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PHARMACEUTICAL GENE DELIVERY AND THEIR TECHNIQUES

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PHARMACEUTICAL GENE DELIVERY AND THEIR TECHNIQUES

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3. Approaches of gene transfer
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4. vector system used for gene delivery
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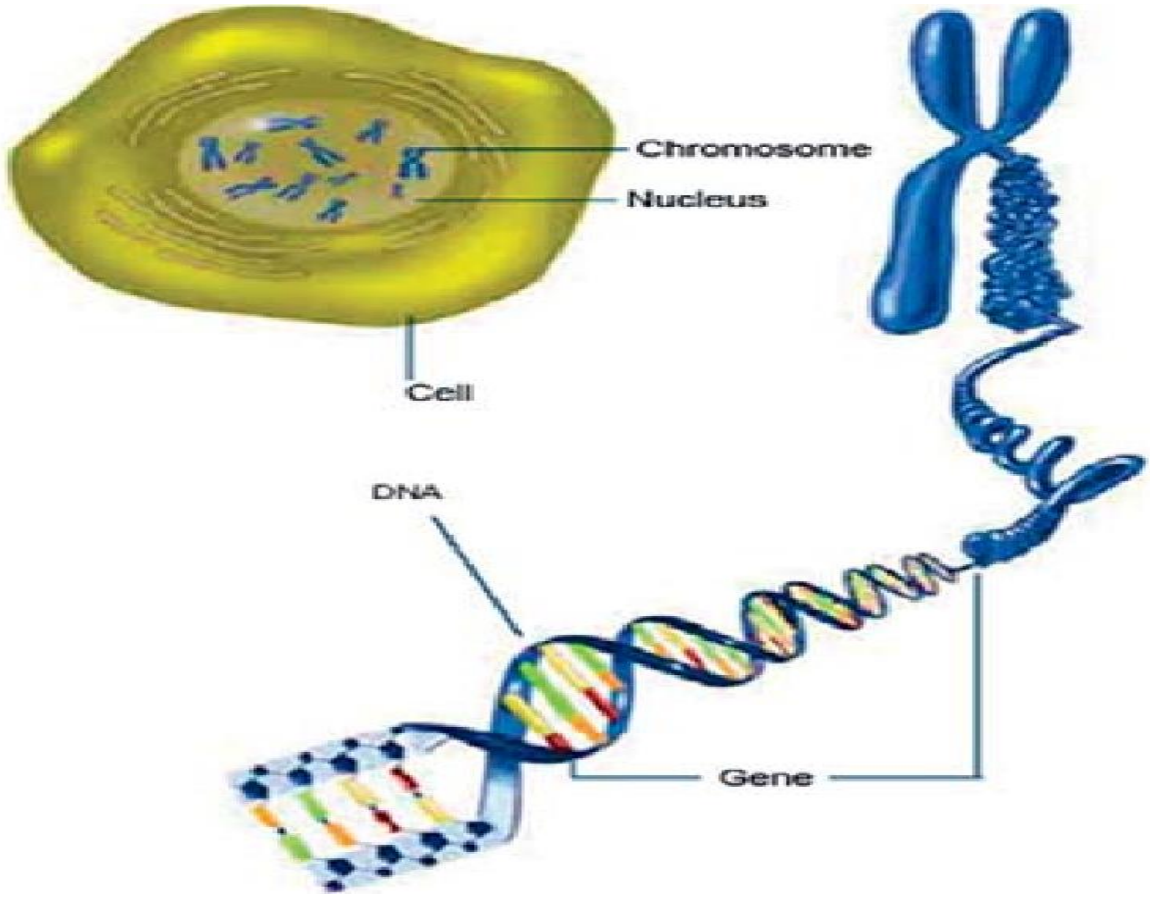
Introduction

- A gene is the basic physical and functional unit of heredity. Genes, which are made up of DNA, act as instructions to make molecules called proteins.
- Genes encode instructions on how to make proteins.
- In humans, genes vary in size from a few hundred DNA bases to more than 2 million bases.
- Every person has two copies of each gene, one inherited from each parent.



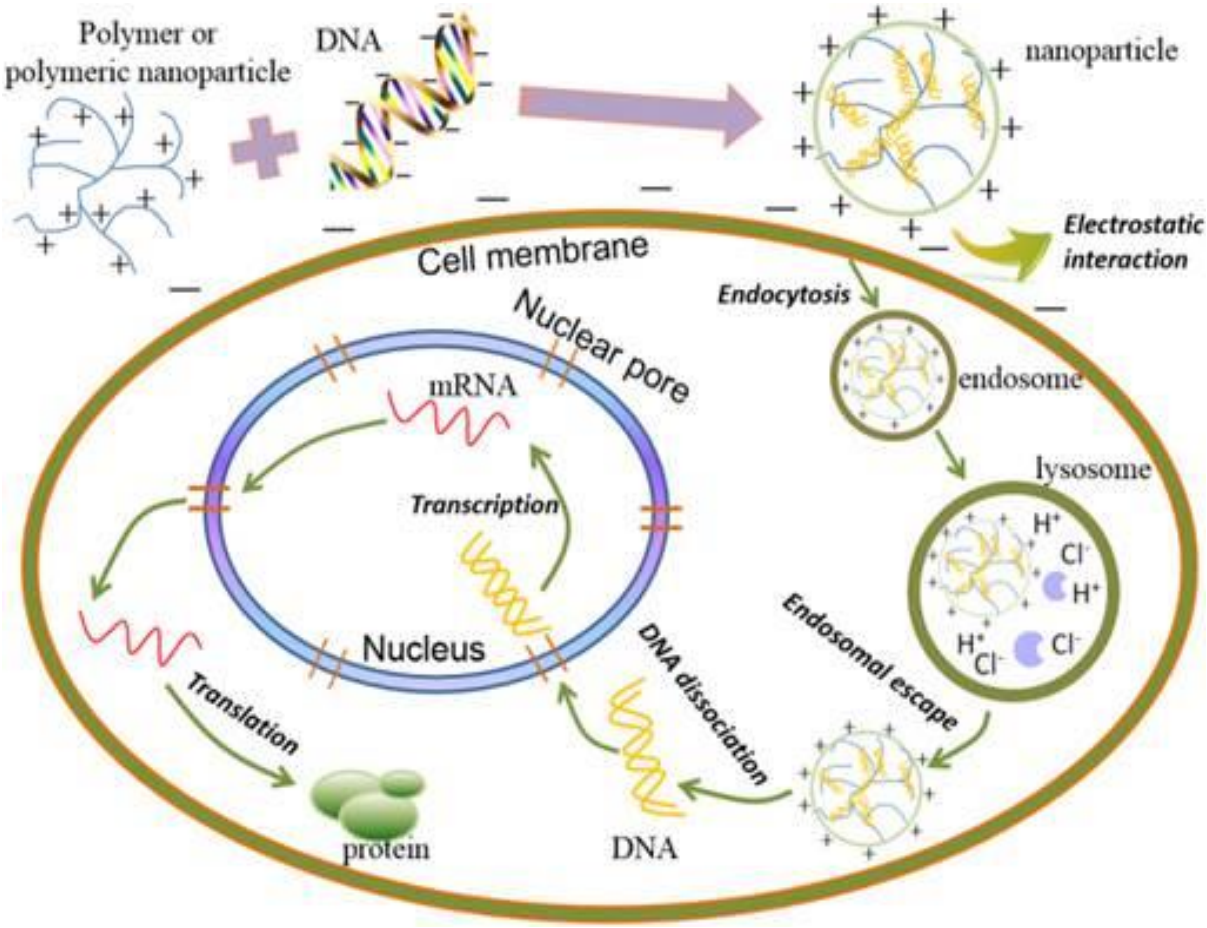
Gene delivery

- "Gene delivery is the process of introducing foreign DNA into host cells. It is also one of the steps necessary for gene therapy."
- In most gene delivery studies a normal gene is inserted into the genome(genetic material) to replace an abnormal disease causing gene.
- A carrier molecule called vector carries the therapeutic gene into patients target cell.

Gene therapy

- "A type of experimental treatment in which foreign genetic material (DNA or RNA) is inserted into a person's cells to prevent or fight disease."
- In the future, this technique may allow doctors to treat a disorder by inserting a gene into a patient's cells instead of using drugs or surgery.
- Researchers are testing several approaches to gene therapy, including:

- 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.



TECHNIQUES OF GENE DELIVERY

- Based on vectors used the gene transfer techniques can be divided into
- Viral method
- Non-Viral methods

VIRAL METHOD

- It is Virus Mediated Gene Transfer.
- In other way the gene can be packed into a virus and allow it to infect the host cell without harming it in any way.
- This method can be used both for the transformation of prokaryotic host cell as well as transfection of eukaryotic host cells.

- In the case of bacterial host cells, the recombinant DNA can be packed into the empty head of a specially designed bacteriophage (e.g., lambda phage) and allow the virion to infect the host cell.

NON-VIRAL METHODS

- Non-viral vectors consist of a nucleic acid, either naked or complexes with a carrier that aids its passage into the target tissue.
- Non-viral vectors using mechanical or chemical approach can efficiently transfect cells in vitro.
- Mechanical methods include direct injection or gene gun to introduce plasmid DNA.

Limitation

- Low level of gene expression
- Inability to use for systemic administration due to presence of serum nuclease.

APPROACHES OF DNA TRANSFER

- Generally there are two approaches for DNA transfer
  - DNA transfer by artificial method
  - DNA transfer by natural method

1. **DNA TRANSFER BY ARTIFICIAL METHOD:**
   - **Physical Method**
     - Microinjection
     - Biolistic OR Micro projectile
     - Sonoporation
   - **Chemical Method**
     - DNA transfer by calcium phosphate method
     - Liposome mediated Transfer
   - **Electrical Method**
     - Electroporation(Electro permeabilization)

PHYSICAL METHODS:

A –Sonoporation

- Sonoporation, or cellular sonication, is the use of sound (typically ultrasonic frequencies) for the transfer of recombinant DNA into the target host cell.
- Sonoporation refers to the formation of small pores in cell membranes by using ultrasound for the transfer of nucleic acid materials.
- It is the use of sound (typically ultrasonic frequencies) for modifying the permeability of the cell plasma membrane.
- This technique is usually used in molecular biology and non-viral gene therapy.

B-MICROINJECTION

- DNA microinjection was first proposed by Dr. Marshall A. Barber in the early of nineteenth century.
- Microinjection is the use of a glass micropipette to inject a liquid substance at a microscopic level. The target is often a living cell.
- Glass Micropipette is usually of 0.5-5 Micrometer.
- It involves delivery of foreign DNA into a living cell (e.g. a cell, egg, oocyte, embryos of animals) through a fine glass micropipette. The introduced DNA may lead to the over or under expression of certain genes.
- This is the direct introduction of the recombinant DNA into the host cell.

C-Biolistic OR Micro projectile:

- Biolistic OR particle bombardment is a physical method that use accelerated micro projectile to deliver DNA or other molecules into intact tissues and cells.
- Biolistic transformation is relatively new and novel technology among physical method of artificial transfer of DNA.
- The Gene gun is a device that fires DNA into target cells.
- The DNA to be transformed into the cells is coated onto microscopic beads of either gold OR tungsten

- In this method DNA is coated with gold particles are then loaded into a device, which is similar to gun and it generates force by which it can penetrate into the cell.

ELECTRICAL METHOD

- **A-ELECTROPORATION**
- Electroporation is a process that uses electrical pulses to produce transient pores and introduce foreign gene into host cell.
- Electroporation is temporary destabilization of the cell membrane targeted tissue by insertion of a pair of electrodes into it so that DNA molecules in the surrounding media of the destabilized membrane would be able to penetrate into cytoplasm.
- Delivery of a voltage (current) across the surfaces will result in the spontaneous creation of pores in the plasma membranes.
- When the current is removed, the pores will spontaneously close, trapping some of the DNA within the cells.

3-CHEMICAL METHODS:

- **A-Liposome Mediated Transfer:**
- Liposomes are the spheres of lipids which can be used to transport molecules into the cell.
- Cationic liposomes usually comprise a formulation of positively charged lipid that is used for the transfer of nucleic acid.
- The positive surface charge of the liposomes mediates the interaction of the nucleic acid and the cell membrane, allowing for fusion of the liposome/nucleic acid transfection complex with the negatively charged cell membrane. The transfection complex is thought to enter the cell through endocytosis.

B-DNA TRANSFER BY CALCIUM PHOSPHATE METHOD:

- This technique is used for the transfection of plant and mostly animal cells.
- The recombinant DNA is mixed with calcium chloride in a phosphate buffer at neutral pH. This results in the formation of recombinant DNA-calcium phosphate complex which appears as a thin precipitate.

- This precipitate is then added to the host cell.
- The precipitate is taken up by the cell by the process of phagocytosis.
- The recombinant DNA enters the nucleus and integrates into the host's genome.
- The transfection efficiency can be increased by exposing the host cell to 10-20% glycerol or Dimethyl sulfoxide.

2-DNA TRANSFER BY NATURAL METHOD:

1. Conjugation
2. Transduction
3. Bacterial transformation

1. CONJUGATION

- Conjugation is a gene transfer process in which a recipient bacterium receives DNA from a donor bacterium by cell-to-cell contact through conjugative pili (hair like appendages on surface of bacteria)
- Conjugation is mediated by certain plasmids.
- To perform conjugation, one bacterium has to carry a transferable plasmid (referred as F+ or R+ plasmid), while the other do not have plasmid (F- or R- plasmid). The transfer of plasmid DNA occurs from the F+ positive bacterial cell to the F-negative bacterium. And make it F+ once transfer is complete.

2. TRANSDUCTION:

- Transduction is the process in which DNA is transferred from one bacterium to another by way of bacteriophage.
- When bacteriophages infect bacteria, their mode of reproduction is to use the DNA replication proteins and mechanisms of the host bacterial cell to make abundant copies of their own DNA.
- These copies of bacteriophage DNA are then packaged into virions, which have been newly synthesized.
- Such virions can then be spread to new bacteria upon subsequent infection.

3. BACTERIAL TRANSFORMATION:

- Genetic transformation is the active uptake of free DNA by bacterial cells and the heritable incorporation of its genetic information.

- Transformation is the genetic alteration of a cell resulting from the direct uptake and incorporation of exogenous genetic material from its surroundings through the cell membrane.
- Transformation occurs naturally in some species of bacteria.

VECTOR SYSTEM USED FOR GENE DELIVERY:

- To transfer the desired gene into a target cell, a carrier is required. Such vehicles of gene delivery are known as vectors.
- Two types of vector system are used for gene delivery
- Viral Vector
- Non- Viral Vector

VIRAL VECTORS

- One of the successful gene therapy systems available today are viral vectors. Remove the viral DNA and using the virus as a vehicle to deliver the therapeutic DNA. All viral vector genomes have been modified by deleting some areas of their genomes so that their replication becomes deranged and it makes them safer.

NON-VIRAL VECTORS:

- Non-viral vectors consist of a nucleic acid, either naked or complexes with a carrier that aids its passage into the target tissue. They have the advantage of a low risk factor due to the absence of a viral component.
- It includes,

➢ Naked DNA

➢ Lipid mediated delivery

A) Naked DNA:

- Naked plasmid DNA enters the cell and express their genetic material.
- It is the direct introduction of therapeutic DNA into target cell.
- The mechanism for naked DNA–mediated gene transfer is not yet clear.
- It was suggested that naked DNA is taken up by parenchymal cells in vivo by an active, receptor-mediated process.

B) Lipid Mediated delivery:

- Transfer of genetic material into the cells takes place via Liposomes, which are the vesicles that can merge with the cell membrane since they are made of phospholipid bilayer.
- Cationic liposomes usually comprise a formulation of positively charged lipid and a co-lipid required for stabilization of the liposome complex.
- The positive surface charge of the liposomes mediates the interaction of the nucleic acid and the cell membrane, allowing for fusion of the liposome/nucleic acid transfection complex with the negatively charged cell membrane. The transfection complex is thought to enter the cell through endocytosis.

Goals

- An important goal for the research of gene delivery system is to develop clinically relevant vectors that use to combat elusive diseases such as AIDS, cancer, Alzheimer, etc.
- DNA-based viral vectors for gene delivery systems utilize the viral vectors to deliver genetic materials to the host cells. The DNA viral vectors are efficient for delivering the genetic materials to the host cells.

Conclusion

- It is concluded that physical techniques can directly transfect cell without carrier vectors, and they are easy to prepare, possible to transfect large molecules, and safe to manipulate.
- The aim of advanced physical techniques to contribute in developing new delivery strategies with high efficiency, high cell viability and minimal risks.