

SIMULTANEOUS IMAGING OF LENTIVIRAL GENOMIC RNA AND Gag IN LIVE CELLS: DECIPHERING THE CELL BIOLOGY OF ENCAPSIDATION

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Genome encapsidation is a critical event in the HIV-1 assembly process. Encapsidation represents a heretofore unexploited therapeutic target and it intersects with important current problems such as drug resistant HIV-1 genome recombination and restriction factor incorporation into particles. While HIV-1 Gag protein and genomic RNA determinants required for encapsidation are well established, where and when encapsidation occurs in the cell are unknown. Here we developed the first MS2 phage coat protein RNA labeling systems for lentiviruses. We use these systems to track the spatial dynamics of lentiviral genomic RNAs (HIV-1, FIV) vis-à-vis Gag in live cells. The genomic RNAs of both lentiviral genera were observed to traffic Rev-dependently into the cytoplasm, with focal nuclear envelope accumulation occurring in transit. Co-localization of Gag with RNA at the nuclear envelope was identified for FIV. This was packaging signal-independent, with either molecule expressed alone similarly targeted, indicating independently directed processes rather than RNA trapping of Gag. However, MS2-labeled HIV-1 and FIV genomes targeted the plasma membrane if and only if they contained the specific packaging signal. Genomes co-localized with Gag there and also co-accumulated with Gag in late endosomal foci, again only packaging signal-dependently, consistent with internalization from the plasma membrane. These results indicate that lentiviral genomic RNAs are trafficked to the plasma membrane by Gag, and suggest Gag-genomic RNA association may initiate at the nuclear envelope.