



BP 605 T. Pharmaceutical Biotechnology (Theory)

Microbial Genetics

Dr Chandresh Sharma

Assistant Professor

Department of Biotechnology

Chhatrapati Shahu Ji Maharaj University, Kanpur



Overview

Introduction to Microbial genes

Mutations and types

Gene transfer



Microbial Genetics

Glossary:

Strain or clone: A clone is a population of cells that are genetically ideal pure culture.

Genome : All the genes present in a cell.

Phenotype: Collection of characteristics that are observable.

Genotype: Specific set of genes it possess.

Gene: A gene is a nucleotide sequence that code for a polypeptide, tRNA or rRNA. Most bacterial genes have at least four major parts promoters, leaders, coding regions and terminator.

Genetic recombination: Two separate genomes are brought together in one unit.

Mutation: Inherited change in the base sequence of nucleic acid - **alteration in the genetic material.**



Gene Expression

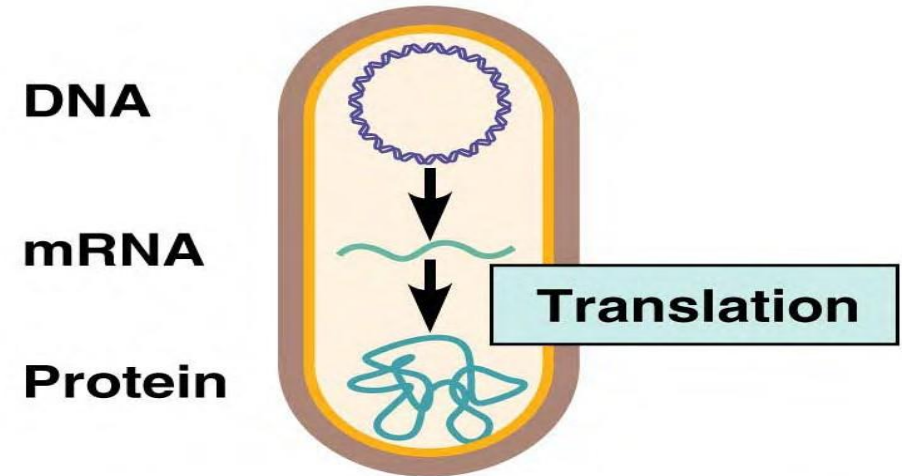
The expression of a gene into a protein occurs by:

1) **Transcription** of a gene into RNA

- produces an RNA copy of the coding region of a gene
- the RNA transcript may be the actual gene product (rRNA, tRNA) or be translated into a polypeptide gene product (mRNA)

2) **Translation** of mRNA transcript into polypeptide

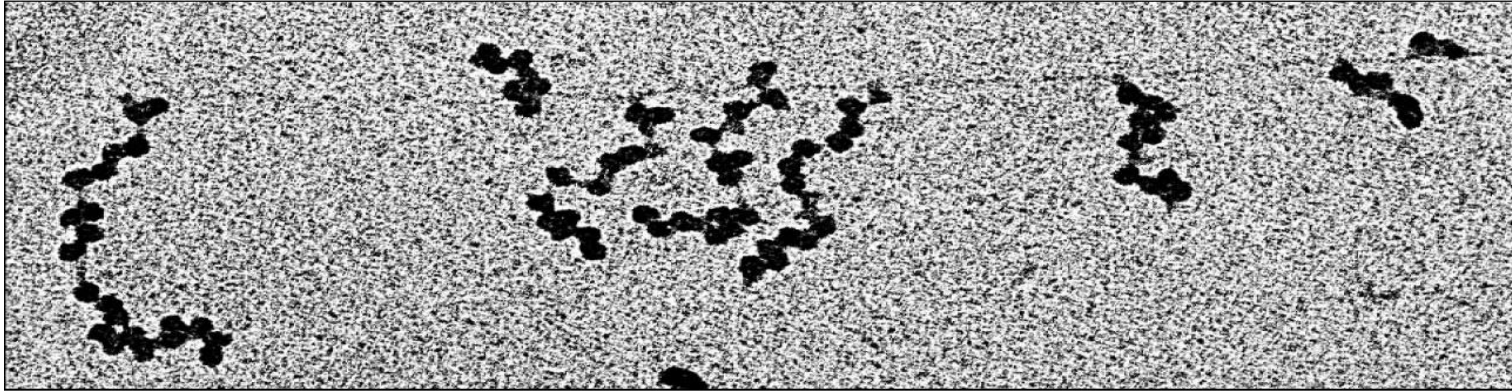
- accomplished by **ribosomes** with the help of tRNA



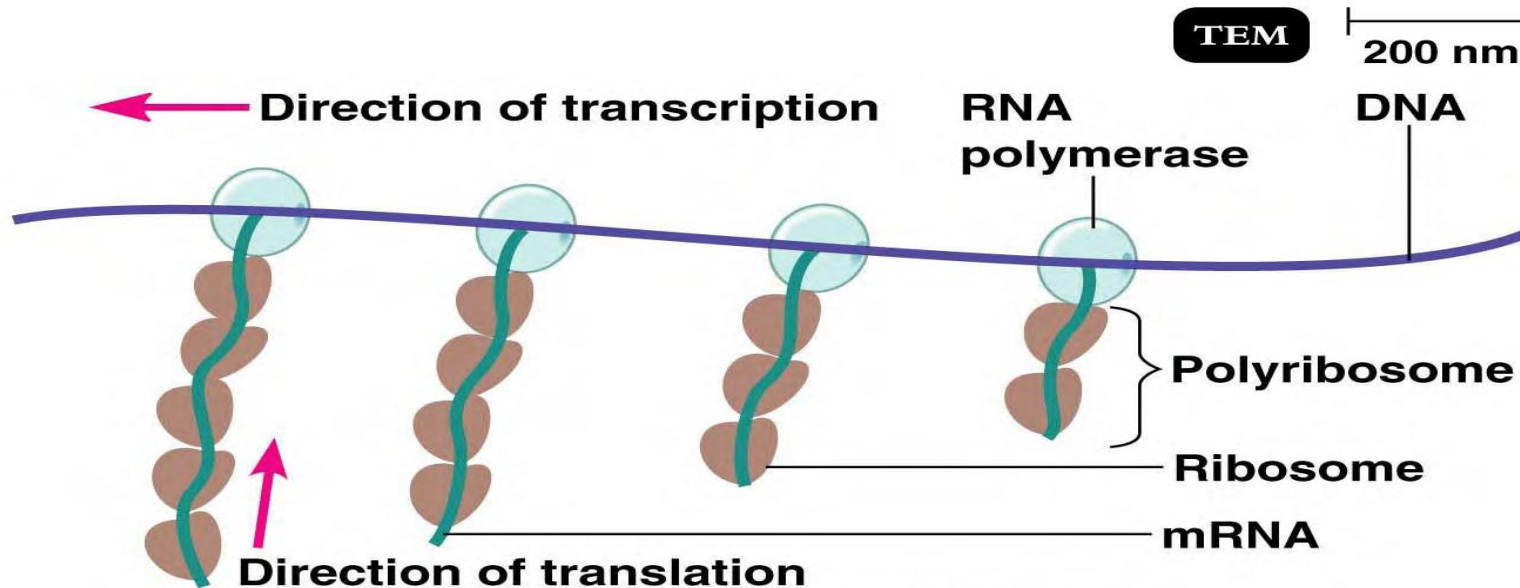
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Gene Expression in Prokaryotes



- gene expression is not necessarily “segregated”

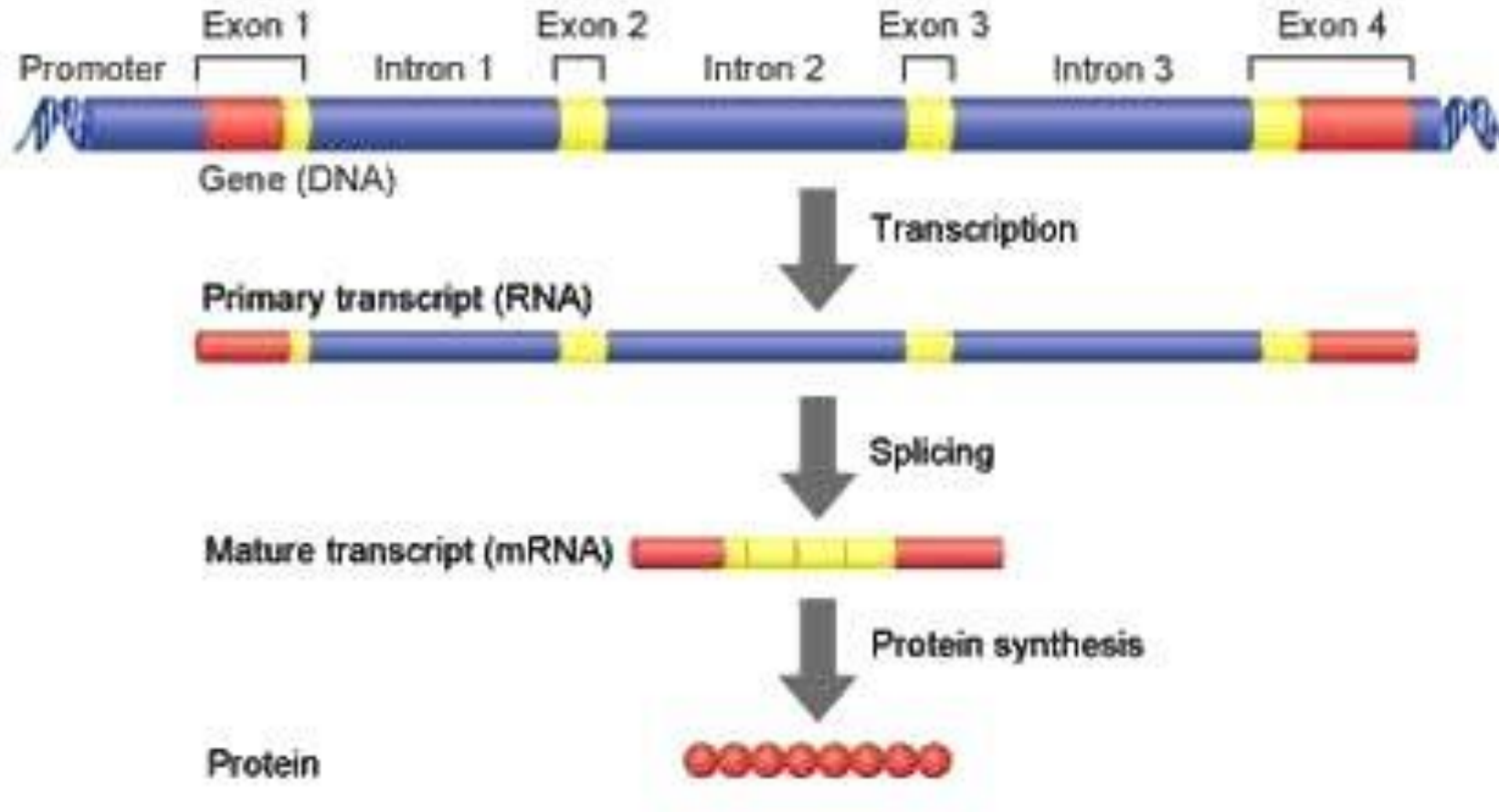


- transcription & translation can occur simultaneously



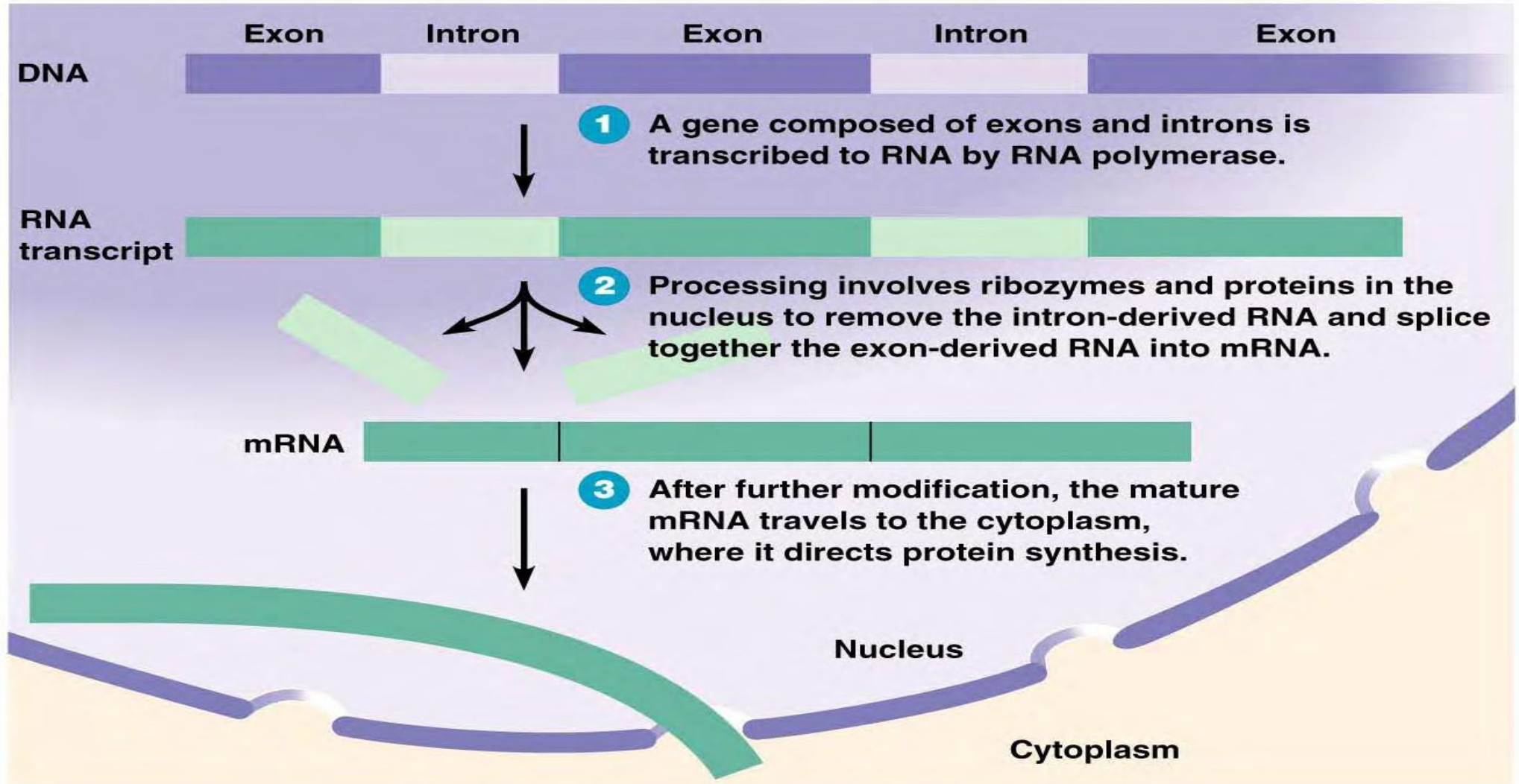
Overview of Gene expression

Structure of a Gene



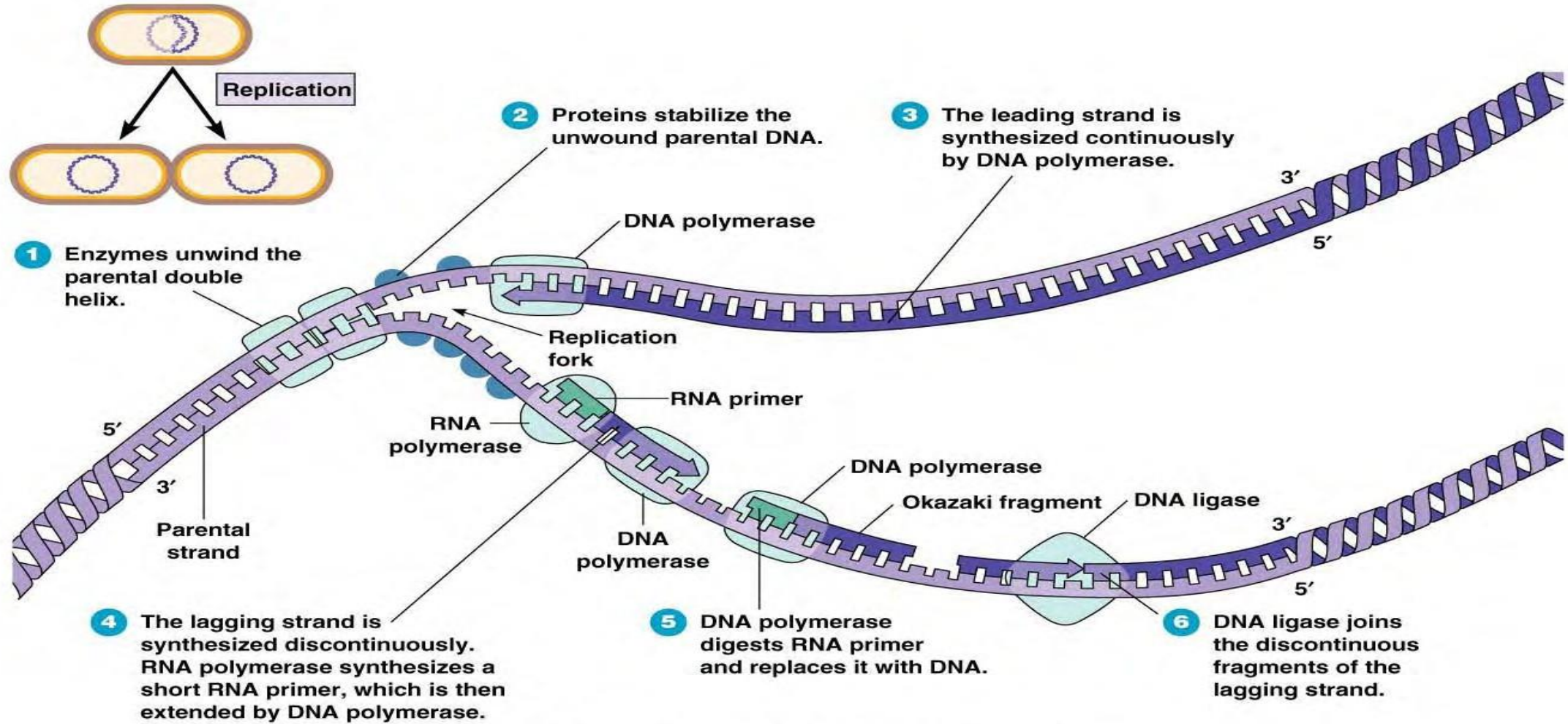


Splicing of Eukaryotic Transcripts





DNA Replication





Features of DNA Replication

Both strands serve as a template:

- synthesis is always 5'-3'
- *leading* strand synthesis is continuous,
lagging strand synthesis is discontinuous

Each new DNA fragment requires an RNA primer:

- DNA synthesis cannot begin without a primer to add to

Some important enzymes:

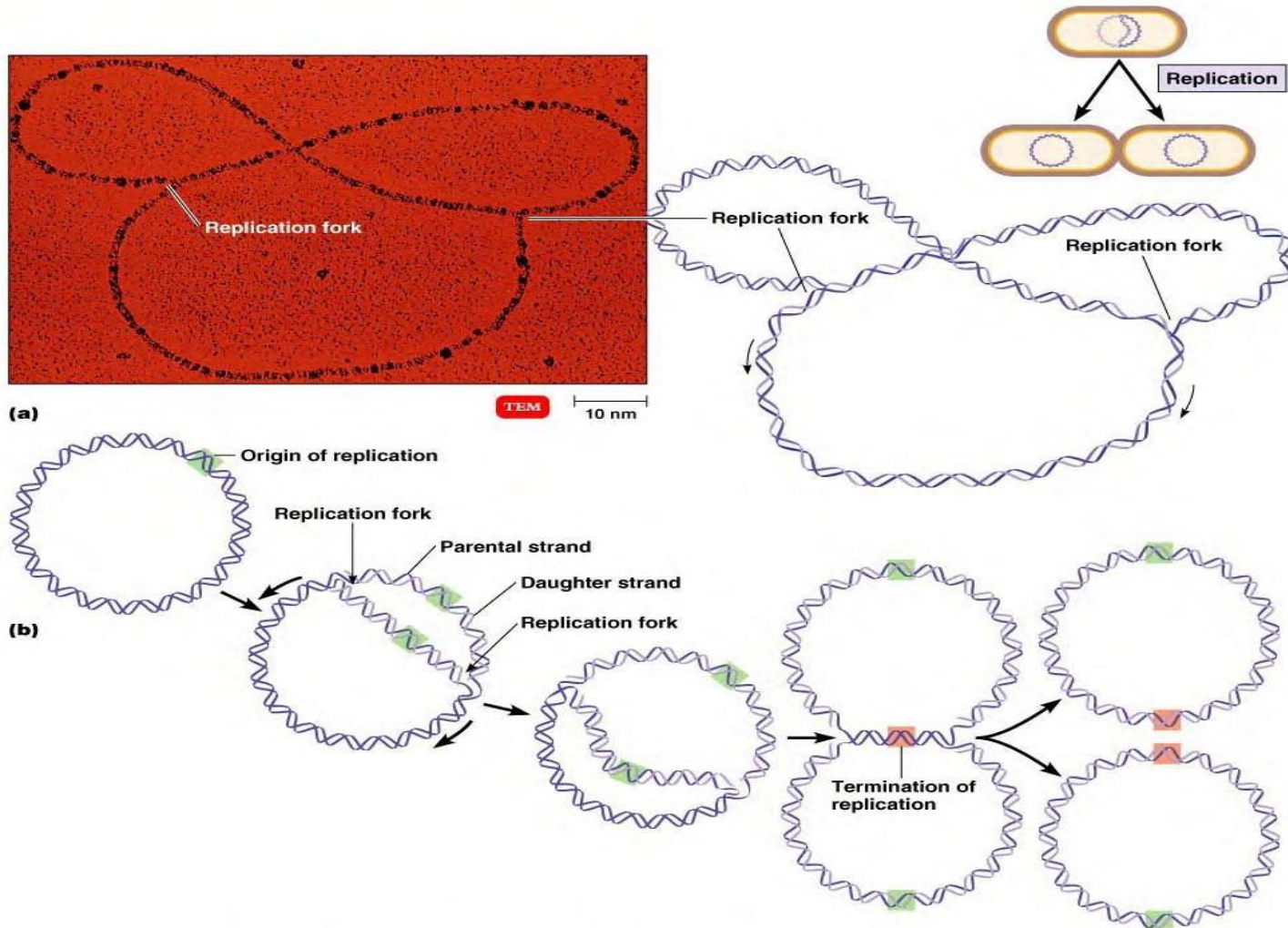
DNA Polymerase (synthesizes new DNA)

Primase (makes RNA primers)

DNA Ligase (“stitches” fragments together)



DNA Replication in Prokaryotes



- begins at the origin of replication (OriC)

- can only be completed if DNA is circular



Mutations

A mutation is *any* change in DNA sequence:

- change of one nucleotide to another
- insertion or deletion of nucleotides or DNA fragments
- inversion or recombination of DNA fragments

What causes mutations?

- errors in DNA replication, DNA repair
- chemical mutagenesis
- high energy electromagnetic radiation
 - UV light, X-rays, gamma rays



Mutation

- ✓ **Mutation** is an inherited change in the base sequence of the nucleic acid comprising the genome of an organism .
- ✓ A strain carrying such changes is called as **mutant**.
- ✓ A **mutant may differ** from its parent strain in genotype (sequence of nucleotides in the DNA of the genome) and sometimes in phenotype (observable properties from its parent) also.
- ✓ A **nutritional mutant** that has a requirement for a growth factor is called an **auxotroph** and the wild-type parent from which the auxotroph was derived is called a **prototroph**.



Mutation

- Mutation can be either **spontaneous or induced**.
- Spontaneous mutation occurs naturally (natural radiation or due to error in pairing of bases during replication).
- Mutation involving one or a very few base pairs are referred to as **point mutations**.
- Mutation involving change in base pairs without causing change in the amino acid that code for is called **silent mutation**. (For eg. Change in **UAC to UAU** would not account for change as both code for tyrosine).



Mutation

- Mutation involving change in base pair which codes for a different amino acid is called **missense mutation**. Eg. (UAC - Tyrosin; AAC- asparagine).
- Some times a mutation may result in premature termination of translation (as the base pair alteration contribute to stop codon TAG - UAG (stop codon) resulting in incomplete protein – such is called **non-sense mutation**.



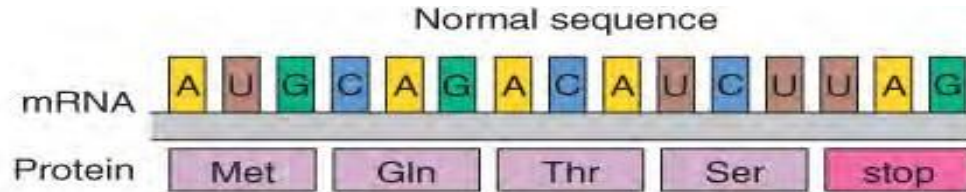
Mutation

	No mutation	Point mutations			
		Silent	Nonsense	Missense	
				conservative	non-conservative
DNA level	TTC	TTT	ATC	TCC	TGC
mRNA level	AAG	AAA	UAG	AGG	ACG
protein level	Lys	Lys	STOP	Arg	Thr

- ✓ Agents that induce mutations are called **mutagens** which may be chemical or physical agents. Eg.
- ✓ **Chemical mutagens** – Nitrous acid (HNO_3), Hydroxylamine (NH_2OH), alkylating agents.
- ✓ Physical mutagens – UV and ionizing radiation (x-rays)

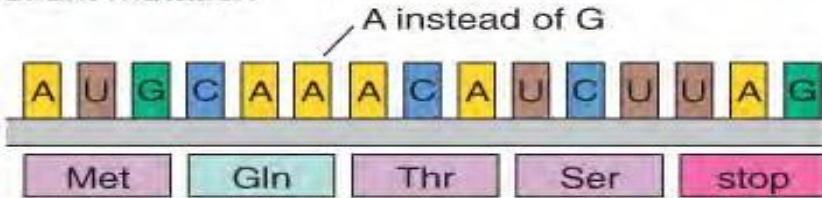


Effects of Mutations

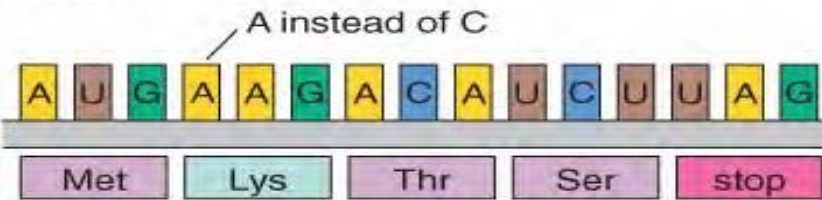


(a) Base-pair substitutions

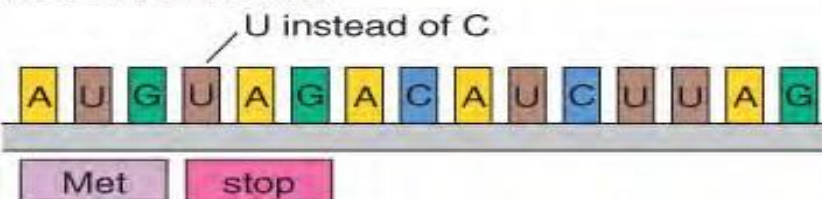
Silent mutation



Missense mutation

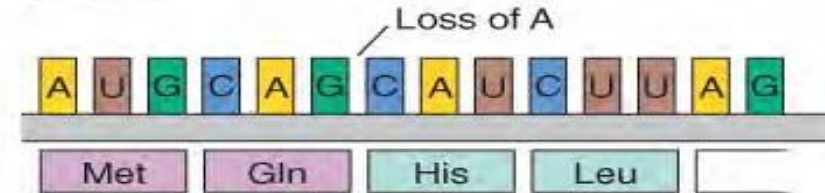


Nonsense mutation

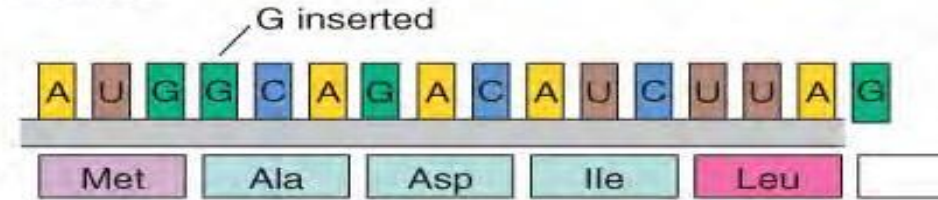


(b) Deletion/insertion

Deletion



Insertion



***insertions & deletions can cause "frame shifts"**



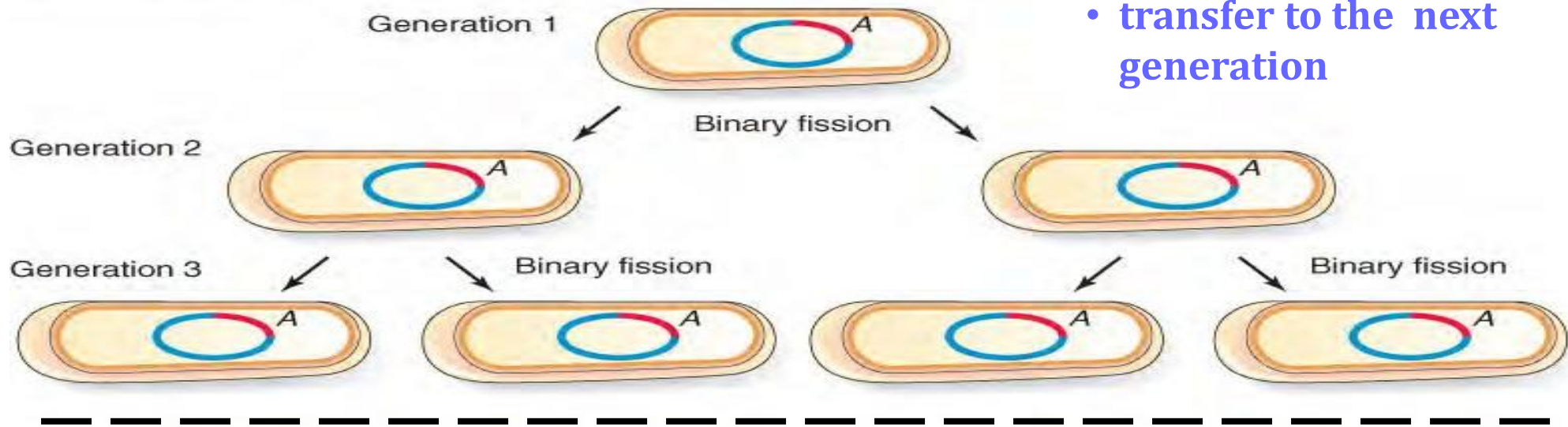
Genetic recombination or Gene Transfer in Bacteria

- ✓ In prokaryotes, genetic recombination occurs because **fragments of homologous DNA from a donor chromosome are transferred to a recipient cell** by any of the three following processes.
- **Transformation** – Transfer of bacterial genes involving free DNA.
 - **Transduction** – Transfer of host genes from one cell to another mediated by a virus.
 - **Conjugation** – Transfer of genes from one cell to another involving cell to cell contact and a plasmid.

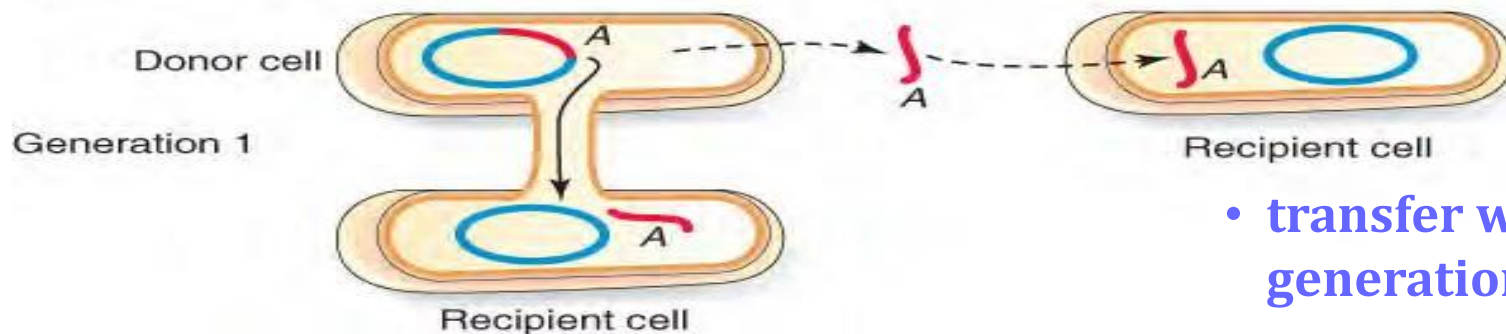


Horizontal vs Vertical Gene Transfer

Vertical



Horizontal (or lateral)





Transformation

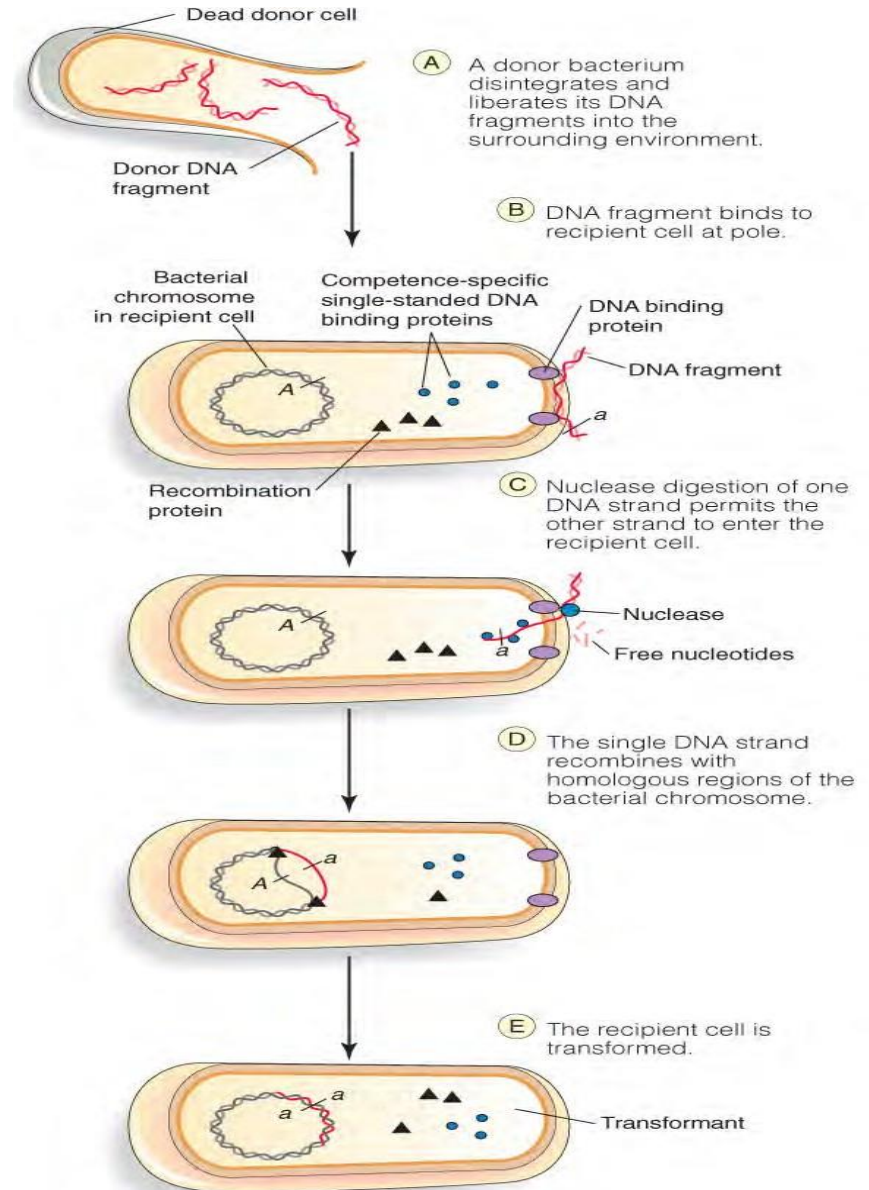
- A cell that is able to take up a molecule of DNA and be transferred is called **competent cell**.
- Bacteria differ in the form in which DNA is taken up. In **Gram negative bacteria** (eg. *Haemophilus*) only DS DNA is taken up into the cell, however only SS – DNA segment is incorporated into the genome.
- In **Gram positive bacteria** (*Streptococcus* sp. and *Bacillus*) only SS – DNA is taken up.



Transformation

Under the right conditions, bacteria can “take in” external DNA fragments (or plasmids) by **transformation**.

- DNA binding proteins transfer external DNA across cell envelope
- homologous recombination can then occur
- bacterial cells capable of transformation are referred to as **competent**



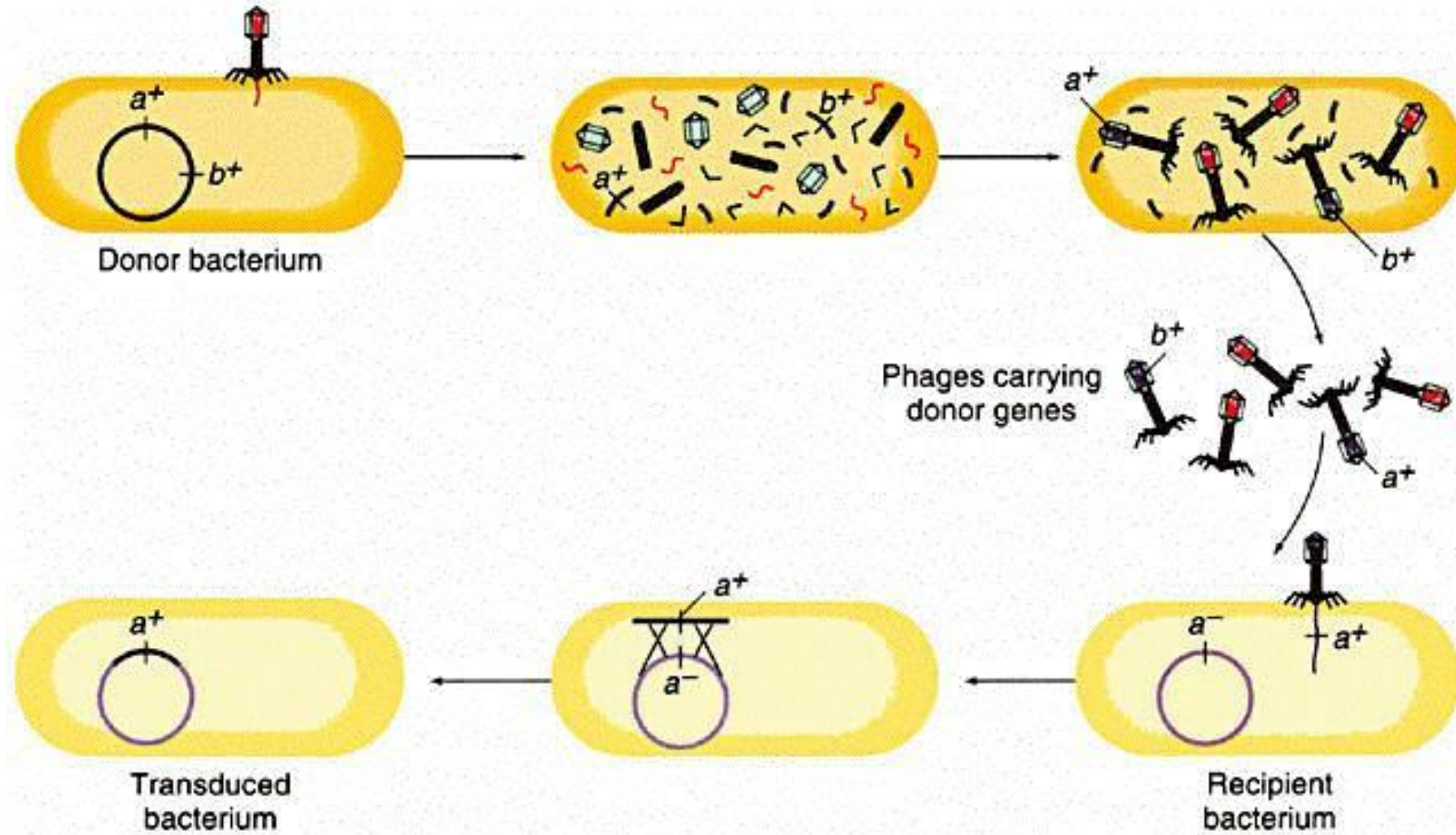


Transduction

- **Not all phages can transduce and not all bacteria are transducible.**
- In **generalized transduction** host DNA derived from any portion of host genome becomes a part of the DNA of the mature virus particle in place of the virus genome, which gets integrated into another cell upon entry.
- In **specialized transduction**, when a lysogenized cell reverts to lytic cycle, a part of host DNA is exchanged for phage DNA, which replicates and forms phage, which when transduced, the new gene gets into another cell.



Bacterial Transduction





Conjugation

- **Conjugation or mating** – involves the transfer of DNA from a donor to a recipient by cell to cell contact through the F (Fertility) pilus, followed by recombination within the recipient bacterial cell.
- **Pili** are involved in **attachment processes**.
- **F pili** specifically join mating bacteria. When an F pilus joins with the mate, there is a **change in plasma membrane permeability** so that DNA can move from one cell to another.
- Bacteria that produce F pili are **donors** and are designated F⁺ strains.

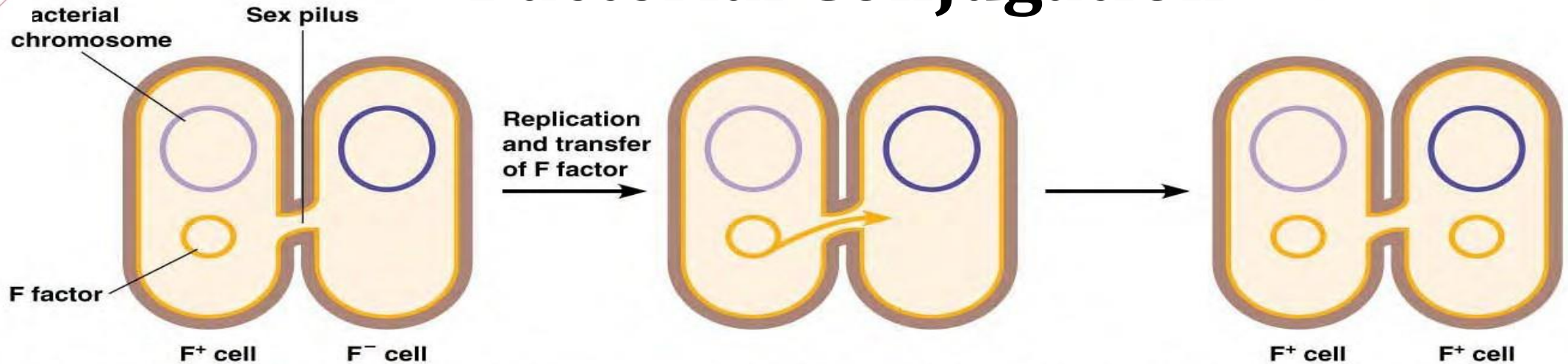


Conjugation

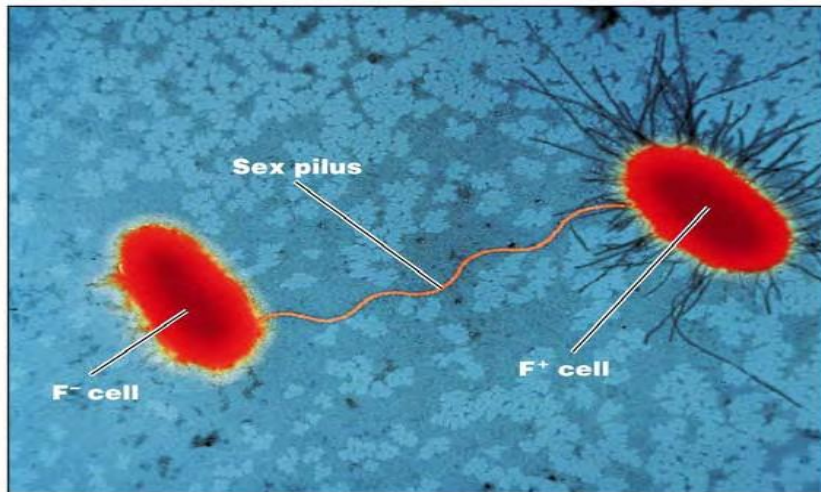
- ✓ **During mating**, a single strand of donor DNA is replicated, and this copy is transferred to the recipient where the complimentary strand is synthesized.
- ✓ Bacteria are designated **Hfr** (high frequency recombinant) if the F plasmid DNA is incorporated into the bacterial chromosome.
- ✓ **Bacteria lacking F pili** are recipient strains and are designated **F⁻ strains**. When F⁺ cell mates with F⁻ cell, the F plasmid DNA is copied and transferred from donor to the recipient. This **results in F⁺ strains**.
- ✓ The F plasmid confers the genetic information for acting as a donor strain.



Bacterial Conjugation



(a) When an F factor (a plasmid) is transferred from a donor (F^+) to a recipient (F^-), the F^- cell is converted into an F^+ cell.



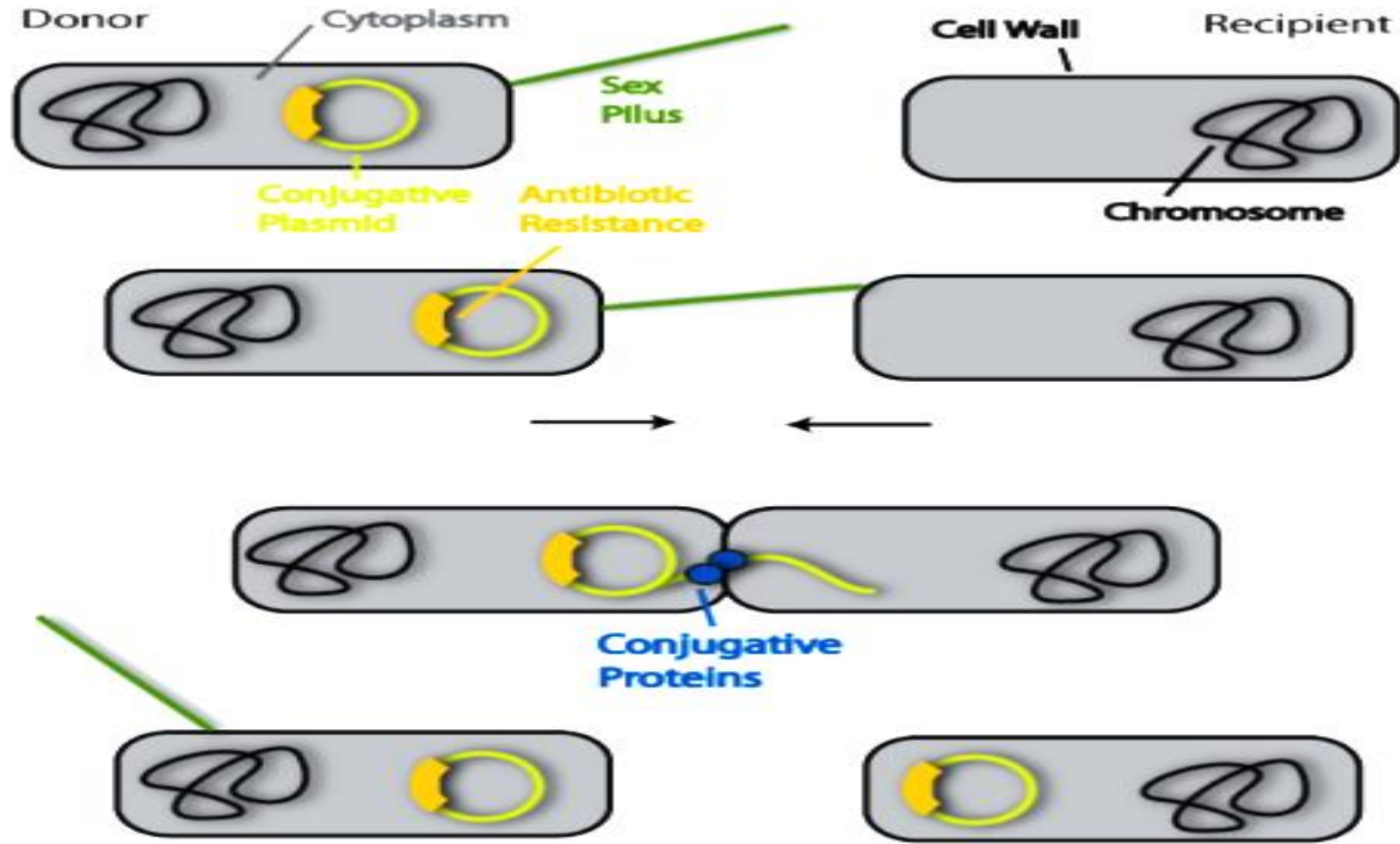
TEM 1 μm

Requires an F factor plasmid

- has all “conjugation genes”
- directs formation of a sex pilus
- single DNA strand produced by DNA replication is transferred to F- cell through the sex pilus, recipient produces 2nd strand



Bacterial conjugation



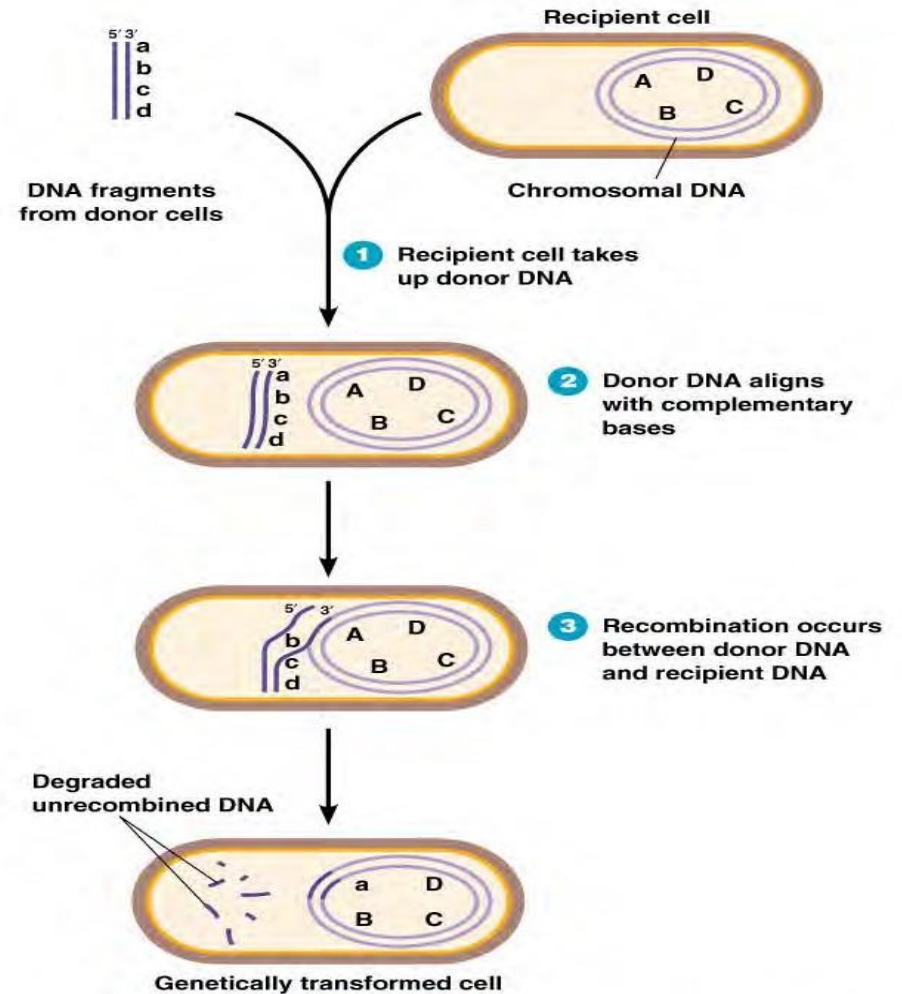


Homologous Recombination

Unless transferred DNA is circular w/Ori (plasmid), it must recombine with host DNA to be retained

Recombination can occur between *homologous* (similar) DNA sequences:

- DNA with “same” genes
- facilitated by special proteins
- original DNA is lost





Plasmid

- Some bacterial cells contain one or more small circular macromolecules of DNA that store additional specialized information. These are called **plasmids (extra chromosomal DNA)**.
- Plasmids contain only **1 - 5%** as much DNA as in the bacterial chromosome (roughly **about 20 genes**) which supplement the essential genetic information contained in the bacterial chromosome.





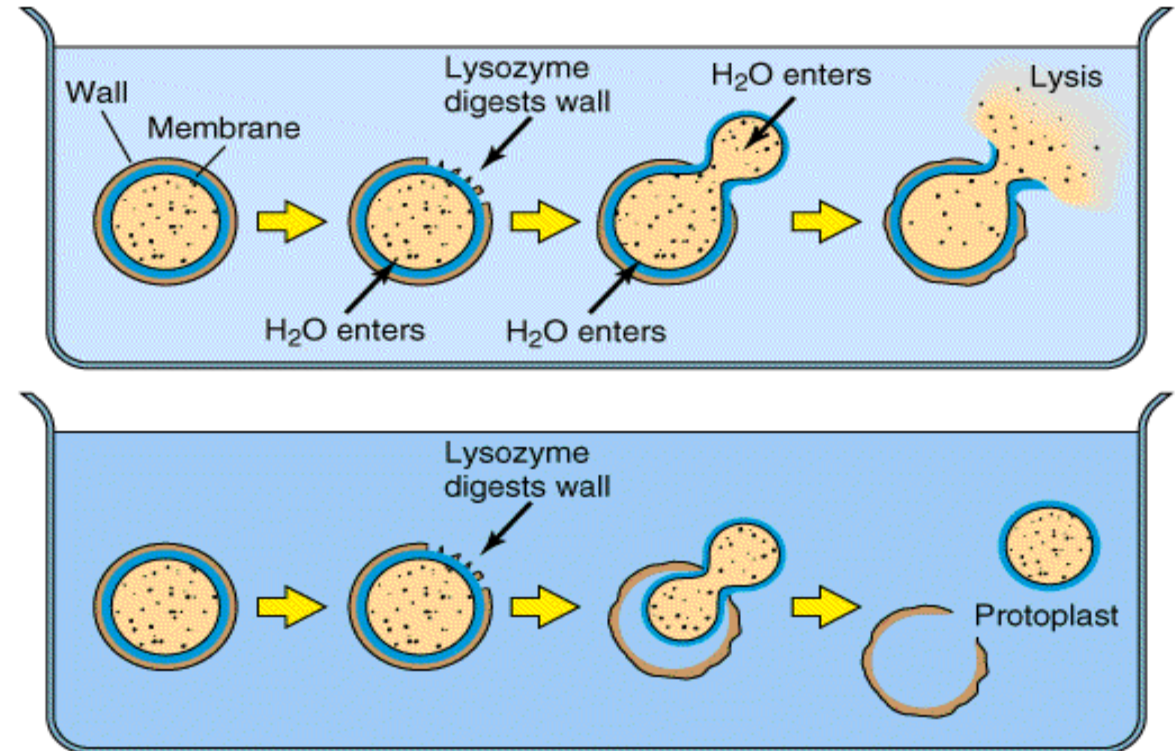
Plasmid

- **Genetic information contained in plasmids can be important**, in establishing characters such as **resistance to antibiotics and tolerance to heavy metals**.
- Thus the **gene products of plasmids may permit the survival of bacteria** under conditions that are normally unfavourable for growth and survival.
- Plasmids can be transferred from one bacterial cell to another, sometimes even from one bacterial species to another.



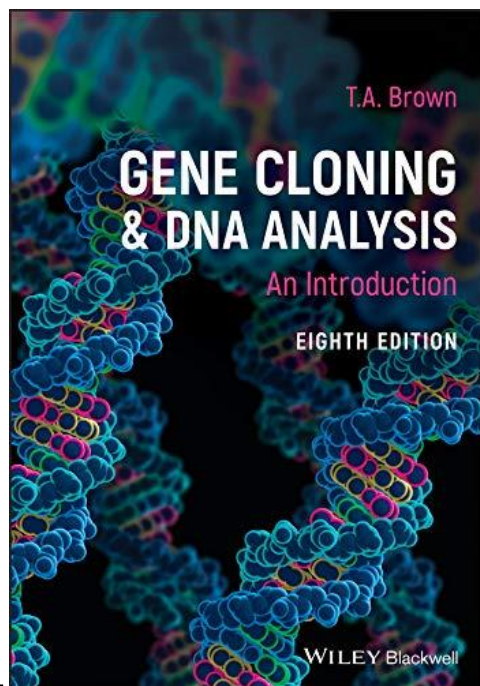
Protoplasts and Spheroplasts

- ✓ When the peptidoglycan layer of the cell wall is digested with lysozyme or when its synthesis is blocked, the cell ordinarily lyses.
- ✓ However, in a hypertonic medium (eg. 20% of sucrose or 0.5M KCl), the **cell survives as an osmotically sensitive sphere**.
- ✓ With gram-positive organisms this product is free of wall constituents and is called a **protoplast**.
- ✓ With gram negative bacteria, these osmotically sensitive spheres retain much of the outer membrane and are called **spheroplasts**.





Acknowledgement

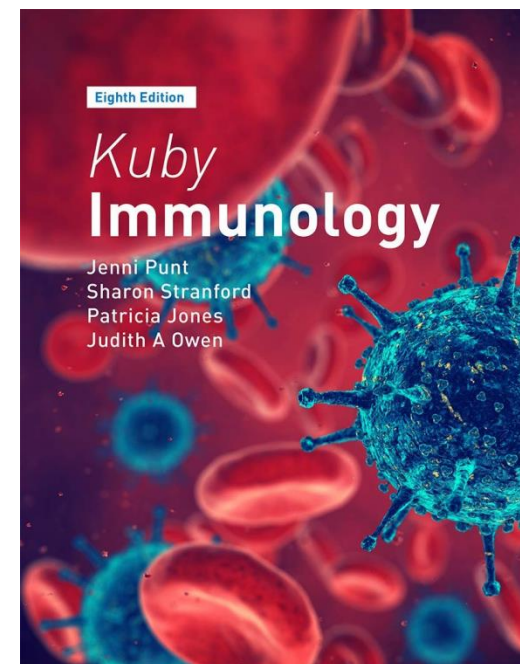


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Gary Walsh
University of Limerick, Republic of Ireland

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For Query

chandreshsharma@csjmu.ac.in; sharmac3001@gmail.com