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Synonymous Recoded Env Gene Induce Lethality and Loss of Protein Expression in HIV-1

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BACKGROUND

During HIV-1 replication, genes can be divided into two groups depending on its temporal expression. The viral envelope (Env) gene protein is expressed late in the virus life cycle, and its mRNA exportation to the cytoplasm and expression need the early virus expressed transinducer Rev. Rev joins to a specific region in the envelope of the incompletely spliced mRNA called Rev Responsive Element (RRE).

The genetic code is redundant and codon usage differs among different species. The codon usage of HIV-1 Env transcripts is different from that of the host human cell. It has been demonstrated that protein expression is affected by the presence of "rare" codons.

OBJECTIVE

We aim here to explore the impact of synonymous codon usage on HIV-1 ENV expression and virus replication capacity.

METHODS

HIV envelope recodification and virus recovery. Six codons (AGG, GAG, CCT, ACT, CTC, and GGG) in HIV-1 gp160 were changed to CGT, GAA, CCG, ACG, TTA, and GGA, respectively (as described by Shin et al, PNAS 2015)[2] , obtaining a HIV envelope (Recoded_env) with 39 mutations (1,5% of gp160). To obtain viral particles, the different parts of HIV-1 genome were PCR amplified and transfected in MT-4 cells. Viral replication was quantified by measuring HIV-1 capsid p24 antigen production in the culture supernatant.

Protein expression. WT and Recoded-env genes were introduced in an expression vector (pcDNA3.1) with a splicing donor (SD) sequence before the ATG and, together with the Rev gene, were transfected in 293T cells. Protein extraction was performed 48 hours after transfection. Western Blot assay was performed to detect envelope expression, using 2F5 antibody against gp41. Rev was detected with Anti-V5 antibody. Hsp90 detection was used as cellular control.

mRNA relative quantification. Relative real-time PCR analysis was performed from transfected 293T cells. Its mRNA was extracted and DNase treated before analysis. A TaqMan probe against RRE was used to detect the envelope´s mRNA. GAPDH probe was used as cellular control.

Sequence analysis: Viral RNA was extracted from supernatant cultures. RNA was reverse transcribed and the consensus sequence of the amplified population was obtained by Sanger.

RESULTS

1.Recoded_env has 39 synonymous mutations, with no changes in the amino acid sequence. The RRE is the region where Rev joins and it is essential for viral viability, so it was not mutated. The beginning and the end of the envelope were not mutated neither, since they overlap with other reading frames.

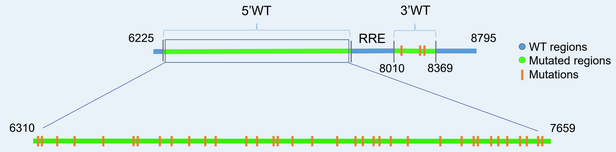


Figure 1. Schematic representation of the mutations in the Recoded_env. Both WT and mutated regions are shown in different colors. 36 of the mutations are in the region before the RRE (5'region). The other 3 mutations are after the RRE (3'region).

After transfection, we observed that Recoded_env was **lethal**. After five blind passages, there was neither detection of p24 nor syncitia.

2. Western blot analysis of Env expression in 293T cells revealed that, in contrast to WT, the Recoded-env mRNA protein was not being translated although there were not significant differences in Env mRNA production.

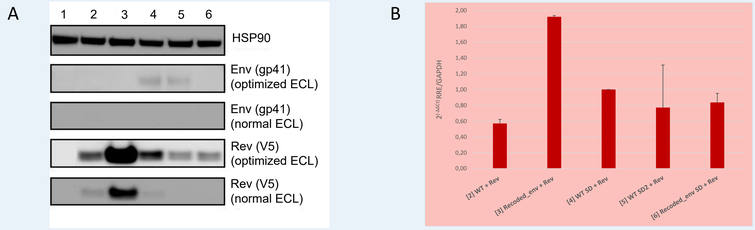


Figure 2. A) Western blot analysis of protein expression after transfection of 293T cells ([1] Negative control (no Env, no Rev). [2] WT + Rev (no SD). [3] Recoded-env + Rev (no SD). [4] WT SD+ Rev. [5] WT SD2 + Rev. [6] Recoded-env SD + Rev). **B)** mRNA analysis by qPCR after transfection in 293T cells.

3. Additional mutants were designed to see which mutations were responsible for this phenotype, without modifying WT_env codon usage (CAI) or codon pair bias (CPB). New mutants included changes in total number substitutions and/or CpG content [4].

Table 1. Resume of the additional designed mutants.

Seq. name	Recovery (days after transfection)	Nº mut	CAI	CPB [a]	CpG [b]	TpA [b]
WT_env	4	-	0,1368950	0,046	26	194
Recoded_env	-	39	0,1315866	0,048	42	192
Recoded_env_mm	8	21	0,1340826	0,046	35	193
Recoded_env_sCpG	5	20	0,1295865	0,053	26	196
Recoded_env_sCpG2	10	36	0,1291150	0,055	26	194
Recoded_env_5'WT	9	3	0,1360499	0,047	27	193
Recoded_env_3'WT	11	36	0,1320015	0,046	41	193

4. All of these new mutants were replicative and were recovered at different days after transfection. Syncitia was observed in cell culture and p24 was detected in their supernatant.

The day of recovery may give an approach of how they could replicate. However, replication assays need to be performed.

Remarkably, the number of synonymous mutations in the Recoded_env_5'WT variant was much lower than in the Recoded_env_3'WT.

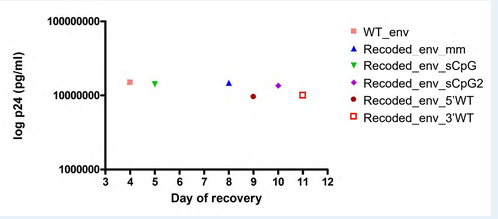


Figure 3. p24 amounts of the different recoded envelopes after viral recovery. Quantities are given in pg/ml.

	A	B
WT_env	GAGTTAGGCAGG	GAGTTAGGCAGG
Recoded_env_mm	.....C.T....	.....C.T.G..
Recoded_env_sCpG	.....	.....
Recoded_env_sCpG2	.....A....	.....A....
Recoded_env_5'WT	.....C.T....	.....

Figure 4. Partial nucleotide alignment of the 3' region of the envelope. **A)** Original design of the sequences of the mutated envelopes. **B)** Sequences generated by the mutated viruses after transfection.

5. After collection of the supernatant of each replicative virus, RT-PCR of the envelopes was performed.

Sequence analysis of the recovered viruses revealed several changes in the 3' region of the envelopes.

CONCLUSIONS

We show here that the HIV-1 replication capacity is affected by the codon usage. Our results also indicate that mutations in the 3' coding region of Env (gp41) can lead to lethality. Ex vivo expression experiments demonstrated that Env mRNA translation was affected. However, it remains to be elucidated whether the interaction of Env mRNA and Rev is affected. Overall, our results emphasize the relevance of synonymous substitutions in shaping virus phenotype.

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