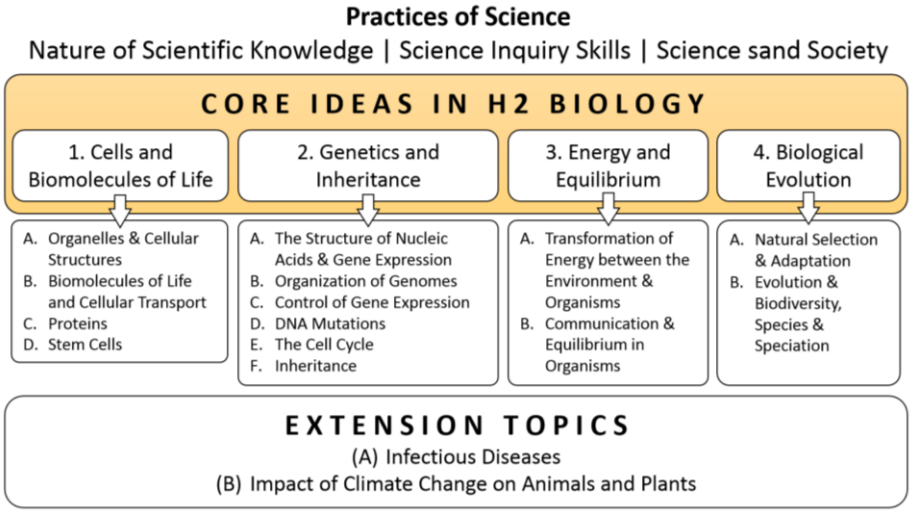
Core Idea 2B | 2C

10. Genetics & Inheritance (VII)

Organization of Genome & Control of Gene Expression in Prokaryotes



SYLLABUS OVERVIEW		
No.	**Overarching Idea**	**Topics**
1	**Core Idea 1** The Cell and Biomolecules of Life	Cell – The Basic Unit of Life
2		Biomolecules of Life and Cellular Transport
3	**Core Idea 3** Energy and Equilibrium	Transformation of Energy – Photosynthesis and Cellular Respiration
4	**Core Idea 2** Genetics and Inheritance	Genetics and Inheritance (I) – The Cell Cycle
5		Genetics and Inheritance (II) – DNA Replication and Gene Expression
6		Genetics and Inheritance (III) – DNA Mutations and their Consequences
7		Genetics and Inheritance (IV) – Molecular Techniques in DNA Analysis
8		Genetics and Inheritance (V) – Organization of Genome & Control of Gene Expression in Eukaryotes *[Includes Core Idea 1D: Stem Cells]*
9		Genetics and Inheritance (VI) – Organization and Inheritance of Viral Genomes
10		Genetics and Inheritance (VII) – Organization of Genome & Control of Gene Expression in Prokaryotes
11		Genetics and Inheritance (VIII) - Inheritance
12	**Core Idea 3** Energy and Equilibrium	Communication and Equilibrium in Multicellular Organisms
13	**Core Idea 4** Biological Evolution	Biological Evolution
14	**Extension Topic A** Infectious Diseases	Immunity and Infectious Diseases
15	**Extension Topic B** Impact of Climate Change on Animals & Plants	Climate Change – Causes and Impacts on Animals and Plants

NARRATIVES

An understanding of *Genetics and Inheritance* that would help make sense of the transition from molecular to organismal level. *Genetics and Inheritance* provides the molecular basis to the understanding of how variations in populations arise and this is important in the study of biological evolution. At the cellular level, expression of genes involves cellular structures such as the nucleus, endoplasmic reticulum and ribosome. Many essential products of gene expression are enzymes involved in biochemical pathways which control physiological functions. As such, mutation of genes may give rise to dysfunctional proteins which in turn could result in diseases. Sickle cell anemia and cancer are raised as examples of a monogenic and a multi-genic disease respectively.

The following questions should help students frame their learning:

- How does the genetic make-up of an organism influence its appearance, behavior and survival?
- How can we ensure continuity of human as a species?

Heritable information, in the form of DNA (and in some cases RNA), provides for continuity of life

Genetic information is stored in an organism's DNA; expression of genes results in the synthesis of functional products, such as rRNA, tRNA and proteins. These products play a role in intra- and extra-cellular biochemical pathways and influence the physiological processes in organisms.

Genomes contain heritable information necessary for continuity of life at all levels: cell, organism and system. This information is stored and passed on to subsequent generations via DNA. Reproduction can occur at the cellular or organismal level; each progeny needs to receive heritable genetic information from its parents.

An understanding of how eukaryotic, prokaryotic and viral genomes are organized has implications on how gene expression in organisms is controlled. The genome of prokaryotes typically comprises a large circular chromosome and smaller plasmids. Generally, structural genes, which code for proteins essential for bacteria survival, are found in the main chromosome while genes that confer advantages to bacteria survival in stressful environments are found in the plasmids. Prokaryotes reproduce by binary fission. In addition, genetic material can be transferred between bacteria through transformation, transduction and / or conjugation. This transfer of genetic material gives rise to genetic variation within a bacteria population.

LEARNING OUTCOMES

Core Idea 2B: Organization of Genomes
In addition to a large, circular chromosome, bacteria also have several plasmids. Even though bacteria reproduce asexually, they exhibit great deal of genetic diversity through mutation and genetic transfer.

Candidates should be able to:

a) Describe the structure and organisation of ~~viral,~~ prokaryotic ~~and eukaryotic~~ genomes (including DNA/RNA, single-/double-stranded, number of nucleotides, packing of DNA, linearity/circularity and presence/absence of introns)

d) 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.

Core Idea 2C: Control of Gene Expression
In prokaryotes, operons, like the trp and lac operons, regulate gene expression using repressible and inducible systems. Regulatory genes encode proteins that control transcription of structural genes.

Candidates should be able to:

a) 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)

LECTURE OUTLINE

1. Structure and Organization of Bacterial Genome
- 1.1 Structure of Bacterial Genome
- 1.2 Organisation of Bacterial Genome

2. Asexual Reproduction through Binary Fission
- 2.1 Process

3. Genetic Variation in Bacteria
- 3.1 Transformation
  - 3.1.1 Natural
  - 3.1.2 Artificial
- 3.2 Transduction
  - 3.2.1 Generalised
  - 3.2.2 Specialised
- 3.3 Conjugation

4. Control of Gene Expression in Prokaryotes
- 4.1 Concept of operon
  - 4.1.1 Structure of Operon
  - 4.1.2 Advantages of Operon
  - 4.1.3 Regulatory Gene
  - 4.1.4 Relationship between Operon and Regulatory gene coding for repressor
- 4.2 Regulation of operon
  - 4.2.1 Negative control
  - 4.2.2 Positive control
- 4.3 *Lac* operon – an inducible operon
  - 4.3.1 Context
  - 4.3.2 Structure of the *Lac* operon
  - 4.3.3 *LacI* regulatory gene
  - 4.3.4 Control of the *Lac* operon
  - 4.3.5 Mechanism of *Lac* operon
  - 4.3.6 Summary on Regulation of *Lac* operon
- 4.4 *Trp* operon – a repressible operon
  - 4.4.1 Context
  - 4.4.2 Structure of *Trp* operon
  - 4.4.3 *TrpR* regulatory gene
  - 4.4.4 Control of the *Trp* operon
  - 4.4.5 Mechansim of *Trp* operon
- 4.5 Inducible system vs repressible system

5. Comparison between Prokaryotes and Eukaryotes
- 5.1 Structure and Organisation of Prokaryotic vs Eukaryotic Genome
- 5.2 Prokaryotic vs Eukaryotic control of gene expression

6. Glossary

TEXTBOOK REFERENCES

1. Biology by Campbell and Reece, 9th Edition, *Pages 556-564 and 352-355*
2. Biological Science by R.Soper, 3rd Edition.
3. Biology of Microorganisms by Brock Madigan Martinko Parker, 7th Edition

Web-links And Animations

Structure and Organisation of Bacterial Genome:

Bacterial chromosome compaction	Supercoiling of bacterial chromosome
https://www.youtube.com/watch?v=30B0wGAID4o	https://youtu.be/5hwaDamU-jo

Binary fission in bacteria

https://www.youtube.com/watch?v=XICA-cdvSvU

Genetic Variation in Bacteria:

Artificial Transformation 0:00-1:25	Transformation, Transduction and Conjugation	What is a plasmid?	Bacterial conjugation
https://www.pbslearningmedia.org/resource/biot11.sci.life.gen.transbact/transforming-bacteria/	https://www.youtube.com/watch?v=7tLV20dk-FM	https://www.youtube.com/watch?v=SdqJFA6mOkI	https://www.youtube.com/watch?v=hm8SZaFmlWg

Control of Gene Expression in Prokaryotes:

Lac Operon	Lac Operon *(highly recommended!)*
http://www.sumanasinc.com/webcontent/animations/content/lacoperon.html	https://www.youtube.com/watch?v=-4Jr84qyKPo&t=231s

1. Structure and Organisation of Bacterial Genome

***Core idea 2B**- Candidates should be able to:*

a) Describe the structure and organisation of ~~viral,~~ prokaryotic ~~and eukaryotic~~ genomes (including DNA/RNA, single-/double-stranded, number of nucleotides, packing of DNA, linearity/circularity and presence/absence of introns)

1.1 Structure of Bacterial Genome

Key Concept 1:
Prokaryotic cells lack membrane-bound organelles. All bacteria contain cytoplasm, ribosomes, a plasma membrane and a nucleoid containing DNA. Almost all bacteria have cell walls.

- Dimension of bacteria:
  - Diameter of ~**1 μm**, length of **0.1 – 10 μm**, as compared to eukaryotic cells with diameter of 10-100 μm.

- Bacteria **do not** have a nucleus; they have a **nucleoid** region (i.e. not membrane-bound) where chromosomal DNA is found (Fig. 1.1a).

- Chromosomal DNA is a **single, circular, double-stranded DNA.**
  - Consists approximately **5 x 10^6 base pairs** and of length 1 mm (NOTE: bacterial genome differs in length between various species). This is considerably less than eukaryotic cells but more than that of viruses.
  - Contains a **single origin of replication.**

- *Some* bacteria may have one or more extrachromosomal DNA called **plasmids** (Fig. 1.1a). They are smaller **circular, double-stranded DNA**.
  - Plasmids **replicate independently** from the bacterial chromosome.
  - Plasmids may contain a few **beneficial, but non-essential genes.**
  - Different types of plasmids have different functions.
    - **F (fertility) plasmids** → carry genes coding for sex pilus (Refer to section 3.3 on Conjugation)
    - **R (resistance) plasmids** → carry antibiotic resistance genes (eg. code for proteins that break down β-lactam in antibiotic penicillin)

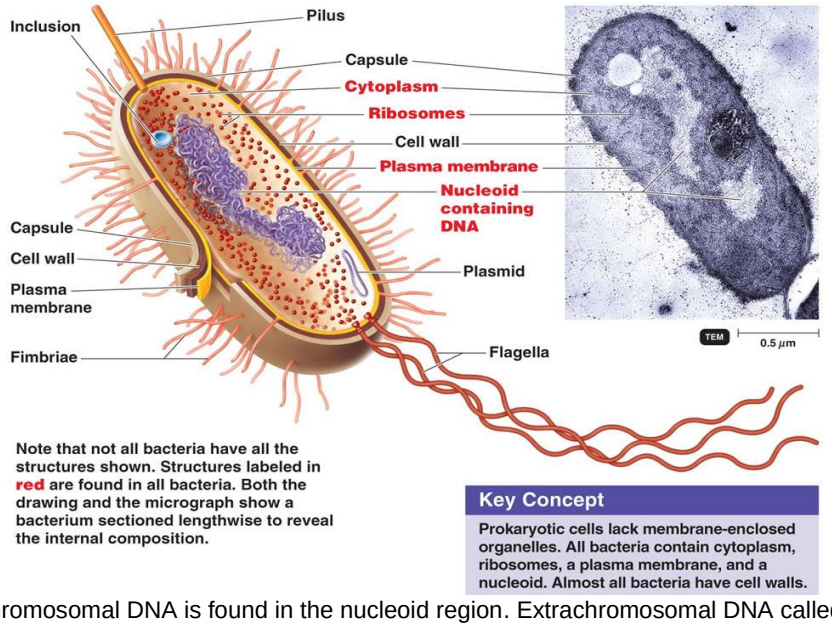


Fig. 1.1a: Bacterial chromosomal DNA is found in the nucleoid region. Extrachromosomal DNA called plasmids may be found in some bacteria.

1.2 Organisation of Bacterial Genome

- Due to the small size of bacteria (diameter of 1 μm, length of 0.1 – 10 μm), bacterial genomes have very compact genome organization and very little space between the genes.

- For the chromosomal DNA to fit into the cell, chromosomal DNA needs to be folded or coiled. This process of DNA packing is called chromosomal compaction. It involves **DNA binding proteins** (nucleoid-associated proteins) that help to form initial **loops**, followed by the **supercoiling** of DNA (Fig. 1.1b).

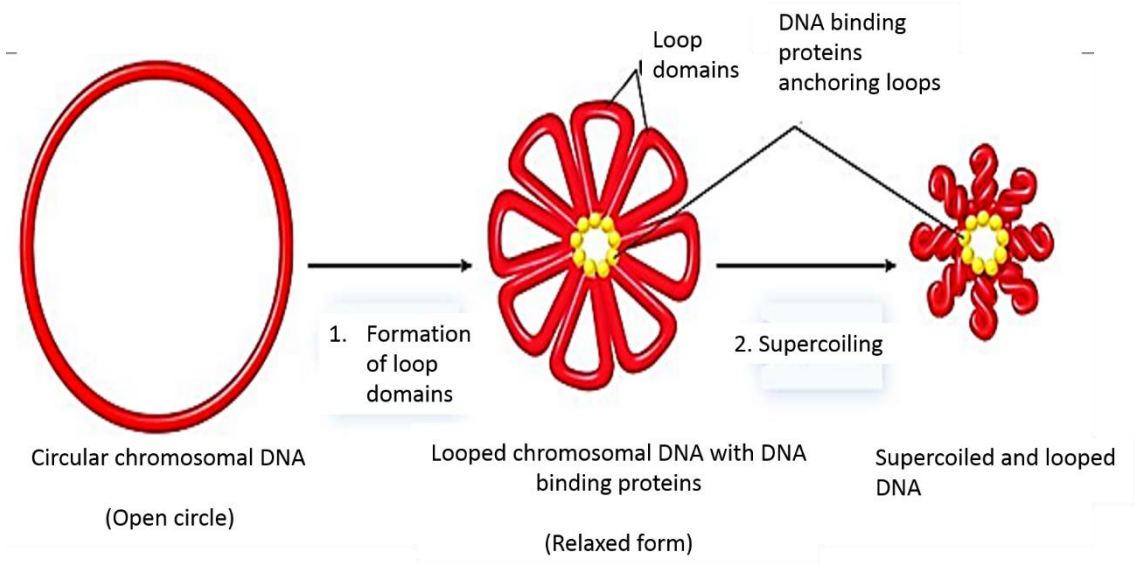


Fig. 1.1b: Bacterial chromosomal DNA compaction. The chromosomal DNA changes from the open circle, relaxed form to the supercoiled, looped form.

- Some features of the prokaryotic genome are:
  - **Absence of introns** in prokaryotic genes. Each gene consists of a continuous coding sequence.
  - **Few repetitive sequences**
  - **Shorter sequence of genes** (2/3 the length of eukaryotic genes)
  - **Related genes** (e.g. genes involved in regulating same metabolic pathway) are organized into an **operon**, and are controlled by a **single promoter** *(Refer to: Section 4.1).*

2. Asexual Reproduction by Binary Fission

***Core idea 2B**- Candidates should be able to:*

d) 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.

Key Concept 2:
Bacteria can reproduce quickly by binary fission to form genetically identical daughter cells.

- Bacterial cells reproduce by **binary fission** (Fig. 2.1a), which is a form of **asexual reproduction.** [NOTE: binary fission is NOT mitosis! Why?]

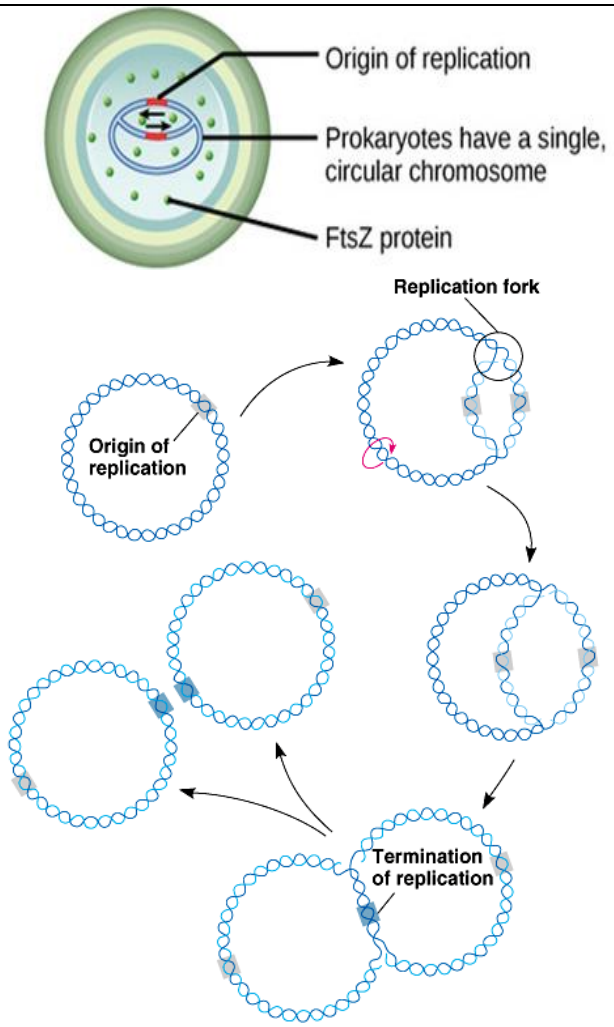
- Significance: Many cycles of binary fission produce a **colony of bacteria** that are **genetically identical** to the **parent cell** (i.e. no genetic variation).

2.1 Process

Step 1: Duplication of Chromosome

- **DNA replication** occurs at **a single origin of replication** to produce 2 origins**.**

- Both strands of DNA act as templates to produce **double amount of DNA** *via* **semi-conservative replication.**

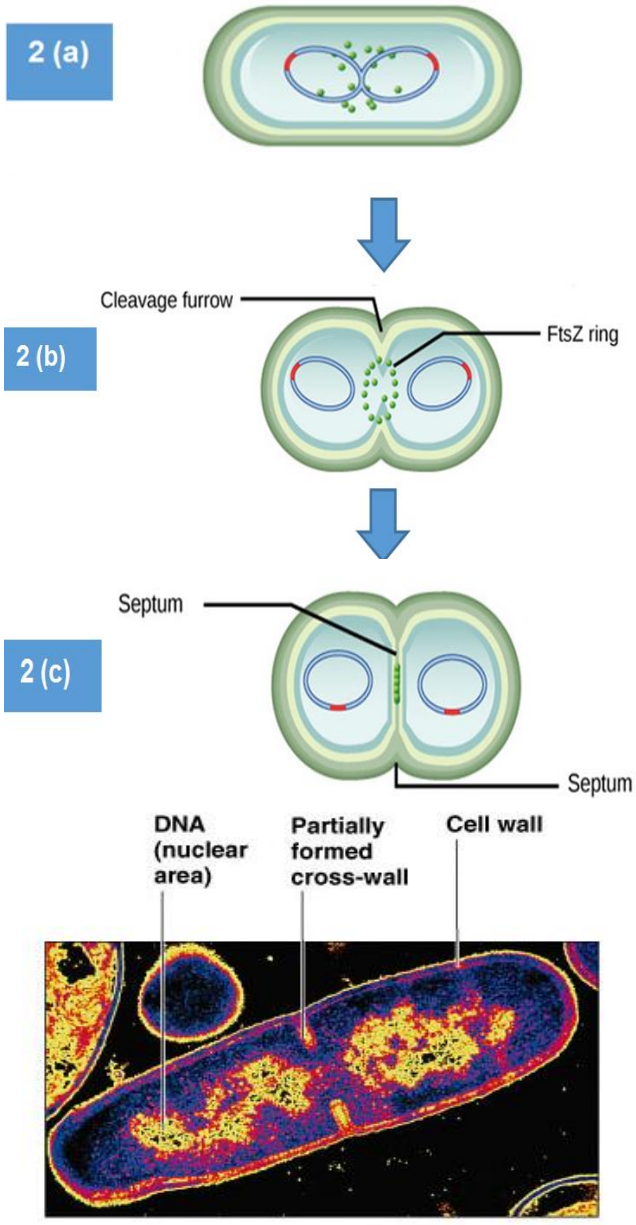


Step 2: Cell Elongation

(a) While chromosome is replicating, the bacterium **elongates** and continues to **grow**.

(b) FtsZ proteins move to the midpoint between chromosomes to form a ring.

(c) The ring directs the formation of a septum that divides the cell. Plasma membrane and cell wall materials accumulate.



Step 3: Division into two cells

- After the septum is complete, the cell separates into two, forming **two genetically identical daughter cells.**

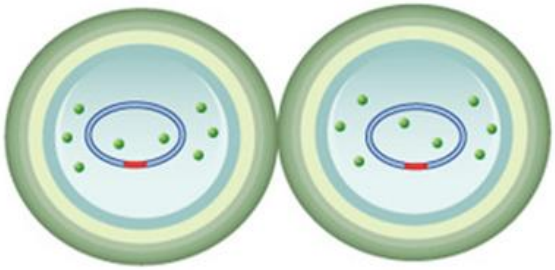


Fig. 2.1a: Process of binary fission in bacteria

Note:

- Plasmids can replicate independently of the chromosomal DNA. Hence, **different numbers** of plasmids may be inherited by the daughter cells after binary fission. This may lead to daughter cells becoming *genetically different* from the parent cell (Fig. 2.1b).

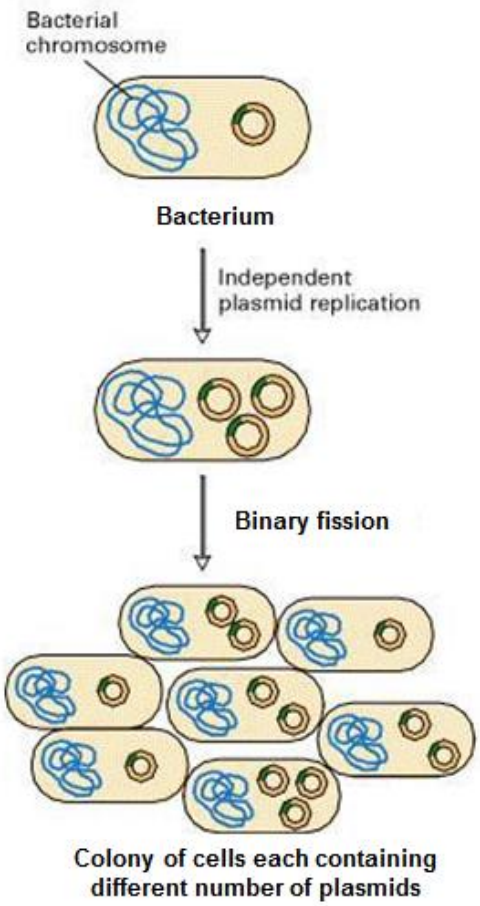


Fig. 2.1b: Different numbers of plasmids in each daughter cell after binary fission.

3. Genetic variation in Bacteria

***Core idea 2B**- Candidates should be able to:*

d) 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.

Key Concept 3:
Mutation and genetic recombination give rise to genetic variation in prokaryotes.

- Unlike eukaryotes, bacteria cannot achieve genetic variation via crossing over during meiosis or through fertilization. Instead, **genetic variation** in bacteria is generated by **mutations** and **genetic recombination**.
    - **Mutations** can increase genetic variation in bacteria quickly as bacteria have **rapid reproduction rate** (e.g. *E.coli* can reproduce in 20 minutes).
    - Genetic variation can also arise from **genetic recombination** i.e. **exchange of genetic material between homologous DNA regions of different bacterial cells**.
    - Genetic variation in bacterial populations allows bacteria to adapt to and survive in different environments.
- Genetic recombination can occur through 3 different processes:
    - **Transformation** (Section 3.1)
    - **Transduction** (Section 3.2)
    - **Conjugation** (Section 3.3)
- Genetic recombination usually **involves homologous recombination** (Fig. 3.1a):
    - **Homologous DNA** sequence contains the **same type of gene** but **different alleles**.
    - **Pairing** of two homologous chromosomes occurs.
    - One **donor DNA strand** is nicked, which then displaces a **recipient DNA strand** at the same region. This results in an **exchange of genetic material** between the two strands of DNA, and new phosphodiester bonds are formed between the donor strand and recipient DNA.
    - Subsequently, the donor DNA strand may act as a template for the synthesis of its complementary strand in the recipient DNA, thereby completing the process (not shown). The cell is now a **recombinant**: Its chromosome contains DNA from **two different cells.**

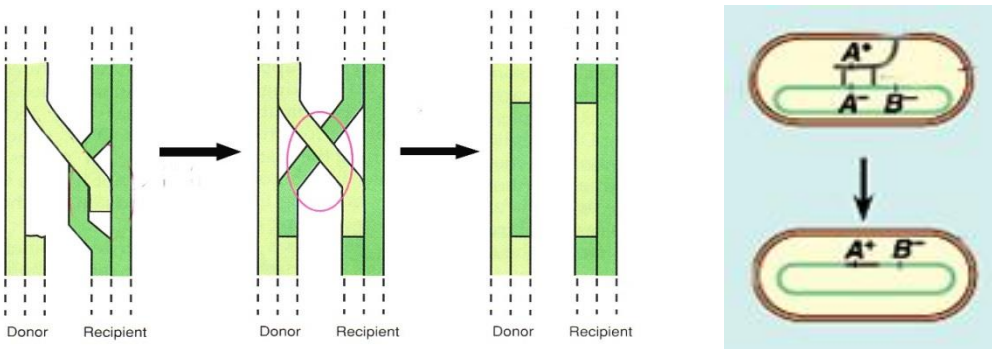


Fig. 3.1a: Left: Homologous recombination between homologous regions of DNA from two different bacterial cells. Right: An example of homologous recombination, resulting in an exchange of A^- allele for A^+ allele.

3.1 Transformation

- **Transformation** is the **incorporation** of **naked DNA** (fragments of DNA) from the **environment** into the **recipient cells** to alter the **bacterial genotype**.
- A bacterial cell that is capable of transformation is called a **competent cell.**
- Bacterial transformation can occur via 2 modes, namely:
    - Natural transformation (in some species of bacteria) – This is a random process which occurs by chance in nature.
    - Artificial transformation – This is induced in the laboratory by scientists.

3.1.1 Natural Transformation (Fig. 3.1b)

1) The DNA of the donor bacterium is **fragmented** and **released** into the environment.
2) One of the fragments is taken up by a **competent** recipient bacterium. The competent cell has **DNA binding proteins** on the **outer surface of the cell wall** to bind to exogenous DNA from closely related species and transport it into the cell.
3) **Homologous recombination** occurs and the **donor DNA is incorporated into the DNA genome of recipient cell.**

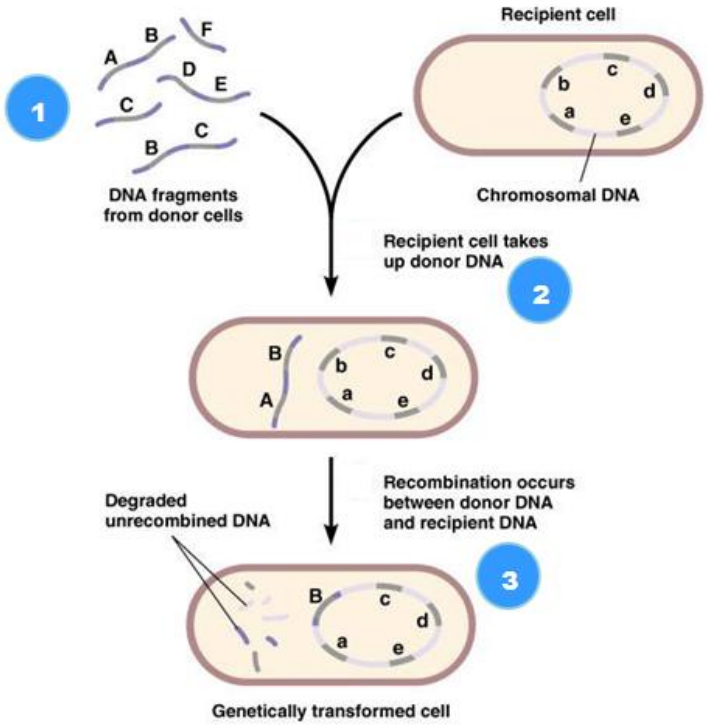


Fig. 3.1b: Natural transformation.

3.1.2 Artificial Transformation (Fig. 3.1c)

1) The bacterial cells are first mixed with calcium chloride ($CaCl_2$) and kept at 0°C.
   - Significance: The addition of calcium ions serves to **neutralize** the repelling **interaction between** the **negatively-charged DNA and** the **poly-anions (negatively-charged ions) of the membrane**. $CaCl_2$ **increases the competence of bacterial cells to take up DNA**.

2) The foreign DNA (eg. plasmid containing ampicillin resistance gene) to be transformed into the bacterial cells are added to these bacterial cells and incubated at 0°C for about 30 min.
   - Significance: **stabilize** the lipid **membrane** and allow for increased interactions between calcium ions and the negative poly-anions of the membrane.

3) The mixture is subjected to **60s** of **heat shock** treatment at **42°C.**
   - Significance: Heat shock **creates transient pores** on the bacterial cell surface membrane, **allowing DNA to enter the cells**. Bacterial cells which have taken up exogenous DNA are said to be **transformed**.
   - Note: **transformation efficiency is usually very low (less than 1%)**. In another words, out of 1000 bacterial cells, less than 10 will take up the exogenous plasmid.

4) The mixture is kept at 0°C for about 2 min.
   - *Significance: 'Recovery' stage. Allows the pores to close.*

5) The bacterial cells are incubated at 37°C overnight.
   - *Significance: Allows binary fission to take place to produce bacteria colonies.*

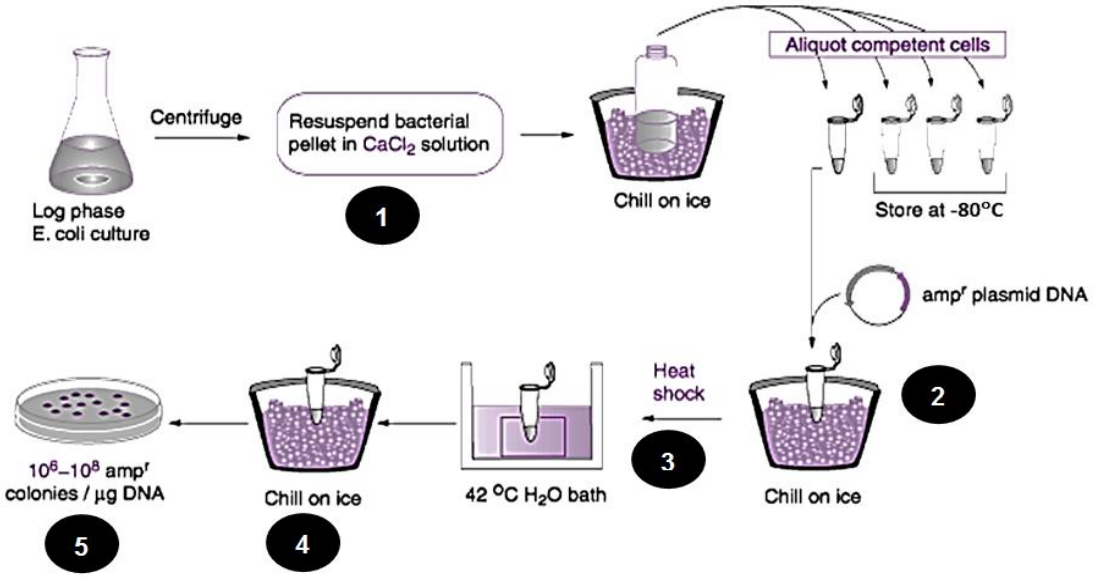


Fig. 3.1c: Artificial transformation.

3.2 Transduction

- **Transduction** is the **transfer of bacterial DNA by bacteriophages** (viruses that infect bacterial cells) **between bacterial cells**.

- There are two types of transduction:
  - Generalized transduction (Section 3.2.1)
  - Specialized transduction (Section 3.2.2)

3.2.1 Generalised Transduction (Fig. 3.2a)

- Generalized transduction occurs during the **lytic life cycle** of a virulent phage such as **T4 phage.**

1. During the adsorption phase, the **virulent phage attaches** to and **infects** a host bacterial cell by **injecting phage DNA** (shown in light blue) into the host cell.

2. The host **bacterial DNA** (shown in black) is hydrolysed / **degraded** into pieces by phage enzymes.

3. During the synthesis phase, **phage** particles **replicate** by using bacterial cell's DNA polymerase and ribosomes to make new viral DNA and proteins.

4. **Assembly** of the phage particles occur when the **viral DNA** is **packaged inside** the **capsids** (protein coat of the virus).

   Occasionally, during encapsidation of viral DNA, a small piece of the **degraded bacterial DNA is randomly packaged within a capsid**, forming a **generalized transducing phage particle** (carries only bacterial DNA).

5. When this generalized transducing phage particle **infects another bacterium** (i.e. second round of infection), it **injects bacterial DNA from the previous host** into its new bacterial host.

6. Some of this bacterial DNA can subsequently replace the homologous region of the new host's cell by **homologous recombination**.

   The recipient bacteria will have a **recombinant DNA** that is derived from **two bacterial cells**.

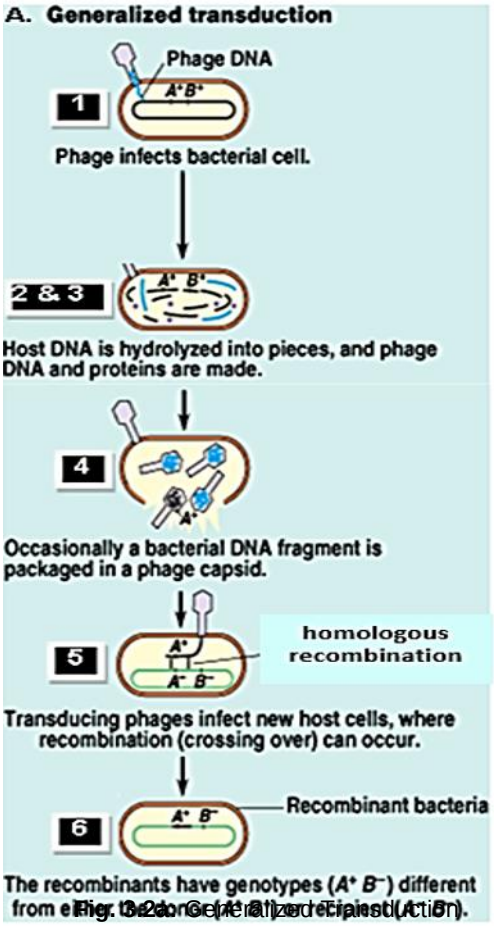


Fig. 3.2a: Generalized Transduction

3.2.1 Specialised Transduction (Fig. 3.2b)

- Specialized transduction occurs during the **lysogenic life cycle** of a temperate phage such as **lambda phage.**

1. When **temperate phage** recognizes, **attaches** and **infects** a **bacterial** host cell, the **viral DNA** (shown in light colour) is **integrated** into the **bacterial chromosome** (shown in black). The integrated phage DNA is known as a **prophage**.

2. Environmental factors (e.g. UV light) can induce a switch in the phage replication mode from **lysogenic to lytic**. When this occurs, the **prophage** is **excised** from the bacterial chromosome to initiate a lytic cycle.

   Occasionally, this **excision** is **imprecise** causing a small region of **adjacent** bacterial DNA to be excised with it. This **prophage with adjacent bacterial genes** are **packaged into** a **capsid** forming a **specialized transducing phage particle** (carries a hybrid of viral and bacterial DNA).

3. When this specialised transducing phage particle **infects another bacterium** (i.e. second round of infection), the **bacterial DNA from the previous host** and the phage genome is **injected** into its new bacterial host.

   Some of this **bacterial DNA** can subsequently **replace** the **homologous region** of the **new host's cell** by **homologous recombination.**

4. The recipient bacteria will have a **recombinant DNA** that is derived from **two bacterial cells**.

   Note: Specialized transduction *only* transfers bacterial genes **near** the prophage integration site on the bacterial chromosome.

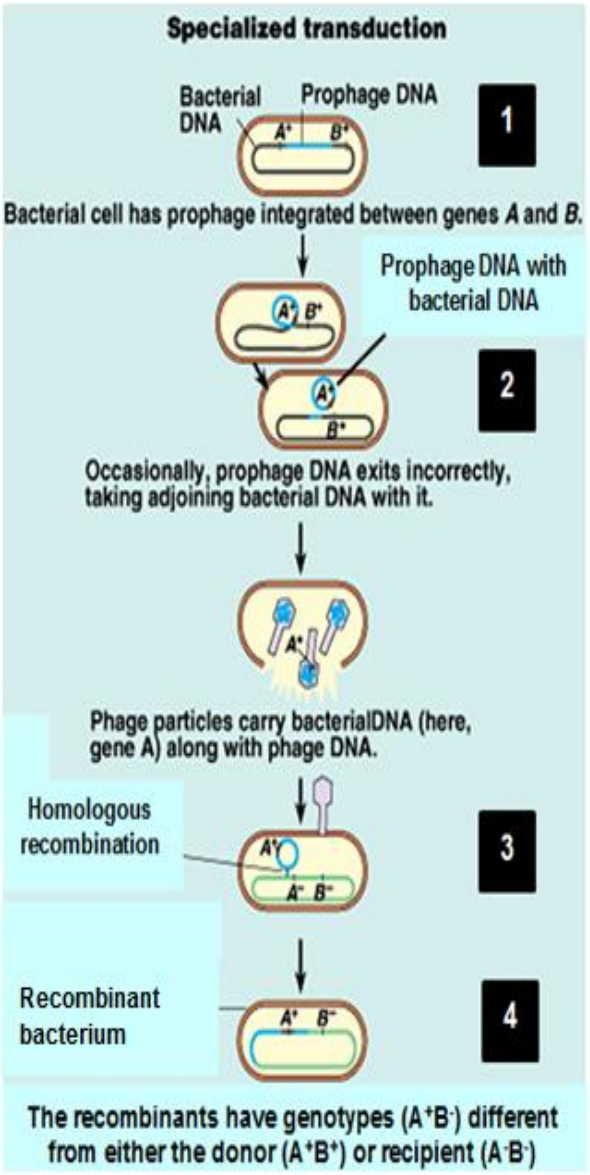


Fig. 3.2b: Specialized transduction

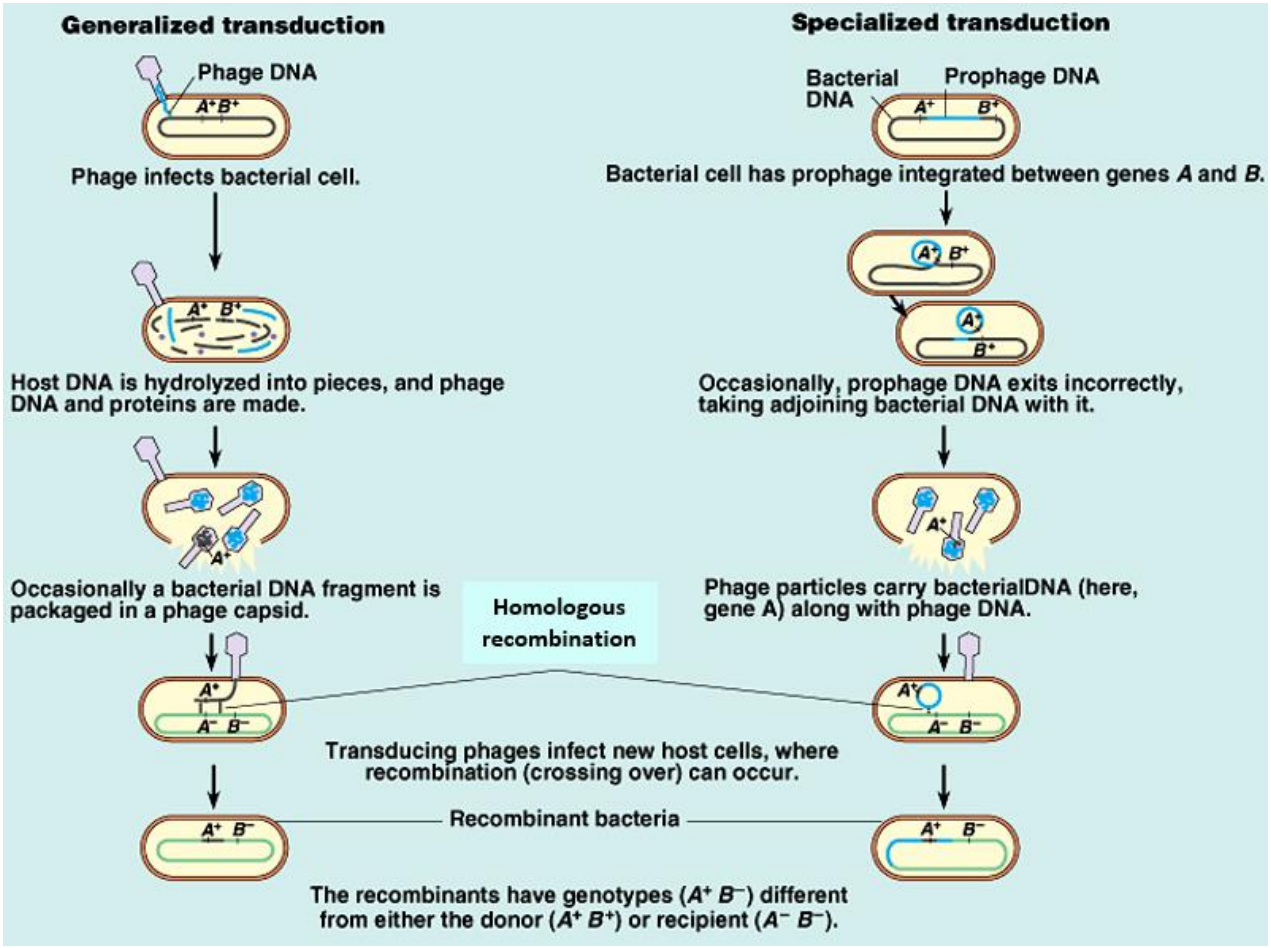


Fig. 3.2c: Comparison between generalized and specialized transduction.
Note that the recombinant bacterium as a result of generalized transduction contains only bacterial DNA, while the recombinant bacterium after specialized transduction contains both bacterial DNA (the new allele A^+) and prophage DNA.

Comparison between Generalised Transduction and Specialised Transduction

Features	**Generalised Transduction**	**Specialised Transduction**
Type of virus		
Effect on host genome	• Host cell's DNA is _____	• Host cell's DNA is _____ during _____cycle • Host cell's DNA is eventually _____ During_____ cycle
Integration of viral DNA into host chromosome	• Viral DNA is _____ into the bacterial chromosome	• Viral DNA is _____into the bacterial chromosome to form _____-
Type(s) of phage cycle	• Occurs during the_____ cycle	• Occurs during the _____ cycle for integration of viral DNA • When under environmental trigger, switches to _____ cycle
Types of DNA in transducing particles	• _____DNA is randomly packaged within a capsid	• _____ with adjacent _____ are packaged into a capsid

3.3 Conjugation

- **Conjugation** is the **unidirectional transfer** of genetic material in the form of an **F (fertility) plasmid** from a donor bacterial cell to a recipient bacterial cell via a **sex pilus** (plural: pili). (Fig. 3.3a)

- The **donor** bacterium, is known as the **F^+ cell (contains F plasmid).** It donates DNA in a form of F (fertility) plasmid.
  - Role of F plasmid: contains the **origin of replication**, an **origin of transfer,** and **genes coding for the formation of sex pilus.**

- The **recipient** bacterial cell, is known as **F^- cell (does not contain F plasmid).** Hence the F^- cell receives genes in a form of F plasmid from the donor bacterium. (Fig. 3.3b)

- A **sex pilus** formed by the F^+ cell temporarily joins the two bacterial cells (enabling **cell-to-cell contact**) and creates a **cytoplasmic bridge** between the bacterial cells.

- Conjugation can occur within the same bacterial species or between different bacterial species.

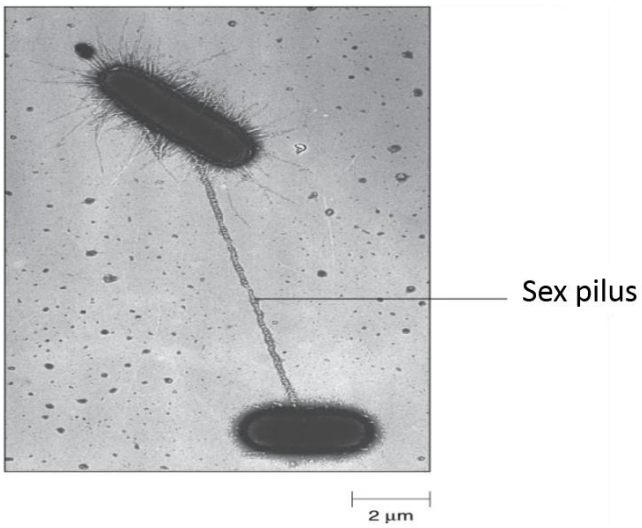


Fig. 3.3a. Sex pilus formed between F^+ and F^- cells.

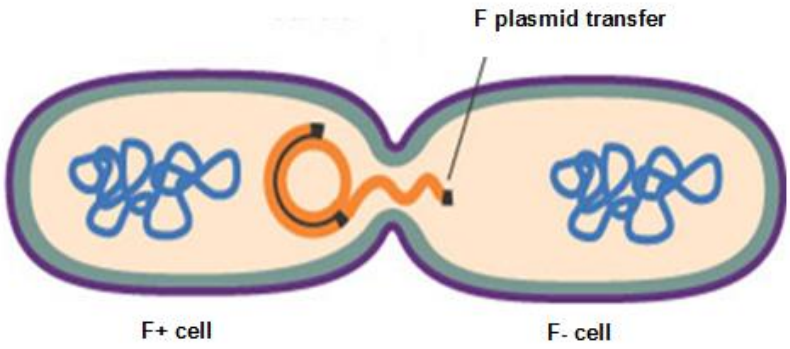


Fig. 3.3b. F plasmid transfer between F^+ and F^- cells.

Process of Conjugation by the Rolling Circle Mechanism (Fig. 3.3d):

1) F^+ cell produces a **sex pilus**.

2) **Sex pilus** attaches to recipient cell (F^- cell) and brings the F^+ cell and F^- cell together.

3) F plasmid replicates by the rolling-circle mechanism. (Fig. 3.3c)

   (a) One of the strands of **F plasmid** is **nicked by relaxosome** at the **origin of transfer (*OriT*)**, forming a 3' OH end and 5' P end.

   (b) F plasmid DNA begins to **unwind** with the help of **relaxosome**.

   (c) The **5' P end of the nicked DNA strand** is then transferred with the help of **transferosome** to the recipient cell.

   (d) **Un-nicked DNA strand** is used as a **template** where DNA polymerase adds deoxyribonucleotides to the **free 3'OH end to synthesize a complementary DNA strand**. This restores the double stranded F plasmid in the **donor cell**.

   Nicked DNA strand is used as a template and to form the complementary DNA strand **discontinuously**. This restores the double stranded F plasmid in the **recipient cell**.

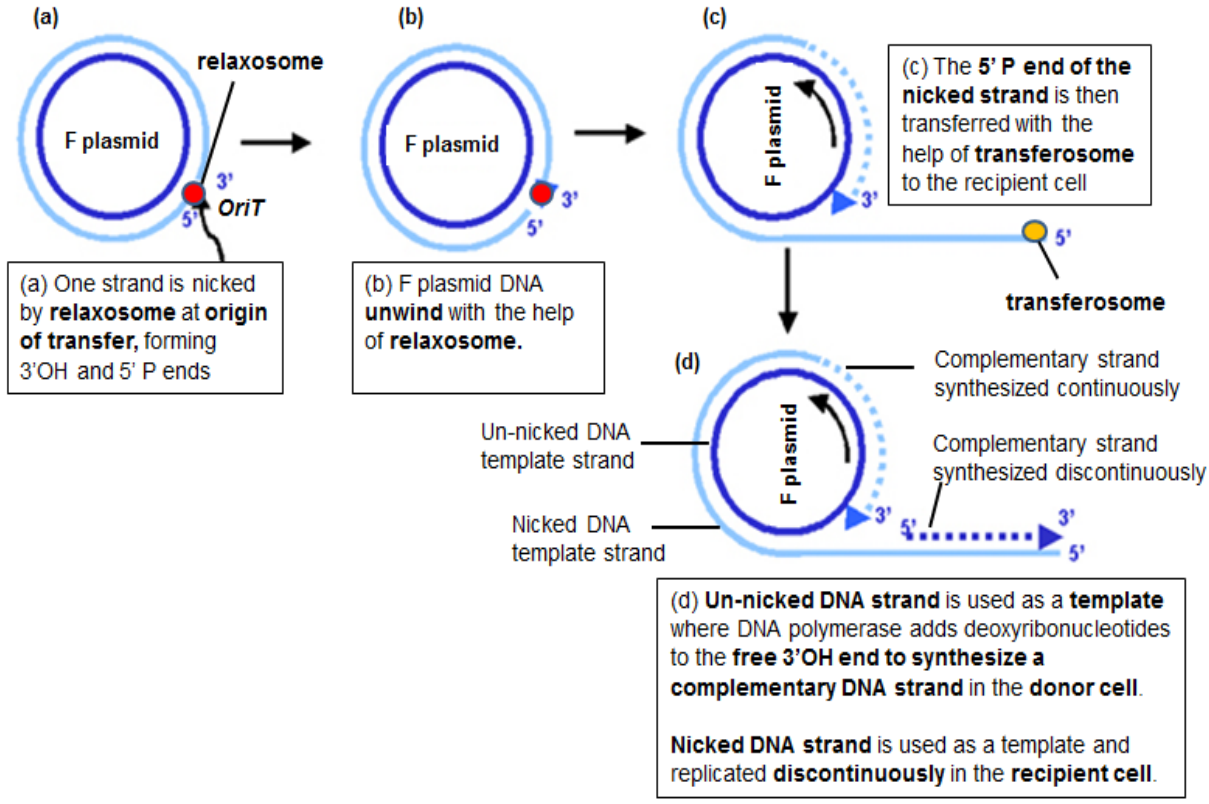


Fig. 3.3c: The rolling circle mechanism

4) The new DNA double helix becomes an **F plasmid** in the F^- recipient cell, hence **converting** the recipient F^- cell to an **F^+ cell** and the cells separate.

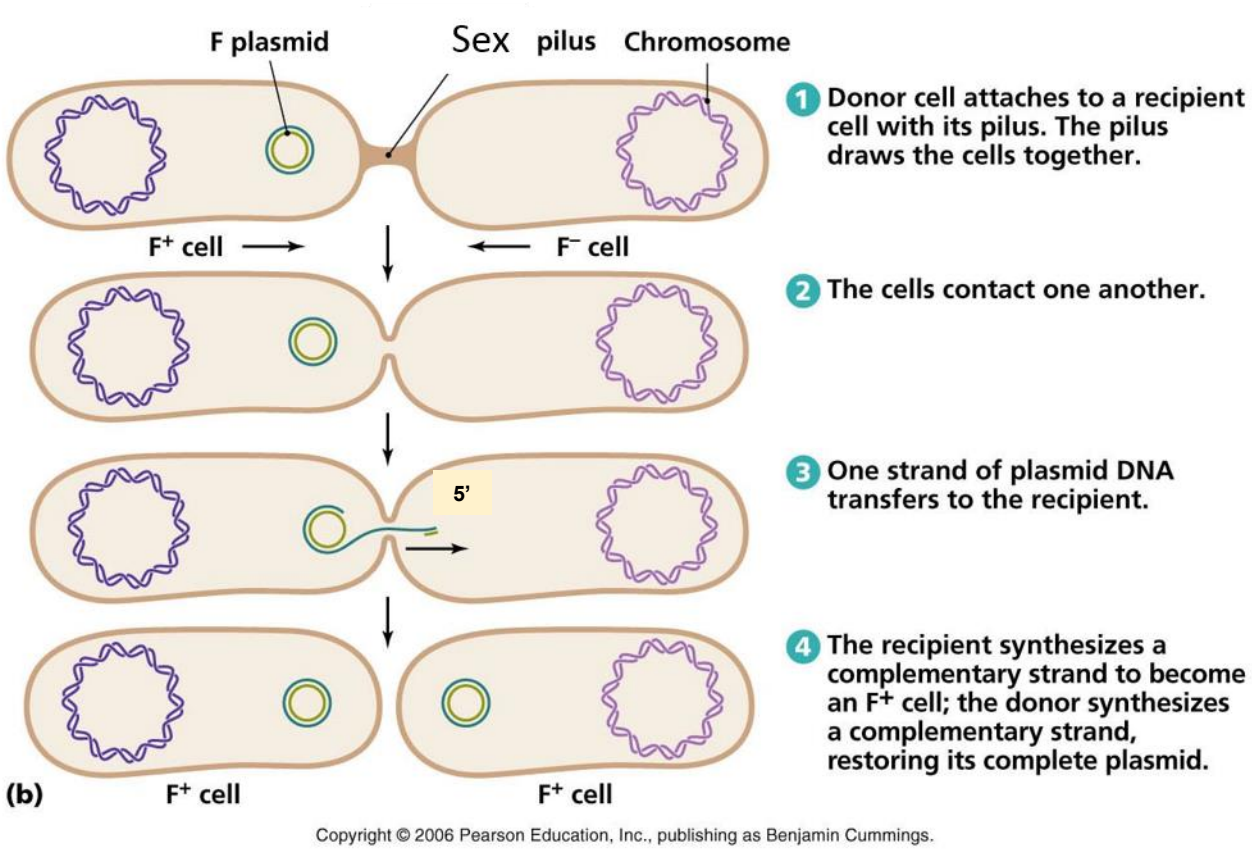


Fig. 3.3d: The process of bacterial conjugation.

Check your understanding

1) What is a plasmid?

2) Is F plasmid a cell?

3) What is a cell containing an F plasmid called? What about a cell without the F plasmid?

4) What is the role of the F plasmid?

Checklist for Sections 1 to 3:

Ensure that you are able to answer these questions confidently.

1. Describe the structure of the bacterial genome.

2. Describe the process of binary fission.

3. Describe the process of bacterial conjugation.

4. Describe the process of bacterial transformation.

5. Describe the process of general transduction and specialized transduction.

6. Suggest how an antibiotic resistance gene can be transferred from one bacterium to another.

7. Suggest how the antibiotic resistance gene confers antibiotic-resistance to the bacteria.

4. Control of Gene expression in Prokaryotes

***Core idea 2C**- Candidates should be able to:*

a) 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)

Evidence for the mechanism of gene regulation in prokaryotes was first obtained from studies into control of enzyme synthesis in *E. coli.* Of the 800 enzymes synthesized by *E.coli,* some are synthesized continuously, while others are synthesized only in the presence of an inducer compound.

Since then many operons have been discovered and the two commonly known examples of operons in *E. coli* are the *Lac* Operon (codes for proteins required to import and digest the disaccharide, lactose), and the *Trp* Operon (codes for proteins required for the synthesis of an amino acid, tryptophan).

4.1 Concept of Operon

Key Concept 4:

- Bacterial genes are organized into operons which allow bacteria to respond/adapt quickly to their environment.
- In prokaryotes, transcription is the most important control point of gene expression.

4.1.1 Structure of Operon

- An **operon** is a single unit of genetic function that consists of three elements (Fig. 4.1a):

1. A **single promoter** region
   - Where **RNA polymerase binds to** and **initiates transcription**
   - Transcription gives rise to **one** long **mRNA,** which is then **translated into several different proteins** (i.e. the **mRNA is polycistronic**).
   - This is possible because the **mRNA is punctuated with start and stop codons** that signal where the coding sequence for each polypeptide begins and ends (Fig. 4.1b).
   - In contrast, **eukaryotic mRNA is monocistronic** i.e. the eukaryotic mRNA is **translated into one protein**.

2. **Structural genes**
   - These are genes that code for proteins with **structural or enzymatic functions.**
   - In bacteria, structural genes in an operon are **clustered together** and transcribed as one mRNA molecule.
   - These genes **code for a series of functionally related proteins** that are involved in **a single biochemical pathway**.
   - e.g. in *Lac* operon, all the structural genes required for the synthesis of enzymes involved in the catabolism of lactose - *lac*Y, *lac*Z, *lac*A are clustered together under a single promoter.

3. An **operator** region
   - Situated **between the promoter and the structural genes**
   - Consists of a specific nucleotide sequence of the DNA where the **repressor protein binds** to. This **blocks** the access of RNA polymerase to the promoter, thus inhibiting mRNA synthesis
   - When bound by a repressor protein, **all the structural genes** in the operon are **simultaneously repressed.**

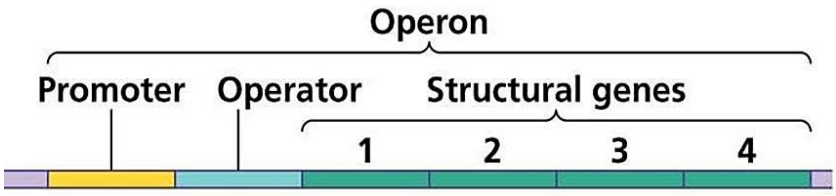


Fig. 4.1a: General structure of an operon

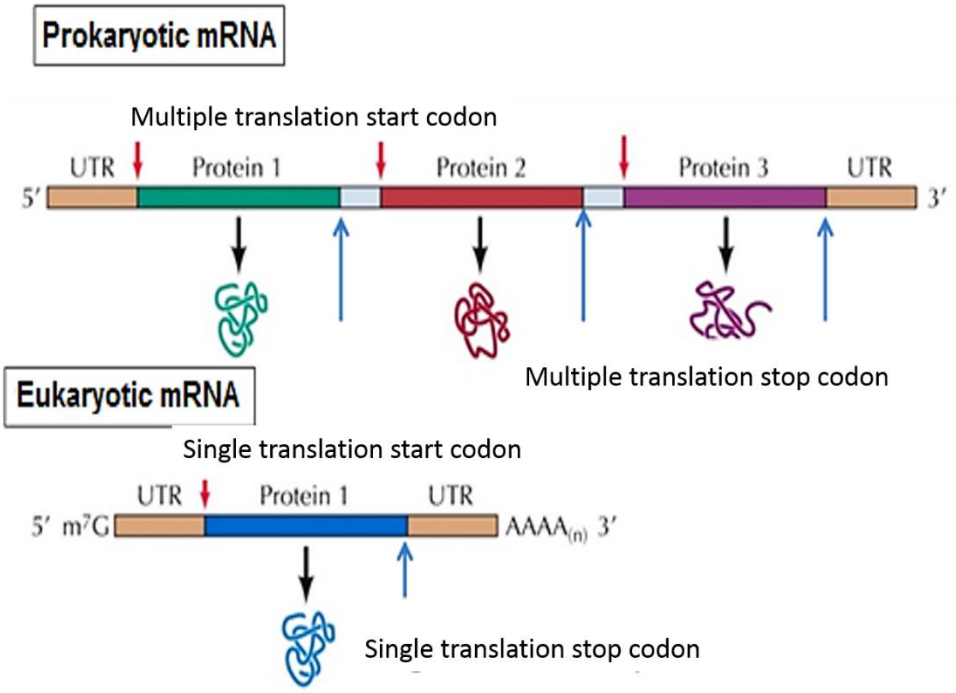


Fig. 4.1b: Polycistronic mRNA in prokaryotes vs. monocistronic mRNA in eukaryotes.
Note the multiple start codons (red short arrows) and multiple stop codons (blue long arrows) in prokaryotic mRNA.

4.1.2 Advantages of Operons

- In operons, **genes that function together or have similar functions** are **regulated together**.

- As prokaryotes are simple unicellular organisms, organizing their genome into operons can allow them to **respond to changes in the environment** (eg. composition of growth medium**) more quickly.**

- Operons ensure that the **cell does not waste energy** synthesizing unneeded enzymes or other proteins. E.g.:
  - The *lac* operon is transcribed only when the substance to be broken down (i.e. lactose) is present.
  - The *trp* operon is transcribed only when the substance required by the cell (i.e. tryptophan) is absent.

4.1.3 Regulatory Gene (Fig. 4.1c)

- A regulatory gene **codes for** a specific protein product that **regulates the expression of structural genes in a particular operon**.
  - E.g. For the *lac* operon, the regulatory gene *lacI* codes for a *lac* repressor protein
  - E.g. For the *trp* operon, the regulatory gene *trpR* codes for a *trp* repressor protein
  - E.g. The regulatory gene that codes for *cyclic AMP receptor protein (CRP)*

- It may be situated **some distance** from the operon it controls.

- Each regulatory gene has its **own promoter.**

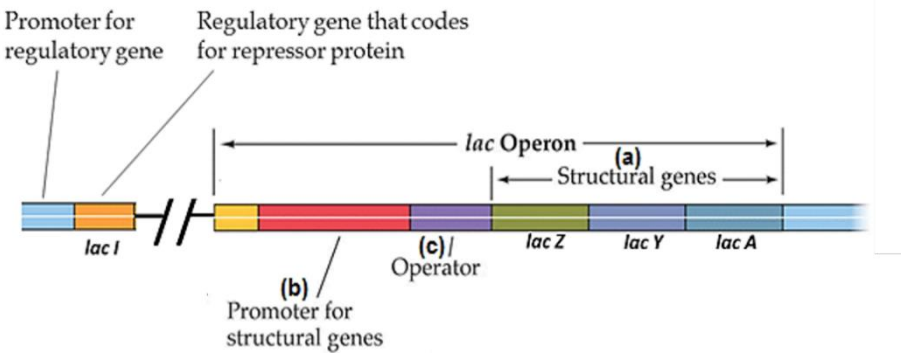


Fig. 4.1c: *Lac* operon and its regulatory gene.
(**NOTE:** A regulatory gene and an operon each has its own promoter).

What is the difference between a structural gene and a regulatory gene?

4.1.4 Relationship between Operon and Regulatory Gene coding for repressor protein (Fig. 4.1d)

- Depending on the operon being regulated, the regulatory gene coding for repressor can **<u>either</u>** code for an

  1) **inactive repressor** protein, or
  2) **active repressor** protein

- Only the **active repressor** protein can **bind to the operator.** (Why?)

- Thus there **are 2 possible scenarios:**

  I. Regulatory gene codes for an inactive repressor (e.g. *trp* operon)
  - Inactive repressor **<u>will not</u>** bind to operator.
  - Thus, the operon is switched **<u>on.</u>**
  - The repressor has to be **<u>activated</u>** via the binding of a small allosteric molecule known as a **<u>co-repressor.</u>** The activated repressor can bind to the operator.

  II. Regulatory gene coding for active repressor (e.g. *lac* operon)
  - Active repressor **<u>will</u>** bind to operator.
  - Thus, the operon is switched **<u>off.</u>**
  - The repressor can be **<u>inactivated</u>** via the binding of a small allosteric molecule known as an **<u>inducer.</u>** The inactivated repressor thus will not bind to the operator.

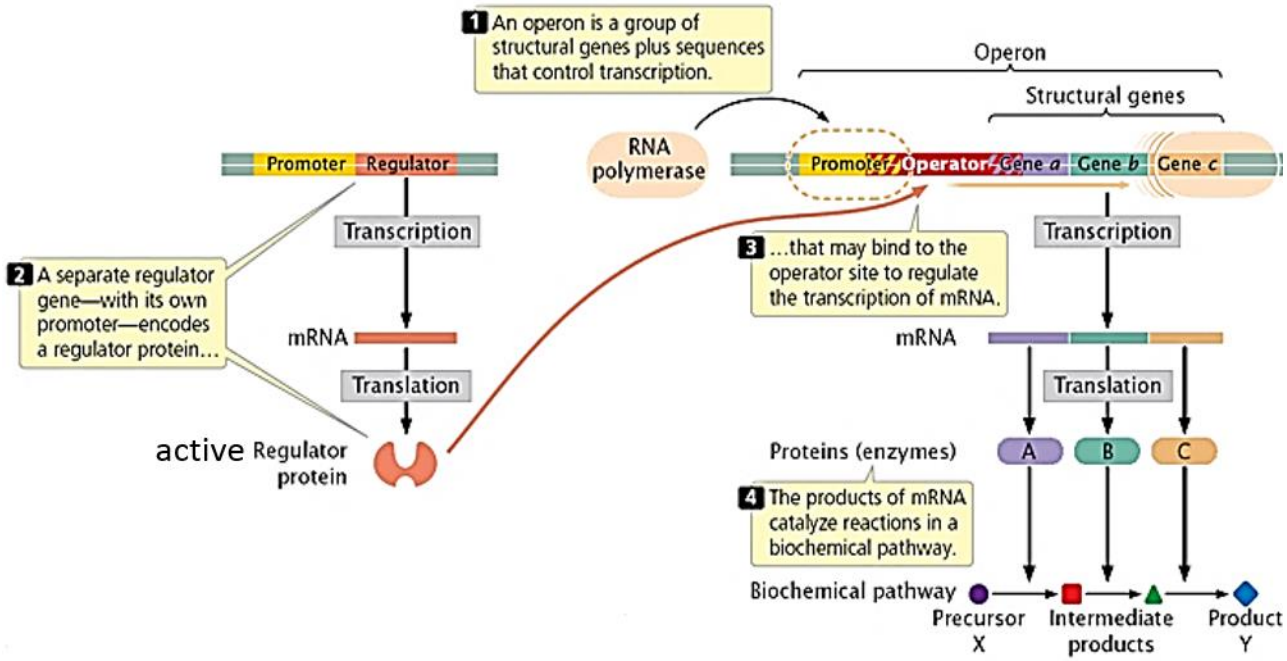


Fig. 4.1d: A regulatory gene codes for a protein product that regulates the transcription of structural genes in a particular operon.

4.2 Regulation of Operon

- Operons can be controlled by positive and/or negative gene regulation.

4.2.1 Negative Control

- Operons are **switched off** by the **active form** of a **repressor.** The repressor is encoded by a **regulatory gene**.

- Eg. of negative control of genes

    - Regulation of *lac* operon: The *lac* operon is **switched off** when the **active *lac* repressor** binds to the **operator**. The repressor is coded for by *lacI* gene, and is normally produced in the active form.

    - Regulation of *trp* operon: The *trp* operon is **switched off** when the **active *trp* repressor** binds to the **operator.** The repressor is coded for by *trpR* gene, and is normally produced in the inactive form.

4.2.2 Positive Control

- The **rate of transcription** of structural genes is **increased** in the presence of an active **activator.** The activator is encoded by a **regulatory gene**.

- Eg. of positive control of genes

    - Regulation of *lac* operon: When the activator **cyclic AMP receptor protein (CRP)** is in its **active** form, it can bind to a **specific CRP-binding site** upstream of the *lac* promoter to **increase the rate of transcription** (Refer to Section 4.3.2).
      Note: CRP is also known as catabolite activator protein (CAP).

4.3 Lac Operon – an inducible operon

Key Concept:

- *Lac* operon is an inducible operon. It is involved in the transport and metabolism of lactose in bacteria.
- The initiation of transcription of the *lac* operon is controlled by the availability of lactose and glucose in the bacterial environment.

4.3.1 Context

- Bacteria such as *Escherichia coli (E.coli)* regulate gene expression in response to changes in environmental conditions.

- Normally in the natural environment of *E.coli* (E.g. in the human gut), glucose from digesting carbohydrates/starch is available. **Glucose** is utilized by bacteria as an energy source, as they are **respiratory substrates** for the energy-producing glycolytic pathway.

- Lactose is not a common component in the human gut. However, when there is little or absence of glucose and lactose is present, lactose will be metabolized into its constituents.

- The *lac* operon is involved in the transport and metabolism of lactose (a disaccharide).

    - The *lac* operon consists of **3 adjacent structural genes *lacZ*, *lacY*, and *lacA***, controlled by a **single promoter and operator** (Fig. 4.3a).

    - These genes code for **enzymes** involved in the **hydrolysis of lactose** into **glucose** and **galactose** (Fig. 4.3b). Glucose can then be utilized by *E.coli* as a respiratory substrate. Galactose can also be further converted to glucose.

- The *lac* operon is an inducible operon.

    - Most of the time, the ***lac* operon is not expressed / switched off,** and the enzymes for lactose utilization are not synthesized.

    - If **lactose becomes available, the lac operon is switched on** (**induced**) and all the 3 genes are expressed together, resulting in a coordinated synthesis of lactose-utilizing enzymes.

    - This ensures that the bacterium **does not synthesize the enzyme until it is needed.** i.e. when substrate **inducer** is present.

- Such **inducible operons** usually function in **catabolic** pathways (breakdown of substances).

4.3.2 Structure of the *Lac* operon

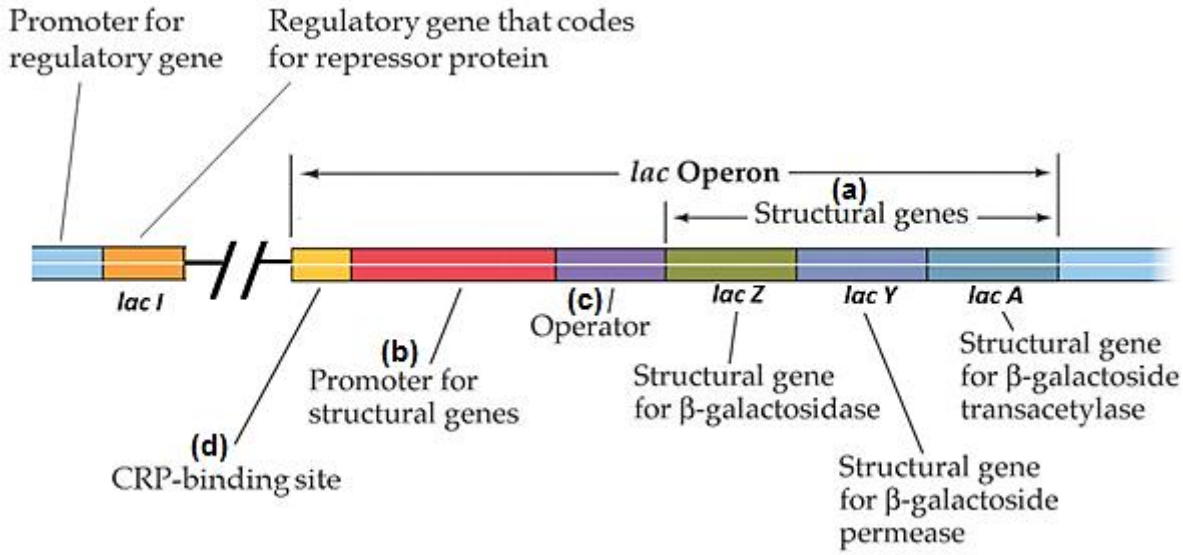


Fig. 4.3a: The *lac* operon and the *lacI* regulatory gene (upstream).

- The *lac* operon (Fig. 4.3a) contains

  a) Three **structural genes**

  - ***lac Z gene*** – codes for an enzyme **β-galactosidase** (Fig. 4.3b) that **catalyses** the **hydrolysis of lactose** to **glucose and galactose**. β-galactosidase is also responsible for the **conversion of lactose** to an isomer, **allolactose.**

  - ***lac Y gene*** – codes for enzyme **permease**, a membrane protein that **transports lactose into the bacterial cell** (Fig. 4.3b).

  - ***lac A gene*** – codes for **transacetylase**, whose function in lactose metabolism is still uncertain.

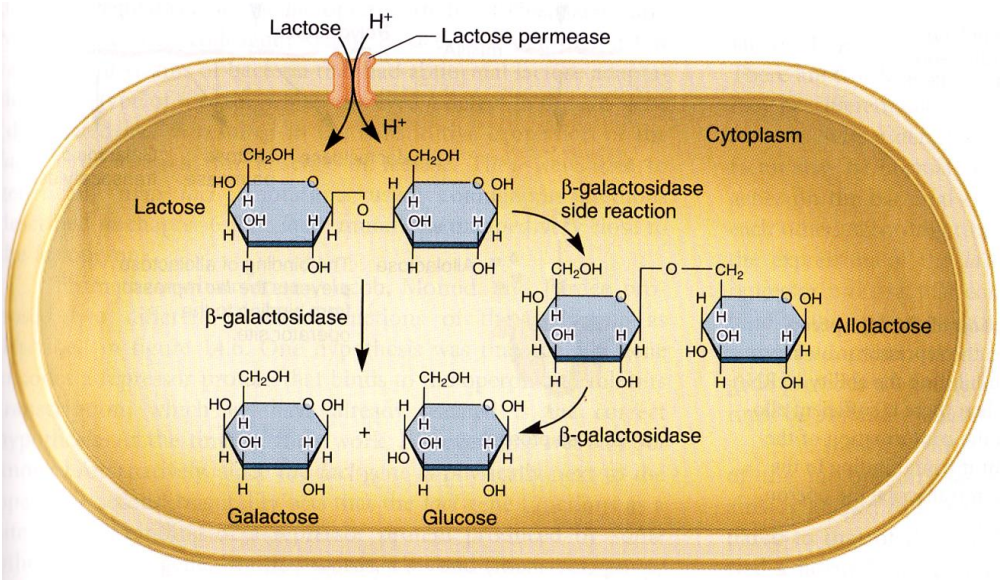


Fig. 4.3b: A diagrammatic illustration of the functions of β-galactosidase and permease in lactose metabolism

a) **Promoter**

- A specific DNA sequence where **RNA polymerase binds to** and **initiates transcription** of the *lac* genes.

b) **Operator**

- A specific DNA sequence where the **active repressor protein binds to,** and **blocks RNA polymerase from binding to promoter.** Hence mRNA synthesis is prevented.

c) **cAMP receptor protein (CRP) binding site**

- A DNA sequence **upstream** of the promoter where **activated cAMP receptor protein (CRP) binds to.**
- The binding of activated CRP to the CRP-binding site causes the **DNA** to **bend about 90^0** and **enhances the binding** of **RNA polymerase to the promoter** (Fig. 4.3c). This **increases the rate of transcription** of *lac* structural genes.
- Hence, **activated CRP** functions as an **<u>activator</u>** protein.
- **<u>Active CRP</u>** is formed when **CRP is complexed with cAMP**.
  - **cAMP is synthesized** from **ATP** by enzyme **adenylyl cyclase** in the plasma membrane (Fig. 4.3d). Adenylyl cyclase activity is **inhibited** by the presence of **glucose.**
  - Therefore, when glucose level is high, cAMP level is low, CRP remains inactive.
  - When glucose level is low, accumulation of cAMP occurs. This allows formation of activated CRP. (Fig. 4.3e)

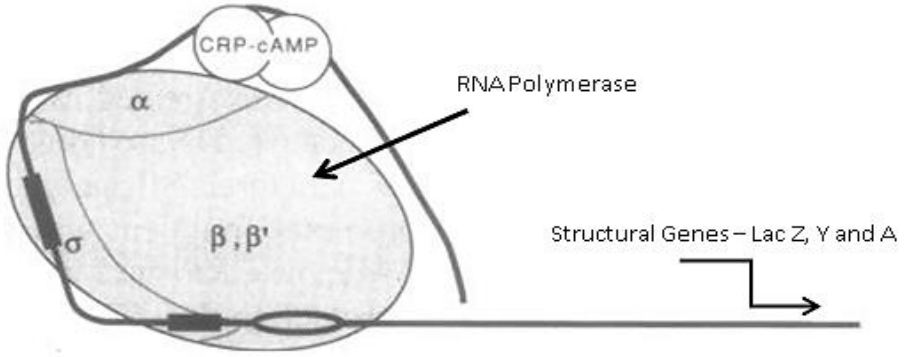


Fig. 4.3c: Activated CRP binding to the CRP-binding site causes DNA to bend, which facilitates binding of RNA polymerase

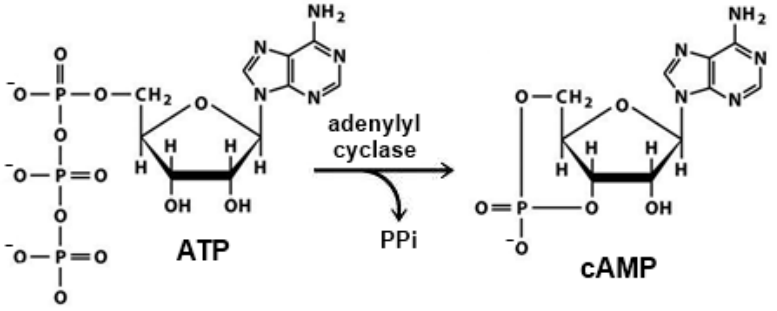


Fig 4.3d: Adenylyl cyclase catalyzes the formation of cAMP from ATP, with the release of pyrophosphate (PPi).

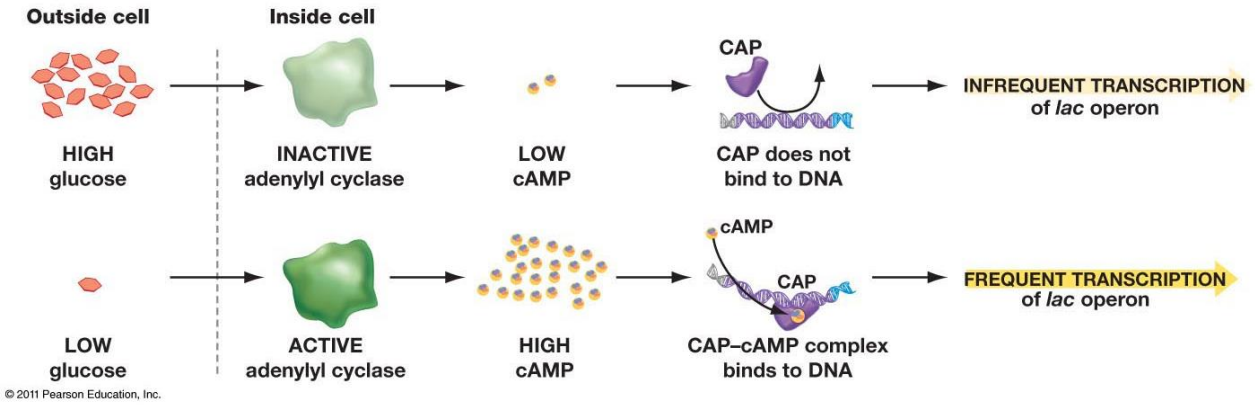


Fig. 4.3e: The amount of cAMP and the rate of transcription of the *lac* operon are inversely related to the concentration of glucose.

4.3.3 *LacI* regulatory gene

- The *lac* regulatory gene, ***lacI***, (Fig. 4.3a) is not part of the *lac* operon, but is located **upstream** of *lac* operon.
- It is responsible for the negative control of the *lac* operon.
    - The *lacI* gene **continuously** codes for an **active repressor protein**.
    - This active repressor binds to the **operator** and **blocks RNA polymerase from binding to the promoter.** This **prevents mRNA synthesis**.

4.3.4 Control of the *lac* operon

- The *lac* operon is under dual control: **negative control** by the **lac repressor protein,** and **positive control** by the **activator protein, cyclic AMP receptor protein (CRP)**.
    - Negative control: In the **absence of lactose**, **enzymes** involved in the hydrolysis of lactose are **not synthesized**. This is because the **active lac repressor protein** is bound to the **operator** to ensure that the ***lac* operon is switched off**.
    - Positive control: In the **absence of glucose**, **active CRP complex (activator)** binds to the **CRP binding site**, thus **increasing the rate of transcription of genes** that encode proteins for lactose utilization.

4.3.5 Mechanism of *Lac* Operon

Scenario 1: Glucose is present, Lactose is absent –
***Lac* operon switched off** (Fig. 4.3f)

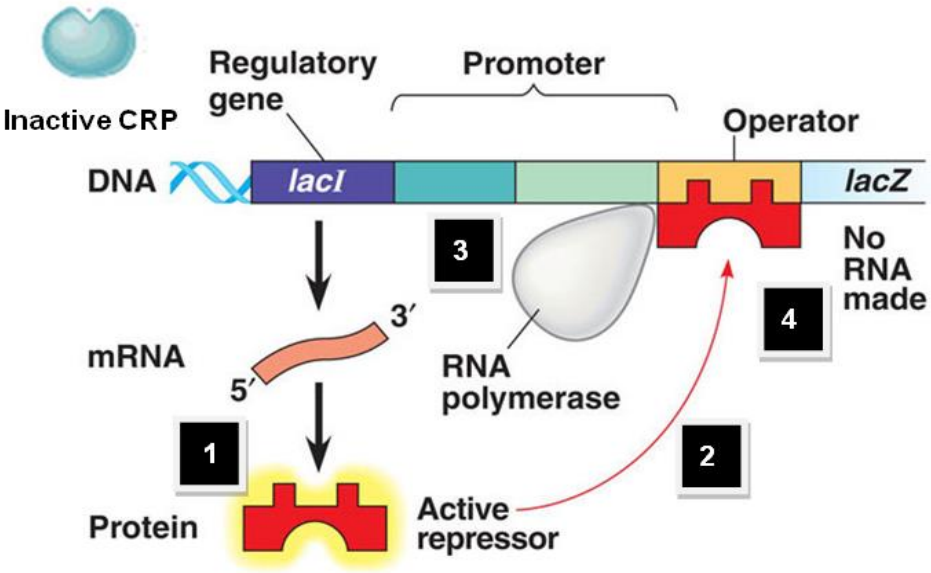


Fig. 4.3f: Lactose absent, thus allolactose is absent. Repressor remains active and binds to operator; operon is switched off. Note that the presence of inactive CRP here does not affect the level of transcription.

- When **glucose is present**, glucose will be taken up for cellular **respiration** to provide energy for the bacteria.

1. In the **absence of lactose**, **no allolactose (inducer) is present** to bind to the active repressor.

2. ***Lac* repressor remains active** and hence able to **bind to the operator.**

3. **RNA polymerase cannot bind to the promoter.** Hence, **transcription of the structural genes** (*lacZ*, *lacY* and *lacA*) **is prevented.**

4. The ***lac* operon is thus switched off**. This helps the bacteria to conserve its resources and energy, since enzymes for the lactose metabolism are not required.

<u>Scenario 2: Glucose is present, Lactose is present –</u>
***Lac* operon switched on at basal/low transcription rate** (Fig. 4.3g)

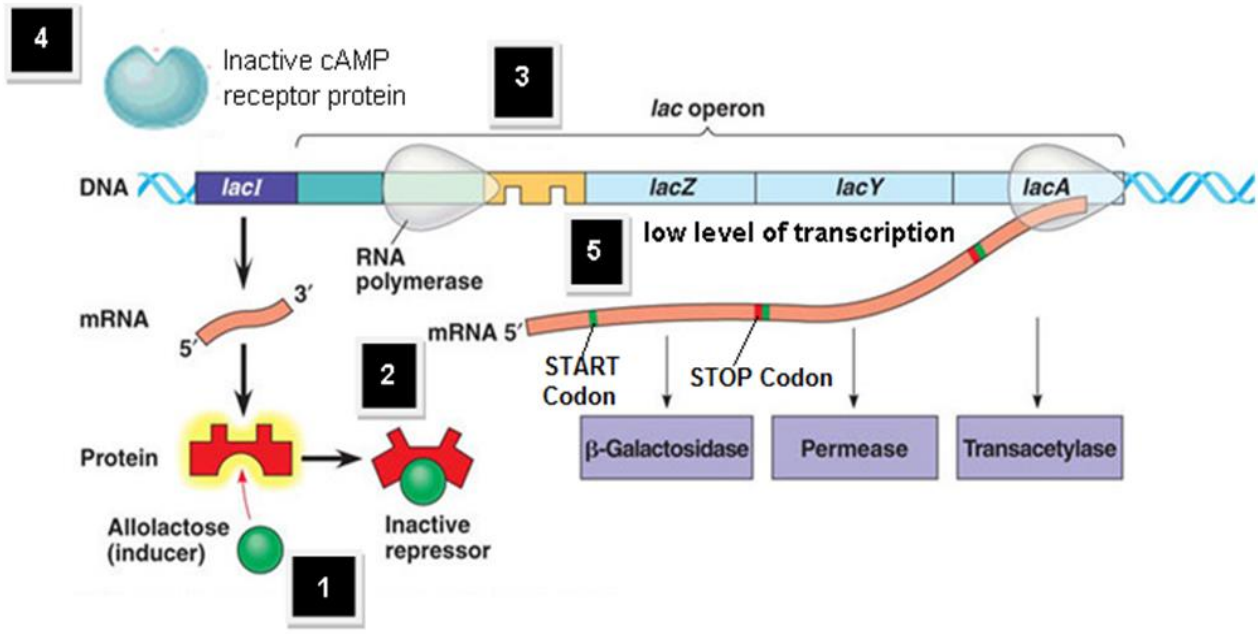


Fig. 4.3g: Lactose present, thus allolactose is present. However, glucose is present, thus cAMP level is low: low level of transcription.

- In *E. coli*, **glucose** is the **preferred source of carbohydrate** as it is the **major substrate for cellular respiration.** Therefore, if lactose and glucose are both present, the cell will **utilize all the glucose before the *lac* operon is turned on.**

1. Lactose taken up by the cell is converted to its isomer**, allolactose (inducer),** which **binds to the active repressor.**
   - *Note: The binding of repressors to operators is reversible. Thus, a small amount of the enzyme β-galactosidase remains in the cell which can convert lactose to its isomer allolactose.*

2. This **inactivates the repressor** and **prevents** it from **binding to the operator**.

3. This allows **RNA polymerase to bind to the promoter** to initiate **low level of transcription** (also known as *basal level transcription*).

4. **Presence of glucose in the cell**
   - **Inhibits the synthesis of cAMP** and **stimulates the transport of existing cAMP out of the cell.**
   - **A low level** of **cAMP** results in a **low level of activated cAMP receptor protein (CRP)**, as CRP can only be activated when complexed to cAMP. Low level of active CRP binds to the CRP binding site.

5. **RNA polymerase binds less efficiently to the promoter,** thus there is only a **low level of transcription** (this is known as the *glucose effect* in some textbooks).

Scenario 3: Glucose is absent, Lactose present-
***Lac* operon switched on with high rate of transcription** (Fig. 4.3h)

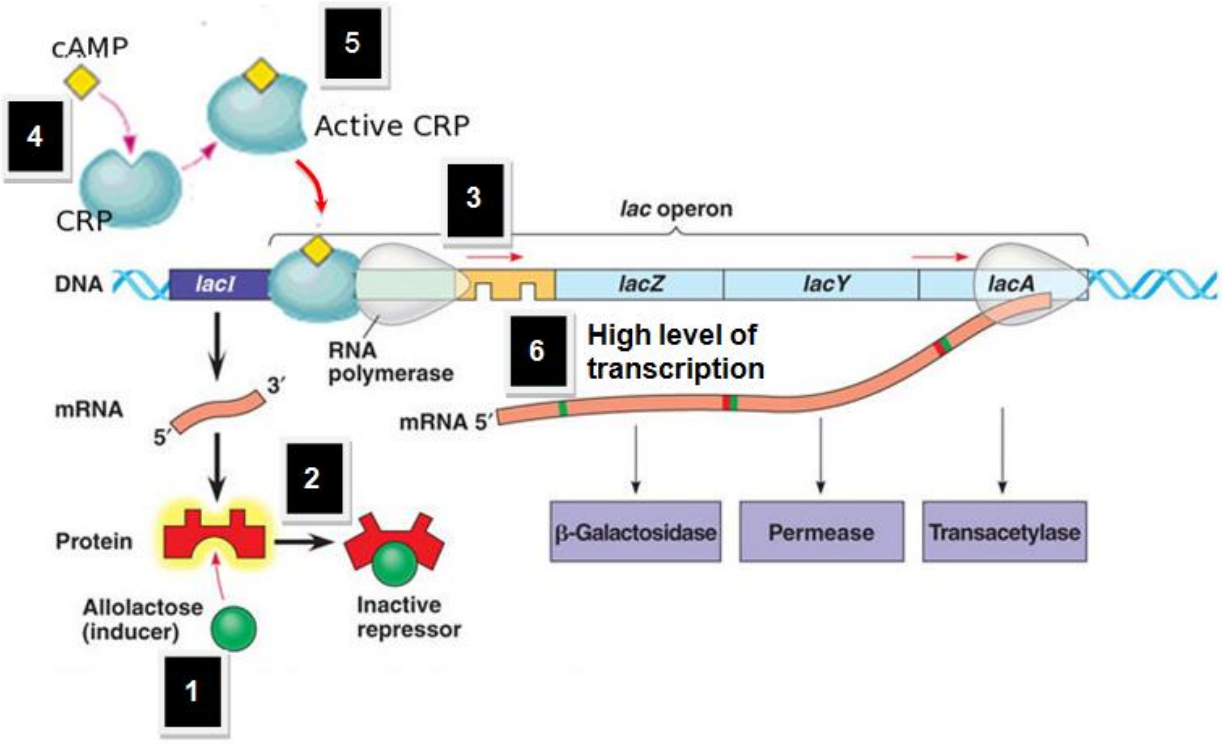


Fig. 4.3h: Lactose is present, thus repressor becomes inactive. Operon is switched on. Glucose is absent, thus active CRP increases rate of transcription of the *lac* operon.

1. Lactose taken up by the cell is converted to its isomer**, allolactose (inducer),** which **binds to the active repressor.**

2. This **inactivates the repressor** and **prevents** it from **binding to the operator**.

3. This allows **RNA polymerase** to **bind to the promoter** to initiate **low level of transcription**.

4. **Absence of glucose in the cell** allows bacterial cell to **synthesise cAMP**.

5. Accumulation of cAMP allows cAMP to complex with CRP to form **high level of activated cAMP receptor protein (CRP).** Activated CRP binds to the **CRP binding site** and causes **DNA to bend**. This **facilitates the binding of RNA polymerase to the promoter** and **upregulates transcription**.

6. **High level of transcription** results in *lacZ*, *lacY* and *lacA* genes being **transcribed** and the resulting mRNA **translated** to produce ***β-galactosidase, permease and transacetylase*** respectively.

Scenario 4: Glucose is absent, Lactose is absent –
***Lac* operon switched off** (Fig. 4.3i)

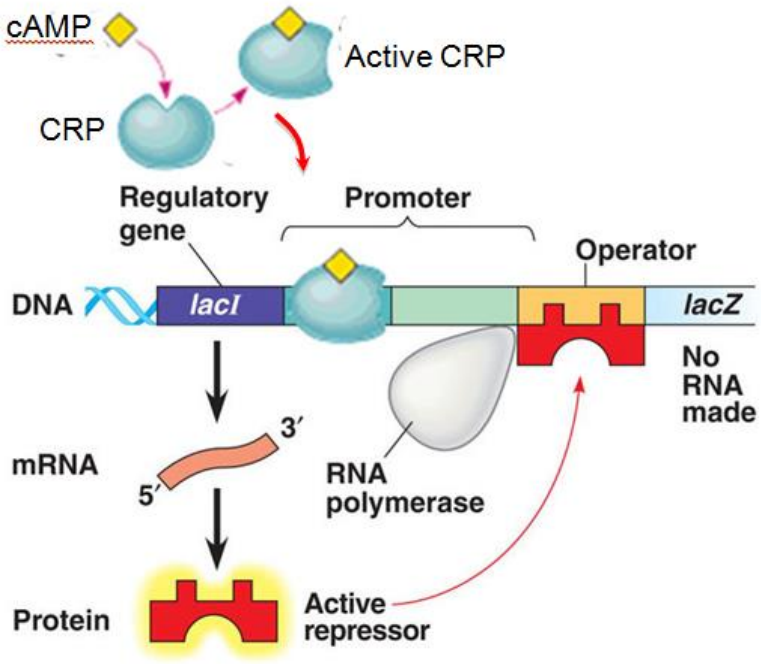


Fig. 4.3i: Lactose is absent, thus allolactose is absent. Repressor remains active and binds to operator: operon is switched off. Note that even with the active CRP bound (because glucose is absent), transcription is not upregulated.

1. Since lactose is unavailable, the operon is switched off via the mechanism described in **Scenario 1**. This conserves energy and resources as the proteins encoded by the *lac* structural genes are not required by the cell.

2. If there are no other energy sources, the cell dies.

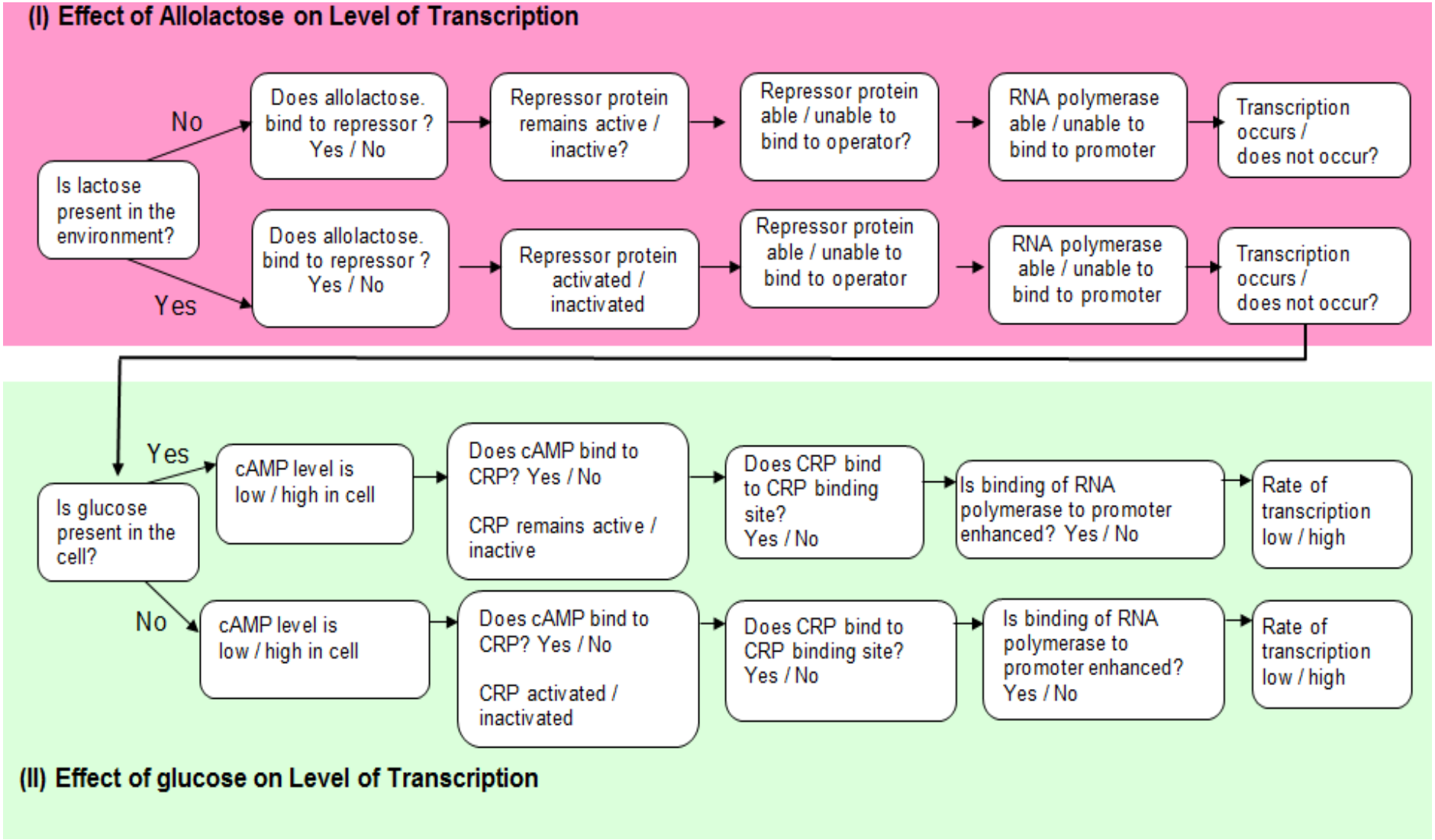
TEST YOURSELF

1. What are the regulatory gene(s) involved in *lac* operon?

2. What type of biological molecules are these?
   - Repressor -
   - Operator -
   - CRP-binding site -
   - CRP –

3. What are the two important conditions required for high levels of transcription of the *lac* structural genes?

4.3.6 Summary on Regulation of Lac operon



4.4 Trp Operon – a Repressible Operon

Key Concept 6:

- *Trp* operon is a repressible operon. It is involved in the synthesis of tryptophan in the bacteria.
- The initiation of transcription of the *trp* operon is controlled by the availability of tryptophan in the environment.

4.4.1 Context

- Bacteria such as *Escherichia coli (E.coli)* regulate gene expression in response to changes in environmental conditions (e.g. growth medium).

- Normally, **tryptophan** (an amino acid) is **absent** or present in low levels in the cell. Hence, the bacteria will synthesize tryptophan by **switching on the operon** that is involved in tryptophan synthesis**.** This sustains the life of the bacteria.

- However, if **tryptophan is present** in the environment, the synthesis of tryptophan is not required. Hence**,** the **operon is switched off.**

- The ***Trp* operon**

    - Consists of **5 adjacent structural genes *TrpE, TrpD, TrpC, TrpB*, and *TrpA*,** controlled by a **single promoter and operator** (Fig. 4.4a)**.**

    - These genes code for **enzymes involved in the synthesis of tryptophan**.

- The *Trp* operon is a **repressible operon.**

    - Most of the time, the *trp* operon is **expressed / switched on,** and the enzymes for tryptophan synthesis are produced.

    - If tryptophan becomes available, the *trp* operon is **switched off / repressed**. Hence, tryptophan functions as a **co-repressor** to switch off the operon**,** via feedback inhibition / end-product inhibition.

    - This **ensures that the bacterium does not synthesize the enzyme until it is needed** (i.e. when product is absent).

- Such **repressible operons** usually function in **anabolic** pathways (production of substances).

4.4.2 Structure of the *Trp* operon

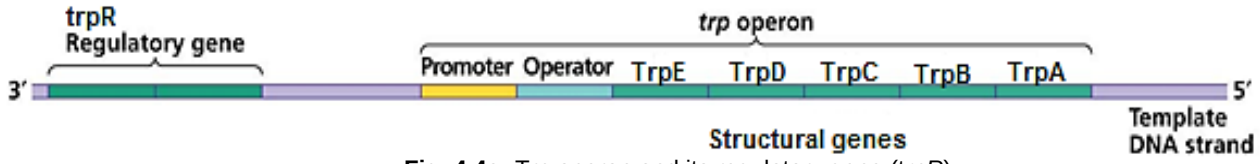


Fig. 4.4a: Trp operon and its regulatory gene (*trpR*).

- The *trp* operon (Fig. 4.4a) contains

  a) Five **structural genes (*TrpE* to *TrpA*)**

  b) **Promoter**

  - A specific DNA sequence where **RNA polymerase binds to** and **initiates transcription** of the *Trp* genes.

  c) **Operator**

  - A specific DNA sequence where the **active repressor protein can bind to** and **block RNA polymerase from binding to promoter.** This prevents mRNA synthesis.

4.4.3 *TrpR* regulatory gene

- The **regulatory gene** of the *trp* operon, ***trpR***, **continuously** codes for an **inactive** repressor protein.
- Hence, **RNA polymerase can bind to the promoter** and **initiate transcription** of structural genes of the operon.
- These structural genes code for **enzymes** involved in the **formation of tryptophan.**

4.4.4 Control of the *Trp* operon

- The *trp* operon is under **negative control** by the ***trp* repressor protein.**
  - In the **presence of tryptophan**, **enzymes** involved in the synthesis of tryptophan are **not synthesized**. This is because the **active *trp* repressor protein** is bound to the **operator** to ensure that the ***trp* operon is switched off**.

4.4.4 Mechanism of Trp Operon

Scenario 1: Tryptophan is absent -
***Trp* operon switched on** (Fig. 4.4b)

a. When **tryptophan is absent** in the cell, the cell needs to **produce enzymes** that synthesise tryptophan.

b. Tryptophan **(corepressor) is absent**. Hence, the **repressor protein remains inactive**.

c. The inactive repressor **cannot bind to the operator**.

d. **RNA polymerase binds to the promoter** and **transcribes the *trp* structural genes**. The encoded enzymes that synthesize tryptophan are produced.

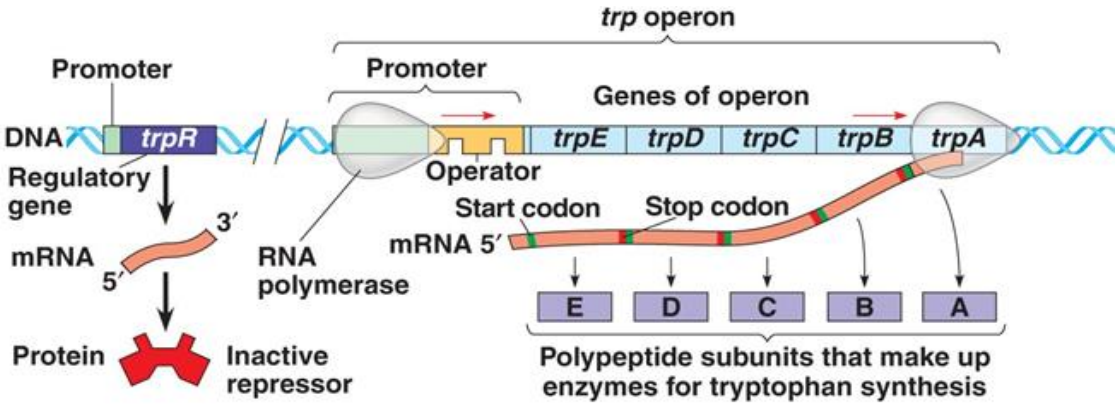


Fig. 4.4b: Trytophan is absent, thus repressor remains inactive: operon is switched on.

Scenario 2: Tryptophan is present -
***Trp* operon switched off** (Fig. 4.4c)

a. When **tryptophan is present** in the cell, the cell needs to **stop synthesizing** the **enzymes** that synthesise tryptophan**.**

b. Tryptophan (a small allosteric molecule) that is present act as a **co-repressor**. It **binds to inactive repressor** protein to **activate** it.

c. The **active repressor** now **binds to the operator. This prevents RNA polymerase from binding to the promoter**.

d. This prevents transcription of the *trp* structural genes. Hence, mRNA and the proteins it codes for are **not synthesized**.

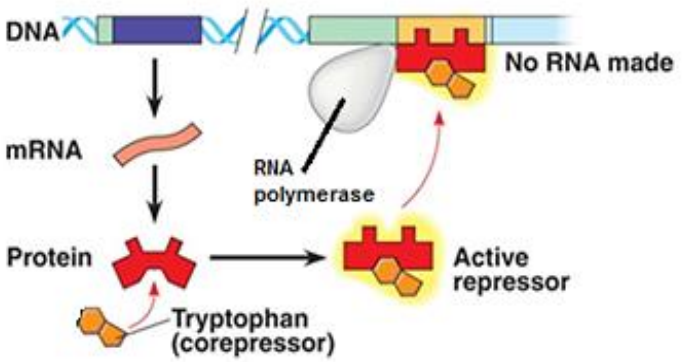


Fig. 4.4c: Tryptophan (co-repressor) is present to activate the repressor: operon is switched off.

4.5 Inducible system vs repressible system

Inducible System (eg. *lac* operon)	Repressible System (eg. *trp* operon)
• Functions in **catabolic pathways**, digesting nutrients to simpler molecules. • Ensures that the cell does not synthesize an enzyme until it is needed.	• Functions in **anabolic pathways**, synthesizing end products. • Ensures that the cell does not waste energy synthesizing unneeded enzymes.
• Allosteric binding by an **inducer molecule** (eg allolactose) makes the active repressor protein inactive (Fig. 4.5a), and the ***operon is switched on***. Inducible operon is **activated**.	• When co-repressor (eg. tryptophan) binds to the inactive repressor protein, this activates the repressor (Fig 4.5a) and ***turns the repressible operon off***. Repressible operon is ***inactivated.***

5. Comparison between Prokaryotes and Eukaryotes

5.1 Structure and Organisation of Prokaryotic vs Eukaryotic Genome

Feature	Prokaryotic genome	Eukaryotic genome
Location	•	• _____, enclosed by _____
Number of Chromosomes	•	•
Nature of DNA (linear vs circular)	• _____ and do not have ends	• _____ i.e. has two ends
Size & Complexity	• _____ **genome** (fewer genes)	• _____ **genome** (more genes)
Are DNA associated with any proteins?	• DNA is bound to _____ _____ _____ which facilitate compaction	• Eukaryotic DNA is complexed with _____ to form chromatin
Origin of Replication	• _____ origin of replication	• _____ origins of replication
How are the genes organized?	• **Genes are organized into an operon** • Each transcription unit consists of **several genes**	• **Genes are not organized into operons**. • Each transcription unit only consists of **one gene**
Promoter	• **Multiple genes** in the **same operon** are controlled by a **single promoter**	• **Each gene** is controlled by a **single promoter**.
Where are the regulatory sequences (control elements) located?	• **Control element (operator)** is situated **between the promoter and the first structural gene**.	• _____**control elements** usually situated **upstream of the promoter**. • _____**control elements** can be **situated anywhere** in the genome.
Are introns present/absent?	• **Introns** _____ • Each gene consists of a continuous coding sequence.	• **Introns** _____ **in each gene**, which separate the coding sequences (exons)
Presence of repetitive sequences	• **Not significantly present**.	• **A significant proportion** of the genome consists of repetitive sequences.

5.2 Prokaryotic vs Eukaryotic control of gene expression

	Eukaryotic genome	Prokaryotic genome
1. At chromosomal level		
	• **histone modifications** can occur • genes in the heterochromatin region, which is more highly condensed, are usually not expressed • **DNA methylation and histone acetylation,** which affect how compact the region is, thus, affect transcription activation	• DNA not associated with histones, hence **no histone modification possible** • Only DNA methylation for protection only.
2. At transcriptional level		
Control at Promoter	• **One promoter** occurs for **each gene**	• **One promoter** occurs for **each operon**, which may **consist of several genes.**
Presence of Other Regulatory sequences?	• **Two types of control elements present**: (I) Proximal control elements – located near the transcription unit (II) Distal control elements – may occur far away from transcription unit	• **Only one type of control element (operator)** present. • Situated **between the promoter and first structural gene**
Induction/ repression in response to external stimuli	• In response to external stimuli, **transcription factors** may bind to enhancer or silencer regions, and activate or silence transcription	• In response to external stimuli (eg. presence of glucose/ lactose), **regulatory proteins** bind to control regions for each operon, inducing or repressing transcription

3. At post-transcriptional level		
	• **Nuclear membrane** separates the processes of transcription and translation • **Post transcriptional modifications** (addition of 5'cap, 3' poly-A tail, splicing) occur • **Alternative splicing** possible • 5'cap and 3' poly-A tail **increase transcript stability / prevent transcript degradation**	• **Transcription and translation occur simultaneously** due to the **absence of a nuclear membrane** • **No post transcriptional modifications occur** • Leads to **lower stability of transcript** and its **degradation** within seconds / minutes
4. At translational level		
	• **Regulatory proteins** bind to the **5' leader sequence of the mRNA** to **stop translation**	• **No regulatory proteins bind to the 5' region of mRNA**, the Shine-Dalgarno sequence, thereby **does not stop translation**
5. At post-translational level		
	• Degradation of proteins by ubiquitin • Chemical modifications/ cleavage	• No degradation of proteins by ubiquitin • No chemical modifications/ cleavage

6. Glossary

Co-repressor
A small molecule that binds to a bacterial repressor protein and changes its shape, allowing it to switch an operon off

Cyclic AMP (cAMP)
Cyclic adenosine monophosphate, a ring-shaped molecule made from ATP, which is a common intracellular signaling molecule. A regulator for some bacterial operons

Operon
A unit of genetic function found in bacteria consisting of a promoter, an operator and a coordinately regulated cluster of genes whose products function in a common pathway

Operator
A sequence of nucleotides in prokaryotes near the start of an operon to which an active repressor binds to. The binding of the repressor prevents RNA polymerase from attaching to the promoter and transcribing genes of the operon

Promoter
A specific nucleotide sequence in DNA that binds to RNA polymerase and starts gene transcription

Regulatory gene
A gene that codes for a protein eg. repressor protein, that controls the transcription of another gene or group of genes

Repressor
A protein that inhibits gene transcription by binding to the operator

Shine-Dalgarno sequence
A ribosomal binding site in bacterial mRNA, generally located around 8 bases upstream of the start codon, AUG. This RNA sequence helps recruit the ribosome to the mRNA to initiate translation by aligning the ribosome with the start codon.

Structural gene
Region of DNA that codes for a protein or an RNA molecule that forms part of a structure that has an enzymatic function. Distinguished from regions of DNA that regulate gene expression.

Checklist for Organization of Genome & Control of Gene Expression in Prokaryotes:

1. Describe the concept of an operon using the *lac* operon.

2. Explain whether the *lac* operon will be transcribed under the following conditions:

   a) glucose present + lactose present

   b) glucose present + lactose absent

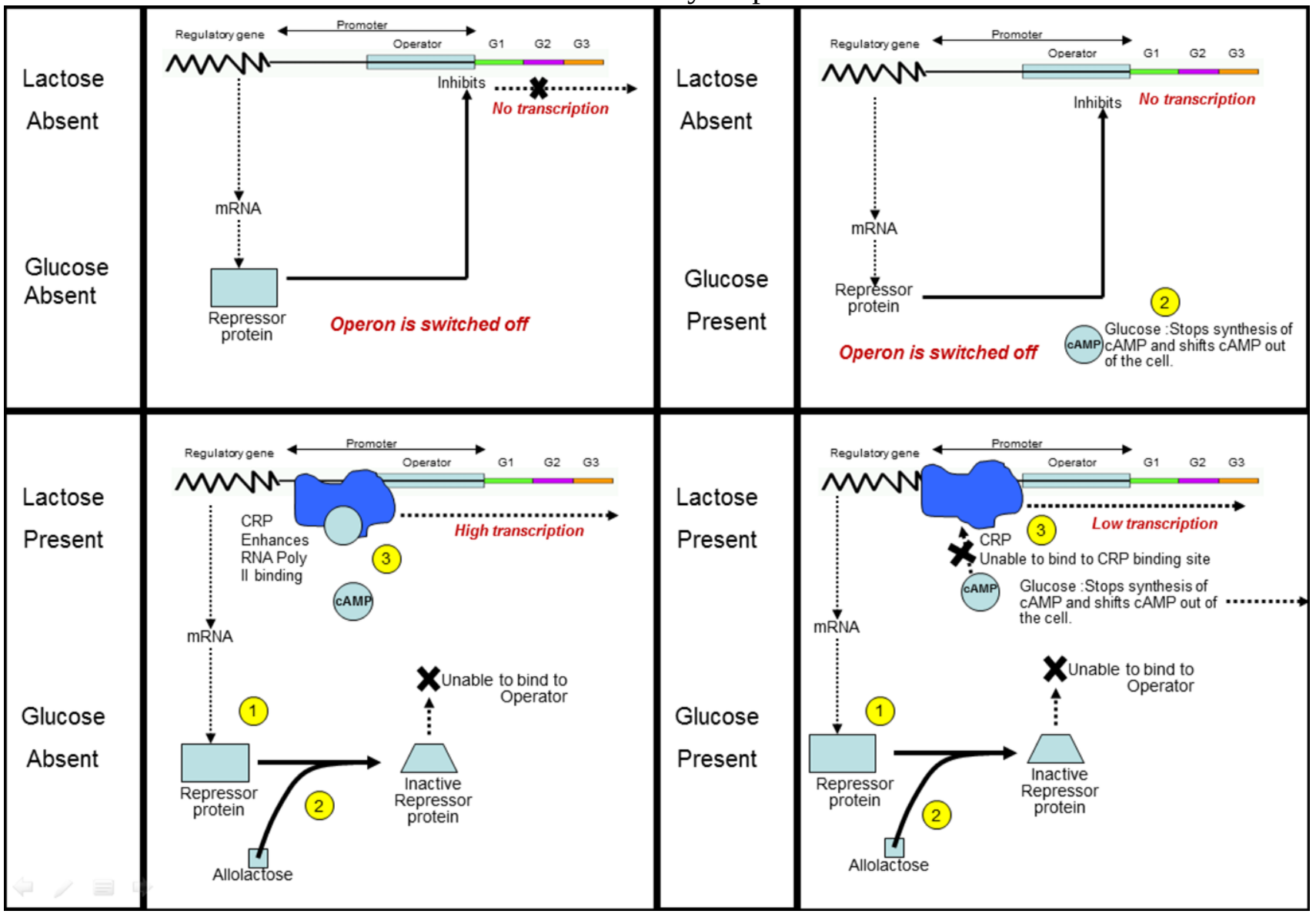
   c) glucose absent + lactose present

   d) glucose absent + lactose present

3. Differentiate between a regulatory gene and a structural gene.

4. Differentiate the concept of an inducible operon and a repressible operon.

Summary map



= End of lecture notes =