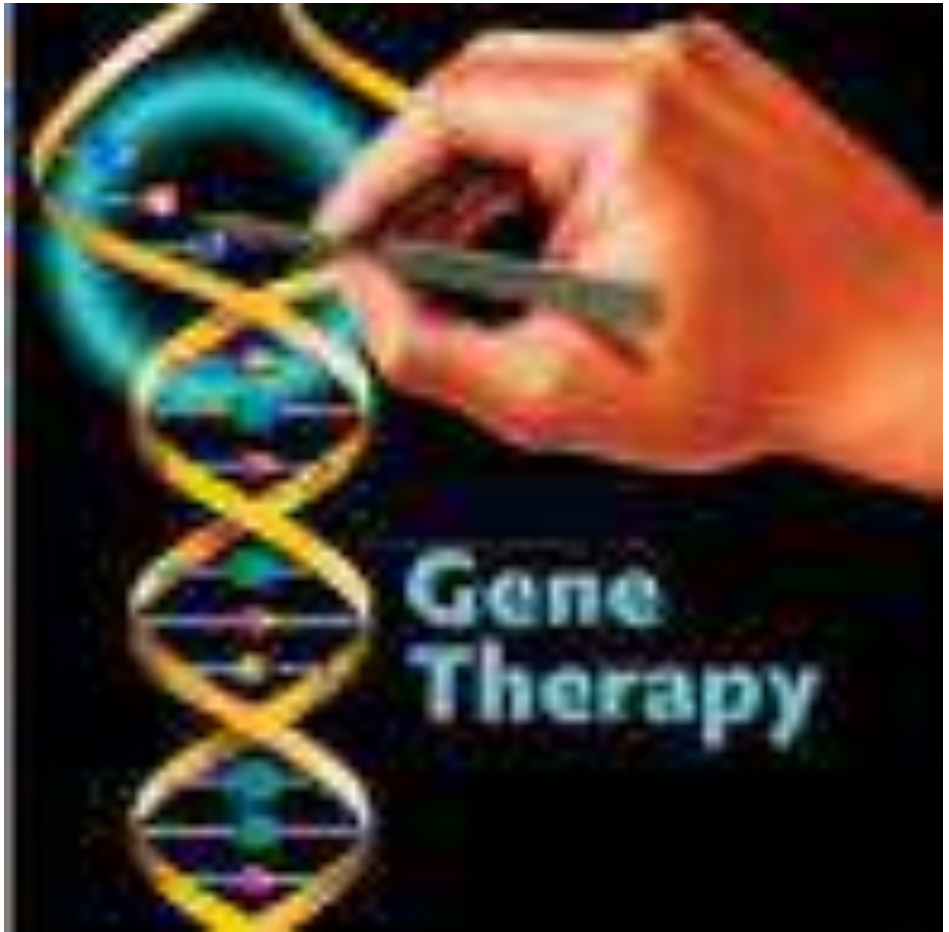
GENE THERAPY



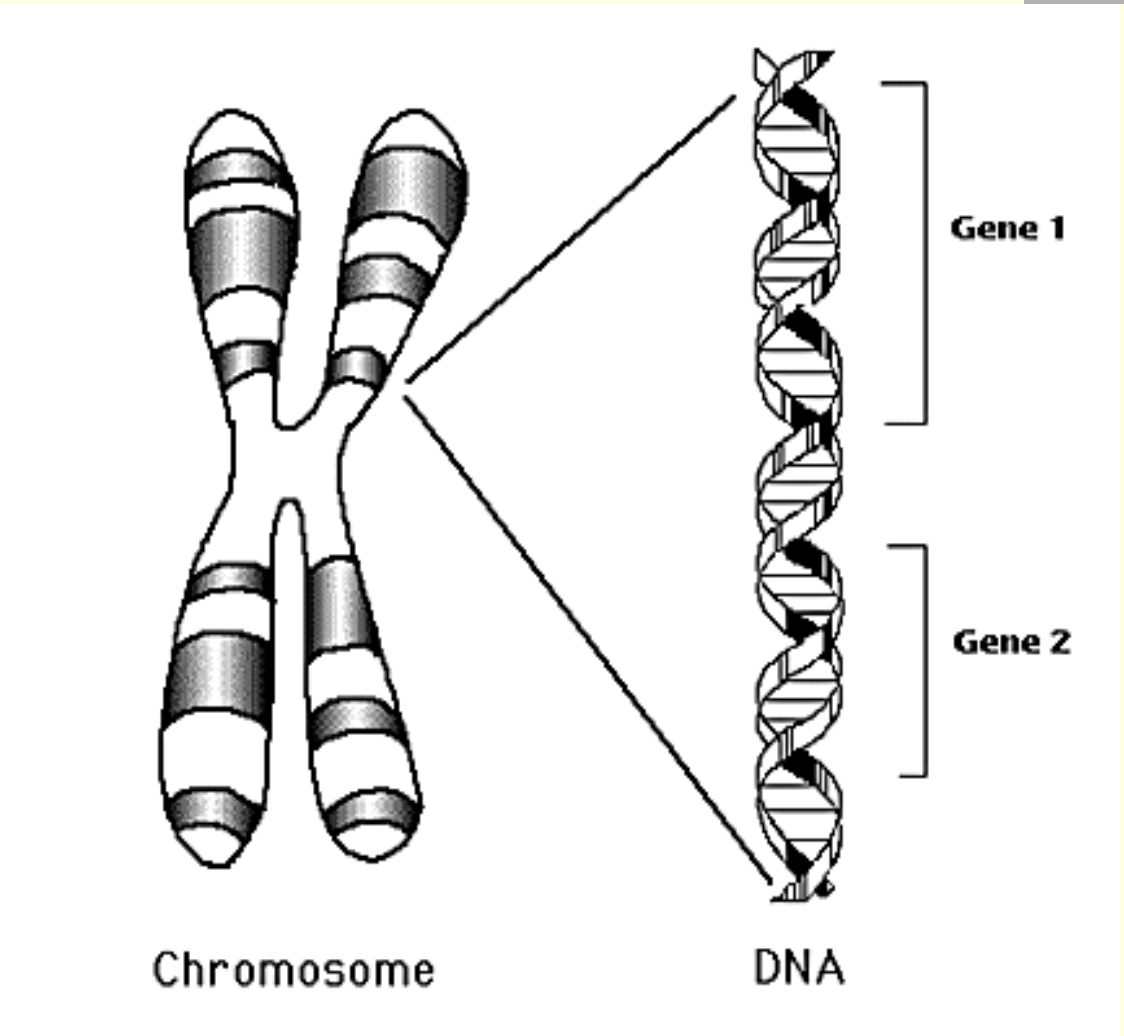
Genes

- Are carried on a chromosome
- The basic unit of heredity
- Encode how to make a protein
  - DNA→RNA →proteins
- Proteins carry out most of life's function.
- When altered causes dysfunction of a protein
- When there is a mutation in the gene,

⟹ It will change the codon,
⟹ will change which amino acid
⟹ will change the conformation of the protein
⟹ will change the function of the protein.

- Genetic disorders result from mutations in the genome.

Picture of a Chromosome



http://www.accessexcellence.org/RC/VL/GG/genes.html

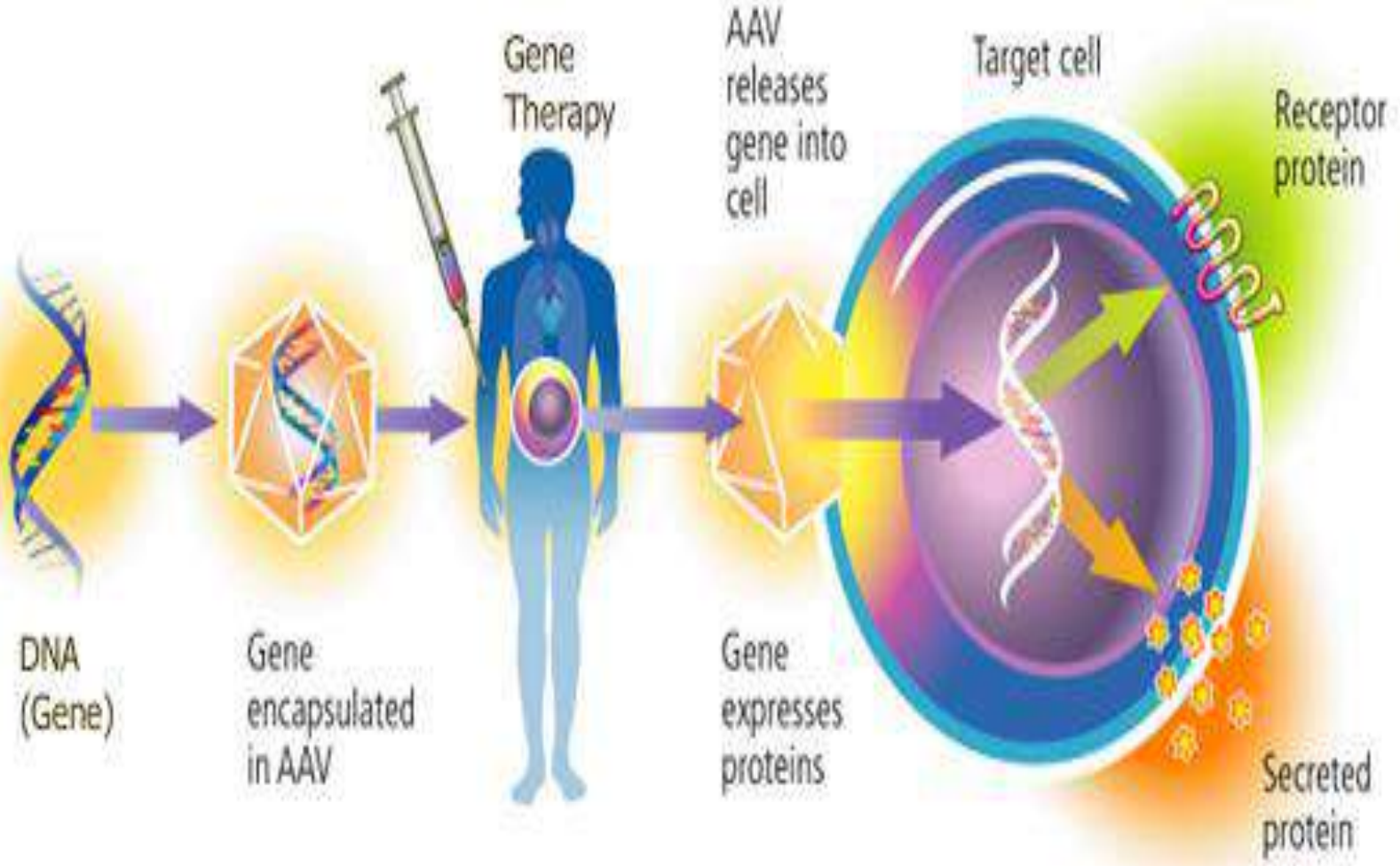
Diseases for applying gene therapy

Disease		Target cell
Severe combined immunodeficiency		Bone marrow cells or T-lymphocytes
Hemophilia		Liver, muscle
Cystic fibrosis		Lung Cells
Cancer		Many cell types
Neurological diseases	Parkinson's/ Alzheimers	Nerve Cells
Infectious diseases	AIDS, hepatitis B	White Blood Cells

What is gene therapy (GT)? Why is it used?

- Gene therapy is the application of genetic principles in the treatment of human disease
- Gene therapy = Introduction of genetic material into normal cells in order to:
  - counteract the effect of a disease gene or
  - introduce a new function
- GT is used to correct a deficient phenotype so that sufficient amounts of a normal gene product are synthesized → to improve a genetic disorder
- Can be applied as therapy for cancers, inherited disorders, infectious diseases, immune system disorders

What is gene therapy?



What is Gene Therapy ?

- It is a technique for correcting defective genes that are responsible for disease development
- There are four approaches:

1. A normal gene inserted to compensate for a nonfunctional gene.
2. An abnormal gene traded for a normal gene
3. An abnormal gene repaired through selective reverse mutation
4. Change the regulation of gene pairs

Gene therapy could be very different for different diseases

- Gene transplantation
  (to patient with gene deletion)
- Gene correction
  (To revert specific mutation in the gene of interest)
- Gene augmentation
  (to enhance expression of gene of interest)

Three types of gene therapy:

- Monogenic gene therapy
  - Provides genes to encode for the production of a specific protein
    - Cystic fibrosis, Muscular dystrophy, Sickle cell disease, Haemophilia, SCID
- Suicide gene therapy
  - Provide ‘suicide’ genes to target cancer cells for destruction
    - Cancer
- Antisense gene therapy
  - Provides a single stranded gene in an ’antisense’ (backward) orientation to block the production of harmful proteins
    - AIDS/HIV

Getting genes into cells

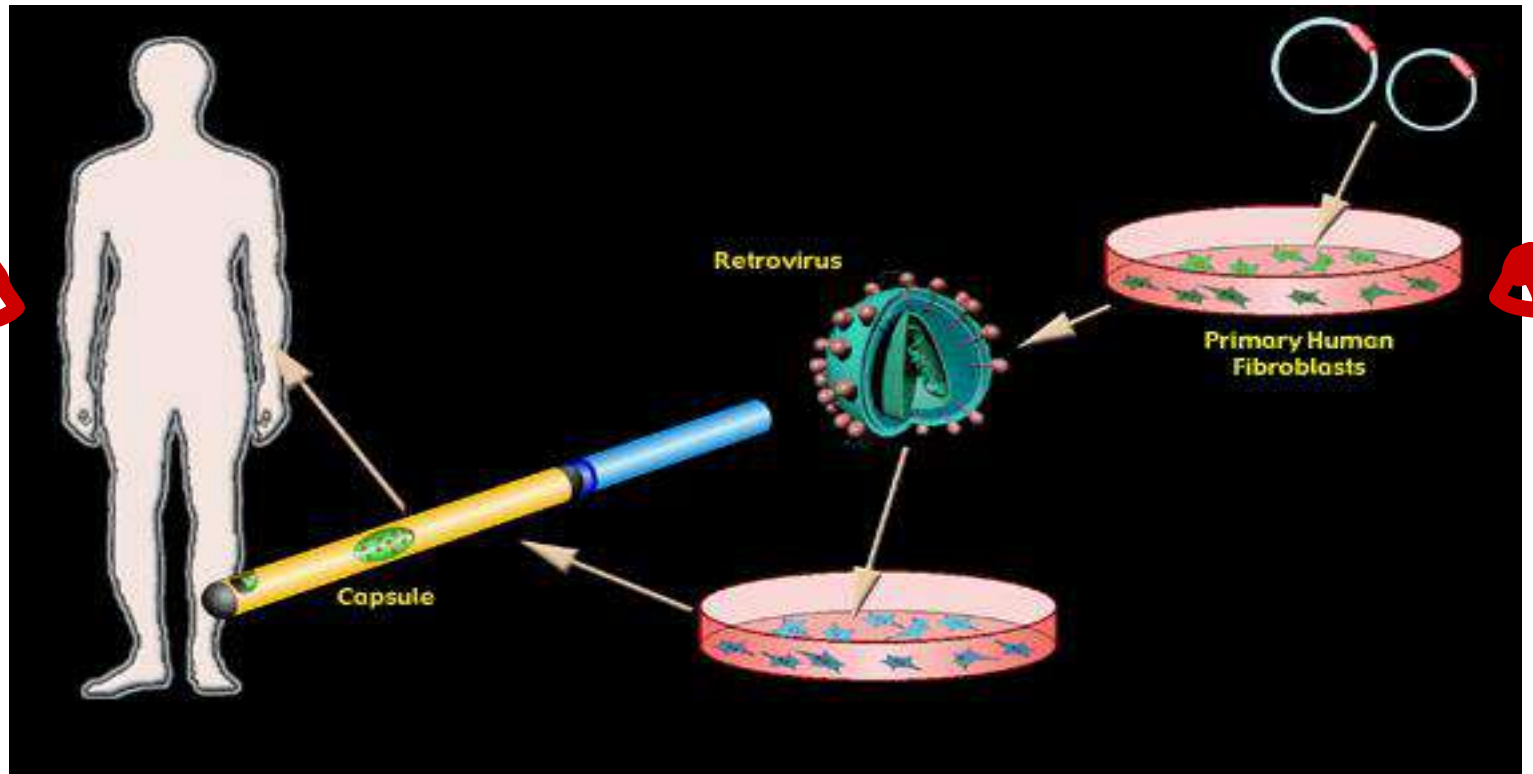
- ***In vivo* versus *ex vivo***
  - *In vivo* = intravenous or intramuscular or non-invasive (‘sniffable’)
  - *Ex vivo* = hepatocytes, skin fibroblasts, haematopoietic cells (‘bioreactors’)

- **Gene delivery approaches**
  - Physical methods
  - Non-viral vectors
  - Viral vectors

Gene therapy

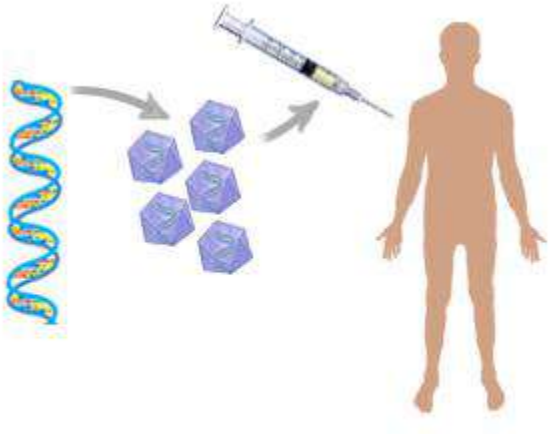
In vivo

Ex vivo

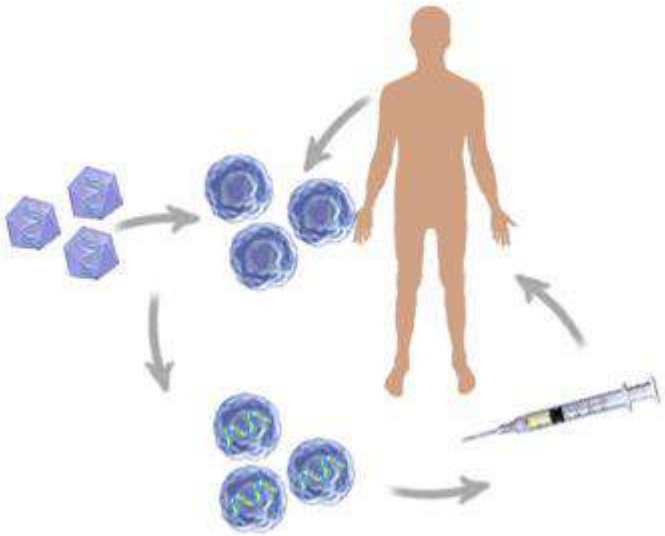


Gene Therapy

- In vivo Gene Therapy



- Ex vivo Gene Therapy



Gene Therapy Video

In vivo gene therapy

1. The genetic material is transferred directly into the body of the patient

2. More or less random process;
small ability to control; less manipulations

3. Only available option for tissues
that can not be grown in vitro;
or if grown cells can not be transferred back

In vivo techniques

- *In vivo* techniques usually utilize viral vectors
  - Virus = carrier of desired gene
  - Virus is usually “crippled” to disable its ability to cause disease
  - Viral methods have proved to be the most efficient to date
  - Many viral vectors can stable integrate the desired gene into the target cell’s genome

  - Problem: Replication defective viruses adversely affect the virus’ normal ability to spread genes in the body
    - Reliant on diffusion and spread
    - Hampered by small intercellular spaces for transport
    - Restricted by viral-binding ligands on cell surface → therefore cannot advance far.

Methods of Gene Therapy

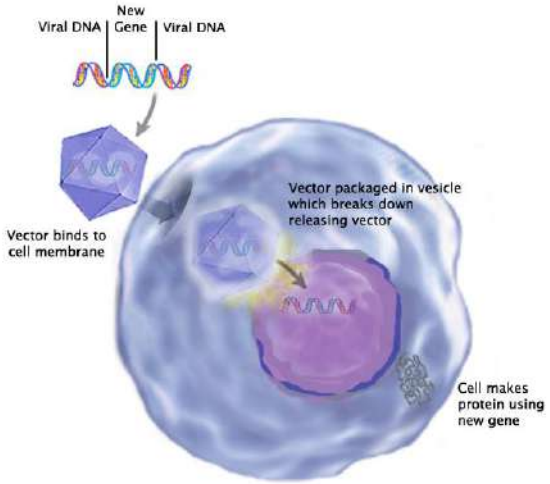
A. **viral vectors – usual *In vivo* techniques**

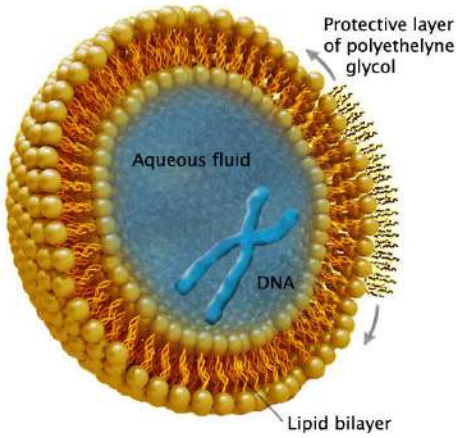
Viruses

B. Non-viral Options -

Gene Therapy

- Vectors for Therapeutic Gene Delivery
  - Viral
  - Non-Viral





Animation: Gene Therapy Vectors

Methods of Gene Therapy

A. viral vectors – usual *In vivo* techniques

Viruses

- Replicate by inserting their DNA into a host cell
- Gene therapy can use this to insert genes that encode for a desired protein to create the desired trait
- Four different types

MOST COMMON VIRAL VECTORS

Retroviruses

can create double-stranded DNA copies of their RNA genomes. Can integrate into genome. HIV for example

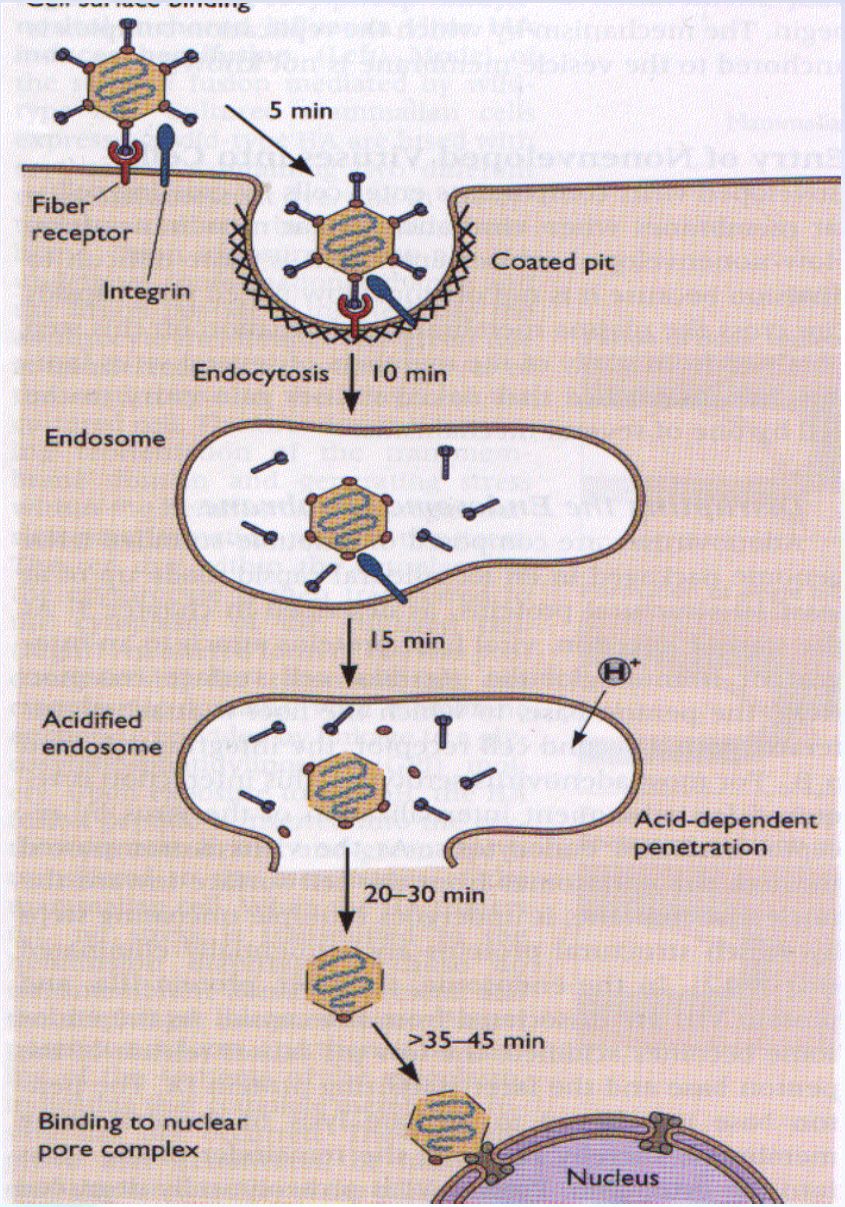
Herpes simplex viruses

dsDNA viruses that infect a neurons. Cold sores virus

Adeno viruses

Adeno Associated viruses

Viral vectors



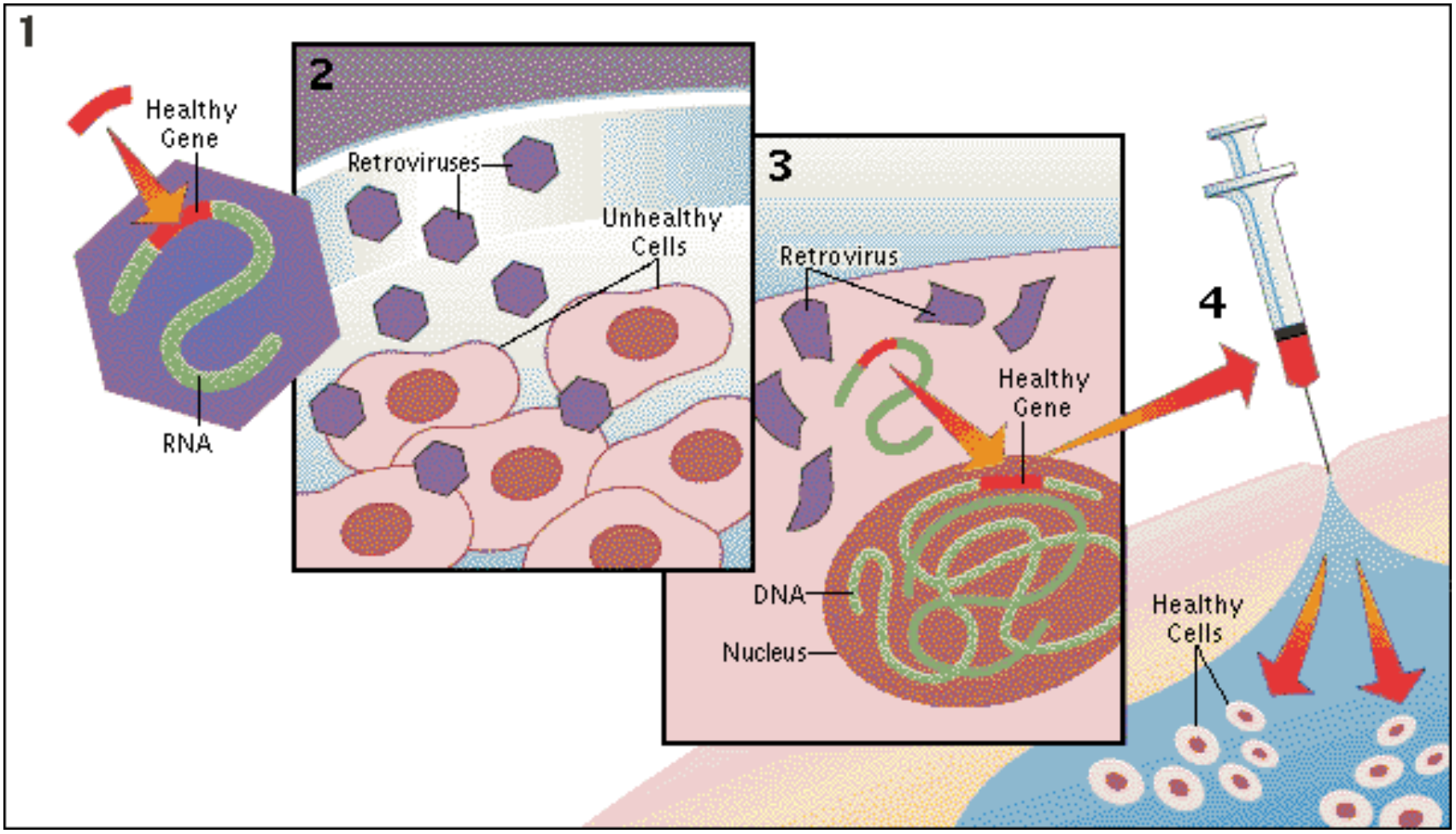
Compared to naked DNA, virus particles provide a relatively efficient means of transporting DNA into cells, for expression in the nucleus as recombinant genes *(example = adenovirus)*.

[figure from Flint *et al.* Principles of Virology, ASM Press, 2000]

How It Works ?

- A vector delivers the therapeutic gene into a patient's target cell
- The target cells become infected with the viral vector
- The vector's genetic material is inserted into the target cell
- Functional proteins are created from the therapeutic gene causing the cell to return to a normal state

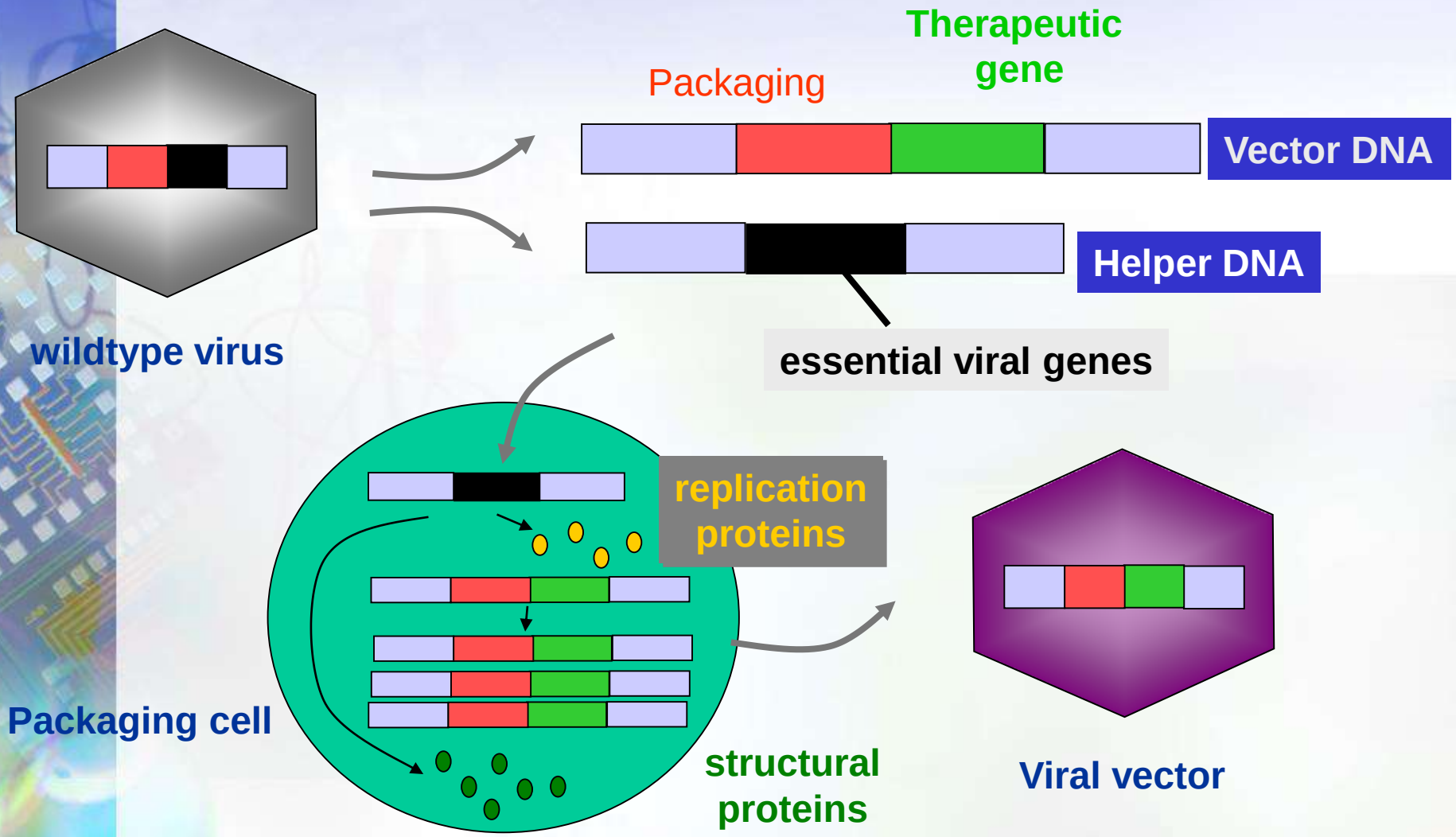
Picture ☺



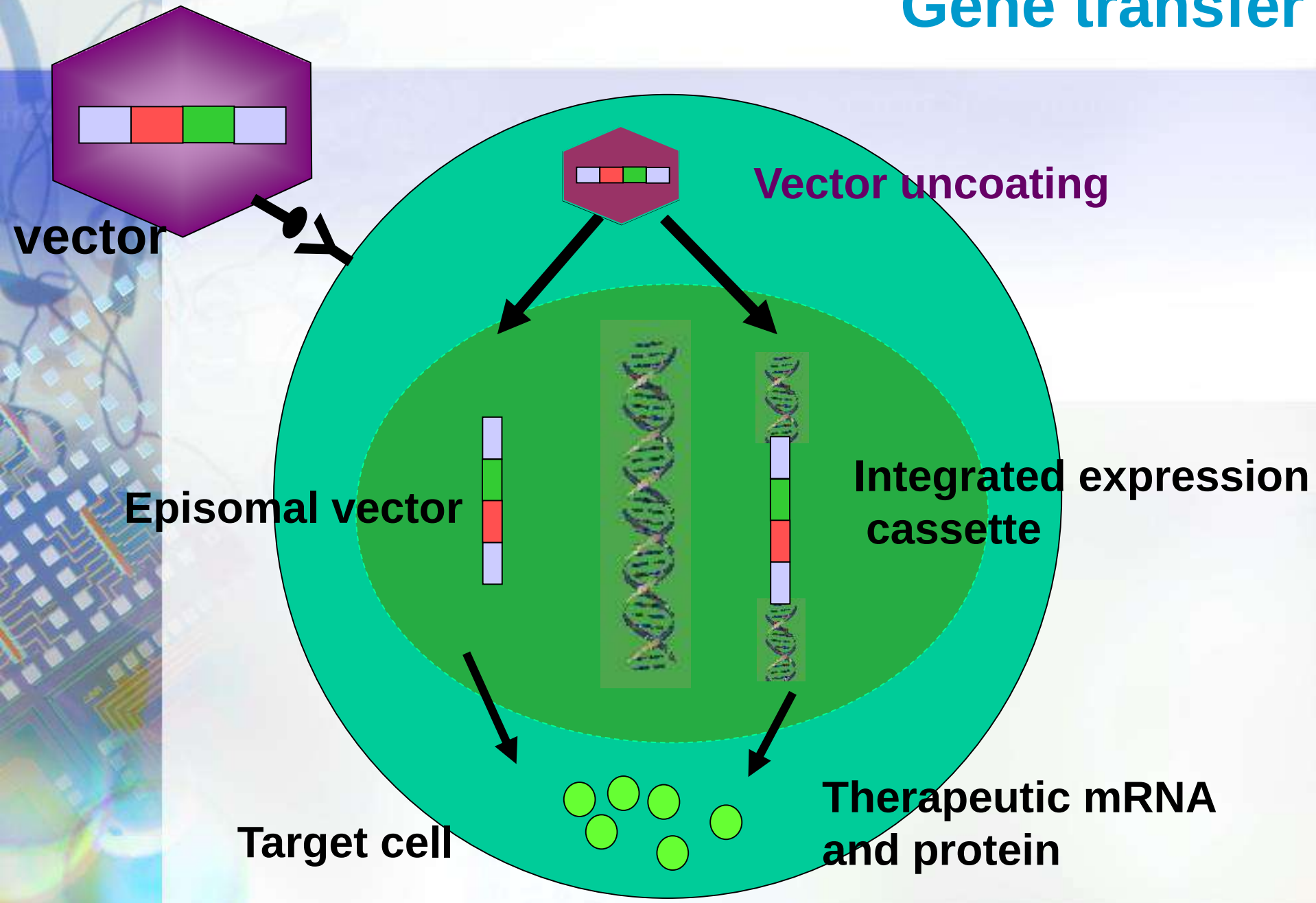
http://encarta.msn.com/media_461561269/Gene_Therapy.html

Engineering a virus into a viral vector

http://www.edu365.com/aulanet/comsoc/Lab_bio/simulacions/GeneTherapy/GeneTherapy.htm



Gene transfer



Delivery System of Choice = Viral Vectors

a. Rendering virus vector harmless

Remove harmful genes → "cripple" the virus

Example – removal of *env* gene → virus is not capable of producing a functional envelope

Vectors needed in very large numbers to achieve successful delivery of new genes into patient's cells

Vectors must be propagated in large numbers in cell culture (10^9) with the aid of a helper virus

Delivery System of Choice = Viral Vectors

b. Integrating versus Non-Integrating Viruses

- Integrating viruses
  - Retrovirus (e.g. murine leukemia virus)
  - Adeno-associated virus (only 4kbp accommodated)
  - Lentivirus
- Non-Integrating viruses
  - Adenovirus
  - Alphavirus
  - Herpes Simplex Virus
  - Vaccinia

b. Integrating versus Non-Integrating Viruses

Integrating vectors have their DNA/RNA delivered **permanently incorporated** into the host chromosomes. The advantage is that when the cell undergoes mitosis, the integrated DNA will be replicated with the chromosome it is housed in, and both daughter cells have the vector DNA inside them. The advantage is in therapy for cell types where you expect the cells to undergo expansion/mitosis at some frequency.

Non-integrating vectors remain episomal, meaning the DNA is **never integrated** into the host chromosomes. The episomal vector DNA retains a unique epigenetic signature. The advantage is no risk of disrupting essential host genes on chromosomes through integration, a process that might otherwise cause oncogenesis. Cell types with low rates of mitosis, such as liver cells, are good matches for this strategy, or therapeutic strategies that desire only transient expression, which can then be lost once the cell divides.

Viral vectors

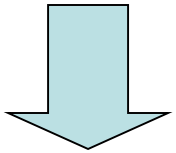
- **1. Retroviruses**
  - eg Moloney murine leukaemia virus (Mo-MuLV)
  - Lentiviruses (eg HIV, SIV)
- **2. Adenoviruses**
- **3. Adeno-associated viruses** (AAV)
- **4. Herpes simplex**

1. Retroviruses

- Created double stranded DNA copies from RNA genome
  - The retrovirus goes through reverse transcription using reverse transcriptase and RNA
  - the double stranded viral genome integrates into the human genome using integrase
    - integrase inserts the gene anywhere because it has no specific site
    - May cause insertional mutagenesis
      - One gene disrupts another gene's code (disrupted cell division causes cancer from uncontrolled cell division)
  - vectors used are derived from the human immunodeficiency virus (HIV) and are being evaluated for safety

Retroviral vectors are able to infect dividing cells only

In dividing cells nuclear membranes are broken down, so viral genome can enter and integrate into the chromosome



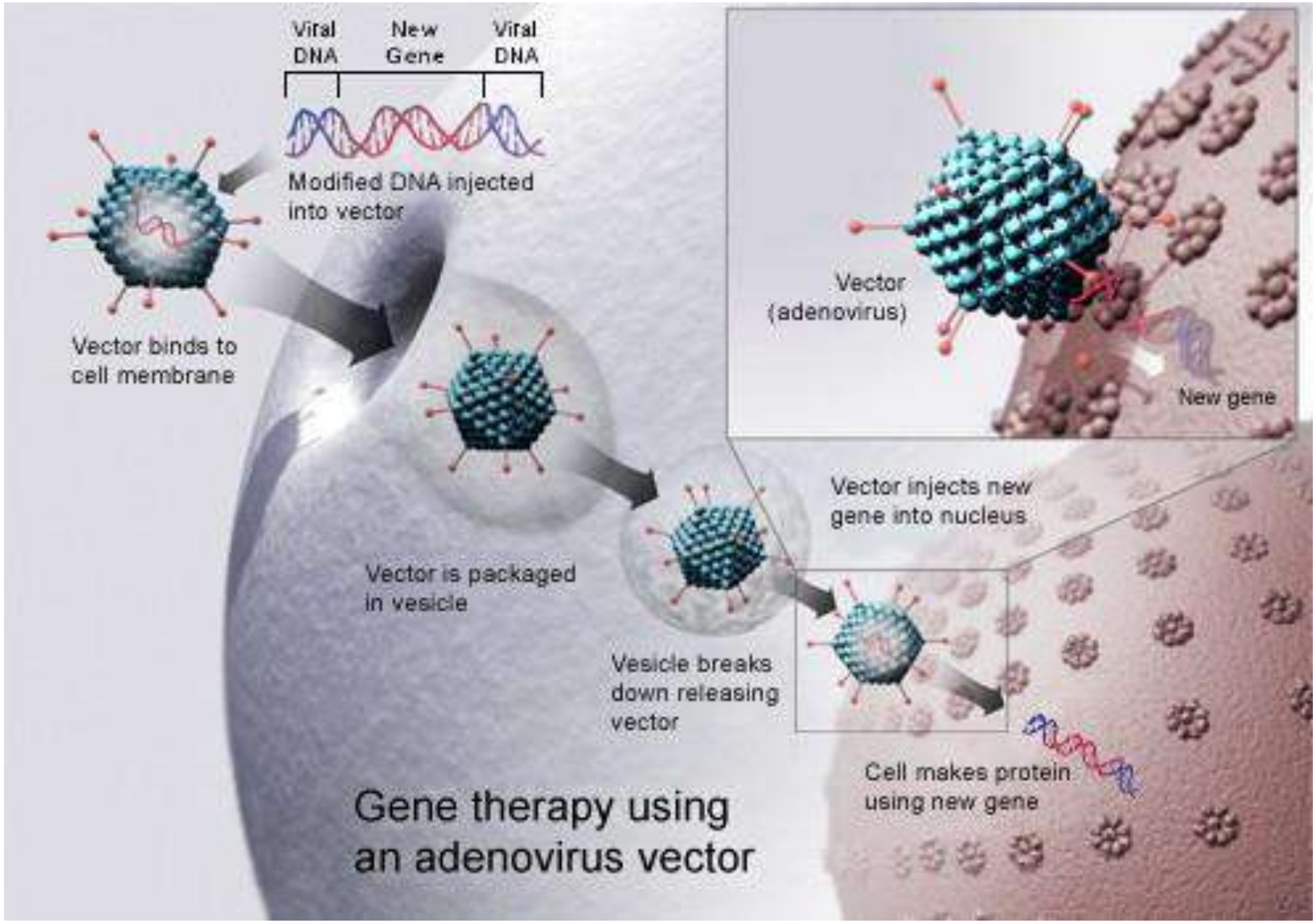
Good for cancer gene therapy

So, retroviruses are most often used vectors for common disease gene therapy

2. Adenoviruses

- Are double stranded DNA genome that cause respiratory, intestinal, and eye infections in humans
- The inserted DNA is not incorporated into genome
- Not replicated though ☹
  - Has to be reinserted when more cells divide
- Ex. Common cold

Adenovirus cont.



http://en.wikipedia.org/wiki/Gene_therapy

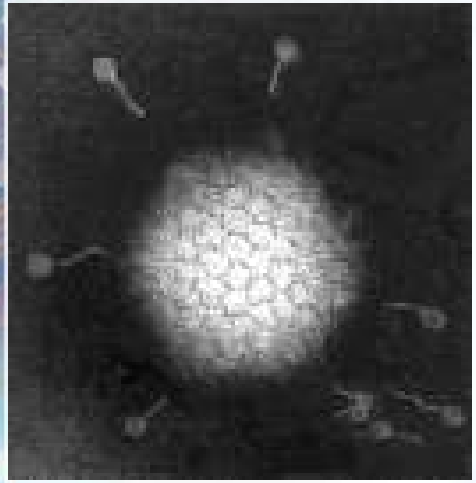
Adenovirus

Advantages

- High transduction efficiency
- Insert size up to 8kb. High viral titer (10^{10}-10^{13})
- Infects both replicating and differentiated cells

Disadvantages

- Expression is transient (viral DNA does not integrate)
- Viral proteins can be expressed in host following vector administration
- In vivo delivery hampered by host immune response



Adenoviruses (members of the family ***Adenoviridae***) are medium-sized (90–100 nm), nonenveloped (without an outer lipid bilayer) viruses with an icosahedral nucleocapsid containing a double stranded DNA genome. Their name derives from their initial isolation from human adenoids in 1953

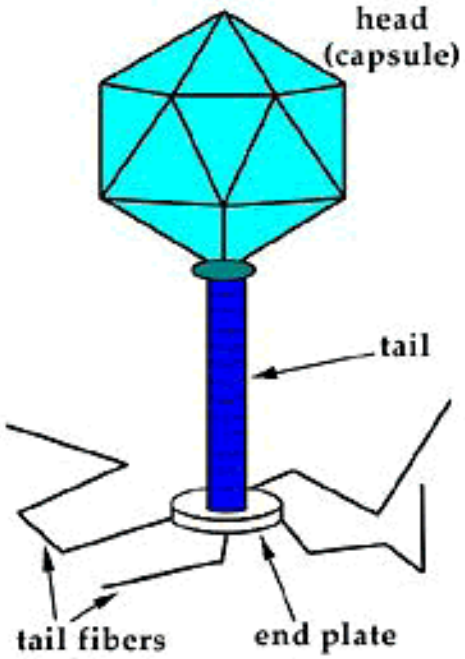
3. Adeno-associated Viruses

- Adeno-associated Virus- small, single stranded DNA that insert genetic material at a specific point on chromosome 19
- From parvovirus family- causes no known disease and doesn't trigger patient immune response.
- Low information capacity
- gene is always "on" so the protein is always being expressed, possibly even in instances when it isn't needed.
- hemophilia treatments, for example, a gene-carrying vector could be injected into a muscle, prompting the muscle cells to produce Factor IX and thus prevent bleeding.
  - Study by Wilson and Kathy High (University of Pennsylvania), patients have not needed Factor IX injections for more than a year

Adeno-associated virus (**AAV**) is a small virus that infects humans and some other primate species. AAV is not currently known to cause disease.

4. Herpes Simplex Viruses

- Double stranded DNA viruses that infect neurons
- Ex. Herpes simplex virus type 1



http://www.ucmp.berkeley.edu/alllife/virus.html

Herpes Simplex Virus

Advantages

- Large insert size
- Could provide long- term CNS gene expression
- High titer

Disadvantages

- System currently under development
- Current vectors provide transient expression
- Low transduction efficiency

B. Non-viral Options

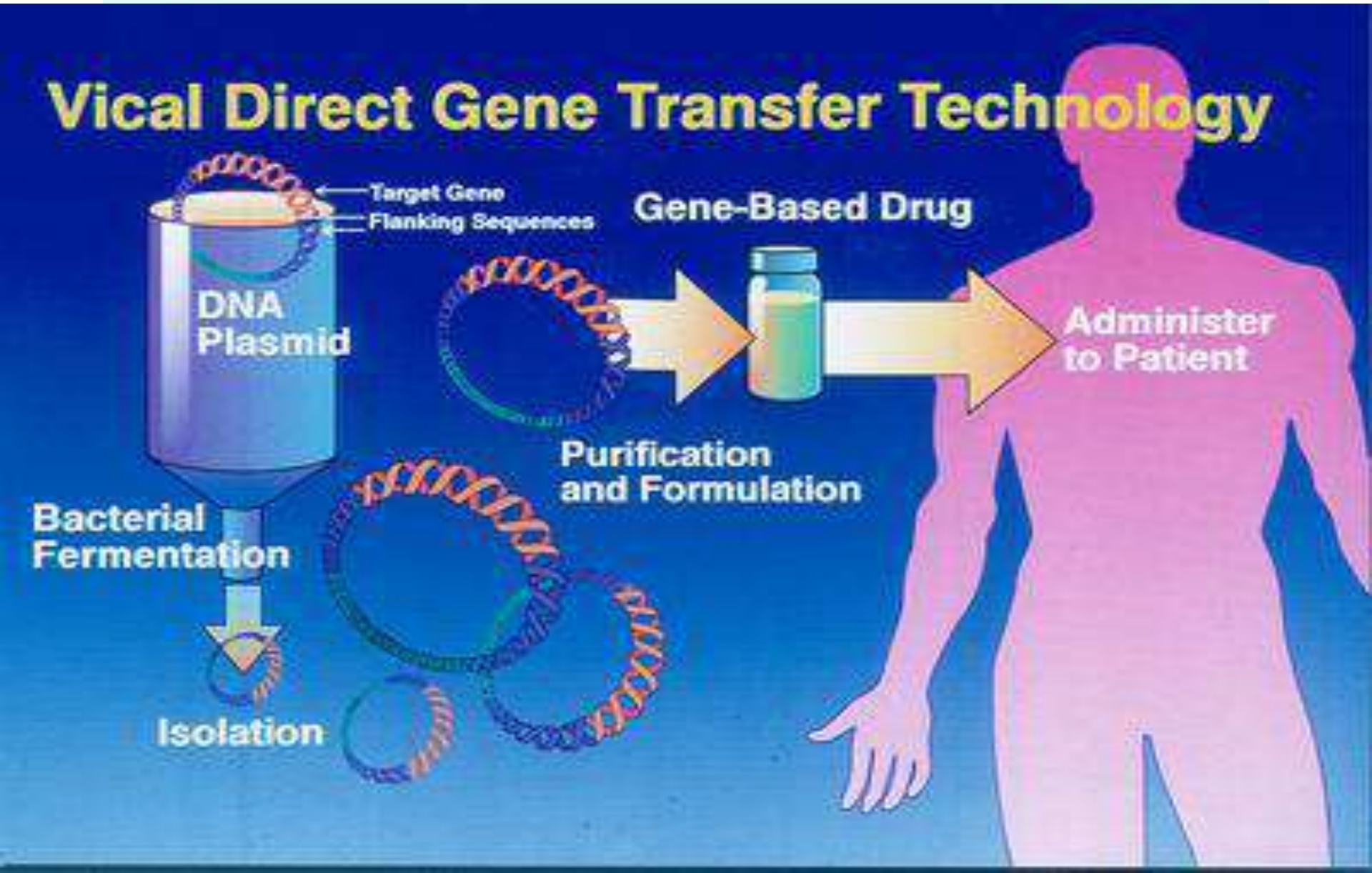
a. Direct introduction of therapeutic DNA
  - But only with certain tissue
  - Requires a lot of DNA

b. Creation of artificial lipid sphere with aqueous core, liposome
  - Carries therapeutic DNA through membrane

c. Chemically linking DNA to molecule that will bind to special cell receptors
  - DNA is engulfed by cell membrane
  - Less effective ☹

d. Trying to introduce a 47th chromosome
  - Exist alongside the 46 others
  - Could carry a lot of information
  - But how to get the big molecule through membranes?

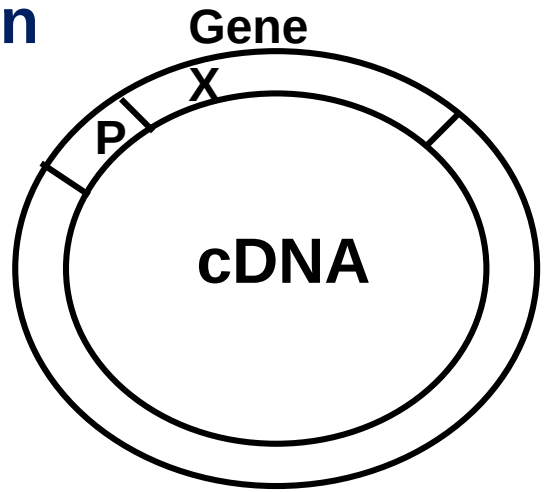
Injections of naked DNA



Non-viral DNA carriers:

a. Naked plasmid DNA injection

Promoter + gene of interest
(P) (gene X)



Expression observed in thymus, skeletal and cardiac muscle, skin.

Non-viral DNA carriers:

b. Cationic liposomes: Positively charged lipids interact with negatively charged DNA. (lipid-DNA complex).

-Transverses cell membranes

Advantages:

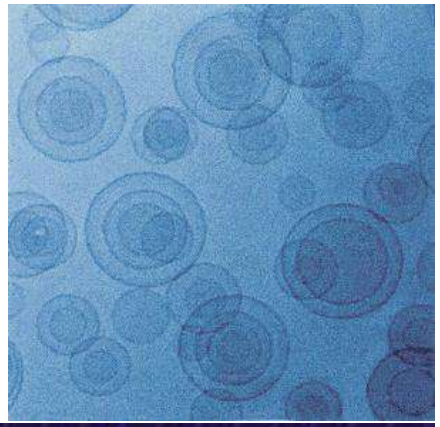
a. Stable complex
b. Can carry large sized DNA
c. Can target to specific cells
d. Does not induce immunological reactions.

Disadvantages:

a. Low transfection efficiency
b. Transient expression
c. Inhibited by serum
d. Some cell toxicity

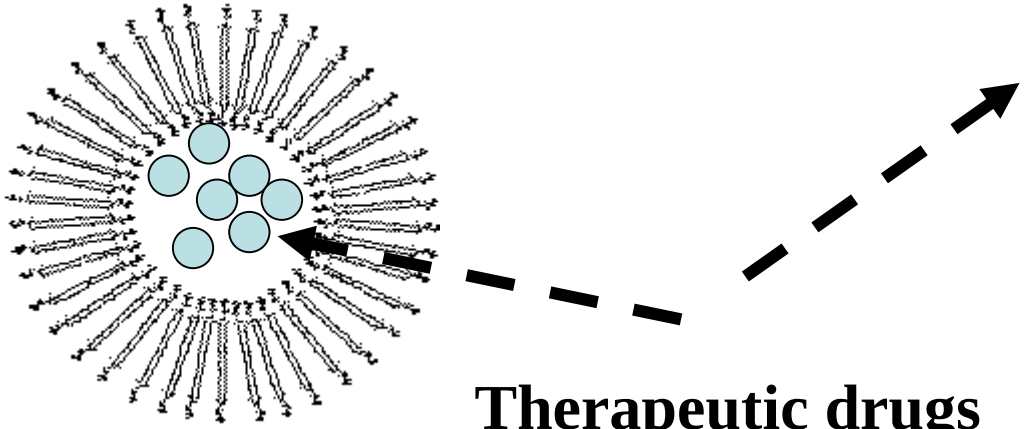
Liposomes

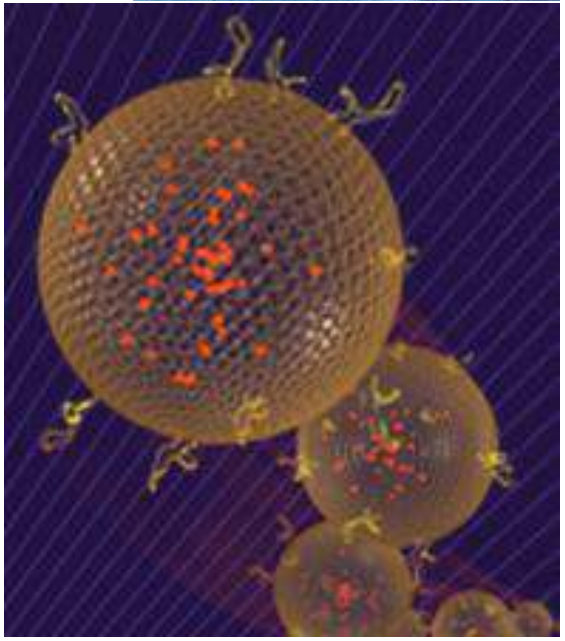
Next level idea – why naked DNA?



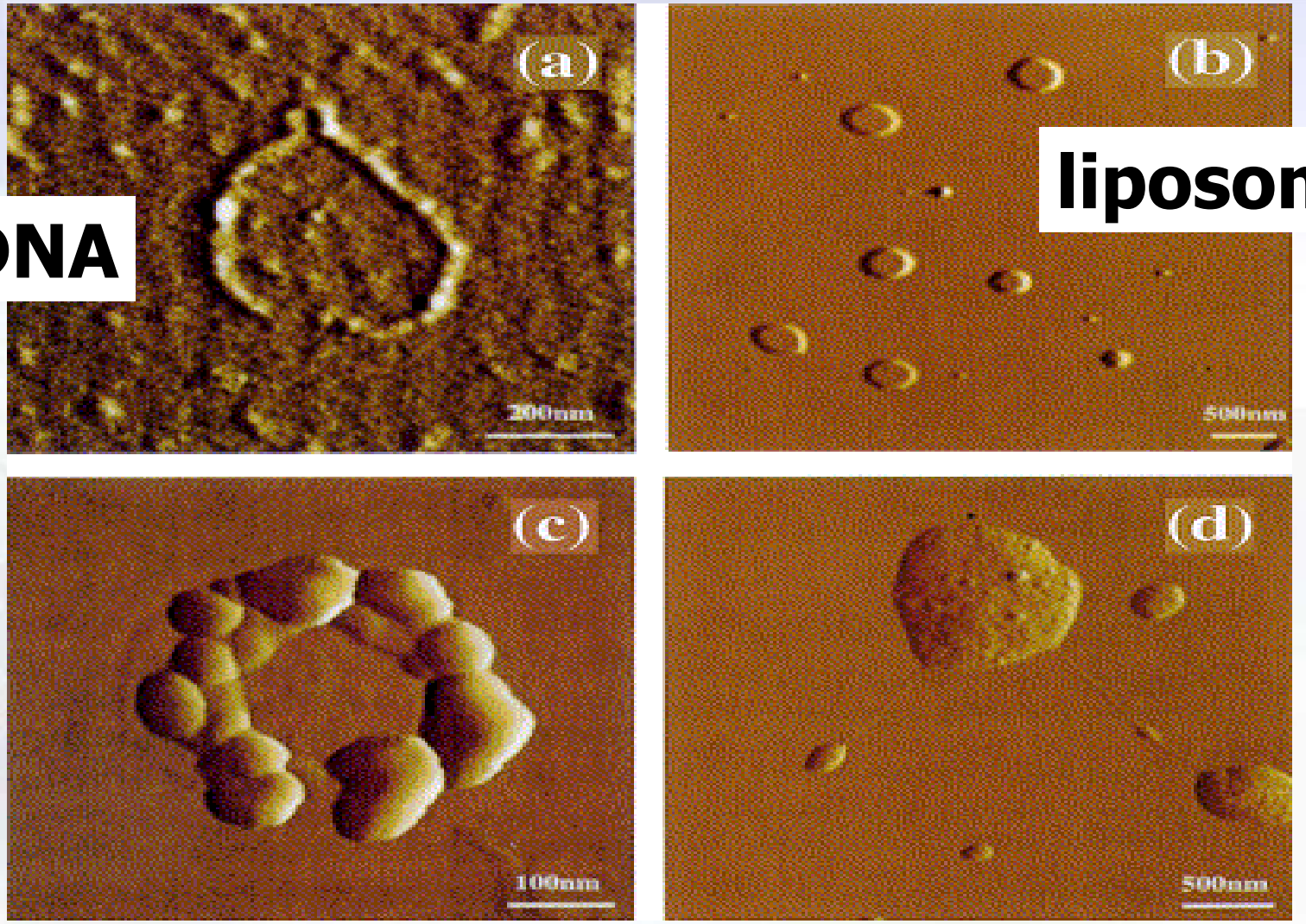
Lets' wrap it in something safe to increase transfection rate

Lipids – are an obvious idea !





Liposomes

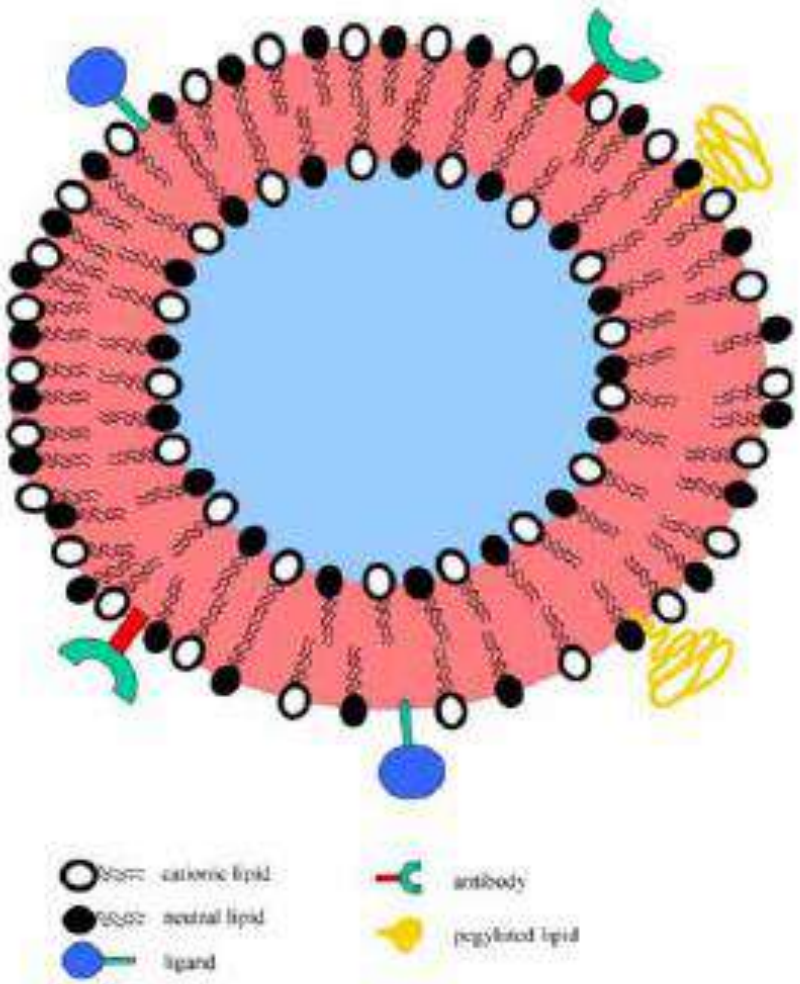


DNA

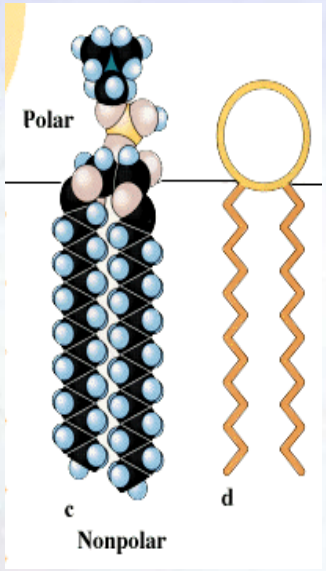
liposome

complexes

Cationic Liposome

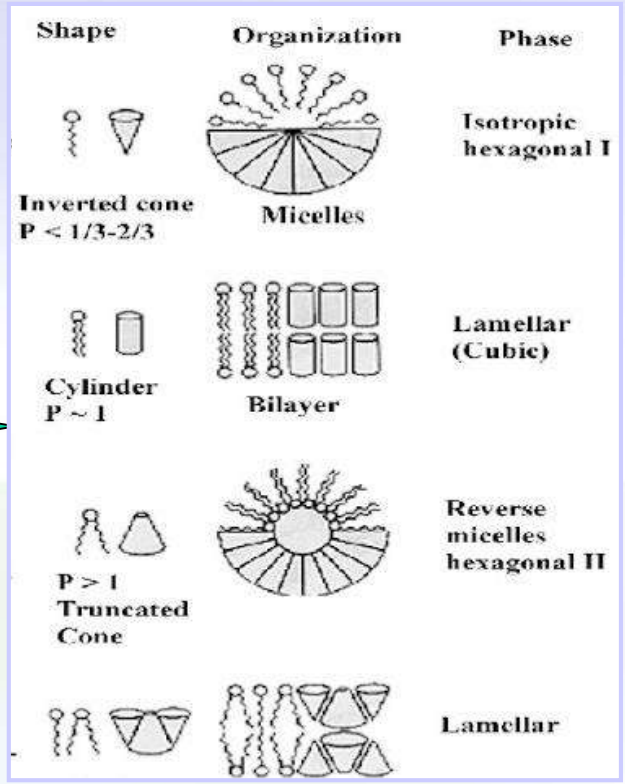


Liposomes



A phospholipid

Lipid Organization



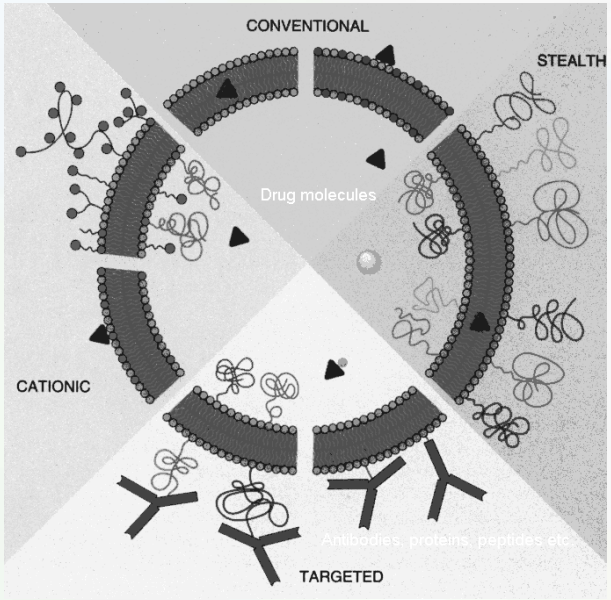
Phopholipid Hierarchal Structures

- In aqueous solution, polar phospholipids form ordered aggregates to minimize hydrophobic interactions
- Lipid shape and conditions of formation affect the final lipid organized structure

Liposomes

- Liposomes are
  - not limited by size or number of genes
  - safe
  - easy to produce
  - short-term expression

- Diverse manners of ‘lysing’ the liposome
  - Temperature sensitive
  - Target sensitive
  - pH sensitive
  - Electric field sensitive

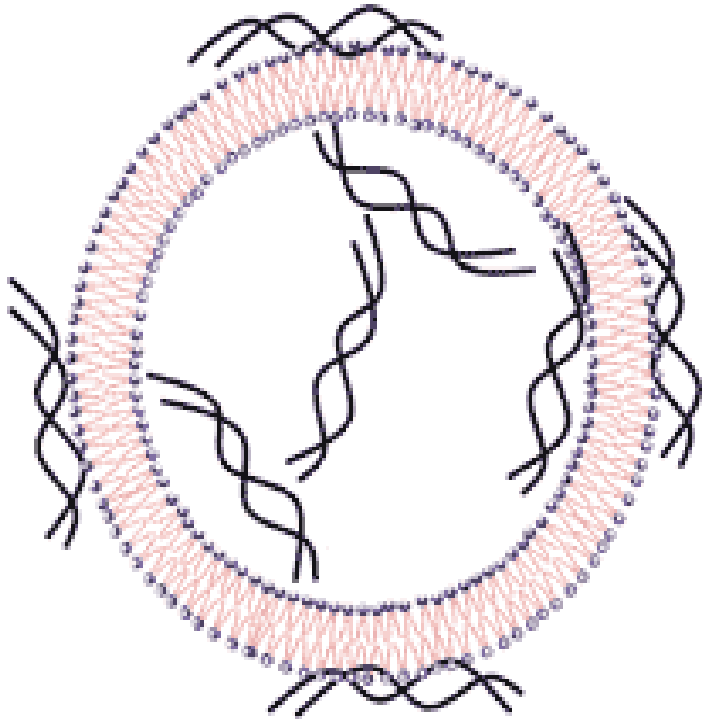


DNA delivery of genes by liposomes

Cheaper than viruses

No immune response

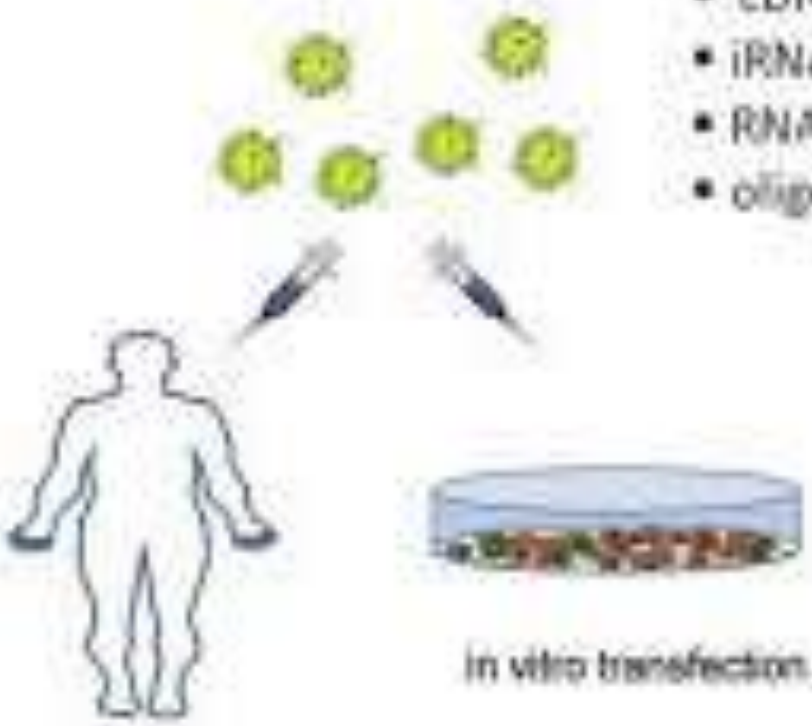
Especially good
for in-lung delivery (cystic fibrosis)



100-1000 times more plasmid DNA needed
for the same transfer efficiency as for viral vector

SLN and NLC-based gene delivery systems:

- cDNA plasmids
- iRNA
- RNAm
- oligonucleotides



in vitro transfection system

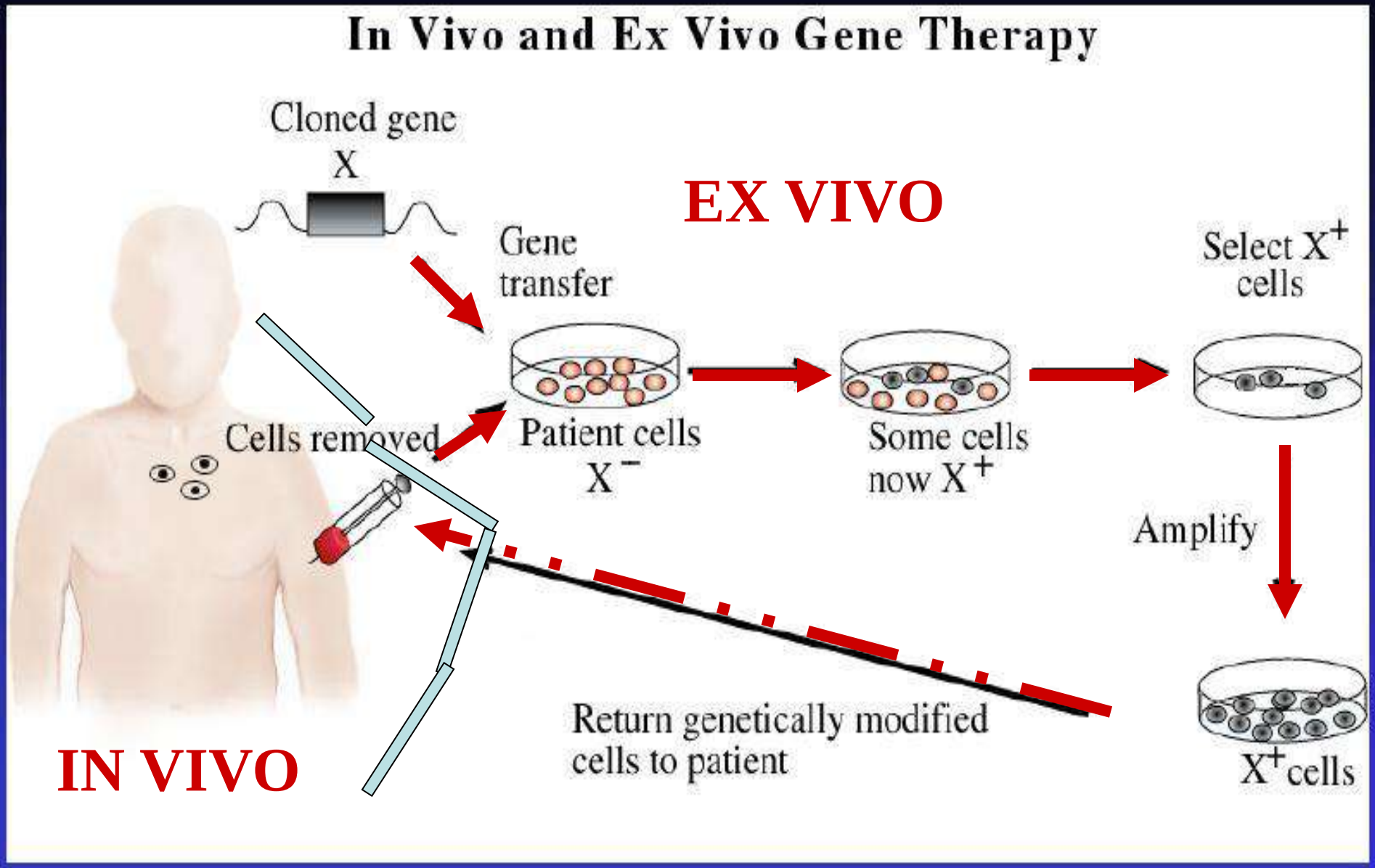
ocular diseases
infectious deseses
lysosomal storage disorders
cancer

Ex vivo gene therapy

1. The genetic material is first transferred into the cells grown in vitro

2. Controlled process;
Genetically altered cells are selected and expanded; more manipulations

3. Cells are then returned back to the patient

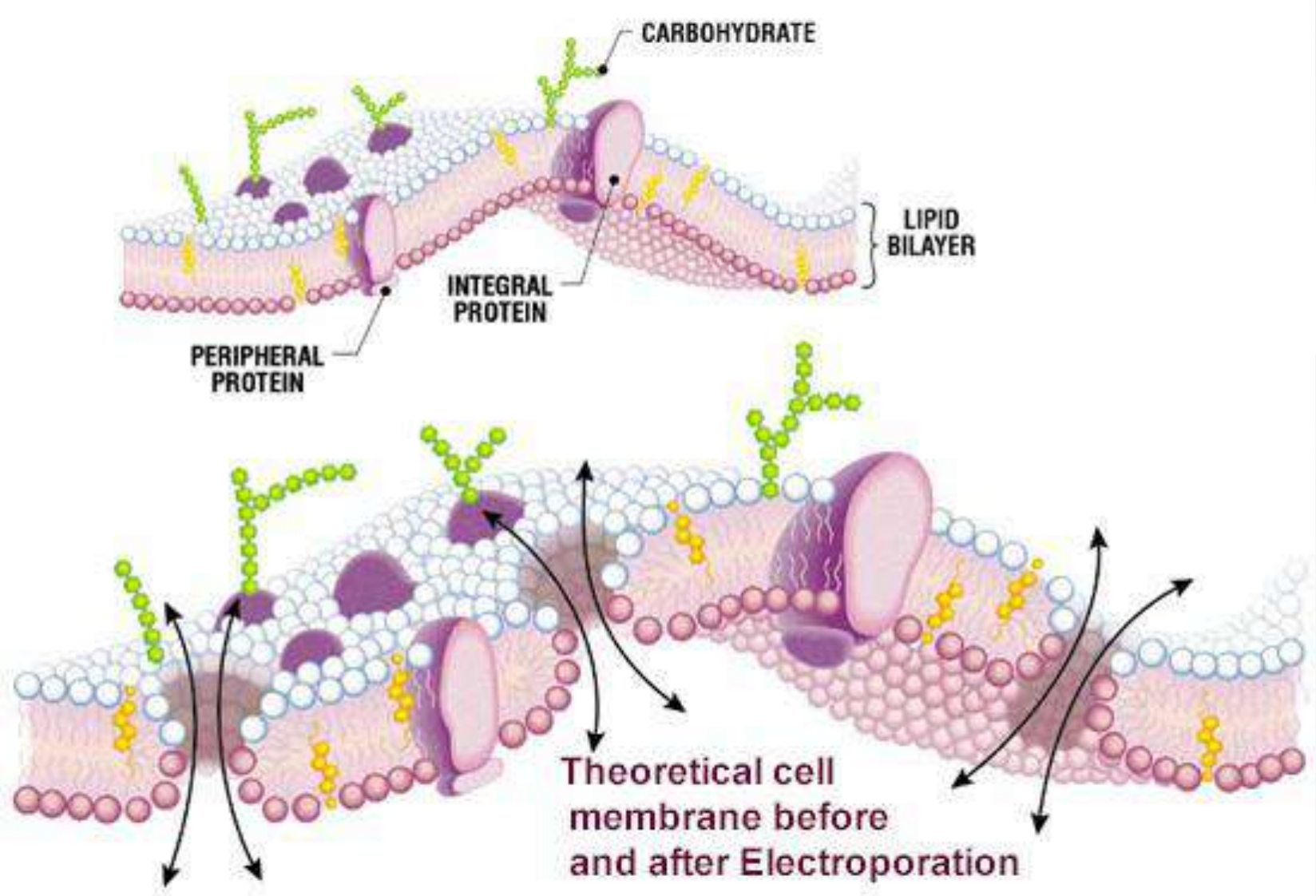


http://laxmi.nuc.ucla.edu:8237/M288/SChow_4_10/sld005.htm

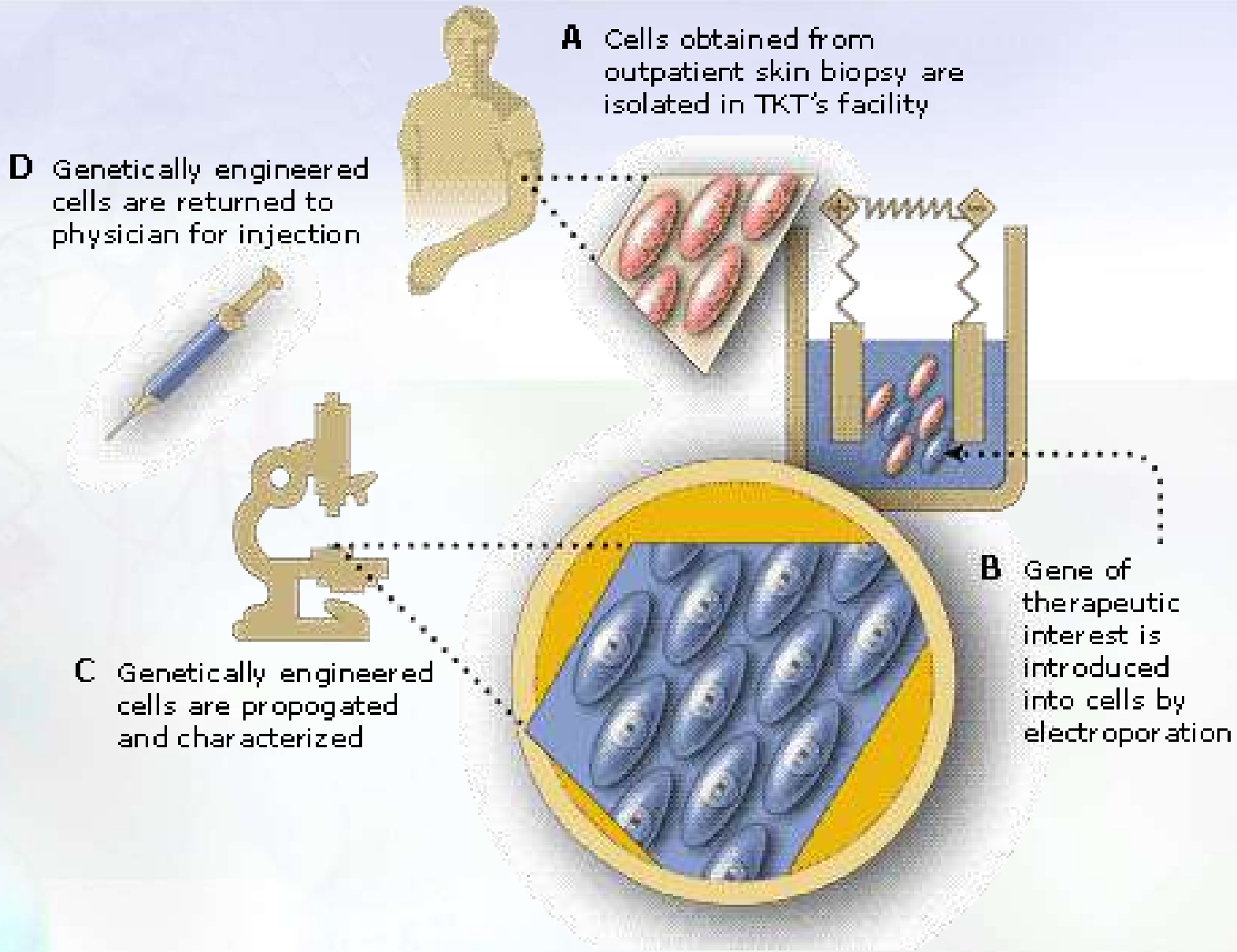
Ex vivo

- *Ex vivo* manipulation techniques
  - Electroporation
  - Liposomes
  - Calcium phosphate
  - Gold bullets (fired within helium pressurized gun)
  - Retrotransposons (jumping genes – early days)
  - Human artificial chromosomes

Electroporation



Ex vivo Electroporation



Limitations of Gene Therapy

- Gene delivery
  - Limited tropism of viral vectors
  - Dependence on cell cycle by some viral vectors (i.e. mitosis required)
- Duration of gene activity
  - Non-integrating delivery will be transient (transient expression)
  - Integrated delivery will be stable

Patient safety

– Immune hyperresponsiveness (hypersensitivity reactions directed against viral vector components or against transgenes expressed in treated cells)

– Integration is not controlled → oncogenes may be involved at insertion point → cancer?

Limitations of Gene Therapy

- Gene control/regulation
  - Most viral vectors are unable to accommodate full length human genes containing all of their original regulatory sequences
  - Human cDNA often used → much regulatory information is lost (e.g. enhancers inside introns)
  - Often promoters are substituted → therefore gene expression pattern may be very different
  - Random integration can adversely affect expression (insertion near highly methylated heterogeneous DNA may silence gene expression)

Limitations of Gene Therapy

- Expense
  - Costly because of cell culturing needs involved in *ex vivo* techniques
  - Virus cultures for *in vivo* delivery
  - Usually the number of patients enrolled in any given trial is <20
  - More than 5000 patients have been treated in last ~12 years worldwide

Gene Therapy Trials in U.S.
(Information from US NIH, Office of Recombinant DNA Activities – 1999)

Diagnosis	# Trials (total = 338)
Genetic disease	18
HIV	21
Cancer	196
Other	3

Limitations of Gene Therapy - Summary

- **Short Lived**
  - Hard to rapidly integrate therapeutic DNA into genome and rapidly dividing nature of cells prevent gene therapy from long time
  - Would have to have multiple rounds of therapy
- **Immune Response**
  - new things introduced leads to immune response
  - increased response when a repeat offender enters
- **Viral Vectors**
  - patient could have toxic, immune, inflammatory response
  - also may cause disease once inside
- **Multigene Disorders**
  - Heart disease, high blood pressure, Alzheimer's, arthritis and diabetes are hard to treat because you need to introduce more than one gene
- **May induce a tumor** if integrated in a tumor suppressor gene because insertional mutagenesis

Gene Therapy of Cancer

Cancer results from multiple mutations

A. Methods for gene therapy of cancer

- Viruses
- Naked DNA (vector-free)
- Liposomes
- Protein-DNA complexes
- Gene gun
- Calcium phosphate precipitation
- Electroporation
- Intracellular microinjection

B. Reasons for lack of clinical success

- Low transduction frequency
- Insufficient expression *in vivo*

C. STRATEGIES

Strengthening of the immune response against a tumor

Recent Developments

- Genes get into brain using liposomes coated in polymer call polyethylene glycol
  - potential for treating Parkinson's disease
- RNA interference or gene silencing to treat Huntington's
  - siRNAs used to degrade RNA of particular sequence
  - abnormal protein wont be produced
- Create tiny liposomes that can carry therapeutic DNA through pores of nuclear membrane
- Sickle cell successfully treated in mice

Recent Developments in Gene Therapy

- Liposomes coated in polymer PEG – can cross the blood-brain barrier (viral vectors are too big) (January 2003)
- Case Western Uni. & Copernicus Therapeutics able to create tiny liposomes 25nm across to carry therapeutic DNA through pores in nuclear membrane
- New gene approach repairs errors in mRNA
  - Thalassaemia
  - Cystic fibrosis
  - Some cancers
  - (Please refer to Newscientist.com)

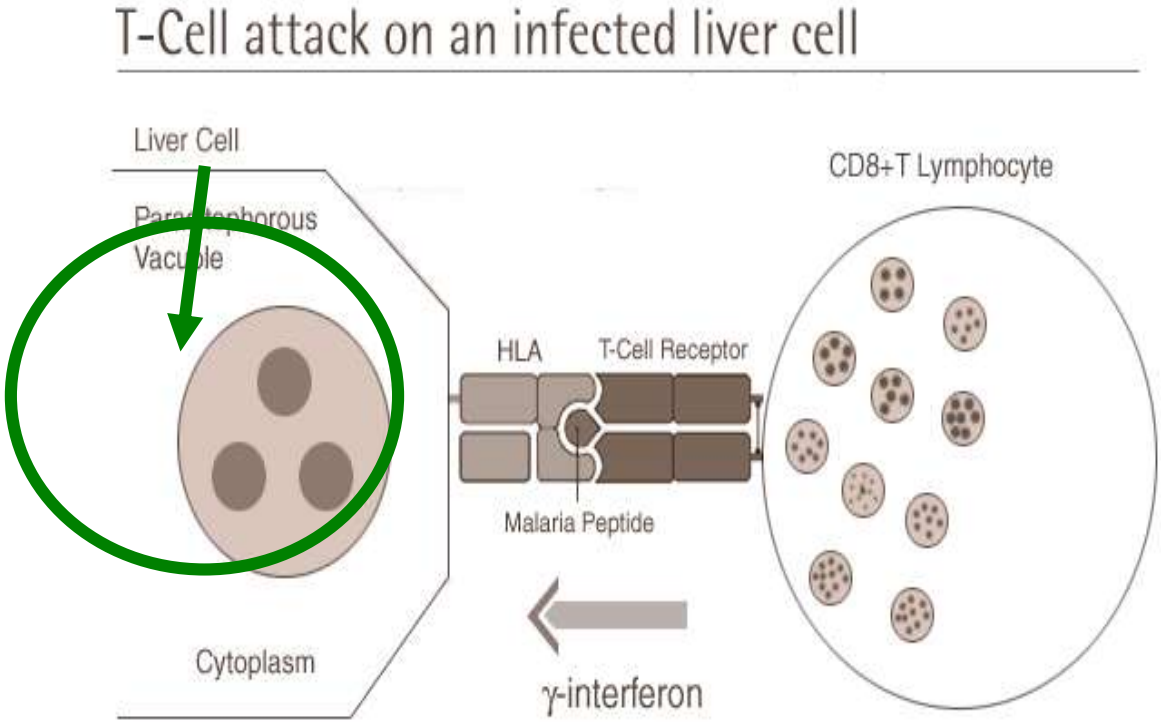
Current attempts with naked DNA vaccination in infectious diseases

HIV
Hepatitis
Influenza

Tuberculosis,

Lyme disease

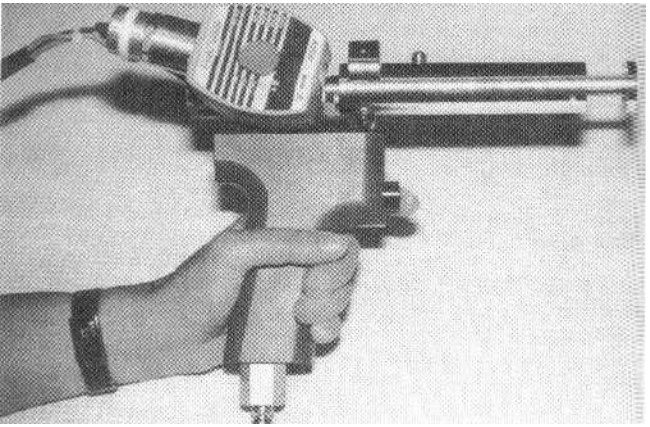
Malaria



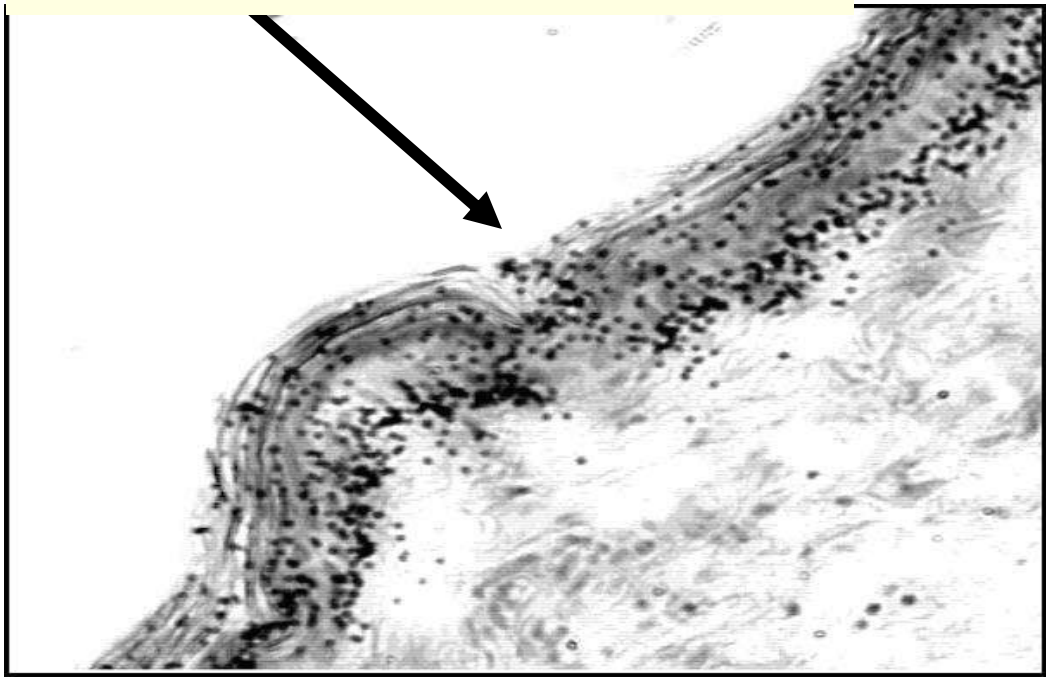
Ballistic DNA Injection (gene guns)

Invented for DNA transfer to plant cells

Fully applicable to eukaryotic cells



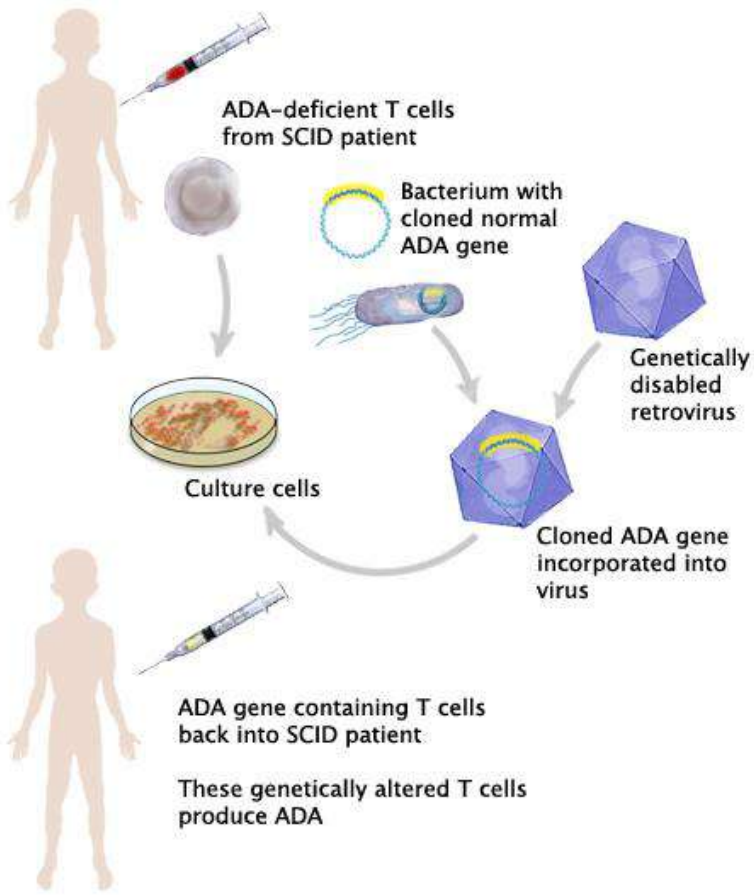
plasmid DNA shown here



http://www.wellesley.edu/Biology/Courses/219/Gen_news/i3_Gene_Therapy.jpg

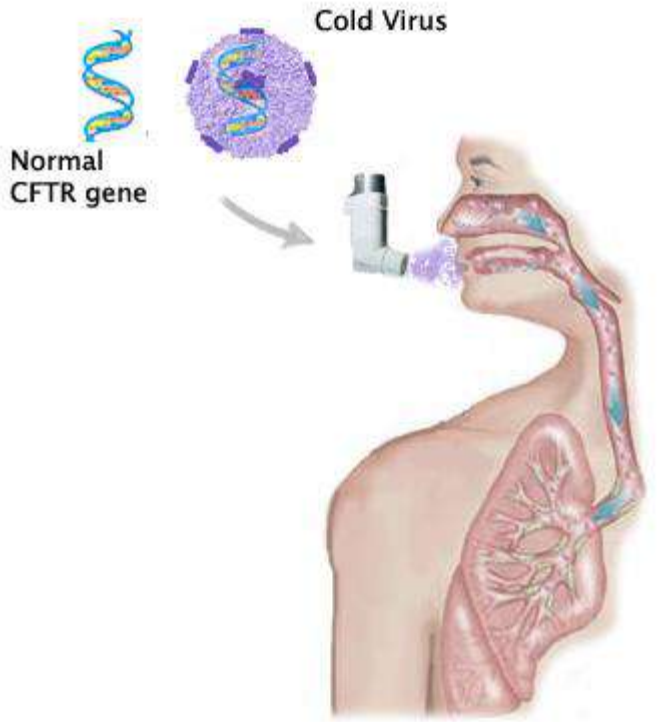
Gene Therapy

- 1990 – 4 year old Ashanti DaSilva had a genetic disorder called severe combined immunodeficiency (SCID)
- Defect in ADA gene results in an accumulation of dATP, which is toxic to certain types of T cells
- Takes down the entire immune system



Gene Therapy

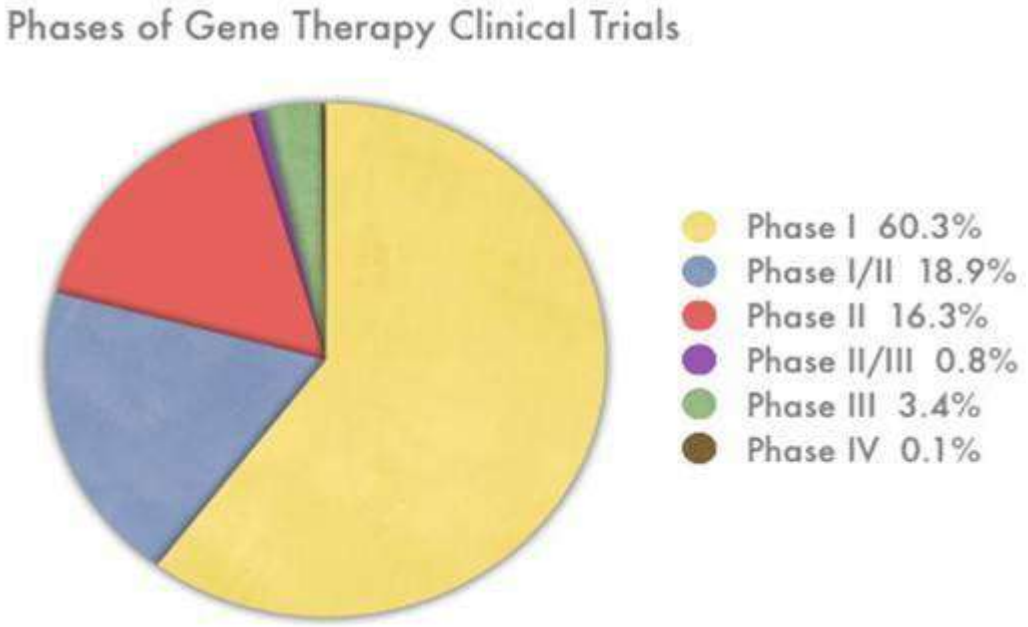
- Case Study: Cystic Fibrosis
  - Defective cystic fibrosis transmembrane conductance regulator (CFTR)
  - Normally it serves as a pump at the cell membrane to move electrically charged chloride atoms out of the cells
  - If cells can't move chloride out, they absorb water trying to dilute the chloride in the cell
  - This leads to the production of THICK sticky mucus



Animation: Cystic Fibrosis Case Study

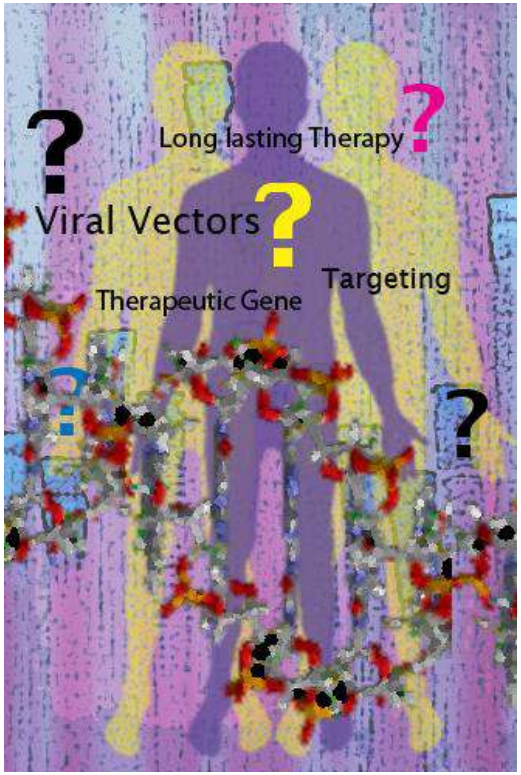
Gene Therapy

- Gene Therapy in Clinical Trials



Gene Therapy

- Challenges
  - Adverse effects of viral vectors
  - Targeting specific cells
  - Controlling expression of the therapeutic gene
  - Long lasting therapy



Video: Challenges of Gene Therapy

CONCLUSIONS

Goal of gene therapy:

1. Management and correction of human diseases
   a. Inherited and acquired disorders
   b. cancer
   c. AIDS/HIV

Good news: Promising advances during the last two decades in recombinant DNA technology.

1. Recent success in treating SCID.

Bad news: (Until recently?) Efficacy in any gene therapy protocol not definitive.

1. Shortcomings in gene transfer vectors.

2. Inadequate understanding of biological interactions of vector and host.

(Jesse Gelsinger case).

END

Lecture 1 and 2