

## DEFINING THE NUCLEATION STEP OF EARLY CAPSID ASSEMBLY

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During retroviral maturation several thousand CA molecules assemble to form a capsid shell. We, along with others, hypothesize that proper capsid assembly is dependent on the formation of a unique intermolecular interaction that initiates and facilitates the growth of a capsid. An in vitro assembly system using purified Rous sarcoma virus (RSV) CA protein was developed to dissect the protein multimerization pathway and define the requirements for initiation of assembly. Relatively high concentrations (500 mM) of sodium phosphate ( $\text{NaPO}_4$ ) at near neutral pH induce monomeric CA to polymerize into capsid-like structures. Followed by turbidity, the assembly kinetics shows a sigmoidal curve typical of a nucleation-driven mechanism. Additionally, we purified correctly folded CA oligomers ranging in size from dimers up to tetramers which may represent intermediate steps along the assembly pathway. When added to an assembly reaction with monomeric CA the oligomers acted as seeds for assembly. The results provide support that the isolated intermediates are on a correct assembly pathway and that an oligomer as small as a dimer can nucleate assembly. Furthermore, we have found that in the presence of low levels (50 mM) of  $\text{NaPO}_4$  monomeric CA protein forms assembly seeds. This study provides strong evidence that the in vitro assembly pathway is dependent upon the formation of a dimeric nucleus. The dimer interface has yet to be determined but may represent a unique interface not previously observed in RSV CA. Preliminary data reveals that the two domains of CA influence nucleation. This is the first report of the isolation of on-path assembly intermediates and demonstration that the rate limiting step of in vitro assembly is the formation of a dimer. The characterization of the CA oligomers may provide important new information about the dynamic behavior of CA during the early steps of capsid formation and provide insight into discrete steps of assembly that can be targeted by retroviral inhibitors. Furthermore, our system describes alternative in vitro conditions for examining the biology of and screening of inhibitors to HIV capsid assembly.