Disruption of a RNA Secondary Structure in HIV-1 gp41 Induces Viral Lethality

Ana Jordan-Paiz*[1], Maria Nevot[1], Kevin Lamkiewicz[2], Marie Lataretu[2], Sandra Franco[1], Manja Marz[2], Miguel Ángel Martínez[1]

[1] IrsiCaixa AIDS Research Institute, Hospital Universitari Germans Trias i Pujol, Badalona, Spain
[2] RNA Bioinformatics & High-Throughput Analysis Jena, Friedrich Schiller Unibersity, Jena, Germany

*ajordan@irsicaixa.es

CROI 2020 Conference on Retroviruses and Opportunistic Infections
Boston, MA
March 8-11, 2020

0180

BACKGROUND

Due to the redundancy of the genetic code, it can be found that for one amino acid, some codons are more frequently used than others. Moreover, this codon usage differs among different species. Based on this, synonymous genome recoding has been widely used as a tool to attenuate several viruses. However, the exact mechanism of attenuation remains unknown.

OBJECTIVE

We aimed here to explore the impact of synonymous codon usage on HIV-1 Env expression and virus repication capacity.

Severe reduction in **Env protein translation** and **viral replication capacity** are observed after synonymous changes in **an evolutionary conserved local RNA secondary structure.**

CONCLUSIONS

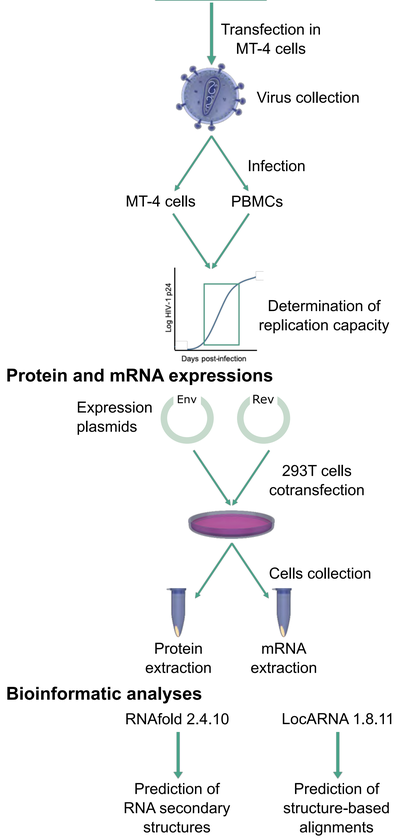
We show here that codon usage of the HIV-1 env strongly impacts the replication capacity of the virus. Moreover, synonymous recoding has allowed to disrupt an evolutionary conserved local RNA secondary structure leading to severe reduction in mRNA translation and virus replication capacity.

This study has been funded by the Spanish Ministry of Economy, Industry and Competitiveness (MINECO) and the European Regional Development Fund (FEDER) under agreement.

METHODS

HIV-1 envelope recodification





Protein and mRNA expressions

Bioinformatic analyses

RESULTS

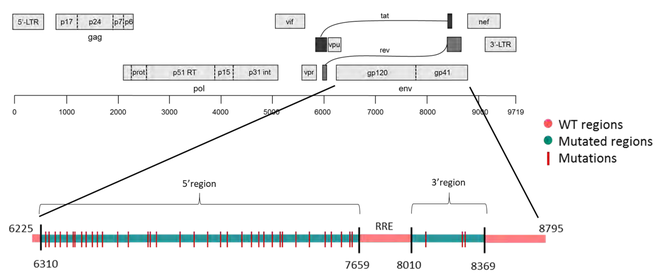


Figure 1. Schematic representation of the mutations in the Recoded_env. A total of 39 mutations were introduced, affecting 34 codons. 36 of the mutations are in the region before the RRE (5'region) and the other 3 are after the RRE (3'region). Transfection of Recoded_env resulted in lethal phenotype.

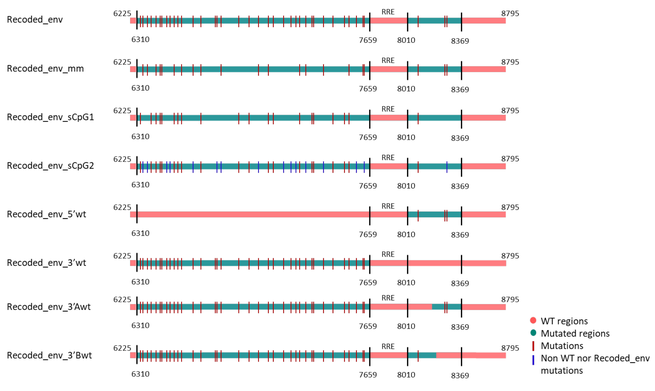


Figure 2. Schematic representation of the distribution of the mutations for the different variants. New variants were designed to know which mutations were responsible for the lethal phenotype. After transfection, Recoded_env_3'Awt did not grow. Moreover, Recoded_env_mm and Recoded_env_5'wt generated new mutations in culture (not shown). Remarkably, Recoded_env_3'Bwt was able to replicate by only reverting two mutations, corresponding to codon 34.

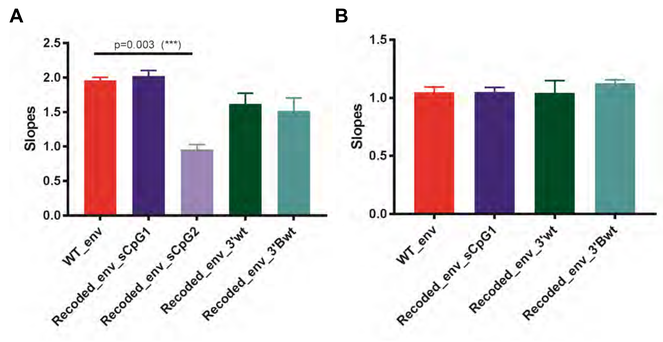


Figure 3. Replication capacity curves of the obtained viruses. A) Replication capacity in MT-4 cells. Recoded_env_sCpG2 had lower replication capacity when compared to WT_env. Remarkably, this variant had one mutation in the modified codon 34. B) Replication capacity in PBMCs. No differences were observed between the different variants when compared to WT_env.

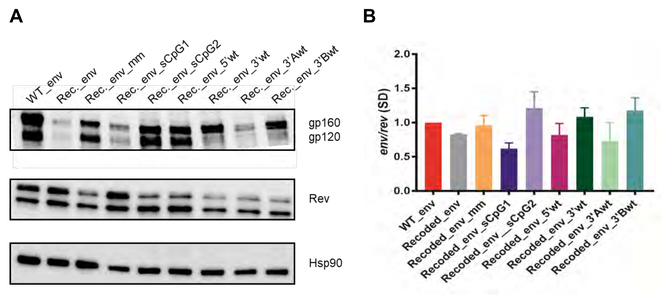


Figure 4. A) Protein expression from 293T cells transfection. Reduced protein production was observed in Recoded_env and Recoded_env_3'Awt, matching their lethal phenotypes. B) mRNA expression from 293T cells. No significant differences were observed.

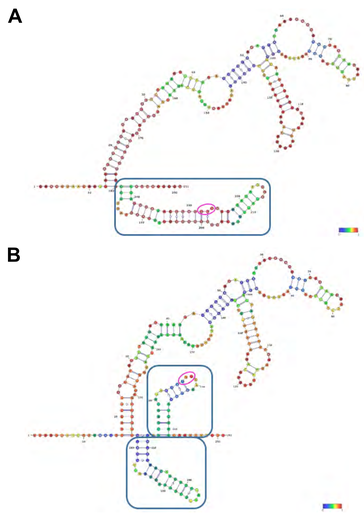


Figure 5. RNAfold probability predictions of the RNA secondary structures of the 3'region partial sequence containing codon 34 (circled in pink). Recoded_env mutations in codon 34 disrupted the WT RNA secondary structure (circled in blue). A) WT_env partial sequence. B) Recoded_env partial sequence.

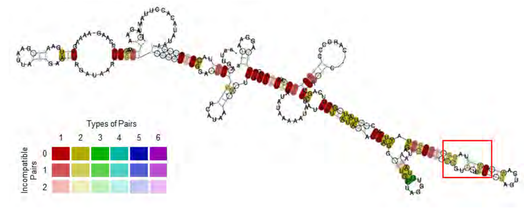


Figure 6. LocARNA prediction for the RNA secondary structure of HIV-1 subtype B sequences alignment containing codon 34 (in red). Sequence of the partial alignment begun in position 1901 and finished in 2201 of *env*. HIV-1 subtype B sequences alignment demonstrated that WT RNA structure is conserved.