Retroviral vectors used in genetic engineering

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These vectors have found wide application in both fundamental biology and gene therapy. For gene therapy, it is necessary for the gene to be stably maintained and continuously expressed. Vectors derived from murine retroviruses meet this condition. Before turning to the principles underlying the design of retroviral vectors, it is necessary to dwell on the main features of the genome structure and life cycle of retroviruses. The wild-type retrovirus genome is represented by two identical single-stranded RNA molecules, each consisting of six sites: The long terminal repeat (5'-LTR), which contains transcription initiation signals, the non-coding sequence $ps^{+}(\psi^{+})$ required to package the RNA into the viral particle; three genes encoding the inner capsid structural protein (gag), a protein with reverse transcriptase and integrase functions (po 1), and the envelope protein (env); the 3'-LTR sequence, which contains the polyadenylation signal.

The life cycle of a retrovirus (Fig. 1) includes the following stages: infection of a target cell, synthesis of DNA-copy of genome by its own reverse transcriptase, transport of viral DNA into nucleus, incorporation of viral DNA into one of chromosomal sites of a host cell, transcription of mRNA from viral DNA under the control of a strong promoter located at 5'-LTR, transcription of Gag, Pol and Env proteins in cytoplasm, formation of viral capsid and packaging of two RNA-chains and reverse transcriptase molecules into it, virion release from the cell.

The natural properties of retroviruses determine the possibility of creating effective vectors based on them for gene transfer and expression in mammalian cells, including human cells.

Retroviral vector DNA can be used for cell transformation on its own, but the efficiency of delivery to the nucleus and integration into the host cell genome is extremely low. Therefore, a technique has been developed for packaging the retroviral vector RNA into intact viral particles that penetrate the cell with high

frequency, which guarantees the incorporation of its corresponding DNA into the host cell genome.

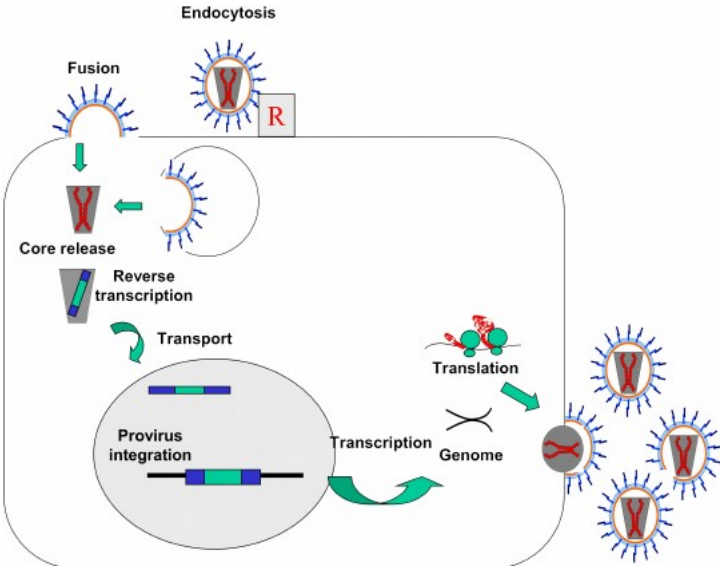

Fig. 1. The life cycle of a retrovirus.

Their transcription is controlled by the 5'-LTR promoter. Both sites lack the ψ^+-sequencing required for viral RNA packaging. Therefore, the packaging cells synthesize viral proteins, but due to the absence of the ψ^+-sequence responsible for packaging in either retroviral RNA and the formation of smaller than normal viral RNA molecules, empty viral particles are produced. After transfection of retroviral vector DNA into such cells, it is incorporated in the chromosomal DNA and is transcribed to form full-sized RNA of the retrovirus which contains the necessary (ψ^+) sequence for packaging. Under these conditions, only the RNA of the vector is packed into the viral capsid. The released viral particles are not able to replicate, i.e. such particles are a kind of single-acting infectious virus. They cannot transfer the genes acquired as part of the vector to uninfected cells horizontally. At the same time, an integrated vector is transmitted to daughter cells by inheritance, i.e. vertically.

The packaging cells do not produce replication competent wild-type retroviruses capable of inserting themselves into genes and leading to uncontrolled proliferation of some cells, i.e. turning them into cancer cells. This is essential, especially if retroviral vector particles are used for gene therapy of human somatic cells.