

**POSTER 26****STABLE TETRAMERIC COMPLEXES OF RETROVIRAL Gag PROTEINS BOUND TO VIRAL RNA**

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Early in assembly, Gag proteins form dimers using RNA as a substrate. However, the virus particles that are ultimately released from the cell contain a hexameric lattice of immature Gag proteins. What factors stimulate the transition from the dimeric to the hexameric state? Moreover, does RNA-binding induce structural changes in Gag that impact higher-order complex formation? In these studies, we sought to characterize early assembly complexes and determine the stoichiometry and binding affinity of Gag to viral and non-viral nucleic acid substrates.

The wild-type Rous sarcoma virus (RSV) Gag polyprotein lacking the PR domain was purified from *E. coli* and analyzed in the absence/presence of nucleic acids using fluorescent anisotropy, tryptophan fluorescence, and crosslinking. In the absence of exogenous nucleic acids, Gag was in monomer-dimer equilibrium. When complexed to a 10-mer oligonucleotide, Gag bound the nucleic acid substrate as a dimer. Using longer nucleic acids resulted in Gag oligomers fitting a complex dimer-tetramer-hexamer binding model. The relative binding affinities of these complexes indicate that Gag favors the dimer-tetramer state and forms hexamers only under conditions of excess protein. Strikingly, very stable Gag tetramers formed when an 800-nucleotide viral RNA sequence was used as the substrate.

What is the role of this stable-tetrameric complex in the viral life cycle? One possibility is the tetrameric complex of Gag with viral RNA acts as a stable intermediate that prevents the formation of larger assembly complexes until higher Gag concentrations exist, possibly at the plasma membrane. Alternatively, interactions with cellular factors may provide signals that induce changes in the oligomeric state or conformation of Gag. Current experiments will test whether inositol phosphates, intracellular transport machinery, or membrane lipids promote transition from dimers to hexamers. Through these studies, we hope to determine how Gag-Gag and Gag-RNA interactions are regulated, aiding in our overall understanding of assembly and leading to the development of novel assembly inhibitors.