Abstract

Replication-incompetent recombinant retroviruses are currently used for gene delivery. The limited efficiency of gene transfer using these vectors hampers implementation of gene therapy. Successful integration of Moloney murine leukemia virus (MMuLV)-derived retroviral vectors into the host cell DNA requires cell division. The time difference between virus entry and cell division is variable and prolonged in slowly dividing cells. Therefore, the rate of intracellular decay of internalized vectors between the time of entry into the target cell and cell division may limit the probability of successful integration following viral entry. We present two methods that measure the intracellular stability of MMuLV derived retroviral vectors in NIH 3T3 cells. The first is based on a temporary interruption of cell cycle progression by using cell detachment. This method provides an estimate, but not a direct measurement, of the half-life. The results show that the MMuLV intracellular half-life is on the order of but shorter than the total cell cycle time. The second method allows the direct measurement of the intracellular half-life by using two cell cycle-specific labels: 5-bromodeoxyuridine, a thymidine analog that labels cells in S-phase; and the viral vector that labels cells in mitosis. By varying the time between the administration of the two labels, the intracellular half-life is measured to be in the range of 5.5 to 7.5 h. Such a short intracellular half-life may restrict the efficiency of gene transfer by retroviral vectors, particularly in slowly dividing target cells.