Generation of VSV-G pseudotyped lentiviral particles in 293T cells

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Lentiviral vectors are powerful gene-transfer tools for transducing non-proliferating cells of laboratory animals, for basic research purposes and for experimental gene therapy. Lentiviral vectors can infect with high efficiency neurons and other terminally differentiated cells; transduced, reverse-transcribed DNA sequences can subsequently integrate into the genome of target cells, and are stably expressed at high levels for extended times [1]. Lentiviral particles produced for gene-transfer purposes are usually pseudotyped with the "G" glycoprotein of the vesicular stomatitis virus (VSV) envelope. To minimize recombination events, HIV and VSV structural genes, as well as the sequences of interest, are transfected separately into packaging cells. 2nd generation lentiviral vectors are commonly produced using a three-plasmid system: (i), a lentiviral construct containing the cDNA sequence of interest; (ii), an expression vector containing the VSV "G" gene; (iii), a "packaging" vector containing HIV *gagpol*, *tat* and *rev* sequences,

transcriptionally controlled by promoters unrelated to HIV (*e.g.*, CMV). Lentiviral systems have been recently implemented in terms of biosafety through the creation of vectors harbouring "self-inactivating", truncated 3' LTRs [2]. Moreover, in safer third generation systems *tat* and *rev* are deleted from helper constructs, *rev* is supplied *in trans* through an additional expression plasmid, and transcription in lentiviral vectors is driven by Tat-independent RSV/HIV or CMV/HIV chimeric LTRs [3]. Here we document by transmission imaging the production of replication-incompetent, VSV-pseudotyped lentiviral particles by human embryo kidney 293T cells, cotransfected with the pMD2G VSV envelope vector, the pCMV-dR8.74 helper construct, and the pWPT lentiviral vector, harbouring the ~2.4kb cDNA encoding the delta isoform of protein kinase C, directionally subcloned under the transcriptional control of an elongation factor 1- α internal promoter. Extracellular release of lentiviral particles is visible in panel A (scale bar, 200 nm): particles bud-off, leaving roundish "coated pits" on the membrane of producer cells. The capsids of budding lentiviral particles shown in this paper appear as spherical structures (panels B and C; scale bars, 100 nm and 120 nm, respectively), closely resembling the core of immature HIV [4, 5].

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