Two HIV-2 Env regions potentially involved in intracellular transport acts at two different moments during viral production

N. Bakouche [1], *P. de Sany* [1] *and J. Ruelle* [1]

[1] Université Catholique de Louvain, Institut de recherche clinique (IREC), pole de microbiologie medical (MBLG), Avenue Hippocrate 54 - B1.54.05, 1200 Bruxelles, Belgique.

Background

HIV-2 possesses the *gag*, *pol,* and *env* main genes like other retroviruses. They respectively code for the structurals proteins, the enzymes and two envelope glycoproteins (gp). When we studied the Env C-terminal sequence (the cytoplasmic tail ; CT) from clinical samples, we observed highly conserved sequences among which the internalisation domain (ID ; YxxV) and a di-leucine motifs. Both sequences are involved in HIV-1 Env intracellular transport. We show here preliminary results defining the phenotypic impact of substitutions in the similar region of HIV-2 Env.

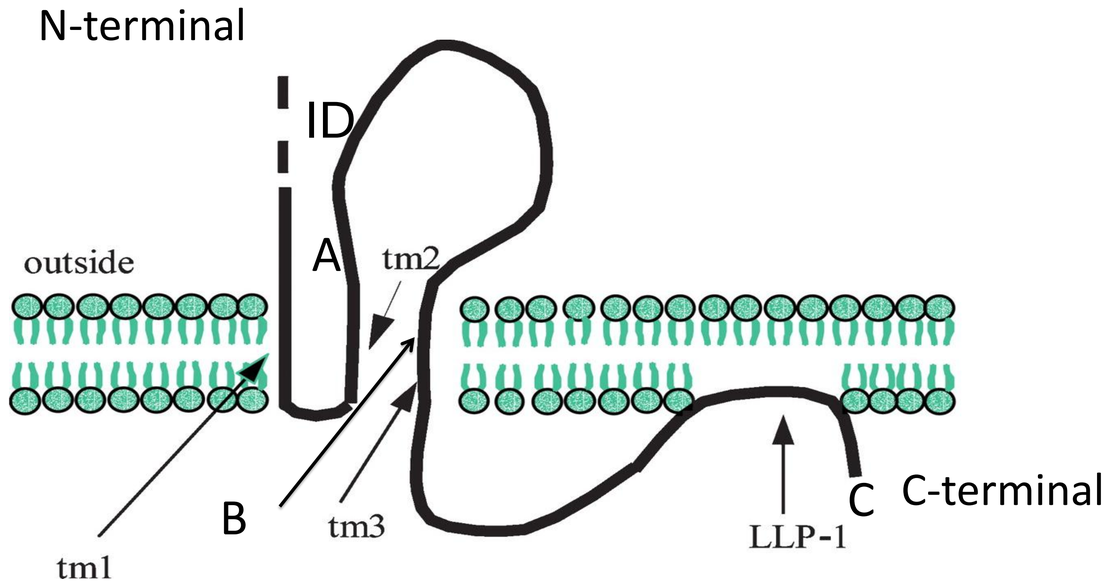

Figure 1. HIV-2 Env C-terminal structure (CT), based on the predicted HIV-1 structure described by Heap *et al*. (2005). The N-terminal side of Env is completely outside the virus (partly represented in the figure). The external N-terminal region (only the end represented here) is followed by two transmembrane regions (tm1 and tm2) and the cytoplasmic tail (CT ; between A and C). The CT harbours a first external loop with the internalisation domain (ID) anchored by the tm3. The C-terminal region of the CT is completely inside the virus and ends with an hydrophobic domain known in the HIV-1 to be the lentiviral lytic peptide 1 (LLP-1). The region between B and C are deleted *in vitro* and then not present in cultured virus.

Materials and methods

We developed a site directed mutagenesis protocol on long plasmids to modify directly the sequence of an infectious HIV-2 group A clone (pKP59-ROD) without subcloning. With this technique, we obtained a virus with full-length Env virus as observed *in vivo* (RODenv1-858), a virus with a partially truncated Env as observed *in vitro* (RODenv1-749) and a virus without CT (RODenv1-701). Then, we changed the ID sequence of the *in vitro* adapted virus (RODenv1-749PV709-710AA) and the di-leucine motif in the *in vivo* like virus (RODgpTM1-858LL857-858AA) by substituing the last two amino-acids of these sequences by two alanines. The mutant plasmids were used for virus production by transfection on 293F cells. Then, the virus produced were use for *in vitro* single cycle infection on MT-2 cells and p24 Ag level were monitored in the culture supernatant.

SRLRK GYR*PV*FSSPPGYIQQIHIHKDRGQPANEETEEDGGSNGGDRYWP
WPIAYIHFLIRQLIRLLTRLYSICRDLLSRSFLTLQLIYQNLRDWLRLRTAFLQ
YGCEWIQEAFQAAARATRETLAGACRGLWRVLERIGRGILAVPRRIRQGAEIA*LL*

Figure 2. Amino acid sequence of the CT. The ID and the LLP-1 are in black and the amino acids subsitued in italics . The proline show in red is the last amino acids found in cultured strain.

Results

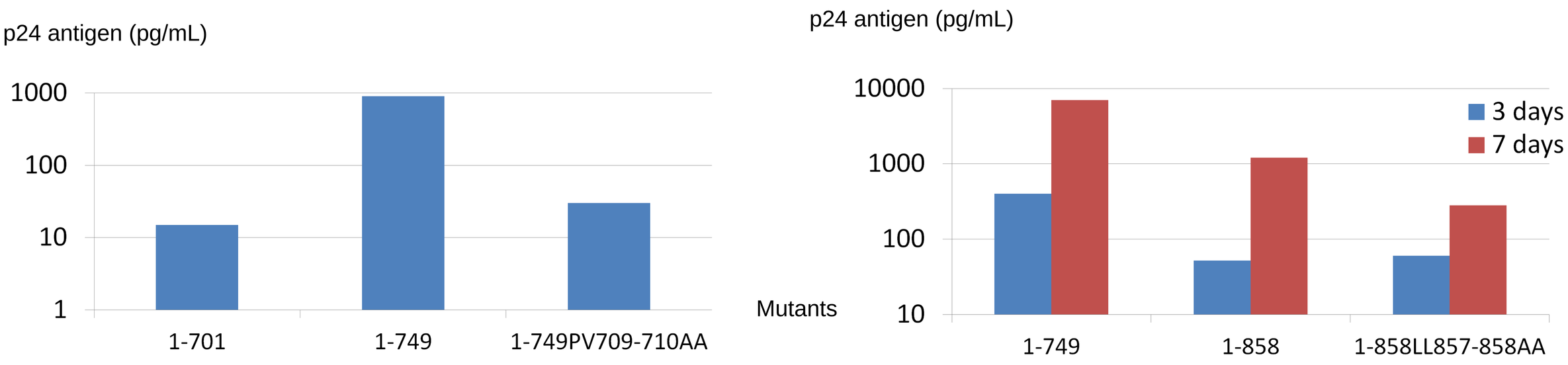

Figure 3. In the left, p24 antigen level after 3 days of infections of the mutants RODgpTM1-701, -749 and -749PV709-710AA. In the right, p24 antigen level after 3 and 7 days of infection in another experiment of the mutant 1-749, -858 and 858LL857-858AA. No viruses were produced after 7 days.

Conclusions

• An HIV-2 ROD *in vitro* variant with a small Env C-Terminal part (CT length = 49 aa ; RODenv1-749) replicates significantly better *in vitro* than the *in vivo* wild type strain (CT length = 158 aa; RODenvTM1-858) and than a virus without CT (RODenvTM1-701). However, a substitution of only two aa inside of the 49 aa of the RODgpTM1-238 cytoplasmic tail (CT) decrease significantly the viral replication at a level nearly identical to a virus without CT. Interestingly, the effects of the modification on viral replication can be seen already in early viral production.

• The CT wild type ROD viral strain delay its viral production compared with other virus. However, when we subsitute the two last aa of the CT (LL) of the RODenvTM1-758 virus, we abolished the late viral production while keeping the early low production.

•Two aminos acids sequences namely the internalisation domain and the di-leucine motif of the Env HIV-1 both play a role in the Env intracellular transport. We show here that these sequences are mandatory for viral replication, but acts at two different times. Those observations led us to hypothetize that these regions has independent roles during protein transport.

Bibliography

Figure 1 was inspired by the figure from **:** C. J. Heap, S. A. Reading and N. J. Dimmock. 2005. An antibody specific for the C-terminal tail of the gp41 transmembrane protein of human immunodeficiency virus type 1 mediates post-attachment neutralization, probably through inhibition of virus–cell fusion. J Gen Virol. 86 :1499-507.