CORE IDEA 2: GENETICS AND INHERITANCE
TOPIC 2.11: GENETICS OF BACTERIA

Learning Outcomes:

Core Idea 1 [Recap – Lecture notes CI 1.1 Organelles and Cellular Structures]

(d) describe the structure of a typical bacterial cell (small and unicellular, peptidoglycan cell wall, circular DNA, 70S ribosomes and lack of membrane-bound organelles)

Core Idea 2:

(d) **[modified]** describe the structure and organisation of **prokaryotic genomes** (including DNA/RNA, single-/double-stranded, number of nucleotides, packing of DNA, linearity/circularity and presence/absence of introns)

(g) outline the mechanism of asexual reproduction by binary fission in a typical prokaryote and describe how transformation, transduction and conjugation (including the role of F plasmids but not Hfr) give rise to variation in prokaryotic genomes

(i) explain how gene expression in prokaryotes can be regulated, through the concept of simple operons (including *lac* and *trp* operons), including the role of regulatory genes, and distinguish between inducible and repressible systems (knowledge of attenuation of the *trp* operon is not required)

Use the knowledge gained in this section in new situations or to solve related problems.

Textbooks and References

Campbell, Urry, Cain, Wasserman, Minorsky, Reece (2018) **Biology – A Global Approach (11th Edition) (Global Edition) Chapter 27: Prokaryotes pg. 625 – 633, Chapter 18: Control of Gene Expression pg. 413 - 417** (Pearson Publication) ISBN-10 1-292-17043-3

Note: This textbook is available in our library. You may wish to borrow them to supplement your reading when necessary.

Contents

1. Bacteria Cell Structure

Learning Outcome 1(d)

Describe the typical structure of a bacterial cell (small and unicellular, peptidoglycan cell wall, circular DNA, 70S ribosomes and lack of membrane-bound organelles)

- Bacteria (singular: bacterium) are **small**, **unicellular** prokaryotes.
- They **lack a true nucleus and membrane-bound organelles** (e.g. rER, Golgi apparatus, mitochondrion, chloroplast).
- They possess one **circular double-stranded DNA** in the nucleoid region and **70S ribosomes** in the cytoplasm.

	Structure	Function
1. Nucleoid	Non-membrane bound. A region of cytoplasm where the bacterial chromosome is found. Appears lighter than the surrounding cytoplasm in electron micrographs.	Contains one double-stranded, circular DNA molecule. Presence of a large amount of RNA and RNA polymerase involved in transcription and translation.
2. Plasmids	Extra-chromosomal genome. Small circular DNA molecule, not part of the bacterial chromosome. Replicates independently from the bacterial chromosome.	Carries a few genes which code for proteins that are not required for survival. These proteins, when expressed, might result in characteristics that confer advantages in stressful environments (e.g., antibiotic resistance). May or may not be present in all bacteria
3. Ribosomes	70S (unlike 80S in eukaryotes). Gives cytoplasm a granular appearance.	Required for polypeptide synthesis.
4. Cell Surface Membrane	Phospholipid bilayer containing proteins.	Selectively permeable barrier that regulates the transport of material in and out of the bacterial cell. Proteins involved in the transport of ions, nutrients, and waste across the membrane. Energy transduction as it contains enzymes involved in ATP synthesis
5. Cell Wall	Consists of peptidoglycan (sugars cross-linked by peptide bonds between short polypeptide chains)	Maintains cell shape Protects bacteria from osmotic lysis

6. Flagella (singular: flagellum)	Hollow cylindrical thread made of protein Bacteria cells may possess none, one or multiple flagella.	For movement
7. Pili (singular: pilus)	Hollow hair-like structure made of protein	Sex Pili – Long conjugation pili that facilitates transfer of genetic material between two bacteria

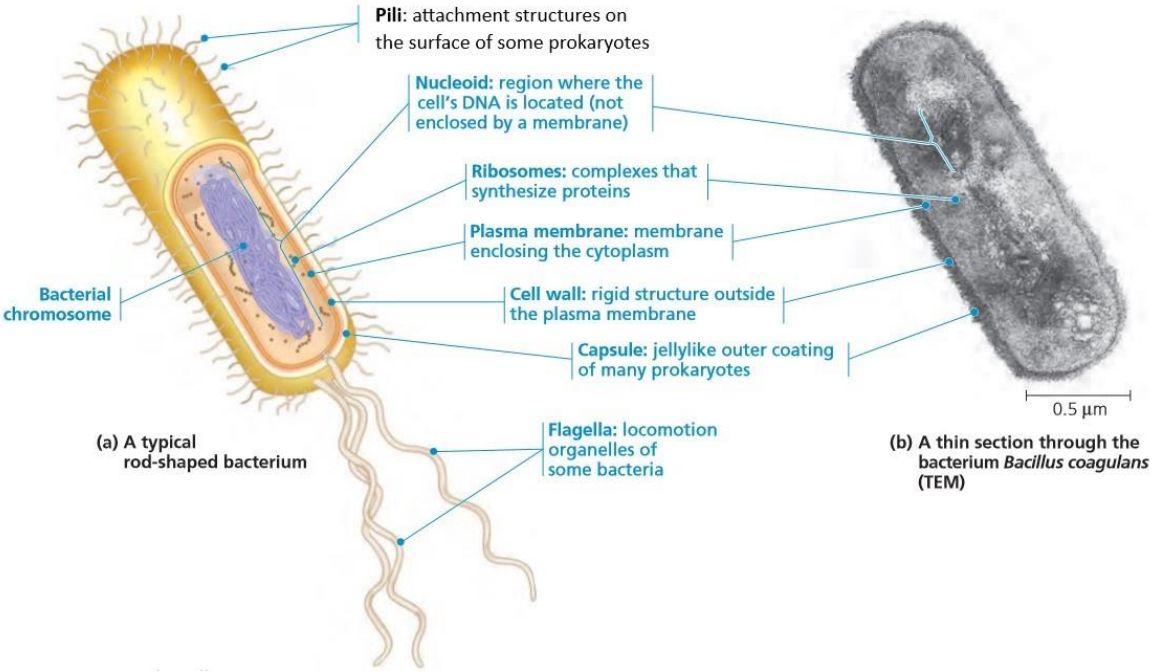


Fig. 1.1 A typical prokaryotic cell

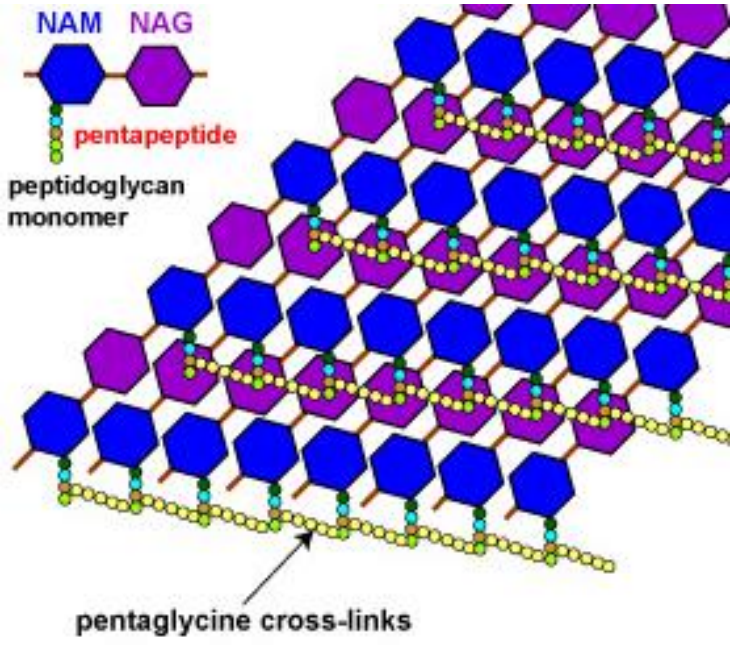


Fig. 1.2 Peptidoglycan Cell Wall in Bacteria;
sugars cross-linked by peptide bonds between short polypeptides

CHECKPOINT 1

1 Which of the options below correctly list the distinctive features of prokaryotes?

A cellulose cell wall, 70S ribosomes, linear DNA
B cellulose cell wall, 80S ribosomes, circular DNA
C peptidoglycan cell wall, 70S ribosomes, circular DNA
D peptidoglycan cell wall, 80S ribosomes, linear DNA

2 The genome of prokaryotes typically comprises a large circular chromosome and smaller plasmids.

TRUE **FALSE**

3 Compare the structure between prokaryotic and eukaryotic genome

Feature	**Prokaryotic**	**Eukaryotic**
Condition		
Nucleus		
DNA		
Plasmids		
Chromosomes		
Genome size		

Learning Outcome 2(d)

[modified] describe the structure and organisation of **prokaryotic genomes** (including DNA/RNA, single-/double-stranded, number of nucleotides, packing of DNA, linearity/circularity and presence/absence of introns)

2. Structure and Organisation of Prokaryotic Genome

2.1 Bacterial Chromosome

- Bacteria have simpler, **smaller genomes than eukaryotes**. For example, *Escherichia coli* bacteria has a genome size of 5 million bases compared to yeast which has 12 million bases.
- Bacterial cells are **monoploid**, with only one set of chromosomes
- Bacterial cell has a single molecule of circular, **double stranded DNA**.
- Circular DNA is present in nucleoid region, not bound by a membrane.
- Circular DNA contains a single origin of replication (oriC).
- Circular DNA contains **very few introns** between genes and totally absent within genes (unlike eukaryotes).
- Circular DNA is associated with **DNA binding proteins** (nucleoid-associated proteins) to result in the DNA bending and coiling to form **loop domains**, which then **supercoil** to form condensed DNA.

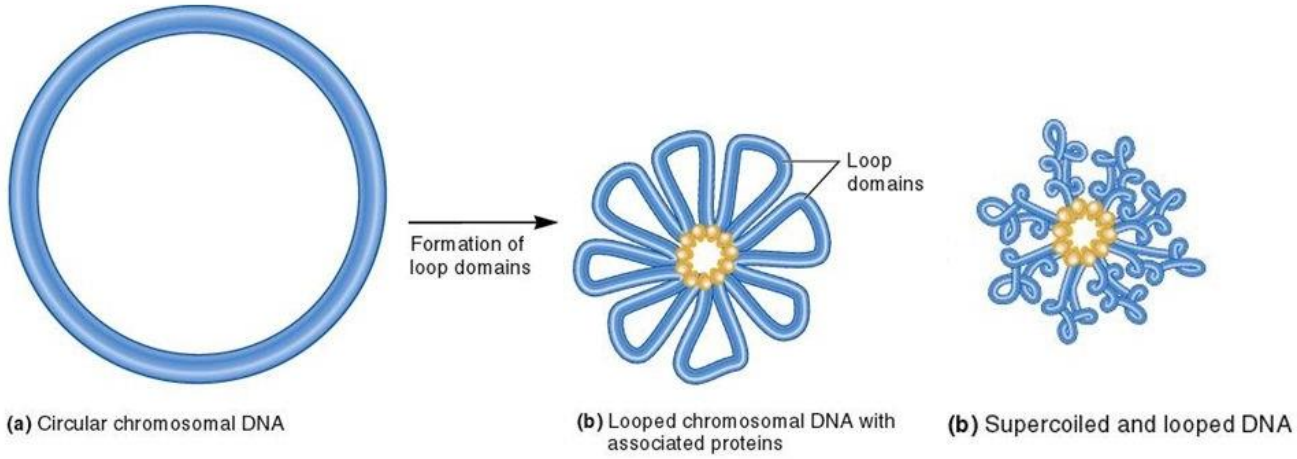


Fig. 2.1.1 Compacting of bacterial chromosome

2.2 Bacterial Plasmid

- Plasmids are small, circular, doubled-stranded, extrachromosomal DNA (outside chromosome) that can self-replicate

- Plasmid DNA contains only a few genes:
  - Genes involved in its **own replication** and control
  - Genes that can **provide survival advantages** for the bacteria under **certain environmental conditions**
  - Examples: resistance to antibiotics and heavy metals, resistance to radiation, ability to utilize certain metabolite, production of toxins.

- Since plasmid contains genes which provide survival advantages, and not genes which are essential for survival, plasmids may or may not be present in bacteria cells.

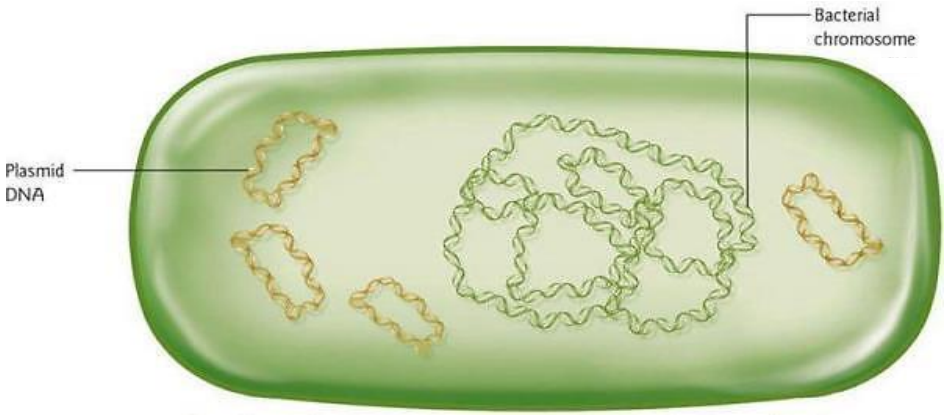


Fig. 2.2.1 Bacterial chromosome and plasmids

CHECKPOINT 2

1 The bacteria chromosome is present in the _____ region of the cell.

2 DNA compacting occurs due to DNA binding _____which bend and coil the DNA to form _____ domains which then _____ to form condensed DNA.

3 The bacterial chromosome contains _____ origin of replication.

4 Plasmids are essential for bacterial growth and survival under all conditions.

TRUE FALSE

5 Plasmids replicate only when the bacterial chromosome replicates.

TRUE FALSE

Learning Outcome 2(g) part (i)

Outline the mechanism of asexual reproduction by binary fission in a typical prokaryote

3. Bacteria Reproduction via Binary Fission

Binary fission (binary − "two parts", fission − "split') is the **asexual** means by which bacterial cells produce **genetically identical offspring**.

Repeated binary fission of bacterial cells results in the formation of a cluster of **genetically identical cells** called a colony.

Binary fission is a form of **vertical gene transfer**, where genetic material is passed from parent to off-spring.

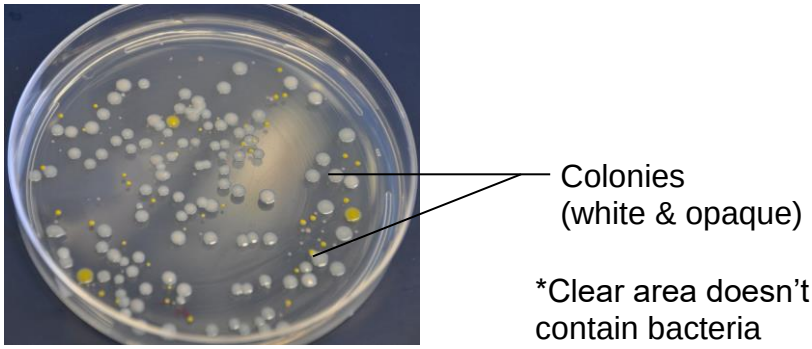


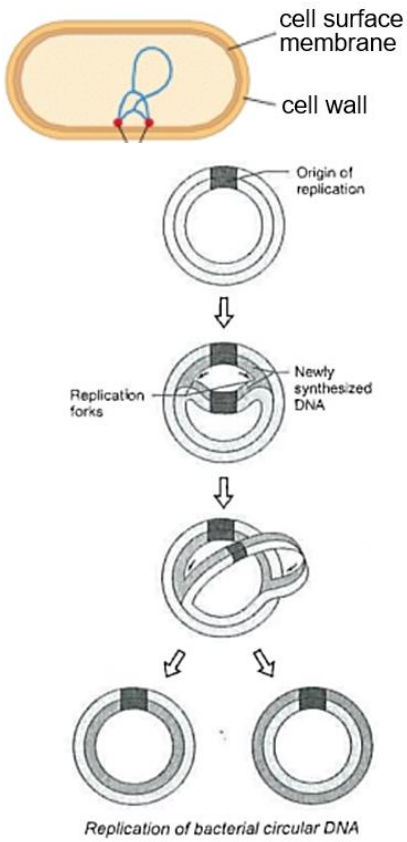
Fig. 3 Bacterial colonies cultured on nutrient medium in a Petri dish

Under optimum conditions, each cycle of binary fission takes around 20 – 30 minutes. This is advantageous in a **stable, favourable environment** as it allows successful phenotypes to rapidly colonise a habitat.

However, bacterial reproduction can be limited by factors such as a lack of nutrients, metabolic waste, poisoning, competition and consumption by other organisms.

PIT FALL*: Binary fission is **NOT** mitosis and vice-versa. The mechanism of binary fission greatly differs from that of mitosis though the outcome is similar (i.e. production of two genetically identical daughter cells).*

3.1 Process of Binary Fission

Stage	Description
1. DNA REPLICATION  *Replication of bacterial circular DNA* * The DNA replication by bacterial chromosome follows <u>semi-conservative replication</u> model	1. Bacterial chromosome is attached to the cell surface membrane at its **origin of replication.** • The DNA double helix unwinds and unzips at the **<u>single</u> origin of replication**, separates to form a replication bubble. • DNA replication proceeds in **both directions** (e.g. **bidirectional replication**). • Both strands of DNA act as **templates** for the synthesis of new daughter strands. • Similar to DNA replication in eukaryotes, each of the two replication forks has a leading and a lagging strand. *(Refer to CI2.2 DNA replication).* • Enzymes like helicase, topoisomerase, primase, DNA polymerase, and DNA ligase are also involved in the DNA synthesis process. 2. The **second origin of replication** (of the newly synthesised DNA molecule) then attaches to the membrane at an adjacent site. ***** In a **eukaryotic cell**, there are **<u>multiple</u> origins of replication (oriR)**, ensuring that DNA replication can be completed in a shorter period.

As the DNA is circular, there are no free ends to the DNA and also no end replication problem. An interlocking structure made up of 2 daughter DNA molecules is formed. **Topoisomerase** is needed to cut, separate and reseal the two DNA molecules.

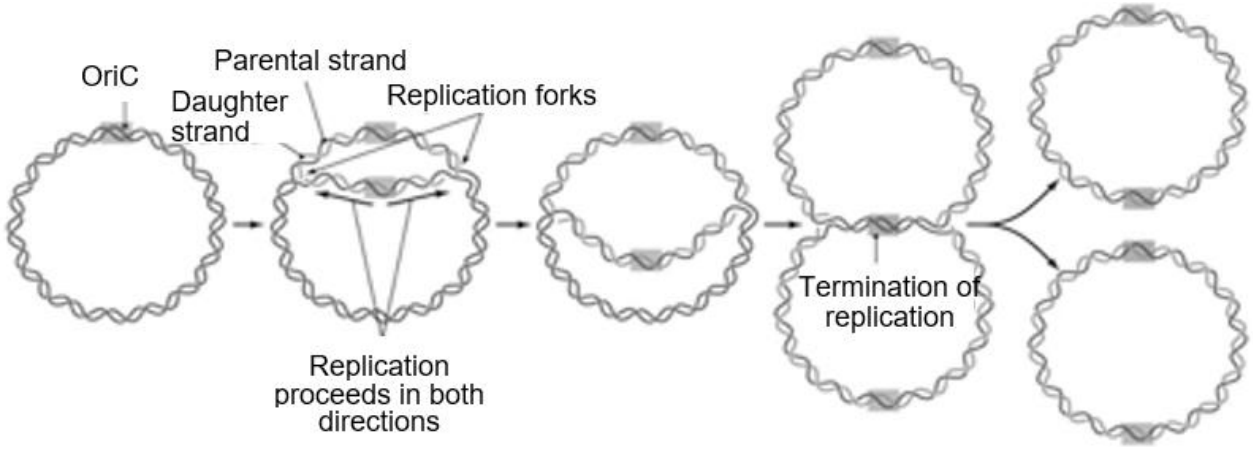
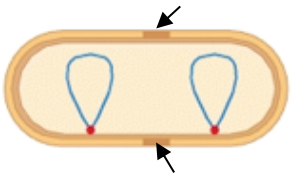
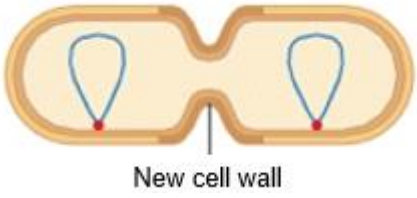
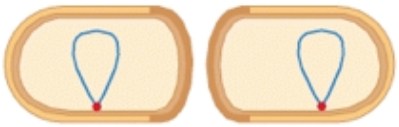


Fig. 3.1 Replication of bacterial chromosome

2. ELONGATION 	• The bacterial cell **elongates** by growing membrane and depositing cell wall material between the 2 chromosomes.
3. NEW CELL WALL FORMATION  	• After the bacterium grows to a sufficient size, a **new cell wall** forms between the two daughter cells.
4. TWO GENETICALLY IDENTICAL DAUGTHER CELLS 	• New cell wall fuses, leading to the formation of two daughter cells. • Each daughter **cell** has bacterial chromosome **<u>genetically identical</u>** to each other.

CHECKPOINT 3

1 Which of the following statements is true for binary fission?

A Binary fission results in the production of two genetically identical daughter cells.
B Replicated chromosomes are separated by attaching to a mitotic spindle.
C Binary fission and eukaryotic mitosis are identical processes.
D Bacterial cells must mature before they are able to perform binary fission.

2 Arrange the following stages of binary fission in the correct sequence

1. After the bacterium grows to a sufficient size, a new cell wall begins to form between the two daughter chromosomes.
2. The bacterial cell elongates by growing the membrane and depositing cell wall material between the two chromosomes.
3. The newly formed cell wall fuses, leading to the separation of two genetically identical daughter cells.
4. The DNA double helix unwinds at the single origin of replication and is replicated in both directions, resulting in two identical copies of the bacterial chromosome.

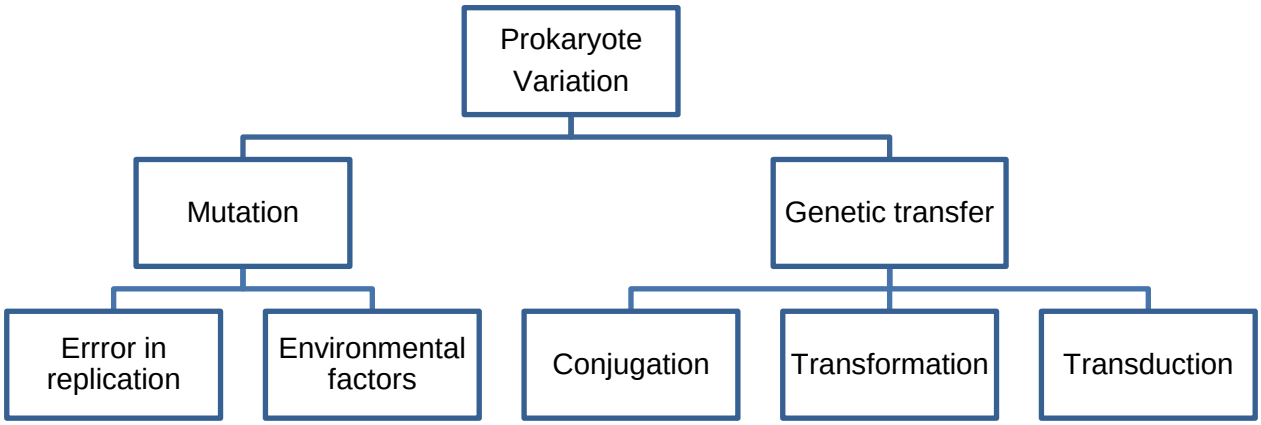
A 4 → 2 → 1 → 3
B 2 → 4 → 1 → 3
C 4 → 1 → 2 → 3
D 2 → 1 → 4 → 3

Learning Outcome 2(g)

Describe how transformation, transduction and conjugation (including the role of F plasmids but not Hfr) give rise to variation in prokaryotic genomes.

4. Genetic Variation in Bacteria

Unlike in eukaryotes where meiosis and fertilisation give rise to genetic variation in offspring, bacteria undergo binary fission, where daughter cells are genetically identical to the parent cell. Genetic diversity in bacteria can be attributed to **mutations** and **genetic transfer**.



4.1 Mutation

- Mutations are the main source of genetic variation in bacteria. Changes in nucleotide sequences of a gene can occur due to:
  - spontaneous **errors in replication** (e.g. insertions, deletions and substitutions)
  - induced by **environment factors** (e.g. exposure to mutagens)

- Relatively high mutation rates are due to:
  - **high asexual reproductive rates**
  - **short generation time**

4.2 Genetic Transfer between Bacteria

- Another source of genetic variation in bacteria depends on the **transfer of genetic material from one bacterium (donor cell) to another (recipient cell).** These genetic transfers result in the acquiring of new abilities to survive in an unfavourable environment. This is also known as **horizontal gene transfer.**

- There are three different types of horizontal genetic transfers:
  (a) **transformation** (b) **transduction** and (c) **conjugation**.

Genetic **variation** is present in the recipient cell **only** when genetic transfer results in:

- **novel** genetic material (e.g. plasmid) present in the recipient cell, **or**
- **genetic recombination** when the DNA from the donor is combined with the recipient cell's genome in the recipient cell.

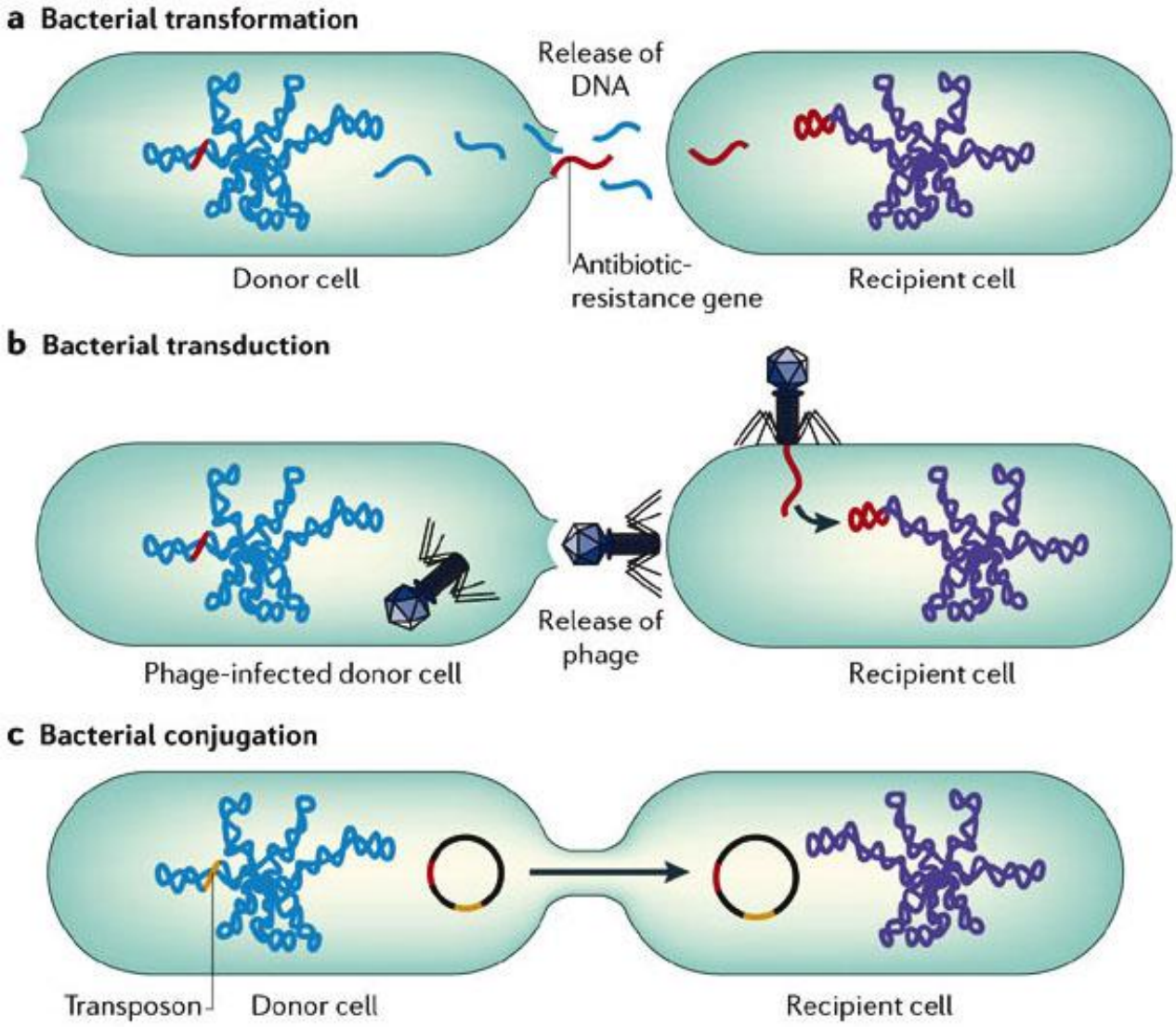
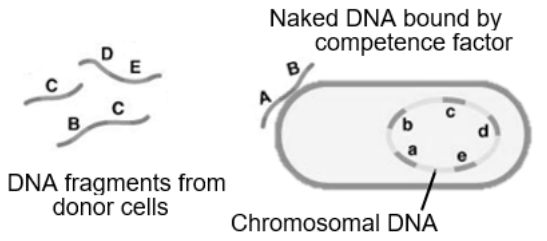
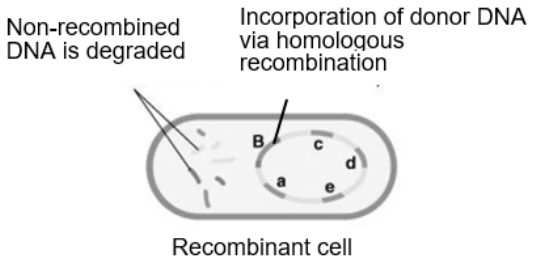


Fig. 4.2 Various ways of bacterial genetic transfer

4.2.1 Transformation

- **<u>Transformation</u>** involves the **uptake** and **incorporation** of foreign DNA acquired from the environment.
    - **<u>Competent</u> bacterial cells** have genes that code for competence factors. Competence factors are cell surface proteins that bind and take up DNA fragments.

Diagram	Description
	1. Foreign DNA fragment of **dead, lysed bacteria (donor cell)** is recognized and bound by **competence factors** on cell surface of the recipient cell.
	2. Upon entry to the cell, the single-stranded DNA fragment <u>aligns itself with homologous regions</u> (similar sequences) of the bacterial genome. It is then **integrated into** recipient cell's genome via **homologous recombination**. 3. Cell is now a **<u>recombinant</u>** cell. Its chromosomes contains DNA derived from two cells. 4. The cell will <u>express</u> the newly incorporated gene and pass it on to offspring during binary fission.

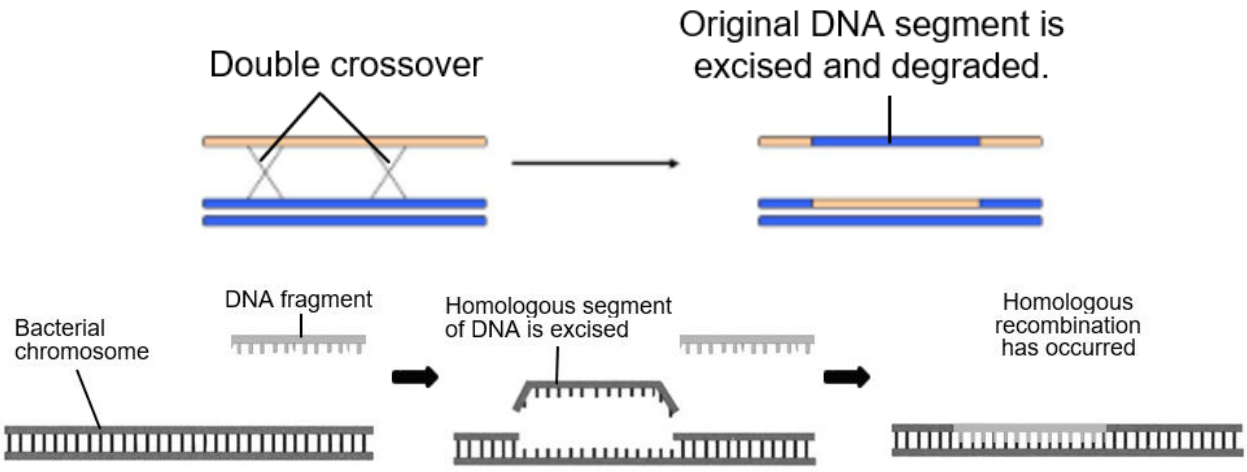


Fig. 4.2.1.1 Homologous recombination.
DNA fragment of similar sequence aligns with bacterial chromosome. Breakage of phosphodiester and hydrogen bonds causes homologous segment to be excised, while the DNA fragment binds to template strand, re-forming of phosphodiester and hydrogen bonds.

CHECKPOINT 4.2.1

Arrange the following stages of bacterial transformation in the correct sequence:

1. Single-stranded DNA integrates into the bacterial genome via homologous recombination.
2. Foreign DNA from a donor bacterium binds to competence factors on the recipient cell surface.
3. The recombinant cell expresses the new gene and passes it on during binary fission.
4. The cell becomes a recombinant with DNA from two sources.

A 2 → 1 → 4 → 3
B 1 → 2 → 3 → 4
C 2 → 3 → 1 → 4
D 4 → 2 → 3 → 1

- **Induced competence**
  - Bacterial cells that do not have competence factors can be induced to be competent via artificial means:
    - Heat shock
      Bacterial cells are incubated with exogenous DNA in cold $CaCl_2$ solution, followed by brief heat shocked to induce formation of transient pores for DNA uptake.
    - Electroporation
      Bacterial cells are subjected to electric shock to create transient pores in the membrane to induce DNA uptake.
  - Induced competence allows scientists to artificially introduce plasmids containing specific genes into bacteria cells for experimental purposes.

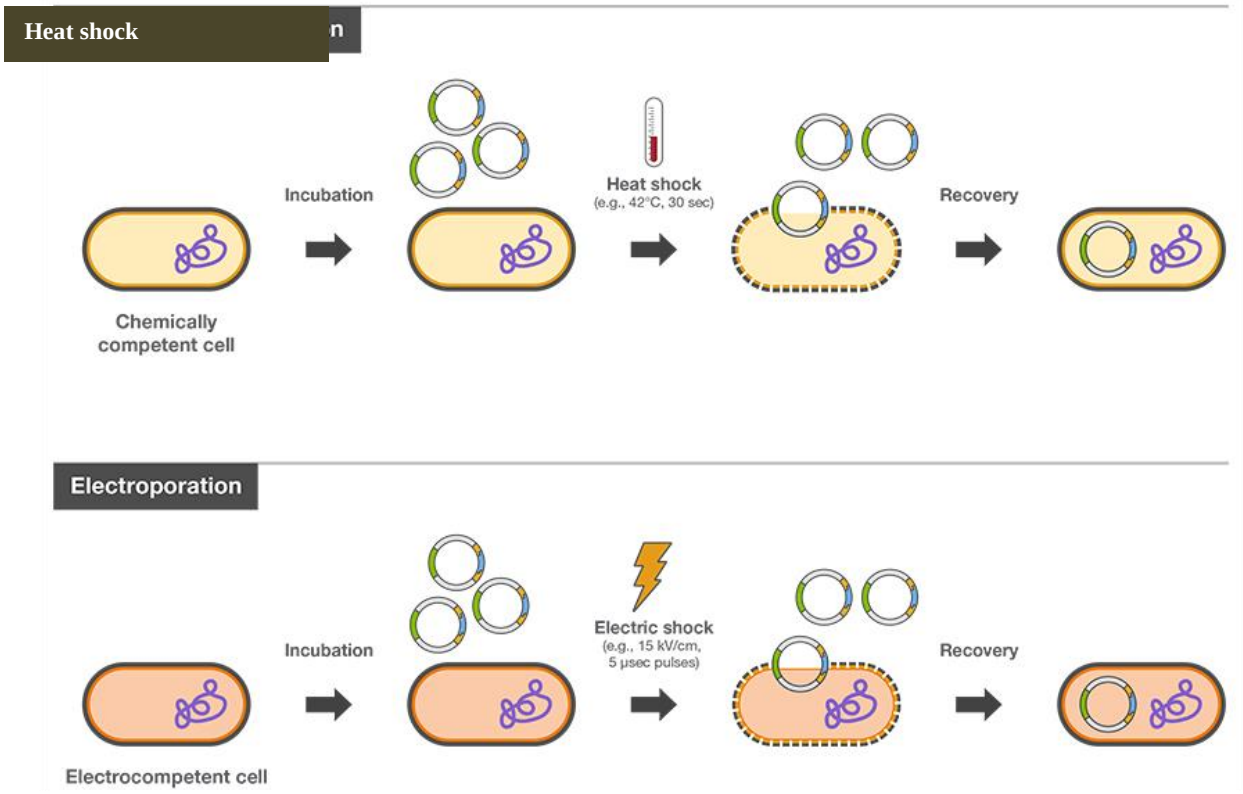


Fig. 4.2.1.2 Induced competence

4.2.2 Transduction

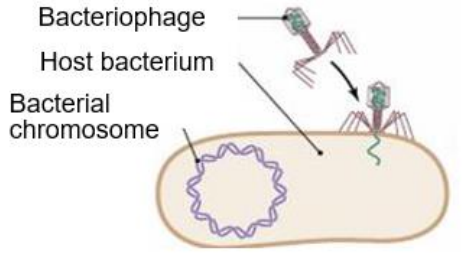
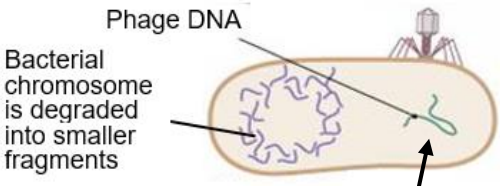
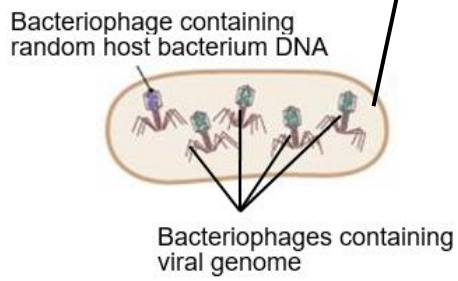
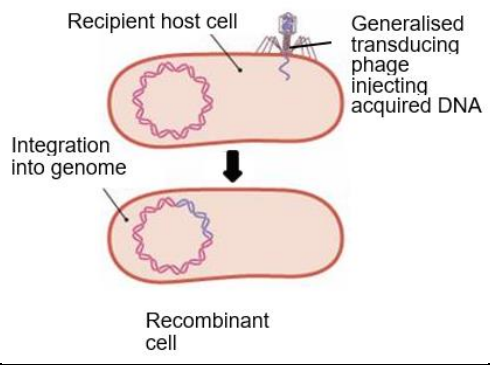
- **<u>Transduction</u>** involves the transfer of DNA taken from a donor cell to recipient cell by **bacteriophages**.

- There are 2 types of transduction:
  (i) Generalised Transduction
  (ii) Specialised Transduction

(i) Generalised Transduction

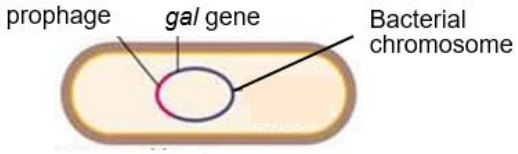
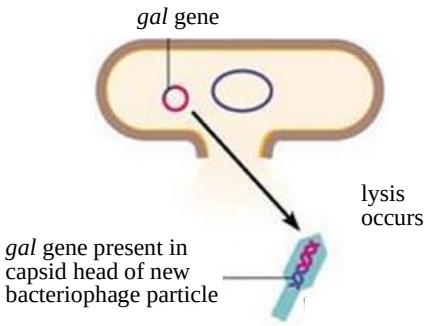
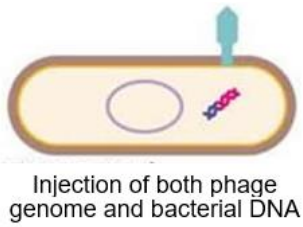
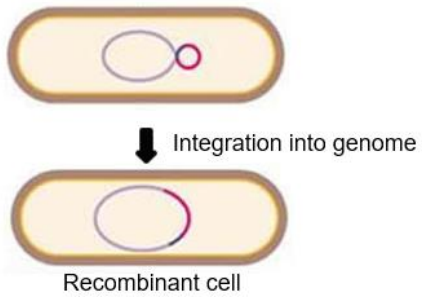
- It occurs primarily through bacteriophages that undergo the **lytic cycle** i.e. virulent phages (e.g. T4 phage).

- It results in the **<u>random transfer</u>** of **<u>any</u> segment of the donor bacterial genome** to the recipient bacterial genome.

Diagram	Description
	***1.*** Bacteriophage (e.g. T4 phage) binds to the host cell receptor and attaches to its host bacterium (e.g. *E. coli*) to **inject its viral genome** into host cell.
	2. Phage **degrades** host bacterial chromosome into small fragments using phage's nuclease.
	3. During assembly of new phages, **<u>random</u> fragments of the lysed bacterial genome** can be **wrongly packaged** into the capsid of the phage. 4. This results in the formation of a transducing phage. Transducing phage is a **defective bacteriophage**.
	5. After lysis of the bacterial host cell, the transducing phage attaches to a new bacterial host bacterium (recipient cell). 6. It then injects its viral DNA, together with fragment of bacterial DNA (donor cell) from the first host, into the second host cell. 7. The bacterial DNA fragment is then integrated into the genome of the recipient cell via the process of **homologous recombination**. This results in **<u>recombinant</u> bacterial cell.**

(ii) Specialised Transduction

- It occurs only through bacteriophages that undergo the **lysogenic cycle** i.e. temperate phages (e.g. lambda phage).
- It results in the transfer of **<u>specific</u> bacterial DNA segments <u>adjacent to the prophage</u>** to a recipient cell.

Diagram	Description
	1. Bacteriophage (e.g. lambda phage) attaches to its host bacterium and injects its viral genome into host cell. 2. Phage DNA is **integrated** into the bacterial genome, forming a **prophage** and enters the lysogenic cycle.
	3. When prophage is **spontaneously induced**, it enters the lytic cycle. 4. The prophage is excised. However, **incorrect excision** sometimes occurs resulting in **host bacterial gene(s) <u>adjacent</u> to it** (e.g. *gal* gene) being **excised together**. 5. Both **phage DNA and bacterial genes** are **assembled into the capsid** resulting in the formation of transducing phage.
	6. After lysis, the released transducing phages attach to a new host bacterium (recipient cell). 7. It then injects its viral DNA, together with bacterial gene (e.g. *gal* gene) from the first host, into the second host cell.
	8. The **viral genome and the bacterial gene** from the first host are then **integrated into the genome** of the recipient cell at the prophage insertion site. 9. This results in **<u>recombinant</u> bacteria**.

CHECKPOINT 4.2.2

1 Arrange the following stages of generalized transduction in the correct sequence:

1. Random fragments of bacterial DNA are packaged into the phage capsid during new phage assembly.
2. The phage injects its viral genome into the bacterial host cell.
3. Phage attaches to a new bacterial host and injects both viral and donor bacterial DNA.
4. Host bacterial chromosome is degraded by the phage's nuclease.
5. The bacterial DNA fragment is integrated into the recipient genome through homologous recombination, leading to recombinant bacterial cell.

A 4 → 1 → 2 → 5 → 3
B 2 → 4 → 1 → 3 → 5
C 1 → 4 → 2 → 3 → 5
D 2 → 3 → 4 → 1 → 5

2 Arrange the following stages of specialized transduction in the correct sequence:

1. The phage injects the viral and bacterial DNA into a new host bacterium.
2. Excision of the prophage from the bacterial genome occurs, sometimes taking adjacent bacterial genes along with it.
3. The phage integrates its DNA into the bacterial chromosome, forming a prophage and entering the lysogenic cycle.
4. The bacterial DNA is incorporated into the new host's genome, leading to recombination.
5. Viral and bacterial DNA are packaged into a newly formed phage.

A 2 → 3 → 5 → 1 → 4
B 3 → 2 → 5 → 1 → 4
C 3 → 5 → 2 → 4 → 1
D 5 → 2 → 3 → 1 → 4

3 Which of the following statements about transduction in bacteria is correct?

A. In generalized transduction, only bacterial genes adjacent to the prophage integration site are transferred.
B. Specialized transduction can transfer any random fragment of the bacterial chromosome.
C. In specialized transduction, excision of the prophage sometimes carries adjacent bacterial genes into new host cells.
D. Generalized transduction requires the phage to undergo the lysogenic cycle to transfer bacterial DNA.

3

4.2.3 Conjugation

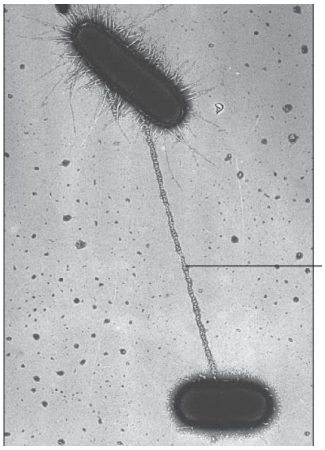
- **Conjugation** involves the **unidirectional** transfer of a **F plasmid** via direct contact from F^+ cell to F^- cells.
    - F+ cells contain F plasmid, also known as donor cells
    - F^- cells do not contain F plasmid, also known as recipient cells

- The **F plasmid** contains genes which are not necessary for survival but **confer advantages**.

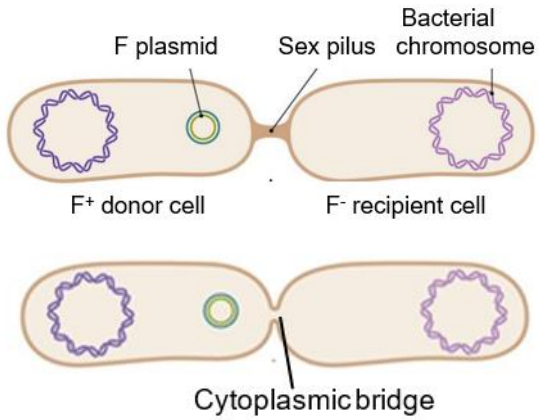
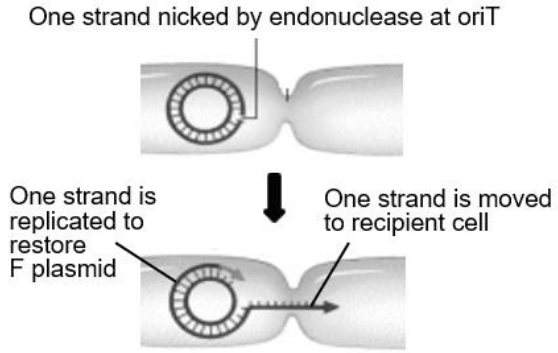
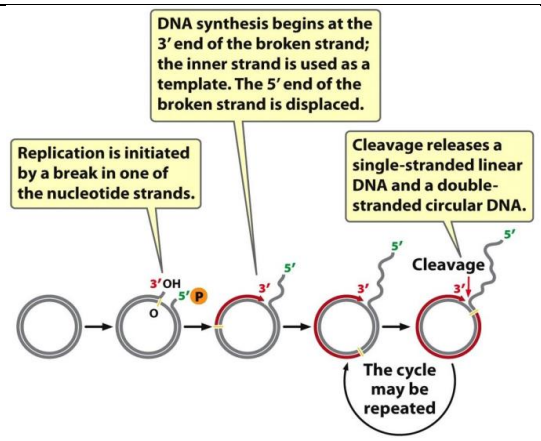
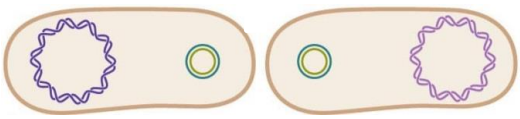
    ✓ **fertility (F) factor**
        - F factor enables the bacterium to produce the **sex pilus** (plural: pili) which is a protein appendage that attaches the donor to the recipient cell for direct cell-to-cell contact.

    ✓ gene that expresses surface **proteins** on the F^+ cell
        - Sex pilus will not attach to other F^+ cells due to the presence of such proteins. This phenomenon known as surface exclusion.

    ✓ origin of replication
        - F plasmid can control its replication and replicates by **rolling-circle mechanism**.



Process of Conjugation

Diagram	Description
	1 **F^+ cell** which contains the F plasmid, synthesizes the **sex pilus**. It then uses sex pilus to attach to **F^- cell.** 2 The sex pilus then retracts and pulls both cells together. A temporary **cytoplasmic bridge** is then formed between the cells.
	3 Double stranded F plasmid **breaks** at **specific point** 4 One strand moves from **donor cell** to **recipient cell** via **temporary cytoplasmic bridge**
	5 As transfer of the single strand F plasmid continues, the DNA strand which remains in the donor cell is replicated via **rolling circle mechanism**. 6 The single strand **F plasmid transferred to the recipient act as template**, synthesizing a daughter strand via complementary base pairing. *For more information about the rolling circle mechanism, and the replication of DNA, refer to the enrichment section.
 Both cells possess a complete copy of the F plasmid; both are now F^+ cells and can act as donor cells	7 Double-stranded plasmid in recipient cell will **circularise.** The F^- cell now contains a F plasmid and is therefore converted to a F^+ cell. It can now act as the donor cell.

CHECKPOINT 4.2.3

1 Conjugation results in the production of

A F^+ cell from a F^- cell
B F^+ cell from a F^+ cell
C Sex pilus
D F plasmids

2 Arrange the following stages of bacterial conjugation in the correct sequence:

1. The F+ cell synthesizes a sex pilus, which attaches to the F– cell.
2. One strand of the F plasmid moves from the donor cell to the recipient cell via a cytoplasmic bridge.
3. The single-stranded F plasmid is used as a template to synthesize a complementary strand via the rolling circle mechanism.
4. The F+ cell retracts the pilus, pulling the cells together, and a cytoplasmic bridge forms.
5. The recipient cell now contains a double-stranded F plasmid and becomes an F+ cell.

A 1 → 2 → 4 → 3 → 5
B 1 → 4 → 2 → 3 → 5
C 1 → 4 → 2 → 3 → 5
D 2 → 1 → 4 → 3 → 5

3 There is only 1 strand of DNA in the F plasmid in both the donor and recipient cell after conjugation.

TRUE FALSE

4 The table shows some aspects of conjugation in bacteria.

Which combination describes conjugation?

	Presence of F plasmid in bacterium	Presence of pili on bacterium	Role of F^+ bacterium in conjugation	Type of DNA donated or received
A	✓	✓	Donates F plasmid	Single stranded
B	✓	×	Donates F plasmid	Double stranded
C	×	✓	Receives F plasmid	Single stranded
D	×	×	Receives F plasmid	Double stranded

Learning Outcome 2(i) [Modified]

Explain how gene expression in prokaryotes can be regulated, through the concept of simple operons (including *lac* and *trp* operons), including the role of regulatory genes.

5. Regulation of Gene Expression in Bacteria

Gene expression is the process whereby a gene that turned on is being transcribed into RNA and translated into specific protein molecules. The amount of protein that a cell expresses depends on the metabolic or physiological state of the cell.

Regulation of gene expression in bacterial cells serves to conserve energy and resources as only the genes whose products are needed by the cell are expressed. This helps the bacteria adapt to the environmental changes. Bacteria regulate gene expression by increasing, decreasing or preventing **transcription** and **translation** of genes.

5.1 The Operon Model

- An operon consists of a cluster of genes that code for enzymes in a related metabolic pathway under the transcriptional control of a single promoter and operator.

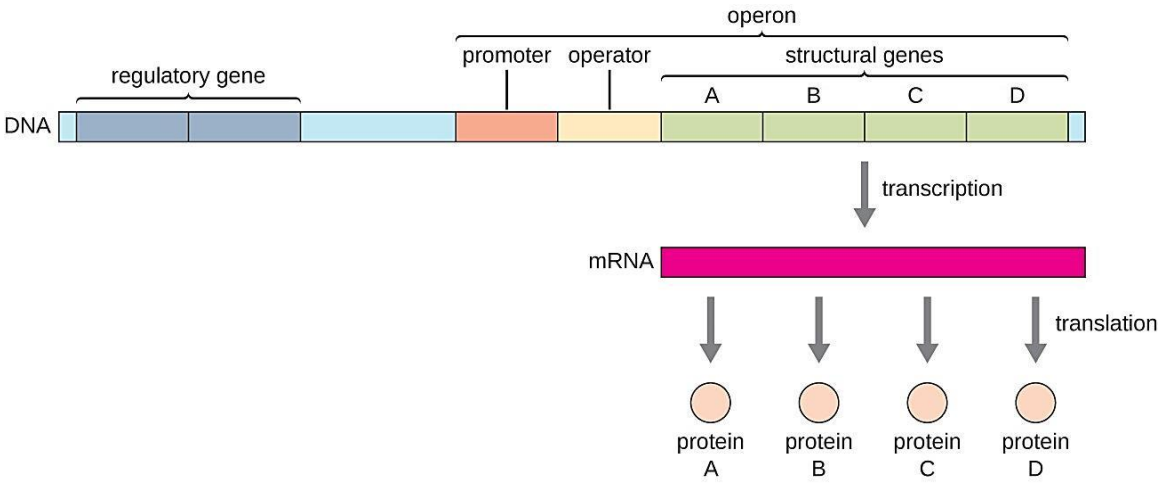


Fig. 5.1 An operon

An **operon** consists of 3 elements:

i. Promoter

- Located upstream of both operator and structural genes.
- A region of DNA sequence where RNA polymerase binds to initiate transcription of structural genes.

ii. Operator

- Located between the promoter and structural gene OR within the promoter
- A region of DNA sequence where active repressor protein binds to inhibit transcription of structural genes.
    - When a repressor protein is bound to the operator, RNA polymerase cannot bind to the promoter, transcription is inhibited, operon is switched off.
    - When a repressor protein is not bound to the operator, , RNA polymerase can bind to the promoter, transcription can occur, operon is switched on.

iii. Structural gene(s)

- A gene that codes for RNA or protein product (e.g. transport proteins, enzymes etc.) other than a regulatory protein.
- Several structural genes are grouped into one operon.

iv. Regulatory genes

- Codes for regulatory proteins that can either inhibit or enhance the binding of RNA polymerase at the promoter when they bind to the operator.
- Regulates the transcription of structural genes is located outside (usually upstream of) the operon.

Advantages of the Operon Model

1. Allows bacterial cells to **rapidly respond** to changes in the environment e.g. presence of respiratory substrates like lactose.
    - Expression of structural genes in the same operon is under control of a single promoter. Clustering genes of related function into one transcriptional unit allows for a single "on-off" switch to control the group of genes.
    - This allows for coordinated gene expression where proteins performing related functions (e.g. enzymes in the lactose metabolic pathway) are synthesised together when required.
    - This increases the speed of response of the bacterial cell to changes in its environment.

2. Aids in **efficient use of cellular resources** by bacterial cells.
    - As it allows the bacterium to produce proteins (e.g. β-galactosidase) required by the cell only when the environmental condition requires them.
    - This prevents wastage of energy and resources and provides a selective advantage for the bacterium during natural selection.

CHECKPOINT 5.1

1 Which of the following statements about operons is correct?

A The operator is located upstream of the promoter and controls the binding of RNA polymerase.
B A repressor protein binds to the structural gene to stop transcription
C The promoter is a DNA sequence where RNA polymerase binds to initiate transcription of structural genes.
D Structural genes are regulatory proteins that control the transcription of the operon.

2 Which of the following statements correctly describes an advantage of the operon model in bacteria?

A Operons allow the simultaneous transcription of multiple regulatory genes, ensuring more control over gene expression.
B Operons enable bacteria to regulate the expression of a cluster of genes that are involved in unrelated metabolic pathways.
C Operons allow bacteria to efficiently coordinate the expression of multiple genes involved in the same metabolic pathway, saving energy and resources.
D The operon model ensures that all genes are constantly transcribed, regardless of environmental conditions.

5.2 Repressible Operon e.g. tryptophan operon (*trp* operon)

- The *trp* operon controls the synthesis of tryptophan (an amino acid) from its precursor molecule

- The *trp* operon is a **repressible operon**
    - In repressible operons, the operon is **normally turned on** as the regulatory protein is synthesised in the inactive form.

    - When the regulatory protein is activated, operon **can be turned off**, thus making the operon "repressible"

    - **Significance** of having repressible operons
        - Mainly found in anabolic pathways (synthesis)
        - Synthesis of biomolecules are needed for survival (hence operon normally on)
        - Need to avoid over-production of biomolecules to prevent wastage (switch OFF)

    - *trp* operon is **usually switched on** and controls some **anabolic pathways**.

- The *trp* operon is subjected to **negative gene regulation**

Negative gene regulation is the type of control in which the active form of regulatory protein (i.e. repressor) turns the operon OFF

    - The trp operon's repressor protein is synthesised in an **inactive form**.
        - It is activated when a co-repressor binds to it.
            - The co-repressor is usually an end-product of the metabolic pathway coded by the operon.
        - The activated repressor protein binds to the operator and turn the operon off

(a) Structure of *trp* operon

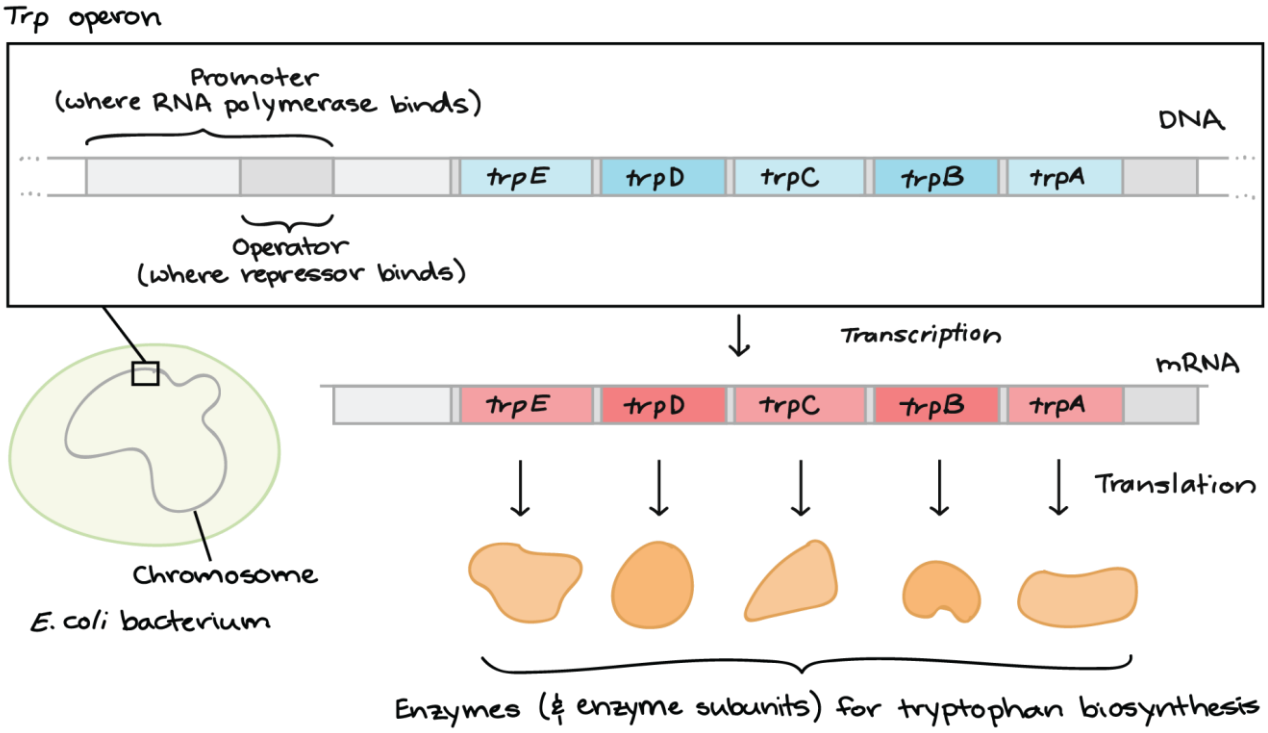


Fig. 5.2.1 Structure of the trp operon

- The *trp* operon consists of:

i. Promoter

ii. Operator

- A region of DNA sequence where active repressor protein (repressor protein which is bound to co-repressor) binds to prevent transcription.

iii. Structural genes

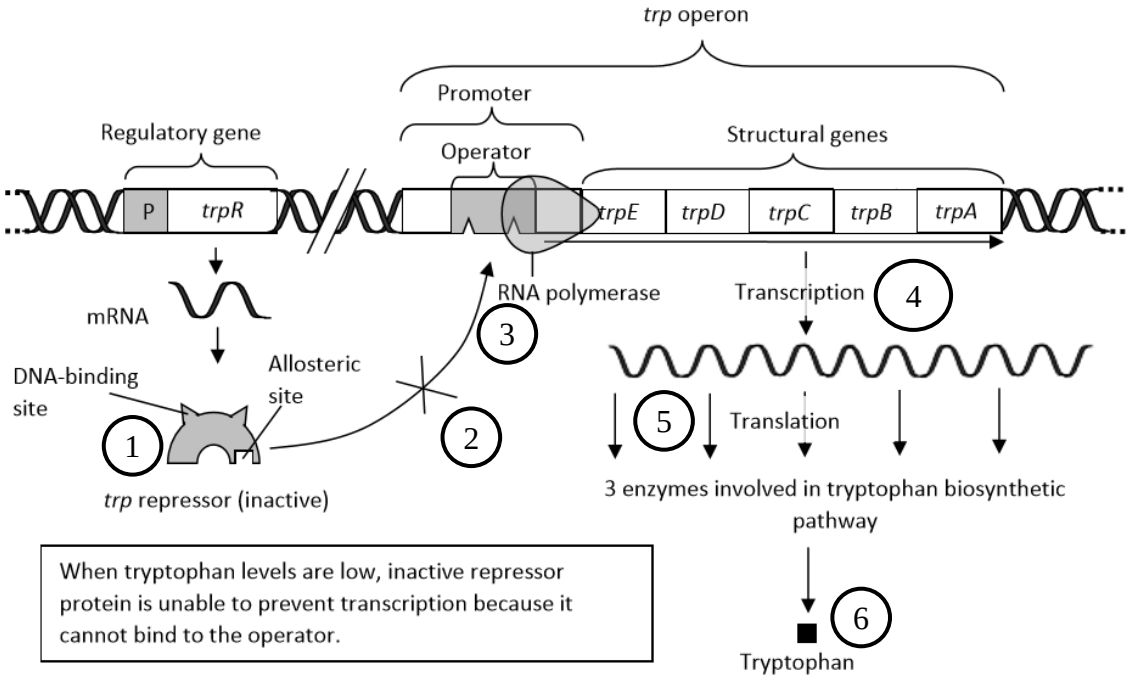
- *trpE*, *trpD*, *trpC*, *trpB*, *trpA* (in this sequence)
- Codes for 5 enzymes involved in the synthesis of tryptophan

Regulatory gene (outside the operon, **NOT part of operon**)

- *trpR* gene
- Codes for an inactive *trp* repressor protein i.e. cannot bind to the operator (on its own)

(b) When tryptophan is ABSENT

1. *trp* repressor is in the ……. form and ……. bind to the operator
2. RNA polymerase ……. bind to promoter
3. Genes of the *trp* operon ……. be transcribed
4. *trp* operon is turned …….
5. Enzymes that synthesize the tryptophan ……. be produced
6. Tryptophan ……. be synthesised



NOTE: The default state of the repressible *trp* operon is "ON"

Fig. 5.2.2 *Trp* operon

(c) When tryptophan is PRESENT

1. **tryptophan accumulates,** acts as a …….. and binds to ……. trp repressor
2. *trp* repressor is in the ……. form and ……. bind to the operator
3. RNA polymerase ……. bind to promoter
4. Genes of the *trp* operon ……. be transcribed
5. *trp* operon is turned …….
6. Enzymes that synthesize the tryptophan ……. be produced
7. Tryptophan ……. be synthesised

**This ensures that the bacterium does not produce the five enzymes when nutrient tryptophan is already available. Hence, this prevents wastage of energy and resources. (This mechanism is a form of end-product inhibition)*

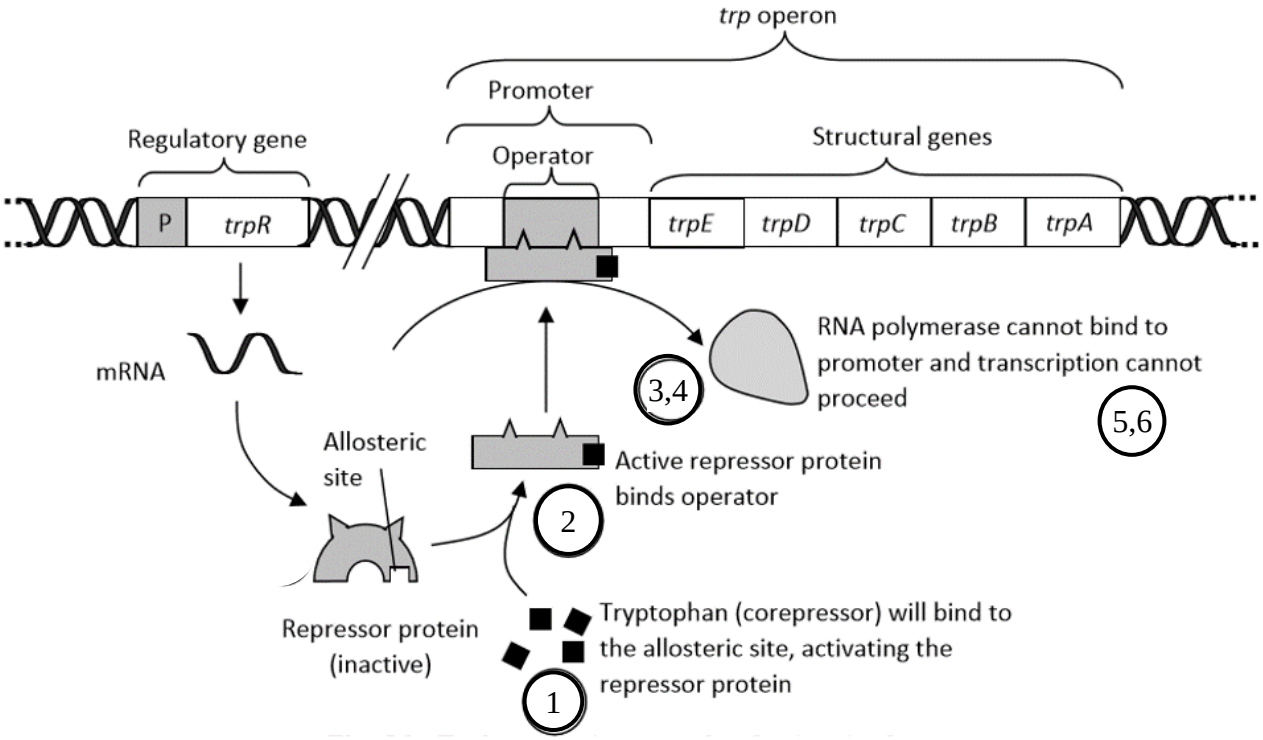


Fig. 5.2.3 *Trp* operon (with excess Tryptophan)

CHECKPOINT 5.2

1 What happens when tryptophan in **absent**?

1. RNA polymerase binds to the promoter.
2. The trp repressor is inactive and cannot bind to the operator.
3. The trp operon is turned on.
4. Genes of the trp operon can be transcribed.
5. Enzymes for tryptophan synthesis are produced.
6. Tryptophan is synthesized.

A 2 → 1 → 4 → 3 → 5 → 6
B 3 → 2 → 1 → 5 → 4 → 6
C 1 → 4 → 2 → 3 → 6 → 5
D 2 → 1 → 3 → 5 → 6 → 4

2 What happens when tryptophan in **present**?

1. Tryptophan accumulates and binds to the inactive trp repressor, acting as a co-repressor.
2. The trp operon is turned off.
3. RNA polymerase cannot bind to the promoter.
4. Enzymes for tryptophan synthesis cannot be produced.
5. The trp repressor is in the active form and binds to the operator.
6. Tryptophan cannot be synthesized.
7. Genes of the trp operon cannot be transcribed.

A 1 → 2 → 3 → 4 → 5 → 6 → 7
B 5 → 1 → 3 → 7 → 6 → 2 → 4
C 3 → 2 → 5 → 4 → 6 → 1 → 7
D 1 → 5 → 3 → 7 → 2 → 4 → 6

3 Which of the following statements correctly describes how trp operon is regulated in E. coli?

A In the absence of tryptophan, the trp operon is turned off by a repressor protein binding to the operator, preventing RNA polymerase from initiating transcription.
B In the presence of tryptophan, the trp operon is turned on, allowing the synthesis of enzymes needed to produce tryptophan.
C The trp operon is negatively regulated, meaning it is turned off when tryptophan binds to the repressor, activating it and allowing it to block transcription.
D The trp operon is positively regulated, and transcription only begins when tryptophan binds to the repressor

5.3 Inducible Operon e.g. lactose operon (*lac* operon)

- The *lac* operon controls the synthesis of enzymes involved in the **hydrolysis of lactose** to glucose (a respiratory substrate) and galactose.

- The lac operon is an **inducible operon.**
    - In inducible operons, the operon is **normally turned off** as the regulatory protein is synthesised in the active form.

    - When the regulatory protein is inactivated, operon can be **turned on**, thus making the operon "inducible"

    - Significance of Inducible Operons
        - Mainly found in catabolic pathways (breakdown)
        - Synthesis of biomolecules are not needed all the time (hence operon normally off)
        - Production of biomolecules on a needs basis (can be switch ON)

    - Lac operon is **usually switched off** and controls some **catabolic pathways**

- Regulation of the *lac* operon is via a **dual-regulation mechanism**:
    - Negative Regulation (through the *lac* repressor protein)
        - Repressor protein is synthesised in an **active** form, which usually binds to operator and prevents transcription.
            - It is **deactivated** when an **inducer** binds to it.
            - The **inducer** is usually a substrate of the metabolic pathway coded by the operon.
    - Positive Regulation (through catabolite activator protein)

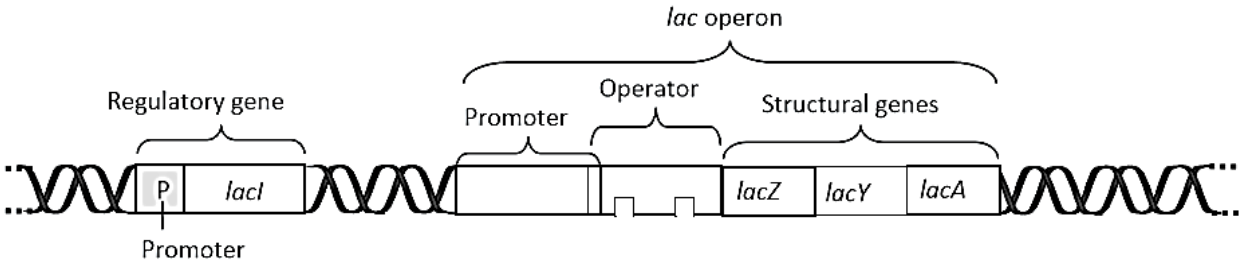


Fig. 5.3.1 Organisation of the *lac* operon and its associated regulatory gene

(a) Structure of *lac* operon

- The *lac* operon controls the **catabolism (breakdown) of lactose** (a respiratory substrate) found in the environment of the bacteria.

- The *lac* operon consists of

i. Promoter

ii. Operator

- A region of DNA sequence where **active repressor protein binds** to prevent transcription.

iii. Structural genes

- *lacZ gene which* codes for **β-galactosidase** which breaks down lactose into glucose and galactose. β-galactosidase also converts lactose to allolactose
- *lacY gene which* codes for **lactose permease**, a protein transporter involved in active transport of lactose into the bacterium from the external environment
- *lacA gene which codes for* enzyme **transacetylase** that may be involved in the removal of toxic by-products from the metabolism of lactose

iv. Regulatory gene (outside the operon, **NOT part of operon**)

- *lacI* gene
- which codes for the lac repressor protein which is made in the active form i.e. can bind to the operator

(b) Scenario N1: When Lactose is ABSENT (negative regulation)

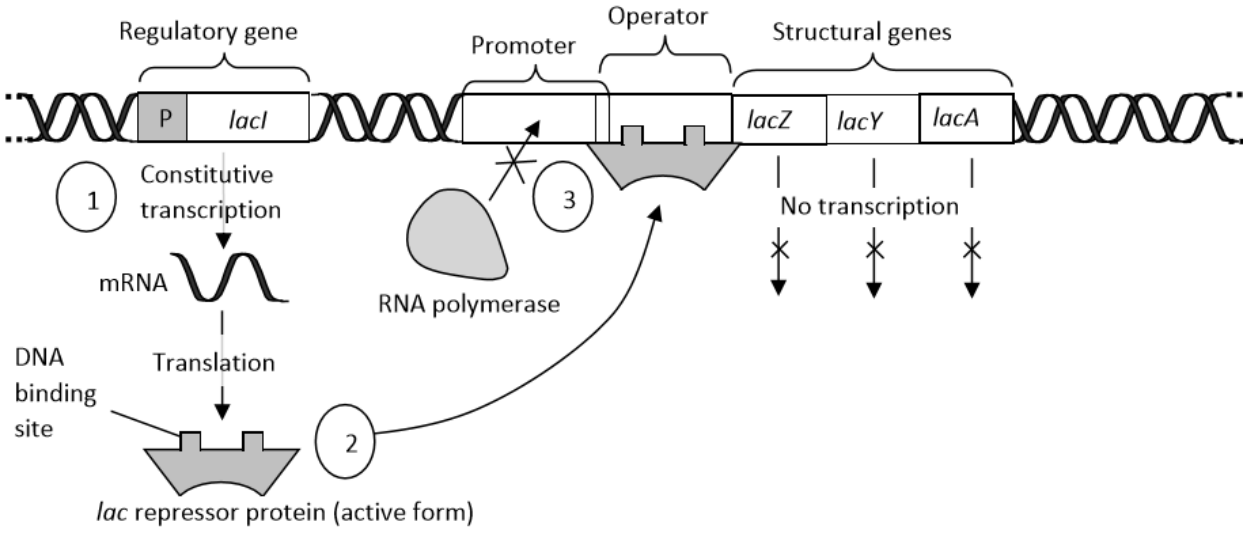


Fig. 5.3.2 Negative regulation of *lac* operon when lactose is absent

1. *lacI* is **……..**, producing lac repressor proteins.
2. *lac* repressor is synthesized in its **……..** conformation.
3. *lac* repressor **……..** bind to the operator.
4. RNA polymerase **……..** bind the promoter.
5. Genes of *lac* operon **……..** be transcribed.
6. Operon is switched **……..**
7. Enzymes for lactose metabolism are **……..** produced.

   *which helps conserve resources since lactose is not present in the environment

NOTE: The binding of the repressor is weak and dissociates at times, resulting in a basal level of expression of *lac* operon structural genes, producing a low amount of galactosidase, permease and transacetylase within the cell. The presence of a low level of these enzymes is necessary for regulation of the lac operon.

(c) Scenario N2: When Lactose is PRESENT (negative regulation)

- Apart from the DNA-binding site, the *lac* repressor protein contains another binding site known as the **allosteric site**. The allosteric site is required for the binding of **allolactose**, a structural isomer from lactose.

- In the <u>presence of lactose</u>, a few molecules of lactose will enter the cell with the help of **permease**

- These lactose molecules are <u>converted to allolactose</u> by the few **β-galactosidase** molecules present.

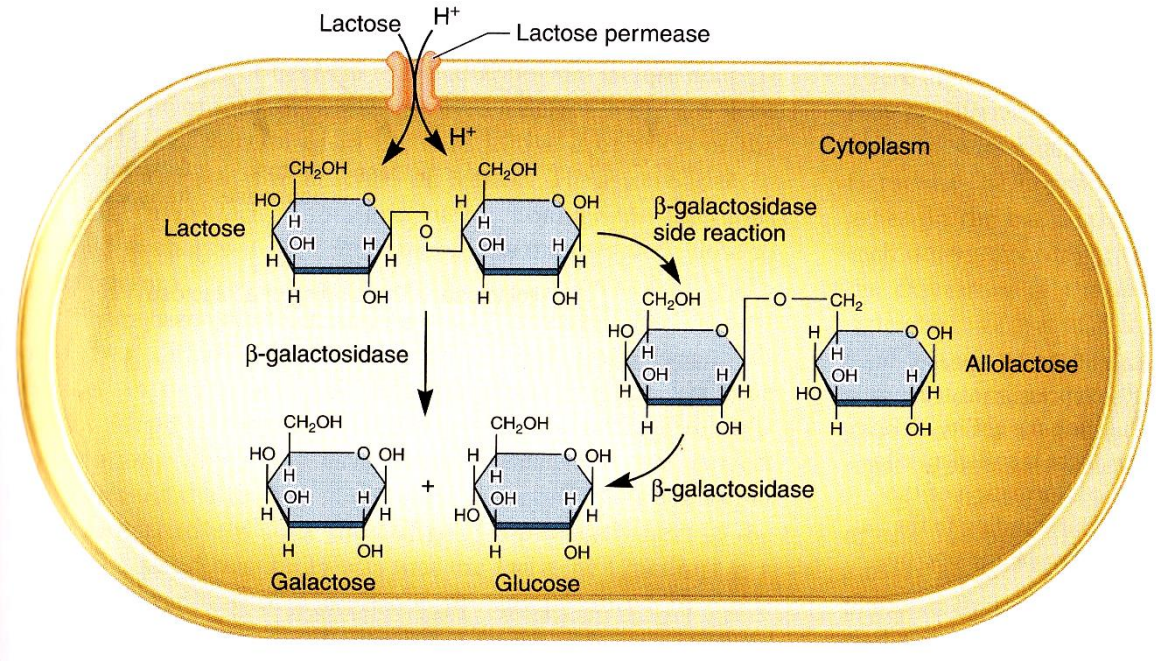


Fig. 5.3.3 Functions of permease and β-galactosidase

NOTE: A **basal level of expression** of the *lac* operon is present even when lactose is absent from the environment. This enables uptake and metabolism of lactose once it is present in the environment.

→ basal level of expression allows for some permease and β-galactosidase to be present in the cell

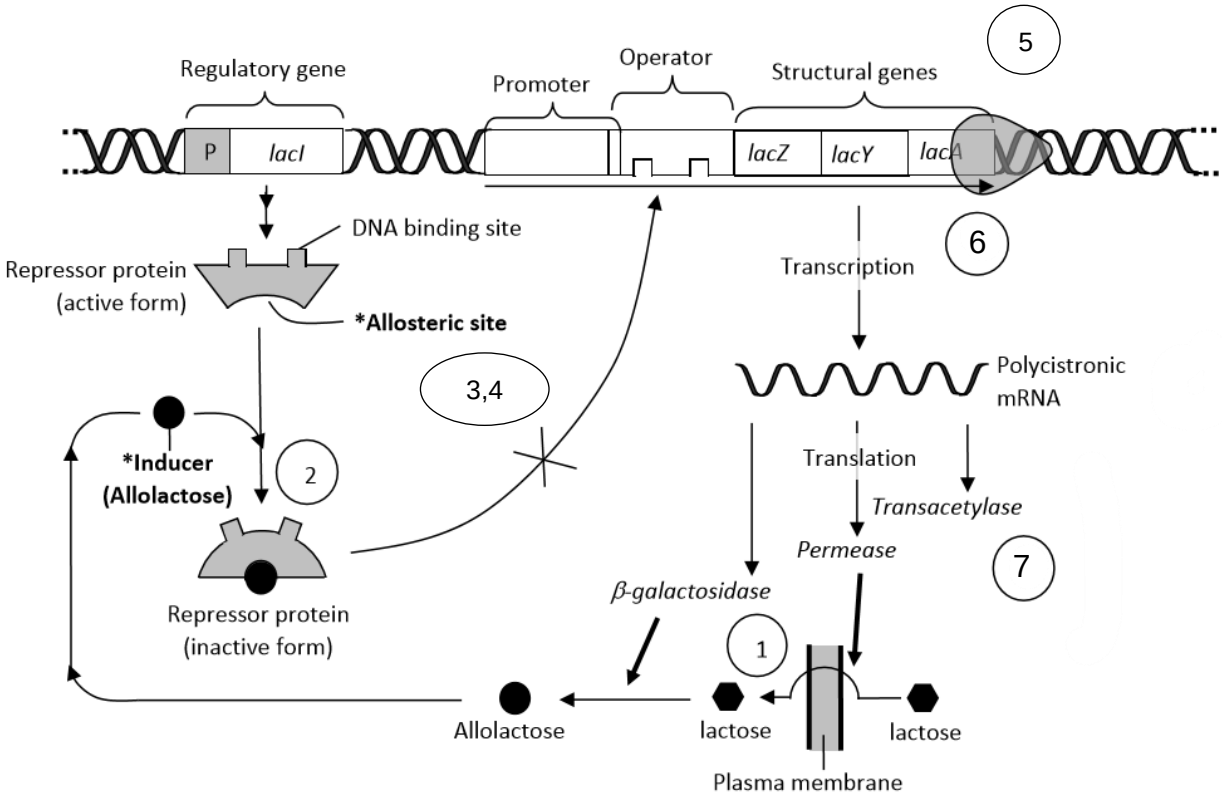


Fig. 5.3.4 Regulation of *lac* operon when lactose is present

1. Lactose can **enter** the cell due to the presence of a few molecules of …….
   Lactose is subsequently converted to ……. by …….……..
2. ……. binds to the ……. site on the lac repressor, causing it to change to an ……. conformation.
3. *lac* repressor ……. bind to the operator of the lac operon.
4. RNA polymerase ……. bind the promoter.
5. Genes of lac operon ……. be transcribed.
6. Operon is switched ……..
7. Enzymes for lactose metabolism ……. produced.
8. Lactose in the environment can be hydrolysed.

CHECKPOINT 5.3

1 Arrange the following steps in the correct sequence when lactose is ABSENT.

1. Enzymes for lactose metabolism are not produced.
2. lac repressor is synthesized in its active conformation.
3. RNA polymerase cannot bind the promoter.
4. lacI is active, producing lac repressor proteins.
5. Operon is switched off.
6. lac repressor can bind to the operator.
7. Genes of lac operon cannot be transcribed.

A 4 → 2 → 6 → 5 → 1→ 3 → 7
B 4 → 2 → 6 → 3 → 7 → 5 → 1
C 2 → 4 → 6 → 3 → 5 → 7 → 1
D 4 → 6 → 2 → 3 → 7 → 1 → 5

2 Arrange the following steps in the correct sequence when lactose is **present**.

1. Operon is switched on.
2. Allolactose binds to the allosteric site on the lac repressor, causing it to change to an inactive conformation.
3. Lactose in the environment can be hydrolysed.
4. Genes of lac operon can be transcribed.
5. Lactose can enter the cell due to the presence of a few molecules of permease. Lactose is subsequently converted to allolactose by β-galactosidase.
6. Enzymes for lactose metabolism are produced.
7. lac repressor cannot bind to the operator of the lac operon.
8. RNA polymerase can bind the promoter

A 5 → 7 → 2 → 4 → 6 → 1 → 3 → 8
B 5 → 2 → 7 → 8 → 6 → 3 → 1 → 4
C 5 → 2 → 7 → 8 → 4 → 1 → 6 → 3
D 2 → 5 → 3 → 8 → 7 → 4 → 6 → 1

3 Which of the following statements about the lac operon is **correct?**

A The lac operon is a repressible operon, meaning it is usually turned on until it is actively repressed.
B In the lac operon, the regulatory protein is synthesized in an inactive form and becomes active only in the presence of an inducer.
C The lac operon is an inducible operon that is typically off, becoming active only when the repressor protein is inactivated by an inducer.
D The lac operon regulates anabolic pathways, where the production of complex molecules is required continuously.

(d) Positive Regulation of lac operon via CAP and cAMP

- *E. coli* and many other bacteria will **metabolise glucose preferentially** in the **presence of lactose** and other sugars.
    - When **glucose is available with lactose**, studies show that *E. coli* metabolises glucose and represses the use of lactose.

- Presence or absence of glucose will therefore determine the rate that the lac operon is expressed through the action of the **catabolite activator protein (CAP)**.↓
    - CAP is an activator of the lac operon.
    - CAP binds directly to the promoter via its DNA binding site to initiate the transcription of structural genes.
    - CAP is synthesised in the **inactive** form.

- A small organic molecule, cyclic AMP (cAMP) can bind to CAP's allosteric site, activating CAP.
    - cAMP is an allosteric activator of CAP
    - When glucose concentration increases, cAMP concentrations decrease
        - cAMP is synthesised from ATP by **adenylyl cyclase**.
        - **Glucose inhibits adenylyl cyclase** → reduces amount of cAMP present → Reduces the amount of cAMP-CAP complex present.

High glucose concentration

Low glucose concentration

*Recall that CAP is a activator protein, binding to the promoter promotes transcription

- The lac operon can also undergo positive **regulation.**

Positive gene regulation is the type of control in which the active form of regulatory protein (i.e. activator) turns the operon ON

    - since the active CAP protein binds to the promoter to initiate transcription, positive regulation takes place.

(e) Scenario P1: When Glucose is ABSENT (and lactose is present)

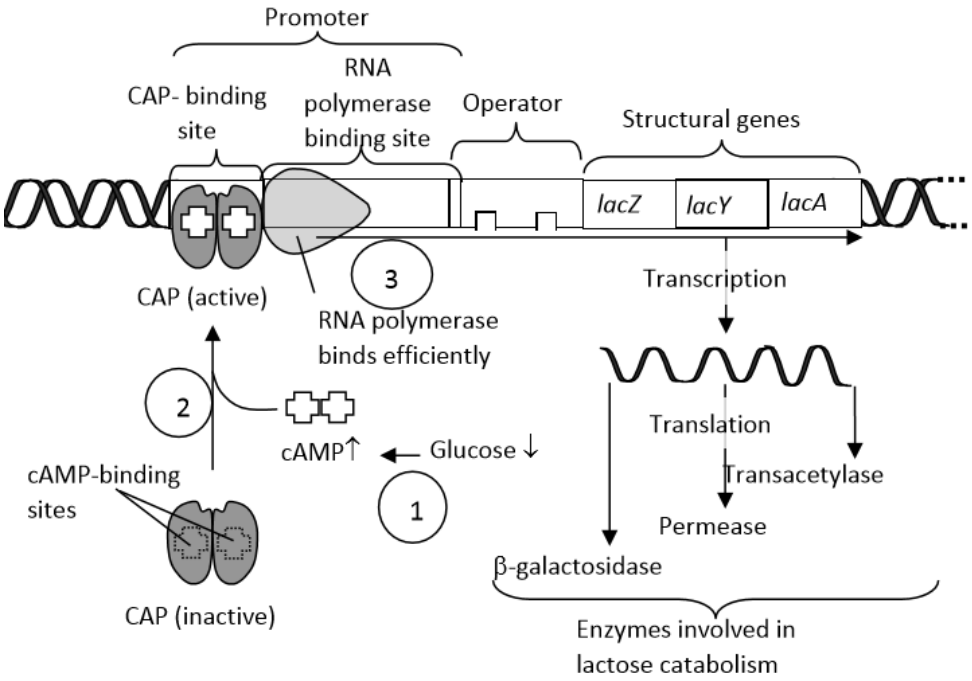


Fig. 5.2.3.5 Transcription of *lac* operon structural genes in the absence of glucose

1. Low glucose concentrations, adenylyl cyclase is **……**, cAMP can be synthesised.
2. cAMP concentration **……** in the cell.
3. cAMP will bind to CAP, causing it to change to an **……** conformation.
4. CAP **……** to *lac* promoter.
5. **……** affinity of RNA polymerase for promoter.
6. **……** rate of transcription of *lac*Z, *lac*Y, and *lac*A genes.

***This scenario complements scenario N2 above, enhancing the binding of RNA polymerase and increased transcription of genes that hydrolyse lactose. This leads to metabolism of lactose.**

(f) Scenario P2: When Glucose is PRESENT (and lactose is present)

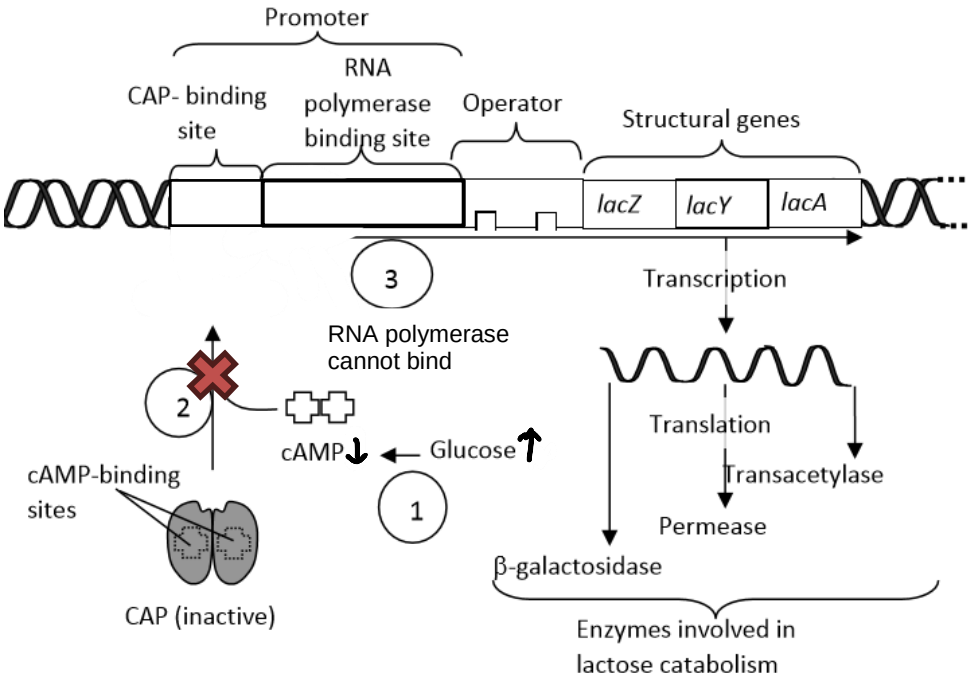


Fig. 5.2.3.6 Transcription of lac operon in the presence of glucose

1. High glucose concentrations ..... adenylyl cyclase and the synthesis of cAMP
2. cAMP concentration ..... in the cell
3. ..... cAMP will bind to CAP, causing CAP to change to an ..... conformation.
4. CAP ..... bind to lac promoter.
5. .....affinity of RNA polymerase for promoter.
6. ..... rate of transcription of *lac*Z, *lac*Y, and *lac*A genes.

***Even though scenario N2 occurs simultaneously with P2, this scenario does not complement scenario N2 as it prevents the efficient binding of RNA polymerase. When RNA polymerase cannot bind well, rate of transcription of structural genes coding for the hydrolysis of lactose decreased. This leads to the preferential metabolism of glucose.**

- This ensures that the preferred carbon source, glucose, is utilised before other alternative carbon sources are used.

- Hence, the *lac* operon is under **dual control**, and can exhibit both negative control by the *lac* repressor and positive control by CAP.

CHECKPOINT 6

1. Summary of *trp* operon (negative regulation)

Tryptophan	*trp* repressor	Bound to operator?	State of *lac* operon
Absent			
Present			

2. Summary of *lac* operon (negative regulation)

Lactose	*lac* repressor	Bound to operator?	State of *lac* operon
Absent			
Present			

3. Summary of *lac* Operon (positive regulation)

Lactose	Glucose	[cAMP]	CAP	State of *lac* operon
Absent	High Concentration			
Present	Low Concentration			

Learning Outcome 2(i) [Modified]
Distinguish between inducible and repressible systems

5.4 Comparing inducible and repressible systems

Type of negative operons	Inducible operon	Repressible operon
Named example	***lac*** operon	***trp*** operon
Default operon expression	Switched **off**	Switched **on**
Type of metabolic pathway	**Catabolic**, e.g. breakdown of *lac*tose	**Anabolic**, e.g. synthesis of tryptophan
Usual state of repressor	**Active** and is bound to the operator, preventing the initiation of transcription by blocking RNA polymerase from binding to the promoter	**Inactive** and does not bind to the operator, allowing transcription initiation when RNA polymerase to bind to the promoter
Effector molecule bound to repressor	**Allo*lac*tose** binds to repressor to inactivate it, by changing it to its inactive conformation. The repressor can no longer bind to the operator, **allowing transcription initiation** when RNA polymerase binds to the promoter.	**Co-repressor (tryptophan**) binds to the repressor to activate it by changing it to its active conformation. The repressor can now bind to the operator, **preventing the initiation of transcription** by blocking RNA polymerase from binding to the promoter.
Conditions under which transcription of structural genes occurs	Availability of substrates	Insufficient amounts of end product
Significance	Ensures that enzymes are only synthesised when required, thus **conserving energy and resources**	Ensures that resources are not used to synthesise products already present in sufficient quantities, thus **conserving available energy and resources**

GLOSSARY OF TERMS FOR OPERON

Repressible Operons (e.g. trp operon)	Inducible Operons (e.g. lac operon)
DEFINITION • In repressible operons, the operon is **normally turned on** as the regulatory protein is synthesised in the inactive form. • When the regulatory protein is activated, operon **can be turned off**, thus making the operon "repressible" *Repressible operons : Can be switched off	**DEFINITION** • In inducible operons, the operon is **normally turned off** as the regulatory protein is synthesised in the active form. • When the regulatory protein is inactivated, operon **can be turned on**, thus making the operon "inducible" *Inducible: Can be switched on
SIGNIFICANCE • Mainly found in <u>anabolic pathways</u> (synthesis) • Synthesis of biomolecules are <u>needed for survival</u> (hence operon normally on) • Need to <u>avoid over-production</u> of biomolecules to prevent wastage (switch OFF)	**SIGNIFICANCE** • Mainly found in <u>catabolic pathways</u> (breakdown) • Synthesis of biomolecules <u>are not needed all the time</u> (hence operon normally off) • Production of biomolecules <u>on a needs basis</u> (can be switch ON)

Negative Gene Regulation	Positive Gene Regulation
DEFINITION • Negative gene regulation is the type of control in which the <u>active form of regulatory protein</u> (i.e. repressor) <u>turns the operon OFF</u> *Negative: Operon turn OFF by regulatory protein	**DEFINITION** • Positive gene regulation is the type of control in which the <u>active form of regulatory protein</u> (i.e. activator) <u>turns the operon ON</u> *Positive: Operon turn ON by regulatory protein
Example: • Regulation of trp operon by trp repressor protein • Regulation of lac operon by lac repressor protein	**Example:** • Regulation of lac operon by catabolite activator protein (CAP) and cyclic AMP (cAMP)

- **Anabolic pathway**: Series of reactions that results in the **synthesis** of one of more specific cellular components. E.g: Tryptophan amino acid synthesis.
- **Adenylyl cyclase:** An enzyme that converts ATP to cyclic AMP (**cAMP**).
- **Catabolic pathway**: Series of reactions that results in the **degradation** of one or more specific cellular components. E.g: Breakdown of *lac*tose into glucose and ga*lac*tose.
- **Co-repressor**: A small molecule that binds to a bacterial repressor protein and changes the

protein's shape, **activating it**, allowing it to bind to the operator and switch an operon off. E.g: Tryptophan

- **Constitutive gene**: Refers to a gene that encodes a product required in the maintenance of basic cellular processes. Also known as housekeeping genes. They are **expressed all the time**. E.g: *Lac*I gene.

- **Inducer**: A specific small molecule that binds to a bacterial repressor protein and changes the repressor's shape, **inactivating it**, so that it cannot bind to an operator, switching an operon on. E.g: Allo*lac*tose

- **Operator:** A **site on DNA at which a repressor protein binds** to prevent transcription from initiating at the adjacent promoter.

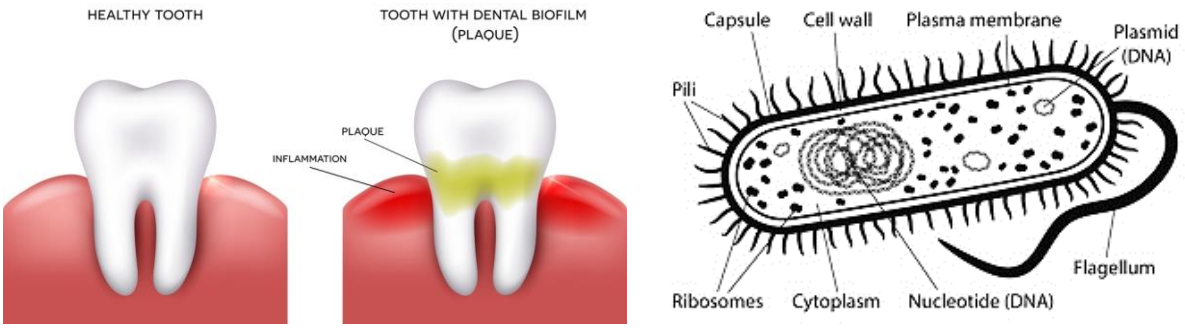
- **Operon**: A unit of genetic function found in bacteria and phages, **consisting of a promoter, an operator, and a coordinately regulated cluster of genes** whose products function in a common pathway. E.g: Trp operon, *Lac* operon.

- **Polycistronic mRNA**: A messenger RNA that contains the base sequence coding for the amino acids sequence of **several proteins**.

- **Regulatory gene**: A nucleotide sequence that codes for a protein involved in **regulating the expression of other genes**. E.g. repressor, CAP.

- **Structural gene**: Any gene that codes for a protein (or RNA) product that forms part of a structure or has an enzymatic function. E.g: TrpE, *Lac*Z.

ENRICHMENT – APPLICATIONS OF BACTERIA IN REAL LIFE



Dental caries is a common chronic infectious, transmissible disease resulting from tooth-adherent specific bacteria, primarily *Streptococcus mutans* that metabolise sugars to produce acid, which over time, demineralises the tooth structure. *S. mutans* is able to synthesise large extracellular mucoid glucans from sucrose which combined with other proteins and bacteria formed a biofilm known as dental plaque on tooth surfaces.

Unlike *S. mutans*, some other prokaryotes have capsules and most capsules are composed of high molecular-weight viscous polysaccharides that are retained as a thick gel outside the cell wall or envelope. An example is *Streptococcus pneumoniae* (the pneumococcus) which is an important human pathogen. Its virulence is largely due to its polysaccharide capsule, which shields it from the host immune system.

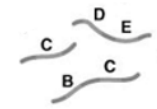
ENRICHMENT – TRANSFORMATION

Transformation involves the uptake of **naked**, **foreign DNA** in the **environment** by **competent** bacterial cells and is incorporated into the genome of the recipient cell.

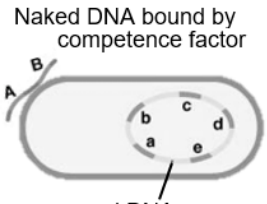
INTAKE OF FOREIGN DOUBLE STRANDED DNA

Earlier in the lecture notes, foreign DNA is taken into the cell. However, in reality, foreign double-stranded DNA is taken in instead. So how is it possible for homologous recombination to take place?

- Naked, foreign **double-stranded DNA fragment** of **dead, lysed bacteria (donor cell)** is recognised and bound by **competence factors** on cell surface of the recipient cell.
- As the double-stranded DNA fragment enters the cell, one of the strands is **degraded by either an exonuclease or endonuclease.**
  → this allows for homologous recombination



DNA fragments from donor cells



Chromosomal DNA

ENRICHMENT – CONJUGATION

Conjugation involves the **unidirectional** transfer of a **F plasmid** via direct contact from F^+ cell (donor) to F^- (recipient) cells. F+ cells contain F plasmid, F^- cells do not.

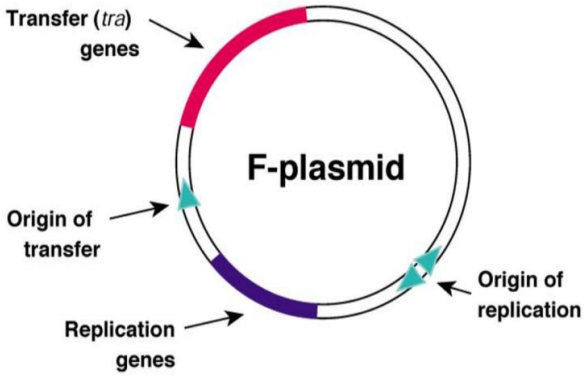
OTHER FEATURES OF THE F PLASMID

- Asides from containing the
  - ✓ **fertility (F) factor**
  - ✓ gene that expresses out **surface proteins** on the F^+ cell
  - ✓ origin of replication

  The **F plasmid** also contains

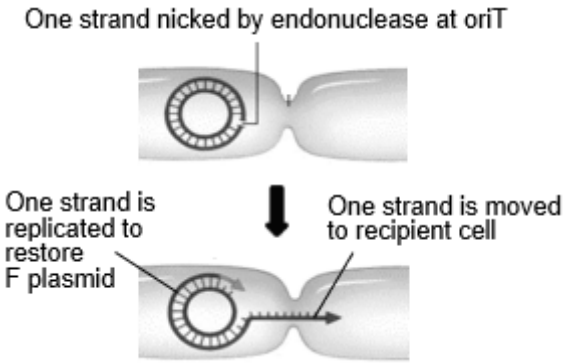
  ✓ gene coding for **endonucleases**
    - Endonucleases cut one strand of the double stranded DNA at a specific site called the **origin of transfer (oriT).**

  ✓ regulatory gene that controls and **regulates its own replication**
    - The number of copies of plasmid per cell is low.



ORIGIN OF TRANSFER

- A single strand of the F plasmid dsDNA is cleaved by endonucleases at its **origin of transfer (oriT)** and transferred to the recipient cell.



ROLLING CIRCLE MECHANISM

1. **Endonuclease** cleaves one strand of double-stranded F plasmid at the **origin of transfer (OriT)** which is transferred from donor cell into the recipient cell;

2. F plasmid is replicated via **rolling circle mechanism** in donor cell;

3. The **free 3' end of the cleaved DNA** strand is **extended by DNA polymerase** for synthesis of a new complementary strand using the intact strand as the template;

4. The **cleaved single stranded F plasmid transferred via the 5' end** (into the recipient cell) acts as template for synthesis of the second DNA strand in the recipient cell;