

Chromosome

DNA

**Genes**

# Genes

- The basic unit of heredity
- Carried on a chromosome
- Encode how to make a protein

DNA → RNA → Proteins

**PROTEINS** carry out most of life's function.

- Mutation in the gene, alteration in codon, which will change which amino acid or its sequence which will change the conformation of the protein thus change the function of the protein.

# 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**

# History

**In the 1980s, Scientists began to look into gene therapy.**

- **Insertion of human genes into a bacteria cell.**
- **Transcription and translation of the information into a protein by Bacteria cell**
- **Introduce the protein into human cells**

# The First Case

**The first gene therapy was performed on September 14<sup>th</sup>, 1990**

- **Ashanti DeSilva was treated for SCID (Sever combined immunodeficiency)**
- **Doctors removed her white blood cells, inserted the missing gene into the WBC, and then put them back into her blood stream.**
- **This strengthened her immune system**
- **Only worked for a few months** 😞

# TYPES

## **Somatic Cell Gene Therapy (SCGP):**

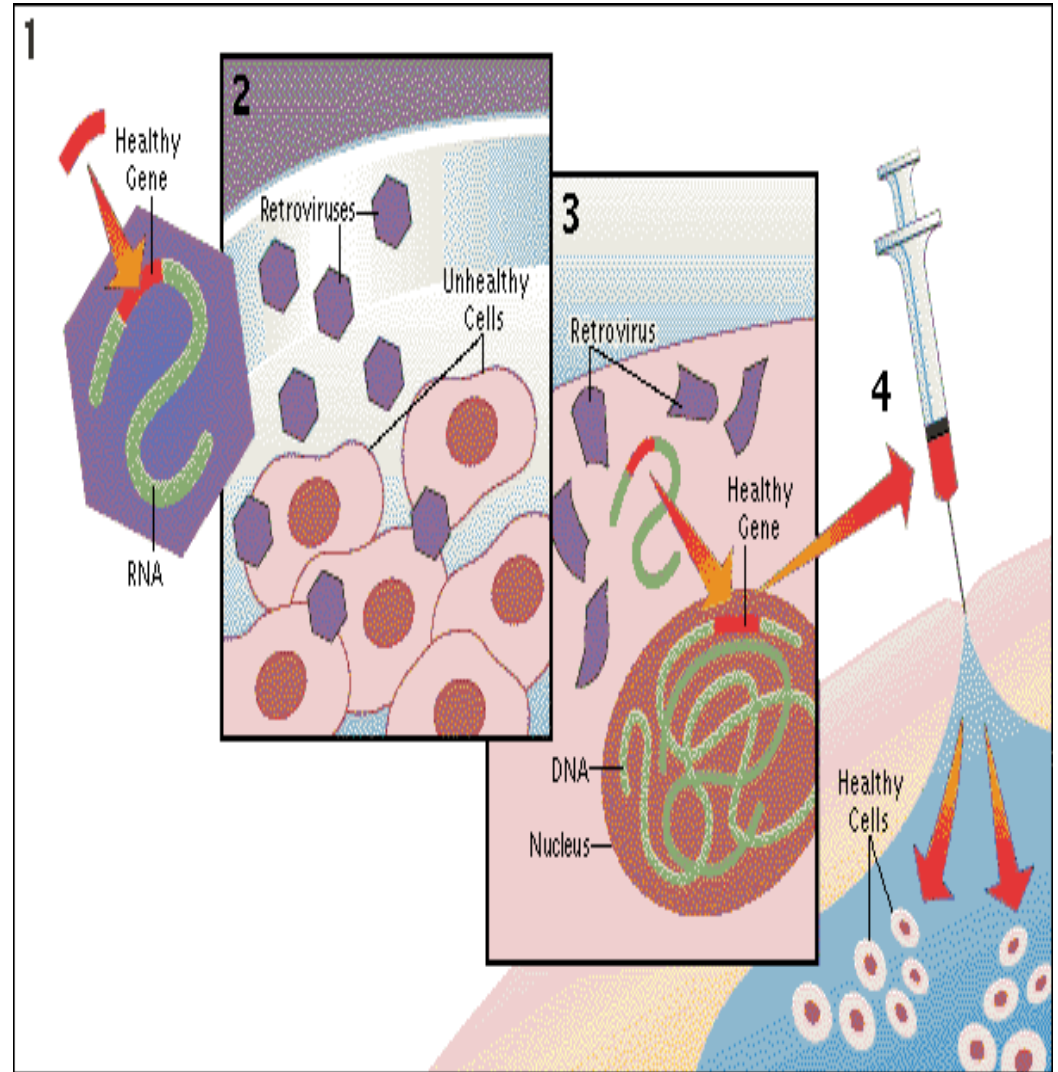
The therapeutic genes are transferred into somatic cell other than a gamete, germ cell, gametocyte or undifferentiated stem cell. It only affect the individual patient only, and are not inherited by offspring.

## **Germline Gene Therapy (GGT):**

Modifying (introduction of functional genes into their genomes) a germ cell causes all the organism's cells to contain the modified gene.

# Mechanism

- 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



# 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



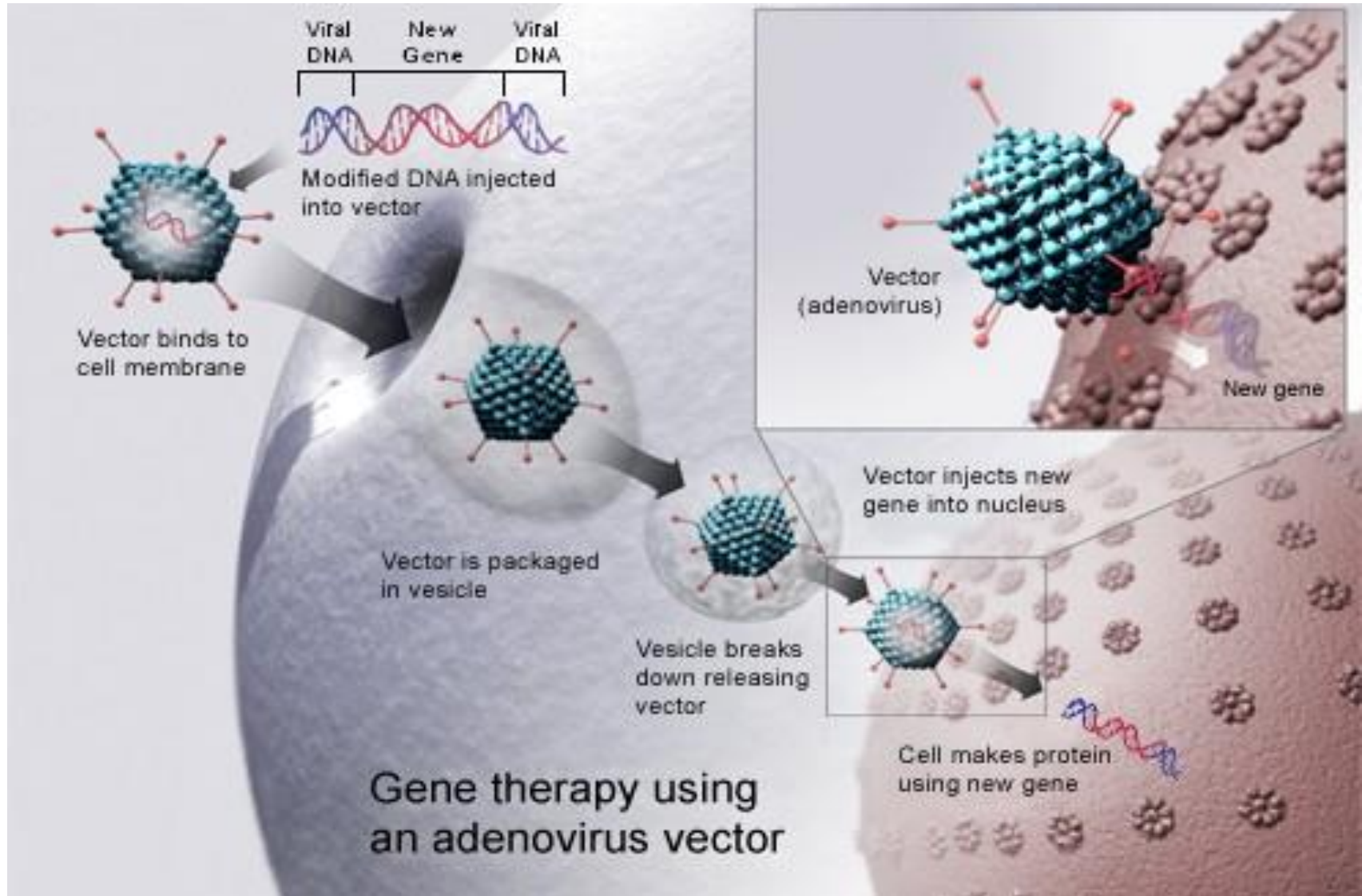
# Retroviruses

- **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**

# Adenoviruses

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

# Adenovirus cont.

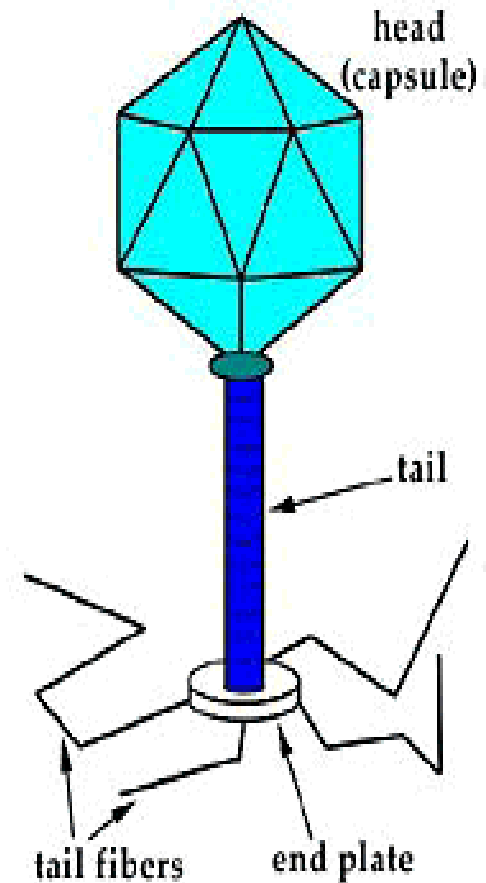


# Adeno-associated Viruses

- 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

# Herpes Simplex Viruses

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



# Non-virus Options

## Advantages:

- large scale production.
- low host immunogenicity.

## Disadvantage:

- Produced lower levels of transfection and gene expression, and thus lower therapeutic efficacy. Later technology remedied this deficiency.

# NON-VIRUS OPTIONS

- **Direct introduction of therapeutic DNA**
  - ✓ only with certain tissue
  - ✓ Requires a lot of DNA
- **Creation of artificial lipid sphere with aqueous core, liposome**
  - ✓ Carries therapeutic DNA through membrane
- **Chemically linking DNA to molecule that will bind to special cell receptors**
  - ✓ DNA is engulfed by cell membrane
  - ✓ Less effective
- **Trying to introduce a 47<sup>th</sup> Chromosome**
  - ✓ Exist alongside the 46 others
  - ✓ Could carry a lot of information
  - ✓ But how to get the big molecule through membranes?

# Transportation Techniques

- **Injection of naked DNA,**
- **electroporation,**
- **the gene gun,**
- **sonoporation,**
- **magnetofection**



# Current Status

- FDA hasn't approved any human gene therapy product for sale
- Reasons:
- In 1999, 18-year-old Jesse Gelsinger died from multiple organ failure 4 days after treatment for ornithine transcarboxylase deficiency.
- Death was triggered by severe immune response to adenovirus carrier
- January 2003, halt to using retrovirus vectors in blood stem cells because children developed leukemia-like condition after successful treatment for X-linked severe combined immunodeficiency disease

# Limitation of Gene Therapy

- **Short Lived**
  - Hard to rapidly integrate therapeutic DNA into genome and rapidly dividing nature of cells prevent gene therapy from long time
  - multiple rounds of therapy
- **Immune Response**
  - Introduction of foreign material leads to immune response
  - increased response when a repeat offender enters
- **Viral Vectors**
  - Can cause toxic, immune, inflammatory response
  - Can cause disease once get 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

# Unsuccessful Gene therapies

- Jesse Gelsinger, a gene therapy patient who lacked ornithine transcarbamylase activity, died in 1999.
- Within hours after doctors shot the normal OTC gene attached to a therapeutic virus into his liver, Jesse developed a high fever. His immune system began raging out of control, his blood began clotting, ammonia levels climbed, his liver hemorrhaged and a flood of white blood cells shut down his lungs.
- One problem with gene therapy is that one does not have control over where the gene will be inserted into the genome. The location of a gene in the genome is of importance for the degree of expression of the gene and for the regulation of the gene (the so-called "position effect"), and thus the gene regulatory aspects are always uncertain after gene therapy

# **Successful Gene Therapy for Severe Combine Immunodeficiency**

- **Infants with severe combined immunodeficiency are unable to mount an adaptive immune response, because they have a profound deficiency of lymphocytes.**
- **severe combined immunodeficiency is inherited as an X-linked recessive disease, which for all practical purposes affects only boys. In the other half of the patients with severe combined immunodeficiency, the inheritance is autosomal recessive — and there are several abnormalities in the immune system when the defective gene is encoded on an autosome.**

# Severe Combined Immunodeficiency Continued

A previous attempt at gene therapy for immunodeficiency was successful in children with severe combined immunodeficiency due to a deficiency of adenosine deaminase.

In these patients, peripheral T cells were transduced with a vector bearing the gene for adenosine deaminase. The experiment was extremely labor intensive, because mature peripheral-blood T cells were modified rather than stem cells, and the procedure therefore had to be repeated many times to achieve success.

# Successful One Year Gene Therapy Trial For Parkinson's Disease

- **Neurologix a biotech company announced that they have successfully completed its landmark Phase I trial of gene therapy for Parkinson's Disease.**
- **This was a 12 patient study with four patients in each of three dose escalating cohorts. All procedures were performed under local anesthesia and all 12 patients were discharged from the hospital within 48 hours of the procedure, and followed for 12 months. Primary outcomes of the study design, safety and tolerability, were successfully met. There were no adverse events reported relating to the treatment.**

# Parkinson's Disease Cont.

The gene transfer procedure utilized the AAV (adeno-associated virus) vector, a virus that has been used safely in a variety of clinical gene therapy trials, and the vehicle that will be used in all of the company's first generation products, including epilepsy and Huntington's disease. In its Parkinson's disease trial, Neurologix used its gene transfer technology.

# 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 Disease.**
  - **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**