

POSTER 29**STUDIES ON THE ROLE OF THE GGA, Arf AND Gas7 PROTEINS IN RETROVIRUS ASSEMBLY**

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Retroviral particle production is mediated by the Gag precursor protein, termed Pr55^{Gag} in the case of HIV-1. Assembly/release of virus particles requires the translocation of Gag from the site of assembly in the cytosol to the plasma membrane. We recently reported that two families of cellular proteins, the Golgi-localized, γ -ear-containing, Arf-binding proteins (GGA1, GGA2, and GGA3) and the ADP ribosylation factors (Arfs) play important roles in retroviral Gag trafficking to the plasma membrane. Overexpression of the GGA proteins, and disruption of the Arfs, led to severe reductions in particle production resulting from defects in Gag–membrane association. Intriguingly, we observed that overexpression of GGA1, but not GGA2 or GGA3, trapped HIV-1 Gag in an internal GGA-induced compartment. The compartment induced by GGA overexpression also sequestered the Arf proteins, leading us to perform a series of experiments that demonstrated a direct role for Arf proteins in retroviral Gag trafficking.

In a yeast two-hybrid screen to uncover additional cellular host factors involved in HIV-1 assembly and release, we identified growth arrest-specific 7, isoform b (Gas7b) as an HIV-1 Gag- and GGA1-interacting protein. The minimal Gas7b-binding domain was mapped to the capsid region of Gag. The Gag–Gas7b interaction could also be visualized in HeLa cells by using bimolecular fluorescence complementation (BiFC) assays. In HeLa cells, Gas7b-HA primarily localized to the cytoskeletal network in the absence of HIV-1 Gag expression. However, in BiFC assays, interaction with Gag shifted the Gas7b localization pattern to that of the wild-type Gag. Moreover, when overexpressed in HIV-1-infected cells, Gas7b-HA was incorporated into the released virions. Interestingly, the predominant form of Gas7b-HA in virions, a C-terminal fragment of ~38 kDa in size, appears to be a product of Gas7b cleavage by the viral protease. Gas7b harbors a variety of domains (e.g., WW, SH-3, coiled-coil, and F-BAR) that could theoretically function during different stages of the assembly/release pathway. Further work will focus on deciphering the functional role of Gas7b, and related proteins, in the retroviral life cycle.