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DELINEATING THE BINDING SITES OF MASON-PFIZER MONKEY VIRUS (MPMV) GAG PRECURSOR POLYPROTEIN ($Pr78^{GAG}$) ON GENOMIC RNA FOR ITS SELECTIVE PACKAGING

by

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Abstract

A key step in retroviral life cycle is the selective packaging of its dimeric RNA genome (gRNA) from a pool of cellular and spliced viral RNAs into nascent virions. This involves binding of the retroviral Gag polyprotein to sequences at the 5' end of the viral genome, the packaging signal. The aim of this study was to identify full-length Gag polyprotein ($Pr78^{Gag}$) binding sites on Mason-Pfizer monkey virus (MPMV) gRNA, a promising candidate for the development of safe human gene therapy vectors. Towards this end, recombinant MPMV $Pr78^{Gag}$-His_6-tagged protein was cloned and expressed in bacterial cells, and purified from the soluble fraction using immobilized metal affinity chromatography (IMAC) followed by size exclusion chromatography (SEC). The biological activity of the purified protein was determined by its ability to assemble virus like particles (VLPs), while its ability to package MPMV specific sub-genomic RNAs was confirmed in eukaryotic cells. Competitive band shift assays demonstrated preferential $Pr78^{Gag}$ binding to unspliced over spliced viral RNA. Further competitive band shift assays were performed using mutants in two purine-rich motifs consisting of a 16-nucleotide stretch of single-stranded purines (ssPurines; U^{191}UAAAAGUGAAAGUAA^{206}) and a partially base-paired purine-rich region (bpPurines; G^{246}AAAGUAA^{253}), previously found to be important for MPMV gRNA packaging. To map the precise $Pr78^{Gag}$ binding sites on the MPMV gRNA, *in vitro* Gag-RNA foot-printing experiments followed by high-throughput selective 2' hydroxyl acylation analyzed by primer extension (hSHAPE) were performed. These revealed that $Pr78^{Gag}$ binds to ssPurines, and the A^{252}AGUGUU^{258} loop, corresponding to two unpaired adenosine residues of the bpPurines and the adjacent region called the "GU-rich region" (G^{254}UGUU^{258}), both of which flank the major splice donor. Hence, ssPurines are present on both the genomic and spliced viral RNAs, while the A^{252}AGUGUU^{258} loop is found only on the gRNA, revealing how MPMV discriminates between genomic and spliced RNAs. Collectively, this study reveals how MPMV $Pr78^{Gag}$ binds in a redundant fashion to the two single-stranded loops (ssPurines and the A^{252}AGUGUU^{258} loop) to bring about selective gRNA packaging over spliced viral RNAs. These results should help in understanding virion assembly and facilitate development of safe and efficient retroviral vectors for human gene therapy.

Keywords: Retroviruses; Mason-Pfizer monkey virus (MPMV); $Pr78^{Gag}$; Genomic RNA (gRNA); Gag/RNA interactions; RNA packaging; Purines; Footprinting; hSHAPE.