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Nuclear trafficking of hiv-1 pre-integration complexes in living cells

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HIV-1 viral particles engineered to incorporate integrase fused to EGFP (HIV-IN-EGFP) have proven effective to study pre-integration complexes (PICs) within nuclei of infected cells [1]. In this study we now report the live imaging analysis of nuclear PICs obtained by time-lapse microscopy.

By monitoring IN-EGFP labeled complex movements within nuclei of infected cells we have been able to follow their trajectories in three dimensions. We observed PICs moving within the nuclear compartment, as well as crossing the nuclear envelope from the outer nuclear volume. Movement within the nucleus was observed primarily within regions of decondensed chromatin. To characterize these movements we calculated the mean squared displacement (MSD) of 5-minute long segment of intranuclear trajectories. In summary we obtained results indicating that 75.2% of PICs displayed active-type movement at some point during the 30 minutes of acquisition, while the remaining 24.8% exhibited only free or constrained diffusion.

To gain insights into the underlying mechanism of the active transport of the IN-EGFP labeled complexes in the nucleus we tested if the active movements transport observed was mediated by actin filaments. To this end, HIV-1 trafficking was analyzed in cells treated with a drug inhibiting actin polymerization (latrunculin B). Preliminary studies revealed that treated cells showed a decrease in the number of PICs moving by active transport (47%),

indicating that disruption of actin function impairs the active nuclear transport of HIV PICs.

In conclusion, here we report a first detailed kinetic analysis of PICs trafficking inside the nuclear compartment. The results show that the movement of HIV PICs can take place by active transport. Because it is known that HIV shows a preference for integration into active genes these observations suggest that HIV utilizes an actin based transport mechanism to actively seek these preferred sites of integration. We believe that the identification of nuclear factors involved in HIV PIC trafficking will shed light on still unexplained aspects of HIV-1 nuclear biology.

References

1. *Plos One*. 2008, **3**(6):e2413.