Differential timing of translocation of HIV-1 subtype B and C Vpu to the ER/Golgi and plasma membrane compartments

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The HIV-1 Vpu protein functions largely to target CD4 molecules for proteasomal degradation, and to enhance virion release. The subcellular localisation of Vpu is related to these functions. Previous studies showed subtype B Vpu localisation at the ER/Golgi complex, while subtype C Vpu localised to the plasma membrane (PM) at 48 hours post-transfection. To determine if subtype C Vpu can localize to the ER/Golgi, we evaluated the cellular localisation of Vpu from two South African subtype C isolates as compared to subtype B Vpu, over time. Codon optimized *vpu* genes from subtype C isolates FV5 and FV15 (which have a six and two amino acid insert in the N-terminal domain, respectively) and a representative subtype B *vpu* were TA cloned into the pcDNA6.2/C-emGFP expression vector. The three Vpu-emGFP recombinant plasmids were cotransfected with pDsRed-ER, pDsRed-Golgi, or pDsRed-Mem into HEK 293T cells, and observed at 24, 48, and 60 hours post-transfection under a confocal microscope to confirm the presence of Vpu at different subcellular compartments. Cotransfection and microscopy conditions were methodically optimised. At 24 hours post-transfection, the subtype C FV5 Vpu had ER/Golgi localisation, but none at the PM. The subtype C FV15 Vpu had weaker ER/Golgi localisation and no PM localisation. In contrast, the subtype B Vpu had strong PM localisation. At 48 hours, FV5 and FV15 Vpu showed PM localisation, while subtype B Vpu was clearly localised at the ER/Golgi. At 60 hours, FV5 Vpu was observed at the PM, whereas FV15 and subtype B Vpu showed ER/Golgi localisation. These findings illustrate the efficient translocation of Vpu between different cellular compartments and for the first time, the difference in timing between subtype B and C Vpu, as well as íntrasubtype differences. This difference in shuttling suggests implications for the timing of viral assembly and release. Further investigations may clarify the impact of this timing on the difference in disease pathogenesis noted between infections with the different subtypes.