



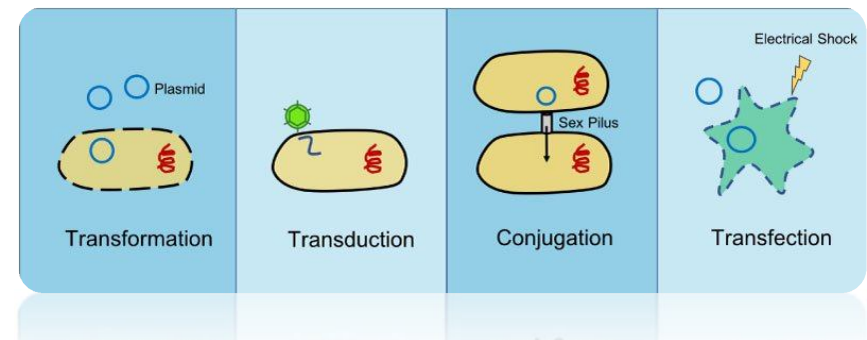
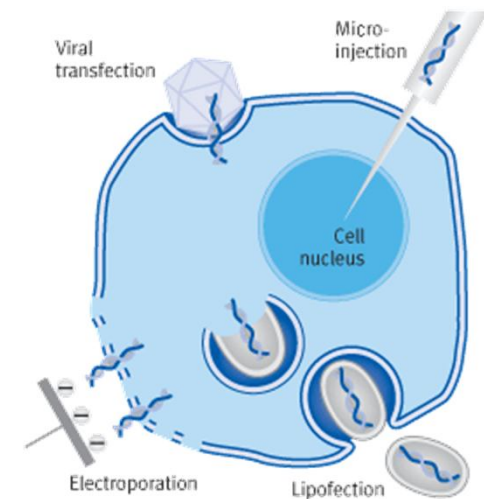
Lecture7. Gene Transfer

Lecture outlines:

- Introduction
- Transformation
 - Chemical (Heat shock) transformation
 - Electroporation
- Transduction
- Conjugation
- Transfection of Eukaryotic Cells

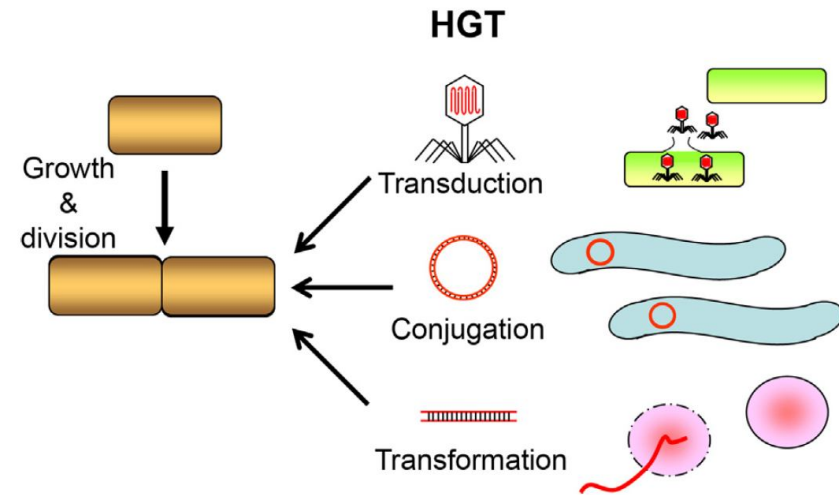
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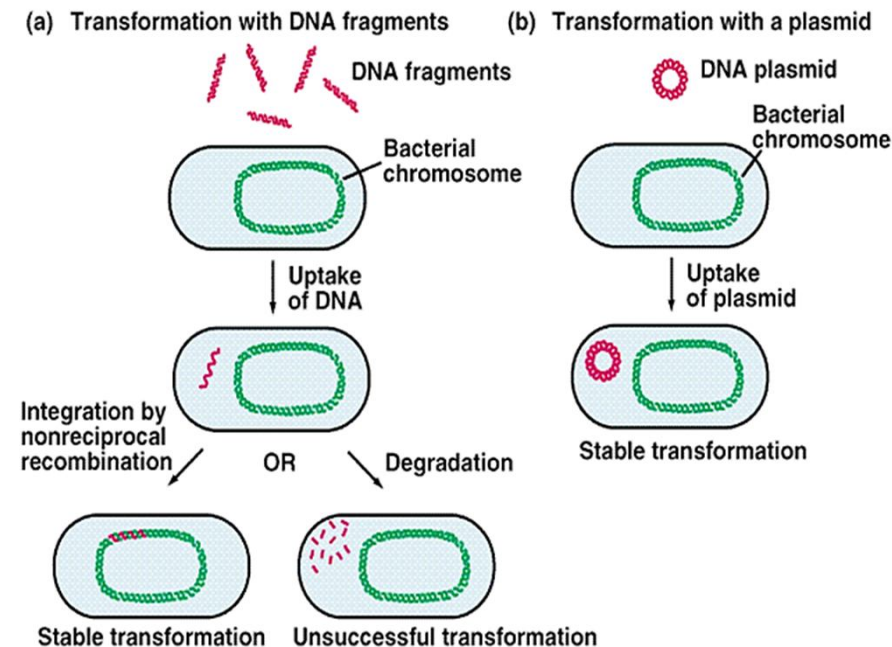
Introduction

- Horizontal gene transfer (HGT) is the movement of genetic material between organisms.
- It plays a key role in bacterial evolution and is the primary mechanism by which bacteria have gained antibiotic resistance and virulence.
- Scientists have studied how HGT occurs in nature and have learned how to introduce genetic materials into cells in the lab.
- The introduction of foreign DNA or RNA into bacteria or eukaryotic cells is a common technique in biotechnology.
- There are multiple ways foreign DNA can be introduced into cells including transformation, transduction, conjugation, and transfection.
- Transformation, transduction, and conjugation occur in nature as forms of HGT, but transfection is unique to the lab.
- Let's take a look at these different methods of DNA insertion.



Transformation

- Transformation is the uptake of genetic material from the environment by bacterial cells.
- In nature, this genetic material often comes from adjacent lysed bacteria and can include plasmid DNA or fragmented DNA released into the environment.
- Several prokaryotes (G+ and G- bacteria) are naturally transformable. However, not all bacteria are naturally competent to take up DNA.
- Competent:** A cell that is able to take up DNA and be transformed.
- Special proteins in the bacterial cell wall play a role in the uptake of foreign DNA.
- Streptococcus*, *Haemophilus*, and *Neisseria* are naturally competent and easy to transform.
- E. coli* are poorly transformed. However, *E. coli* become adequately competent if they are treated with high concentrations of calcium ions and then chilled for several minutes.

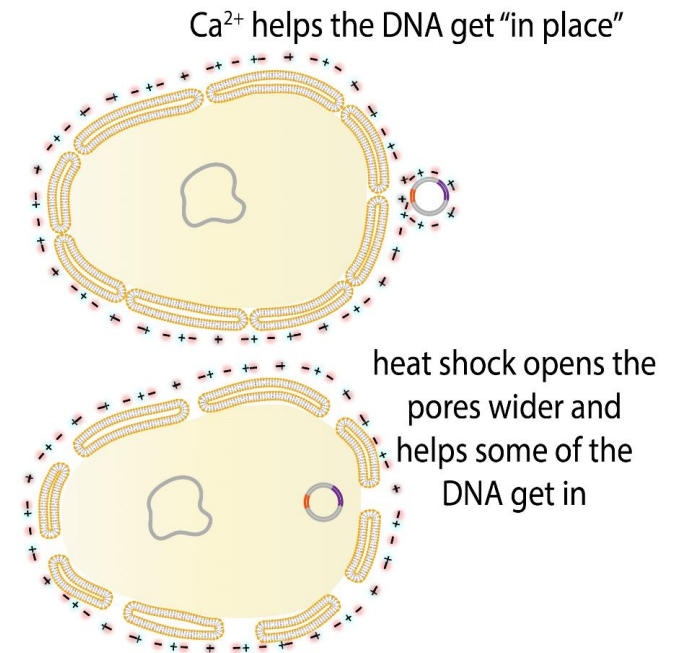


Laboratory Methods of Transformation

- Artificial competence can be induced in laboratory procedures that involve making the cell passively permeable to DNA by exposing it to conditions that do not normally occur in nature such as **Chemical (Heat shock)** or **Electroporation methods**.

A. Chemical (Heat shock) transformation

- Typically the cells are incubated in a solution containing divalent cations often calcium chloride (**CaCl₂**) under cold conditions, this will facilitate binding of DNA to the surface of the cell, before being exposed to a heat pulse (heat shock).
- Calcium chloride partially disrupts the cell membrane, which allows the recombinant DNA to enter the host cell. Cells that are able to take up the DNA are called competent cells.



B. Electroporation

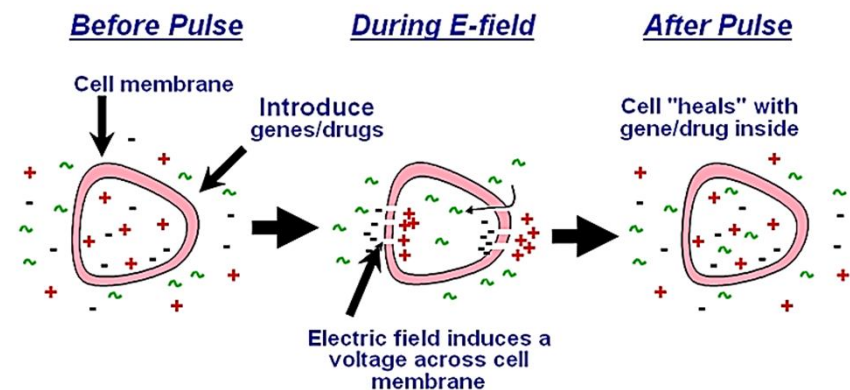
- **Electroporation** is a physical technique used to get DNA into organisms that are difficult to transform.
- In Electroporation, cells are mixed with DNA and then exposed to brief high-voltage electrical pulses.
- This makes the cell envelope permeable and allows entry of the DNA.
- In this method the cells are briefly shocked with an electric field of 10-20 kV/cm, which is thought to create holes in the cell membrane through which the plasmid DNA may enter.
- After the electric shock, the holes are rapidly closed by the cell's membrane-repair mechanisms.



Electroporation cuvette

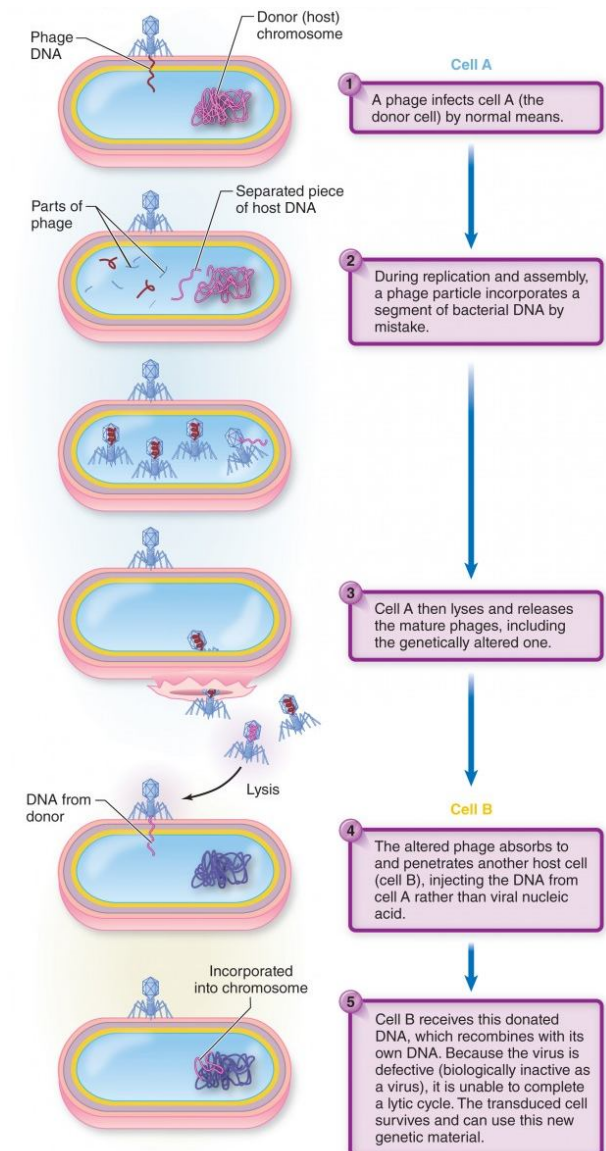


Gene Pulser electroporators



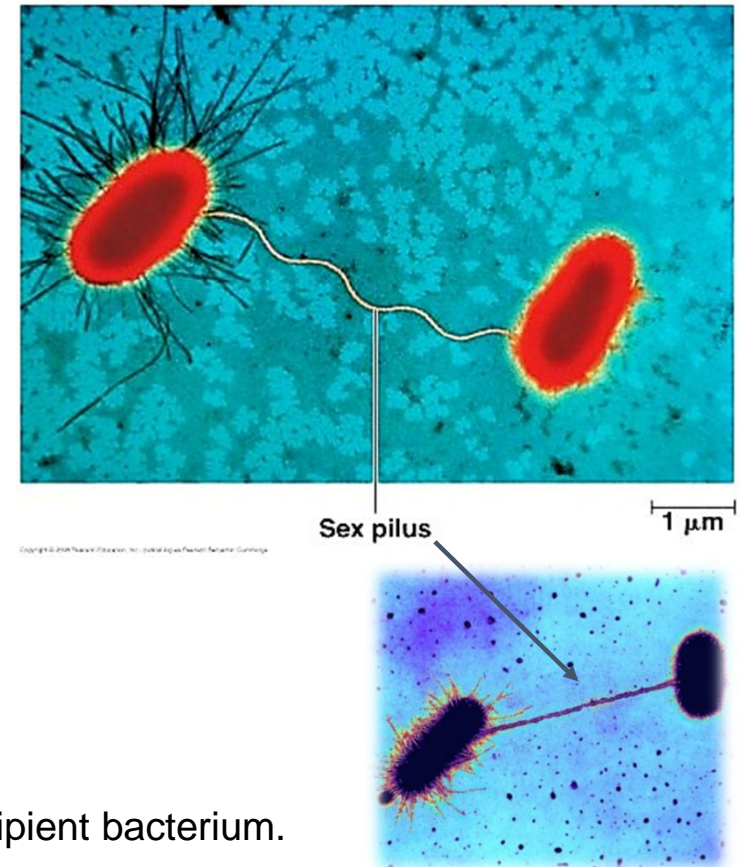
Transduction

- Is a process by which bacterial virus (bacteriophage) transfers genetic material from one bacterium to another.
- Not all phages can transduce, and not all bacteria are transducible, but the phenomenon is sufficiently widespread that it likely plays an important role in gene transfer in nature.
- Transferred DNA once inside the infected bacterium can either exist as transient extrachromosomal DNA, like a plasmid, or it can integrate into the host genome at a particular integration site.
- Transduction is a common tool used by scientists to introduce different DNA sequences of interest into a bacterial cell or a host's genome.
- Scientists also use transduction to introduce foreign DNA into eukaryotic cells. This can be done with lentiviral and Adeno Associated Viruses (AAV).



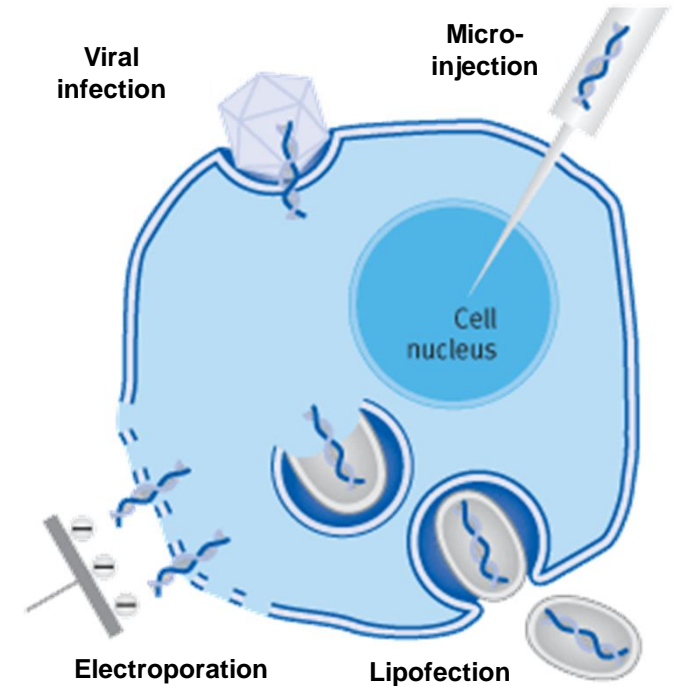
Bacterial conjugation

- Bacterial conjugation (mating) is a mechanism of genetic transfer that involves cell-to-cell contact.
- During conjugation, genetic material is transferred from a donor bacterium to a recipient bacterium through direct contact.
- The donor bacterium contains a DNA sequence called the Fertility factor (F-factor).
- The F-factor is found on an episome, a piece of DNA that can replicate on its own or be integrated within a bacterial chromosome and allows the donor bacterium to make a small “bridge” or sex pilus that attaches to the recipient cell drawing it close.
- Once in contact the donor can transfer genetic material to the recipient bacterium.
- The genetic material transferred is commonly a plasmid and can confer genetic advantages such as antibiotic resistance.



Transfection

- Unlike the last three methods which can be used in prokaryotes, transfection is only done in eukaryotic cells.
- Transfection is the process by which foreign DNA is deliberately introduced into a eukaryotic cell **through non-viral methods** including both chemical and physical methods in the lab.
- Chemicals like calcium phosphate and diethylaminoethyl (DEAE)-dextra neutralize or even impart an overall positive charge on DNA molecules so that it can more easily cross the negatively charged cell membrane.
- Physical methods such as electroporation or microinjection actually pokes holes in the cell membrane so DNA can be introduced directly into the cell.
- Microinjection requires the use of a fine needle to deliver nucleic acids to individual cells.



Lipofection

- Lipofection, also known as “lipid transfection” or “liposome-based transfection,” **uses a lipid complex to deliver DNA to cells.**
- Lipids are a broad class of fat-soluble biomolecules, such as fats, oils, and waxes.
- In aqueous solutions, cationic lipids form vesicles with a bilayer lipid sheet, known as liposomes. When liposomes encounter nucleic acids they re-form into nucleic acid lipid complexes called lipoplexes which can be actively taken up by eukaryotic cells by means of endocytosis.

