Fluorescent imaging of engineered lentivirus for targeted gene delivery

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Developing methods to label viruses with fluorescent moieties has its merits in elucidating viral infection mechanisms and exploring virus-associated therapeutics. The endocytic pathways used by some viruses have been exploited. But many critical properties of endocytic traffic and entry mechanisms of virus still remain unclear.

We constructed lentivirus encoding green fluorescent (GFP) fused to HIV accessory protein Vpr and imaged by confocal laser scanning microscopy in order to unravel viral targeting and endocytic pathways via tracking a single viral particle. Using other immunofluorescence labeling, it was demonstrated that antibody and fusogenic protein were co-incorporated into a single lentiviral particle. To determine that antibody on the virus surface recognizes a specific surface antigen of the desired cell type, we examined the colocalization of viral particles on the surface of the antigen-expressed cell (293T/CD20). To investigate receptor-mediated virus entry, the yellow fluorescent protein-tagged human CD20 antigen was constructed and expressed in 293T cells (293T/CD20-YFP). For further studies of intracellular behavior, we will examine the dependency of clathrin-coated-pits endocytic pathway, microtubule-associated transport, and endosomal fusion events involved in fusogenic molecules in our virus system.