Bacteria

1. Learning Outcomes

Candidates should be able to:

1d Describe the structure of a typical bacterial cell (small and unicellular, peptidoglycan cell wall, circular DNA, 70S ribosomes and lack of membrane-bound organelles). (Covered in JC1 Cell Biology)

2d Describe the structure and organisation of prokaryotic genome (including DNA, double-stranded, number of nucleotides, packing of DNA, circularity and absence of introns). (Covered in JC1 Cell Biology)

2g 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.

2i 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. (Attenuation of trp operon is not required).

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

2. References

Campbell and Reece, BIOLOGY, 9th Ed.

Brooker, Widmaier, Graham and Stiling, BIOLOGY

Green, Stout and Taylor, BIOLOGICAL SCIENCE Vol 1 and 2, 3rd Ed.

Soloman, Berg and Martin BIOLOGY 7th Ed.

Tortora, Funke and Case, MICROBIOLOGY, 6th Ed.

Alberts, Bray, Lewis, Raff, Roberts and Watson, MOLECULAR BIOLOGY OF THE CELL, 3rd Ed.

Raven, Johnson, Losos, Mason and Singer, McGraw-Hill, Biology, 8th Edition

3. Contents

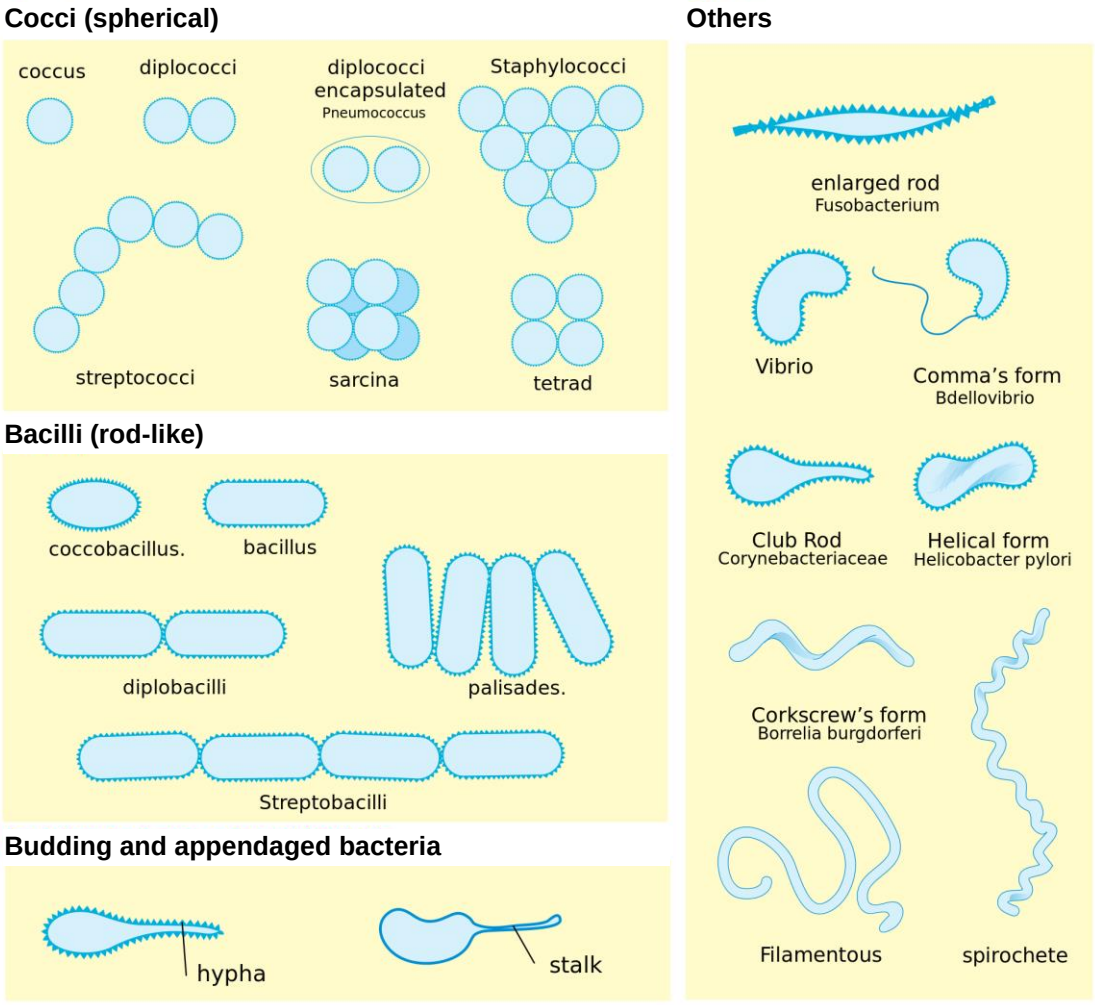
4. Bacterial Cells (Prokaryotic Cells)

Prokaryotes generally lack membrane-bound organelles and the endomembrane systems; but they still survive and reproduce. In the endosymbiont theory, organelles like mitochondria and chloroplasts represent formerly free-living prokaryotes that were taken inside another cell, and this could explain the link between the eukaryotes and prokaryotes.

A. Morphology

Most bacterial cells are very **small** (about 0.2µm in diameter and 2-8µm in length) and are **unicellular**. Bacteria may be classified based on their morphology ("*morphe*" = "form", "shape", "outward appearance"), amount of peptidoglycan in the cell wall or phylogeny (phylogeny = the organization of species to show their evolutionary relationships). Based on morphology, bacteria may be classified into: (a) coccus (spherical), (b) bacillus (rod-like), (c) spiral and (d) filamentous (elongated).

Fig. 1 - Different morphology of bacterial cells.



B. General Bacterial Structure (Ultrastructure)

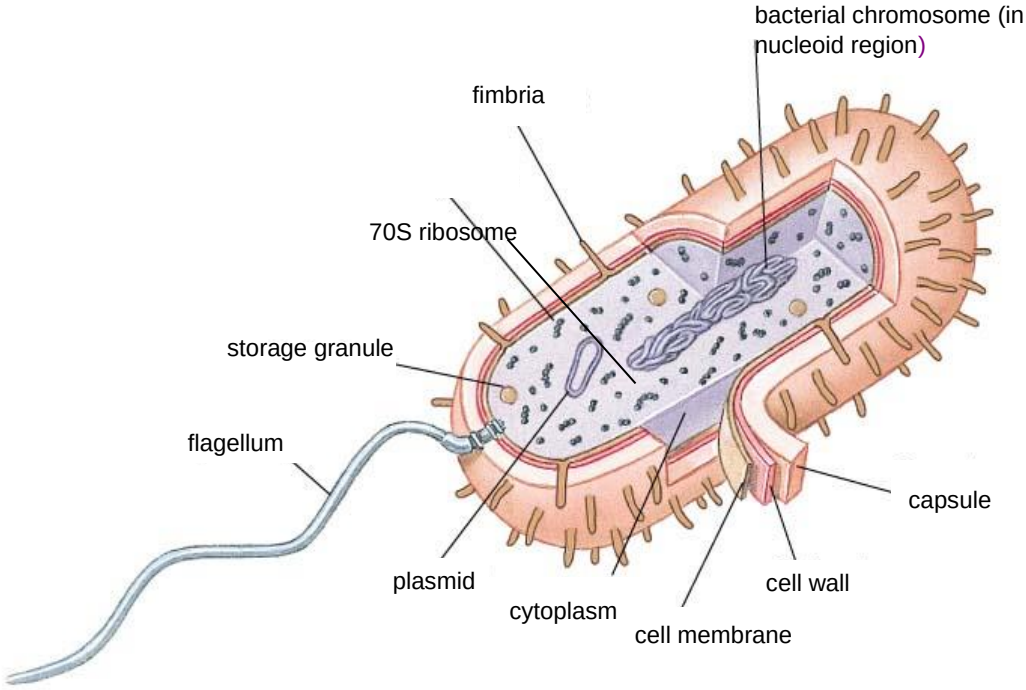


Fig. 2 - Diagram of a bacterial cell

Notes to Self:

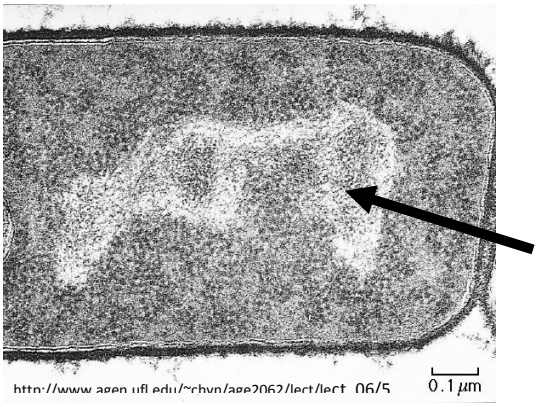
Internal Structure

Bacteria are prokaryotes and have a very simple internal structure with no membrane-bound organelles. Structures present include:

- The main component of the genome in most bacteria is one **double-stranded, circular DNA molecule** that is **associated with proteins** (they are not called histones!).
- The DNA forms **loop domains** with the proteins, followed by further **supercoiling,** forming highly condensed DNA

  (NB: stretched out, the DNA of *E. coli* would measure about 1mm in length, 500 times longer than the cell).
- The entire structure is referred to as the **bacterial chromosome**. Bacterium being prokaryote has **no intron** in its chromosome (non-coding sequences within the gene).
- The bacterial chromosome makes up a dense region within the cell called the **nucleoid**, which is not bound by a membrane.
- In addition to the chromosome, some bacteria may also have **plasmids**, which are much smaller rings of **autonomously replicating circular DNA**.

a)



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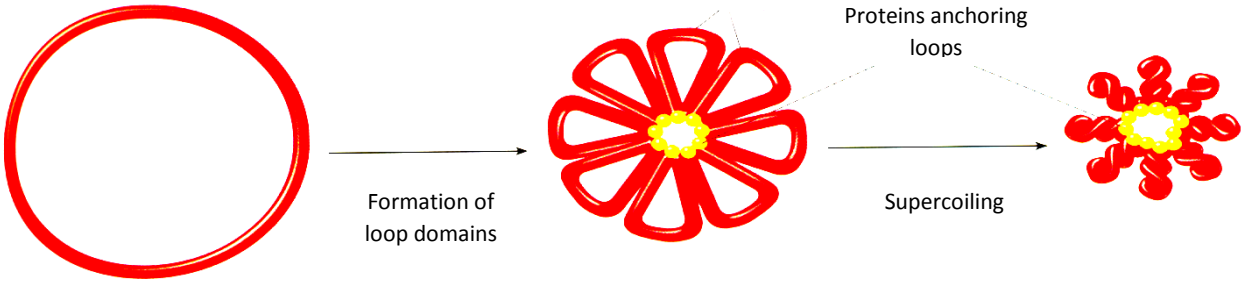


Fig. 3 **a)** The nucleoid region of a bacterial cell is stained less densely than the surrounding cytoplasm

b) Condensation of the bacterial DNA is made possible through its association with proteins

- **Nucleoid:**
  - Region in the bacterial cell where chromosomal DNA is generally confined to.
  - It is not bound by a membrane but is visibly distinct from the rest of the cell interior.

Notes to Self:

- **Ribosomes:**
  - **70S** (vs. 80S in eukaryotes). They are needed for protein synthesis.
  - The ribosomes give the cytoplasm of bacteria a granular appearance in electron micrographs.

- **Storage granules:**
  - Nutrients and chemical reserves may be stored in the cytoplasm in the form of granules e.g. granules of glycogen, lipids and ions like phosphorous and magnesium.

- **Plasmid(s) (may be present)**
  - A small, **circular autonomously replicating DNA molecule.** (This is not the bacterial chromosome.)
  - The plasmid contains genes which may confer advantages on bacteria living in stressful environments e.g. antibiotic resistance genes.
  - Multiple copies are usually present in a cell.
  - Plasmids are used extensively in genetic engineering as vectors for carrying and expressing foreign DNA in bacterial cells.
  - Different bacteria can have different plasmids.
  - Bacterial genome includes both the bacterial chromosome and the plasmid (if present).

Surface Structure

Notes to Self:

- **Cell Membrane**
  - A phospholipid bilayer similar the cell membrane of other cells.
  - In addition to the roles of a cell membrane which you have learned (See Notes on *Cell Membranes*), the membrane of a bacteria is also where the electron transport chains, as well as the enzyme ATP synthase are embedded to produce ATP during photosynthesis and/or respiration. (How is this different from an eukaryotic cell?)

- **Cell Wall**
  - Consists of a polymer called **peptidoglycan** – long chains of sugars cross-linked by short peptide chains. (compare: what is the cell wall in plants made up of?)
  - It protects the cell from osmotic lysis.
  - Bacteria may be classified as gram-positive or gram-negative bacteria (depending on whether they get stained by Gram stain which indicates the nature of the cell wall).

(For info)

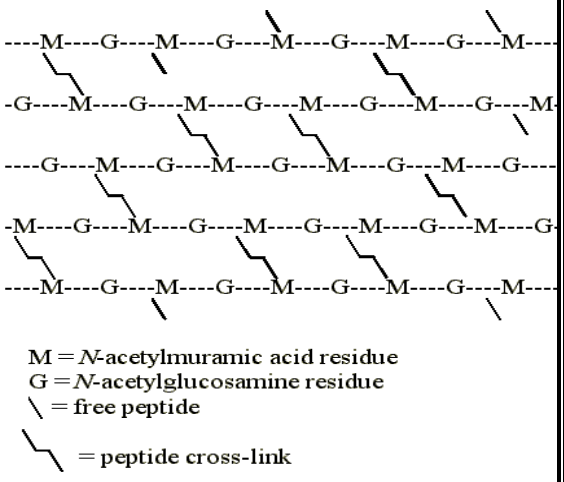
Peptidoglycan

> the sugar component is made up of alternating units of NAG (N-acetylglucosamine) and

NAM (N-acetylmuremic acid).

> the sugar is linked by short peptide chains as shown on the right

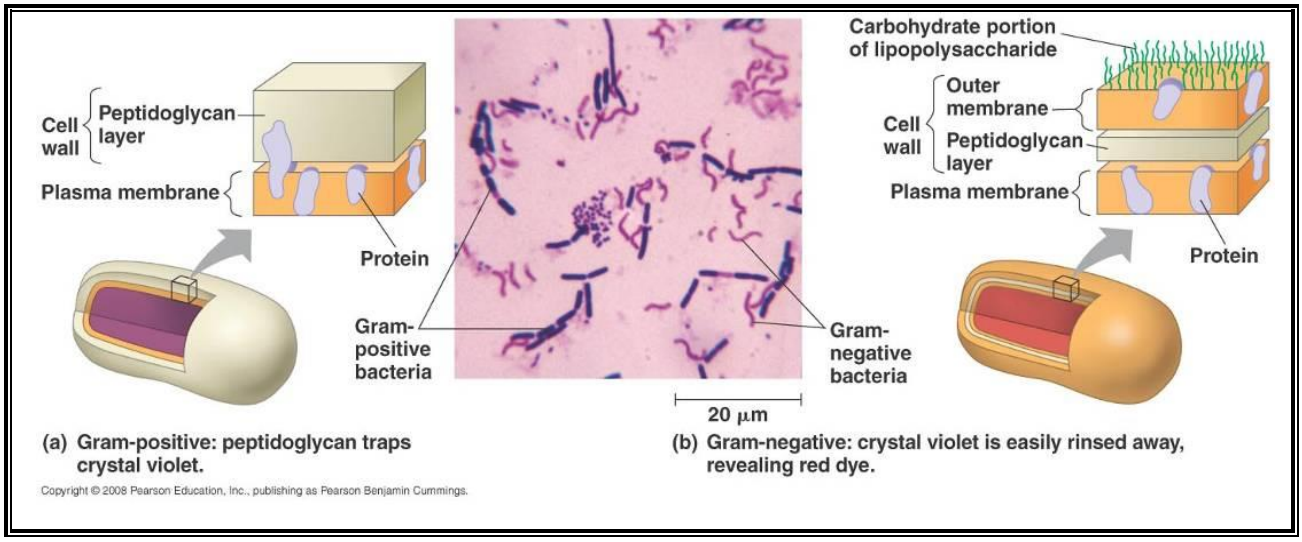
Diagram from http://www.mikeblaber.org/oldwine/bch5425/lect10/lect10.htm



> In gram-positive bacteria, the cell wall is a thick peptidoglycan layer.

> In gram-negative bacteria, the cell wall includes a thin peptidoglycan layer, followed by an **additional outer membrane.**

(For the purpose of the syllabus, only peptidoglycan is considered as the constituent of bacterial cell wall unless the bacteria is specified.)



- **Capsule** (may be present in some bacteria)
  - Some bacteria have a layer of polysaccharides known as glycocalyx (= sugar coat) to the exterior of the cell wall.
  - The glycocalyx can be a distinct layer, referred to as the **capsule** seen in Fig. 2, or exists it is a diffused mass known as the **slime layer**.
  - The capsule may also contain proteins.
  - <u>Functions:</u>

    > Often, the capsule protects the bacteria from being taken in via phagocytosis by the white blood cells which are unable to recognize the bacteria due to the capsule

    > It also enables bacteria to adhere to one another or to particular surfaces e.g. mucous membrane.

Notes to Self:

(3) <u>Appendages</u> (may be present in some bacteria)

Both fimbriae and pili are hollow, hair-like structure composed of protein.

- **Fimbriae** (singular: fimbria)
  - These are short, bristle-like fibres extending from the cell surface and are usually evenly distributed over the entire cell surface or at poles of cells;
  - <u>Function:</u> for attachment to surfaces or other bacteria/organisms

- **Pili** (singular: pilus)
  - pili are longer and fewer in numbers than fimbriae.
  - <u>Function:</u> involves in motility and DNA transfer

    > motility; a pilus makes contact with a surface and retract to pull the bacteria forward in a jerky, intermittent movement

    > DNA transfer; a specialised pilus like the sex pilus, allows two bacterial cells to be drawn close to each other so that a mating bridge can be formed for the transfer of genetic material.

- **Flagella** (singular: flagellum)
  - Long appendages for **motility**.
  - The bacterial flagellum is a hollow cylindrical protein thread that **propels** the bacterium by rotation.
  - Some bacteria possess more than one flagellum and they may be found distributed all over the cell, at one pole or at opposite poles of a cell.

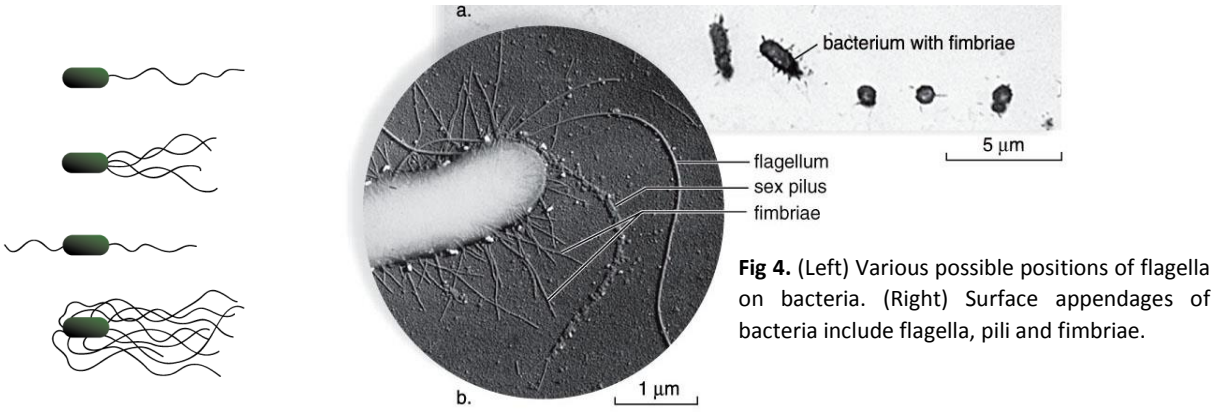


Fig 4. (Left) Various possible positions of flagella on bacteria. (Right) Surface appendages of bacteria include flagella, pili and fimbriae.

Structural features of Bacteria

Structural feature	Information
Cell wall	Prevents osmotic lysis of cell protoplast and confers rigidity and shape to cells - composed of peptidoglycan
Location of chromosome	Found within nucleoid region; no true nucleus
Chromosome	A single, circular, double helix DNA – supercoiled
DNA-associated proteins	Proteins anchoring loop domains present
Plasmids	Extra-chromosomal DNA that replicates autonomously; quantity can range from 5 - 100s!
Organelles	No membrane-bound organelles
Ribosomes	70S (vs 80S in eukaryotes)
Appendages:	
a) Fimbriae	Attachment to surfaces and to other bacteria/organisms

b) Pili	(i) Mediates DNA transfer during conjugation (sex pilus) (ii) Motility by retraction
c) Flagella	Swimming movement; propulsion
Capsules (organised mass of glycocalyx)	protection against phagocytic engulfment; attachment to surfaces; contains water to prevent desiccation - composed of polysaccharides and sometimes polypeptides
Slime layers (diffused mass of glycocalyx)	Attachment to surfaces; to form biofilm - composed of polysaccharides and sometimes polypeptides

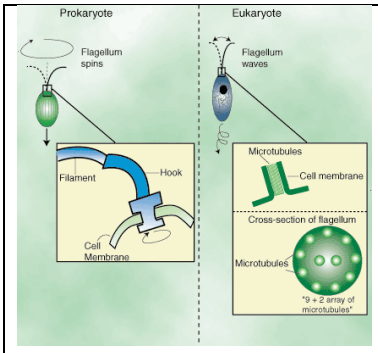
Question: List 4 differences between a bacterial and a eukaryotic chromosome? [4]

Point of comparison	*Eukaryotic Chromosome*	*Bacterial Chromosome*
Location	*located within membrane-bound nucleus*	*Located within nucleoid region; not enclosed by a membrane*
Structure of DNA	*Linear DNA*	*Circular DNA*
No. of chromosomes in a cell	*Have several different chromosomes*	*Only one chromosome*
Intron	*Presence of introns within the genes*	*Intron is absent*
Associated proteins	*Associated with large amount of histone proteins*	*Associated with small amount of proteins*

Structural Features that Distinguish a Prokaryotic Cell from a Eukaryotic Cell

(This will be covered in greater detail in topic: Prokaryotic and Eukaryotic genome)

Feature	Prokaryotic cell	Eukaryotic cell
Cell size	Smaller	Larger
Nucleus	No true nucleus / No nuclear envelope	Nucleus present / Nucleus with nuclear envelope present
Genetic material	Circular DNA lying naked in a region in the cytoplasm known as the nucleoid; DNA is associated with small amount of histone-like proteins	Linear DNA found within membrane-bound nucleus; DNA is associated with large amounts of histones proteins
Ribosome for protein synthesis	70S; No ER present for ribosomes to attach	80S; Ribosomes may be attached to ER or may be free in the cytosol
Organelles	Few e.g. ribosomes No membrane bound organelles	Many; Membrane bound organelles present e.g. nucleus, mitochondria; ➔ double membrane e.g. Golgi apparatus, lysosomes, vacuoles, endoplasmic reticulum ➔ single membrane
Cell walls	Composed of peptidoglycan (murein)	Composed of cellulose in plants Composed of chitin in fungi
Flagella ***(if any)***	Simple;	Complex; composed of tubulin; each is made up of several strands with a 9+2

	No microtubules; composed of protein flagellin instead; each is a single strand of protein; Extracellular (not enclosed by plasma membrane);	arrangement of microtubules (read Campbell if interested) Intracellular (surrounded by plasma membrane);
Photosynthesis	Involves plasma membrane of bacteria	Involves chloroplast
Respiration		Involves mitochondrion and cytoplasm

5. Binary Fission

- Binary fission is the means by which bacteria replicate and produce offspring. (NB: this process is different from division through mitosis).

- Before one bacterial cell splits into two independent cells, the bacterial chromosome must first replicate.

Bacteria chromosome replication

DNA replication begins at the **origin of replication (*ori*)**, made up of a specific sequence of nucleotide bases (Fig. 5).

1. There, the double helix separates to form a **replication bubble** made up of two single DNA strands. Replication takes place outward from the origin in both directions forming 2 replication forks.
   Each replication fork will have both a leading strand and a lagging strand being synthesized, just as in eukaryotes.
2. As the chromosome replicates, the 2 newly formed *ori* move to **opposite poles** of the cell and attach to the plasma membrane.
3. The cell also **elongates** to prepare for division.
4. But because the DNA is circular with no free ends, an interlocking structure made up of the 2 daughter DNA molecules will be formed with the completion of replication.
   Enzyme **topoisomerase** is needed to cut, separate and reseal the two DNA molecules (Fig. 6).
5. When the daughter DNA molecules are separated, the bacterium will have reached twice its initial size. **Invagination** of the plasma membrane and the deposition of new cell wall (also known as division septum) eventually divide the parent cell into two daughter cells, with each inheriting a complete genome (genetically identical).

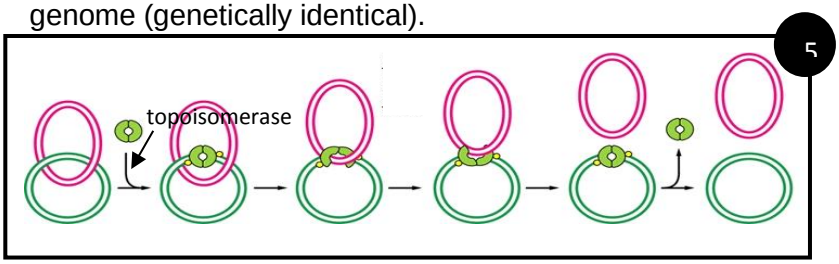


Fig. 6 - Topoisomerase helps to separate 2 entangled DNA molecules.

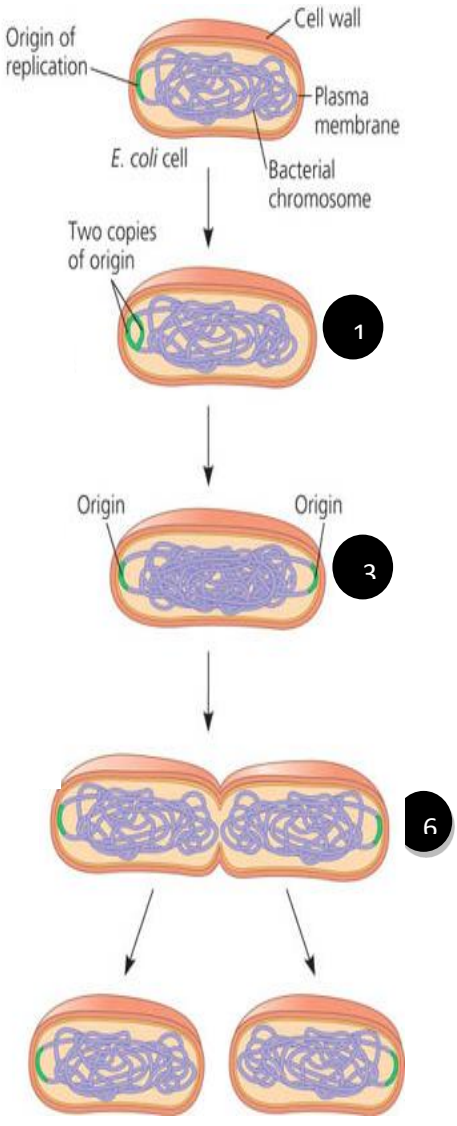


Fig. 5 - The process of binary fission

Binary fission is the <u>asexual means</u> by which bacterial cells produce **genetically identical offspring**. →This can be a selective advantage in a stable, favourable environment as it allows successful genotypes to rapidly reproduce and colonise a habitat.

Question: Can you describe how binary fission is different from the process of mitosis?

Notes to Self:

No spindle fibres are involved; no specific positioning of linear chromosomes in the cell that characterizes the different stages of mitosis, no nuclear division.

6. Ways to generate genetic variation in bacterial genomes by DNA introduction

In a rapidly changing environment, **generating genetic variation** through forming **new combination of new alleles** becomes crucial for **enhancing reproductive success** i.e. at least some individuals may be selected for and survive to reproduce (Theory of Natural Selection to be covered under *Evolution*). In eukaryotes, sexual reproduction increases genetic variable within a population.

In bacterial cells which undergo asexual reproduction, 3 processes, **transformation**, **transduction** and **conjugation** help to increase genetic variation by bringing together DNA from different individuals.

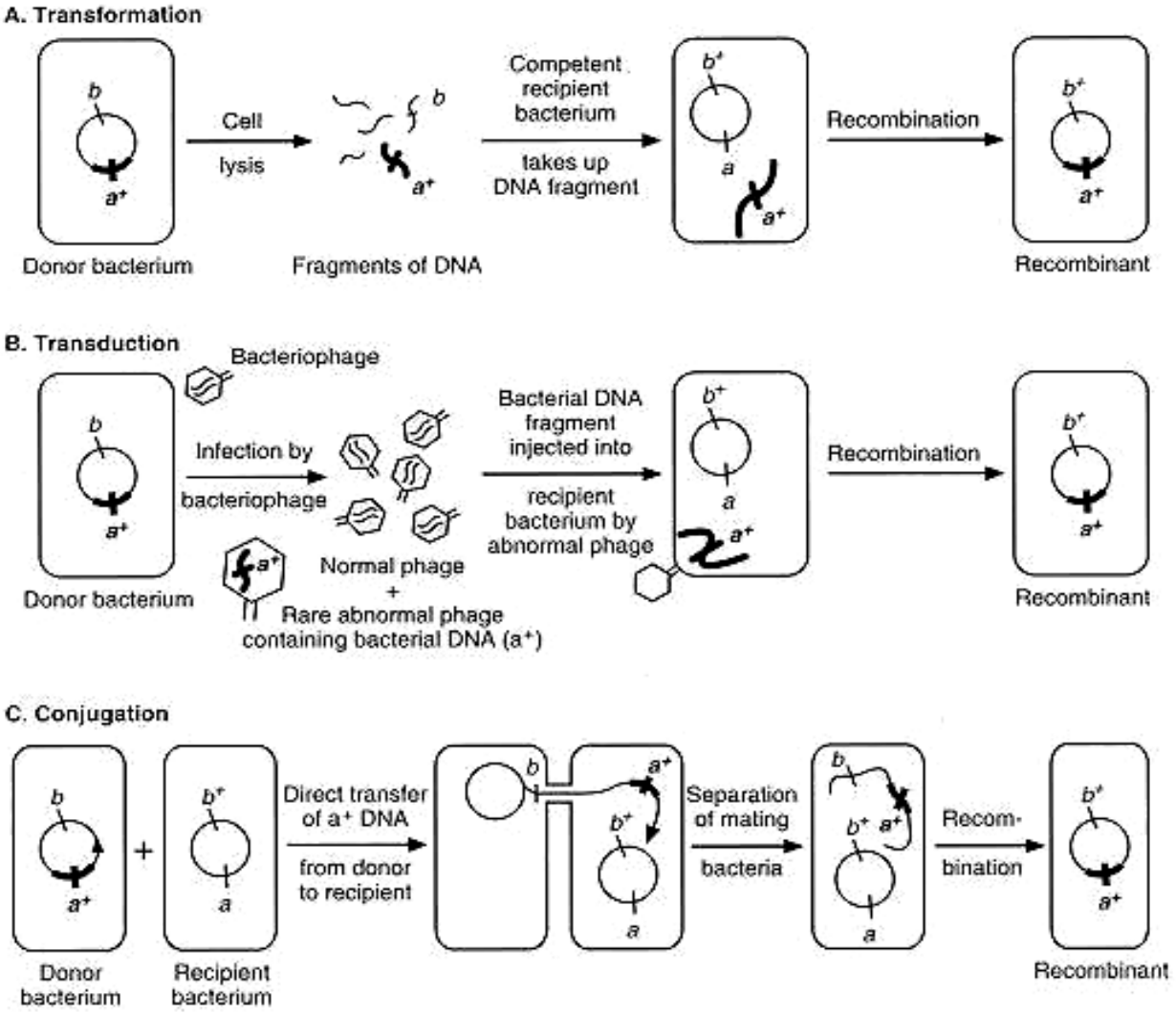


Fig. 7 - Methods by which DNA are introduced into bacteria

A. Transformation

- Transformation refers to the **uptake of naked, foreign DNA** from the surrounding environment, resulting in a **change of the bacterial cell's genotype and phenotype.**

- The foreign DNA may have come from **dead lysed neighbouring** cells in the medium.

- Some bacteria possess **cell-surface proteins** that can **bind to and transport DNA** into the cell. Such cells with the natural ability to take up foreign DNA are described as **competent cells**.

- Bacterial cells that lack these surface proteins can be made artificially competent through immersion in a culture medium with high concentrations of $CaCl_2$ followed by a heat shock treatment. This technique is used in genetic engineering to introduce foreign genes into the *E. coli* genome.

> *For info – but can be tested*
>
> Bacterial cells are permeable to chloride ions but not the calcium ions. The chloride intake is accompanied by an influx of water into the cells, causing the cells to swell and priming it for the heat shock treatment. The heat shock treatment induces the formation of transient pores which allows for the uptake of DNA from the surrounding medium.
>
> Also as a cation, calcium can bind to both the negatively charged DNA and the cell membrane, which also has a negative charge. This neutralization of charges enhances the ability of the cell to take up the DNA.

- The foreign DNA can then be incorporated into the chromosome through **crossing over at 2 homologous regions** found on the bacterial chromosome (i.e. **homologous recombination**). (Note: If no crossing over occurs, the foreign DNA will not be incorporated into the bacterial chromosome. It will be degraded.)

- The resultant cell is a **recombinant cell**. If different alleles for a gene were exchanged, there will be a permanent change in the organism's **phenotype as the new allele is expressed**. The bacterium's recombinant genome will be passed on to all subsequent offspring through binary fission.

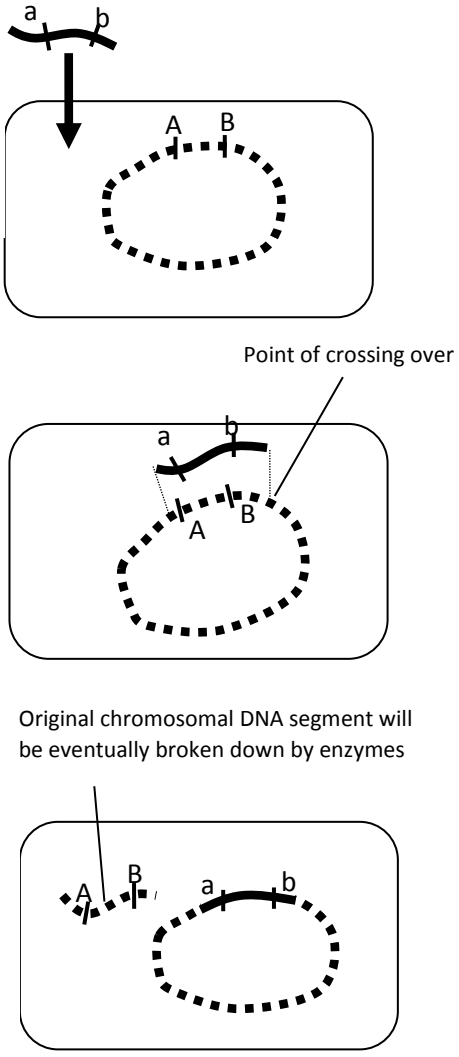


Fig. 8 - Bacterial transformation, including crossing over at a region containing alleles A / a, and alleles B / b

B. Transduction

In this process, **phages** (viruses that infect bacteria) randomly **carry bacterial genes from one host cell (donor cell) to a recipient cell** as a result of aberrations in the phage reproductive cycle.

2.1 Generalised Transduction

- When a phage undergoes the **lytic cycle**, phage enzymes may **hydrolyse the bacterial chromosome** into small pieces of DNA.
- During assembly of the phage genome within the phage capsid, **a small piece of the host cell's degraded DNA is randomly packaged** within a capsid (instead of the phage's own genetic material).
- Following lysis of the host cell (donor cell), the **defective phage** is released and can infect another bacterium (the recipient). The piece of bacterial DNA acquired from the host cell (donor cell) injected into the recipient cell.
- Since viral genes have been replaced by bacterial genes in the defective phage, no new phages can be synthesized (sometimes) in the recipient cell.
- The foreign bacterial DNA can subsequently replace the homologous region of the recipient cell's chromosome if **crossing over** and **homologous recombination** takes place.
- The recipient cell with the new alleles integrated into its genome becomes a **recombinant cell**, which expresses new characteristics.
- As any **random portion of the bacterial DNA** may be transferred, this process is called **generalized transduction**.

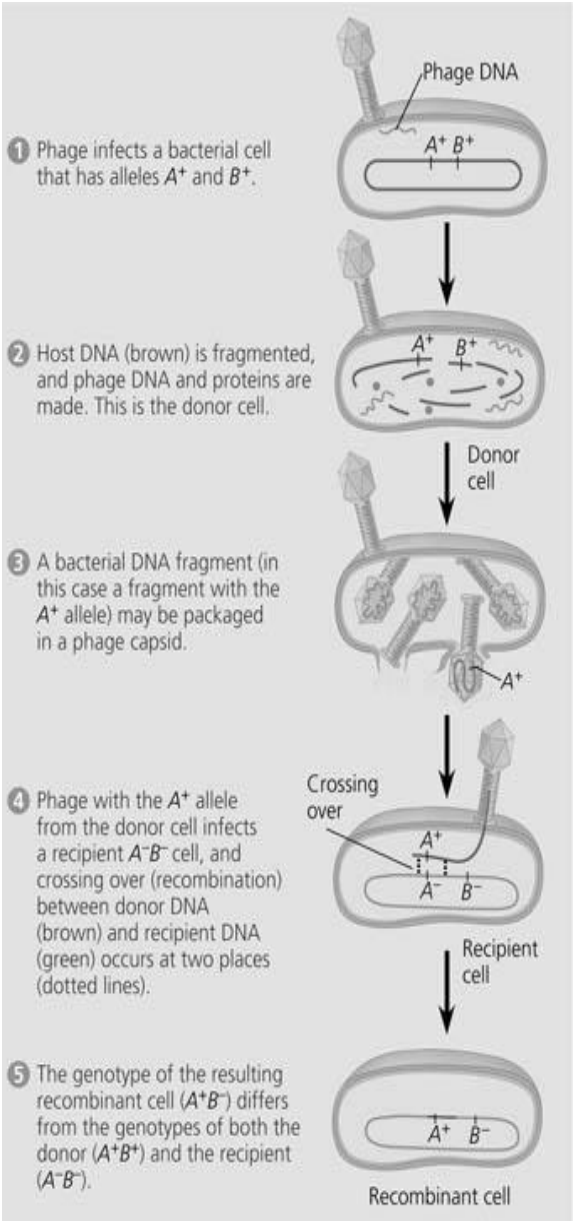


Fig. 9 - The process of generalised transduction

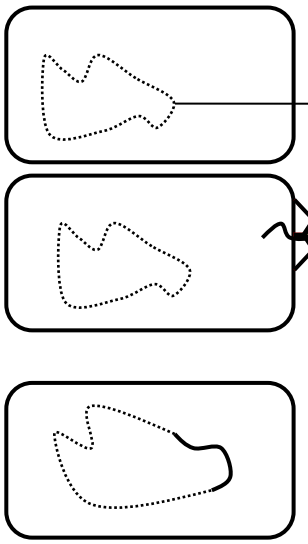
2.2 Specialised Transduction

- This process is carried out by **temperate phages.** (undergoes lysogenic cycle to integrate their genome into the bacterial chromosome, forming a **prophage** …refer to *Virus* notes)

- During specialised transduction, the bacterial DNA that is transferred is **restricted to bacterial genes adjacent to the integrated prophage.** Thus, it is called **specialized transduction**.

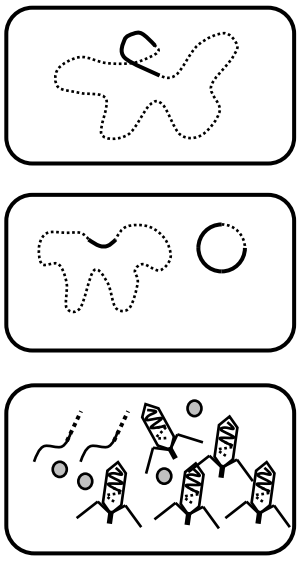
Process (including lysogenic cycle):

Bacterial Cell 1



(1) Phage attaches to host cell and injects viral genome into host (donor cell).

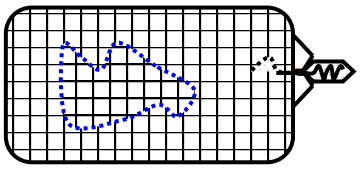
(2) Phage DNA integrates into the bacterial chromosome, becoming a **prophage** → phage enters the **lysogenic pathway.** As the cell divides by binary fission, a single infected cell can give rise to a large population of bacteria carrying the prophage.



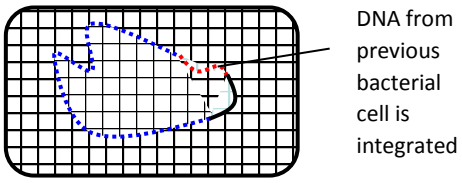
(3) Upon **induction**, the prophage exits the bacterial chromosome, i.e. switches from the lysogenic cycle to the lytic cycle, but the viral DNA may be **improperly excised**, such that **bacterial DNA** adjacent to the prophage is **excised** as well.

(4) The **phage-host hybrid DNA is replicated**, and packaged in new viral progeny. All the viral progeny, which are recombinant phages are capable of infecting other bacteria (transducing particles).

Bacterial Cell 2



(5) the **recombinant phage** subsequent infects another new host cell (recipient cell)



(6) New alleles can be incorporated into the bacterial genome of recipient cell by:

i) crossing over and homologous recombination at homologous regions – refer to Fig. 8.

ii)

OR

iii) integration of the phage-bacterial hybrid DNA into genome of recipient cell, as defective phage enters the

Fig. 10 - The process of specialised transduction

Notes to Self:

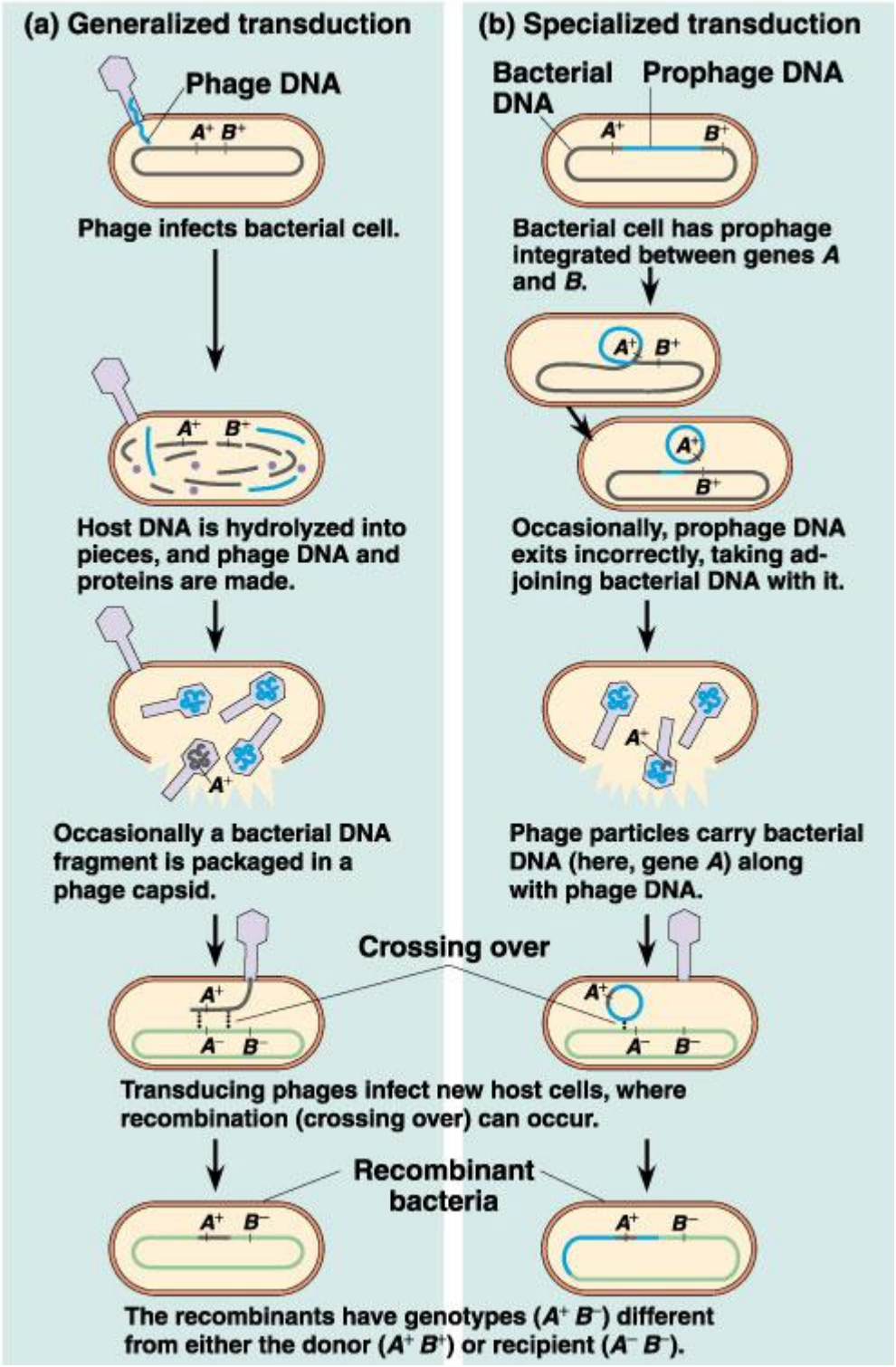


Fig. 11 - The process of generalized and specialised transduction

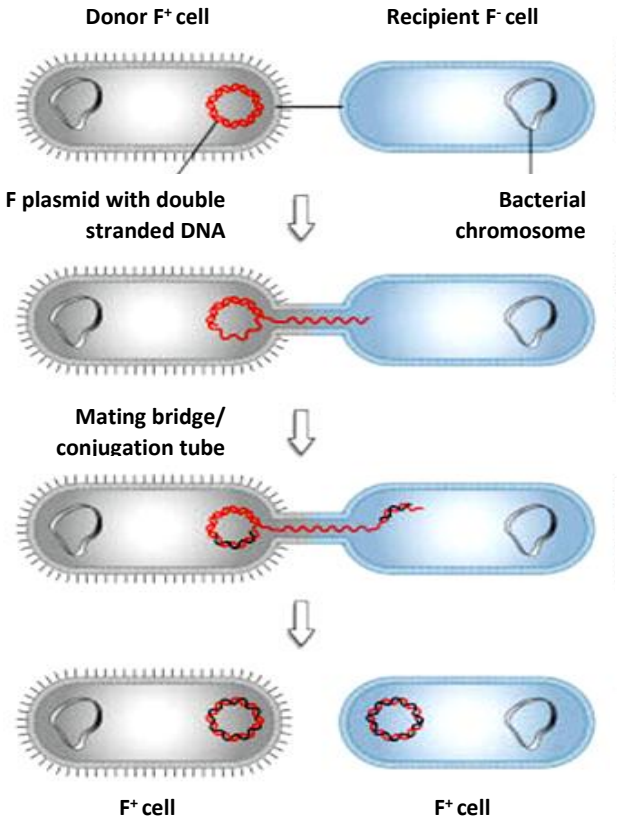
C. Conjugation

Notes to Self:

- Conjugation refers to the **direct transfer** of genetic material from **one bacterial cell to another**, through a **temporary link** between the two cells (refer to Fig. 11).

- The transfer of DNA is always **one-way** → from **donor cell** (called an **F^+ cell)** to **recipient cell** (called an **F^- cell**) in that it possesses an **F plasmid**.
  - ➔ On the F plasmid is a segment of DNA called an **F factor** (F = fertility) that carries **genes coding for sex pili.**
  - ➔ Due to the presence of F factor, the donor cell is able to produce appendages called **sex pili** to attach itself to the recipient cell.

Process:



(1) Conjugation begins when **sex pilus** on the surface of an **F^+ cell** make contact with an **F^- cell**.

(2) After the sex pilus attaches, it retracts, pulling the two cells closer**.** The hollow pilus then forms a temporary **mating bridge/conjugation tube.**

A **single strand of plasmid DNA** passes through the bridge and is replicated via the **rolling circle DNA replication**. (see below)

(3) The single strand of plasmid DNA in the recipient cell recircularises and serves as **template** for the synthesis of a complementary daughter stand

(4) Through semi-conservative replication, both cells now contain a double-stranded F plasmid. **Both cells are now F^+.**

Fig. 12 - Conjugation involving an F^+ and F^- cell; recipient cell becomes F^+

- Bacterial conjugation can occur between bacteria of the same species or of different species. Success rate decreases with decreasing relatedness between species.

Rolling circle DNA replication

- For the transfer to take place, **one strand of the double-stranded F plasmid is nicked by a nuclease.**

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

- The **newly synthesized strand displaces the nicked strand which is transferred concurrently**, via the 5' end, across the mating bridge into the recipient cell.

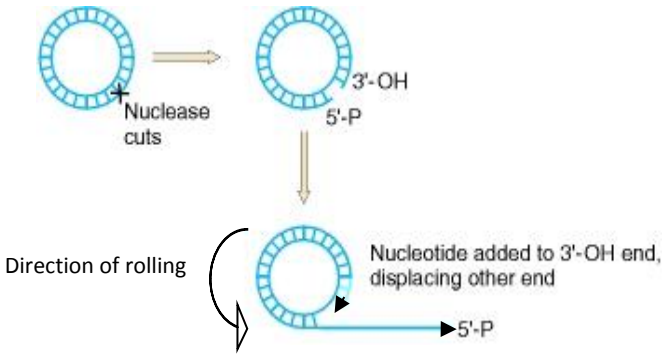


Fig. 13 - Rolling circle DNA replication

- Upon completion of a unit length of the plasmid DNA (after 1 round), **another nick occurs to release the original** strand and end the replication of the newly synthesized strand;

- This form of DNA replication for the plasmid is known as **rolling circle DNA replication** and is also found in some phage genomes. In this case, it is coupled to the transfer of DNA to another cell.

- In the **recipient cell**, the **single strand of F plasmid DNA re-circularises** and **serves as a template** for the synthesis of a complementary daughter stand

Notes to Self:

For info (but it is recommended that you have a brief understanding of Hfr cells)

Conjugation involving an Hfr cell (refer to Fig. 14)**:**

- Sometimes, a donor cell's **F plasmid** can be **integrated** into the **bacterial chromosome**.
- Such a cell is called an ***Hfr* cell** (for ***H****igh* ***f****requency of* ***r****ecombination*); it will similarly function as the donor during conjugation.
- During transfer, DNA replication is initiated at the Ori within the F factor DNA.
- A single strand of DNA moves into the F^- cell.
- Usually, the fragile mating bridge is broken before an entire strand of bacterial chromosome and the rest of the F factor can be transferred to the recipient cell.
- The single strand of DNA serves as the template for the synthesis of a new complementary DNA strand in both cells.
- If part of the newly acquired DNA crosses over with a homologous region on the F^- chromosome, a **recombinant F^- cell** will result.

Question: Conjugation has sometimes been called 'bacterial sex'. However, this term is misleading. Can you explain how conjugation is different from sexual reproduction?

Process does not involve equal contributions of genetic material from 2 gametes. No offspring are produced. Rather, it is a form of genetic transfer where the genetic composition of the recipient cell is altered.

Question: Describe the differences which occur during conjugation when the donor cell is an F^+ cell, compared to an Hfr cell.

Point of Comparison	F^+ Cell	Hfr Cell
Type of DNA transferred to recipient	The entire strand of F plasmid DNA is transferred across.	Part of the F plasmid DNA and some neighbouring bacterial chromosomal DNA is transferred across.
Subsequent change in genotype of recipient cell	The recipient changes from F^- to F^+	The recipient remains F^- but can still become a recombinant.

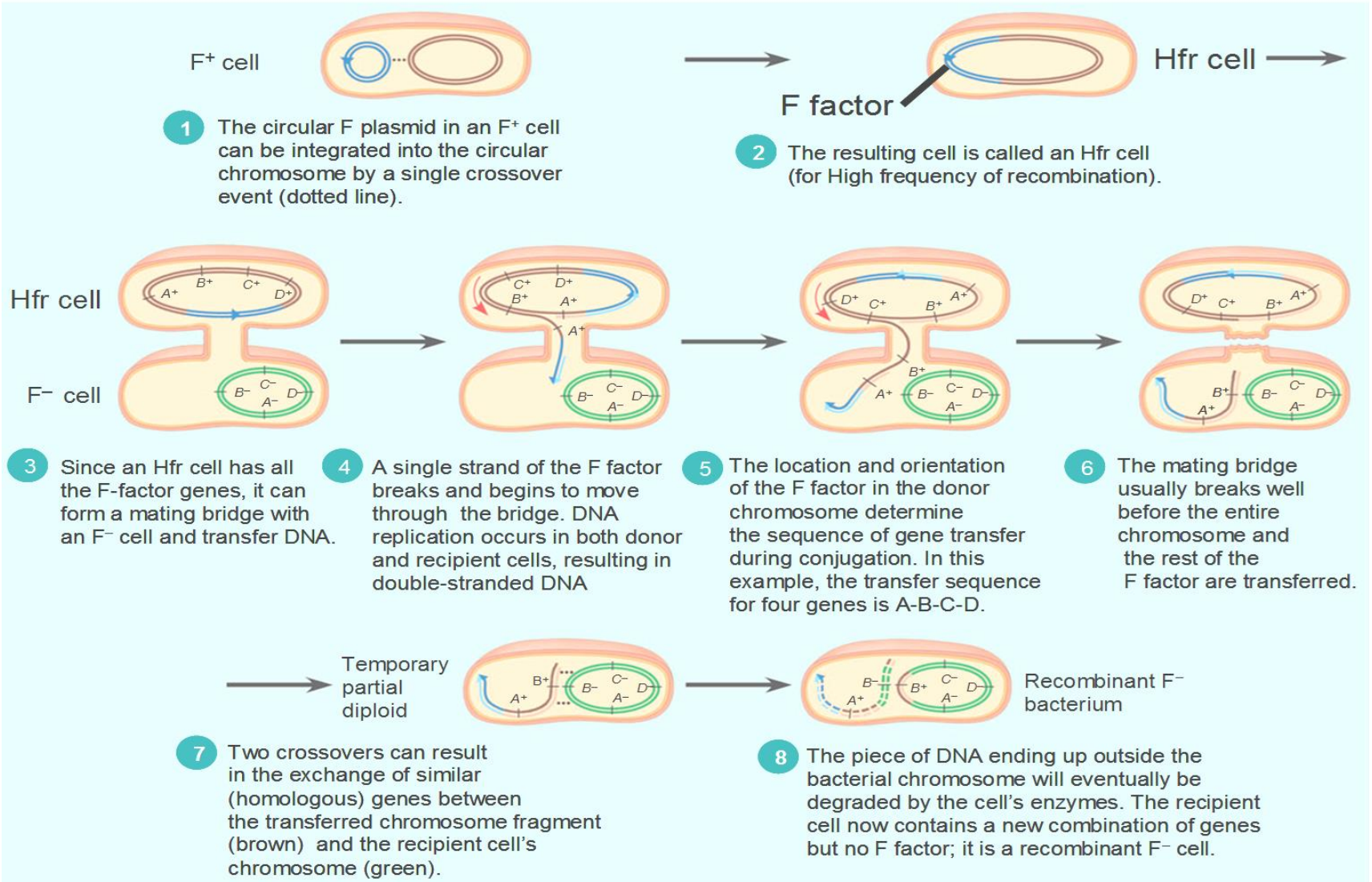


Figure 14. Conjugation involving an Hfr and F- cell; recipient cell remains F- at the end of the conjugation process

Notes to Self:

7. Gene Regulation in Bacteria – An Introduction

Notes to Self:

- All somatic cells of an organism carry **identical genes**. Despite this, cells in a **multicellular organism show a wide variation in structure and function.** For example, a liver cell have different structure and function from a white blood cell even though they share the same genetic makeup.

- Even within a single cell, the rate at which certain protein molecules are synthesized varies according to **circumstances and demand.**

Question: Why do different cell types have different structure and function?

- *This is a result of regulation of gene expression.*
- *In each specialized cell type, **certain sets of genes are expressed**, hence **certain set of tissue-specific proteins are synthesized**, which determine the **specialized function** of the cell.*
- *Hence certain subsets of the total genetic information are expressed in any given cell, allowing cells to **specialise.***
- *Gene regulation can also be **influenced by the environment,** allowing the cell to be **responsive** to changes in the environment.*

- Some proteins are **synthesized continuously at a constant rate** and genes coding for such proteins are said to be **constitutively** expressed.

- However not all proteins are constitutively expressed. The expression of other genes are **regulated** and there are several mechanisms by which this is done:

Legend: ⩚ = *point of control*

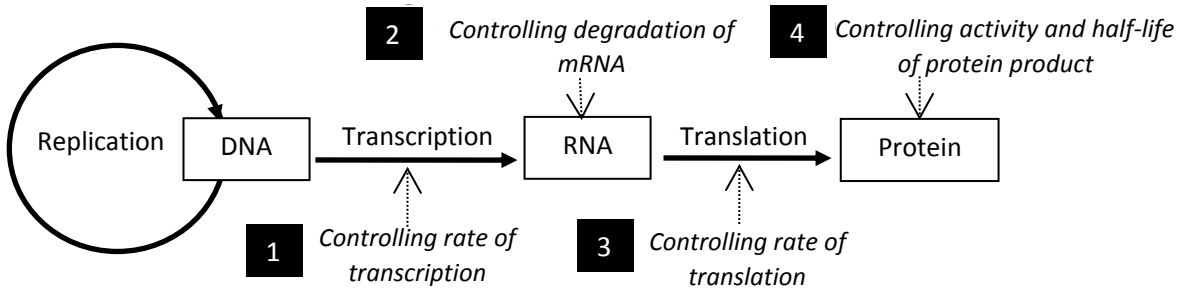


Fig. 15 - The Central Dogma and various points of gene regulation

Q: Can you suggest which level of gene regulation control predominates? Why?

***Transcriptional-level control** predominates as it is the most **efficient** mechanism with **minimal wastage**.*

*[For this topic we will be focusing on **regulation at the level of transcription**.]*

8. The *lac* Operon in *E. coli*

Notes to Self:

(1) Background

- *Escherichia coli* (***E. coli***) is a bacterium commonly found in the **intestines** of humans and other mammals.

- *E.coli* living in the colon of an adult cow is NOT normally exposed to the milk sugar lactose, a disaccharide. However, the *E. coli* living in a calf will be exposed to lactose from the mother's milk.

- This situation presents a dilemma.
  - Should a bacterial cell invest energy and materials to produce lactose-metabolizing enzymes *just in case* it ends up in the digestive system of a calf?
  - Given that the average life span of an actively growing *E. coli* cell is about 30 minutes, such an evolutionary strategy appears wasteful. And yet, if *E. coli* cells cannot produce those enzymes, they might starve in the middle of an abundant food supply.

- This dilemma is overcome because *E. coli* is able to **regulate the expression of genes coding for various enzymes,** thereby allowing them to make use of available organic molecules efficiently.

Question: What benefit is there for the bacteria to regulate its genes?

- ***Economical use of energy*** *and* ***resources*** *→ gene is expressed and protein produced only when necessary*
- *Enables bacteria to* ***respond appropriately and rapidly to changes in the environment****.*
- *Ability to do the above confers a* ***selective advantage*** *to such bacteria over those that can't regulate gene expression*

(2) Organisation an operon

- The basic mechanism for this type of control of gene expression in bacteria, described as the operon model, was discovered in 1961 by Nobel prize winners, Francois Jacob and Jacques Monod at the Pasteur Institute in Paris.

  Their work involved the use of bacteria ***E. coli.*** **mutants**. Through their investigations, they found out that genes involved in **lactose metabolism** in *E. coli* were clustered together in a region of the bacterial chromosome known as the **operon**.

- An **operon** is a **cluster of genes with related functions**, regulated in such a way that all the genes in the cluster are turned on and off together (see Fig. 14). It includes a common **promoter**, an **operator**, and **more than one structural genes** that are **controlled as a unit** to produce a **single polycistronic messenger RNA** (mRNA).

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

→ **Regulatory gene** codes for a protein that is involved in the regulation of the expression of other genes eg. repressor, CAP.

Notes to Self:

→ **Structural gene** is any gene that codes for a protein (or RNA) product that forms part of a structure or has a metabolic function, e.g.enzyme.

- Operons occur primarily in **prokaryotes** such as *E. coli* and certain simple eukaryotes e.g. nematodes.

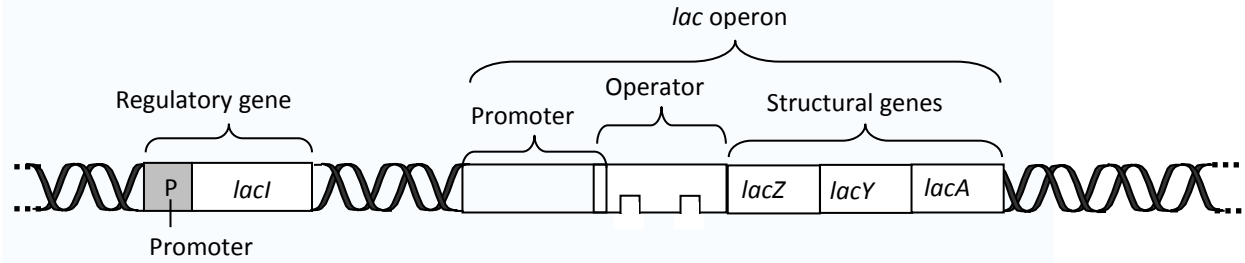


Fig. 16 - Organisation of the *lac* operon and its regulatory gene

Structural Gene	***lacZ***	***lacY***	***lacA***
Enzyme coded for	β-galactosidase	Permease	Transacetylase
Function	Hydrolyses lactose to glucose and galactose	A membrane transport protein that enables cells to take up lactose efficiently	Function not well known. Metabolises certain disaccharides.

Table 1. Genes found within the *lac* operon

<u>Organisation of the *lac* operon (an inducible operon) in *E.coli:*</u>

Notes to Self:

i) Within the *lac* operon are three structural genes arranged in the sequence ***lac Z, lac Y* and *lac A*** as seen in Figure 14. The enzymes which they encode are given in Table 1.

ii) The three structural genes are under the control of one common **promoter** sequence upstream **for RNA polymerase to bind and initiate transcription.**

iii) The **operator** is located between the promoter and the structural genes to **control the transcription of the structural genes by controlling access of RNA polymerase to the genes**, therefore turning the genes "on" or "off" like a switch.

- A short distance upstream of the operon is its **regulatory gene*, lacI*** (pronounced "lac-i") which has its own promoter and terminator sequences.
  → The ***lacI* gene** codes for the ***lac* repressor protein**.

  → Note that the regulatory gene is normally **NOT** considered to be part of the operon.

- The *lac* operon is an example of an **inducible operon** because **the expression of the 3 genes** (***lac Z, lac Y* and *lac A***) is usually "off" but can be **induced** and hence is turned "on" in the presence of an **inducer** molecule**.** In fact, ***lacI*** is named as such because *"I"* stands for "inducibility".

  → The **inducer** molecule for the *lac* operon is **lactose**, or more accurately, its isomer **allolactose**.

  → Lactose that is transported into the cells and converted into the inducer, allolactose.

Notes to Self:

(3) Regulation of the expression of the *Lac* operon

Since the Lac operon produces enzymes involved in the metabolism / hydrolysis of lactose to glucose (to be used as respiratory substrate) and galactose, it makes sense that Lac operon is expressed in the (i) presence of lactose & (ii) absence of glucose.

Hence the expression of the *Lac* operon is regulated by:

(i) **Negative regulation**: turned off by **repressor protein** in the absence of lactose.
(ii) **Positive regulation**: upregulated by the **CAP protein** in the absence of glucose.

(i) NEGATIVE REGULATION

(a) Default mode of *lac* operon (absence of lactose & glucose)

- By default, the *lac* operon is considered **repressed** i.e. "off"

1. The **regulatory gene *lacI*,** is **constitutively transcribed**, resulting in continued production of small amounts of the **lac repressor protein** (see Fig. 16).

2. The **repressor protein** is produced in the **active form** and **binds specifically to the *lac* operator** sequence via its **DNA-binding site**.

3. **In the absence of lactose**, the **repressor** binds to the **operator site, denying RNA polymerase access to the promoter**.

4. Transcription of the structural genes of the *lac* operon is hence blocked. This has the effect of switching the ***lac* operon off** (i.e. the operon is **repressed**).

- However, the binding of the repressor to the operator is mediated by weak interactions. As such, the repressor sometimes dissociates from the operator, resulting in a basal level of lac operon products i.e. galactosidase, permease and transacetylase within the cell, and whose presence is equally important and necessary for the regulation of *lac* operon as shall be discussed later.

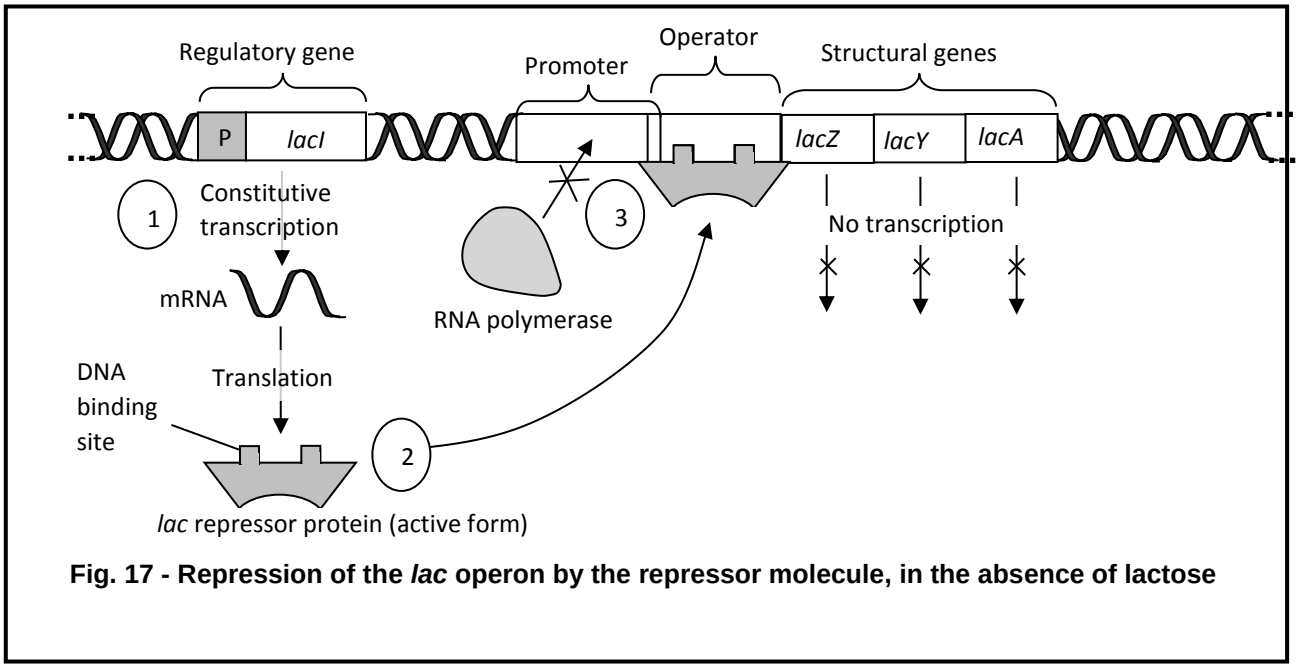
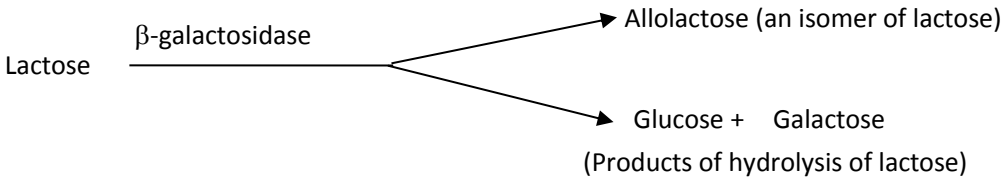


Fig. 17 - Repression of the *lac* operon by the repressor molecule, in the absence of lactose

(b) *lac* operon in the presence of lactose

- The *lac* repressor protein contains another functional region apart from its DNA-binding site. Known as the **allosteric site**, for the specific binding of **allolactose**, a structural isomer from lactose.

1. In the presence of **lactose**, a few molecules of lactose will enter the cell with the help of permease and are converted to allolactose by the few β-galactosidase molecules present. (Lactose is sometimes called an **inducer / effector** molecule as it causes a response.)



Even though the lac operon may be repressed, the repression is somewhat leaky/not 100% efficient, resulting in a low basal level of lac operon products i.e. permease, β-galactosidase (and transacetylase) within the cell.

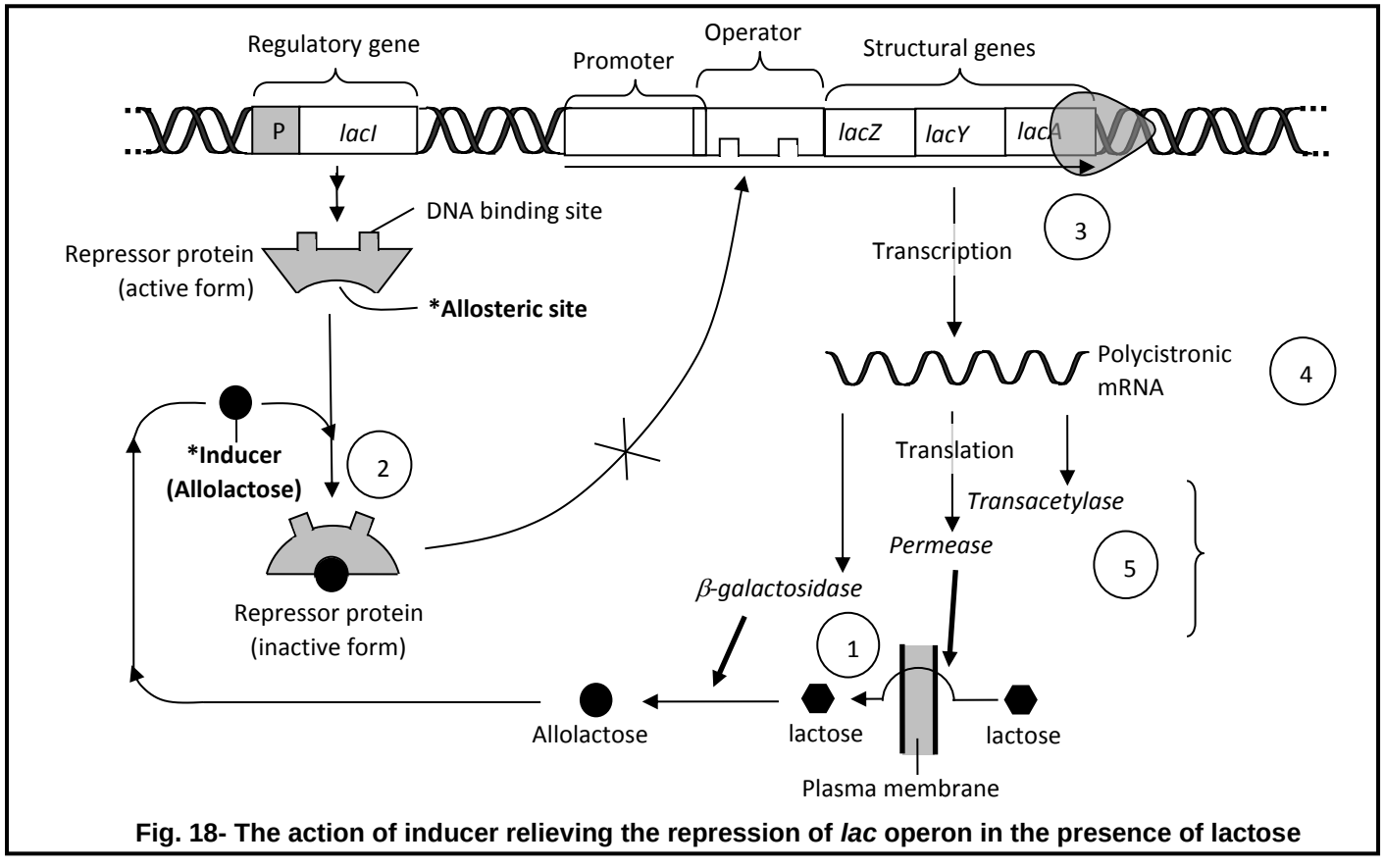


Fig. 18- The action of inducer relieving the repression of *lac* operon in the presence of lactose

Notes to Self:

2. Binding of allolactose to the **allosteric site inactivates the repressor** by altering the tertiary structure of the repressor so that its **DNA-binding site is no longer complementary to and cannot bind to the operator.**

3. **RNA polymerase** can **access and bind** to the **promoter** to initiate the transcription of the structural genes of the operon.

4. The structural genes are transcribed as a single **polycistronic mRNA**. Polycistronic mRNA is a messenger RNA that contains the base sequence coding for the amino acids sequence of several proteins.

5. All three enzymes are translated from a single mRNA molecule. Thus, all the genes in an operon are always expressed (or not expressed) in unison. *(The enzymes are translated separately because each has its own start and stop codon on the mRNA.)*

- The *lac* operon is thus an **inducible operon** that exhibited **negative gene regulation** (by default), a regulatory mechanism in which the **DNA-binding regulatory protein is a repressor that turns off transcription of the gene(s).**

- Inducible genes or operons usually code for enzymes that are part of **catabolic pathways**, which **break down** molecules. Hence the enzymes are expressed on only when lactose if present

(ii) POSITIVE REGULATION

- So far we studied how lactose regulates the expression of the *lac* operon but that is only part of a big picture. A second metabolite, **glucose**, is also **involved in the regulation of the *lac* operon**.

- If you look at the metabolic pathway in Fig.19, all sugars are converted to glucose before they enter the respiratory pathway to yield energy in the form of ATP.

-

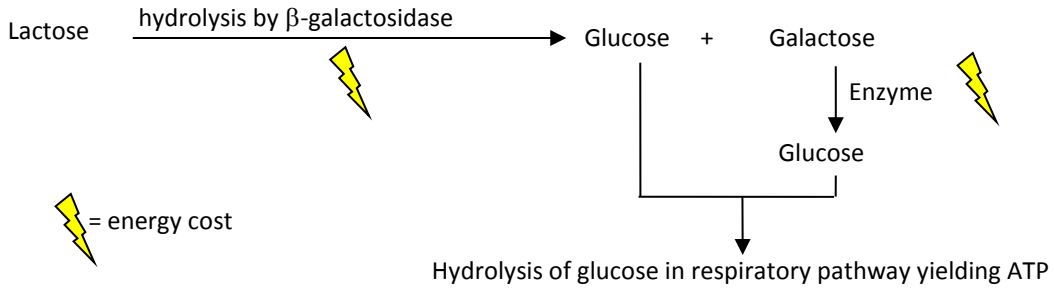


Fig. 19 - The lactose metabolic pathway

- Since considerable energy expenditure is required to synthesize additional lactose-metabolising enzymes such as ***β*-galactosidase**, it makes more

sense for *E. coli* cells to **utilise all available supplies of glucose first, before they start metabolising lactose.**

Notes to Self:

- So what regulatory mechanism ensures that the *lac* operon is "OFF" when glucose is present?

(c) *lac* operon in the presence of lactose and glucose

1. In reality, the ***lac* operon promoter** has a **low affinity for RNA polymerase**. Therefore, even in the presence of lactose, which inactivates the repressor, the *lac* operon is not fully activated on its own is unable to fully activate the *lac* operon. (Fig. 20)

2. A **second regulatory mechanism** that is **sensitive to the presence of glucose** is involved in the regulation of the *lac* operon.

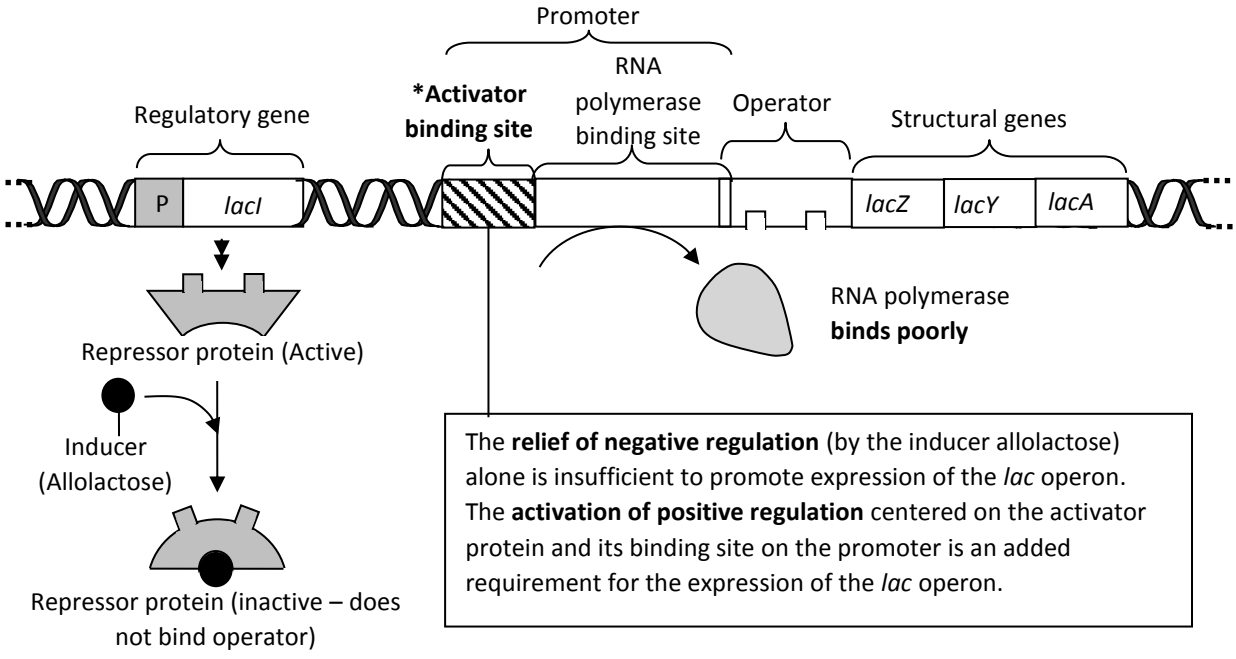


Fig. 20 - Efficient transcription of *lac* operon requires more than the presence of lactose

- **Positive gene regulation** of *Lac* operon therefore involves up-regulation by activator protein, **catabolic activator protein, CAP,** which binds to DNA and stimulate the transcription of the gene(s).

(d) *lac* operon in the presence of lactose but absence of glucose

- Similar to the repressor protein, the **activator protein** has a **DNA-binding site** and an **allosteric site**.
- The activator protein is the **catabolite activator protein (CAP)**.
  - Its **DNA-binding site** allows it to bind to the **activator / CAP-binding site** situated within the **promoter.**
  - Its **allosteric site** is specific for binding of **cAMP, or cyclic AMP**, an alternative form of AMP (adenosine monophosphate).
    (CAP is thus sometimes referred to as cAMP receptor protein (CRP).

1. In the absence of glucose, cAMP levels will increase.

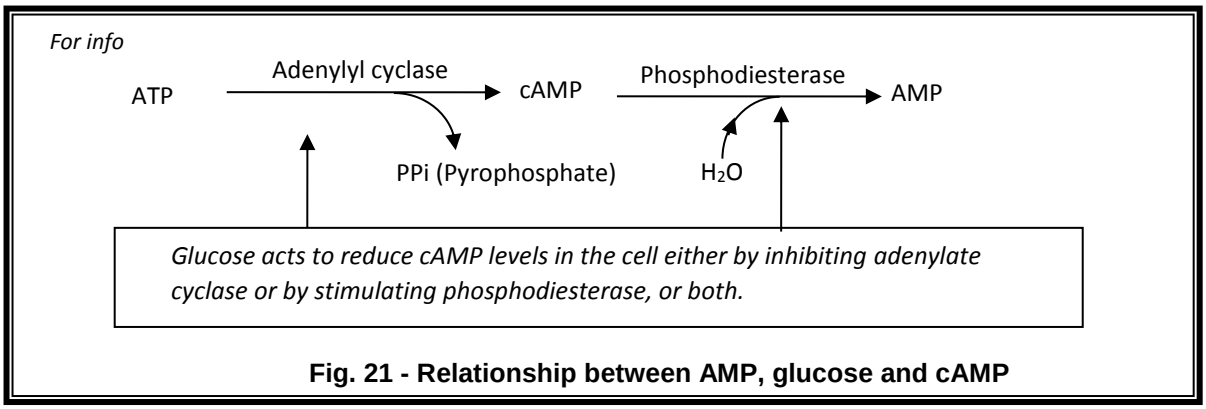
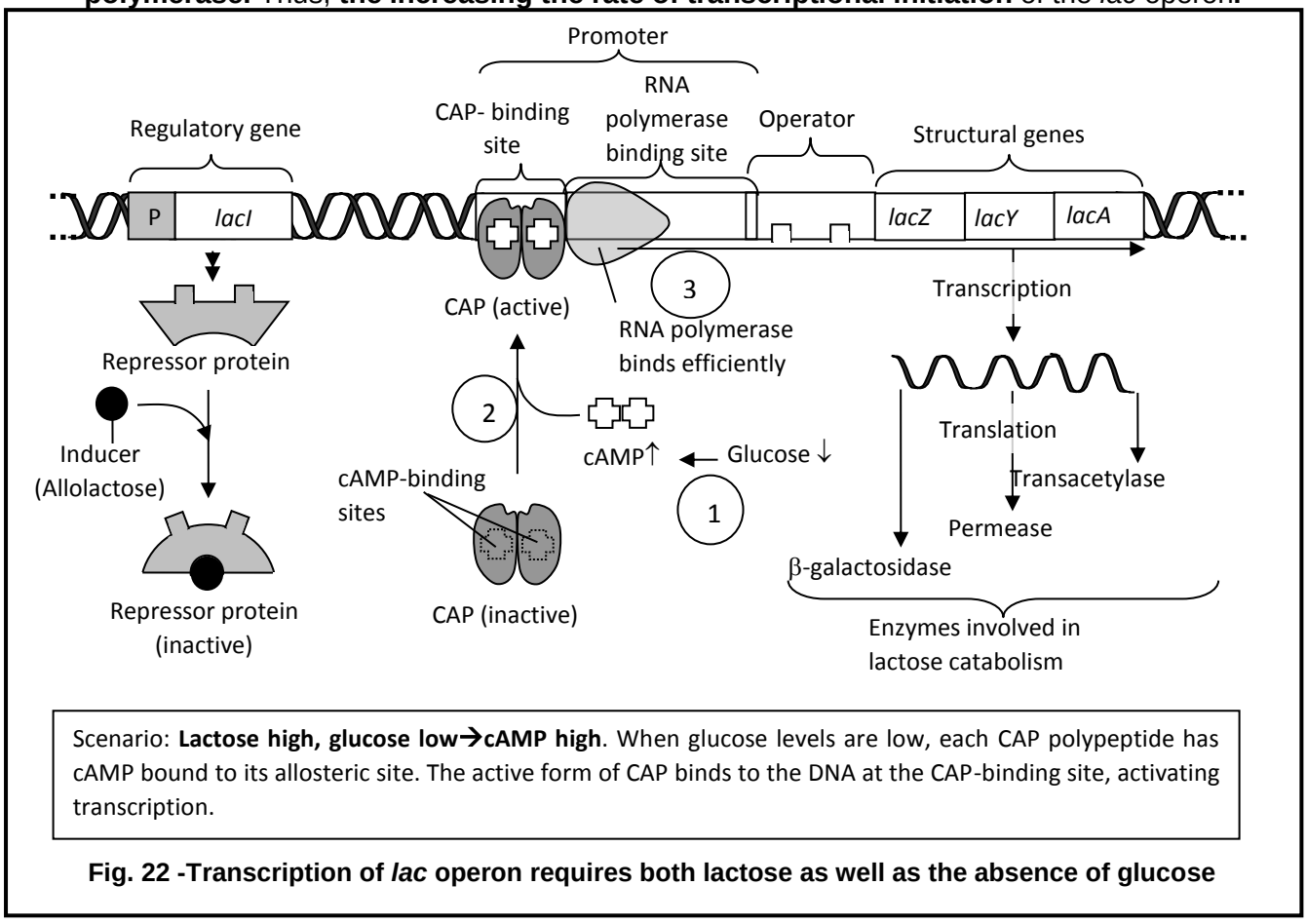


Fig. 21 - Relationship between AMP, glucose and cAMP

*** (Need to know that ↑**glucose** → ↓**cAMP** & ↓**glucose** → ↑**cAMP**)*

2. The **cAMP** binds to the **allosteric site** of CAP, **activating CAP** which binds to the **CAP-binding site within the promoter**.

3. This binding of activated CAP **increases the affinity of the promoter region for RNA polymerase.** Thus, **the increasing the rate of transcriptional initiation** of the *lac* operon**.**



Scenario: **Lactose high, glucose low→cAMP high**. When glucose levels are low, each CAP polypeptide has cAMP bound to its allosteric site. The active form of CAP binds to the DNA at the CAP-binding site, activating transcription.

Fig. 22 -Transcription of *lac* operon requires both lactose as well as the absence of glucose

- So for **lactose-metabolising enzymes** to be produced in **appreciable quantity**, it is not sufficient for **lactose** to be **present** in the bacterial cell. The other requirement is that **glucose** must be in **short supply**.

- This ensures that the preferred carbon source, glucose, is used before other alternative carbon sources are used i.e. if both glucose and lactose are present, rate of transcription of the operon genes will still be low.

Notes to Self:

- Thus the *lac* operon is under **dual control**: **negative regulation by the *lac* repressor and positive regulation by CAP.**

- Hence, the state of the *lac* repressor (with or without bound allolactose) determines whether the *lac* operon's genes undergo transcription or not; the state of CAP (with or without bound cAMP) controls the rate of transcription when the operon is repressor-free.
  (It is as though the operon has both an on-off switch and a volume control.)

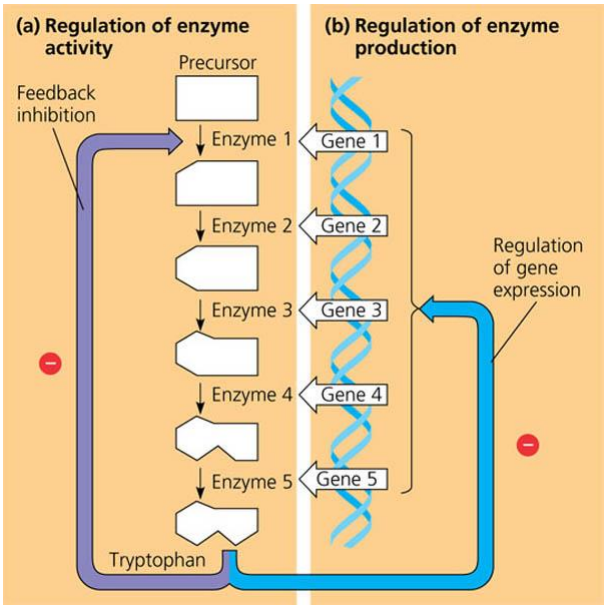
9. The *trp* operon (an example of a repressible operon)

The **tryptophan (*trp*) operon** *(trp* is pronounced "trip") is an example of a **repressible operon**.

- Repressible operons in bacteria are associated mainly with **anabolic** pathways which involve the synthesis of amino acids, nucleotides etc. from simpler materials.

- Repressible operons are normally turned "on" by default and they are turned "off" in the presence of an effector molecule. In most cases, this effector molecule is the **end product** of the **anabolic pathway** so as to avoid devoting resources to unnecessary synthetic activities once the end product has accumulated to sufficient amounts.

- Gene products of the ***trp* operon** in *E. coli* are involved in the **synthesis of the amino acid tryptophan** which is essential for protein synthesis. Hence the operon is usually "ON". (Fig. 23)



- However, when concentration of tryptophan rises e.g. when the host mammal consumes a protein rich diet, enzyme synthesis will be repressed. Since the ***trp* operon is turned 'off' in the presence of tryptophan,** tryptophan is thus the **effector** molecule.

- This process of regulation is called **end-product repression** or often just **repression**. This should be clearly **distinguished from end-product inhibition**. (Repression always occurs at the **level of transcription** of the enzyme while inhibition often involves **inhibition of enzyme activity**.)

Notes to Self:

- Tryptophan does not act directly on the operator but works together with a repressor molecule to repress the transcription of the *trp* operon.

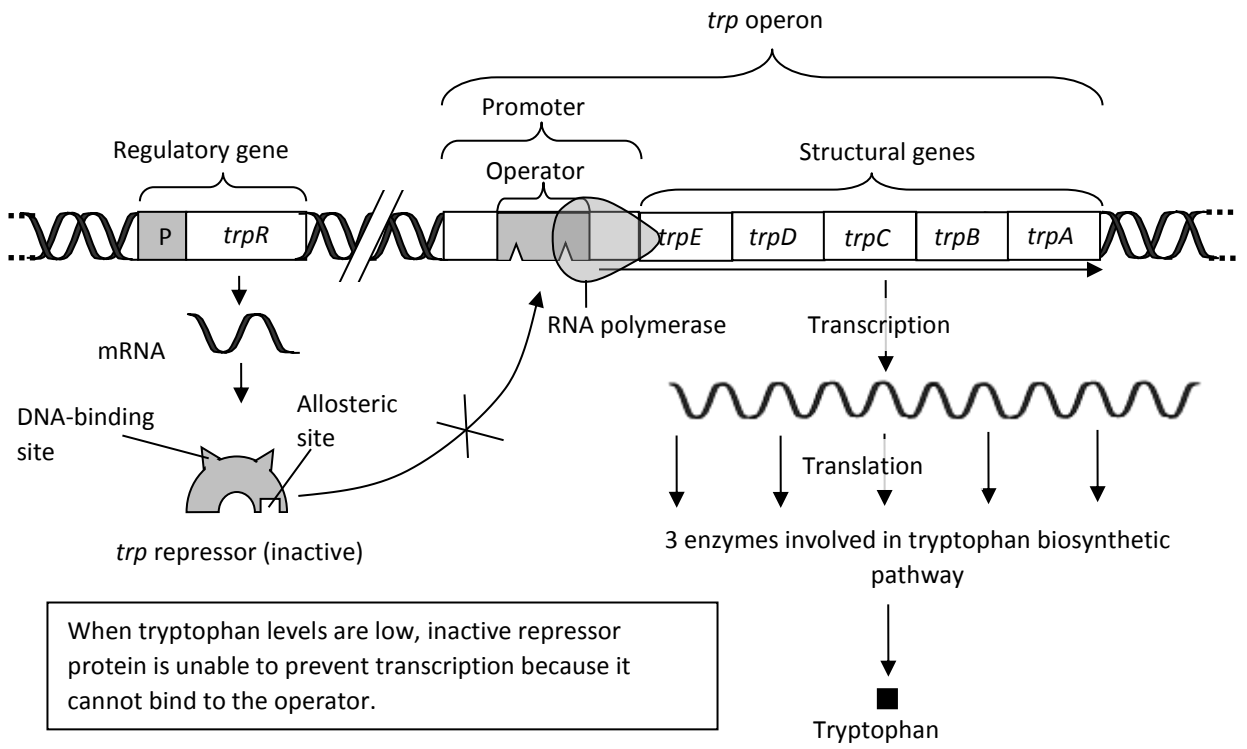


Fig. 23 - Organisation of the repressible *trp* operon; Default 'On'

Notes to Self:

1. The tryptophan repressor is **synthesised in its inactive form** with little affinity for the *trp* operator, which lies within the *trp* promoter. (See Fig. 23)

2. As tryptophan accumulates, it binds to the **allosteric site of the *trp* repressor, activating** the repressor.

3. The **activated repressor protein** binds to the **operator** at its DNA-binding site.
   - Tryptophan therefore serves as a **corepressor**, which works together with a repressor protein (by activating it) to switch an operon off.

4. With repressor bound to operator, RNA polymerase cannot bind to promoter and transcription cannot proceed, hence turning the <u>operon off</u>.

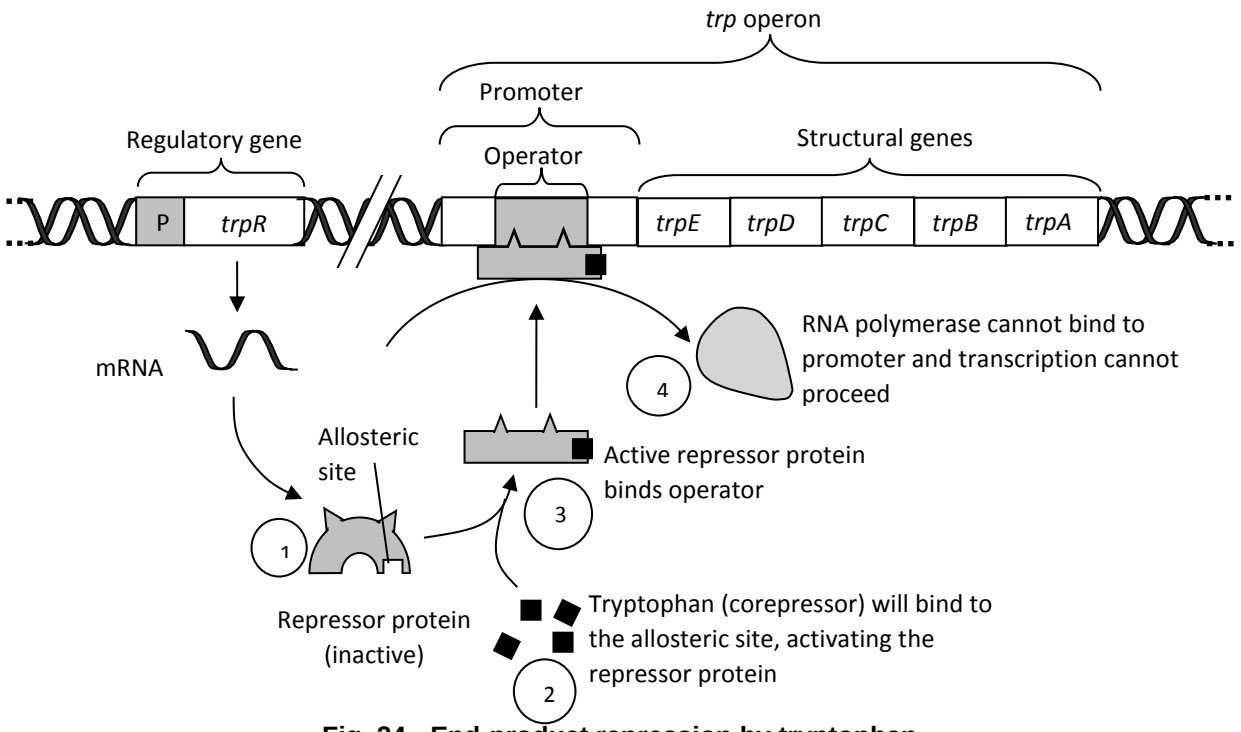
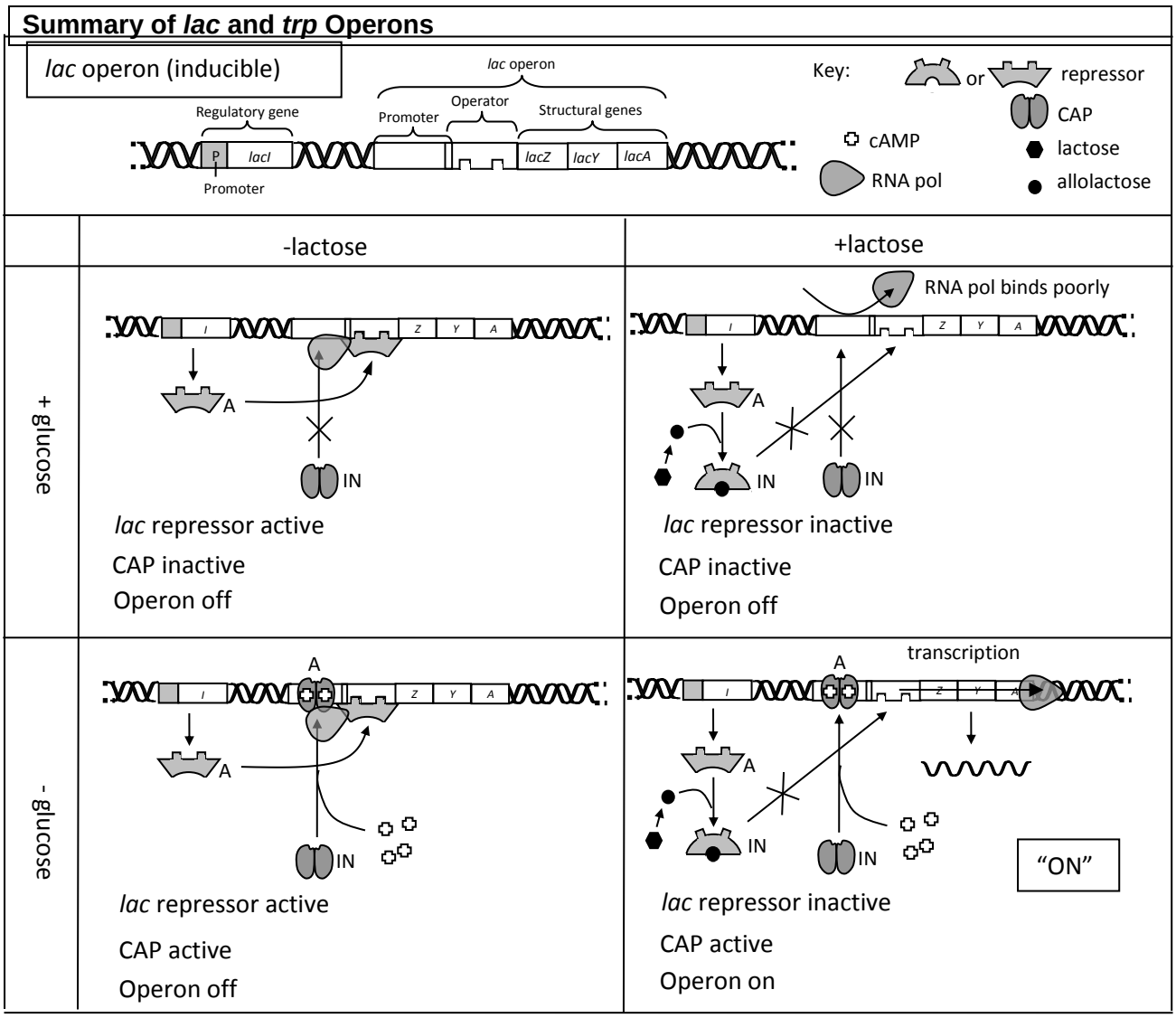


Fig. 24 - End-product repression by tryptophan

	Repressible operon	**Inducible operon** (consider just negative gene regulation)
Named example	*trp operon*	*lac* operon
Type of metabolic pathway	Anabolic pathways	Catabolic pathways
Effector molecule	Corepressor tryptophan, an end product	Inducer lactose, the substrate
Effect of effector on operon	Turns off structural genes	Turns on structural genes
Repressor synthesised in active/inactive form	Inactive form	Active form
When does repressor bind operator?	When complexed with corepressor	On its own
When does repressor not bind operator?	On its own	When complexed with inducer
Default operon expression	ON	OFF
Operon expression when effector molecule is present	OFF	ON

Table 2. Comparison between a repressible operon and an inducible operon

Summary of *lac* and *trp* Operons



Note: A = active; IN = inactive

10. Glossary

Notes to self

***Anabolic pathway**: Series of reactions that results in the synthesis of one of more specific cellular components.*

***Catabolic pathway**: Series of reactions that results in the degradation of one or more specific cellular components.*

***Colon:** Lower and larger part of the intestine.*

***Constitutive gene:** Refers to a gene that encodes a product required in the maintenance of basic cellular processes or cell architecture. Also known as housekeeping genes. They are expressed all the time.*

***Inducible enzymes**: Enzymes for which synthesis is regulated by the presence or absence of its substrate.*

Metabolic pathway**: Series of enzymatic reactions that convert one molecule to another via a series of intermediates. It is the sum of **catabolic and **anabolic*** pathways.*

***Motility**: The ability of an organism to move by itself; different from mobility.*

***Operator**: A site on DNA at which a repressor protein binds to prevent transcription from initiating at the adjacent promoter. (Genes VII, Lewin)*

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

***Recombination:** The formation of a new combination of genes on a chromosome as a result of crossing over.*

***Regulatory gene**: Any of several kinds of nucleotide sequence involved in the control of the expression of structural genes. It codes for a protein involved in regulating the expression of other genes e.g. repressor, CAP. (Genes VII, Lewin)*

***Repressible enzymes**: An enzyme whose synthesis is regulated by the presence or absence of a specific metabolite.*

***Structural gene**: Any gene that codes for a protein (or RNA) product that forms part of a structure or has an enzymatic function. (Genes VII, Lewin. Molcular Biology of the Cell, Bruce Alberts)*

***Terminator**: A regulatory sequence that signals the end of transcription*

11. Links

The topic of cell structure is relevant to the following topics and learning outcomes in the A level Biology syllabus. The links also become clearer when you have gone through the other topics.

Topic	Topic	How it is linked to Bacteria
1	Cell division	Binary fission is a means by which bacterial DNA replicates and cell divides. This is not to be confused with mitosis and cytokinesis.
2	DNA and genomics	DNA replication occurs during binary fission.
3	Virus and bacteria	Generalised and specialized transduction.
4	Prokaryotic and eukaryotic genome / Control of gene expressions	Bacteria are prokaryotes. Gene expression in bacteria using the operon system is regulated at the level of transcription.
5	Immunology	The mode of transmission and infection of bacterial pathogen. The modes of action of antibiotics, including penicillin, on bacteria.

Some Keywords

Notes to self

Structural gene	Generalised transduction	*lac Z*	*lac* repressor
Sex pilus	Specialised transduction	*lac A*	Negative gene regulation
Mating bridge/ conjugation tube	Prophage	*lac Y*	Positive gene regulation
F^+ plasmid	Template	*lac I*	Permease
Regulatory gene	Conjugation	*lac* operon	Transacetylase
Constitutive	Homologous recombination	*trp* operon	β-galactosidase
Peptidoglycan cell wall	Recombinant	Repressible operon and enzyme	Allolactose/lactose
Circular DNA	Lytic cycle	Inducible operon and enzyme/s	Allosteric site
70S ribosomes	Lysogenic cycle	Operator	Catabolite activator protein (CAP)
Binary fission	Transformation	Promoter	CAP binding site
Regulatory gene	RNA polymerase	Polycistronic mRNA	cAMP
Inducer/ Co-inducer	Co-repressor	Catabolic	Anabolic