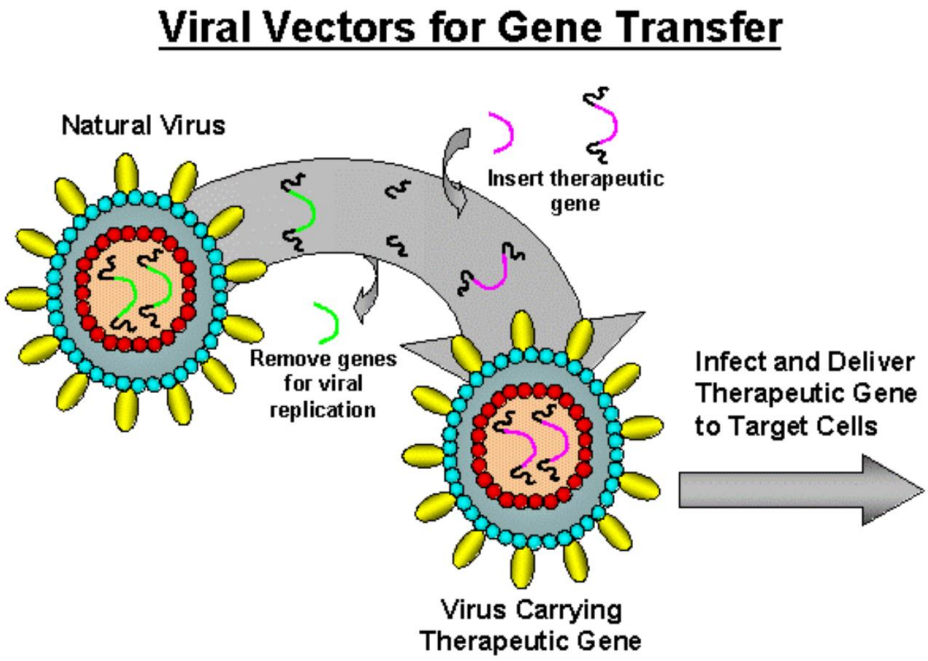
VIRAL MEDIATED GENE TRANSFER TECHNIQUES

Viral vectors are tools commonly used by molecular biologists to deliver genetic material into cells. This process can be performed inside a living organism (*in vivo*) or in cell culture (*in vitro*). Viruses have evolved specialized molecular mechanisms to efficiently transport their genomes inside the cells they infect. Delivery of genes by a virus is termed transduction and the infected cells are described as transduced.

Key properties of a viral vector:

- *Safety*: Although viral vectors are occasionally created from pathogenic viruses, they are modified in such a way as to minimize the risk of handling them. This usually involves the deletion of a part of the viral genome critical for viral replication. Such a virus can efficiently infect cells but, once the infection has taken place, requires a helper virus to provide the missing proteins for production of new virions.
- *Low toxicity*: The viral vector should have a minimal effect on the physiology of the cell it infects.
- *Stability*: Some viruses are genetically unstable and can rapidly rearrange their genomes. This is detrimental to predictability and reproducibility of the work conducted using a viral vector and is avoided in their design.
- *Cell type specificity*: Most viral vectors are engineered to infect as wide a range of cell types as possible. However, sometimes the opposite is preferred. The viral receptor can be modified to target the virus to a specific kind of cell. Viruses modified in this manner are said to be pseudotyped.
- *Identification*: Viral vectors are often given certain genes that help identify which cells took up the viral genes. These genes are called Markers. A common marker is antibiotic resistance to a certain antibiotic. The cells can then be isolated easily as those that have not taken up the viral vector genes do not have antibiotic resistance and so cannot grow in a culture with antibiotics present.



TYPES OF VIRAL VECTORS:

RETROVIRUSES:

A retrovirus is a virus that carries its genetic material in the form of RNA rather than DNA. Retroviruses are used as vectors to transfer genetic material into the host cell. The result is a chimera, an organism consisting of tissues or parts of diverse genetic constitution. Chimeras are inbred for as many as 20 generations until homozygous genetic offspring are born.

To increase the probability of expression, gene transfer is mediated by means of a carrier or vector, generally a virus or a plasmid. Retroviruses are commonly used as vectors to transfer genetic material into the cell, taking advantage of their ability to infect host cells in this way. Offspring derived from this method are chimeric, i.e., not all cells carry the retrovirus. Transmission of the transgene is possible only if the retrovirus integrates into some of the germ cells.

Recombinant retroviruses produce virions, which are used to infect animal cells and mice embryos. Generally, early 4-16 celled embryos are used. But when embryos beyond the 4-celled stage are used, often all the cells in an embryo may not be infected by the retrovirus, resulting in chimeric mice.

Retroviral vectors can either be replication-competent or replication-defective. Replication-defective vectors are the most common choice in studies because the viruses have had the coding regions for the genes necessary for additional rounds of virion replication and

packaging replaced with other genes, or deleted. These virus are capable of infecting their target cells and delivering their viral payload, but then fail to continue the typical lytic pathway that leads to cell lysis and death.

Conversely, replication-competent viral vectors contain all necessary genes for virion synthesis, and continue to propagate themselves once infection occurs. Because the viral genome for these vectors is much lengthier, the length of the actual inserted gene of interest is limited compared to the possible length of the insert for replication-defective vectors. Depending on the viral vector, the typical maximum length of an allowable DNA insert in a replication-defective viral vector is usually about 8–10 kB. While this limits the introduction of many genomic sequences, most cDNA sequences can still be accommodated.

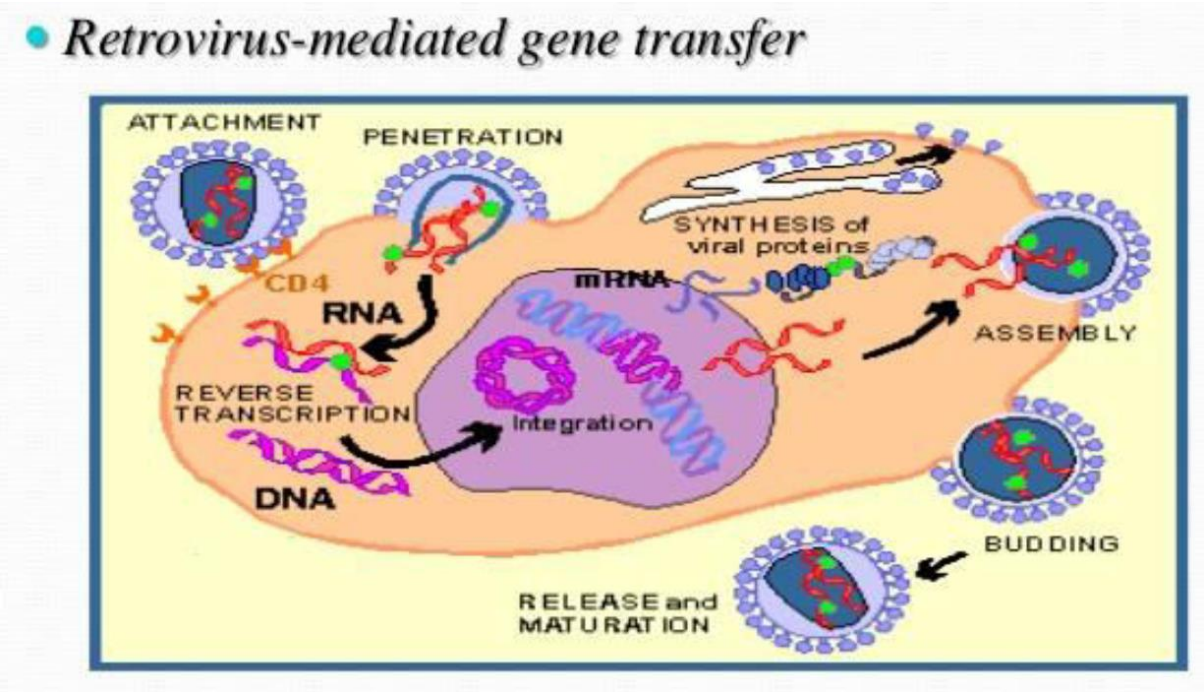
A chimera is an individual, which has in its body cells of two or more genotypes. Chimeric individuals produced by transfection arise when some cells of an embryo become stably transfected, while its other cells are not transfected; as a result, the individual developing from such an embryo will have cells of two genotypes in its body.

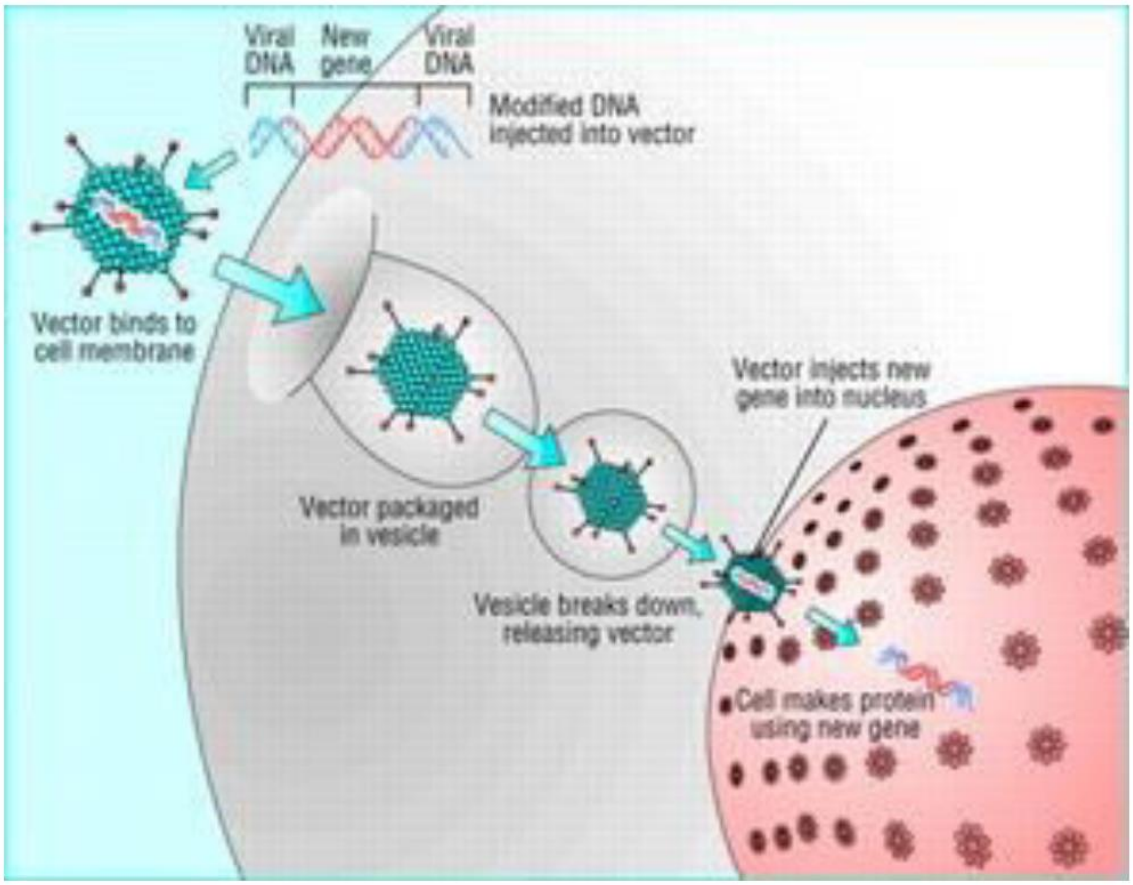
The recombinant retrovirus RNA genome is copied by reverse transcriptase to yield a DNA copy (reverse transcription), which becomes integrated into the cell's gemone. The reverse transcriptase is encoded by the retrovirus, and is produced immediately after infection.

However, the reverse transcription can occur only in those cells, which go through S phase, i.e., are mitotically active. The DNA copy of the recombinant retrovirus integrates into the cellular genome at random sites, and usually is not accompanied with deletions or rearrangements.

The chief advantage of this transfection method is its technical simplicity. Its main limitations, however, are as follows: (1) the additional steps needed for construction of recombinant retroviruses, (2) limits on the size of DNA insert, (3) masaicism of the recovered animals, and (4) possible interference of the proviral LTR sequences with the expression of foreign genes.

METHODS OF CREATION OF TRANSGENIC ANIMALS

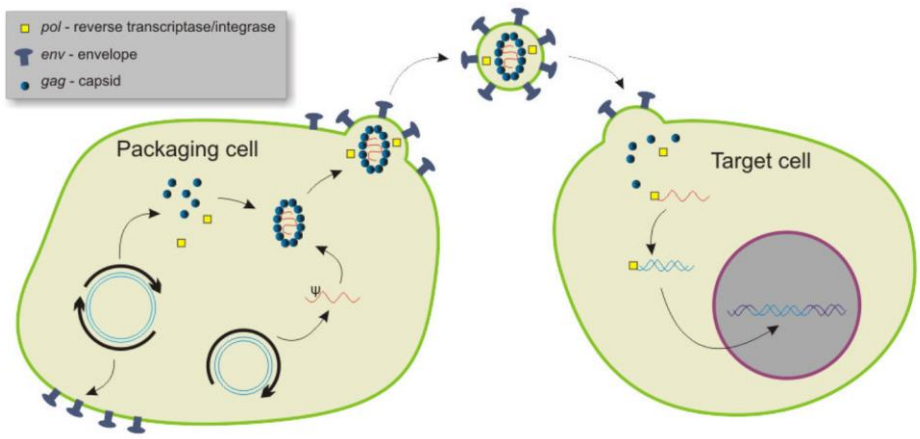




LENTIVIRUSES:

Lentiviruses are a subclass of Retroviruses. They have recently been adapted as gene delivery vehicles (vectors) thanks to their ability to integrate into the genome of non-dividing cells, which is the unique feature of Lentiviruses as other Retroviruses can infect only dividing cells. The viral genome in the form of RNA is reverse-transcribed when the virus enters the cell to produce DNA, which is then inserted into the genome at a random position (recent findings actually suggest that the insertion of viral DNA is not random but directed to specific active genes and related to genome organisation) by the viral integrase enzyme. The vector, now called aprovirus, remains in the genome and is passed on to the progeny of the cell when it divides. The site of integration is unpredictable, which can pose a problem. The provirus can disturb the function of cellular genes and lead to activation of oncogenes promoting the development of cancer, which raises concerns for possible applications of lentiviruses in gene therapy. However, studies have shown that lentivirus vectors have a lower tendency to integrate in places that potentially cause cancer than gamma-retroviral vectors. More specifically, one study found that lentiviral vectors did not cause either an increase in tumor incidence or an earlier onset of tumors in a mouse strain with a much higher incidence of tumors. Moreover, clinical trials that utilized lentiviral vectors to deliver gene therapy for the treatment of HIV experienced no increase in mutagenic or oncologic events.

For safety reasons lentiviral vectors never carry the genes required for their replication. To produce a lentivirus, several plasmids are transfected into a so-calledpackaging cell line, commonly HEK 293. One or more plasmids, generally referred to as packaging plasmids, encode the virion proteins, such as the capsid and the reverse transcriptase. Another plasmid contains the genetic material to be delivered by the vector. It is transcribed to produce the single-stranded RNA viral genome and is marked by the presence of the ψ (psi) sequence. This sequence is used to package the genome into the virion



ADENOVIRUSES:

Adenoviral DNA does not integrate into the genome and is not replicated during cell division. This limits their use in basic research, although adenoviral vectors are still used in *in vitro* and also *in vivo* experiments. Their primary applications are in gene therapy and vaccination. Since humans commonly come in contact with adenoviruses, which cause respiratory, gastrointestinal and eye infections, majority of patients have already developed neutralizing antibodies which can inactivate the virus before it can reach the target cell. To overcome this problem scientists are currently investigating adenoviruses that infect different species to which humans do not have immunity.

ADENO-ASSOCIATED VIRUSES:

Adeno-associated virus (AAV) is a small virus that infects humans and some other primate species. AAV is not currently known to cause disease and consequently the virus causes a very mild immune response. AAV can infect both dividing and non-dividing cells and may incorporate its genome into that of the host cell. Moreover, AAV mostly stays as episomal; performing long and stable expression. These features make AAV a very attractive candidate for creating viral vectors for gene therapy. However, AAV can only bring up to 5kb which is considerably small compare to AAV's original capacity.

Furthermore, because of its potential use as a gene therapy vector, researchers have created an altered AAV called Self-complementary adeno-associated virus (scAAV). Whereas AAV packages a single strand of DNA and requires the process of second-strand synthesis, scAAV packages both strands which anneal together to form double stranded DNA. By skipping second strand synthesis scAAV allows for rapid expression in the cell. Otherwise, scAAV carries many characteristics of its AAV counterpart.

MOLECULAR PHARMING:

Pharming, a portmanteau of "farming" and "pharmaceutical", refers to the use of genetic engineering to insert genes that code for useful pharmaceuticals into host animals or plants that would otherwise not express those genes, thus creating a genetically modified organism (GMO).

Pharming is also known as molecular farming, molecular pharming or biopharming. The products of pharming are recombinant proteins or their metabolic products.

The production of pharmaceuticals in GM animals is another area of intensive research. The preferred bioreactor is the lactiferous gland. So far, more than 20 pharmaceuticals have been synthesised in the milk of mammals. Pharmaceutical agents can also be produced in chickens' eggs, blood, urine, and sperm. Since 2008 the first drug produced by transgenic animals is availiable in the EU. Antithrombin is a substance that blocks blood clotting and can be extracted from the milk of genetically modified goats.

Pharming in mammals: Historical development:

Milk is presently the most mature system to produce recombinant proteins from transgenic organisms. Blood, egg white, seminal plasma, and urine are other theoretically possible systems, but all have drawbacks. Blood, cannot store high levels of stable recombinant proteins, and biologically active proteins in blood may alter the health of the animals. Expression in the milk of a mammal, such as a cow, sheep, or goat, is a common application, as milk production is plentiful and purification from milk is relatively easy. Hamsters and rabbits have also been used in preliminary studies because of their faster breeding.

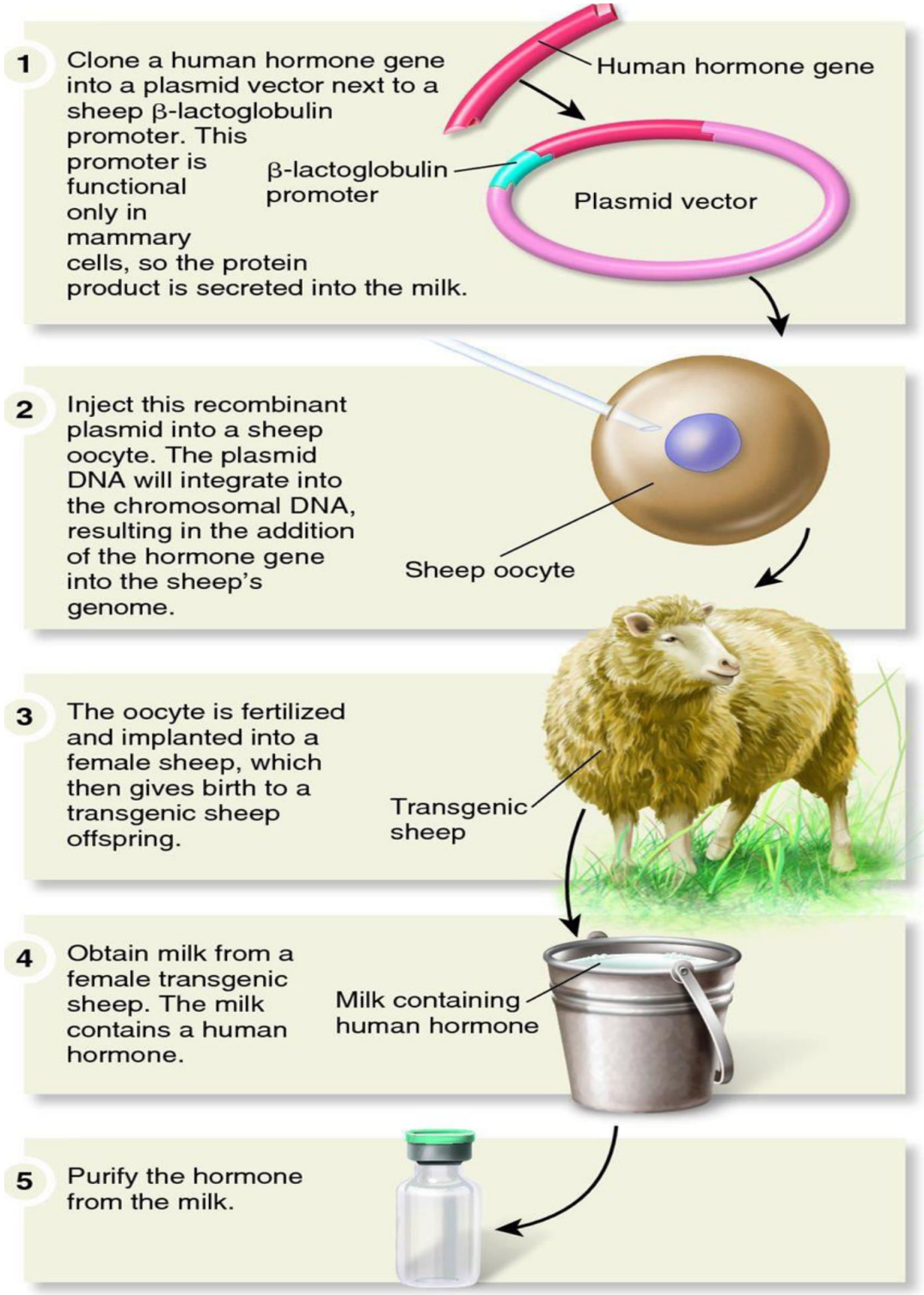
One approach to this technology is the creation of a transgenic mammal that can produce the biopharmaceutical in its milk (or blood or urine). Once an animal is produced, typically using the pronuclear microinjection method, it becomes efficacious to use cloning technology to create additional offspring that carry the favorable modified genome. The drug is called ATryn, which is antithrombin protein purified from the milk of genetically modified goats. Marketing permission was granted by the European Medicines Agency in August 2006.

Patentability issues regarding pharming:

Some mammals typically used for food production (such as goats, sheep, pigs, and cows) have been modified to produce non-food products, a practice sometimes called pharming. Use of genetically modified goats has been approved by the FDA and EMA to produce ATryn, i.e. recombinant antithrombin, ananticoagulant protein drug. These products "produced by turning animals into drug-manufacturing 'machines' by genetically modifying them" are sometimes termed biopharmaceuticals.

The patentability of such biopharmaceuticals and their process of manufacture is uncertain. Probably, the biopharmaceuticals themselves so made are unpatentable, assuming that they are

chemically identical to the preexisting drugs that they imitate. Several 19th century United States Supreme Court decisions hold that a previously known natural product manufactured by artificial means cannot be patented. An argument can be made for the patentability of the process for manufacturing a biopharmaceutical, however, because genetically modifying animals so that they will produce the drug is dissimilar to previous methods of manufacture; moreover, one Supreme Court decision seems to hold open that possibility.



GENE THERAPY

Gene therapy is the therapeutic delivery of nucleic acid polymers into a patient's cells as a drug to treat disease. Gene therapy is an experimental technique that uses genes to treat or prevent disease.

Gene therapy is when DNA is introduced into a patient to treat a genetic disease. The new DNAusually contains a functioning gene to correct the effects of a disease-causing mutation.The DNA is carefully selected to correct the effect of a mutated gene that is causing disease. Gene therapy may be a promising treatment option for somegenetic diseases[?], including muscular dystrophy[?] and cystic fibrosis .

- Replacing a mutated gene that causes disease with a healthy copy of the gene.
- Inactivating, or "knocking out," a mutated gene that is functioning improperly.
- Introducing a new gene into the body to help fight a disease.
- Gene therapy is designed to introduce genetic material into cells to compensate for abnormal genes or to make a beneficial protein. If a mutated gene causes a necessary protein to be faulty or missing, gene therapy may be able to introduce a normal copy of the gene to restore the function of the protein.
- A gene that is inserted directly into a cell usually does not function. Instead, a carrier called a vector is genetically engineered to deliver the gene. Certain viruses are often used as vectors because they can deliver the new gene by infecting the cell. The viruses are modified so they can't cause disease when used in people. Some types of virus, such as retroviruses, integrate their genetic material (including the new gene) into a chromosome in the human cell. Other viruses, such as adenoviruses, introduce their DNA into the nucleus of the cell, but the DNA is not integrated into a chromosome.
- The vector can be injected or given intravenously (by IV) directly into a specific tissue in the body, where it is taken up by individual cells. Alternately, a sample of the patient's cells can be removed and exposed to the vector in a laboratory setting. The cells

containing the vector are then returned to the patient. If the treatment is successful, the new gene delivered by the vector will make a functioning protein.

CELL TYPES: There are two different types of gene therapy depending on which types of cells are treated.

Gene therapy may be classified into two types:

Somatic cell:

In somatic cell gene therapy (SCGT), the therapeutic genes are transferred into any of any cell other than a gamete, germ cell, gametocyte or undifferentiated stem cell. Any such modifications affect the individual patient only, and are not inherited by offspring. Somatic gene therapy represents mainstream basic and clinical research, in which therapeutic DNA (either integrated in the genome or as an external episome or plasmid) is used to treat disease.

Germline:

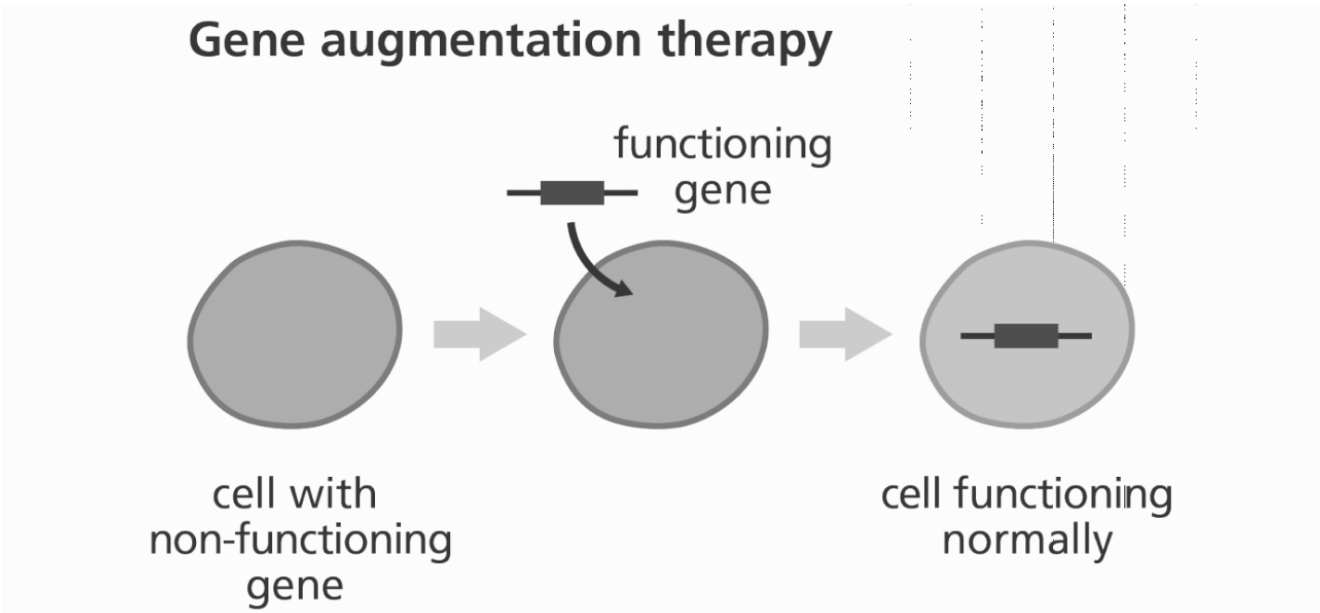
In germline gene therapy (GGT), germ cells (sperm or eggs) are modified by the introduction of functional genes into their genomes. Modifying a germ cell causes all the organism's cells to contain the modified gene. The change is therefore heritable and passed on to later generations.

Gene Therapy Techniques- **There Are Several Techniques For Carrying Out Gene** Therapy. These Include:

Gene augmentation therapy:

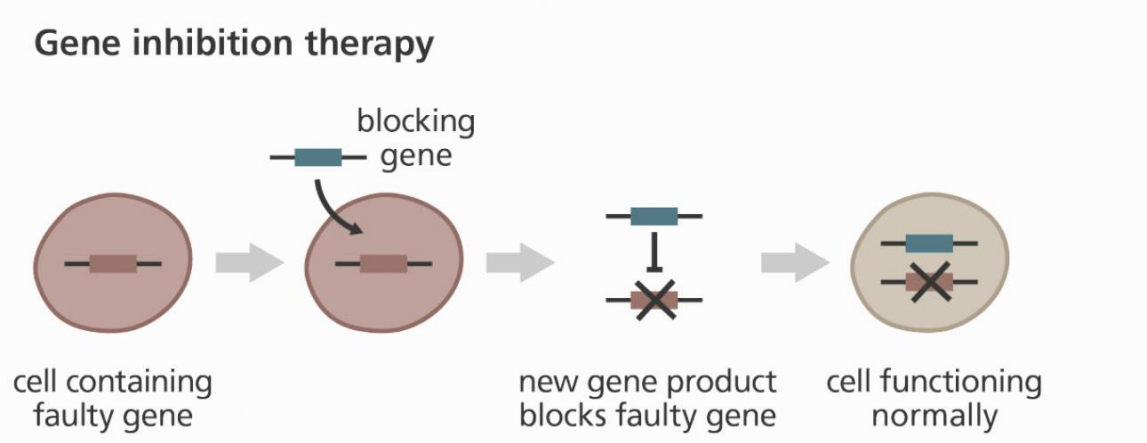
This is used to treat diseases caused by a mutation that stops a gene from producing a

- functioning product, such as a protein .
- This therapy adds DNA containing a functional version of the lost gene back into the cell.
- The new gene produces a functioning product at sufficient levels to replace the protein that was originally missing.
- This is only successful if the effects of the disease are reversible or have not resulted in lasting damage to the body.
- For example, this can be used to treat loss of function disorders such as cystic fibrosis by introducing a functional copy of the gene to correct the disease (see illustration below).



Gene inhibition therapy:

- Suitable for the treatment of infectious diseases, cancer and inherited disease caused by inappropriate gene activity.
- The aim is to introduce a gene whose product either:
  - inhibits the expression of another gene
  - interferes with the activity of the product of another gene.
- The basis of this therapy is to eliminate the activity of a gene that encourages the growth of disease-related cells.
- For example, cancer is sometimes the result of the over-activation of an oncogene (gene which stimulates cell growth). So, by eliminating the activity of that oncogene through gene inhibition therapy, it is possible to prevent further cell growth and stop the cancer in its tracks.



The aim is to insert DNA into a diseased cell that causes that cell to die.

Suitable for diseases such as cancer that can be treated by destroying certain groups of cells.

This can be achieved in one of two ways:

the inserted DNA contains a “suicide” gene that produces a highly toxic product which kills the diseased cell

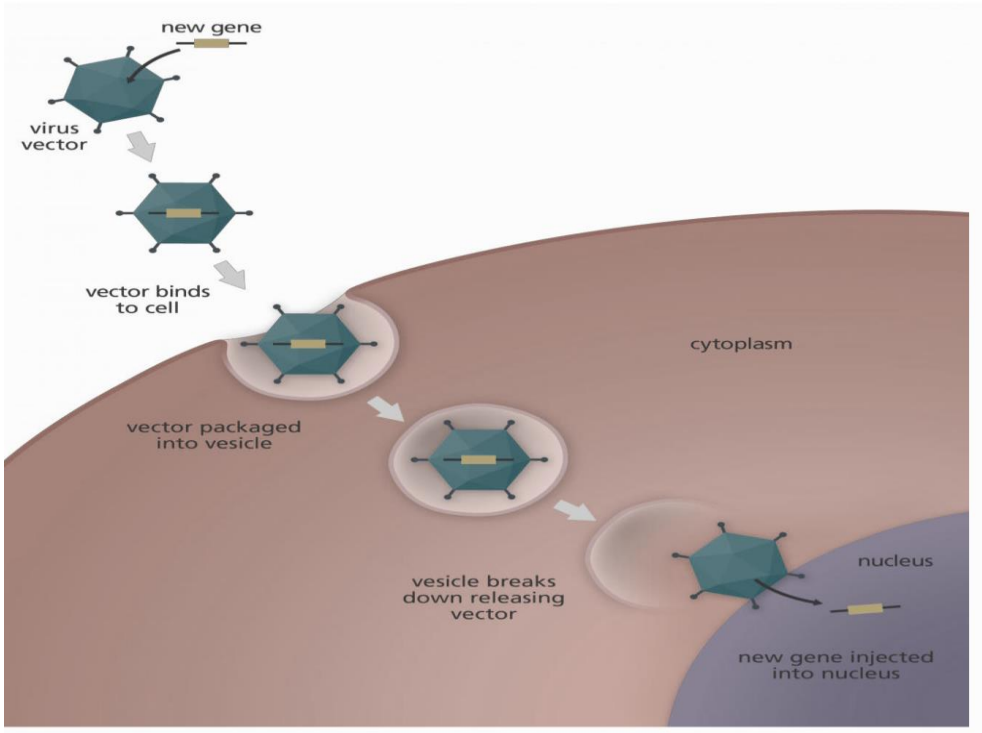
the inserted DNA causes expression of a protein that marks the cells so that the diseased cells are attacked by the body’s natural immune system.

It is essential with this method that the inserted DNA is targeted appropriately to avoid the death of cells that are functioning normally

How is DNA transfer done?

- A section of DNA/gene containing instructions for making a useful protein is packaged within a vector, usually a virus ,bacterium or plasmid .
- The vector acts as a vehicle to carry the new DNA into the cells of a patient with a genetic disease.

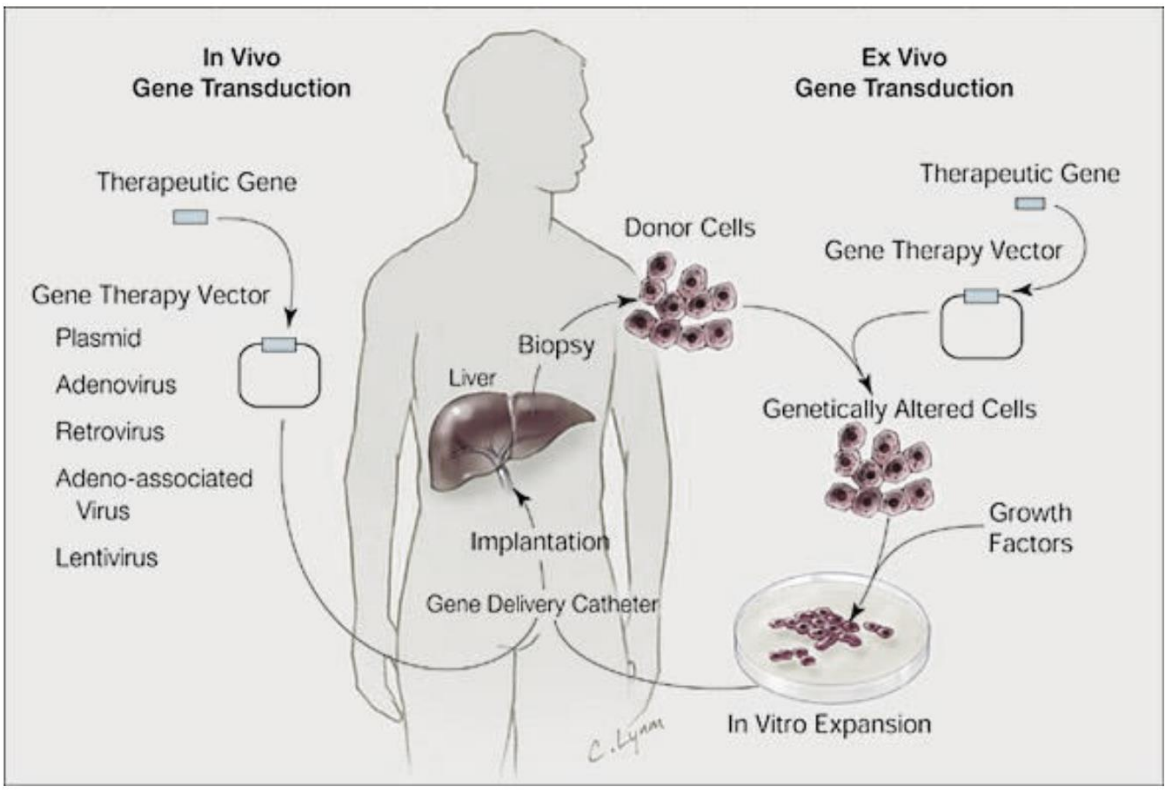
- Once inside the cells of the patient, the DNA/gene is expressed by the cell’s normal machinery leading to production of the therapeutic protein and treatment of the patient’s disease.



Somatic gene therapy can be broadly split into two categories:

Ex Vivo- which means exterior (where cells are modified outside the body and then transplanted back in again). In some gene therapy clinical trials, cells from the patient's blood or bone marrow are removed and grown in the laboratory. The cells are exposed to the virus that is carrying the desired gene. The virus enters the cells and inserts the desired gene into the cells' DNA. The cells grow in the laboratory and are then returned to the patient by injection into a vein. This type of gene grow in the laboratory and are then returned to the patient by injection into a vein. This type of gene therapy is called ex vivo because the cells are treated outside the body.

In Vivo, which means interior (where genes are changed in cells still in the body). This form of gene therapy is called in vivo, because the gene is transferred to cells inside the patient's body.

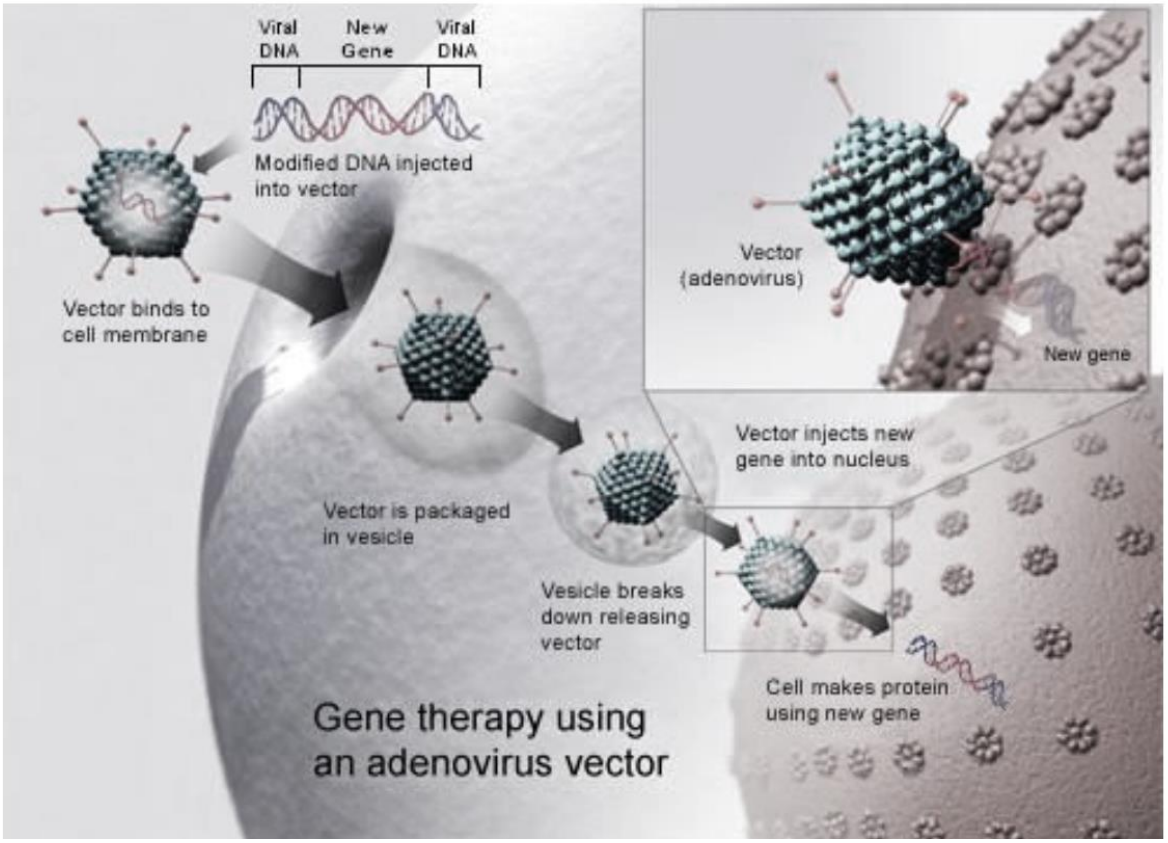


VECTORS:

The delivery of DNA into cells can be accomplished by multiple methods. The two major classes are recombinant viruses (sometimes called biological nanoparticles or viral vectors) and naked DNA or DNA complexes (non-viral methods).

Viruses

In order to replicate, viruses introduce their genetic material into the host cell, tricking the host's cellular machinery into using it as blueprints for viral proteins. Scientists exploit this by substituting a virus's genetic material with therapeutic DNA. (The term 'DNA' may be an oversimplification, as some viruses contain RNA, and gene therapy could take this form as well.) A number of viruses have been used for human gene therapy, including retrovirus, adenovirus, lentivirus, herpes simplex,vaccinia and adeno-associated virus. Like the genetic material (DNA or RNA) in viruses, therapeutic DNA can be designed to simply serve as a temporary blueprint that is degraded naturally or (at least theoretically) to enter the host's genome, becoming a permanent part of the host's DNA in infected cells.



Non-viral:

Non-viral methods present certain advantages over viral methods, such as large scale production and low host immunogenicity. However, non-viral methods initially produced lower levels of transfection and gene expression, and thus lower therapeutic efficacy. Later technology remedied this deficiency.

Methods for non-viral gene therapy include the injection of naked DNA, electroporation, the gene gun, sonoporation, magnetofection, the use of oligonucleotides, lipoplexes, dendrimers, and inorganic nanoparticles.

Reference and further reading

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