

FEW MUTATIONS IN THE 5' LEADER REGION MEDIATE FITNESS RECOVERY OF DEBILITATED HUMAN IMMUNODEFICIENCY TYPE 1 VIRUSES.

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Abstract. We recovered viral fitness in 4 debilitated HIV-1 clones by repeated transfers of large virus populations. Viral fitness increased in just 11 large population passages and in one case increased from a pre-extinction viral passage. Analysis of the entire genomic nucleotide sequences of these populations showed that few mutations, from two to seven per clone, mediated fitness recovery. Strikingly most of the mutations accumulated during fitness recovery were located in the 5'-untranslated leader region of the genome and more specifically in the primer-binding-site (PBS loop). Mutations appearing in coding regions were mainly non-synonymous mutations (75%). Also 25% of the mutations were reversions. This fact together with the presence of non-synonymous mutations could indicate a strong positive selection for optimal HIV-1 replication *in vitro*.

Background. Human immunodeficiency virus type 1 (HIV-1) manifested a drastic fitness loss after a limited number of plaque-to-plaque transfers in MT-4 cells (6,7). The decrease in fitness due to repeated bottlenecks has been interpreted as the result of Muller's ratchet effect (4). Losses were drastic and rapid when compared with the fitness losses experienced by other RNA viruses (5). We now show the increase in fitness of four debilitated HIV-1 clones by repeated large population passages (Figures 1, 2 and Table 1).

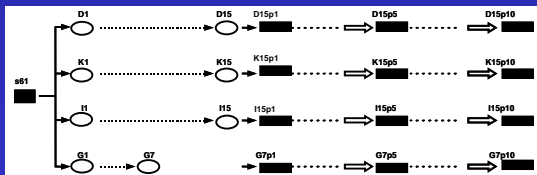


FIGURE 1. Scheme of passages of HIV-1 clones subjected to plaque-to-plaque transfers (open circles) and to high population passages (filled rectangles) in MT-4 cells. HIV-1 clones are indicated by letters followed by a number that gives the total number of plaque-to-plaque transfer and p1 to p10 indicating the number of high population transfers. In total 26 passages were carried out.

Methods. Large virus populations passages were carried out in 5x10⁶ MT-4 cells with a multiplicity of infection of 0.1 PFU/cell. Fitness determination of the viral populations were performed by growth competition experiments with a reference clone. Quantification of viruses was achieved by a Heteroduplex Tracking Assay (HTA). Complete genome sequences were determined on the two cDNA strands from cultures supernatant. Viral RNA was extracted, and amplified using RT-PCR and a nested PCR.

Results. Comparison of the complete genomic nucleotide sequences of initial and final viral populations showed that few mutations, from two to seven per clone, mediated fitness recovery. Eight out of the 20 mutations affected coding regions, mainly by the introduction of non-synonymous mutations (75%). 25% of the overall mutations observed were reversions. Most of the mutations accumulated during fitness recovery (12 out of 20) were located in the 5'-untranslated leader region of the genome and more specifically in the primer-binding-site (PBS) loop (3) (Figure 3). Two of the viruses incorporated the same mutation in the primer activation signal (PAS) in the PBS loop which is critical for tRNA^{Lys}-mediated initiation of reverse transcription (1, 2).

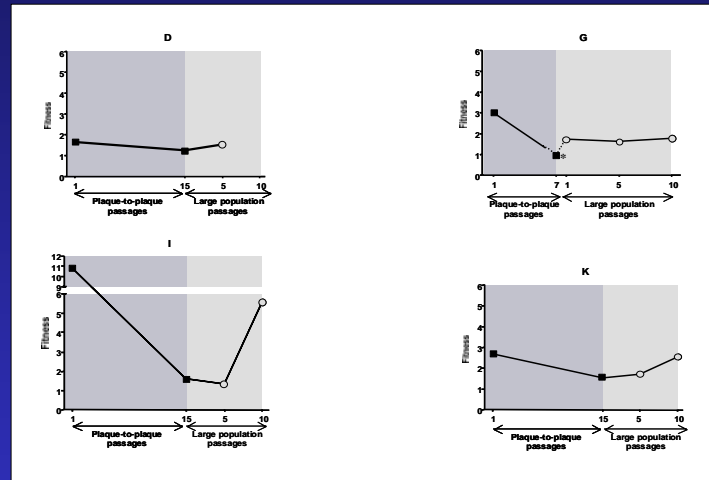


FIGURE 2. Fitness evolution of each clone during plaque-to-plaque and large population passages. Fitness values were calculated from the exponential slope of the corresponding fitness vectors (7). Plaque-to-plaque transfers are indicated by filled squares and high population passages are indicated by open circles. Fitness of clone D15p10 could not be calculated. The asterisk indicates a debilitated clone (G7) that could not produce plaques over plaque-to-plaque transfer 7. The fitness of this clone could not be determined by competition passages.

Clone	Final plaque to-plaque population ^a	Fitness values			Maximal fitness increase ^b
		Large population passages			
		1	5	10	
D	1.23	n.d.	1.57	n.d. ^c	0.34 (+28%)
K	1.54	n.d.	1.70	2.5	n.d. (+62%)
I	1.61	n.d.	1.32	5.64	4.03 (+250%)
G	<0.001^d	1.73	1.61	1.78	1.78 (+178000%)

TABLE 1. Fitness values of clones after 15 plaque-to-plaque transfers and after 1, 5 and 10 high population passages.

^a All clones were plaque passaged fifteen times except clone G that went to extinction in passage seven.

^b Maximal fitness increase determined between the plaque-to-plaque and large population passages. The values used for the comparison are marked in bold. Values in brackets are in percentage for all clones.

^c We were unable to determine the fitness of clone D15p10. This clone displayed a mixture of variants and the heteroduplex tracking assay after the competition passages could not be analysed.

^d Limit of detection.

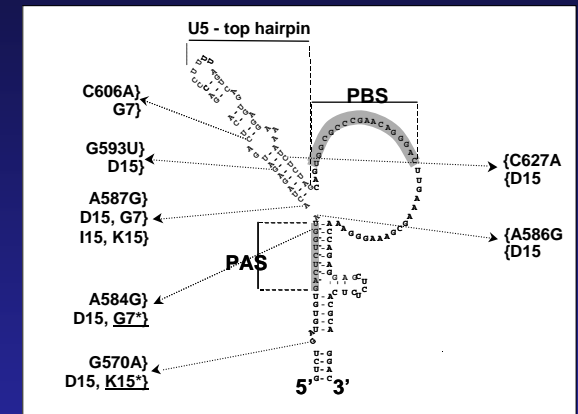


FIGURE 3. Localization of the mutations accumulated in the U5-PBS loop during large population passages. The PAS and PBS sequences are shadowed. Secondary structure model for the U5-PBS leader region of HIV-1 of LAI sequence modified from (2). The location of the primer-binding-site and the primer activation signal is indicated. The positions for the mutations accumulated during fitness recovery in all the final p10 viral populations are marked. Watson-Crick base pairing are marked with dashes. Clones underlined and with asterisks indicate reversions to the sequence present before fitness loss.

Conclusions.

- Large population passages promoted fitness recovery of four debilitated HIV-1 populations.
- Mutations tend to occur predominantly in the 5'-untranslated leader region (PBS loop).
- Two convergent mutations in the evolving populations were found.
- The high frequency of reversions, non-synonymous mutations and unequal distribution of mutations indicate the operation in this experimental conditions of a strong positive selection acting on HIV-1.

References.

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