

**POSTER 23**

## SELECTION OF THE HIV-1 DIMERIZATION INITIATION SIGNAL IN CELL-BASED SYSTEMS

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One HIV-1 particle contains two copies of viral genome in a dimeric form. The dimerization initiation signal (DIS), a 6-nt sequence located toward the 5' end of the viral genome, is thought to be the point where the two RNA molecules initiate dimerization. Most HIV-1 strains contain either GCGCGC or GTGCAC in their DIS sequences. We sought to delineate the selection pressure exerted on the HIV-1 DIS during virus replication. To approach this experimental question, we generated a library of replication-competent subtype B HIV-1 with randomized DIS sequences. We then infected target cells with viruses generated from the library and monitored the DIS sequences of the virus population during various passages. Our randomized DIS library contains 20,000 independent clones, which should cover most of the possible 4096 ( $4^6$ ) sequences. We have sequenced 300 clones from the library; our results confirmed the complexity of the library and we did not observe the wild-type DIS sequence (GCGCGC), suggesting that this sequence occupies a very small portion of the library. We have performed the selection in a T cell line and in PBMC for multiple passages between 100 to 200 days. In the initial passages, multiple random sequences were present in the DIS sequences of the viral population. After several passages, the viral populations had fewer variants, and variants with certain DIS sequences were amplified whereas others were diminished. The wild-type DIS is among the amplified sequences; additionally, there are other sequences that are selected and sustained at high efficiency. Most of the amplified non-wildtype sequences contained GC-rich palindromes, indicating such sequences are selected in DIS. Preliminary studies comparing these selection conditions indicated that there are similarities and differences among virus populations generated from these two target cells. These results reveal the selection pressure and constraints of the HIV-1 genome during virus replication.

\*Equal contribution