2^{nd} year Medical Students - JU

Bacterial genetics

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Bacterial genetics

ILOs:

- bacterial genome and replication
- Mutations and effects on bacteria
- Genetic exchange and role in virulence
- Transposons definition and significance

The bacterial genome:

- Chromosome (a must)
- Plasmids
- Bacteriophage
- Transposons

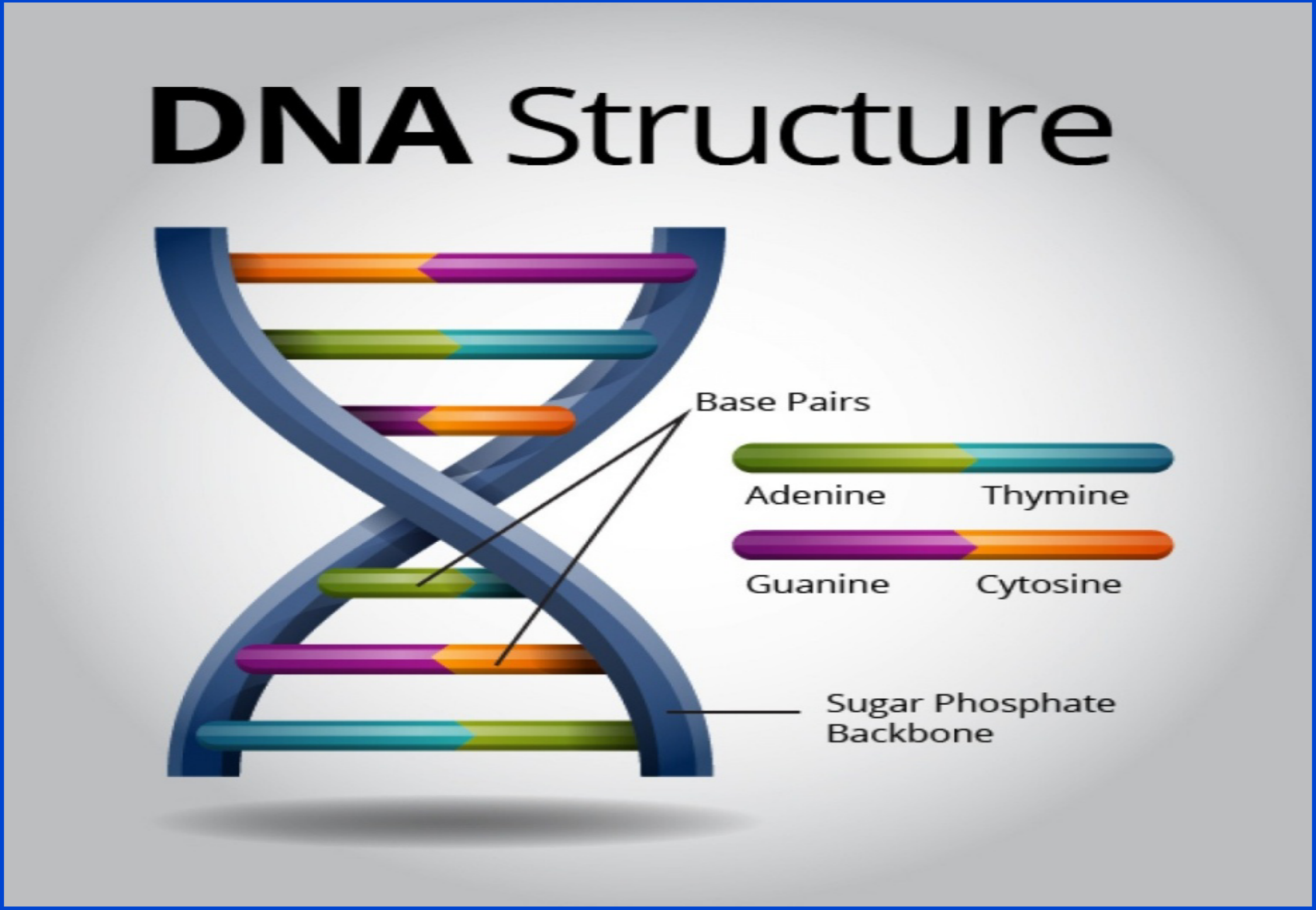
Bacterial Genetics

Significance of bacterial genetics?

- ✔ Structure, Pathogenicity and virulence
- ✔ Antibiotic resistance
- ✔ Toxins
- ✔ Enzymes
- ✔ Molecular cloning

Bacterial genetics

- **Bacterial Genome**: **Chromosome,** single circular double-stranded DNA.



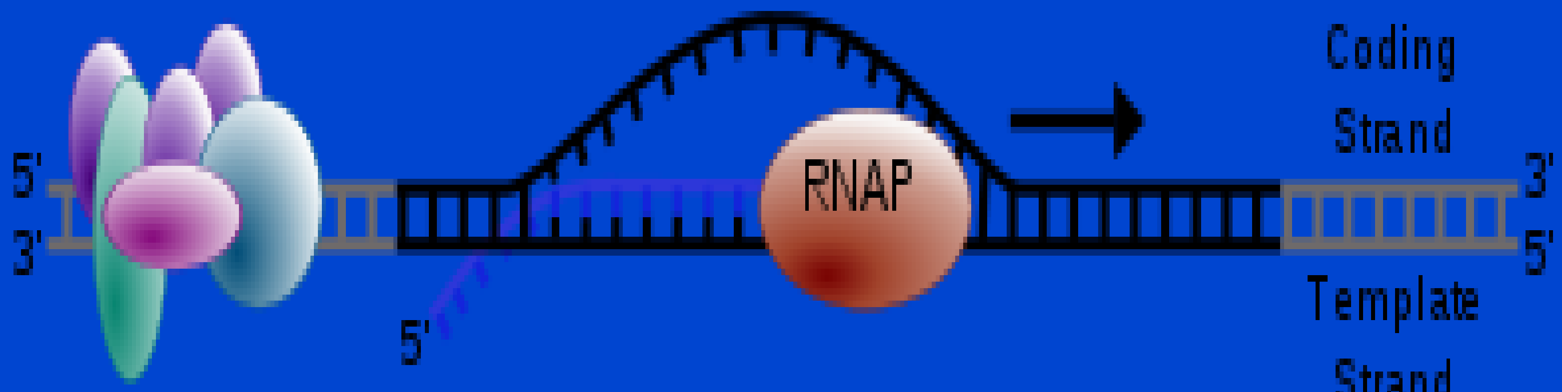
Gene expression

- is the process by which information from a gene is used in the synthesis of a functional gene product. These products are often proteins, but in non-protein coding genes such as transfer RNA (tRNA) or small nuclear RNA (snRNA) genes, the product is a functional RNA

- Several steps in the gene expression process may be modulated, including the transcription, RNA splicing, translation, and post-translational modification of a protein. Gene regulation gives the cell control over structure and function, and is the basis for cellular differentiation, morphogenesis and the versatility and adaptability of any organism.

- In genetics, gene expression is the most fundamental level at which the genotype gives rise to the phenotype, i.e. observable trait.

Transcription



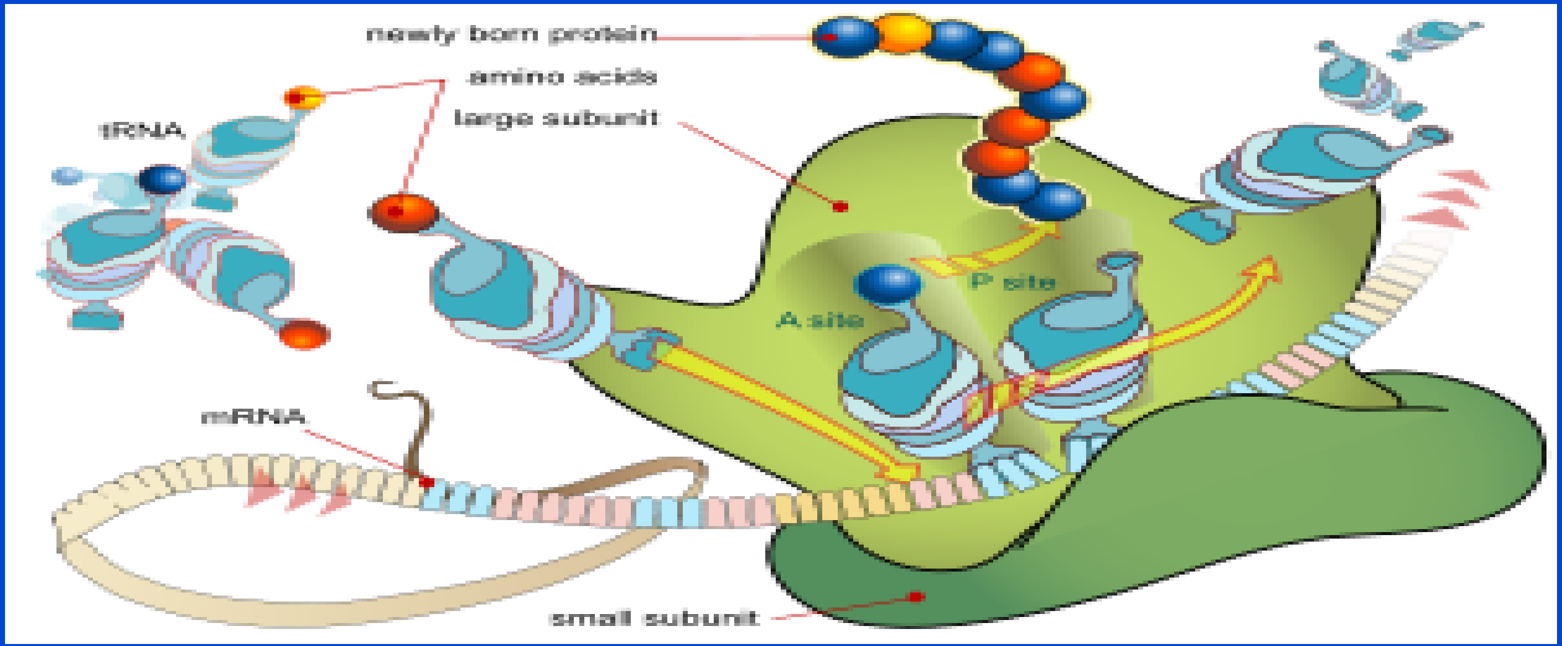
The process of transcription is carried out by RNA polymerase (RNAP), which uses DNA (black) as a template and produces RNA (blue).

RNA processing

- While transcription of prokaryotic protein-coding genes creates messenger RNA (mRNA) that is ready for translation into protein, transcription of eukaryotic genes leaves a primary transcript of RNA (pre-mRNA), which first has to undergo a series of modifications to become a mature mRNA.

Translation

- Is the process of translating the sequence of a messenger RNA (mRNA) molecule to a sequence of amino acids during protein synthesis. The genetic code describes the relationship between the sequence of base pairs in a gene and the corresponding amino acid sequence that it encodes.



During the translation, tRNA charged with amino acid enters the ribosome and aligns with the correct mRNA triplet. Ribosome then adds amino acid to growing protein chain.

In prokaryotes translation generally occurs at the point of transcription (co-transcriptionally), often using a messenger RNA that is still in the process of being created.

Gene regulation in bacteria

- Bacterial genes are often found in operons (specific regulatory molecules that control whether a particular gene will be transcribed into mRNA. Often, these molecules act by binding to DNA near the gene and helping or blocking the transcription enzyme, RNA polymerase

Genes in an operon are transcribed as a group and have a single promoter. Each operon contains regulatory DNA sequences, which act as binding sites for regulatory proteins that promote or inhibit transcription.

Regulatory proteins often bind to small molecules, which can make the protein active or inactive by changing its ability to bind DNA.

Some operons are inducible, meaning that they can be turned on by the presence of a particular small molecule. Others are repressible, meaning that they are on by default but can be turned off by a small molecule.

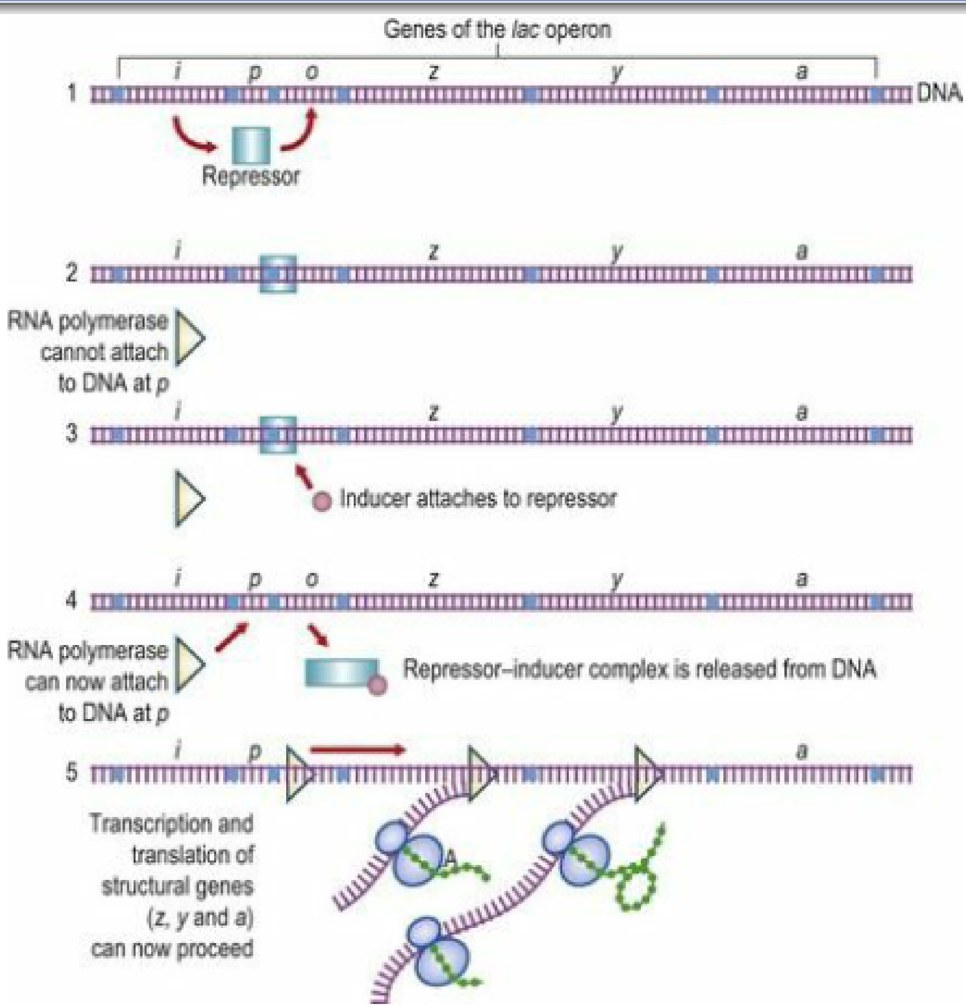
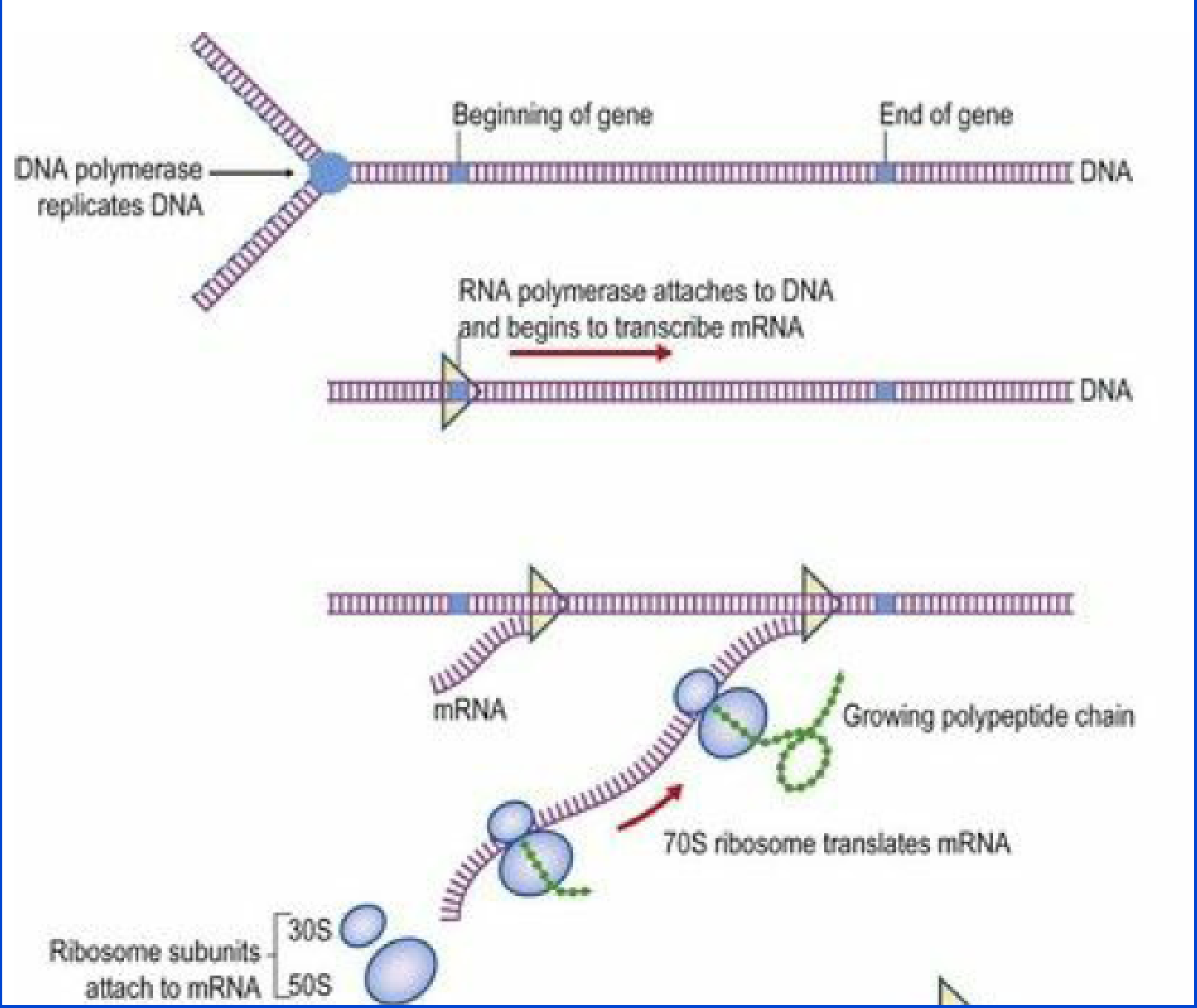


Fig. 6.2 The *lac* operon of *Escherichia coli*. (1) The *lac* repressor is produced from the *i* gene. (2) Binding of the repressor to the operator site (*o*) prevents transcription of the genes *z* (β-galactosidase), *y* (galactoside permease) and *a* (transacetylase). (3) The inducer (lactose, or a closely related derivative) can bind specifically to the repressor. (4) The repressor molecule is thereby altered at its operator-binding site and the repressor–inducer complex is released from the DNA. (5) RNA polymerase can now attach to the promoter site (*p*) and transcribe the structural genes of the *lac*


Beginning of gene
End of gene
DNA polymerase
replicates DNA
DNA
RNA polymerase attaches to DNA
and begins to transcribe mRNA
DNA
mRNA
Growing polypeptide chain
70S ribosome translates mRNA
30S
Ribosome subunits
attach to mRNA
50S

- **Genotype / Wild Type :** Represents all potential genes of bacteria cell.. Its genome.. All Inherited essential biological features & growth patterns.

- **Phenotype:** The expressed genes..The observed characteristics of the of the individual bacteria species/strain. Expressed by physical & biochemical properties. Growth patterns, Fermentation products, Antibiotic resistance, Toxins production. .etc.

• 1. Mutation:

- **inaccuracies during DNA replication lead to change in the base sequence of DNA that may result in insertion of amino acid into a protein and phenotypic alteration**

- **Spontaneous or induced**

- **Spontaneous:**

a. Happens every 10^4-10^9 cell division (1 colony is 10^{10})

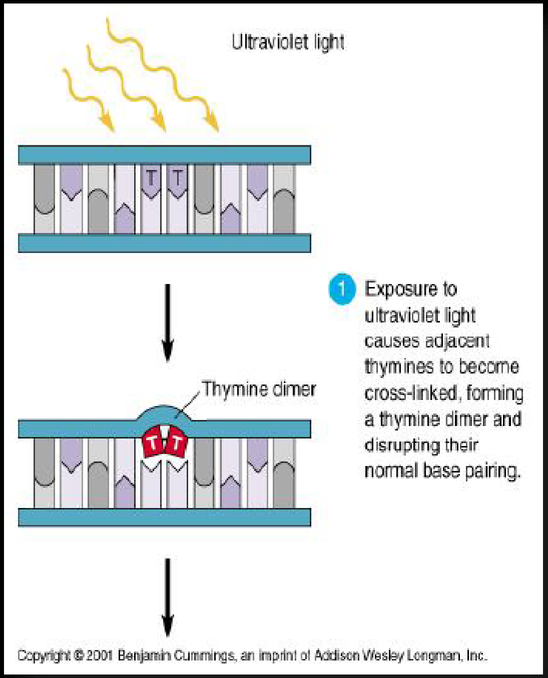
b. happen all of the time, regardless of growth conditions.

- **Induced mutations**

- **Chemicals, X-rays, UV light Viruses..**
- ✔ **Direct damage of nucleotides**
- ✔ **Alteraion of the nucleotide bonds**

Physical : (Radiation)

Ultraviolet (UV) radiation can cause mutations. During a time when the DNA is single stranded, UV can excite the electrons in side-by-side Thymines. This can cause these Thymines to bond covalently to each other, forming a Thymine dimmer.



- **Mutations outcome:**

✔ No effect / silent

✔ Change in protein structure such as an antibiotic target causing resistance to antibiotics

✔ change or inhibition of a functional protein synthesis which can be lethal to bacteria

		Second letter				
		U	C	A	G	
First letter	U	UUU Phe UUC Phe UUA Leu UUG Leu	UCU Ser UCC Ser UCA Ser UCG Ser	UAU Tyr UAC Tyr UAA Stop UAG Stop	UGU Cys UGC Cys UGA Stop UGG Trp	U C A G
	C	CUU Leu CUC Leu CUA Leu CUG Leu	CCU Pro CCC Pro CCA Pro CCG Pro	CAU His CAC His CAA Gln CAG Gln	CGU Arg CGC Arg CGA Arg CGG Arg	U C A G
	A	AUU Ile AUC Ile AUA Ile AUG Met	ACU Thr ACC Thr ACA Thr ACG Thr	AAU Asn AAC Asn AAA Lys AAG Lys	AGU Ser AGC Ser AGA Arg AGG Arg	U C A G
	G	GUU Val GUC Val GUA Val GUG Val	GCU Ala GCC Ala GCA Ala GCG Ala	GAU Asp GAC Asp GAA Glu GAG Glu	GGU Gly GGC Gly GGA Gly GGG Gly	U C A G
						Third letter

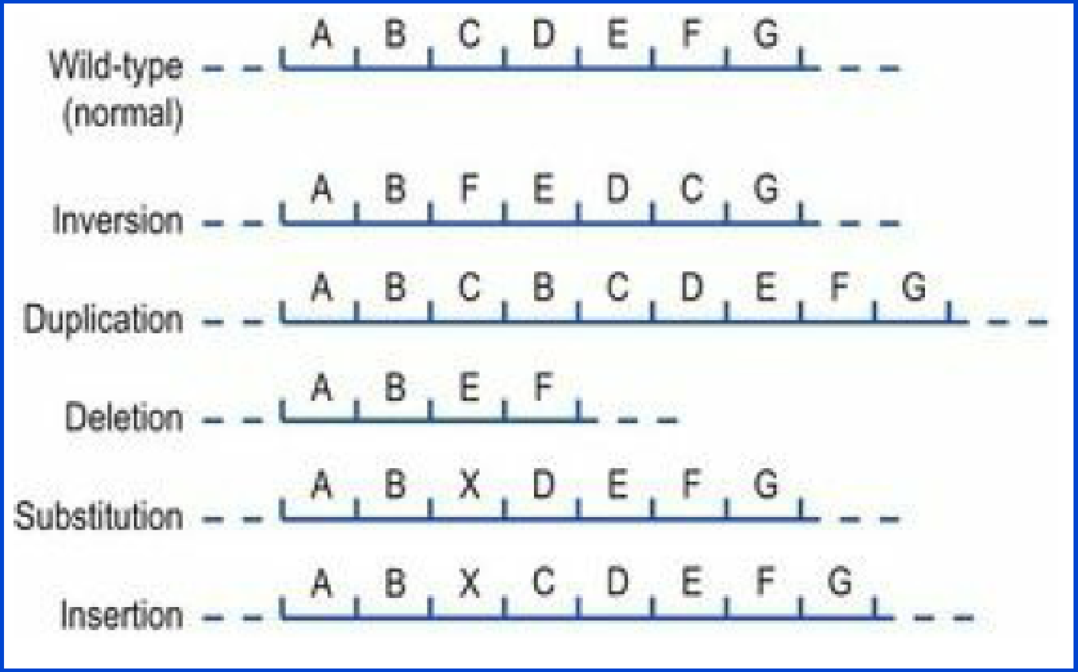
Types of mutations

- Multisite: many nucleotides

✔ Inversion, duplication or deletion

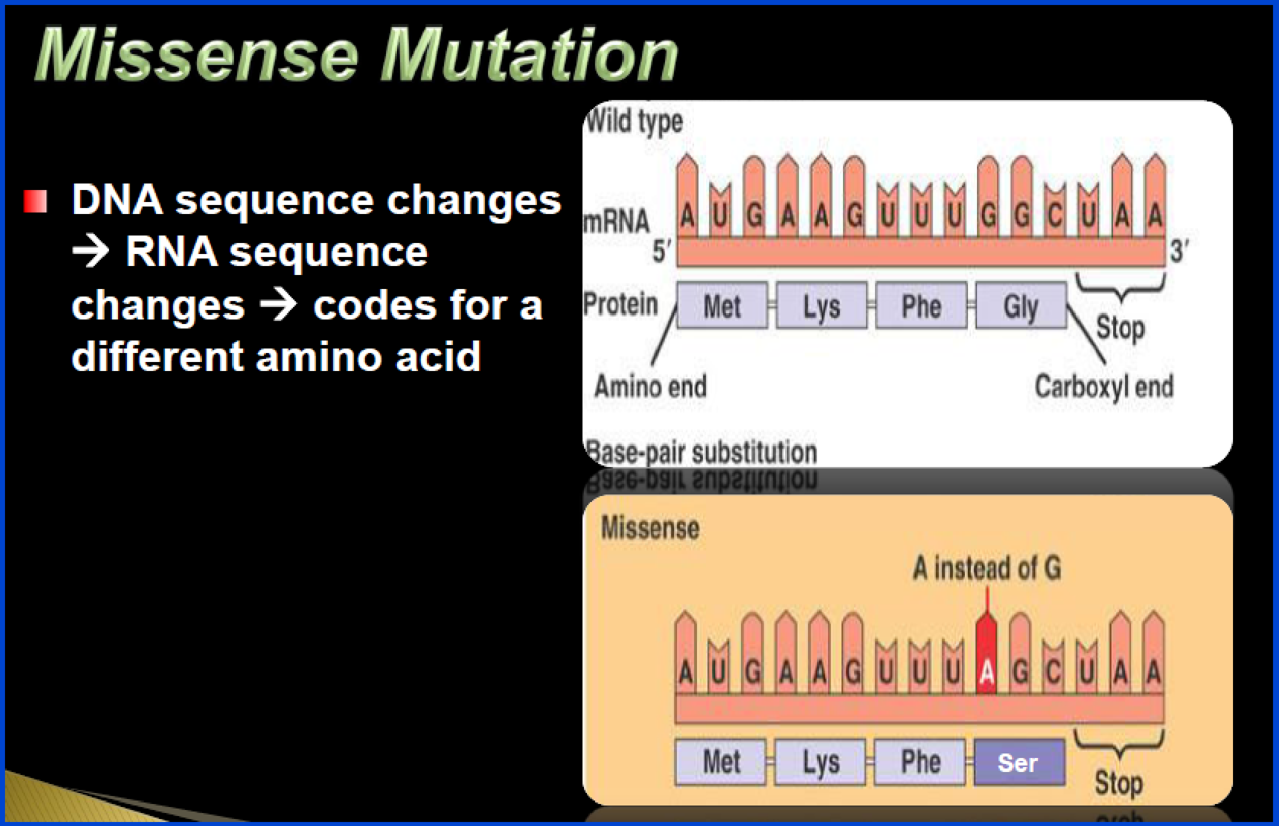
- Point mutation: single or few bases

✔ Insertion, substitution or deletion



Description of mutations

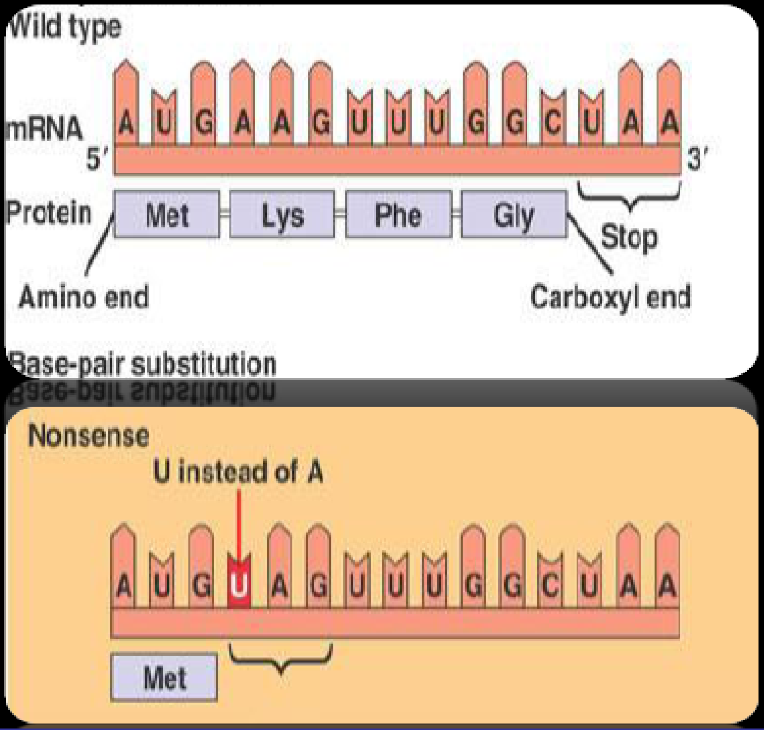
Missense mutation: result in different amino acids being inserted in protein



Nonsense mutation: change a codon encoding an amino acid into stop codon that result in failure of protein synthesis

Nonsense Mutation

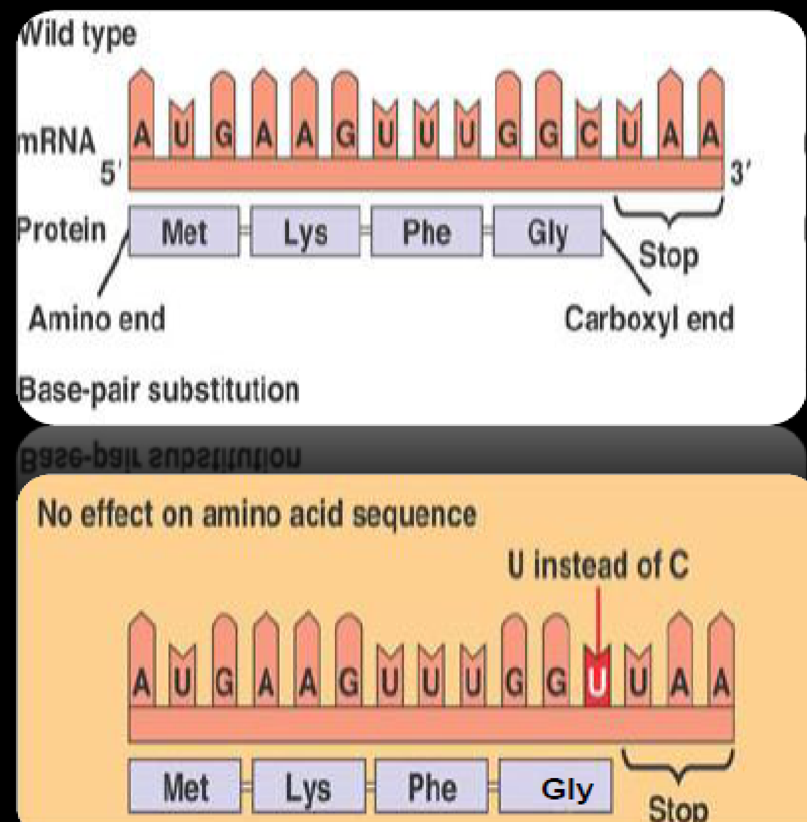
- DNA sequence changes → RNA sequence changes → early stop codon introduced
- Translation stops → Protein is incomplete



Silent mutation: the change in the nucleotide sequence doesn't result in a change in protein sequence

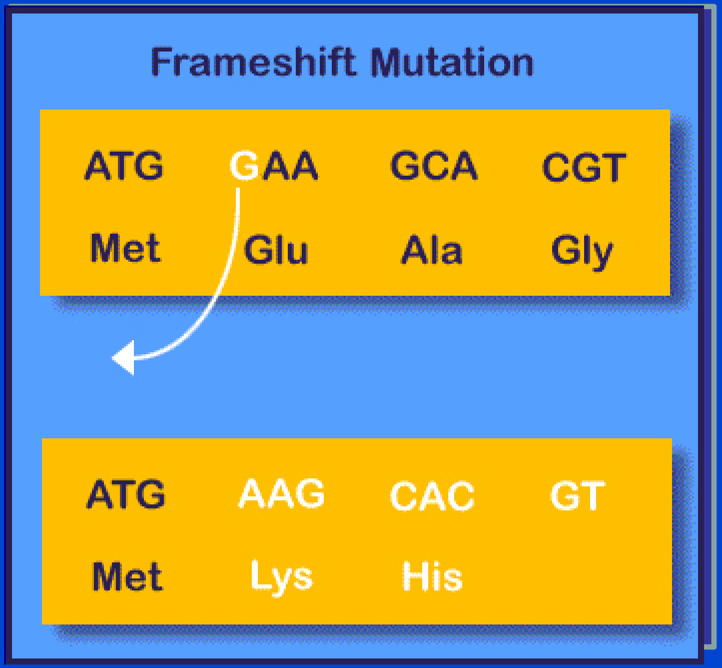
Silent Mutation

- DNA sequence changes → RNA sequence changes → still codes for the same amino acid
- No effect on the amino acid sequence



2. Frame shift mutation:

- One or more base are added or deleted
- Shift in the reading frame



2. Genetic exchange:

Importance:

a. moving **antibiotic resistance** genes among bacteria
b. moving **virulence** gene among bacteria
c. changing the **antigenic make-up** to avoid immunity

Mechanisms

1. **Transformation - uptake of naked DNA**
2. **Transduction - bacteriophage as vectors**
3. **Conjugation - plasmids moved by cell-cell contact**
4. **Transposons**

- 1. **Transformation**

    a. recipient cell must be **competent**

competence is the ability of a cell to alter its genetics by taking up extracellular ("naked") DNA from its environment in the process called transformation.

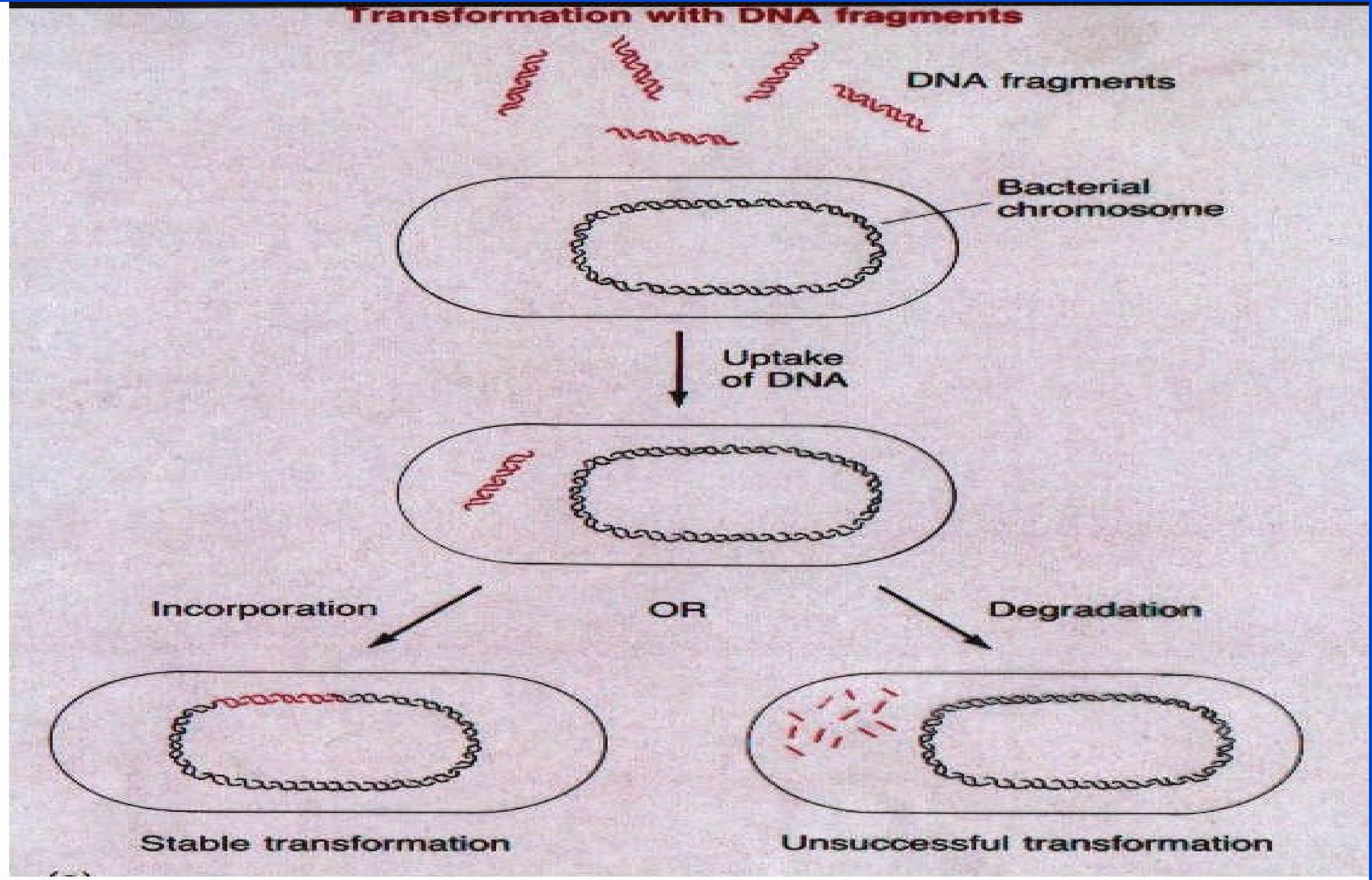
b.Naked DNA will be uptaken

c. natural competence versus artificial competence

**** Natural competence, a genetically specified ability of bacteria which is thought to occur under natural conditions as well as in the laboratory.**

**** Induced or Artificial competence, which arises when cells in laboratory cultures are treated to make them transiently permeable to DNA.**

d. only **certain bacteria** are naturally transformable e.g ***Streptococcus pneumoniae, Haemophilus influenzae, Neisseria gonorrhoeae, Vibrio***


Transformation with DNA fragments
DNA fragments
Bacterial chromosome
Uptake of DNA
Incorporation
OR
Degradation
Stable transformation
Unsuccessful transformation

2. Transduction

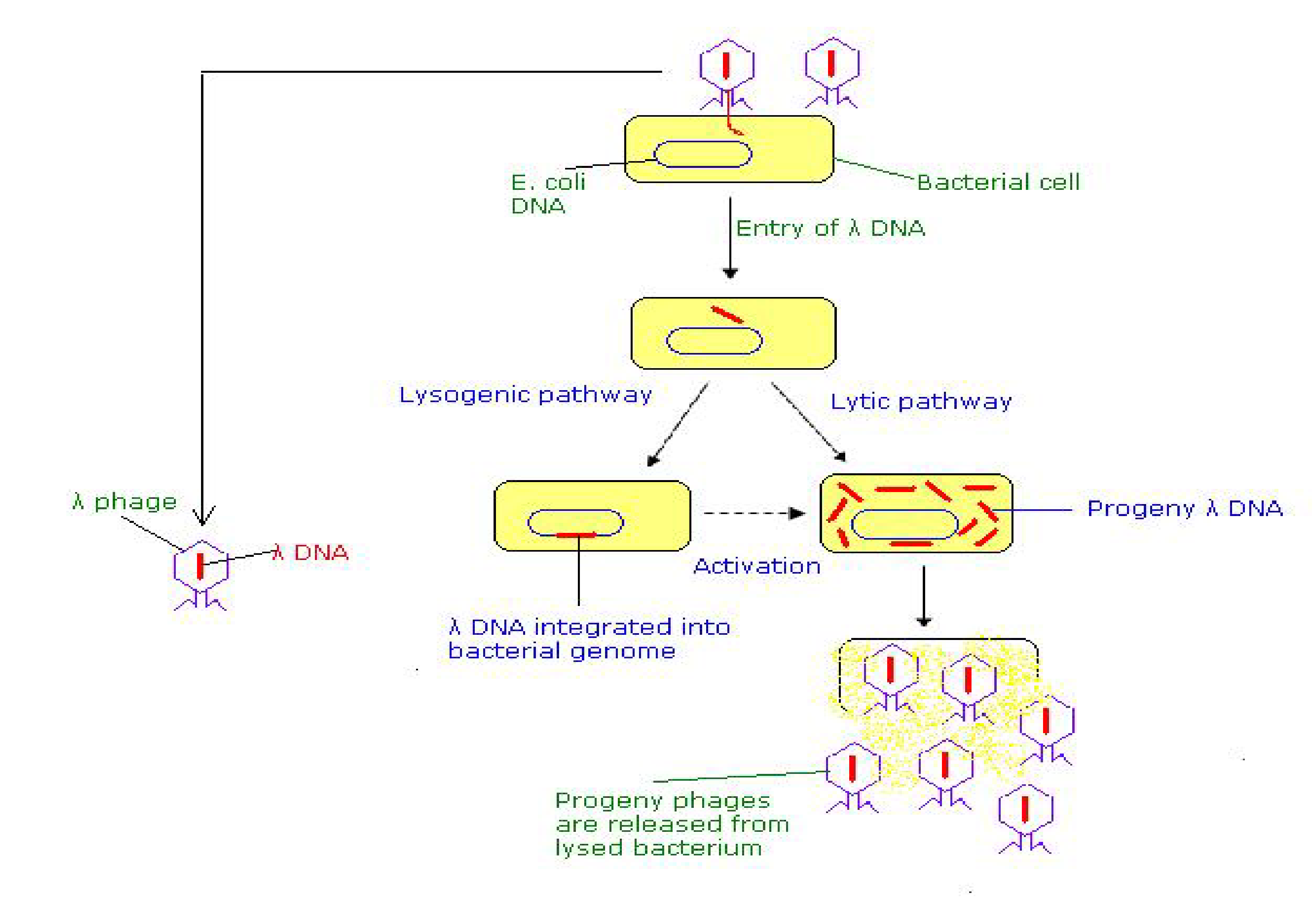
** **bacteriophage (phage)** are viruses of bacteria - can be either **lytic** or **temperate (Lysogenic)**

* **lytic** - always lyse (kill) host bacterial cell

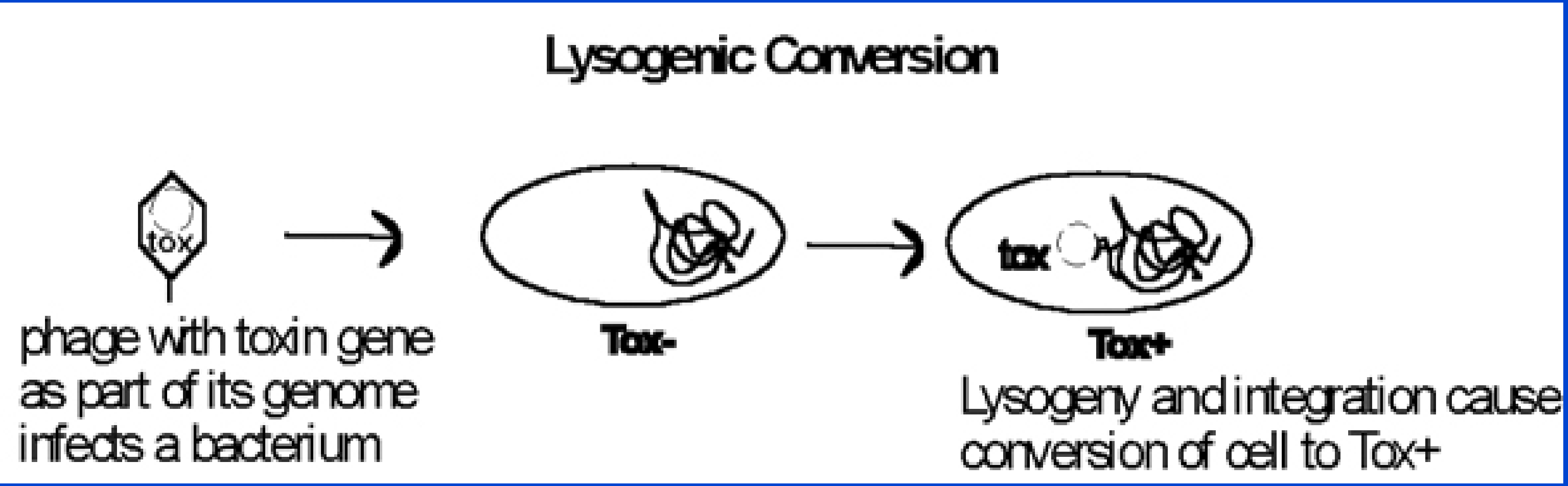
* **temperate** - can stably infect and coexist within bacterial cell (**lysogeny**) until a **lytic phase** is induced

* the phage genome during lysogeny is called the **prophage**, and the bacterial cell is called a **lysogen**

if the phage genome encodes an **observable function**, the lysogen will be altered in its phenotype - **lysogenic conversion** **(e.g.,cholera toxin, group A streptococcus, botulinum toxin diphtheria toxin in *Corynebacterium diphtheriae*)**



Lytic: replicate and burst
Lysogenic: prophage <> lytic



Diphtheria, Cholera, botulinum and erythrogenic toxins

1. Specialized transduction:

**In specialized transduction, bacteriophage transfer only a few restricted gene (DNA fragments) from donor bacteria to recipient bacteria. Specialized transduction is carried only by temperate bacteriophage .

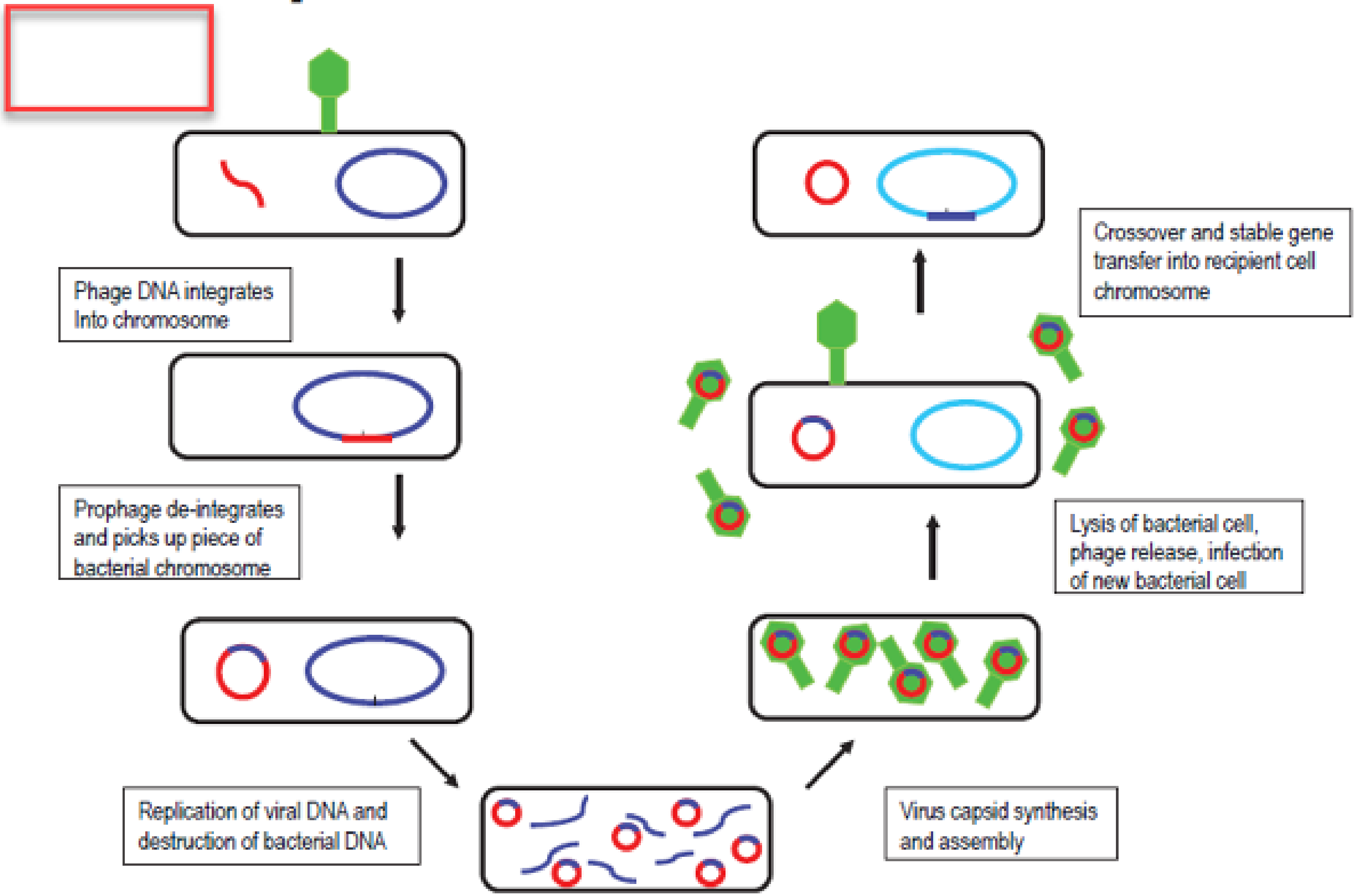
* At first temperate bacteriophage enter into donor bacteria and then its genome gets integrated with host cell's DNA at certain location and remains dormant and pass generation to generation into daughter cell during cell division.

* When such lysogenic cell is exposed to certain stimulus such as some chemicals or UV lights, it causes induction of virus genome from host cell genome and begins lytic cycle.

* On induction from donor DNA, this phage genome sometimes carries a part of bacterial DNA with it.

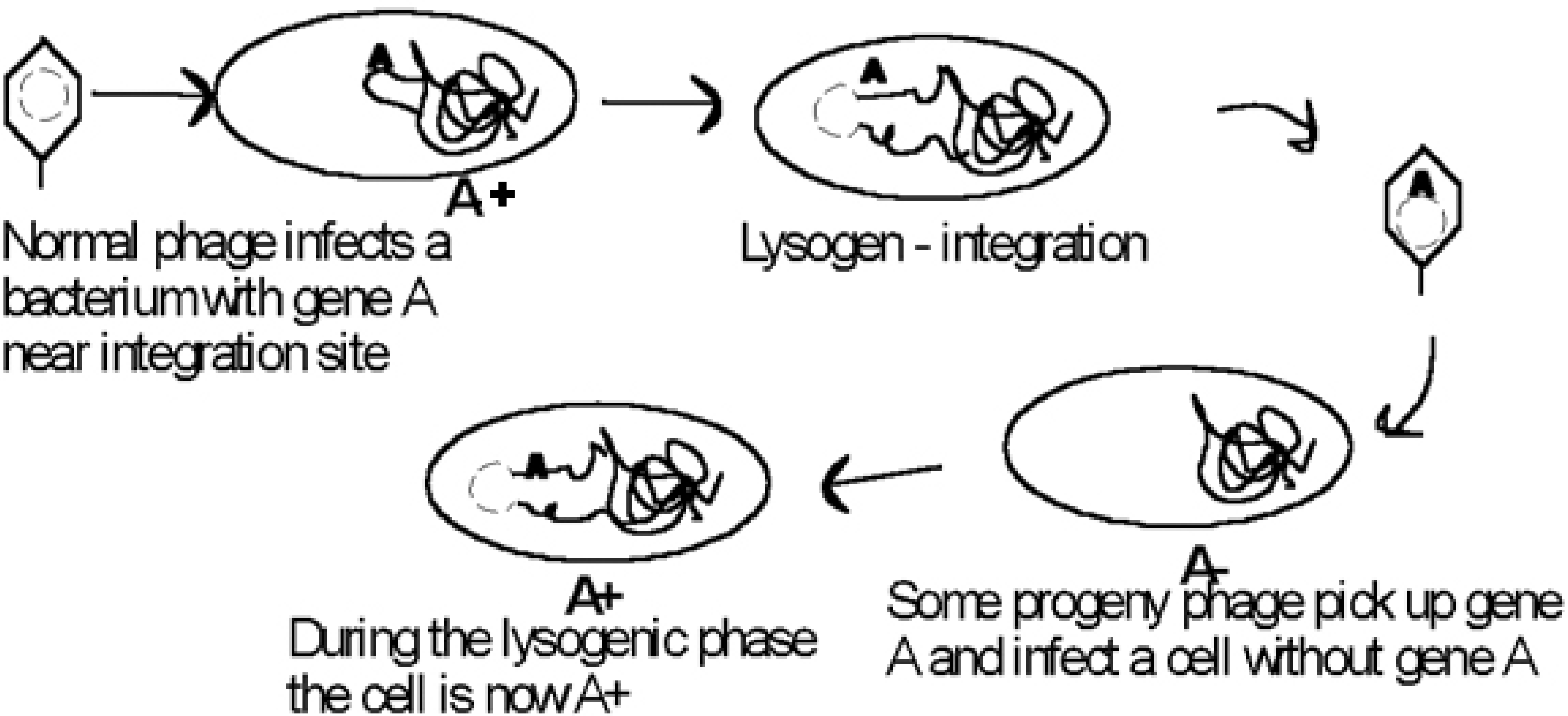
* When such bacteriophage carries a part of donor bacterial DNA infects a new bacteria, it can transfer that donor DNA fragments into new recipient cell. So, in this specialized transduction only those restricted gene are situated on the side of integrated viral genome have a chance to enter into recipient cell.

Specialized transduction



Courtesy of M. Mulks (MSU)

Specialized Transduction



2. Generalized transduction

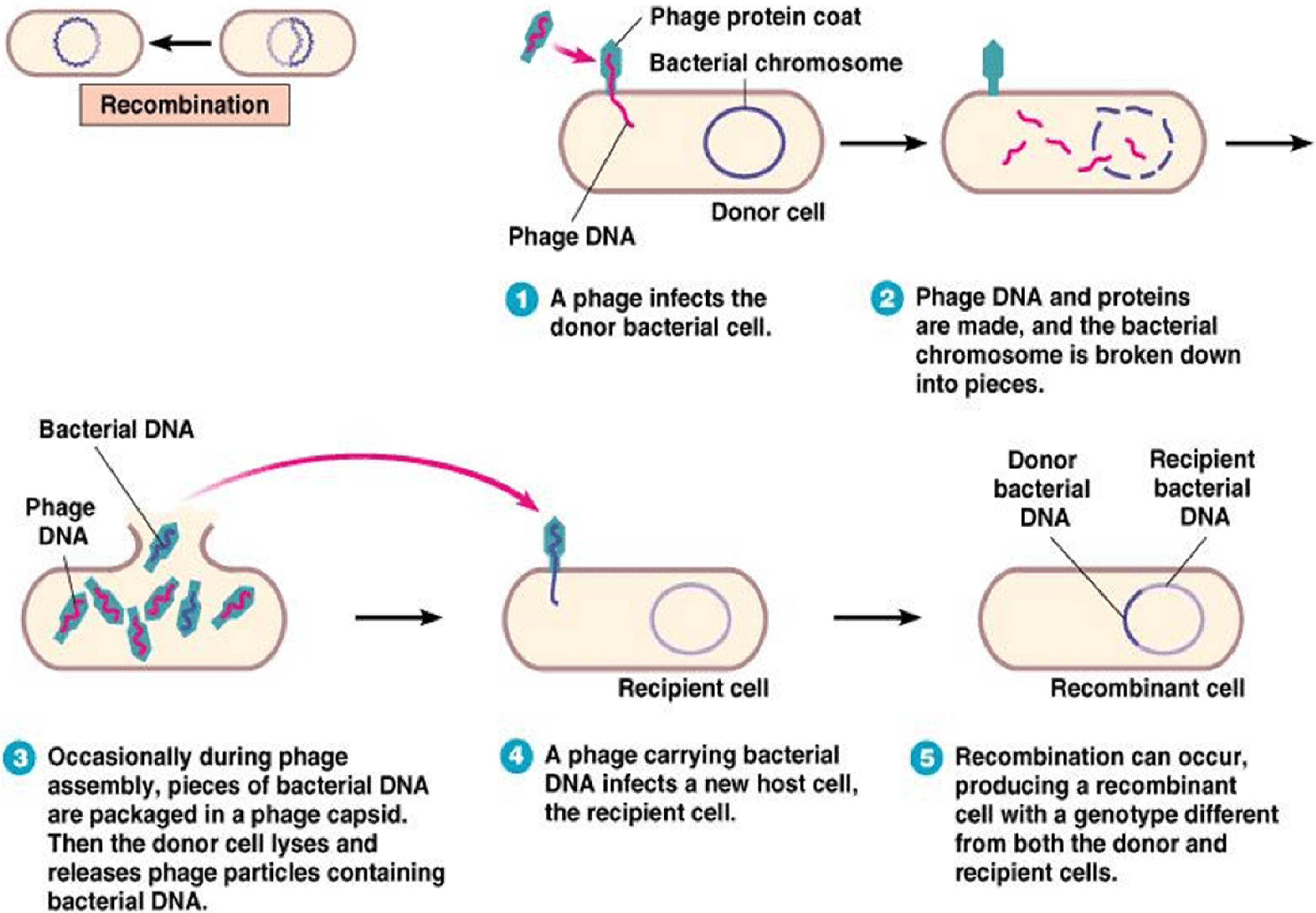
* If all the fragments of donor DNA from any region of chromosome have a chance to enter into transducing bacteriophage then it is known as generalized transduction.

* In this type of transduction, at first bacteriophage infects donor cell and begins lytic cycle.

* When virus enter into bacterial cell, virus hijack host cell and synthesize virus components such as genome, enzymes, capsid, head tail and tail fibers. Then viral enzyme hydrolyses host cell DNA into small fragments.

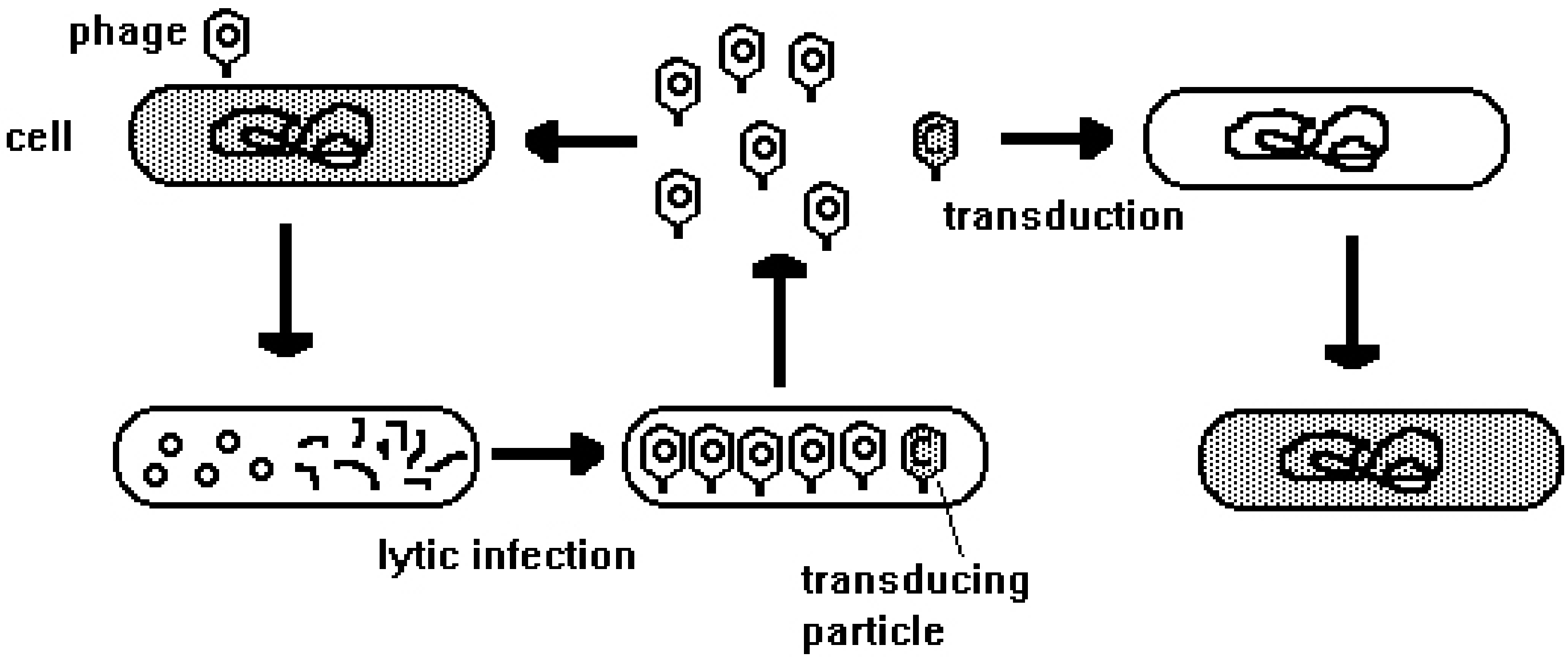
* During assembly of virus component to form progeny viruses, sometime any of the fragments of donor DNA get incorporated into the virus capsid (bacteriophage head). Such abnormal bacteriophage when infects a new cell, it can transfer this donor DNA into new bacteria. Since this donor DNA is not viral DNA, it does not replicates inside recipient bacteria but undergoes homologous recombination with recipient cell's chromosomal DNA forming recombinant cell.

** toxins and antibiotic resistance genes can be moved by generalized transduction



Recombination
Phage protein coat
Bacterial chromosome
Donor cell
Phage DNA
1 A phage infects the donor bacterial cell.
2 Phage DNA and proteins are made, and the bacterial chromosome is broken down into pieces.
Bacterial DNA
Phage DNA
Recipient cell
Donor bacterial DNA
Recipient bacterial DNA
Recombinant cell
3 Occasionally during phage assembly, pieces of bacterial DNA are packaged in a phage capsid. Then the donor cell lyses and releases phage particles containing bacterial DNA.
4 A phage carrying bacterial DNA infects a new host cell, the recipient cell.
5 Recombination can occur, producing a recombinant cell with a genotype different from both the donor and recipient cells.

GENERALIZED TRANSDUCTION



C. Conjugation (mediated by plasmids)

What is a plasmid?

A small DNA molecule that is physically separate from, and can replicate independently of bacterial chromosome / circular double stranded

Three possible states for plasmid conjugation

i. **conjugative** - the plasmid encodes all of the functions for conjugation and can move itself from the donor cell to the recipient cell

ii. **mobilizable** - the plasmid cannot move itself, but can be moved with help from a conjugative plasmid

iii. **non-transmissible** - can't move by conjugation

Conjugation steps in general

i. synthesis of sex **pilus**

ii. **cell to cell contact** via pilus

iii. **copying plasmid DNA and transfer** of copy into recipient cell

- **bacteria containing a conjugative plasmid are called donor, male, (F+)**

- **bacteria receiving the plasmid are called recipient, female, (F-)**

- importance of conjugation - **moving plasmids encoding multiple antibiotic resistance genes (R plasmids)** among diverse bacterial

Types of conjugation;

1 . F+ conjugation

Genetic recombination in which there is a transfer of an F+ plasmid but not chromosomal DNA from a male donor bacterium to a female recipient bacterium. Other plasmids present in the cytoplasm of the bacterium, such as those coding for antibiotic resistance, may also be transferred during this process.

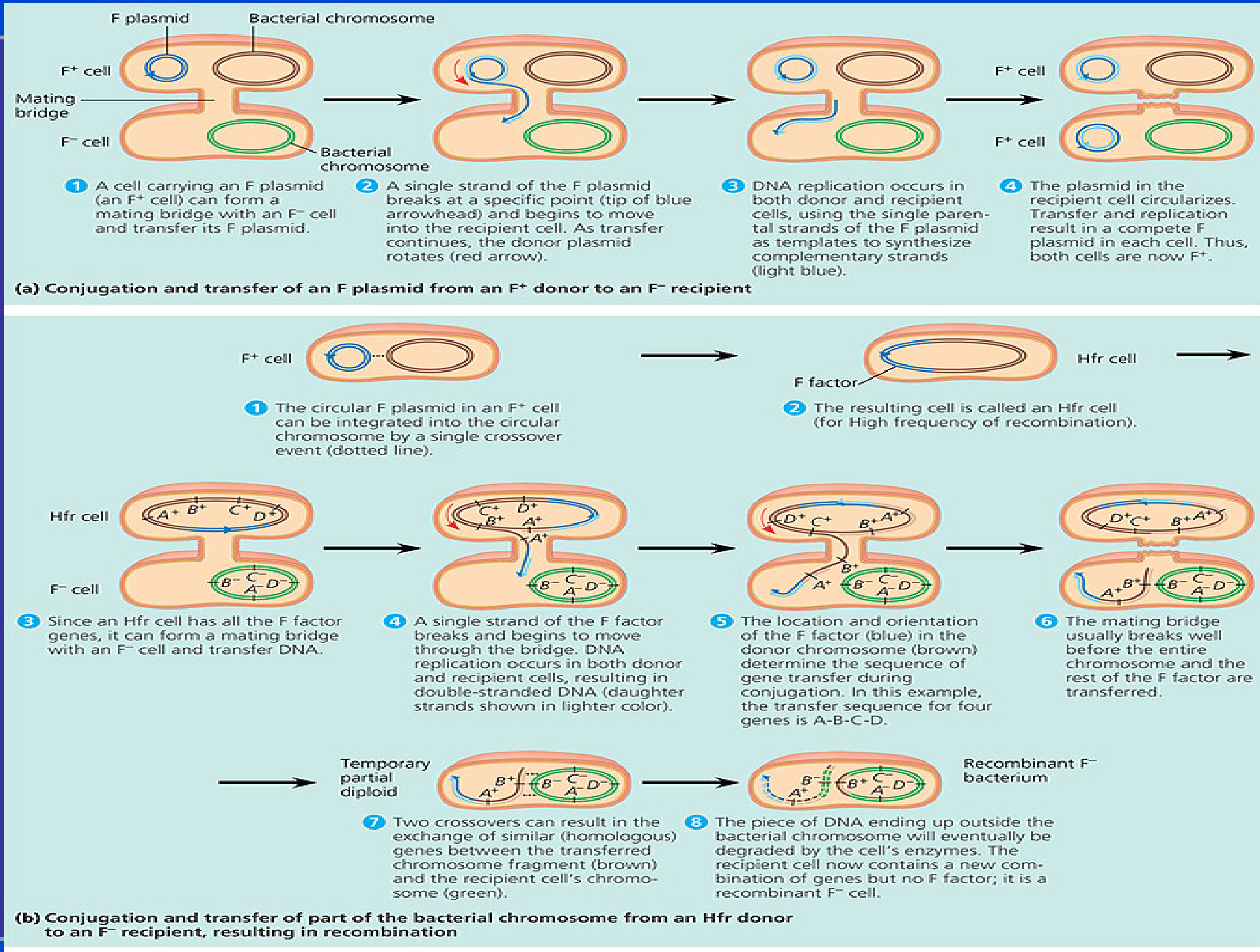
2 . High frequency recombination (Hfr)

Genetic recombination in which fragments of chromosomal DNA from a male donor bacterium are transferred to a female recipient bacterium following insertion of an F+ plasmid into the nucleoid of the donor bacterium.

terms of interest:

- Occasionally, the F factor integrates into a random position in the bacterial chromosome. When this happens, the bacterial cell is called Hfr instead of F+.

- Hfr bacteria are still able to initiate conjugation with F- cells, but the outcome is completely different from conjugation involving F+ bacteria. IT IS called an F’ instead

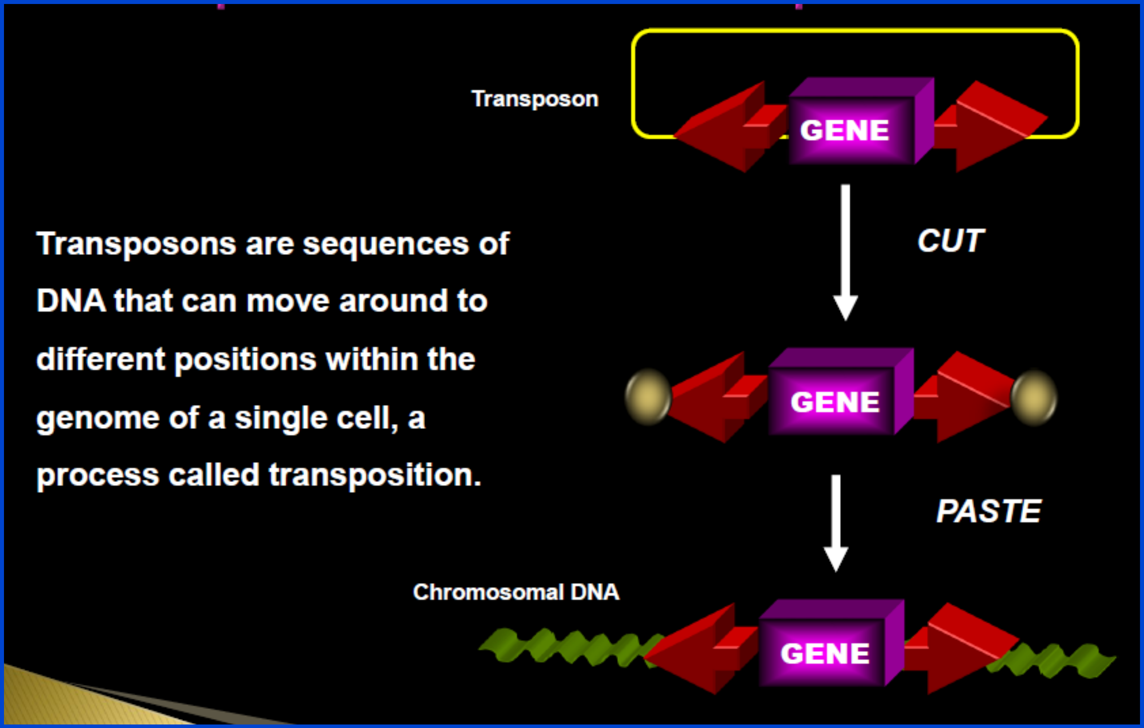


(a) Conjugation and transfer of an F plasmid from an F^+ donor to an F^- recipient

(b) Conjugation and transfer of part of the bacterial chromosome from an Hfr donor to an F^- recipient, resulting in recombination

3. Transposons:

- Jumping genes/copy and paste (Class1) or cut and paste (2)
- Between plasmids or between chromosomes and plasmids
- **medical importance** since many **antibiotic resistance genes** are encoded by transposons in antibiotic **resistance plasmids**



- The End