ASSEMBLY PROPERTIES OF HIV-1 Gag-LEUCINE ZIPPER CHIMERAS: IMPLICATIONS FOR RETROVIRAL ASSEMBLY

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Expression of a single retroviral protein, Gag, in mammalian cells is sufficient for assembly of virus-like particles (VLPs). RNA seems to play an essential role in both the assembly and structure of VLPs (3, 4, 6), but the nature of its contribution to the assembly and structure of retrovirus particles is not yet fully understood. As one approach to further understanding the role nucleic acid binding plays in the assembly process, several groups have previously replaced the principal nucleic acid-binding domain of the Gag protein, nucleocapsid (NC), with either dimerizing or trimerizing zipper domains, and shown VLP production to be largely unaffected (1, 5, 7).

We have extended the analysis of the assembly properties of these HIV-1 Gag-Z chimeras, utilizing dimerizing (Gag-Z_{Leu}) and trimerizing (Gag-Z_{Ile}) constructs. VLPs formed *in vivo* were extensively analyzed. The Gag-Z VLPs were morphologically very similar, though not identical, to wild-type (WT) VLPs, and have a slightly lower buoyant density. They appear to lack RNA. Interestingly, the Gag-Z proteins can co-assemble with WT Gag.

The Gag-Z proteins have also been expressed in and purified from *E. coli*; the fact that they are soluble implies that, like WT Gag, they require a co-factor(s) for assembly. We found that they can bind to nucleic acids, despite the absence of the NC domain, and that nucleic acid can promote VLP assembly *in vitro*. Remarkably, inositol phosphates (IPs), which do not induce assembly of WT Gag but which modulate its assembly on nucleic acids (2), do support assembly of the Gag-Z proteins. Further, the "dimerizing" Gag Z_{Leu} forms very small VLPs in IP6 alone or nucleic acid alone, but correctly sized particles in IP6 + nucleic acid. In contrast, the "trimerizing" Gag-Z_{Ile} forms full-sized particles in IP6 alone. Thus, a trimerizing motif at the C-terminus of the protein can replace nucleic acid in assembly. This finding suggests that nucleic acid acts as a scaffold for trimerization of Gag during normal particle assembly.

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References:

1. Accola, M. A., B. Strack, and H. G. Göttlinger. 2000, J. Virol. 74:5395-5402.
2. Campbell, S., R. J. Fisher, E. M. Towler, S. Fox, H. J. Issaq, T. Wolfe, L. R. Phillips, and A. Rein. 2001, Proc. Natl. Acad. Sci. USA 98:10875-10879.
3. Campbell, S., and A. Rein. 1999, J. Virol. 73:2270-2279.
4. Campbell, S., and V. M. Vogt. 1995, J. Virol. 69:6487-6497.
5. Johnson, M. C., H. M. Scobie, Y. M. Ma, and V. M. Vogt. 2002, J. Virol. 76:11177-11185.
6. Muriaux, D., J. Mirro, D. Harvin, and A. Rein. 2001, Proc. Natl. Acad. Sci. USA 98: 5246-5251.
7. Zhang, Y., H. Qian, Z. Love, and E. Barklis. 1998, J. Virol. 72:1782-1789.