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Vpu-mediated Intracellular Targeting of HIV-1 Core Protein Precursor $Pr55^{gag}$ and Association of $Pr55^{gag}$ with Lipid Microdomains

AKADEMISK AVHANDLING

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ABSTRACT

HIV-1 has been suggested to use lipid rafts for assembly and budding. Lipid rafts can be separated from the bulk membranes by extraction with non-ionic detergents, such as Triton X-100 at cold, followed by a density gradient centrifugation. The analysis of intracellular $Pr55^{gag}$ on iodixanol density gradients yielded intermediate density buoyant complexes while raft-associated proteins floated to the light-density fractions. Extracellular virus-like particles (VLPs) showed a similar intermediate flotation pattern after TX-100 extraction suggesting that $Pr55^{gag}$ was not a genuine raft-associated protein. The lipid analysis of TX-100 extracted VLPs suggested that the intermediate buoyant complexes were largely devoid of membrane cholesterol and phospholipids. We also tested the extractability of the membranes with Brij98 which has been shown to detect rafts at physiological temperatures. Our analyses showed that intracellular $Pr55^{gag}$ as well as VLPs were largely resistant to Brij98-extraction. We concluded that $Pr55^{gag}$ associates with lipid microdomains distinct from the classical Tx-100-resistant lipid rafts.

We also analyzed the intracellular targeting of $Pr55^{gag}$. It has been under an intense debate at which cellular membrane the productive assembly takes place, as $Pr55^{gag}$ has been seen to accumulate at both the internal membranes and the plasma membrane depending on the cell type. To resolve this issue, we performed our analyses in HeLa H1-cells, where the $Pr55^{gag}$ can be found both at the plasma membrane and the internal membranes. We employed pulse-chase studies and a subcellular fractionation assay by which an efficient separation of the plasma membrane from the internal membrane fraction is achieved. The kinetic analyses revealed that in the HeLa H1-cells newly synthesized $Pr55^{gag}$ is exclusively targeted to the plasma membrane. To our surprise, the plasma membrane-associated $Pr55^{gag}$ was subsequently endocytosed, if the viral accessory protein Vpu was defective in our proviral construct. Our work was the first to implicate, that Vpu had an important role in the subcellular localization of $Pr55^{gag}$.

We probed by which mechanism the $Pr55^{gag}$ is taken up into the cells in the absence of Vpu. Our analyses implicated that neither clathrin-mediated endocytosis nor macropinocytosis was involved in $Pr55^{gag}$ uptake. In contrast, the cholesterol depletion affected the uptake of Gag thus suggesting the possibility that cholesterol-mediated uptake might be involved. However, cholesterol depletion had a more pronounced effect if it was applied before the maximal membrane insertion and at least some level of the higher-order multimerization of $Pr55^{gag}$ were achieved. This implicated that the cholesterol-dependent uptake pathway was only indirectly associated with the endocytosis of $Pr55^{gag}$ as cholesterol depletion most likely interfered with Gag multimerization and virus assembly.

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