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A New Foamy Virus-Based Protein Delivery Platform and Characterization of a Novel Gag Motif Essential for Chromatin Binding and Nuclear Genome Targeting and Integration

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Foamy viruses (FVs) are distinct retroviruses with features suitable for the development of novel retroviral gene transfer and vaccine antigen delivery vectors, for instance their desirable safety profile, large transgene capacity, ability to persist in quiescent cells, wide range of potential host cell and high particle stability. In addition, no pathology has been directly linked to FV infection. However, retrovirus vectors, including FVs, have the potential to cause insertional mutagenesis and thus cancer.

In order to circumvent the risks of insertional mutagenesis, subviral FV particles are interesting potential candidates for protein delivery and vaccination. A protein delivery platform consisting of subviral particles (SVPs) of FFV Env and C-terminally truncated Gag proteins that fused to the eGFP cargo protein have been developed. Different production, concentration and purification systems have been studied and compared. By using a correspondingly optimized Gag-eGFP SVPs production protocol, delivery of substantial amounts of Gag-eGFP into targeted cells by SVPs was achieved.

A highly conserved motif QPQRYG essential for capsid assembly and particle budding was found during the development of SVPs delivery platform. Characterization of the function of this motif as well as the conserved Y416 and R419 residues of the directly upstream located potential chromatin binding site CBS previously described for PFV Gag was performed. The data presented here confirmed that chromatin binding by FV Gag is a shared feature and mediated by this motif. Additionally, the mutagenesis of both, critical residues in CBS and the QPQRYG motif negatively affect the nuclear accumulation of Gag of incoming particles as well as provirus DNA in targeted cells and the subsequent integration of the proviral DNA into host genome thus leading to severely reduced viral titers. While the CBS is active using

intracellularly expressed Gag as well as incoming particle Gag, mutagenesis of QPQRYG residues is mostly suppressing nuclear accumulation early during infection. Both motifs are obviously not essential during late events including virus assembly, release, maturation and reverse transcription of viral genomes.

The study enhances the molecular understanding of FV Gag activities during early events and may allow optimization of FV-based vectors for protein and gene delivery.