

POSTER 24**HIV-1 NONSECRETORY *gag* mRNA PARTITIONS TO MEMBRANE-BOUND RIBOSOMES IN A SIGNAL SEQUENCE-INDEPENDENT AND TRANSLATION-DEPENDENT MANNER**

Amit Sharma¹, Eric O. Freed², Kathleen Boris-Lawrie¹

¹Department of Veterinary Biosciences and Molecular Genetics, The Ohio State University, Columbus, OH 43210; ²HIV Drug Resistance Program, National Cancer Institute, Frederick, MD 21702

All eukaryotic cells exhibit partitioning of mRNAs between the cytosol and endoplasmic reticulum (ER) compartments. Enrichment of mRNAs encoding secretory and integral membrane proteins on ER-bound ribosomes is directed by the signal recognition particle (SRP) pathway that recognizes signal sequence-bearing proteins in the cytosol. The mRNA/ribosome/nascent polypeptide chain complex is trafficked to the ER for completion of polypeptide synthesis.

We have identified HIV-1 *gag* mRNA that encodes nonsecretory protein can be synthesized on ER ribosomes. Polysome analysis and fractionation experiments show that a significant fraction of HIV-1 *gag* mRNA partitions to ER-associated ribosomes and the *gag* mRNA/ER ribosome complexes are translationally active. siRNA experiments determined that ER targeting of *gag* mRNA occurs independently of the canonical signal recognition particle (SRP) pathway, whereas *env* mRNA is SRP-dependent. Mutagenesis was used to identify the minimal target sequence in the HIV-1 genome necessary for ER targeting of *gag* mRNA. The *gag* mRNA targeting to ER is not changed by deletion of the 5' untranslated region. By contrast, *gag* mRNA targeting to ER is disrupted by mutagenesis of specific residues of the Gag polypeptide, including the conserved N-terminal myristylation signal. Our results indicate *gag* mRNA targeting to the ER is not directed by a 5' UTR RNA zipcode, but is instead dependent on translation of residues of the *gag* open reading frame. Results will be presented on the relationship between *gag* mRNA ER targeting and the outcome of the virus assembly process; specifically packaging, release and/or infectivity. Our results corroborate a growing body of evidence that subsets of cellular mRNAs are non-canonically partitioned to the ER and implicate an evolutionarily conserved, non-canonical novel translation control mechanism.