DETECTION OF A SIV MATRIX REGION CRUCIAL FOR A CORRECT TARGETING OF THE GAG PROTEINS

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The Gag proteins of retroviruses play a central role in virion assembly and budding. Gag proteins of retroviruses like HIV and SIV are initially synthesized as myristylated polyprotein precursors which are targeted to the inner face of the plasma membrane where they can direct particle assembly and budding, even in the absence of other viral proteins.

Differences between the 87 first amino acids of Gag proteins induce different behaviour for the SIVsmm PBJ14 (PBj) and the SIVmac32H (J5) strains which could be in connection with the contrasted pathogenicity of these two strains[1]. Indeed, using electron microscopy, we noticed that the PBj virus buds efficiently at the plasma membrane and in vacuoles while the J5 virus buds mainly in the endoplasmic reticulum[1].

The 'Receptor Binding Domain' prediction[2] according to the Eisenberg's method[3] detects an important putative interacting domain in the amino terminal part of the matrix protein sequence. This 'Receptor Binding Domain' ('RBD') appears to be weaker for the J5 strain than for the PBj strain. The difference is correlated to the presence of one non-conservative mutation (K30M) and one conservative mutation (R28K) between PBj and J5 strains respectively. The molecular hydrophobic potentials around the molecules were calculated[6] after calculation of 3-D models corresponding to the two strains with MODELLER[4] and validation of these models with PROCHECK[5], . These potentials show that the difference between the 'RBDs' of the two strains coincides with an important modification of the hydropathic environment of both proteins. The mutation of the 30th amino acid seems to be the most influent. A Gag protein with K30M mutation is predicted to have a weaker N-terminal 'RBD'.

Truncated Gag proteins missing the N-terminal 'RBD' are synthesized by initiation of translation at internal methionine codons. These proteins may prevent correct targeting of the full length Gag precursors to the plasma membrane.

The amino terminal 'RBD' seems to be crucial for a correct targeting of the Gag proteins to the plasma membrane. To test the two hypotheses described above, the effect of two mutations of the 30th amino acid of J5 were theoretically and experimentally evaluated: the methionine was substituted by a lysine or by a cysteine residue. Both mutations abolish the translation of an internal initiation product from the 30th methionine codon. The M30K mutation restores a 'RBD' strength and a hydrophathic profile similar to that of PBj. The M30C mutation doesn't modify the 'RBD' strength and the hydropathy of J5.

References

1. Zhang, J. (1998) Thesis ULB.
2. De Loof, H., Rosseneu, M., Brasseur, R. and Ruysschaert, J.M. (1986) *Proc.Natl.Acad.Sci.U.S.A.* **83**, 2295-2299.
3. Eisenberg, D., Weiss, R.M. and Terwilliger, T.C. (1982) *Nature* **299**, 371-374.
4. Sali, A. and Blundell, T.L. (1993) *J.Mol.Biol.* **234**, 779-815.
5. Laskowski, R.A., MacArthur, M.W., Moss, D.S. and Thornton, J.M. (1993) *J.Appl.Cryst.* **26**, 283-291.
6. Brasseur, R. (1991) *J.Biol.Chem.* **266**, 16120-16127.