Specific Insertions of Zinc Finger Domains into Gag-Pol Yields Engineered Retroviral Vectors with Selective Integration Properties

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The clinical use of retroviruses as vectors raises the concerns of insertional mutagenesis and potential oncogenesis due to genomic integration preferences for transcriptional start sites (TSSs). We have developed retroviral vector systems with altered integration specificity by generating a library of viruses with zinc finger domain (ZFD) inserted at random positions throughout murine leukemia virus Gag-Pol, then selecting for variants that are viable and exhibit selective integration properties. We found seven permissive ZFD insertion sites throughout Gag-Pol. Comprehensive genome integration analysis revealed that several ZFD insertions yielded retroviral variants with integration patterns that did not favor TSSs. Furthermore, two variants strikingly integrated primarily into four common sites out of 3.1x10^9 possible human genome locations (P=4.6x10^-29). Our findings demonstrate that insertion of DNA binding motifs into Gag-Pol can make considerable progress toward engineering safer retroviral vectors.