Previous Class

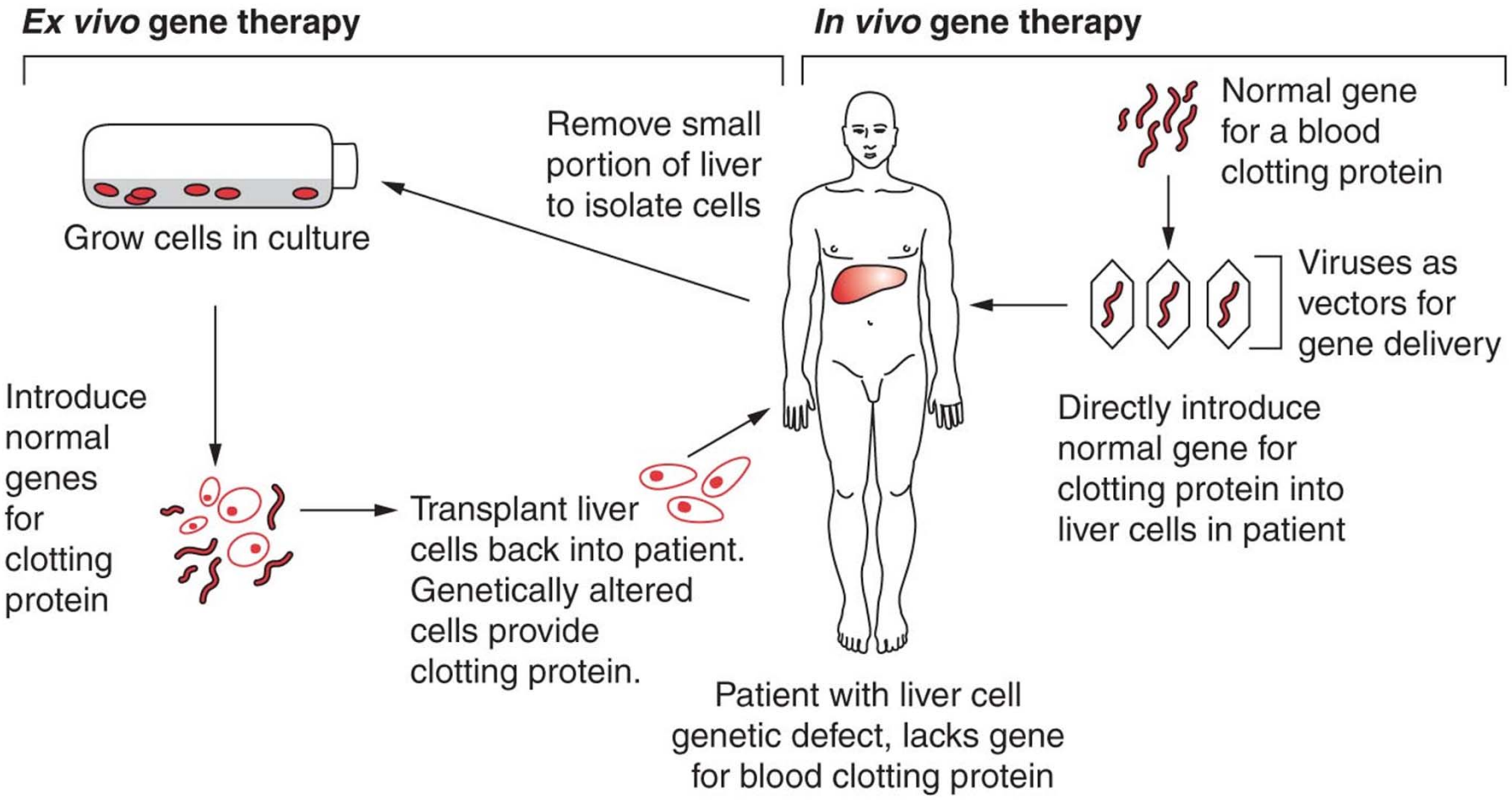
- Identifying novel drugs and developing new ways to treat disease
- Pharmacogenomics
- Improved Drug Delivery
- Nanomedicine
- Artificial Blood
- Vaccines and Therapeutic Antibodies

Gene Therapy

- **Gene therapy** is the delivery of therapeutic genes into the human body to correct disease conditions created by a faulty gene or genes
  - How are genes delivered?
  - How can genes be sent to the proper tissues and organs?
  - Can it be effective and safe?

- How is Gene Therapy done?
- Two primary strategies
  - *Ex vivo* gene therapy
    - Cells are removed from the patient, treated with techniques similar to transformation, and then reintroduced to the person
  - *In vivo* gene therapy
    - Introducing genes directly into tissues and organs in the body
    - Challenge is delivering genes only to intended tissues and not tissues throughout the body

Gene Therapy



In either mode, genes may be introduced into cells as DNA packaged into viruses or as naked DNA

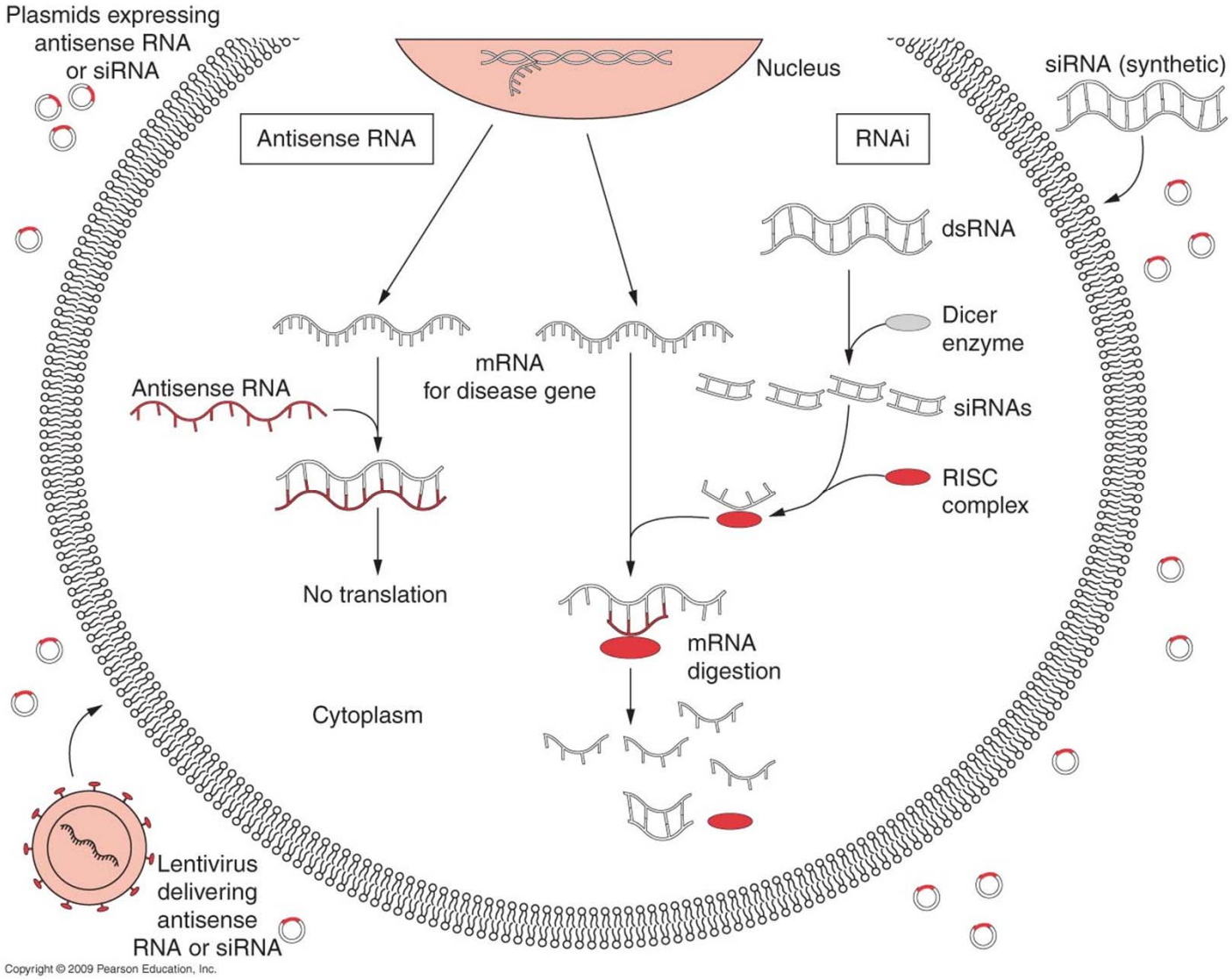
- Vectors for Gene Delivery
  - Rely on viruses as vectors
    - Use viral genome to carry a therapeutic gene or genes and use virus itself to infect human cells, introducing the gene
    - Adenovirus (common cold)
    - Influenza virus (flu)
    - Herpes virus (cold sores, some cause STD)
  - Must make sure the virus has been genetically engineered so that it can neither produce disease nor spread throughout the body

- Viral Infection of Human Cells
  - Bind to and enter cells; release genetic material (DNA/RNA) into nucleus or cytoplasm
  - Human cell now acts as a host to reproduce the viral genome and to produce viral RNA and proteins
- Make Good Vectors
  - Efficient at infecting many types of human cells
  - Retroviruses (HIV) permanently insert (**integrates**) their DNA into host cell genome
  - They can permanently integrate therapeutic genes into the DNA of human host cells, thereby providing lasting gene therapy
  - Some viruses infect only certain types of cells – good for targeted gene therapy
  - For example, herpesvirus infects only cells of central nervous systems
  - Herpesvirus can be effective way or treating genetic disorders of brain such as Alzheimer disease, Parkinson disease, brain tumors (?)

- Other Gene Delivery Options
  - Naked DNA – DNA by itself that is injected directly into body tissues
  - Effective in the liver and in skeletal muscle
  - Only relatively small number of cells take up injected DNA
  - There may not be enough cells expressing the therapeutic gene

- Liposomes – small, hollow particles made of lipid molecules
    - Packaged with gene and injected or sprayed into tissues
- Tiny gold nanoparticles coated with DNA shot into host cells
- Biodegradable gelatin particles
- Gene pills – pills deliver DNA to intestines. They absorb DNA and express therapeutic protein and secrete it into bloodstream

Antisense RNA technology and RNA interference for gene therapy



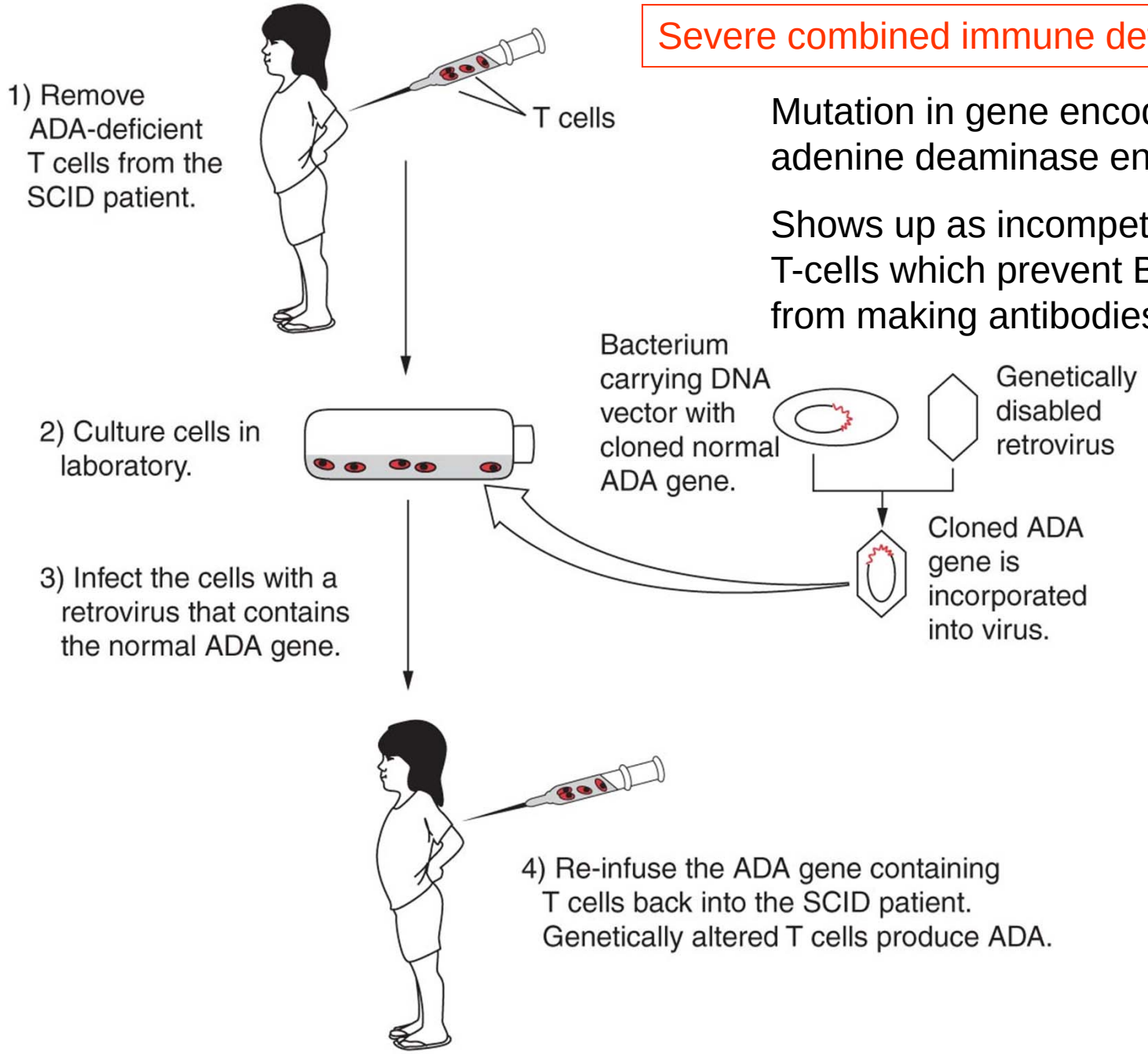
- Problems:
- In vivo delivery
- Tough to penetrate cells and target right tissues
- Most complex diseases are multi-gene problems

- First human gene therapy – SCID patient in 1990
  - SCID is severe combined immunodeficiency
  - Defect in gene called adenosine deaminase (ADA)
    - Produces an enzyme involved in the metabolism of nucleotide dATP
    - Accumulation of dATP is toxic to T cells
    - Without T cells, B cells cannot recognize antigen and make antibodies
  - *Ex vivo* gene therapy successful

Severe combined immune deficiency

Mutation in gene encoding adenine deaminase enzyme

Shows up as incompetent T-cells which prevent B-cells from making antibodies



- Cystic Fibrosis – two defective copies of a gene encoding a protein called **cystic fibrosis transmembrane conductance regulator (CFTR)**
  - Normal protein serves as a pump to remove chloride ions from cell
  - Produced by many cells in the body – skin, pancreas, liver, digestive tract, male reproductive tract, and respiratory tract
    - Extremely thick sticky mucus in airways; infertility; extremely salty sweat

Treating Cystic Fibrosis by Gene Therapy

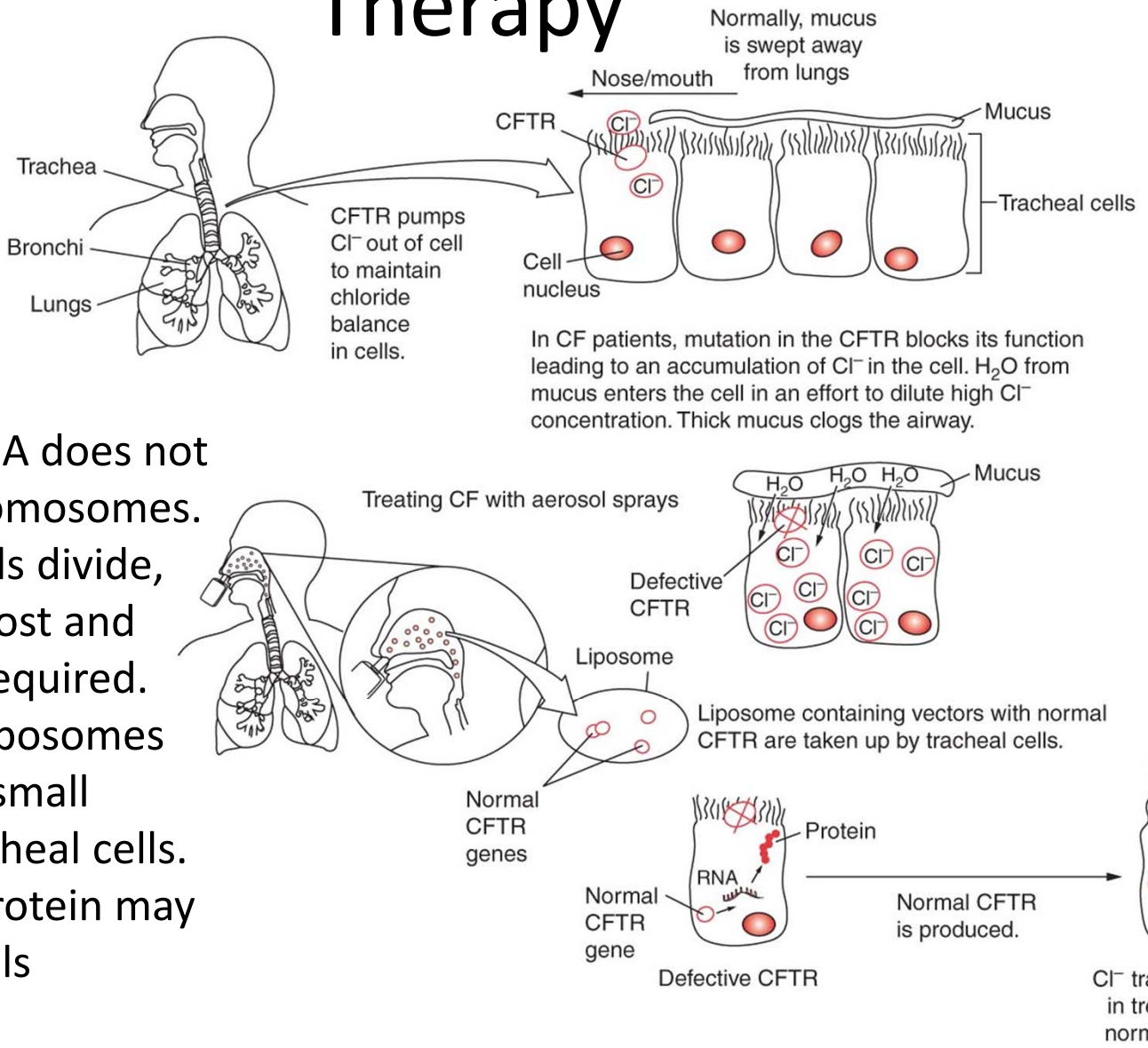
Limitations:

Expensive

Require multiple applications as DNA does not integrate into chromosomes. When tracheal cells divide, delivered gene is lost and more spraying is required.

CFTR-containing liposomes are taken up by a small percentage of tracheal cells.

Expressed CFTR protein may also be toxic to cells



- Challenges Facing Gene Therapy
  - Potential risks of viruses as vectors
    - Death of Jesse Gelsinger in 1999 due to complications related to adenovirus vector
    - Death of 2 children in France in 2002
    - Temporary cessation of a large number of gene therapy trials and FDA stopped most retroviral studies
    - Greater patient monitoring

- Challenges Facing Gene Therapy
  - Can gene expression be controlled?
  - Can we safely and efficiently target only the cells that require the gene?
  - How can gene therapy be targeted to specific regions of the genome?
  - How long will therapy last?
  - Will immune system reject?
  - How many cells need to be corrected?

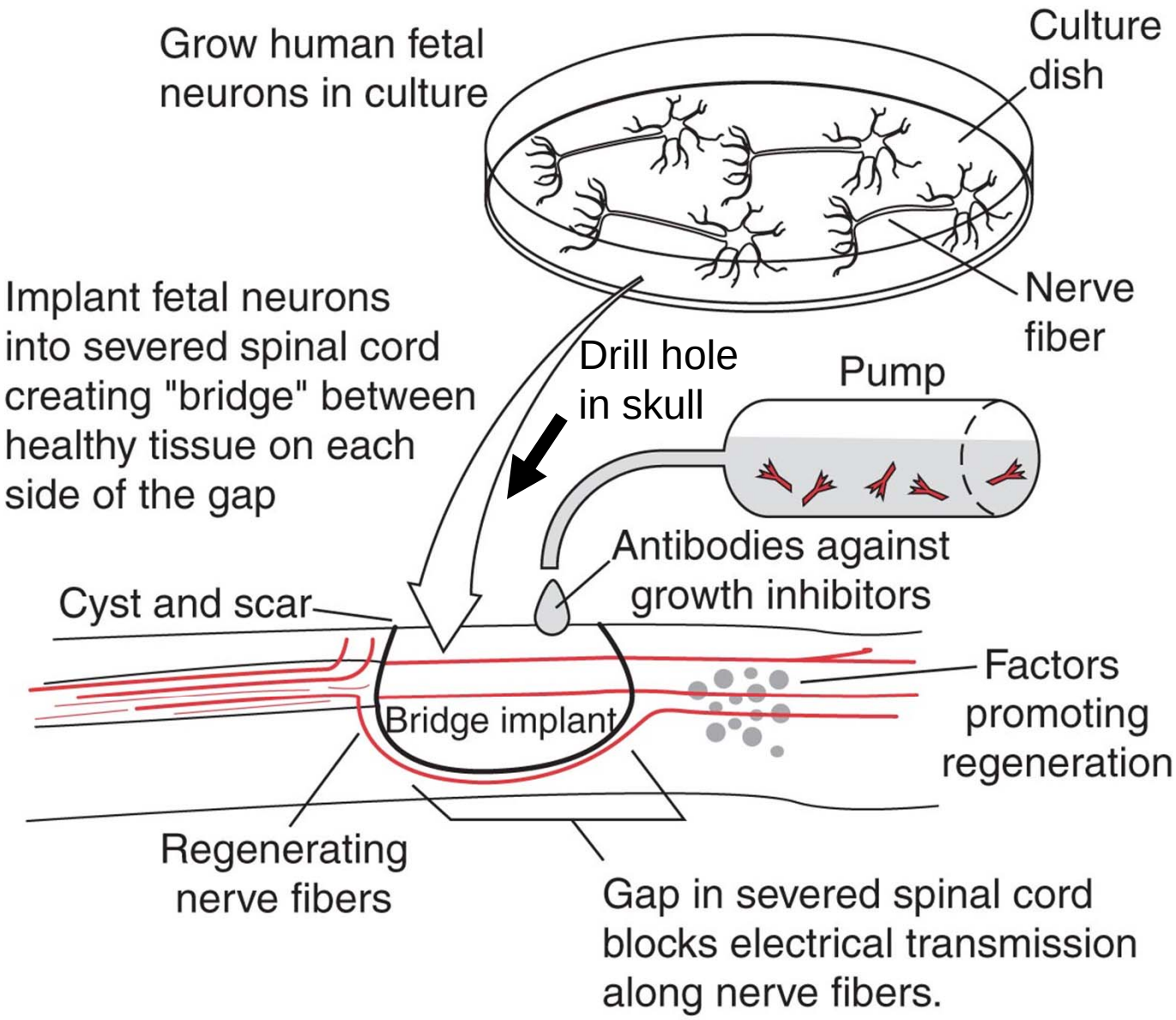
The Potential of Regenerative Medicine

- **Regenerative Medicine** – growing cells and tissues that can be used to replace or repair defective tissues and organs
- Cell and Tissue Transplantation
  - Fetal tissue grafts
  - Organ transplantation
  - Cellular therapeutics

Fetal tissue grafts

- Neurodegenerative diseases – Alzheimer disease and Parkinson disease
- Parkinson disease is due to loss of cells deep inside brain (*substantia nigra*). Neurons in this region produce a chemical *dopamine*, a neurotransmitter or signaling chemical used by neurons to signal.
- Results in tremors, loss of balance, dexterity, etc.
- Drugs become ineffective after long treatment
- Adult neurons may not divide or repair themselves. It may be useful to transplant neurons.
- Introduce fetal neurons which can establish connections with other neurons, replace the damaged brain cells, and restore brain function.
- ~ 100 patients have received fetal neuron transplants and symptoms have improved in 40% of cases

Cell and tissue transplantation



Organ Transplantation

- **Autografting**
  - Transplantation of a patient's own tissue from one region of the body to another
  - Coronary artery bypass: involves removing segments of a vein from the patient's leg and connecting it surgically to arteries in the heart as a bypass around obstructed vessels
  - But for heart or liver transplant, a donor must be found

Rejection of donated organs

- Rejection occurs when recipient's immune system recognizes that the **donor organ is foreign**
- Matching organs for transplantation involves **tissue typing** to check if donor organ is compatible for a recipient.
- Tissue typing is based on marker protein (group of > 70 genes called **major histocompatibility complex** (**MHC**)) present on surface of every cell in the body
- Immune system cells (B-cells and T-cells) recognize **HLAs** (a type of MHC proteins) on all body cells present since birth as “self” (belonging to same individual)

Rejection of donated organs

- Any other cells are "**nonself**" or foreign cells that may be attacked by the immune system and destroyed
- **Some HLAs are common** on most human tissues, and **others are unique** to a given individual
- For successful transplantation of an organ from one human to another a close match of several types of HLAs between donor organ and recipient cells is required; otherwise the recipient will reject the transplanted organ
- Immunosuppressive drugs are used to weaken recipient's immune system and minimize organ rejection