Mechanisms of Genetic Exchange

TRANSFORMATION

- Gene transfer refers to the movement of genetic information between organisms
- 2 types – horizontal & vertical
- Horizontal Gene Transfer : transfer of genes between two independent organisms
- There are three mechanisms of horizontal gene transfer in prokaryotes – transformation, conjugation & transduction
- Vertical Gene Transfer : transfer of genes from parents to offspring; eg. Binary fission of prokaryotes

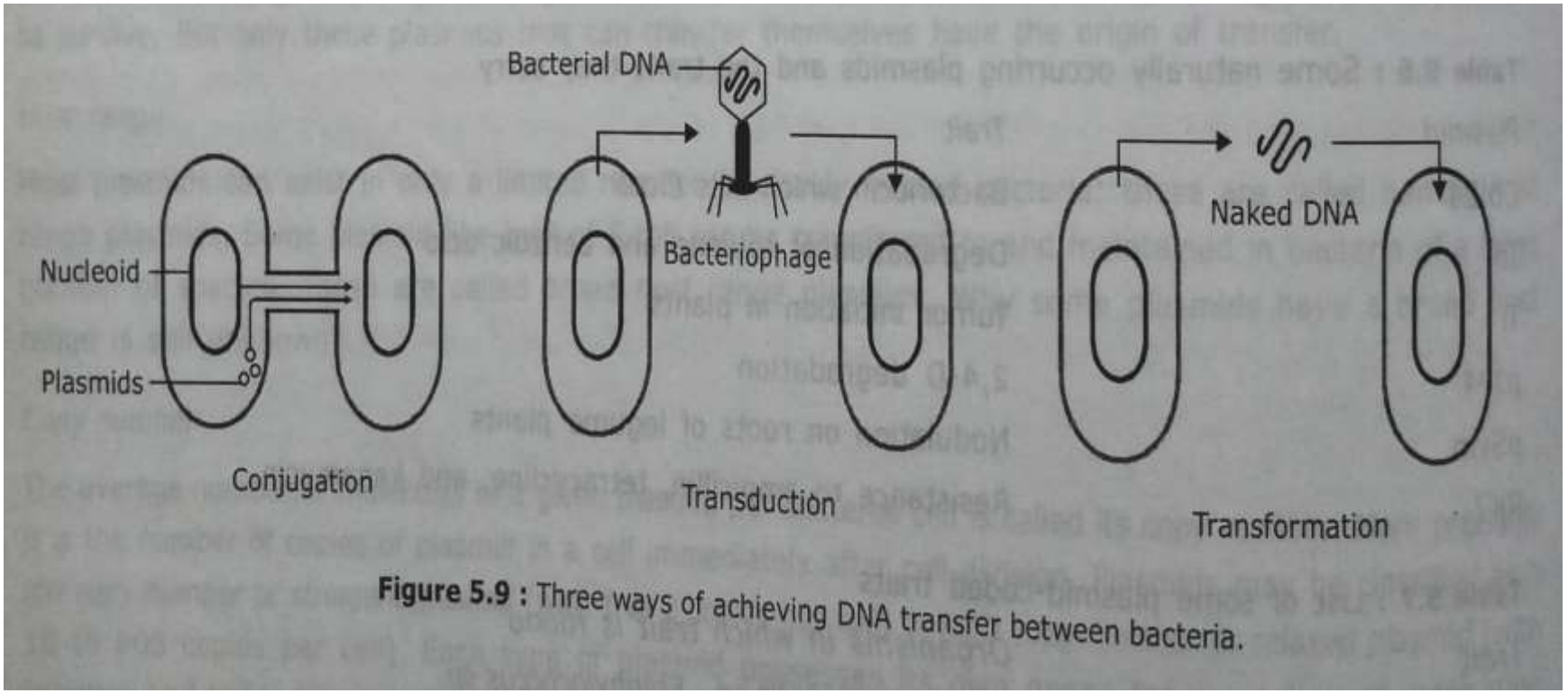


Figure 5.9 : Three ways of achieving DNA transfer between bacteria.

- Exogenote : The fragment of DNA that has been transferred during horizontal gene transfer from a donor cell to a recipient cell
- Endogenote : The recipient bacterial cell's own genetic material into which the donor DNA can integrate
- Merozygote (partial diploid or merodiploid) : A bacterial cell that has received an exogenote is initially diploid for part of its genome and is said to be a merozygote
- In merozygotes, genes (alleles) of exogenote may or may not substitutes alleles of endogenote

- Genetic recombination: The process of replacement of one allele by different alleles from the same genes by preserving the structure of genes
- Genetic recombination generally requires that the two molecules be homologous – that they have very similar (not necessarily identical) DNA sequences
- If they are non-homologous (no sequence homology) due to originating from different species, recombination is very unlikely
- Since exogenotes degraded rapidly, there is a competition between the process of degradation of exogenote and recombination

- Transformation was the first horizontal gene transfer mechanism to be discovered in bacteria
- In transformation, DNA is directly taken up by cells
- Transformants : Bacteria that have taken up DNA are called transformants
- Competence: The ability of a recipient bacterium to take up free DNA and become transformed

  It is an inheritable characteristic of certain strains of bacteria

  some species of bacteria are naturally competent

Transformation can occur at a high frequency since most cells of a naturally competent population can take up environmental DNA at any time

competent bacteria that can take up DNA encode proteins called competence factors that facilitate the binding of DNA fragments to cell surface & uptake of DNA into the cytoplasm

- Examples – *Bacillus subtilis*, *Streptococcus pneumoniae* (Gm +ve)

  Haemophilus influenzae, Neisseria gonorrhoeae, Acinetobacter calcoaceticus, Helicobacter pylori (Gm -ve)

  Cyanobacteria from the genus *Synechococcus*

General steps of Natural Transformation

- Binding of ds DNA to the outer cell surface of the bacterium (binds to membrane-bound dsDNA-binding protein)
- Movement of the DNA across the membranes and cell wall of the bacterium
- Degradation of one of the two DNA strands (exogenote) by a nuclease
- Translocation of the remaining single strand of DNA into the cytoplasm of the cell across the inner membrane
- Stable integration by homologous recombination (mediated by RecA protein) of the translocated single strand into the recipient chromosome

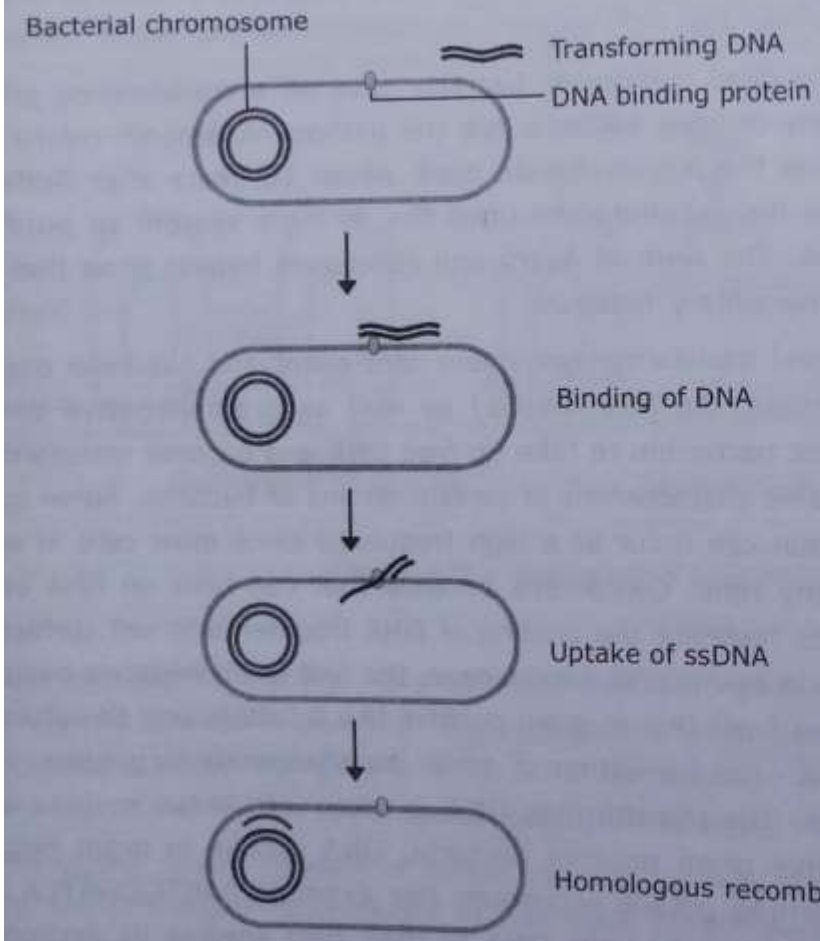


Figure : 5.10
Mechanism of transformation in a bacterium.

Discovery of Transformation

- In 1928, Fred Griffith found that one form of the pathogenic pneumococci *(Streptococcus pneumoniae)* could be mysteriously transformed into another form
- Griffith's experiments were based on the fact that *S. pneumoniae* makes two different types of colonies –

  1. pathogenic smooth colonies
  2. nonpathogenic rough colonies

- Pathogenic strains form smooth colonies on agar plates as they secrete a polysaccharide capsule which allows them to survive in a vertebrate host (mice)
- Rough colony forming strains cannot make capsule and are nonpathogenic in mice
- However, sometimes the rough-colony-forming strains arise from the smooth colony formers in mice

Experimental Protocol

Mice

↓

Rough colony forming bacteria

↓

Mice survived

Mice

↓

Mixture of dead (heat-killed) smooth-colony formers & live rough-colony formers

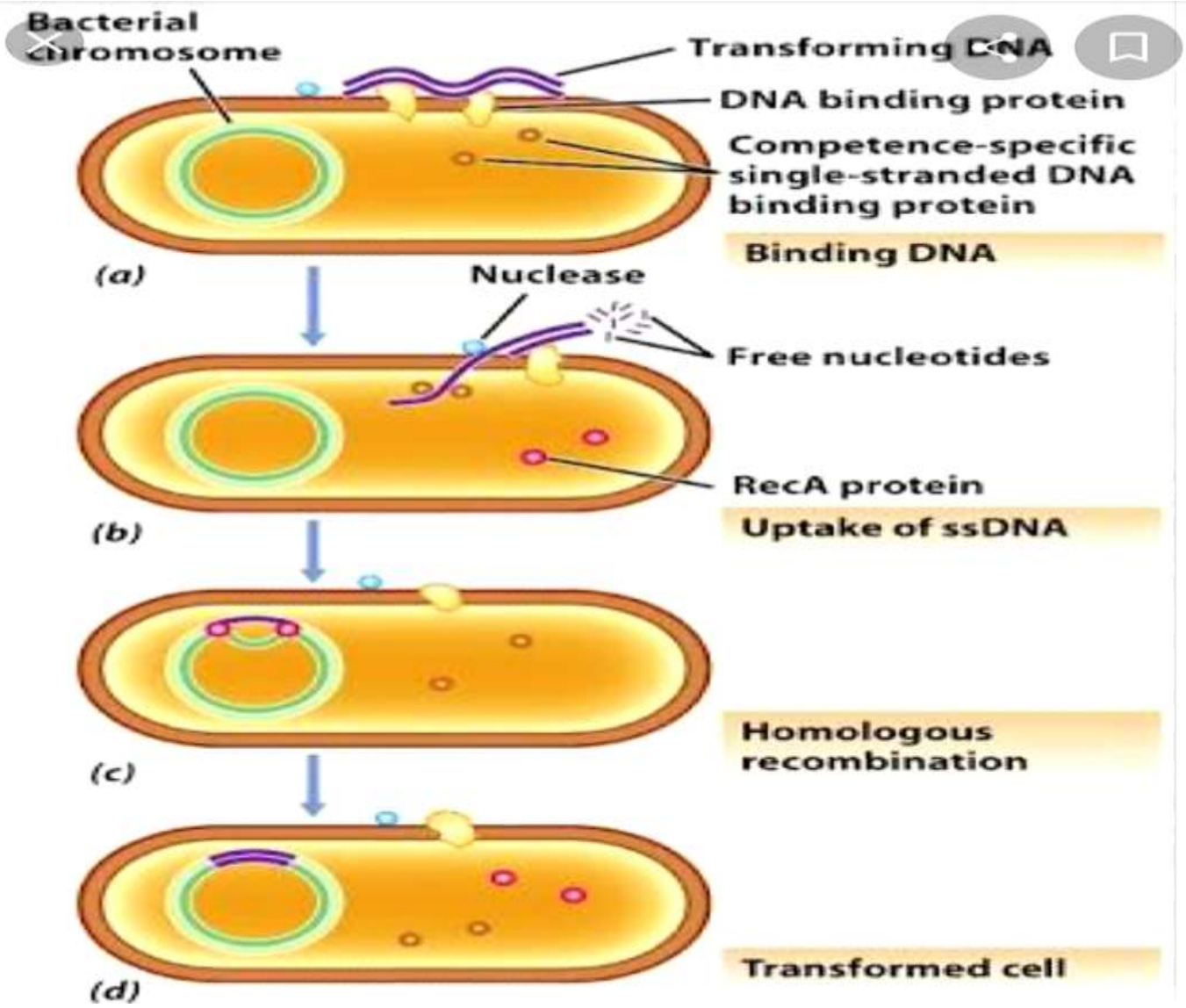
↓

Mice died

- Furthermore, Griffith isolated live smooth-colony-forming bacteria from the blood of the dead mice
- Therefore Griffith concluded that the dead pathogenic bacteria gave off a *transforming principle* that changed the live nonpathogenic rough-colony-forming bacteria into the pathogenic smooth-colony form
- He speculated that this transforming principle was the polysaccharide itself
- About 16 years later, Oswald Avery & his collaborators used the *in vitro* system to purify the *transforming principle* – DNA
- It is the first direct evidence that DNA is the hereditary material, not proteins or other factors in the cell

Competence

- Competence refers to the state in which some bacteria can take up free DNA from their environment
- This capability is genetically programmed
- The process of DNA uptake is called natural transformation
- The length of ssDNA incorporated into the recipient chromosome are about 8.5 – 12 kb, as shown by cotransformation of genetic markers; the incorporation takes only a few minutes to be completed


Bacterial chromosome
Transforming DNA
DNA binding protein
Competence-specific single-stranded DNA binding protein
Binding DNA
(a)
Nuclease
Free nucleotides
RecA protein
Uptake of ssDNA
(b)
Homologous recombination
(c)
Transformed cell
(d)

Competence in Gm +ve bacteria

- 2 well studied Gm +ve bacteria are *Bacillus subtilis* & *Streptococcus pneumoniae*
- The proteins involved in transformation were discovered on the basis of isolation of mutants – lacking completely the ability of DNA uptake
- The affected genes in the mutants were named ***com***, for *competence defective*
- The com genes are organized into several operons
- *comA* & *comK* are regulatory genes
- *comE*, *comF* & *comG* encode structural proteins for uptake

- *comEA* (1st gene of comE operon) encodes the protein that directly binds extracellular dsDNA
- *comF* operon genes encode proteins that translocate the DNA into the cell
- *comG* operon genes encode proteins that provide pore or channel-like structures to allow DNA to move through the thick peptidoglycan cell wall and across the cytoplasmic membrane
- The *comE*, *comF & comG* operons are all under the transcriptional control of ComK protein (a transcriptional factor). ComK protein is regulated by ComA protein

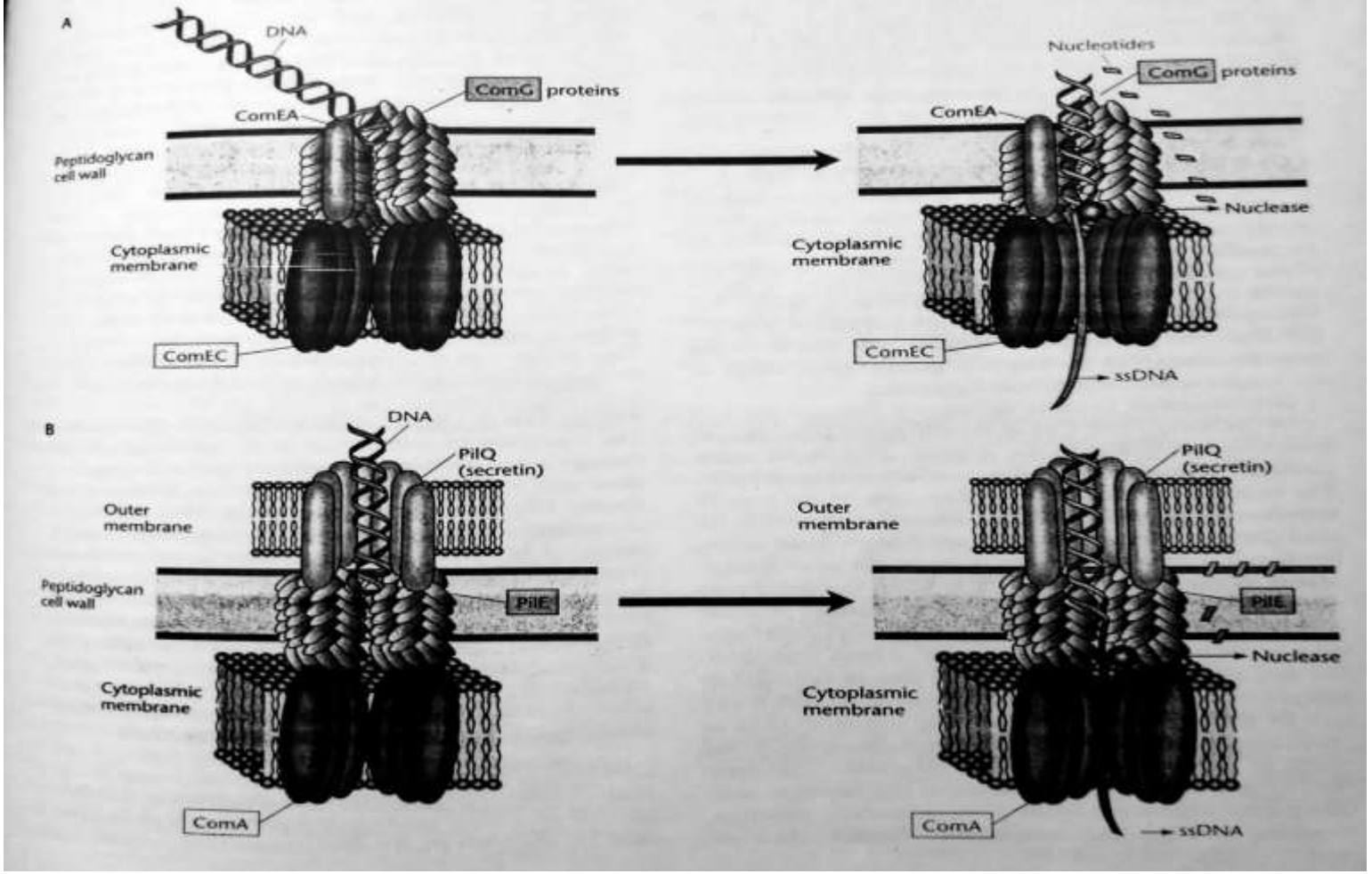
- Other examples of proteins with multiple roles include –

1. *nucA* gene product – nuclease activities make double-stranded breaks in extracellular DNA

2. SSB (single-stranded-DNA binding) proteins –

3. RecA protein – functions in the recombination of transforming DNA with the chromosome

Figure 6.2 Structure of DNA uptake competence systems in gram-positive (A) and gram-negative (B) bacteria. Shown are the proteins involved and the channels they form. The nomenclature in panel A is based on *B. subtilis*, and that in panel B is based on *N. gonorrhoeae*. Some of the *B. subtilis* ComG proteins are analogous to the *Neisseria* PilE protein (shaded boxes). The *B. subtilis* ComEC protein is an orthology of the *Neisseria* ComA protein (unshaded boxes). Ss, single-stranded. Details are given in text. Adapted with permission from D. Dubnau, *Annu. Rev. Microbiol.* **53**:217–244, 1999. Copyright 1999 by Annual Reviews www.annualreviews.org.



Regulation of Competence in *Bacillus subtilis*

- ComP, a sensor protein in the membrane, registered the information that the cell is running out of nutrients & the population is reaching a high density
- High cell density is required for competence in *Bacillus* because of small peptides – competence pheromones – excreted by the bacteria as they multiply
- Cells become competent only in the presence of high concentrations of these peptides which are reached only when the concentration of cells giving them is high

- The high cell density causes ComP to phosphorylate itself
- This phosphate is then transferred to ComA – a response regulator protein, from ComP
- In the phosphorylated state, ComA protein acts as a transcriptional activator of *srfA* operon, required for competence
- Examples of two competence pheromone are *comX* & *comQ* gene products
- Another competence peptide produced by *Bacillus* is – *competence-stimulating factor* (CSF) which is transported into the cell by the oligopeptide permease SpoOK

Competence in Gm -ve bacteria

- They utilize 2 different types of DNA uptake systems –

1. The PSTC transformation pathway – *Haemophilus, Neisseria, Acinetobacter*

2. The type IV Secretion – Related pathway – *Helicobacter pylori*

The PSTC Transformation Pathway

- The term “PSTC” has been applied to some of the proteins indicating their multiple roles in *p*ilus formation, *s*ecretion, *t*witching motility and *c*ometence
- These proteins are related to the ComG protein (*B. subtilis*) & their relatives in *S. pneumoniae*
- These proteins are called **Pil** (rather than Com) because they were discovered on the basis of their involvement in *pilus* formation

- They also function in competence providing structural & translocation functions allowing passage of DNA through the peptidoglycan and cytoplasmic membrane layers
- To overcome an additional obstacle to macromolecular uptake – the outer membrane – Gm –ve bacteria utilize Secretin class of proteins to form pores so as to allow the passage of DNA

The Type IV Secretion – Related Pathway

- Proteins related to conjugation in *Agrobacterium* are utilized for DNA translocation through the membranes and cell walls
- This system can function as a two-way DNA transfer system, moving DNA both into & out of the cell

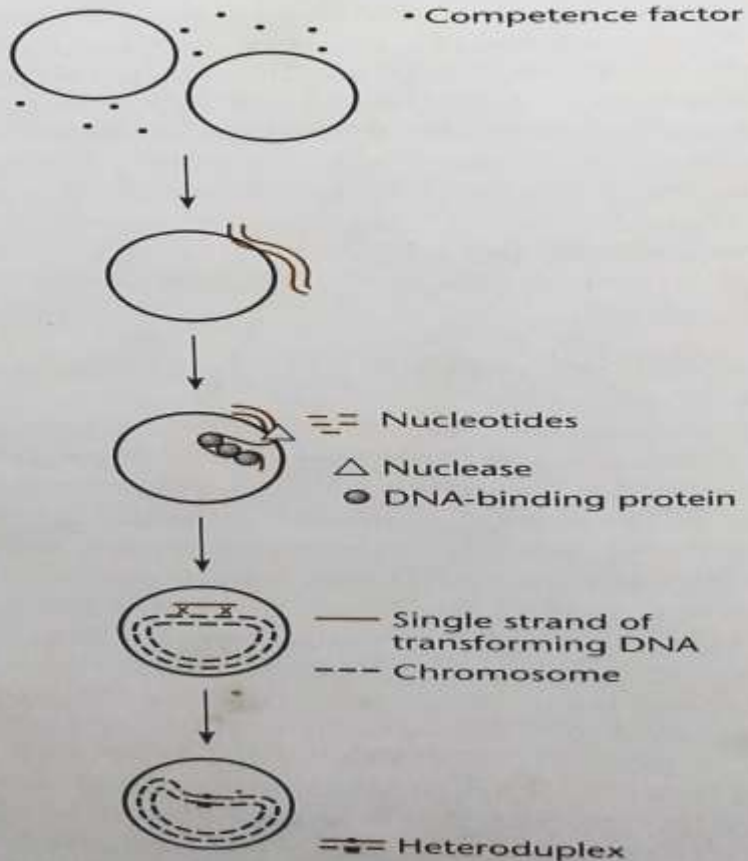
Transformation in *Streptococcus pneumoniae*

1. dsDNA released by lysis of the donor bacteria is bound to specific receptors on the cell surface of the recipient bacterium
2. Competence factors accumulates as the cell reaches a high density (~ 10^7 to 10^8 cells/ml). It also stimulates the production of 8 – 10 new proteins required for transformation
3. The bound DNA is then broken into smaller pieces by endonucleases

4. One of the two complementary strands is degraded by an exonuclease & the remaining strand associates with small proteins & moves through the plasma membrane

5. The transforming DNA integrates into the cellular DNA by replacing the strand of the same sequence (homologous region) in the chromosome, creating a “heteroduplex” in which one strand comes from the donor and one comes from the recipient

Figure 6.6 Transformation in *Streptococcus pneumoniae*. Competence factors accumulate as the cells reach a high density. Double-stranded DNA binds to the cell, and one strand is degraded. The remaining single strand replaces the strand of the same sequence in the chromosome, creating a "heteroduplex" in which one strand comes from the donor and one comes from the recipient.

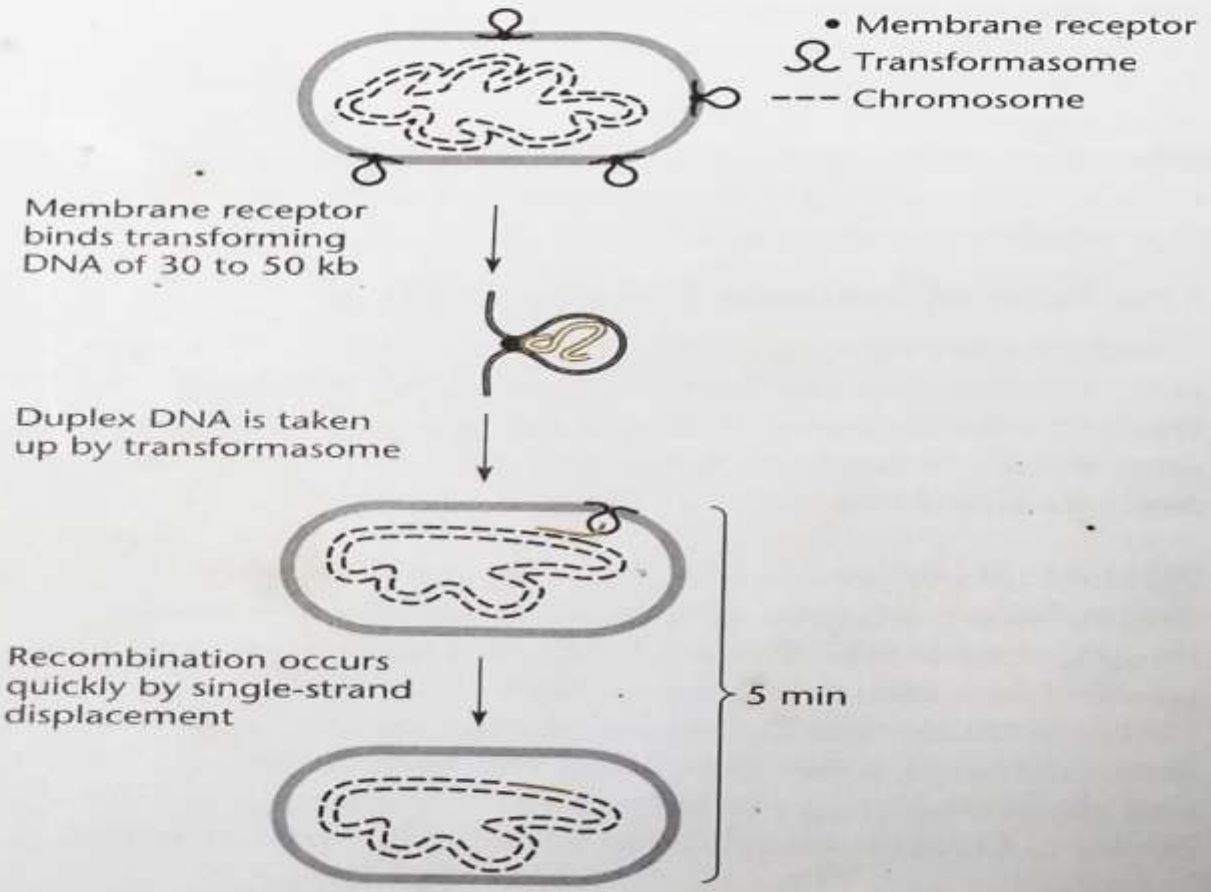


Transformation in *Haemophilus influenzae*

- *Haemophilus* does not produce a competence factor to stimulate the development of competence
- It also takes up DNA from closely related species. The specificity of *Haemophilus* transformation is due to a special 11 bp sequence – 5'AAGTGCGGTCA3'
- This sequence is repeated very frequently in *Haemophilus influenzae.* DNA must have this sequence to be bound by a competent cell

- dsDNA, complexed with proteins, is taken in by membrane vesicles called **transformasomes**
- The new DNA may not become single stranded until it enters the cytoplasm
- Then one strand is degraded & the other strand invades the chromosome, displacing one chromosome strand

Figure 6.7 Transformation in *Haemophilus influenzae*. Double-stranded DNA is first taken up in transformasomes. One strand is degraded, and the other strand invades the chromosome, displacing one chromosome strand.



Plasmid Transformation & Transfection of Naturally Competent Bacteria

- Natural transformation requires breakage of the dsDNA & degradation of one of the two strands so that a linear single strand can enter the cell
- Plasmids and phage DNAs are usually double stranded & must be double stranded to replicate autonomously
- Pieces of ss plasmid or phage DNAs cannot recyclize or make the complementary strand if there are no repeated or complementary sequences at their ends

- Therefore, plasmids or phage DNAs cannot be introduced efficiently into naturally competent cells because they must recyclize to replicate
- Transformation of naturally competent bacteria with plasmid or phage DNA usually occurs only with DNAs that are **dimerized**
- In a dimerized DNA, two copies of the molecule are linked head to tail
- When a dimerized DNA is cut only once, it will have complementary sequences at its ends that can recombine to recyclize the plasmid

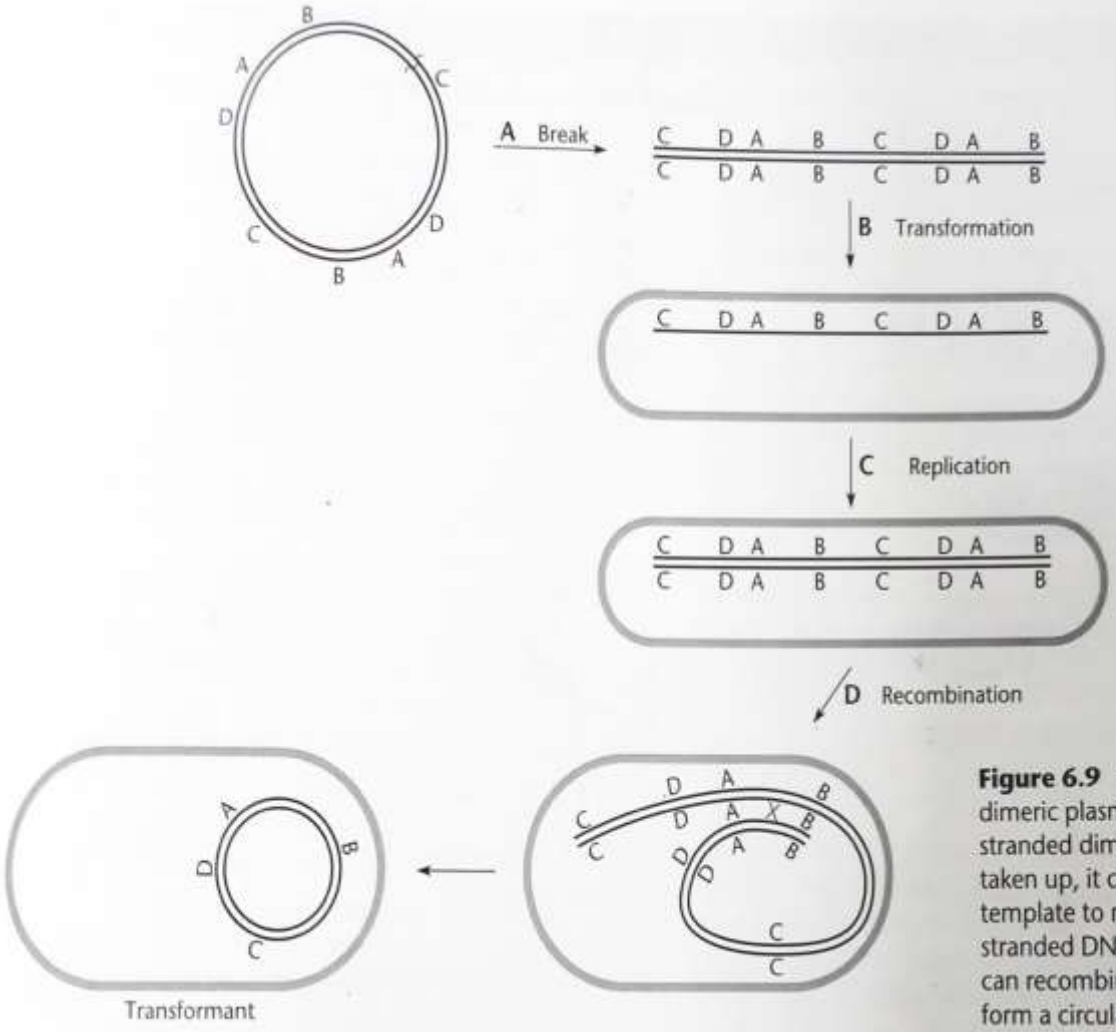


Figure 6.9 Transformation by dimeric plasmids. After the single-stranded dimeric plasmid DNA is taken up, it can serve as a template to make the double-stranded DNA. The repeated ends can recombine with each other to form a circular plasmid.

Transfection

- Viral DNA or genomic RNA can also be introduced into cells by transformation, thereby initiating a viral infection
- When the cell is transformed with viral DNA to initiate an infection, the process is called transfection
- To detect transfection, the potentially transfected cells are usually mixed with indicator bacteria & plated
- If transfection is successful, a plaque will form
- Here, the transfected cells produced phage, which then infected the indicator bacteria resulting in plaque formation

Role of Natural Transformation

1. Nutrition : organisms may take up DNA as a source of carbon & nitrogen

2. Repair : cells may take up DNA from other cells to repair damage to their own DNA

   A region containing a thymine dimer (TT) induced by UV irradiation is replaced by the same, but undamaged, sequence from the DNA of a neighbor killed by the radiation. This mechanism allow survival of the species

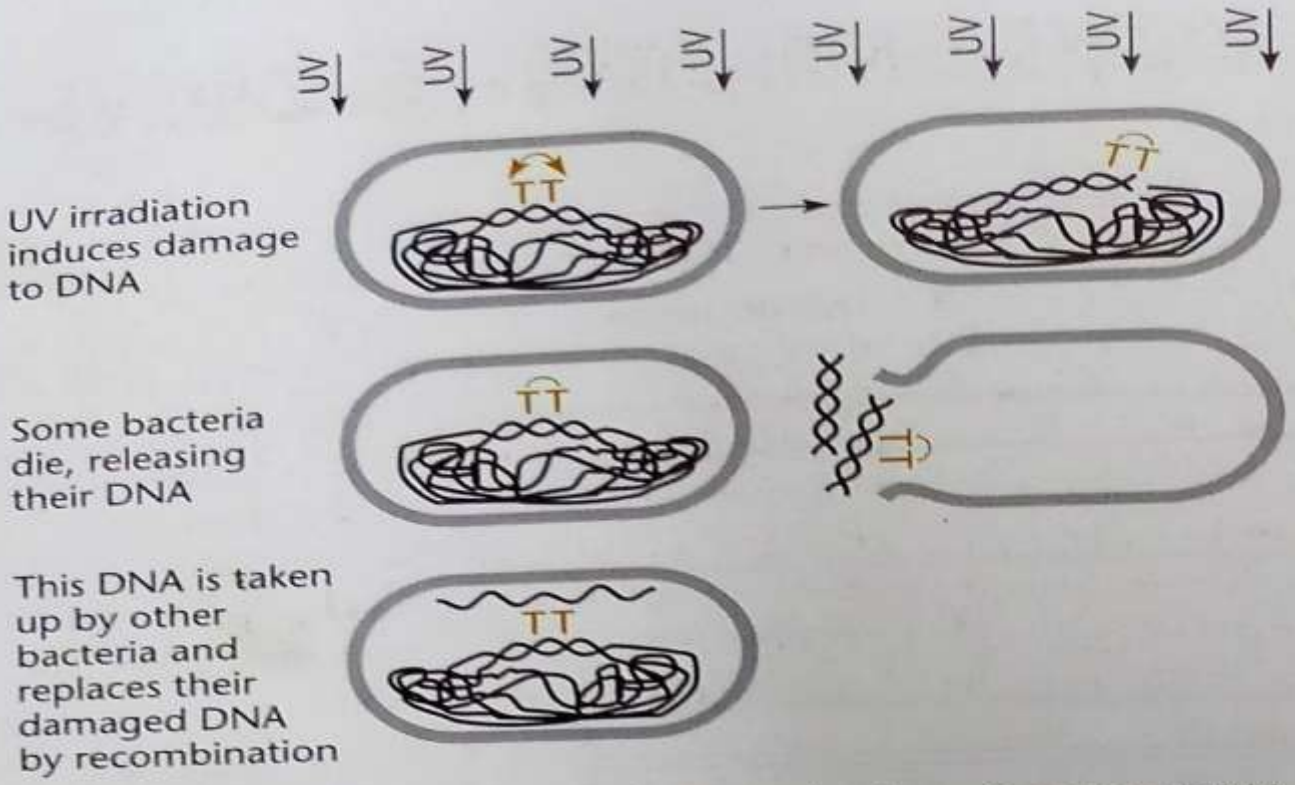


Figure 6.10 Repair of DNA damage by transforming DNA. A region containing a thymine dimer (TT) induced by UV irradiation is replaced by the same, but undamaged, sequence from the DNA of a neighbor killed by the radiation. This mechanism could allow survival of the species.

3. Recombination : due to recombination, transformation is often the best way to reintroduce experimentally altered DNA into cells

   It is also used to map genetic markers in chromosomes

   In *Neisseria gonorrhoeae*, transformation may enhance antigenic variability allowing the organism to avoid the host immune system