

POSTER 33**CHARACTERIZATION OF ELEMENTS IN HTLV-1 ENVELOPE THAT REGULATE TRAFFICKING**

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Envelope (Env) trafficking is an important determinant of cell-to-cell transmission of HTLV-1, in order to regulate envelope incorporation into virus like particles (VLP) and to prevent cell-cell fusion. Viral proteins have hijacked many cellular trafficking pathways by using the sorting signals typical of cellular proteins. The 21-amino acid cytoplasmic domain of HTLV-1 Env contains two important trafficking motifs: 1) a tyrosine based motif (YSLI), which interacts with adaptor proteins (AP), is widely involved in internalization and sorting of cell surface receptors and 2) a PDZ-binding motif (ESSL), which can interact with numerous PDZ proteins. We examined mutated Envs and silenced cellular factors by siRNA methods. HTLV-1 Env mutants with a defective YXX Φ motif (YSLK) or substituted with motifs from transferrin receptor (YTRF), HIV Env (YSPL), or VSV-G (YTDI), all reached the cell surface and induced cell-cell fusion like WT Env. However, the altered Envs showed extremely low infectivity in cell-to-cell and cell-free transmission assays and some of them were poorly incorporated into virions. These results indicate that the sequence/structure of the YXX Φ motif is involved in Env targeting and determines the fate of the internalized protein. Mutation or truncation of the PDZ-binding motif (PBM) decreased the total amount of Env in the cell and caused Env to localize mainly in the Golgi. Mutation of the PBM could result in the rapid degradation of Env directly from the Golgi network or rapid internalization and degradation after reaching the plasma membrane. We believe that the Env PBM is involved in the latter, because Env with mutations in both PBM and YSLI motifs accumulated at the plasma membrane. Furthermore, siRNA silencing of either AP2, which is involved in clathrin-dependent endocytosis, or AP3, which mediates endosomal-lysosomal sorting, caused an increase in the accumulation of the PBM-mutated Env. These results indicate that the PBM-mutated Env is delivered to the cell surface. Future work will focus on screening for cellular proteins that interact with the Env PDZ-binding motif and testing the possibility that silencing of the interacting PDZ-protein leads to rapid recruitment of dynamin and formation of vesicles that are targeted for rapid protein degradation.