Mammalian and Plant Cell Culture
Module 5
Transfection and Infection of Mammalian Cells Handout

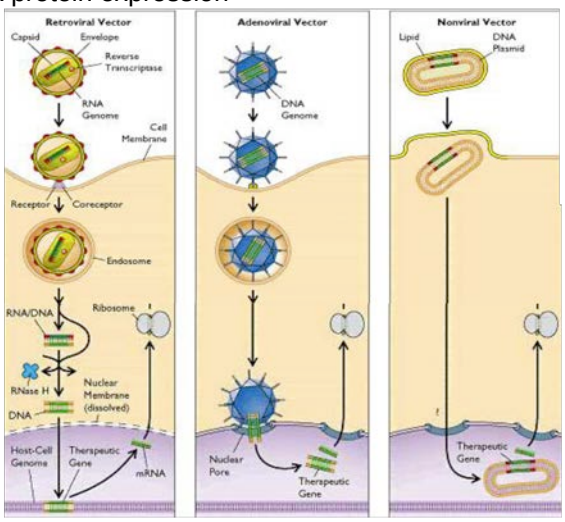
What is transfection?

- The delivery of DNA or RNA into eukaryotic cells
  - ✓ Powerful tool to study and control gene expression
    - Typically done using plasmid DNA, viral packages or short regions of RNA
    - Express a foreign gene or gene regulatory elements
    - RNA interference (RNAi) can be used to block protein expression
  - ✓ Two types: Stable and transient

Gene Transfer

Viral and Non-Viral

- Infection (viral partial mediated)
  - ✓ Retroviral – Murine leukemia, HIV and Lentiviral
  - ✓ DNA Viruses – Adenovirus, Herpes simplex and Adeno-associated virus
- Tranfection (chemical-non viral)
  - ✓ Liposomes (chemical)
  - ✓ Electroporation



Viral Vectors

PROS:

- High Transfection Efficiency
- Natural Tropism (ability to infect different cells)
- Evolved mechanisms for endosomal escape
- Natural transportation mechanism of DNA into nucleus

CONS:

- Strong immune reactions against viral proteins prohibit multiple administrations
- Possibility of chromosomal insertion and protooncogene Activation
- Complicated synthesis process
- Limitation on gene size
- Toxicity, contamination of live virus

Transfection

- The process of introducing nucleic acids into cells by non-viral methods is defined as transfection.
- Desirable Traits include:
  - ✓ High efficiency transfer
  - ✓ Low toxicity and interference of biochemical function
  - ✓ Ease of use
- The name is meant to distinguish the process from the concept of infection, which is the viral mechanism of nucleic acid introduction into cells.
- All transfections are initiated by introducing DNA into the cytoplasm of the cells that will be genetically altered.
- This DNA is part of a construct that typically includes:
  - ✓ a promoter
  - ✓ the gene of interest
  - ✓ occasionally a reporter gene.

Transient Transfection

- Typically, the transfection only impacts the cells that directly receive the transfected DNA.
  - ✓ The plasmid remains in nucleus but is not incorporated into the genome for replication
- The transfected DNA is not passed from generation to generation during cell division and therefore the genetic alteration is not permanent.
- Cells are typically harvested 24 to 78 hours post transfection

Stable Transfection

- In a very low number of cases, the transfected DNA will integrate into a chromosome.
- This allows the transfected DNA to be carried stably from generation to generation.
- Cells must be treated with selective antibiotic which kills non-gene transformed cells
  - ✓ Often times only a portion of the plasmid is integrated – thus surviving cells may not express the transfected gene!

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Non-Viral Chemical Transfection

- DEAE-Dextran – early reagent for transfection
  - ✓ Large cationic (positive charged) carbohydrate polymer which forms ionic bonds to phosphate backbone of DNA
  - ✓ Neutral (or w/excess dextran) or positive charged complex then binds to negative charged lipid on surface of cell.
  - ✓ Endocytosis results in plasmid DNA delivery to cell and presumably the nucleus.
- Calcium Phosphate – precipitates DNA which is taken up by cells via endocytosis
  - ✓ A cheep alternative to other techniques

Cationic liposomes: ***Positively charged lipids interact with negatively charged DNA. (lipid-DNA complex).*** – ***Complex traverses cell membranes***

- Artificial liposomes – able to transfect more cells with higher efficiency than ppt or dextran methods
- Can be used on living tissue
- Wide variety of fusogenic lipids have been created

Advantages:

- ✓ Stable complex
- ✓ Can carry large sized DNA
- ✓ Can target to specific cells
- ✓ Does not induce immunological reactions.

Disadvantages:

- ✓ Low transfection efficiency
- ✓ Transient expression
- ✓ Inhibited by serum
- ✓ Some cell toxicity

Lipophilic Transfection Reagents

- Uses a combination of cationic lipids and neutral lipids.
  - ✓ The cationic lipids associate with the nucleic acid, which is negatively charged.
- The complex of lipid and DNA ends up with a neutral or net negative charge.
- The neutral lipid used aids in the insertion of the liposome into the cells.
- One of the common lipids used is DOPE
  - ✓ Dioleoyl phophatidylethanolamine
  - ✓ Classified as a fusogenic lipid.
  - ✓ Aids in the fusion of the liposomes with the plasma membrane.
- Routinely used for both transient and stable transfections of a variety of cell types.

Mechanism

- The cationic portion of the transfecting reagent associates with the nucleic acid that is negatively charged.
  - ✓ The cationic portion can be a lipid, dendrimer, or enhancer.
  - ✓ For cultured cells an overall net positive charge of the transfecting reagent-DNA complex generally results in higher transfer efficiency.
- Following the uptake of DNA into the cells, the complexes appear in the endosomes and later in the nucleus.
  - ✓ It is unclear how the nucleic acids are released from the endosomes and transverse the nuclear envelope.

Factors Influencing Transfection Efficiency

- Cell Health
- Degree of Confluency
- Contamination
- DNA Quality
  - ✓ Needs to be free of protein, RNA, and Chemical contamination
- DNA Quantity
  - ✓ the optimal amount of DNA to use will vary with type of DNA and cell line

Essential to Optimize

- Charge ratio of transfection reagent to DNA.
- The amount of transfected DNA.
- The lengths of time cells are exposed to the transfection reagent.
- The presence or absence of serum.

- The presence or absence of antibiotics.

Charge Ratio of Transfection Reagent to DNA

- The amount of positive charge contributed by the cationic component of the transfection reagent should equal or exceed the negative charge of the DNA.
- Charge ratios from 1:1 to 4:1 are commonly used.
- Thus the result is a net neutral or net positive charge.
- Initially a 2:1 or 3:1 is commonly recommended.

Deoxyribonucleic acid (DNA)

- The optimal amount of DNA to use in transfection will vary depending of DNA type and cell line.
- Highly supercoiled DNA appears to be better for transient transfections.
- Linear DNA is better for stable transfections, but the uptake of linear DNA is less than supercoiled DNA.
- For adherent cells most protocols suggest to initially test 0.50 to 2.0 μg of DNA/ml of transfecting solutions.
- Increasing the amount of DNA does not necessarily increase the transfection efficiencies.

Timing of Transfection Process

- Optimal times will vary depending on transfection agent, DNA type and cell type.
  - ✓ Optimal mixing times for the formation of DNA-Transfecting reagent complexes is generally given by the company.
  - ✓ It is essential to follow these instructions accurately.
- For incubation with DNA complex with the cells it is generally recommended that one begin with a 1 to 4 hour transfection times.
  - ✓ Optimize by testing time broader time intervals based on the type of transfecting agent.
- Cell morphology should be observed during transfection.
  - ✓ This is primarily important if transfection will be done in serum-free medium as cells typically lose viability under these conditions.

Serum

- Many common protocols recommend serum-free medium for optimal or enhanced performance.
- One of the major areas companies are working to improve their transfection products is for them to be used in serum.
  - ✓ Having serum present typically yields healthier cells.

Plating Cells

- Typically cells will be confluent by the time they are assayed for transfection success.
- The level of confluency on day of transfection should be optimized.
  - ✓ General guideline is to place cells day before transfection so they will be 50 – 80% confluent the day of transfection.
- Another major area where companies are trying to improve their transfecting reagents is in the area of confluency.
  - ✓ This is especially important for transient transfections. A higher transfection efficiency should be maintained if the cells are over 80% confluent when transfected.

Transfection Efficiency

- The goal is to optimize transfection.
- Two issues:
  - ✓ Number of cells transfected
  - ✓ The level of protein expression
- % Transfection Efficiency
  - ✓ The fraction of cells that received the gene insertion.
  - ✓ = Number of Cells Expressing the Desired Protein
    Total Number of Cells in Population
- For many experiments, particularly biochemistry experiment, you need to impact the activity of the majority of cells.
- Easily accomplished using microscopy.

Level of Protein Expression

- Goal is to have the cells produce the greatest amount of protein possible.
- May accept lower transfection efficiency if the cells produce enough protein.
  - ✓ Accept 50% transfection efficiency
  - ✓ Each cell produces 3x as much protein
- Need a quantitative measure of the amount of protein produced.
- Fluorescence Plate Reader

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Transfection Efficiency

- For some experiments you will need both.
  - ✓ Use of dominant-negative constructs to block signaling pathway.
- Challenge
  - ✓ Want to block signaling in as many cells as possible in the population.
  - ✓ To block signaling you need to have a 5 or 10 fold excess of mutant protein to normal protein.

Electroporation

voltage discharges through the liquid of the cell -10,000-100,000 V/cm (varying with cell size) in a pulse lasting a few microseconds to a millisecond is necessary for electroporation.

This electric pulse disturbs the phospholipid bilayer of the membrane and causes the formation of temporary aqueous pores. The electric potential across the membrane of the cell simultaneously rises by about 0.5-1.0 V so that charged molecules (such as DNA) are driven across the membrane through the pores in a manner similar to electrophoresis.

Advantages:

Versatility: Electroporation is effective with nearly all cell and species types (Nickoloff, 1995)

Efficiency: A large majority of cells take in the target DNA or molecule. In a study on electrotransformation of E. coli, for example, 80% of the cells received the foreign DNA

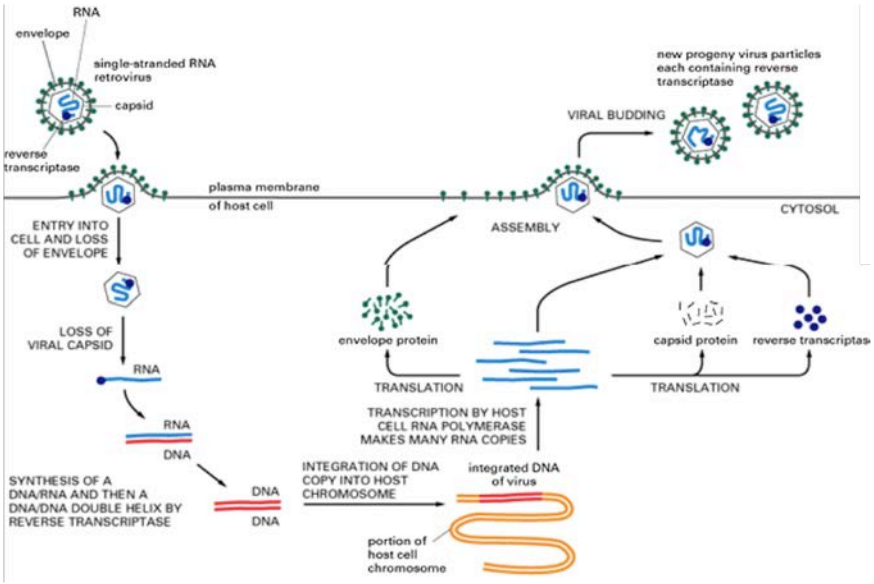
Small Scale: The amount of DNA required is smaller than for other methods

In vivo: The procedure may be performed with intact tissue

Retrovirus

The first infectious agents implicated in tumors (Chicken sarcomas, identified by Peyton Rous, 1906)

- Several types of retrovirus systems/particles are in use
  - ✓ Good for dividing and nondividing cells – use in tissue and primary cell culture
  - ✓ Gene passed on between mother – daughter cells
- Need to maintain virus by culturing infectious Particles.



Retroviral Infection

- Murine Leukemia Virus
  - ✓ Stable integration into host genome at random sites
  - ✓ Long term expression
  - ✓ Non-pathogenic
  - ✓ Small insertion sites for genes
  - ✓ Some mutagenesis occurs upon insertion
  - ✓ Needs a receptor on host cell to infect May only infect dividing cells
- HIV
  - ✓ Infect dividing and non-dividing cells
  - ✓ Random insertion
  - ✓ Various level of gene expression
  - ✓ Often mutated upon insertion
- Lentiviral Infection
  - ✓ Often used for RNA interference technology
  - ✓ A “slow” retrovirus that can infect and replicate in dividing and non-dividing cells