

TRAFFICKING AND DIMERIZATION OF HIV-1 RNA: INVESTIGATIONS INTO ITS SUBCELLULAR LOCALIZATION AND EXPORT REQUIREMENTS

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HIV-1 virions contain two copies of genomic RNA held together as a dimer. Our previous genetic studies demonstrated that HIV-1 genomic RNA must be copackaged into virions as a dimer, not as two monomers. Thus, dimerization of HIV-1 genomic RNA occurs prior to virus budding at the plasma membrane. However, the subcellular localization of the initial RNA-RNA contact and dimerization has yet to be defined. One consequence of packaging two copies of viral RNA in one particle is that frequent recombination can occur during reverse transcription of the viral genome, although only progeny produced from particles packaging RNA from two different viruses can generate recombinants with genotypes distinct from that of the two parents. Therefore, we use recombination as a tool to probe the HIV-1 dimerization process. Using an HIV-1 envelope-driven cell fusion system, we measured the recombination rate between two viruses integrated within different nuclei. The level of recombination observed was similar to that between two viruses integrated within the same nuclei. This result demonstrates that the majority of HIV-1 RNA dimerization occurs within the cytoplasm. We further investigated the importance of the transport pathway used to exit the nucleus on the subsequent ability of two RNAs to copackage and therefore recombine. We created a Rev-RRE-independent HIV-1 virus that instead uses the constitutive transport element (CTE) from Mason-Pfizer monkey virus for RNA transport. This results in the genomic RNA of the CTE virus being exported through the Tap/Nxf1 pathway instead of the usual Crm1 pathway. The recombination rate between two CTE-dependent viruses was found to be similar to that of two RRE-dependent viruses, indicating that the ability of HIV-1 to randomly assort its genomic RNA is independent of the transport pathway used to exit the nucleus. We are currently studying the ability of the CTE-dependent virus to recombine and therefore dimerize with an RRE-dependent virus. To our knowledge, this study is the first to reveal the subcellular localization of the HIV-1 RNA dimerization process and also provides novel insights into this critical step of HIV-1 replication.