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I. The rare revertants of an ASV-transformed rat cell have lost proviral DNA or bear mutations in src.

The molecular events responsible for reversion of cells from a transformed to a normal phenotype may provide important clues to an understanding of oncogenesis and its control. We have isolated and characterized spontaneous revertants of a clonal line of rat-1 cells transformed by the B77 strain of avian sarcoma virus (ASV). This cell line (B31) carries a single, normal ASV provirus, yields wild type ASV efficiently upon fusion with chicken cells, and has an extremely stable transformed phenotype. Using selective procedures to kill transformed cells, we have isolated subclones of B31 which have morphological and growth properties of uninfected rat-1 cells (revertants); these are present in the B31 cultures at a frequency of about 10^{-4} to 10^{-6} .

The revertants have been found to fall into two classes. (i) About one-fourth of the revertants have lost the entire ASV provirus, as demonstrated by hybridization to restriction endonuclease digests of cellular DNA; the mechanism by which the provirus was lost is not known. (ii) The other revertants have acquired mutations in the viral gene (src) required for transformation: they retain a normal provirus; contain the usual species of viral RNA at the same concentrations as in B31 cells (tests performed by N. Quintrell, University of California, San Francisco, [UCSF]); can be retransformed with ASV; yield non-conditional, transformation-defective mutants after fusion with chicken cells; and encode products of src which are abnormal in stability, structure, immunoreactivity, and/or protein kinase activity (done in collaboration with H. Oppermann and A. Levinson, UCSF). In some cases, reversion of the src mutations to wild type occurs at low frequency as manifest by spontaneous retransformation of the rat cells or by recovery of transforming virus during propagation of the mutant viruses in chicken cells. The independence of the mutations has been documented by recovery of wild type recombinant viruses after mixed infections of chicken cells with pairs of mutant viruses. Detailed analysis of about 30 such src mutations and their aberrant products is underway in collaboration with Drs. Oppermann and Levinson.