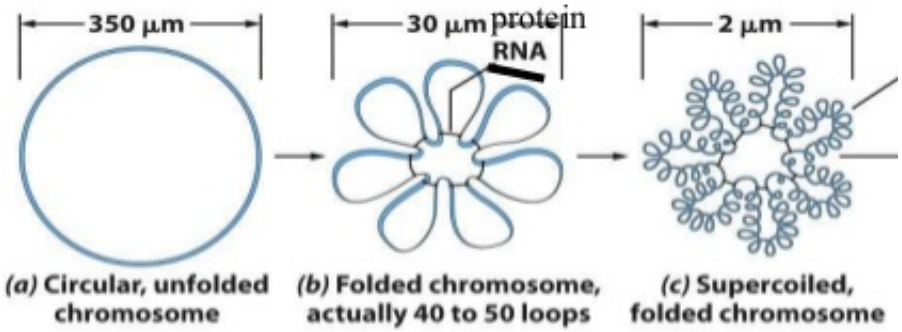
Bacteria

Structure

- Prokaryote ⇒ No membrane-bound organelles
- **Bacterial Chromosome
    - Only **1** circular dsDNA molecule associated with histone-like proteins
    - Forms loop domains with histone-like proteins ⇒ Supercoiling ⇒ Highly condensed DNA
    - Found in **nucleoid region** which is not membrane bound (Less densely stained than cytoplasm)



- May contain plasmids
    - Smaller rings of autonomously replicating circular DNA
    - May contain genes which confer advantages on bacteria living in stressful environment
- 70S Ribosomes
    - Give a granular appearance
- Storage granules containing nutrients and chemical reserves
- Cell membrane
    - No mitochondria ⇒ ATP synthase and ETC required for respiration found on cell membrane
- Cell wall
    - Made of peptidoglycan
    - Protects cell from osmotic lysis
- Capsule
- Appendages
    - Fimbriae
    - Pili
    - Glagella

Asexual reproduction in bacteria

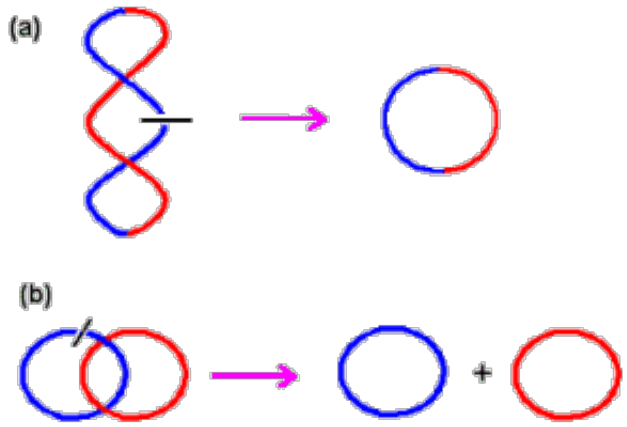
- Produces genetically identical offspring from a single parent
- Selective advantage in a stable, favourable environment as successful genotypes can

rapidly colonise a habitat

Binary Fission

1. DNA replication begins at **origin of replication**
2. Double helix unzips to form replication bubble
    - Replication takes place outwards from ori

1. As chromosome replicates, 2 newly formed origins of replication move to opposite poles of the cell and attach to plasma membrane
2. Cell elongates to prepare for cell division
3. **Topoisomerase** cuts, separates and reseals the 2 interlocking DNA molecules that are formed after replication
4. Plasma membrane invaginates and new cell wall is deposited, dividing the parent cell into 2 daughter cells with each genetically identical to the parent cell



Genetic Variation in Bacteria
Trans**f**ormation (**F**ragments)

- Uptake of **fragments of naked, foreign DNA** by competent bacteria via surface proteins
    - Artificial method: Placed in concentrated $CaCl_2$ culture medium followed by heat shock treatment
- Foreign DNA is incorporated into bacterial chromosome through **crossing over and homologous recombination**
- If foreign DNA with different allele is expressed in recombinant cell ⇒ Bacterial cell is transformed

Trans**d**uction (**D**ouble agent)

- Phages randomly carry bacterial genes from one host cell to another due to aberrations in the phage reproductive cycle

Generalised transduction (any bacteriophage):

1. **Phage** infects a **bacterial cell**, injecting its viral genome
2. During lytic cycle, phage enzymes degrade bacterial DNA into small fragments
3. During **assembly** of phage genome within phage capsid, a fragment of host cell DNA may be randomly packaged into a **capsid** head
4. Upon cell lysis, the defective phage is released and can infect another bacterium, injecting bacterial DNA from the previous host cell into the new bacterium
5. Foreign bacterial DNA can replace the homologous region in the recipient cell's chromosome if **crossing over and homologous recombination** takes place
6. Foreign DNA with different allele may be expressed in recombinant cell

Specialised transduction (temperate bacteriophage):

1. **Temperate phage** infects a **bacterial cell**, injecting its viral genome
2. Phage undergoes lysogenic cycle ⇒ Phage DNA integrated into bacterial chromosome by **integrase** forming a **prophage**
3. Upon spontaneous **induction**, prophage may be improperly excised, picking up the adjacent segment of bacterial gene
4. Phage-host hybrid DNA replicated and may be packaged into the capsid head of new viral progeny during spontaneous **assembly**
5. Upon cell lysis, the defective phage is released and can infect another bacterium, injecting phage-host hybrid DNA into the new bacterium
6. Foreign bacterial DNA can replace the homologous region in the recipient cell's chromosome if **crossing over/homologous recombination** takes place
7. OR: **Integration** of phage-bacterial hybrid DNA into new host cell genome by **integrase**
8. Foreign DNA with different allele may be expressed in recombinant cell

Conjugation (**C**ontact)

- Direct transfer of genetic material from one bacterial cell to another through a temporary link between 2 cells
- One directional transfer of DNA from F^+ cell to F^- cell
    - F^+ cell carries F plasmid which carries genes to produce sex pili

Steps:

1. **F^+** bacterial cell able to produce a sex pilus

2. **Sex pilus** of F^+ cell makes contact with an F^- cell and retracts to bring the F^- cell closer
3. Temporary cytoplasmic **mating bridge** with the F^- cell is formed
4. One strand of the double-stranded F plasmid is nicked by a **nuclease** and transferred from the F^+ cell to the F^- cell through the cytoplasmic mating bridge
    - **Rolling circle mechanism:
        1. Free 3' end of the nick extended by **DNA polymerase** for the synthesis of new complementary strand using the intact strand as a template
        2. Newly synthesised strand displaces nicked strand which is transferred concurrently via the 5' end across the mating bridge
        3. After 1 round, another nick occurs to release the original strand and end replication
5. In the recipient cell, single stranded F plasmid DNA re-circularises and serves as a **template** to synthesise a complementary daughter strand ⇒ Double-stranded F plasmid ⇒ Both cells now F^+ cells

Difference between F plasmid and bacterial chromosome

- F plasmid is much smaller than bacterial chromosome
- F plasmid contains non-essential genes which may confer advantages such as antibiotic resistance whereas bacterial chromosome contains essential genes

Benefits for recipient bacteria:

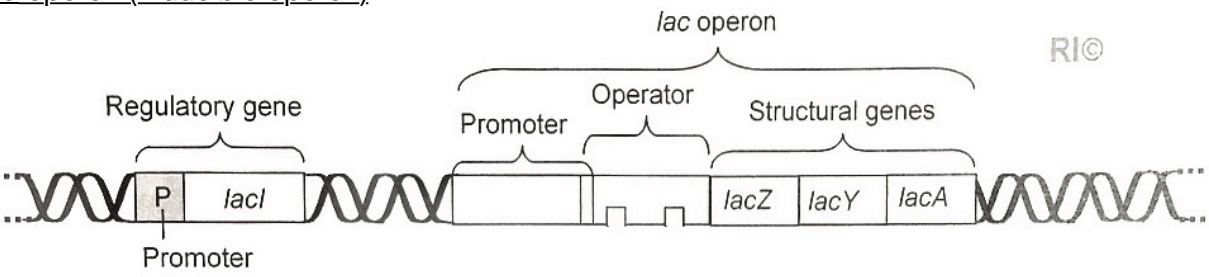
- Gains new alleles that when expressed, allow it to survive in a different environment e.g. **antibiotic resistance**
- Codes for an enzyme that allows for the use of new metabolites/resources

Operons

- A cluster of genes with related functions that are regulated in a way that they are turned on and off together
- Contains:
    - Common promoter
    - Operator
        - Adjacent to the promoter
        - Site on DNA that repressor protein binds to prevent transcription from initiating by restricting access to promoter
    - Structural genes
        - Codes for a protein or RNA molecule that forms part of a structure or has an enzymatic function
        - If multiple structural genes, produces a single polycistronic mRNA
    - Regulatory genes
        - Codes for a specific protein product that regulates the expression of the

structural genes
        - Located far upstream from the operon - NOT in operon
- Benefits
    - Economical use of energy and resources ⇒ Gene is only expressed when necessary
    - Allows bacteria to respond appropriately and rapidly to changes in environment

<u>*lac* operon (Inducible operon)</u>



- Structure:
    - Regulatory gene
        - Promoter region
        - *lacI* coding for lac repressor protein
    - Structural genes
        - *lacZ* coding for **β-galactosidase**
        - *lacY* coding for **permease**
        - *lacA* coding for **transacetylase**
        - Each have independent start and stop codon
    - Promoter region
        - Where RNA polymerase binds and initiates transcription
        - Controls all 3 structural genes
    - Operator
        - Controls access of RNA polymerase to the genes hence controlling transcription of the structural genes
        - Where lac repressor protein binds to in order to switch off the lac operon
- Function:
    - Control lactose metabolism in E. coli
- Type of operon:
    - Inducible operon with -ve and +ve gene regulation
        - Inducible operon = Default mode of operon is off and can be turned on
        - -ve gene regulation = Regulation by repressor proteins
        - +ve gene regulation = Regulation by activator proteins
- Default mode:
    1. *lacI* regulatory gene constitutively transcribed resulting in continued production of the lac repressor protein
    2. ***lac* repressor protein** produced in active form and binds specifically to ***<u>lac</u>* <u>operator</u>** sequence via its DNA binding site

3. In the absence of lactose, the repressor protein occupies the operator site ⇒ Denies RNA polymerase access to the promoter ⇒ Prevents transcription of the operon ⇒ Switches the operon **off**

- Regulated by:
  - lac repressor protein (negative gene regulation)
    1. Absence of lactose, basal levels of **β-galactosidase** and **permease** present as repression of lac operon by lac repressor protein is leaky
    2. In presence of lactose, small amount of **permease** present is able to transport **lactose** from the surrounding medium into the cell
    3. **Lactose** is converted into **allolactose** by **β-galactosidase** in the cell
    4. **Allolactose** acts as an **inducer** molecule which binds to the **lac repressor protein** at its allosteric site
    5. This inactivates the lac repressor protein, altering the conformation of its DNA-binding site such that it is no longer complementary in shape and charge to the operator and thus cannot bind to the operator
    6. RNA polymerase is now free to bind to the promoter and can actively transcribe the structural genes to form a single polycistronic mRNA
  - Catabolite activator protein (positive gene regulation)
    1. lac operon promoter has low affinity for RNA polymerase even when the repressor protein is inactivated by allolactose
    2. In the absence of glucose, **cAMP** levels will increase
    3. **cAMP** binds to the allosteric site of **CAP** ⇒ CAP activated ⇒ CAP able to bind to CAP-binding site within the promoter of the lac operon
    4. ↑ Affinity of the promoter region for RNA polymerase ⇒ ↑ Frequency of transcription of the lac operon
    5. Operon is now turned on with increased synthesis of **β-galactosidase**, **permease** and **transacetylase** for metabolism of lactose
    6. In the absence of glucose and presence of lactose, increased uptake of lactose due to increased levels of permease and increased levels of lactose metabolism to **glucose** and **galactose** due to increased levels of **β-galactosidase**

trp operon (Repressible operon)

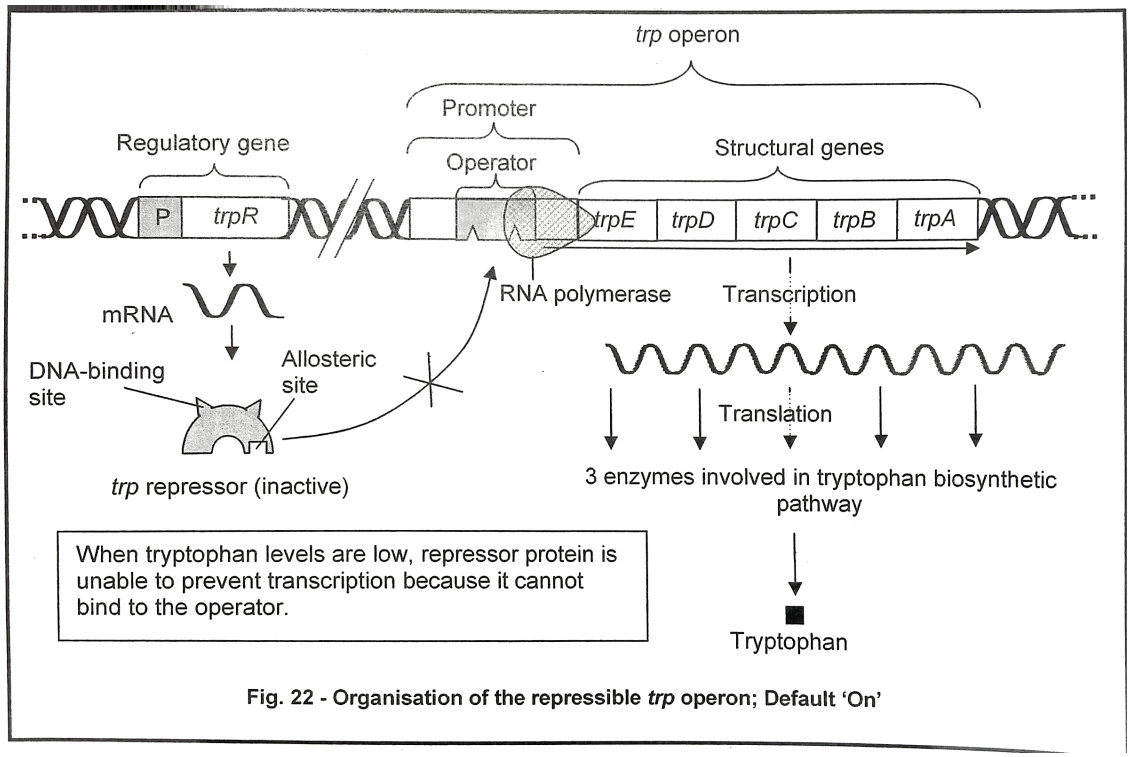


Fig. 22 - Organisation of the repressible *trp* operon; Default 'On'

- Type of operon:
  - Repressible operon with end-product repression
    - Repressible operon = Default mode is on and can be turned off
    - End product repression = End product acts as the effector molecule to turn off the operon
- Regulated by:
  - *trp* repressor protein
    - ***trpR*** regulatory gene constitutively transcribed resulting in continued production of the *trp* repressor protein in its inactive form
    - At high levels of tryptophan, **tryptophan** acts as a **corepressor** which binds to **trp repressor** at its allosteric site
    - This alters the conformation of the trp repressor such that it is complementary to the operator site ⇒ trp repressor activated ⇒ trp repressor able to bind to the operator ⇒ Prevents RNA polymerase from binding to promoter ⇒ Prevents expression of the trp operon

IMPORTANT:

	Repressible operon	**Inducible operon (-ve gene regulation)**

Metabolic pathway	Anabolic pathway (synthesis of product from simpler materials)	Catabolic pathway (breaks down molecule into simper products)
Effector molecule	Corepressor e.g. Tryptophan (End product)	Inducer e.g. Lactose (Substrate)
Effect of effector on operon	Turns **off** structural genes	Turns **on** structural genes
Repressor synthesised in active/inactive form	Inactive	Active
When does repressor bind to operator	When bound to corepressor	On its own
When does repressor not bind to operator	On its own	When bound to inducer
****Default operon expression**	On	Off
****Operon expression when effector molecule is present**	Off	On
E.g.	trp operon	lac operon

	Prokaryote	Eukaryote
Functionally related genes are expressed together	Functionally related genes in an operon are expressed as a set and are dedicated to a particular biochemical pathway.	A protein may be involved in several biochemical pathways and thus, it is more efficient to express each gene more independently than to have multiple copies of the same gene in several different operons.
	Bacteria are unicellular and exposed to the fluctuating	In multicellular eukaryotes,

Fast response time	environment. Operons having related genes expressed under the control of one promoter allows for a fast response to environmental changes.	there are homeostatic mechanisms that keep the internal environment stable. Thus, fast response time is not so critical.
Simple straightforward regulation by one promoter	Bacteria being unicellular and simple in organisation does not require complex gene regulatory mechanisms and the operon model is adequate.	Multicellular eukaryotes have cells that carry the same set of genes but not all are expressed at the same time and place. This allows for differentiation of cells and developmental regulation which cannot be carried out by an operon system.