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INSIGHTS INTO THE PACKAGING OF MOUSE MAMMARY TUMOR VIRUS (MMTV) GENOMIC RNA BY IDENTIFYING $PR77^{GAG}$ BINDING SITES INVOLVED DURING ITS SELECTIVE ENCAPSIDATION

by

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Virtual: Click here to join the meeting

Abstract

Selective encapsidation and/or packaging of retroviral genomic RNA (gRNA) by Gag during retrovirus assembly is a crucial step for generating infectious virus particles. Despite having been studied extensively, the mechanism by which the retroviral Gag precursor selects and packages the retroviral genome remains largely unclear. Therefore, to understand the molecular mechanism(s) of mouse mammary tumor virus (MMTV) gRNA packaging, as a first step, we expressed full-length recombinant $Pr77^{Gag}$-His_6-tag fusion protein in bacteria. The recombinant Gag protein was then purified from the soluble fractions of bacterial cultures using immobilized metal affinity chromatography (IMAC) and size exclusion chromatography (SEC). The purified recombinant $Pr77^{Gag}$-His_6-tag protein retained the ability to assemble *in vitro* into virus-like particles (VLPs). In parallel, the VLPs made *in vivo* following expression of the recombinant $Pr77^{Gag}$-His_6-tag fusion protein in eukaryotic cells could package MMTV subgenomic RNAs. Next, RNA binding and footprinting assays using the purified protein and in cell gRNA packaging experiments identified two critical, non-redundant $Pr77^{Gag}$ binding sites. These binding sites include: i) a stretch of purines in a hairpin loop immediately adjacent to the dimerization initiation site (DIS) hairpin, thus forming a bifurcated stem-loop structure and ii) the primer binding site (PBS). Despite the presence of the packaging signals on both unspliced and spliced RNAs, $Pr77^{Gag}$ specifically bound to unspliced RNA, which is the only one that can adopt the native bifurcated stem-loop structure. Together this study demonstrates the minimal packaging elements at both sequence and structural levels required to initiate MMTV gRNA packaging. Unlike purine rich regions, the direct involvement of PBS in retroviral gRNA packaging has not been documented in retroviruses. These findings add to our knowledge of retroviral gRNA packaging and assembly, making it a potential target for novel therapeutic approaches as well as the development of safer gene therapy vectors.

Keywords: Retrovirus, mouse mammary tumor virus (MMTV), RNA-protein interaction, virus assembly, protein expression, RNA packaging, RNA-Gag interactions, $Pr77^{Gag}$, footprinting; purine-rich sequence.