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Effects of mutations on the subcellular distribution and localization of the HIV-1 VPU

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Vpu is a small type 1 integral membrane phosphoprotein of the human immunodeficiency virus type 1 (HIV-1), predominantly localized in the endoplasmic reticulum (ER)/Golgi compartments of infected or vpu-transfected cells. Although localized in the membrane of the ER, there is no obvious sequence element identified for its retention in the ER or its distribution in the Golgi apparatus. In the present study, we assess the role of the carboxyl-terminal tetrapeptide sequence, VDDL, and the consensus glycosylation signal sequence, NES, on the subcellular distribution and localization of the Vpu protein of HIV-1LAI (subtype B) in HeLa cells. We fused the wild-type Vpu gene and Vpu genes that carry either the VDDL deletion (Vpu) and/or a glycosylation site mutation, to the enhanced green fluorescent protein (EGFP) gene, and monitored the subcellular distribution of the proteins in co-localization and fluorescence confocal microscopy studies. We found that both the EGFP-wtVpu and EGFP-Vpu proteins co-localized with an ER marker, while a species of the EGFP- Vpu protein also co-localized with a Golgi marker. However, alteration of the putative glycosylation signal in the EGFP- Vpu background resulted in the accumulation of Vpu in the Golgi. Our results suggest that the enhanced Golgi accumulation of Vpu proteins harboring both the VDDL deletion and a point mutation in NES sequence may have resulted from the removal of a restriction that would otherwise retain Vpu to the ER compartment of the cell. (Supported by RCMI Grant #5G12RR017581-05 from NCRR-NIH).