# Retrovirus Vectors: Promise and Reality

HOWARD M. TEMIN

THERE ARE MANY REASONS TO INTRODUCE DNA INTO vertebrate cells including study of the introduced DNA and use of the DNA as a marker. There are three primary experimental modalities used: introduction of DNA into cells for a short time (study of transient expression); stable introduction of DNA into cells in culture by nonhomologous or homologous recombination (these cells may in turn be injected into animals); and stable introduction of DNA into somatic or germ line cells in animals.

In their normal life cycle, retroviruses are capable of both inserting foreign DNA into cells and expressing that DNA with high efficiency. Thus, it has long been suggested that retroviruses should be useful as vectors for stable introduction of DNA into cells in culture and animals for genetic engineering of vertebrates, including humans. Several laboratories constructed retrovirus vectors in the early 1980s, and in 1983 the first retrovirus helper cells or packaging cell lines were made, which could provide functions missing in oncogenic or genetically engineered retroviruses. The future seemed bright for the use of retrovirus vectors in genetic engineering of animals and humans (*1*). However, in 1989, there is only one approved use of retrovirus vectors in humans, although they are widely used in mice. Transfection, electroporation, and injection of cells in culture, sometimes followed by selection of homologous recombinants and injection of DNA into fertilized eggs are widely used, especially with animals (see *2*). While alternatives to retroviral vectors are being used, these methods can have problems with efficiency, alteration of added DNA, and the form and expression of stably integrated DNA.

A recent article on progress toward human gene therapy continues to be optimistic about human gene therapy with retrovirus vectors (*3*). Furthermore, retrovirus vectors are still the best agents for stable introduction of DNA into animal cells in culture, and they are useful for the introduction of DNA into animals either directly or after infection of cells in culture.

The fundamental advantages of retrovirus vectors are efficient infection of and expression in most kinds of cells, efficient and accurate integration of a single vector copy into chromosomal DNA, a wide choice of different vectors with different host ranges, and the ease of manipulation of the retrovirus genome. The disadvantages are that retrovirus integration is not homologous and, therefore, cannot directly correct an aberrant gene; retrovirus vector structure has potential instability, so that investigators must confirm that the retrovirus provirus has and continues to have the expected structure; many different vectors may need to be tested to find a satisfactory one because interactions exist between control sequences in close juxtaposition in retrovirus vectors; the size of retrovirus vectors is limited, which means that added sequences must be less than about 8 kbp; and as infection by retroviruses requires cell replication, nondividing cells cannot be targets. There are also some experimental problems with the use of retrovirus vectors: contamination of the vector with replication-competent virus; relatively low vector virus titers; and low expression of inserted genes. These problems are nuisances to persons who want to use these vectors, while they are a source of new phenomena for study for those who are primarily interested in retrovirus replication.

Some problems may be solved by altering experimental conditions and by new vector design. There are new helper cells with little homology between their virus structural genes and the vectors, removing the possibility of recombination to yield replication-competent virus (*4, 5*); there are vectors with additional sequences that result in higher titers (*5, 6*); and insertion of genes in different places in the vector (*7*) or with additional control sequences, along with better infection of stem cells, increases gene expression. Several groups have reported excellent expression of adenosine deaminase and β-globin in mice (*8*). Users of retrovirus vectors must try different vector constructions and be aware of possible artifacts.

What have been the actual uses of retrovirus vectors? Although from the early 1970s there were hopes of using them for treatment of genetic illness in humans and for germ line genetic engineering in farm animals, these hopes are still essentially not realized. However, for laboratory uses, retrovirus vectors are the vectors of choice for stable integration and expression of foreign DNA in vertebrate cell genomes, and they are useful for infection of preimplantation embryos. The experiences with retrovirus vectors have also served as a guide for the development of vectors from other animal viruses.

Retrovirus vectors are the laboratory analogs of highly oncogenic retroviruses. Thus, the most common use of retrovirus vectors is to introduce genes into cells to test whether or not their expression transforms the cells. Genes tested in this way include known or modified viral oncogenes, genes for growth factors or receptors, and even unknown coding sequences. Other successful uses of retrovirus vectors have been in studying retrovirus replication itself. A more novel use of retrovirus vectors has been to mark cells for lineage studies in embryology or to mark tumor-infiltrating lymphocytes for experimental cancer therapy (*9*). The latter is the only human use of retrovirus vectors approved by the Food and Drug Administration and the National Institutes of Health and indicates a switch from the goal of treating single-gene human diseases to that of treating somatic diseases such as cancer and AIDS.

Work with farm animals, notably chickens, illustrates the status of germ line engineering with retrovirus vectors to introduce useful genes. Salter and co-workers (*10*) established a line of chickens producing an envelope protein from a leukemia-inducing retrovirus after infection of eggs with a replication-competent retrovirus vector that became replication-defective during the infection process. These chickens became resistant to infection with the leukemia-inducing virus. Bosselman and co-workers (*11*) infected chicken embryos directly with replication-defective retrovirus vectors. More than 2000 eggs had to be injected to find a few with germ line integrations of the vector, illustrating the difficulty and inefficiency of the procedure.

We have not seen the end of genetic engineering of retrovirus vectors, and we have only seen the beginnings of genetic engineering of organisms with retrovirus vectors. New vectors and new modes of infection are continually being developed and will further extend the range and usefulness of these vectors.

The author is a professor at the McArdle Laboratory for Cancer Research, University of Wisconsin Medical Center, Madison, WI 53706.

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