

ON THE ROLE OF RNA IN HIV-1 PARTICLE ASSEMBLY: IS SP1 AN ALLOSTERIC SIGNAL TRANSMITTER?

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Expression of the Gag protein in a mammalian cell is sufficient for efficient assembly and release of retrovirus particles (VLPs). However, several lines of evidence indicate that nucleic acid (NA) is required for VLP assembly. This requirement is not understood. NA cannot simply be a scaffold upon which Gags are aligned, since quite short NA molecules, only long enough to bind a few Gags, support assembly. These observations suggest that NA-binding induces a conformational change in Gag, exposing interfaces for the Gag-Gag interactions leading to assembly. The interfaces are believed to be within the CA domain of Gag. In HIV-1 Gag, a short spacer, "SP1," lies between the NC and CA domains. Thus a change in NC induced by NA-binding might traverse SP1 in order to alter CA conformation.

We have performed a detailed mutational analysis of the role of SP1 in HIV-1 VLP assembly. We find that assembly is extraordinarily sensitive to changes in SP1: even minute changes in the protein, such as removing a methyl group by replacing an alanine with a glycine, completely destroy the ability of Gag to assemble correctly in mammalian cells.

The sequence of SP1 is suggested to be compatible with α -helix formation (Accola, 2000). However, SP1 is largely unstructured in aqueous solution (Newman, 2004) although it is α -helical in 30% trifluoroethanol (Morellet, 2005). We have analyzed this sequence in media of varying dielectric constant by molecular-dynamics simulations; these studies indicate that SP1 would undergo a concerted change from coil to α -helix as the dielectric constant is reduced.

Other studies have shown that chimeric molecules, in which the NC (and more C-terminal) domains of Gag are replaced with an oligomerizing leucine-zipper motif, also assemble efficiently in mammalian cells. Taken together, these results raise the possibility that NA-binding serves to concentrate Gag molecules into oligomers; the high local protein concentration within the oligomers reduces the local dielectric constant; this in turn induces a shift to α -helicity in SP1; and this conformational change is propagated into CA, exposing interfaces for VLP assembly. Experiments testing these hypotheses are under way.