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II. Retroviruses as mutagens: isolation of revertants with deletions and/or insertions in the ASV provirus in B31 cells after superinfection with MuLV.

The DNA of retroviruses can integrate into many sites in the genomes of their host cells, suggesting that retroviruses might be able to cause mutations by disruption of genes, in the manner demonstrated for Mu-1 bacteriophage and transposable elements of procaryotes. I have tested this possibility by asking whether a non-transforming retrovirus can insert its DNA into a previously-established, transforming provirus and thus inactivate its biological effects. For these experiments, I used selective techniques to obtain revertants of the ASV-transformed rat-1 line B31 following infection at high multiplicity with the Moloney strain of murine leukemia virus. Most of the ca. 35 revertants isolated from the MuLV-infected B31 cells belong to the two classes of spontaneous revertants characterized previously (loss of proviral DNA or mutations in src; see above), but a few exhibited curious lesions in the single ASV provirus present in B31 cells.

ASV proviruses can be denoted "CELL DNA-3'5'-gag-pol-env-src-3'5'-CELL DNA". "3'5'" is a repeated sequence of 300 nucleotide pairs composed of sequences from both ends of viral RNA; the viral genes are written from left to right in their order from the 5' to 3' ends of viral RNA. All of the observed lesions involved regions of provirus less than 1500 nucleotides from the left hand end. In one case, approximately 10^6 daltons of the left end of the provirus was deleted, and 5-6x10^6 daltons of new DNA was inserted. In the second case, no deletion was evident by restriction mapping, but an insert of 5-6x10^6 daltons was located about 500 bases from the left end of the provirus. In the third case, no insertion was identified, but ca. 1.1x10^6 daltons of DNA, including the left end of the provirus and flanking cellular DNA, was deleted. Studies in collaboration with N. Quintrell (University of California, San Francisco) have demonstrated that these lesions interfere with the transcription of the ASV provirus, including the src gene, by either removing or interrupting signals for synthesis and/or processing of viral RNA.

I am now attempting to define the nature of the insertions and the boundaries of the deletions in these three cases and to explore the significance of the localization of these lesions to the left end of the ASV provirus.