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GENE THERAPY: Promises and Problems

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■ **Abstract** Gene therapy can be broadly defined as the transfer of genetic material to cure a disease or at least to improve the clinical status of a patient. One of the basic concepts of gene therapy is to transform viruses into genetic shuttles, which will deliver the gene of interest into the target cells. Based on the nature of the viral genome, these gene therapy vectors can be divided into RNA and DNA viral vectors. The majority of RNA virus-based vectors have been derived from simple retroviruses like murine leukemia virus. A major shortcoming of these vectors is that they are not able to transduce nondividing cells. This problem may be overcome by the use of novel retroviral vectors derived from lentiviruses, such as human immunodeficiency virus (HIV). The most commonly used DNA virus vectors are based on adenoviruses and adeno-associated viruses. Although the available vector systems are able to deliver genes in vivo into cells, the ideal delivery vehicle has not been found. Thus, the present viral vectors should be used only with great caution in human beings and further progress in vector development is necessary.

INTRODUCTION

Biologists will remember Monday, June 19, 2000, as an historic day. Flanking Bill Clinton, the 42nd President of the United States of America, were Francis Collins of the National Institutes of Health (NIH), leader of the publicly funded Human Genome project, and Craig Venter, CEO of Celera Genomics of Rockville, Maryland, to announce the near-completion of the sequencing of the human genome. Imagine: The entire 3 billion nucleotides of our genome are decoded—an impossible task just a few years ago. The estimate of the number of genes ranges from a low of 35,000 to a high of more than 100,000.

What a bonanza for gene therapy. The science of gene therapy relies on the introduction of genes to cure a defect or slow the progression of the disease and thereby improve the quality of life. Therefore, we need genes. Suddenly, we have tens of thousands of them at hand. Though gene therapy holds great promise for the achievement of this task, the transfer of genetic material into higher organisms still remains an enormous technical challenge. Presently available gene delivery vehicles for somatic gene transfer can be divided into two categories: viral and

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nonviral vectors. Viruses evolved to depend on their host cell to carry their genome. They are intracellular parasites that have developed efficient strategies to invade host cells and, in some cases, transport their genetic information into the nucleus either to become part of the host's genome or to constitute an autonomous genetic unit. The nonviral vectors, also known as synthetic gene delivery systems (45), represent the second category of delivery vehicles and rely on direct delivery of either naked DNA or a mixture of genes with cationic lipids (liposomes). In this review, we focus on viral vectors and highlight some examples of their use in clinical trials. A complete, constantly updated list of human gene therapy trials in the United States is available at the Office of Biotechnology Activities, NIH (http://www4.od.nih.gov/oba/rdna.htm).

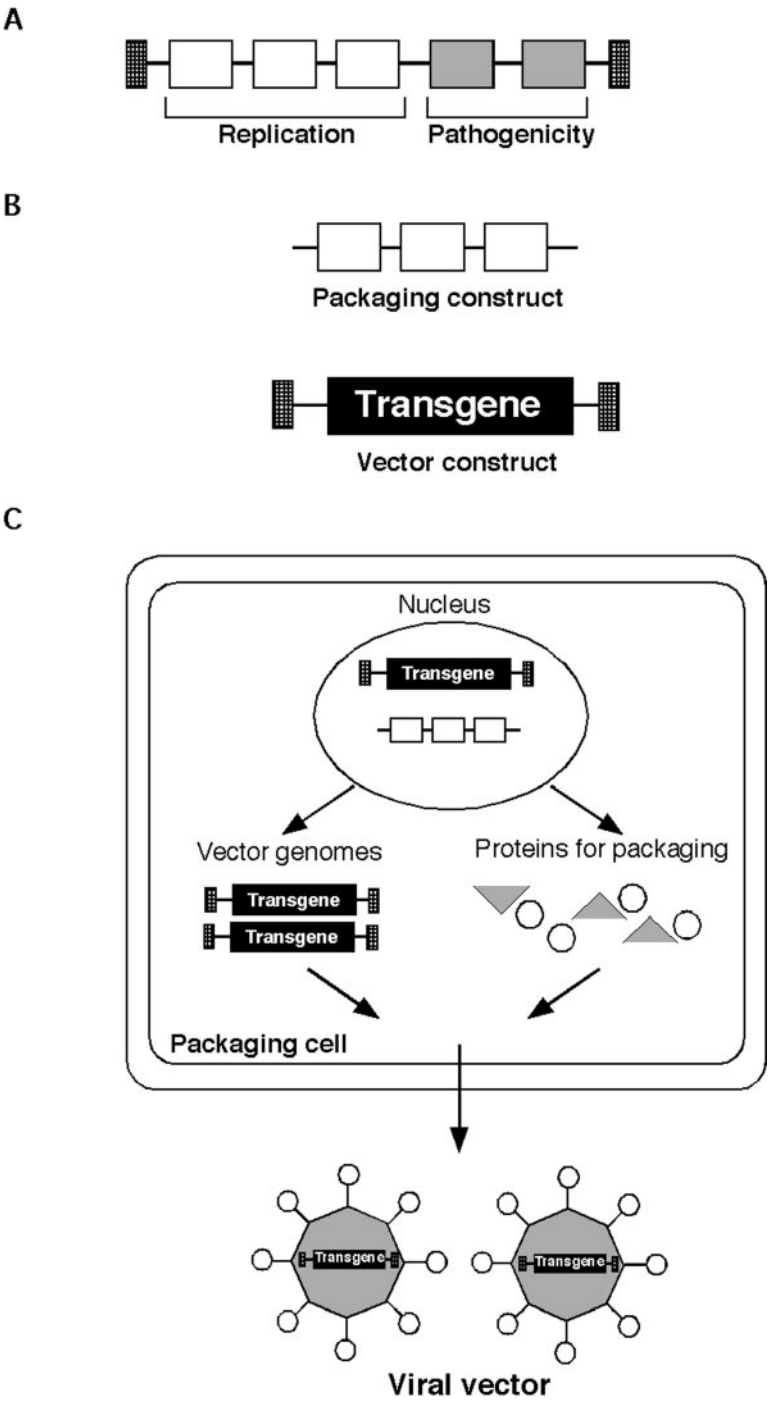
General Concept of Viral Vectors

The first step in viral vector design is to identify the viral sequences that are required for the assembly of viral particles, the packaging of the viral genome into the particles, and the delivery of the transgene to the target cells. Next, dispensable genes are deleted from the viral genome to reduce patho- and immunogenicity. The residual viral genome and the gene of interest (also termed transgene) are integrated into the vector construct (Figure 1).

Viral vectors can be divided into two general categories: (*a*) integrating vectors, capable of providing life-long expression of the transgene, and (*b*) nonintegrating vectors. Examples for integrating vectors are retroviral and adeno-associated virus (AAV)–derived vectors. The major nonintegrating vector currently employed is based on adenoviruses, and the viral DNA is maintained as an episome in the infected cell. Each of these vectors has specific advantages and major limitations. What, then, would be an ideal vector? We believe that it should fulfill the following requirements (147):

1. Efficient and easy production: High-titer preparations of vector particles should be reproducibly available. The efficient transduction of cells within tissues is only possible if a sufficient number of infectious particles reaches the target cells. For the widespread use of viral vectors, facile production procedures have to be developed.

Figure 1 Basic principal of viral vector design. (*A*) Structure of a generic viral genome. (*B*) Strategy of gene therapy vectors. The viral genome is separated into the packaging construct, which contains the viral sequences encoding proteins required for packaging of the vector genome and its replication. The vector construct contains the transgene and *cis*-acting sequences (*hatched boxes*) that are essential for encapsidation of the vector genome and for viral transduction of the target cell. (*C*) The vector and packaging constructs are expressed in the packaging cells, which produce the recombinant viral particles.



2. Safety aspects: The vector should neither be toxic to the target cells nor induce unwanted effects, including immunological reactions against the viral vector or its cargo. The latter carries not only the threat of eliminating the vector and/or the infected cells but also may lead to life-threatening complications, such as septic shock.
3. Sustained and regulated transgene expression: The gene delivered by the viral vector has to be expressed in a proper way. Permanent or even life-long expression of the therapeutic gene is desired only in a minority of diseases (e.g., treatment of hemophilia). Controlled expression of the transgene in a reversible manner would be highly desirable in many cases (e.g., gene therapy for insulin-dependent diabetes mellitus).
4. Targeting of the viral vectors: Preferential or exclusive transduction of specific cell types is very desirable.
5. Infection of dividing and nondividing cells: Because the majority of the cells in an adult human being are in a postmitotic, nondividing state, viral vectors should be able to efficiently transduce these cells.
6. Site-specific integration: Integration into the host genome at specific site(s) could enable us to repair genetic defects, such as mutations and deletions, by insertion of the correct sequences. Thus, replacing defective gene expression by introducing foreign genes and cDNAs would be unnecessary.

RNA VIRUS VECTORS

RNA viruses are a large and diverse group of viruses (150) that have either a single-stranded or a double-stranded RNA genome. They can infect a broad spectrum of cells, ranging from prokaryotes to many eukaryotic cells. Among the RNA-containing viruses, one group has attracted much attention as a gene delivery vehicle: the *Retroviridae* (30). Retroviruses comprise a diverse family of enveloped RNA viruses and can be divided into two categories according to the organization of their genome: simple and complex retroviruses (29). All retroviruses contain three major viral proteins: *gag*, *pol*, *env* [Figure 2, *right*; for review see (30, 160)]. *Gag* encodes the structural virion proteins that form the matrix, capsid, and the nucleoprotein complex. *Pol* codes for the essential viral enzymes reverse transcriptase and integrase. *Env* encodes the viral glycoproteins that are displayed on the surface of the virus. Moloney murine leukemia virus (MLV), a prototypic simple retrovirus, carries only a small set of genetic information, whereas the complex retroviruses like lentiviruses [e.g., human immunodeficiency virus (HIV)] contain additional regulatory and accessory genes. Initially, gene therapy vectors were developed from simple retroviruses. The lessons learned from simple retroviral vectors provided an invaluable basis for the development of vectors derived from complex retroviruses. Emerging vectors based on other RNA viruses, such as alphaviruses, are reviewed elsewhere (71).

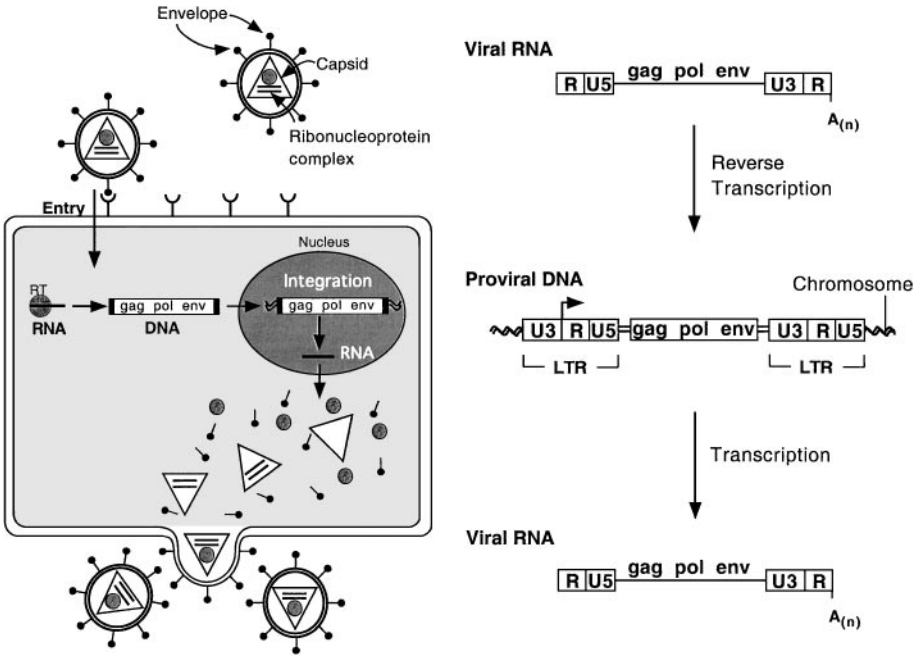


Figure 2 Retroviral lifecycle. (*Left*) Overview of the replication cycle of a prototypic retrovirus, MLV. (*Right*) Organization of the retroviral genome and its transition from RNA → DNA → RNA during viral replication. The prototypic retroviral genome contains the *gag*, *pol*, and *env* genes flanked by the R/U5 region at the 5′ end and the U3/R region at the 3′ end. Reverse transcription results in the proviral DNA that contains long terminal repeats (LTRs) at each end. The LTRs comprise U3, R, and U5 elements in the provirus. Transcription between (not including) the 5′ U3 and the 3′ U5 regions generates the identical organization of the terminal domains as in the parental virus (*top*). $A_{(n)}$, polyA tail.

Retroviral Life Cycle

Knowledge of the viral life cycle was crucial for the development of retroviral vectors. Following infection of the cell, the genomic RNA is reverse transcribed into linear double-stranded DNA by the virion reverse transcriptase (156). Reverse transcription involves two jumps of the transcriptase enzyme from the 5′ end to the 3′ end of the viral template, causing a duplication of the sequences located at the ends of the viral RNA. Thus, the viral DNA is significantly longer than the viral genome at both the 5′ and 3′ ends. The resulting tandem repeats in the viral DNA are termed long terminal repeats (LTRs) (Figure 2). Reverse transcription takes place in the cytoplasm and the viral DNA is translocated into the nucleus.

Simple and complex retroviruses enter the nucleus of the host cell by two different mechanisms: Nuclear entry of simple retroviruses can only occur when the nuclear membrane is disassembled and is, therefore, mitosis dependent. In

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