MODULE 8- LECTURE 1

GENE THERAPY: INTRODUCTION AND METHODS

8-1.1 Introduction

Gene therapy is a novel treatment method which utilizes genes or short oligonucleotide sequences as therapeutic molecules, instead of conventional drug compounds. This technique is widely used to treat those defective genes which contribute to disease development. Gene therapy involves the introduction of one or more foreign genes into an organism to treat hereditary or acquired genetic defects. In gene therapy, DNA encoding a therapeutic protein is packaged within a "vector", which transports the DNA inside cells within the body. The disease is treated with minimal toxicity, by the expression of the inserted DNA by the cell machinery. In 1990 FDA for the first time approved a gene therapy experiment on ADA-SCID in the United States after the treatment of Ashanti DeSilva. After that, approximately 1700 clinical trials on patients have been performed with various techniques and genes for numerous diseases. Many diseases such as ADA-SCID, X-linked SCID, Leber's congenital amaurosis (a retinal disease), Parkinson's disease, multiple myeloma, chronic and acute lymphocytic leukemia, adrenoleukodystrophy have reported of successful clinical trials. But these are still not approved by FDA. Some other diseases on which gene therapy based research is going on are Haemophilia, Tyrosinemia, Hyperbilirubinemia (Criglar-Nijjar Syndrom), Cystic Fibrosis and many other cancers. After 30 years of research and clinical trials, only one product called Glybera got approval in November 2012 which may be available in market in late 2013. It has the ability to cure lipoprotein lipase deficiency (LPLD) a very rare disease.

8-1.2 Types of gene therapy

There are several approaches for correcting faulty genes; the most common being the insertion of a normal gene into a specific location within the genome to replace a non functional gene. Gene therapy is classified into the following two types:

1. Somatic gene therapy

2. Germ line gene therapy

8-1.2 .1 Somatic Gene Therapy

In somatic gene therapy, the somatic cells of a patient are targeted for foreign gene transfer. In this case the effects caused by the foreign gene is restricted to the individual patient only, and not inherited by the patient's offspring or later generations.

8-1.2 .1 Germ Line Gene Therapy

Here, the functional genes, which are to be integrated into the genomes, are inserted in the germ cells, i.e., sperm or eggs. Targeting of germ cells makes the therapy heritable.

8-1.3 Gene Therapy Strategies

8-1.3.1 Gene Augmentation Therapy (GAT)

In GAT, simple addition of functional alleles is used to treat inherited disorders caused by genetic deficiency of a gene product, e.g. GAT has been applied to autosomal recessive disorders. Dominantly inherited disorders are much less amenable to GAT. Figure 8-1.3.1 shows the GAT strategy

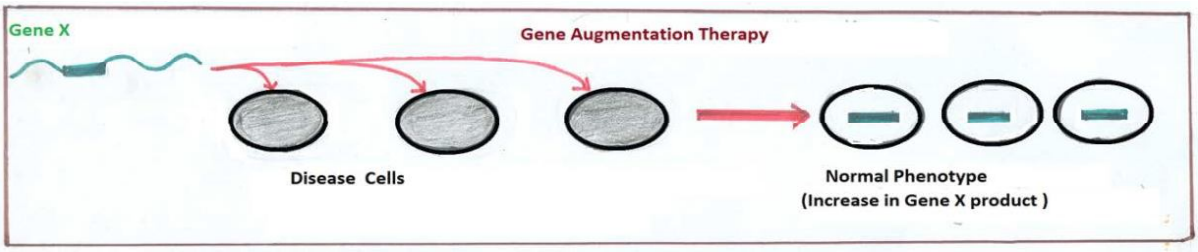

Figure 8-1.3.1: A gene therapy vector has been designed to treat the diseased cells with a gene X. This vector was introduced inside the diseased cells by various gene transfer methods. After a successful homologous recombination the treated cells will show the presence of gene X product as well as normal phenotype.

8-1.3.2 Targeted Killing of Specific Cells

It involves utilizing genes encoding toxic compounds (**suicide genes**), or **prodrugs** (reagents which confer sensitivity to subsequent treatment with a drug) to kill the transfected/ transformed cells. This general approach is popular in cancer gene therapies. This is shown in figure 8-1.3.2a & 8-1.3.2b

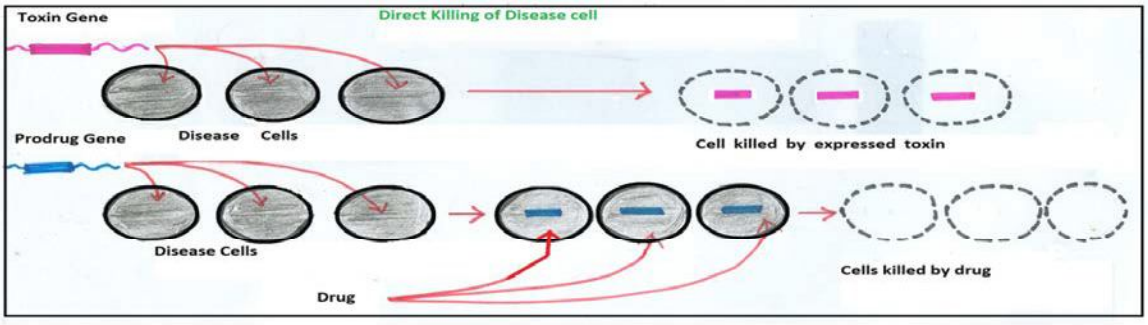

Figure 8-1.3.2: a) Direct killing of diseased cells by two methods. The first method is the introduction of toxin gene into the diseased cell which when expresses toxin protein the cells die. The second method involves incorporation of a certain gene (e.g. TK) in the gene therapy vector which shows a suicidal property on introducing certain drug (e.g. ganciclovir).

Thymidine kinase (TK) phosphorylates the introduced prodrug ganciclovir which is further phosphorylated by endokinases to form ganciclovir triphosphate, an competitive inhibitor of deoxyguanosine triphosphate. Ganciclovir triphosphate causes chain termination when incorporated into DNA.

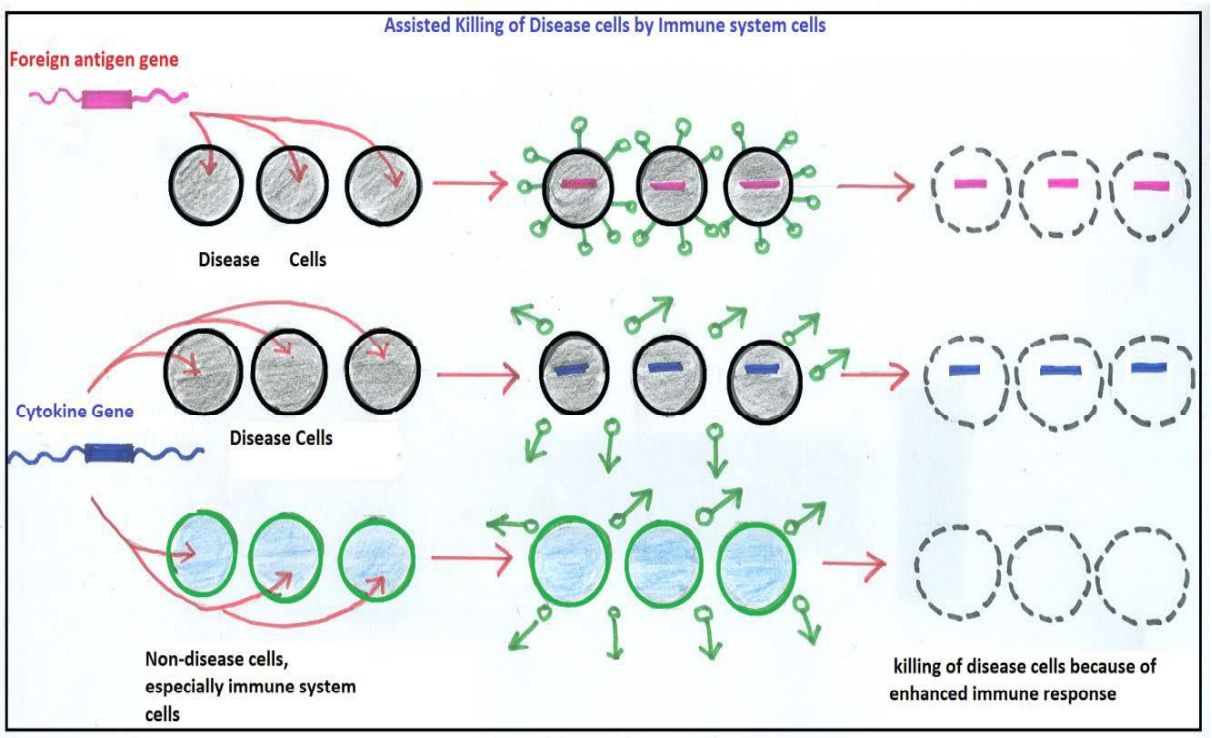

Figure 8-1.3.2: b) Assisted killing is another strategy of killing diseased cells. Here one method is to insert a well known foreign antigen coding gene which induces immune cells for the killing of the diseased cells. Few more methods are based on immune cells activation in which a certain cytokine encoding gene incorporated into gene therapy vector and inserted into either diseased cells or non-diseased cells. This will lead to enhanced immune response followed by killing of diseased cells.

8-1.3.3 Targeted Inhibition of Gene Expression

This is to block the expression of any diseased gene or a new gene expressing a protein which is harmful for a cell. This is particularly suitable for treating infectious diseases and some cancers.

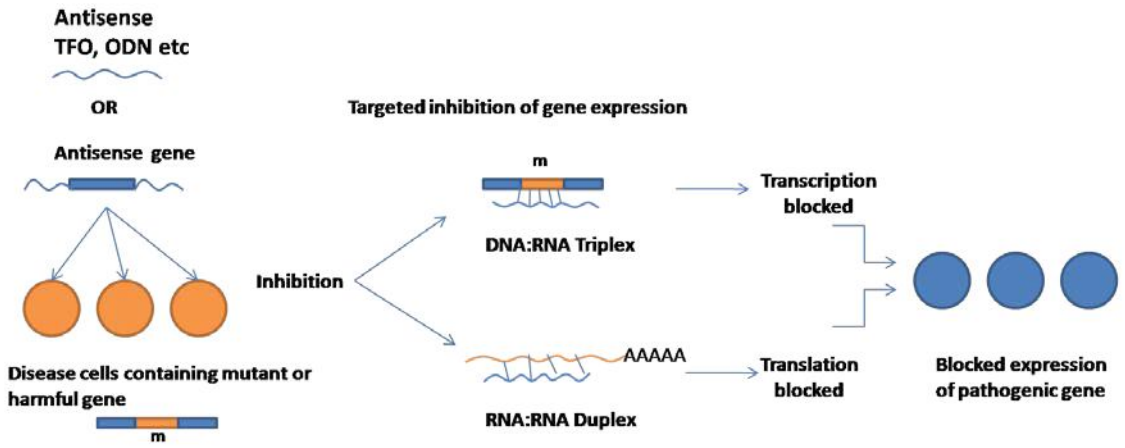

Figure 8-1.3.3 To inhibit the target gene expression in diseased cell the antisense mRNA coding gene inserted vector or triplex-forming oligonucleotides (TFO) or antisense oligonucleotide (ODN) can be introduced which will inhibit the gene expression either by forming DNA:RNA triplex inside the nucleus or forming RNA:RNA duplex by forming complementary mRNA strand of disease protein coding mRNA. This may lead to blocking of disease causing protein expression.

8-1.3.4 Targeted Gene Mutation Correction

It is used to correct a defective gene to restore its function which can be done at genetic level by homologous recombination or at mRNA level by using therapeutic ribozymes or therapeutic RNA editing.

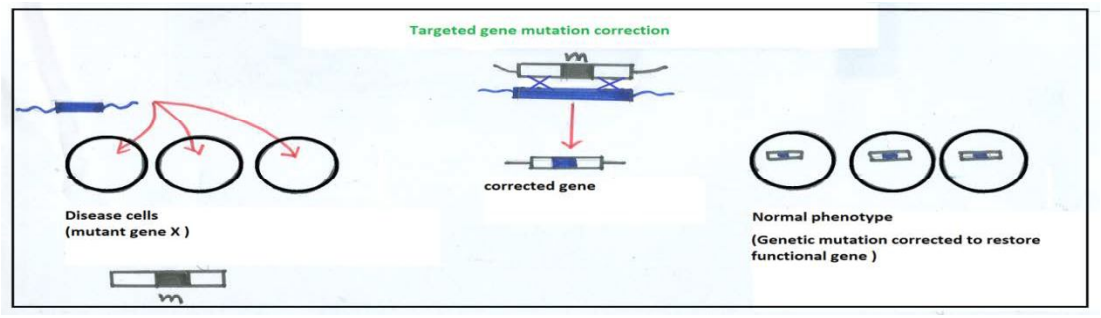

Figure 8-1.3.5 This is used for disease caused by mutation. The corrected gene will be swapped by the mutant gene X (m). Then diseased cells will become normal after the correction of mutation by gene therapy.

(Source: http://www.ncbi.nlm.nih.gov/books/NBK7569/figure/A2871/?report=objectonly)

8-1.4 Gene Therapy Approaches

8-1.4 .1 Classical Gene Therapy

It involves therapeutic gene delivery and their optimum expression once inside the target cell. The foreign genes carry out following functions.

- Produce a product (protein) that the patient lacks;
- Produces toxin so that diseased cell is killed.
- Activate cells of the immune system so as to help in killing of diseased cells.

8-1.4 .2 Non-classical gene therapy

It involves the inhibition of expression of genes associated with the pathogenesis, or to correct a genetic defect and restore the normal gene expression.

8-1.5 Methods of gene therapy

There are mainly two approaches for the transfer of genes in gene therapy:

1**.** Transfer of genes into patient cells outside the body (*ex vivo gene therapy*)

2. Transfer of genes directly to cells inside the body (*in vivo*).

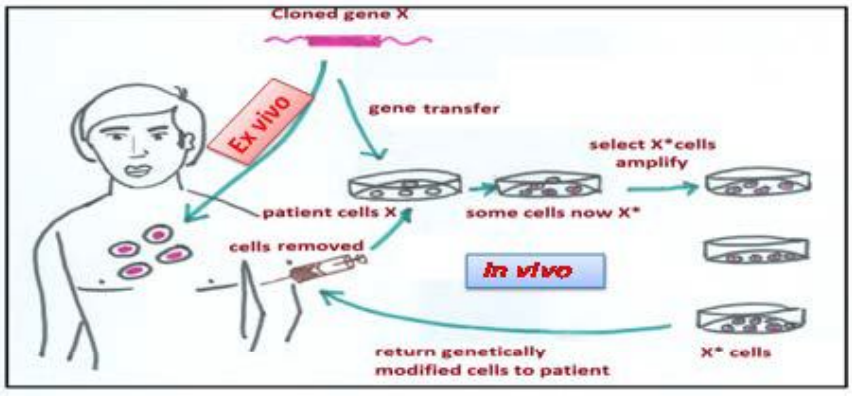

Figure 8-1.5 Gene Therapy using autologous cells: Cells are used, i.e. cells are removed from the patient, cultured *in vitro*, before being returned to the patient's body. In this figure *in vivo* and *ex vivo* gene therapy is diagrammatically explained.

(Source: *http://www.ncbi.nlm.nih.gov/books/NBK7569/figure/A2897/?report=objectonly*)

8-1.5.1 *Ex vivo* gene therapy

- In this mode of gene therapy genes are transferred to the cells grown in culture, transformed cells are selected, multiplied and then introduced into the patient.
- The use of autologous cells avoids immune system rejection of the introduced cells.
- The cells are sourced initially from the patient to be treated and grown in culture before being reintroduced into the same individual.
- This approach can be applied to the tissues like hematopoietic cells and skin cells which can be removed from the body, genetically corrected outside the body and reintroduced into the patient body where they become engrafted and survive for a long period of time.
- Figure 8-1.5.1 shows a self explanatory schematic diagram for e*x vivo* gene transfer.

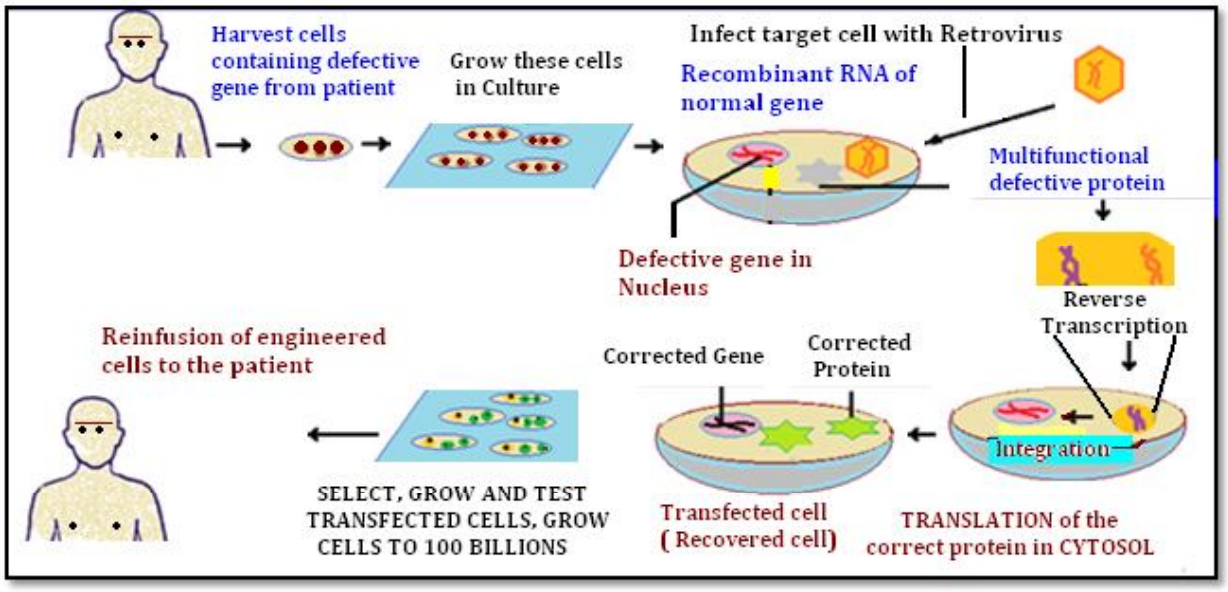

Figure 8-1.5.1 *Ex vivo* therapy involves tightly regulated cellular manipulation in harvested cells

8-1.5.2 *In Vivo* Gene Therapy

- *In vivo* method of gene transfer involves the transfer of cloned genes directly into the tissues of the patient.
- This is done in case of tissues whose individual cells cannot be cultured *in vitro* in sufficient numbers (like brain cells) and/or where re-implantation of the cultured cells in the patient is not efficient.
- Liposomes and certain viral vectors are employed for this purpose because of lack of any other mode of selection.
- In case of viral vectors such type of cultured cells were often used which have been infected with the recombinant retrovirus *in vitro* to produce modified viral vectors regularly. These cultured cells will be called as vector-producing cells (VPCs)). The VPCs transfer the gene to surrounding disease cells.
- The efficiency of gene transfer and expression determines the success of this approach, because of the lack of any way for selection and amplification of cells which take up and express the foreign gene.
- Figure 8-1.5.2 shows various steps of *in vivo* gene transfer.

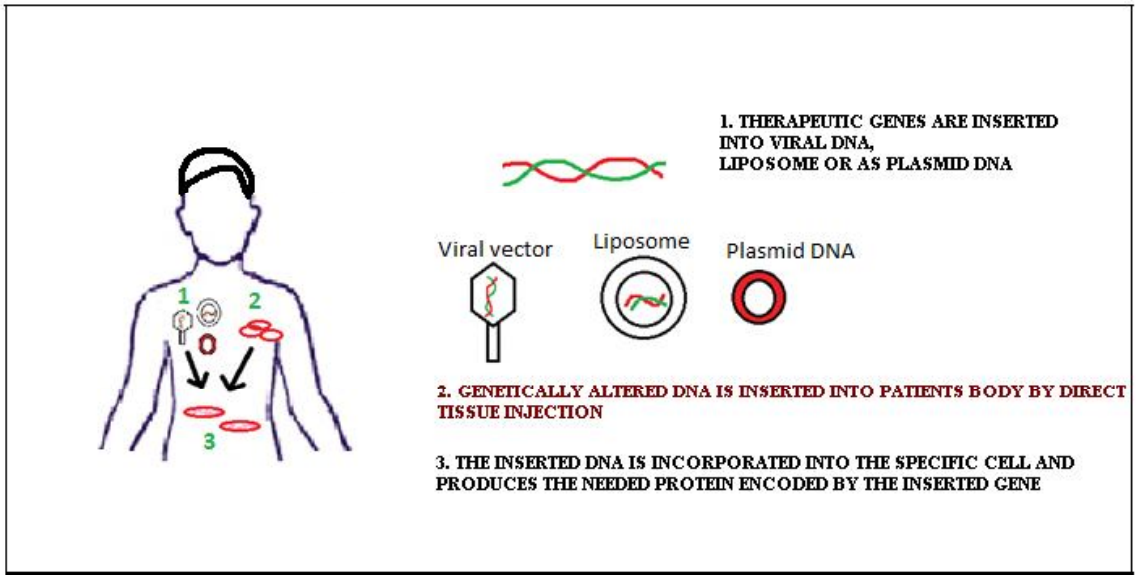

Figure 8-1.5.2 various steps of *in vivo* gene transfer

Table 8-1.5.1: Differences between In Vivo and Ex Vivo gene therapy

Difference Between *in vivo* and *ex vivo* Gene Delivery Systems	
In vivo	***Ex vivo***
Less invasive	More invasive
Technically simple	Technically complex
Vectors introduced directly	No vectors introduced directly
Safety check not possible	Safety check possible
Decreased control over target cells	Close control possible

8-1.6 Target sites for Gene Therapy

Therapeutic genes have to be delivered to specific target sites for a specific type of disease. This table describes the list of such disease and their target sites for gene therapy.

Table 8-1.6.1: Target cells for gene transfer

Target cells for gene transfer	
Disease	**Target Cells**
Cancer	Tumor cells, antigen presenting cells (APCs), blood progenitor cells, T cells, fibroblasts, muscle cells
Inherited monogenic disease	Lung epithelial cells, macrophages, T cells, blood progenitor cells, hepatocytes, muscle cells
Infectious disease	T cells, blood progenitor cells, antigen presenting cells (APCs), muscle cells
Cardiovascular disease	Endothelial cells, muscle cells
Rheumatoid arthiritis	Sinovial lining cells
Cubital tunnel Syndrome	Nerve cells

8-1.7 Vectors for gene therapy

Vectors for gene therapy can be classified into two types:

1. Viral vectors
2. Non-viral

Note: Table 2 shows vectors used in gene therapy. It is adapted From AR Prabhakar in *Gene Therapy and its Implications in Dentistry*. International Journal of Clinical Pediatric Dentistry, 2011; 4(2):85-92

Table 8-1.7: Vectors used in gene therapy

Vectors used in gene therapy	
Viral Vector	**Non-viral Vectors**
Adenovirus	Lipid complex
Retrovirus	Liposomes
Adeno- Associated Virus	Peptide/protien
Lentivirus	Polymers
Vaccinia virus	
Herpes simplex virus	
• Direct gene transfer methods like mechanical, electroporation, gene gun are also ued to transfer genes into target cells.	

8-1.7.1 Viral vectors

Retroviruses, adenoviruses and adeno-associated viruses (AAV) some commonly used viral vectors whereas some less commonly used viral vectors are derived from the Herpes simplex virus (HSV-1), the baculovirus etc.

- **Adenoviral vectors**

Adenoviruses are large linear double-stranded DNA viruses that are commonly used for preparing gene transfer vectors. Adenovirus vectors are known to be the second most popular gene delivery vector for gene therapy of various diseases like cystic fibrosis and certain types of cancer. Figure 8-1.7.1.1 shows how the adenoviruses enter cells by receptor-mediated endocytosis. A primary cellular receptor binds to viral fiber then the virus interacts with secondary receptors which are responsible for its internalization. Coxsackie and Adenovirus Receptor (CAR), Heparan sulphate glycosaminoglycans, sialic acid, CD46, CD80, CD86, alpha domain of MHC I are the primary receptors which are specific for specific strains of adenovirus. Integrins are the secondary receptors which helps in the internalization of viral particles. Some adenovirus directly interacts with integrins like in the case of fiber deficient Ad2 virions.

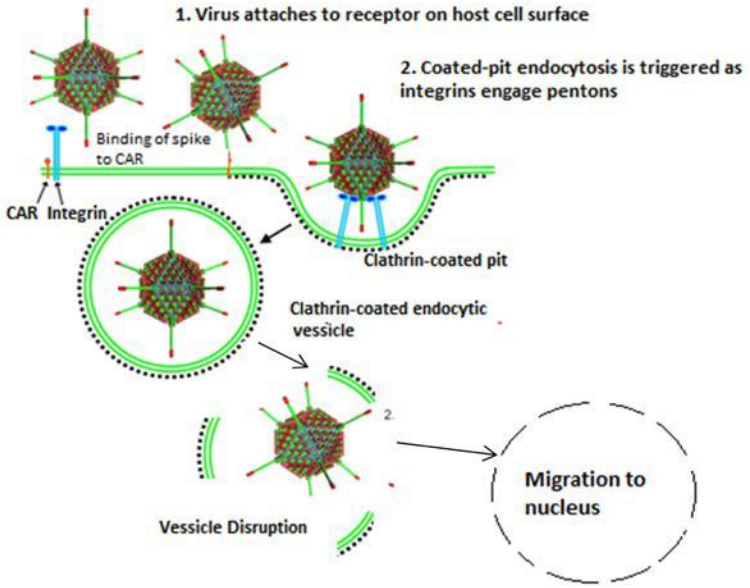

Figure 8-1.7.1.1a

***Adapted and modified from*: http://www.ncbi.nlm.nih.gov/books/NBK7569/figure/A2918/?report=objectonly**

The adenoviral DNA has inverted terminal repeats (ITRs) and a terminal protein (TP) is attached covalently to 5' termini. The adenoviral genome is classified as early and late regions based on the proteins they express. Proteins encoded by early region (E1, E2, E3, E4) genes are involved in viral DNA replication, cell cycle modulation and defense system. The late region genes (L1, L2, L3, L4, L5) encodes the viral structural proteins. Three classes of adenoviral vectors namely first, second and third generation viral vectors are developed for gene therapy purpose.

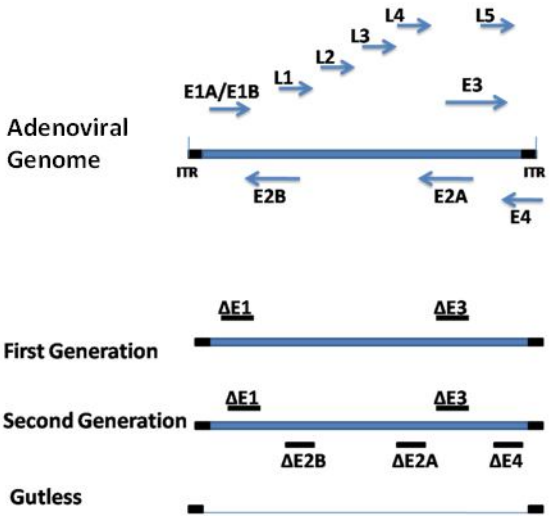

Figure 8-1.7.1.1b Map of Adenoviral genome and construction of different types of adenoviral vectors

Adapted and Modified from: R Alba, A Bosch and M Chillon (2005). Gutless adenovirus: last-generation adenovirus for gene therapy. Gene Therapy, 12, S18-S27

First generation adenoviral Vectors

These vectors are constructed by replacing the E1/E3 expression cassette and inserting our candidate gene of 3-4kb size. E1 encodes proteins responsible for expressions of other viral genes required for viral growth. So cell lines that can provide E1 proteins *in trans* are required for the replication of the E1 deleted viral vectors.

Advantages:

- They are human viruses produced at very high titers in culture.
- They can infect a wide range of human cell types including non- dividing cells.

- They enter into cells by receptor mediated endocytosis with a very high transduction efficiency reaching upto 100% *in vitro*
- Their large size enables them to accept large inserts.

Disadvantages:

- Expression of foreign gene is for short period of time as they do not integrate into the chromosome.
- These vectors may generate immune response causing chronic inflammation.

Second generation adenoviral Vectors

These vectors have been developed to overcome these difficulties. Here of E1/E2 or E3/E4 expression cassettes are called deleted and replaced. The E1/E2 or E3/E4 proteins are required for viral DNA replication. Similar to first generation viral vector, cell lines which can complement both E1and E2 or E3 and E4 are needed. It can carry DNA insert upto 10.5kb

Advantages:

- It has improved safety and increased transgene expression.

Disadvantages:

- These viral vectors are associated with immunological problems.
- Construction of these vectors is difficult.

Third generation adenoviral Vectors

These vectors are otherwise called as **gutless adenovirus.** These are also known as helper dependent adenovirus as they lack all the coding sequences and require helper virus which carries all the coding sequences. Helper virus for example AAV, or artificially disabled viruses provide the viral functions needed for successful infection like viral DNA replication, viral assembly and infection of new cells etc. The size of insert DNA can be 36kb and hence called as high

capacity adenoviruses. They carry only 5' inverted terminal repeats (ITR) and 3' packaging signals (ψ).

Advantages:

- These are non-integrative and high-capacity vectors.
- It can be produced in high titer and the construction of these vector is easy.
- It shows longer stability and reduced immune response.

Disadvantages:

- Helper virus contamination contamination can cause diseases like conjunctivitis, pharyngitis, cold and respiratory disease.

➢ **Adeno- Associated Virus (AAV)**

Adeno-associated viruses (AAVs) are a group of small, single-stranded DNA viruses which cannot usually undergo productive infection without co-infection by a helper virus, such as an adenovirus.

- The insert size for AAV is 4.5 kb, with the advantage of long-term gene expression as they integrate into chromosomal DNA.
- AAVs are highly safe as the recombinant adeno associated vectors contains only gene of interest and 96% viral genes are deleted.

Adeno-associated viruses are explained in detail in Module 5-Lecture 1.

➢ **Retroviral Vectors**

Retroviruses are RNA viruses which possess a reverse transcriptase activity, enabling them to synthesize a complementary DNA. Following infection (transduction), retroviruses deliver a nucleoprotein complex (pre-integration complex) into the cytoplasm of infected cells. The viral RNA genome is reverse transcribed first and then integrates into a single site of the chromosome.

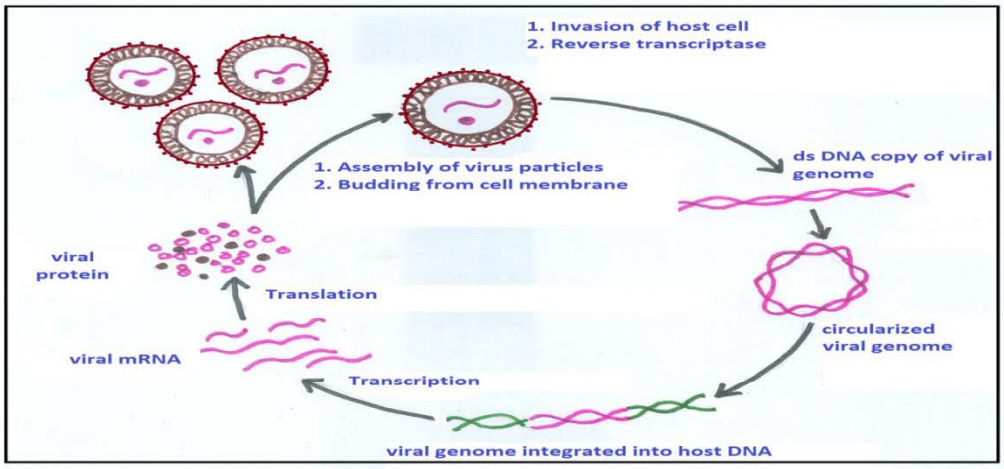

Figure 8-1.6.1.1c

Adaptedand modified from: http://www.ncbi.nlm.nih.gov/books/NBK7570/figure/A2357/?report=objectonly

- **Tumor retroviruses**, example Moloney's murine leukemia virus (MoMuLV), is widely used for the generation of recombinant vectors. these are produced at low titers as all the viral genes are deleted.
- **Oncoretroviruses:** The cells that divide shortly after infection can only be transduced by oncoretrovirus. The preinitiation complex is excluded and their entry is restricted in to the nucleus as they can only enter when nuclear membrane dissolves during cell division the target cells for this viral vector is limited

- **Recombinant lentiviruses** are being developed that are non- pathogenic to humans and have the ability to transduce stationary cells.

➢ **Other Viral Vectors:**

These include herpes simplex virus vectors and baculovirus.

Herpes simplex virus vectors: Herpes simplex virus-1 (HSV-1) is a 150 kb double-stranded DNA virus with a broad host range that can infect both dividing and non-dividing cells. the insert size is comparatively larger (>20kb) but have a disadvantage of short-term expression due to its inability to integrate into the host chromosome

Baculovirus: They can take up very large genes and express them highly efficiently. They help in recombinant protein expression in insect cell. They can infect hepatocytes as an only mammalian cell type and the gene expresses under the control of either mammalian or viral promoter.

Simian Virus 40 Vectors (SV40): SV40 are icosahedral papovavirus with a circular double stranded DNA of 5.2kb size as genetic material. The genome encodes for early proteins viz; large T antigen (Tag) and small t antigen (tag) and late protein viz; a regulatory protein agnoprotein and three structural proteins (VP1, VP2, VP3). The Tag gene is removed as it is responsible for inducing immunogenicity in the recombinant SV40 vector. All the structural proteins except the major capsid protein VP1 is removed resulting in a genome of 0.5kb size which includes origin of replication (ori) and encapsidation sequence. Recombinant SV40 vectors allows expression of transduced gene

8-1.7.2 Non- viral vectors

It involves chemical and physical methods such as direct injection of naked plasmid DNA (particle bombardment), receptor-mediated endocytosis and gene transfer through liposomes, polymers, nano particles etc.

Some non viral methods

- **Direct injection/particle bombardment:**

DNA can be injected parenterally which can be considered for Duchenne muscular dystrophy (DMD). An alternative approach uses particle bombardment (‘gene gun’) technique, in which DNA is coated on to metal micro particles and fired from a ballistic gun into cells/tissues. This technique is used to transfer the foreign DNA and its transient expression in mammalian cells *in vitro* and *in vivo* as well. It can cross the physical barriers like skin, muscle layer for which it is used for vaccination. Particle bombardment is used to deliver drugs, fluorescent dyes, antigenic proteins etc.

Advantage: Simple and comparatively safe.

Disadvantage:

- Poor efficiency of gene transfer.
- A low level of stable integration of the injected DNA. Repeated injection may cause damage in the proliferating cells.

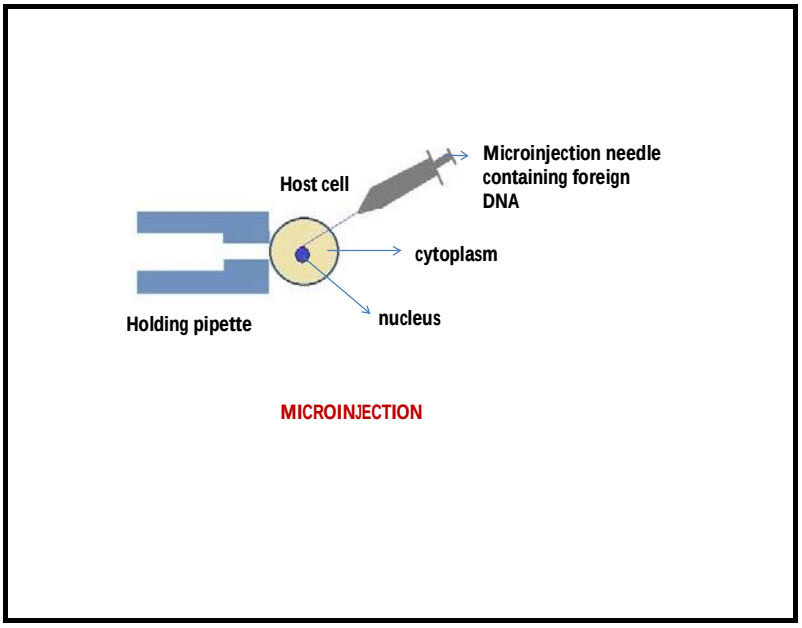

Figure 8-1.7.2.1 Microinjection process

Microinjection involves the delivery of foreign DNA, by the help of glass micropipette into a living cell. The cell is held against a solid support or holding pipette and micro neeedle containing the desired DNA is inserted into the cell. The tip of the pipette used is about 0.5 to 5 micro meter diameter which resembles an injection needle. For this, glass micropipette is heated until the glass becomes somewhat liquefied and is quickly stretched to ressemble a injection needle. The delivery of foreign DNA is done under a powerful microscope (micromanupulator).

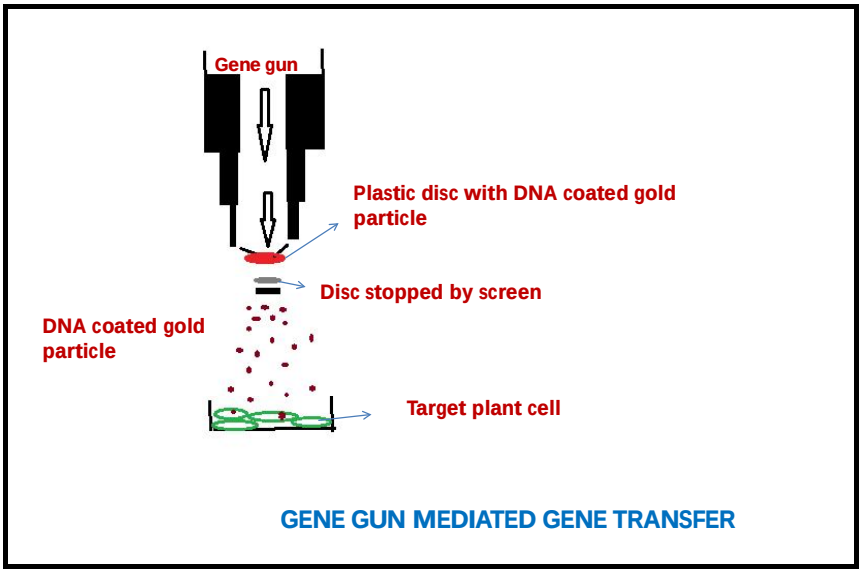

Figure 8-1.7.2.2 Gene gun mediated gene transfer

In **particle bombardment** method, the tungsten or gold particles (micro projectiles) are coated with the foreign DNA. Micro-projectile bombardment uses high-velocity metal particles to deliver biologically active DNA into the target cells. The macroprojectile is coated with the coated particles and is accelerated with air pressure and shot into plant the target tissue. A perforated plate is used, which allows the micro-projectiles to pass through to the cells on the other side of the plate and stops the macropojectile. Particle coated with the foreign gene releases the foreign gene when enters into the target cell and integrates into the chromosomal DNA. This technique is also used to transfer genes in mammalian cells. Mammalian cell lines like HEK 293, MCF7 showed gene expression when transfected with luciferase and green fluorescent genes and their gene expression was dependent on helium pressure, size and amount of gold particle and DNA load on each particle. Cell viability depends on helium pressure.

(See lecture 3 of module 5 for advantages and limitations)

- **Liposomes Mediated**

Liposomes are spherical vesicles which are made up of synthetic lipid bilayers which imitate the structure of biological membranes. DNA to be transferred is packaged into the liposome *in vitro* and transferred to to the targeted tissue. The lipid coating helps the DNA to survives *in vivo* and enters into the cell by endocytosis. Cationic liposomes,

where the positive charge on liposomes is stabilized by binding of negatively charged DNA, are popular vehicles for gene transfer *in vivo.* For more detail see lecture 2 of module 5.

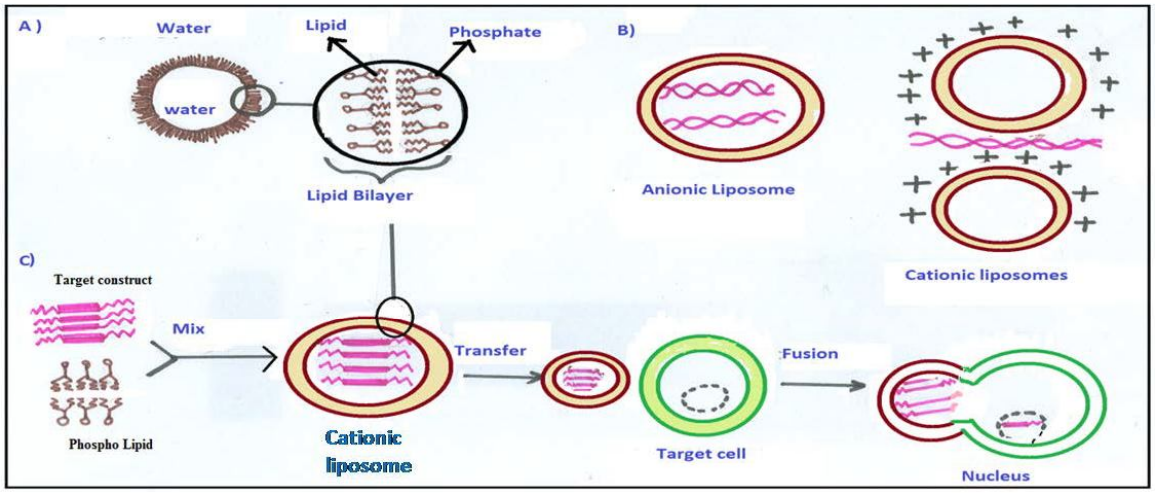

Figure 8-1.7.2.4: In vivo liposome mediated gene transfer- (A) formation of lipid bilayer in water (B) Structure of anionic and cationic liposome (C) Use of liposome to transfer genes into cells.

Adapted and modified from: http://www.ncbi.nlm.nih.gov/books/NBK7569/figure/A2924/?report=objectonly

Advantage:

- The liposomes with the foreign DNA are easy to prepare.
- There is no restriction in the size of DNA that is to be transferred.

Disadvantage:

- Efficiency of gene transfer is low and transient expression of the foreign gene is obtained as they are not designed to integrate into the chromosomal DNA.

➢ **Electroporation**

In electroporation, the external electric field is applied to the protoplast, which changes the electrical conductivity and the permeability of cell membrane; and thus the exogenous molecules found in the medium are taken up to either the cytoplasm (transient transfection) or into the nucleus (stable transfection).The efficiency of electroporation can be increased by giving the cell a heat shock, prior to the application of electric field

or by using small quantity of PEG while doing electroporation. See lecture 3 of module 5 for detailed explanation.

Advantage:

- By electroporation large numbers of cells can be processed at once, and thus the amount of time spent processing cells can be cut down.

Disadvantages:

- If the voltage applied is not calculated properly, the cells may damage.
- If electroporation does not occur in controlled environment, the potentially harmful substances can enter the cell or the impurities from solution may enter. This is because there is no way to control what enters the cell membrane.

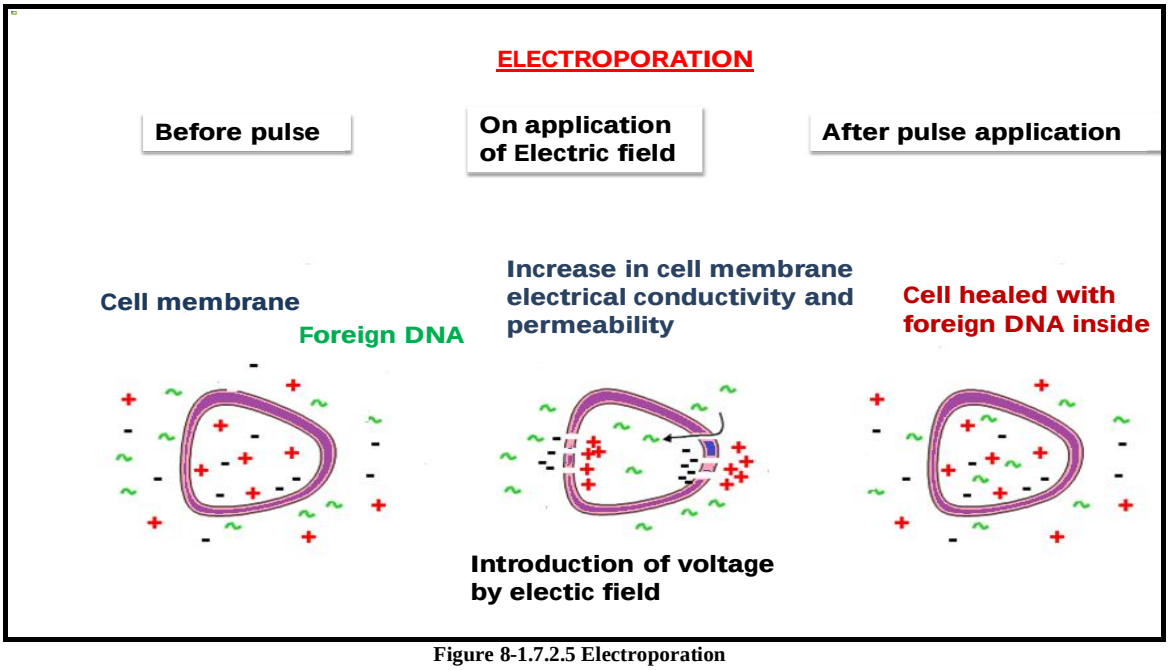

Figure 8-1.7.2.5 Electroporation

- **Sleeping Beauty Transposition**

This is a non viral method of gene transfer which offer the advantage of stable DNA integration into the chromosomes of vertebrates. The sleeping beauty transposition system consists of a sleeping beauty transposon and a sleeping beauty transposase. The SB transposon consists of two terminal inverted repeats present each at the ends of the gene of interest and the SB transposase mediates excision of the SB transposon and its integration into a site of chromosome having a dinucleotide dimer TA by cut and paste mechanism.

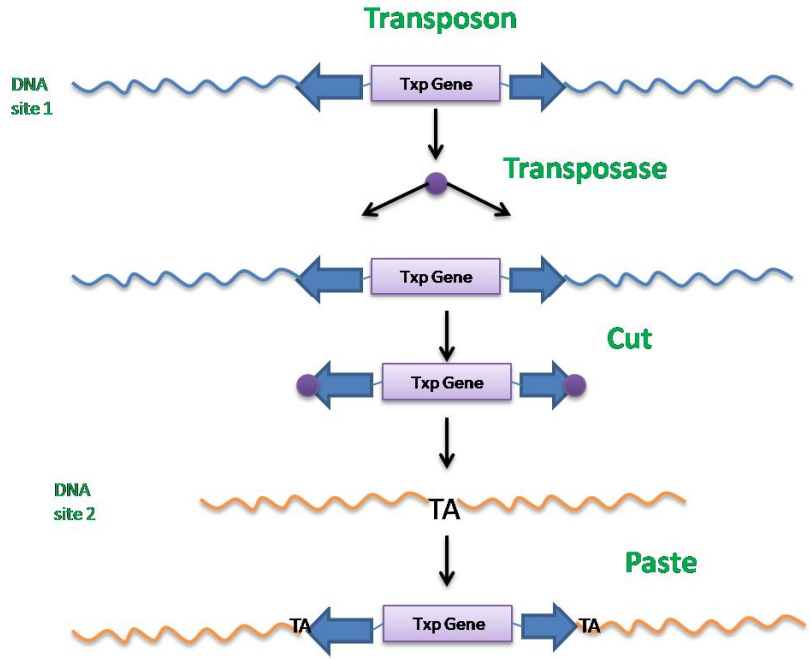

Figure 8-1.7.2.6 Cut and paste mechanism of a DNA transposon by a transposase (txp gene)

Adapted and modified from: **http://www.discoverygenomics.net/pdf/SBT_ProlongedExpressions.pdf**

For gene therapy transposase gene is replaced by our gene of interest and transposase is provided in trans as shown in Figure8-1.7.2.7.

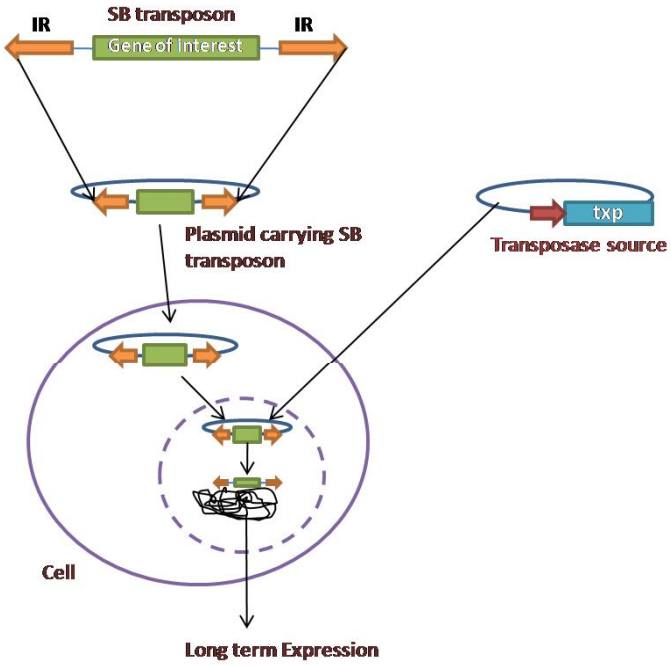

Figure8-1.7.2.7 Mechanism of gene transfer by sleeping beauty transposition

Adapted and modified from: **http://www.discoverygenomics.net/pdf/SBT_ProlongedExpressions.pdf**

- **RNA-DNA Chimera**

This RNA- DNA chimera also known as chimeroplast is used to correct point mutations by mismatch repair. This 68 nucleotides long double stranded nucleic acid molecule comprises of one strand DNA and another strand consisting of two 10 nucleotides long 2'-O methyl RNA stretch separated by a 5 nucleotide long DNA stretch as shown in Figure 8-1.7.2.8. The pentameric chimeric DNA carries the mismatch and the other DNA strand has its complementary bases. Additionally this RNA-DNA chimera consists of two hairpin loop and a GC clamp.

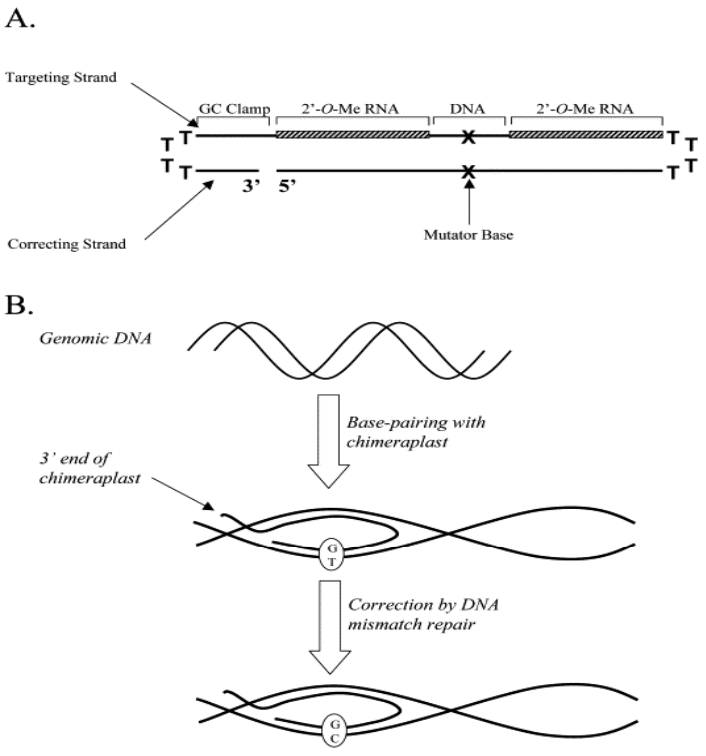

Figure 8-1.7.2.8: Structure and mechanism of action of RNA DNA chimera

Source: **http://www.sciencedirect.com/science/article/pii/S0925443902000686**

- **Receptor-Mediated Endocytosis**

It can be both viral and non-viral mediated gene transfer. Viral vectors attach to the surface receptors through viral surface components and internalized. In non viral mode of receptor mediated endocytosis DNA is first coupled to a ligand that binds specifically to a cell surface receptor and causes transfer of the DNA into cells by endocytosis. Coupling is done by linking the receptor molecule with polylysine followed by reversible binding of the negatively charged DNA to the positively charged polylysine component. Transferrin receptor which is comparatively abundant in proliferating cells and hematopoietic cells is utilized as a target and transferin as a ligand in this approach.

Advantage: Gene transfer efficiency may be high.

Disadvantage:

- It does not allow integration of the transferred genes. Also, the protein— DNA complexes are not stable in serum.

Coupling of inactivated adenovirus to the DNA-transferin complex can increase gene transfer efficiency which help in receptor mediated endocytosis and lysosomal escape.

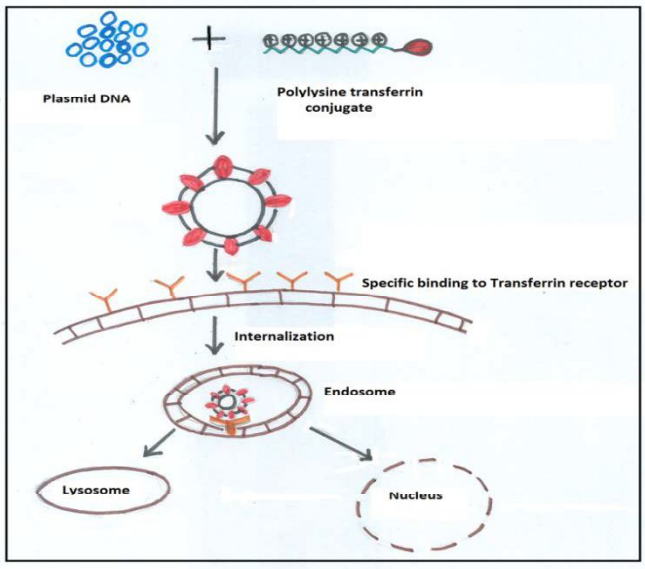

Figure 8-1.7.2.3 Receptor-Mediated Endocytosis

Adapted and modified from: http://www.ncbi.nlm.nih.gov/books/NBK7569/figure/A2927/?report=objectonly

8-1.8 Endosomal/Lysosomal Escape

In gene therapy the gene becomes entrapped in the endocytic pathway and is degraded by the hydrolytic enzymes which are a major limiting step in gene therapy. There are various approaches to escape this endocytic pathway for both viral and non-viral gene delivery systems.

- **For viral gene delivery system**

  Enveloped virus can penetrate through endosome membrane and non enveloped viruses escape by forming pores or lyse the vesicular membrane.

- **For non-viral gene delivery system**

There are various strategies developed non-viral vector system for endosomal escape.

- **The 'proton sponge' hypothesis**: 'Proton sponge' effect has been seen in some cationic polymers which contain protonable amine groups with pKa close to endosomal/lysosomal pH can be used for gene delivery During acidification of endosomes the cationic polymers become protonated so that the H^+ influx is increased and thereby Cl^- concentration is increased inside the endosome leading to water influx. As a result the endosome swells and eventually ruptures.

- **Flip-Flop mechanism:** Due to electrostatic interaction of cationic lipoplexes and anionic lipids of endosomes facing towards cytoplasm there is lateral diffusion of lipids resulting in displacement of nucleic acids from lipoplexes to cytoplasm.

- **Pore formation**: In a peptide based gene delivery system a peptide named GALA which can undergo conformation change at low pH forms a pore in the vesicular membrane when incorporated.

- **Photochemical internalizaton**: This technique uses photosensitizers which can bind to and localize in the plasma membrane. These photosensitizers become confined to endosomal membrane during

endocytosis and remain inactive. When irradiated with light of a particular wavelength they produce reactive oxygen species resulting in lysis of endosomes.

8-1.9 Advantages of Gene Therapy

- Gene therapy can cure genetic diseases by addition of gene or by removal of gene or by replacing a mutated gene with corrected gene.
- Gene therapy can be used for cancer treatment to kill the cancerous cells.
- Gene expression can be controlled.
- Therapeutic protein is continuously produced inside the body which also reduces the cost of treatment in long term.

Bibliography

Bainbridge, J. W. B.; Smith, A. J.; Barker, S. S.; Robbie, S.; Henderson, R.; Balaggan, K.; Viswanathan, A.; Holder, G. E. et al. (2008). "Effect of Gene Therapy on Visual Function in Leber's Congenital Amaurosis". New England Journal of Medicine 358 (21): 2231–2239.

Cartier, N.; Aubourg, P. (2009). "Hematopoietic Stem Cell Transplantation and Hematopoietic Stem Cell Gene Therapy in X-Linked Adrenoleukodystrophy". Brain Pathology 20 (4): 857–862.

Cideciyan, A. V.; Hauswirth, W. W.; Aleman, T. S.; Kaushal, S.; Schwartz, S. B.; Boye, S. L.; Windsor, E. A. M.; Conlon, T. J. et al. (2009). "Vision 1 Year after Gene Therapy for Leber's Congenital Amaurosis". New England Journal of Medicine 361 (7): 725–727.

Coghlan, Andy (11 December 2013) Souped-up immune cells force leukaemia into remission New Scientist, Retrieved 15 April 2013

Coghlan, Andy (26 March 2013) Gene therapy cures leukaemia in eight days The New Scientist, Retrieved 15 April 2013

Ferrua, F.; Brigida, I.; Aiuti, A. (2010). "Update on gene therapy for adenosine deaminase-deficient severe combined immunodeficiency". Current Opinion in Allergy

and Clinical Immunology 10 (6): 551–556. doi:10.1097/ACI.0b013e32833fea85. PMID 20966749.

Fischer, A.; Hacein-Bey-Abina, S.; Cavazzana-Calvo, M. (2010). "20 years of gene therapy for SCID". Nature Immunology 11 (6): 457–460. doi:10.1038/ni0610-457. PMID 20485269.

Griffiths A.J.F, Miller J.H., Suzuki D.T., et al. *An Introduction to Genetic Analysis*, 7th ed.; New York: W. H. Freeman; 2000.

Guha C., Ghosh S.S., Lee S.W., Chowdhury N.R., Chowdhury J.R. *Madame Curie Bioscience Database [Internet];* Austin (TX): Landes Bioscience; 2000.

Ledford, H. (2011). "Cell therapy fights leukaemia". Nature. doi:10.1038/news.2011.472. edit

Lewitt, P. A.; Rezai, A. R.; Leehey, M. A.; Ojemann, S. G.; Flaherty, A. W.; Eskandar, E. N.; Kostyk, S. K.; Thomas, K. et al. (2011). "AAV2-GAD gene therapy for advanced Parkinson's disease: A double-blind, sham-surgery controlled, randomised trial". The Lancet Neurology 10 (4): 309–319.

Maguire, A. M.; Simonelli, F.; Pierce, E. A.; Pugh, E. N.; Mingozzi, F.; Bennicelli, J.; Banfi, S.; Marshall, K. A. et al. (2008). "Safety and Efficacy of Gene Transfer for Leber's Congenital Amaurosis". New England Journal of Medicine 358 (21): 2240–2248.

Simonelli, F.; Maguire, A. M.; Testa, F.; Pierce, E. A.; Mingozzi, F.; Bennicelli, J. L.; Rossi, S.; Marshall, K. et al. (2009). "Gene Therapy for Leber's Congenital Amaurosis is Safe and Effective Through 1.5 Years After Vector Administration". Molecular Therapy 18 (3): 643–650.

Strachan T., Read A.P. *Human Molecular Genetics,* 2nd ed.; New York: Wiley-Liss, 1999; Chapter 22.

Wink M. *An Introduction to Molecular Biotechnology*, 2nd ed.; WILEY- VCH Verlag GmbH & Co. KGaA, 2006; pp 523-542.

Ute Fischer , Sabine Steffens, Susanne Frank, Nikolai G Rainov, Klaus Schulze-Osthoff and Christof M Kramm (2005). Mechanism of thymidine kinase/ganciclovir and cytosine deaminase/5-fluorocytosine suicide gene therapy-induced cell death in glioma cells. *Oncogene* 24, 1231–1243.

Danthinne X and Imperiale M J(2000). Production of first generation adenovirus vectors: a review. *Gene Therapy,* 7, 1707-1714

Martin Andreas MückHäusl(2011), *Genetic engineering of adenoviral vectors for improved therapeutic applications.*

Min Wang, Xinyu Zheng, Xiao-Mei Rao, Hongying Hao, Yanbin Dong, Kelly M. McMasters, and H. Sam Zhou. (2005) Adenoviral vector systems for gene therapy. *Gene Ther Mol Biol,* Vol 9, 291-300

R Alba, A Bosch and M Chillon (2005). Gutless adenovirus: last-generation adenovirus for gene therapy. *Gene Therapy,* 12, S18-S27

Cynthia Lyle and Frank McCormick (2010), Integrin αvβ5 is a primary receptor for adenovirus in CAR-negative cells. *Virology Journal*, **7**:148

Yuanming Zhang and Jeffrey M. Bergelson (*2005*) Adenovirus Receptors. *J Virol.*, 79(19): 12125–12131

Masaki Uchida, Xiong Wei Li, Peter Mertens, H. Oya Alpar (2009). Transfection by particle bombardment: Delivery of plasmid DNA into mammalian cells using gene gun**.** *Biochimica et Biophysica Acta* 1790, 754–764

David S. Strayer,Lokesh Agrawal, Pierre Cordelier, Bianling Liu, Jean-Pierre Louboutin, Elena Marusich, Hayley J. McKee, Carmen N. NiGongyi Ren, and Marlene S. Strayer. (2006). Long-Term Gene Expression in Dividing and Nondividing Cells Using SV40-Derived Vectors. *Molecular Biotechnology,* Vol. 34, 257-270

Andrew Mountain. (2000) Gene Therapy: the first decade. *Trends in Bioetchnology.* Volume 18, Issue 3, 1 March 2000, Pages 119–128.

Rashmi Sharma, Ruchi Khajuria, C.L. Sharma, B. Kapoor, K.C. Goswam, K. Kohli. (2004). Gene Therapy: Current Concepts, *J.K. Science.* Vol. 6 No. 2, page 62-66

Perry B. Hackett, Stephen C. Ekker, David A. Largaespad, R. Scott McIvor. (2005) *Sleeping Beauty* Transposon-Mediated Gene Therapy for Prolonged Expression. *Advances in Genetics.* Volume 54, Pages 189–232.

Ian R. Graham, George Dickson. (2002) Gene repair and mutagenesis mediated by chimeric RNA–DNA oligonucleotides: chimeraplasty for gene therapy and conversion of single nucleotide polymorphisms (SNPs). *Biochimica et Biophysica Acta (BBA) - Molecular Basis of Disease.* Volume 1587, Issue 1, Pages 1–6.

Wanling Liang and Jenny K. W. Lam (2012) Endosomal Escape Pathways for Non-Viral Nucleic Acid Delivery Systems. *Molecular Regulation of Endocytosis* . Chapter 17