Inhibition of SIV Gag particles assembly at the plasma membrane by Gag products translated from internal methionine codons

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Retrovirus particle assembly is driven by the Gag polyprotein and occurs in various intracellular compartments depending on the virus strain. Simian and human immunodeficiency virus (SIV and HIV) particles assemble at the plasma membrane where budding and release of the particles occur. The virus particles are then matured by the viral protease, which cleaves the Gag precursor into the matrix (MA), capsid (CA), and nucleocapsid (NC). Targeting of Gag to the plasma membrane is primarily mediated by the NH2-terminal matrix domain. Myristilation of the NH2 terminal glycine residue of MA is essential for stable association with the plasma membrane. In addition, a cluster of basic amino acids in the NH2-terminal domain of MA identified as a receptor binding domain according to the method of Eisenberg, may stabilize membrane association.

To study the processes involved in the targeting and the assembly of the Gag precursor to the plasma membrane. we expressed the Gag genes from two SIV isolates by using a Semliki Forest virus (SFV) expression vector. These two SIV isolates induce in infected monkeys either an acute wasting syndrome (SIVsmmPBj14) or an immunodeficiency (SIVmac J5) similar to that induced by HIV in humans. We observed that the Gag precursors of these two viruses are targeted to different cellular membranes. The PBj14 Gag precursor was targeted more efficiently to the plasma membrane where budding particles were identified by confocal and electron microscopy. The J5 Gag precursor was essentially budding in the endoplasmic reticulum. This differential targeting correlated with the presence of a lysine or a methionine residue at position 30 in the basic domain of MA. The presence of a lysine residue at this position increases the strength of receptor binding domain. However, we provide evidences that the differential membrane targeting does not correlate with the strength of the receptor binding domain but may rather be attributed to the inhibitory action of Gag products that are translated from the internal methionine codon at position 30 in the SIVmac J5 strain. Thus, the substitution of the methionine residue in the J5 strain by either a lysine or a cysteine leads to efficient plasma membrane targeting as observed with the PBj14 strain. In addition, coexpression of the full length PBj14 Gag precursor with the truncated product initiated at methionine 30 led to the loss of membrane targeting. Dual immunofluorescence staining indicated that the full length Gag precursor was sequestered with the truncated product inside the cytoplasm and no budding particles were observed. Gag products translated from internal initiation codons at positions 118 and 200 may also have a similar inhibitory action on plasma membrane targeting.

These observations may help better understand the role of the localization of particle assembly on SIV pathogenesis. Vaccination protocoles using SFV vectors able to produce and release virus like Gag particles may also favor the development of both humoral and cytotoxic responses directed against SIV.