

HIGH EFFICIENCY OF HIV-1 GENOMIC RNA PACKAGING AND HETEROZYGOTE FORMATION REVEALED BY SINGLE VIRION ANALYSIS

Jianbo Chen¹, Olga Nikolaitchik¹, Jatinder Singh^{2,4}, Andrew Wright², Craig E. Bencsics^{2,5}, John M. Coffin², Na Ni¹, Stephen Lockett³, Vinay K. Pathak¹, and Wei-Shau Hu¹

¹HIV Drug Resistance Program, National Cancer Institute, Frederick, MD 21702; ²Department of Microbiology, Tufts University, Boston, MA 02111; ³Optical Microscopy and Analysis Laboratory, Science Application International Corporation, Frederick, MD 21702; ⁴Present address: Department of Biomedical Sciences and Pathobiology, Virginia-Maryland College of Veterinary Medicine, Virginia Tech, Blacksburg, VA 24061; ⁵Present address: Department of Medical Oncology, Dana Farber Cancer Institute, Boston, MA 02111

A long-standing question in retrovirus biology is how RNA genomes are distributed among virions. In the studies presented in this report, we addressed this issue by directly examining HIV-1 RNAs in virions using a modified HIV-1 genome that contained recognition sites for BglG, an antitermination protein in the *Escherichia coli bgl* operon, which was coexpressed with a fragment of BglG RNA binding protein fused to a fluorescent protein. Our results demonstrate that the majority of virions (>90%) contain viral RNAs. We also coexpressed HIV-1 genomes containing binding sites for BglG or the bacteriophage MS2 coat protein along with 2 fluorescent protein-tagged RNA binding proteins. This method allows simultaneous labeling and discrimination of 2 different RNAs at single-RNA-detection sensitivity. Using this strategy, we obtained physical evidence that virions contain RNAs derived from different parental viruses (heterozygous virions) at ratios expected from a random distribution, and we found that this ratio can be altered by changing the dimerization sequences. Our studies of heterozygous virions also support a generally accepted but unproven assumption that most particles contain 1 dimer. This study provides answers to long-standing questions in HIV-1 biology and illustrates the power and sensitivity of the 2-RNA labeling method, which can also be adapted to analyze various issues of RNA biogenesis including the detection of different RNAs in live cell imaging.