

GAG NC/P1 PROTEASE RESISTANCE MUTATIONS CAN CAUSE SELECTION OF ADDITIONAL NC/P1 CHANGES TO OPTIMIZE CLEAVAGE EFFICIENCY AND REPLICATIVE CAPACITY

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BACKGROUND: Substrate-based protease inhibitor (PI) resistance due to mutations in NC/p1 is caused by an enhanced processing of the *gag* protein. We investigated the effect of enhanced *gag* processing due to NC/p1 resistance mutations on replicative capacity (RC) and the consequences for evolution in the absence of PI pressure.

METHODS: A set of four recombinant viruses containing NC/p1 mutations conferring different levels of PI resistance were generated: HXB2^{431V}, HXB2^{437V}, HXB2^{437T} and HXB2^{436E+437T}. To investigate the effect of enhanced *gag* processing on RC, viral replication curves were generated. To investigate the potential evolutionary pathways in the absence of PI pressure, multiple individual *in vitro* evolution experiments were performed, after which complete Gag and protease were sequenced. From the viruses that were selected, RC, *gag* processing (quantitative immunoblot analysis) and PI susceptibility (MTT assay) were assessed.

RESULTS: Single NC/p1 mutants that displayed only a slight increase in PI resistance did not show an obvious change in RC compared with wild type. This was also reflected in the *in vitro* evolution experiments where the single NC/p1 mutants showed no signs of evolution, with the exception of the selection of A429K in 1/5 experiments for HXB2^{431V}. In contrast, the double NC/p1 mutant (HXB2^{436E+437T}), which displayed a clear increase in processing efficiency and PI resistance, also demonstrated a clear reduction in RC. Interestingly, in all evolution experiments, amino acid changes in/near the NC/p1 site were observed (2/5, -436E; 2/5, +435R; 1/5, +438R). These selected changes restored the processing efficiency and RC. Furthermore, it was observed that due to the normalization of *gag* processing efficiency, in parallel, PI susceptibility returned to wild-type level.

CONCLUSIONS: The results from this study clearly demonstrate that there is an optimum rate for HIV-1 *gag* cleavage. When enhanced *gag* processing due to PI resistance mutations in NC/p1 reduces RC, HIV-1 can modulate the NC/p1 sequence by selection of additional changes to restore *gag* cleavage and RC.

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