undergoes a conformational change that recruits the host CRM1/RanGTP export complex. The Gag:viral RNA:CRM1:RanGTP ribonucleoprotein complex (RNP) is transported to the nuclear pore for export into the cytoplasm. CRM1-dependent nuclear export requires four key hydrophobic residues in the Gag p10 domain, and alteration of each of these residues interferes with nuclear export of Gag. Gag NES mutants (L219A and NES-A) accumulate in punctate foci within nucleoli and in the nucleoplasm. Because CRM1 mediates intranuclear transport of host RNAs, we hypothesized that it might be involved in the transport of Gag from the nucleoplasm to nucleoli prior to engaging the nuclear pore complex.

To test this hypothesis, we co-expressed human CRM1 and L219A.Gag in avian cells. No change in intranuclear Gag localization was observed. However, when the viral RNA packaging signal was expressed in *trans*, L219A.Gag nuclear localization was greatly reduced, and instead, prominent Gag foci were observed at the plasma membrane. We expected that replacing the entire NES sequence with alanine residues (NES-A.Gag) would abrogate CRM1-mediated plasma membrane transport. To our surprise, the NES-A.Gag protein was also relocalized to the plasma membrane in the presence of the viral 5' UTR sequence. These results suggest that may be a second NES in Gag or that CRM1 binds to the viral 5' UTR, either directly or indirectly, to facilitate Gag trafficking to the plasma membrane. Ongoing experiments will test whether chicken CRM1 exerts the same effect as human CRM1 and if the facilitated membrane transport of RSV Gag is cell-type specific. We propose a model whereby CRM1 and/or facilitated nuclear pore egress prepares the Gag-RNA ribonucleoprotein complex for the next part of its journey—transport to the cell periphery for virion assembly.