

## HIV-1 and MLV Particle Assembly: There Are Many Ways to Get Together

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Retroviral assembly is mediated by the viral Gag protein. *In vitro*, association of Gag molecules into virus-like particles requires the presence of nucleic acid. It has been suggested that cooperative binding to nucleic acid brings Gag molecules close together, and that juxtaposition of Gags changes their conformation, exposing new interfaces for Gag-Gag interaction leading to particle assembly (Rein, TiBS 2011).

Recombinant Gag proteins of HIV-1 and murine leukemia virus (MLV) are remarkably different in several respects. One is that HIV-1 Gag dimerizes spontaneously in solution with a  $K_d$  of  $\sim 0.01$  mM (Datta, JMB 2007), while oligomerization of MLV Gag is barely detectable (Datta, JV, in press). *In vivo*, MLV particle assembly seems significantly more dependent upon RNA than HIV-1 assembly. Thus, detergent-stripped immature MLV particles are disrupted by RNase (Muriaux, PNAS 2001) while those of HIV-1 are not (Campbell, PNAS 2001, Klein, JV 2011). Further, HIV-1 Gag lacking NC, the principal RNA-binding domain, can assemble into (somewhat aberrant) particles *in vivo* (Ott, JV 2003), which appear to lack cellular RNA (O'Carroll, submitted), while MLV Gag lacking NC does not assemble (Muriaux, JV 2004).

Does the stronger protein-protein interaction observed with HIV-1 Gag enable it to assemble *in vivo* without RNA? We found that a mutation ablating the dimer interface in HIV-1 Gag still allows HIV-1 Gag to assemble *in vivo* into structures with some resemblance to virus particles, as does the removal of NC. However, combining these two genetic defects completely destroys its assembly capability. In other words, the dimer interface and the NC domain are functionally redundant with respect to particle assembly. Similarly, HIV-1 Gag with mutations interfering with plasma-membrane targeting assembles into (aberrant) virus-like structures within the cytoplasm; again, this ability is lost if these mutations are combined with defects in either the dimer interface or RNA-binding.

In summary, the data suggest that there are (at least) three mechanisms by which HIV-1 Gag molecules are brought together in cells: protein dimerization; RNA-binding; and membrane association. Interfering with any one of these mechanisms does not completely prevent virus-like particle assembly, but interfering with any two does.

This work was supported in part by the Intramural Research Program of the NIH, National Cancer Institute, Center for Cancer Research, in part by the NIH Intramural AIDS Targeted Antiviral Program, and in part with federal funds from the National Cancer Institute, National Institutes of Health, under contract HHSN26120080001E.