

# Dynamics of HIV-1 Recombination in its Natural Target Cells.

David N. Levy<sup>†</sup>, Grace M. Aldrovandi<sup>†§</sup> and George M. Shaw<sup>†\*</sup>

<sup>†</sup>Department of Medicine, <sup>†</sup>Department of Pediatric Infectious Disease, The University of Alabama at Birmingham, and <sup>\*</sup>The Howard Hughes Medical Institute, Birmingham, AL

<sup>§</sup>Current address: Department of Pediatrics, Children's Hospital of Los Angeles, CA

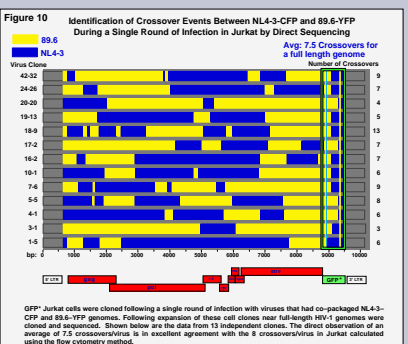
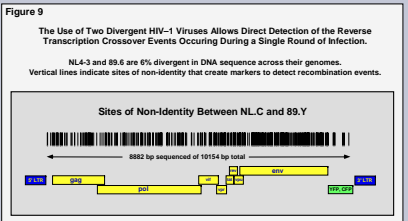
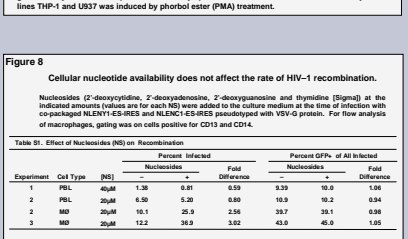
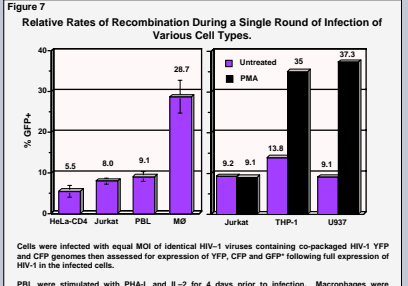
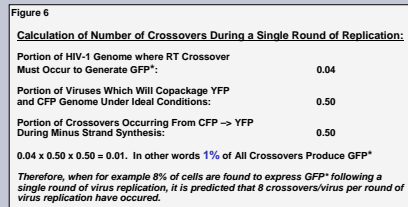
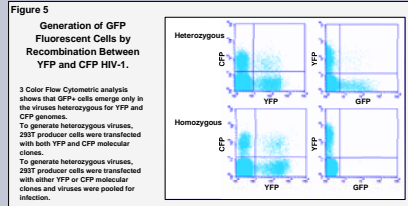
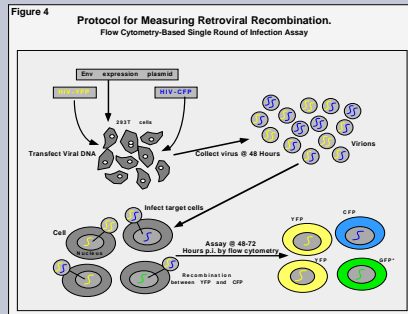
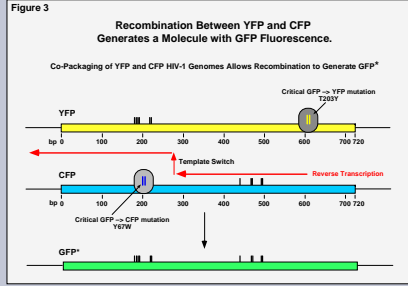
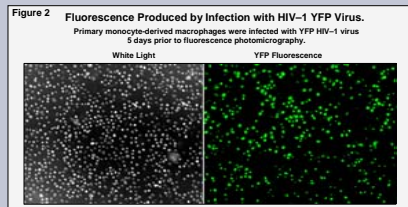
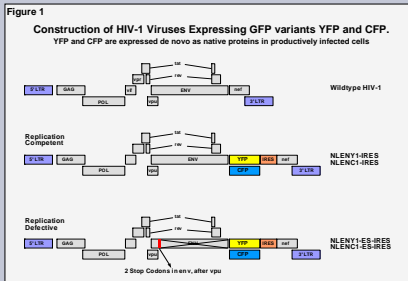
**Background:** Extensive diversification and rapid evolution are hallmarks of HIV infection. Recombination aids HIV diversification, confounding vaccine development and fostering the emergence of variants resistant to multidrug therapy. A better understanding of recombination is crucial, yet recombination rates during infection of relevant primary targets of HIV infection are unknown. Important aspects of HIV-1 recombination remain poorly defined, including the propensity of HIV-1 to productively infect cells with more than one virion (a necessary precondition for recombinational diversification) and the frequency of recombination events during infection of the natural target cell population.

**Methods:** Because prior methods for analysis of recombination in tissue culture have employed antibiotic drug resistance markers to select and enumerate infected cells, these studies have been limited to single round infection of fibroblastic cell lines. We have developed methods using HIV-1 reporter viruses bearing genes for Yellow and Cyan Fluorescent Proteins (Figure 1-2) which allows immediate and simultaneous quantification by flow cytometry of the rate of virus infection, co-infection and recombination within any cell type, including primary T cells and macrophages as well as the SCID-hu (ThyLiv) mouse model. The principle of the method is that recombination between incorporated YFP and CFP genes generates a GFP variant called GFP\* (Figure 3-5). The proportion of cells expressing GFP\* can then be used to calculate the frequency of recombination within the viral population (Figure 6).

**Results:** 1. Frequency of HIV-1 recombination is profoundly influenced by the infected cell. The frequency of recombination in T cells is nearly twice the frequency in HeLa-CD4 fibroblasts. Infection of primary monocyte-derived macrophages results in a frequency of recombination that is 3-4 times the frequency in primary and transformed T cells (Figure 7-10). 2. Differentiation of two monocytic cell lines, THP-1 and U937 results in an enhancement of recombination rates from levels observed in T cells to those found in primary macrophages (Figure 7). 3. Multiround infection assays in primary CD4+ T cells and in SCID-hu (ThyLiv) mice reveal that co-infection of cells proceeds according to nearly the square of the infection frequency, indicating little functional inhibition of co-infection (Figure 11). 4. Since co-infection is a necessary prerequisite for recombinational diversification, the generation of recombinants also proceeds according to this rule, and independently of target cell numbers, input MOI, the number of rounds of viral replication. Nearly identical results are obtained in both the tissue culture and SCID-Hu systems, suggesting that generally applicable relationships are described.

**Conclusions:** If operative in human infection, these results suggest that increases in viral load may confer a compounding increased risk of virus escape via recombinational diversification. We postulate that lentiviruses have evolved high rates of recombination in order to optimize diversification for the exploitation of evolutionary niches and to facilitate immune escape. Since macrophages are among the first cells infected, high recombination in this cell type may aid establishment of diversity early in infection.

Levy et al., 2004 PNAS in press.



## Kinetics of HIV-1 replication, multiple infection and recombination in multiround infection.

