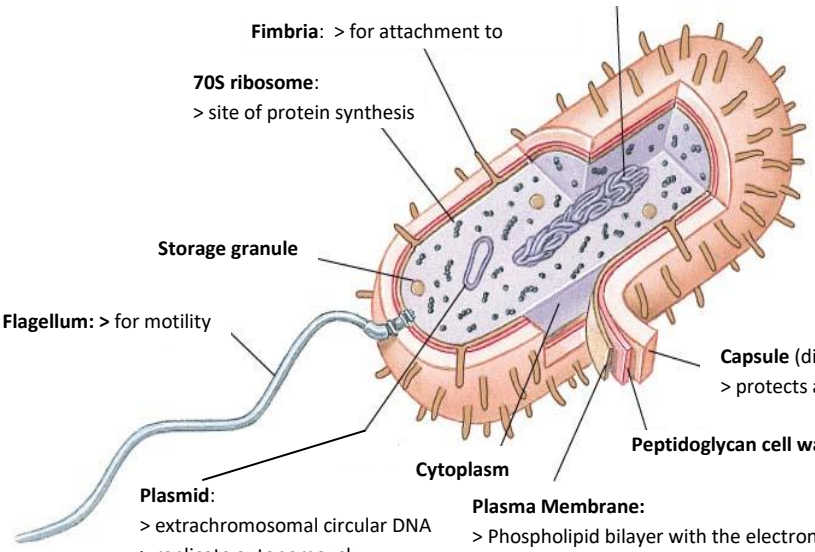
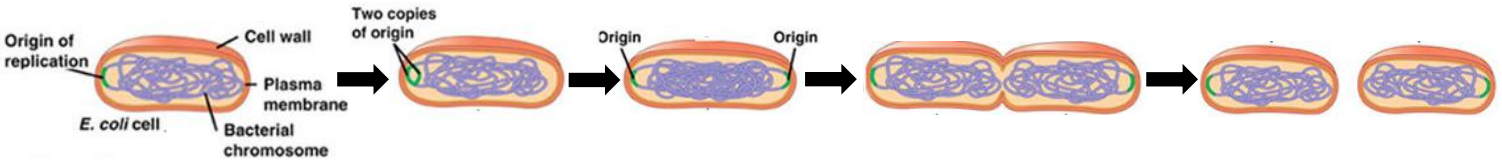
Structure of Bacterial Cell



Binary Fission – asexual reproduction produces genetically identical/clonal bacteria

1. DNA replication begins at the **origin of replication (*ori*)** where DNA is unzipped by breaking hydrogen bonds between bases of the 2 strands to form a replication bubble
2. DNA replicates by **semi-conservative replication** where each original strand serves as **template** for synthesis of daughter strands by **complementary base pairing**
3. 2 newly formed *ori* move to opposite poles of the cell and attach to the plasma membrane
4. Cell elongates to prepare for division.
5. DNA is circular with no free ends, and the 2 daughter DNA molecules will be interlocked with the completion of replication.
6. Enzyme **topoisomerase** cut, separate and reseal the two DNA molecules
7. **Invagination** of the plasma membrane and the deposition of new cell wall (division septum) eventually divide the parent cell into two daughter cells
   ➔ each inherits a complete genome (genetically identical)



Compare Binary Fission & Mitosis

➔ Produces genetically identical offspring: selective advantage in a stable, favourable environment as it allows successful genotypes to rapidly colonise a habitat

Point of Comparison	Binary Fission	Mitosis
End Product	2 genetically identical cells	2 genetically identical cells
Amount of DNA	Same in each daughter cell as compared to parent cell	Daughter cells have the same amount of DNA as parent cells
DNA Replication	DNA replication occurs during binary fission	DNA replication occurs during S phase of interphase before mitosis
Behaviour of Chromosomes	Attachment of chromosomes to plasma membrane	No attachment of chromosomes to plasma membrane
	Formation of entangled rings made up of 2 DNA molecules to be separated	DNA molecules are not entangled
	No chromosome condensation into sister chromatids	Chromosome condensation into sister chromatids
	No specific positioning of chromosomes in the cell that characterizes the different stages	Specific positioning of chromosomes in the cell characterize the different stages
Spindle Fibres	No spindle fibres involved	Spindle fibres involved

Genetic variation in bacteria arise from lateral gene transfer: Transformation, Conjugation & Transduction
➔ resulting in change of the bacterial cell's genotype and phenotype

Transformation

Definition:
Transformation is the **uptake of naked, foreign DNA** from the surrounding environment, resulting in a change of the bacterial cell's genotype and phenotype

Process:
1. **Fragments** of foreign **naked DNA** from dead lysed bacterial cells
2. **Naturally competent** bacteria with **cell-surface proteins** bind and transport DNA into the cell.
3. Artificially bacteria can be **made competent** through immersion in a medium with **$CaCl_2$** followed by a heat shock treatment
4. Foreign **DNA incorporated into chromosome** through **crossing over at 2 homologous regions** found on the bacterial chromosome
5. Result: **recombinant** cell
6. If different alleles for a gene were exchanged, the **new allele** will be **expressed ➔** permanent change in **genotype & phenotype**
7. Recombinant genome will be passed on to all subsequent offspring through binary fission

Conjugation

Definition:
Direct transfer of **genetic material** from one bacterial cell to another through a **mating bridge** between the two cells via the transfer of F plasmid from an **F^+ donor to F^- recipient cell**

Process:
1. **Sex pilus** (coded for by F factor**)** of **F^+** bacterial cell **makes contact** with a **F^-** cell and **retracts** to bring the 2 cells closer
2. The hollow pilus then acts as a **cytoplasmic mating bridge** between the 2 cells
3. One of the 2 strands of the plasmid DNA is nicked and **transferred from the F^+ cell to the F^- cell** through the bridge
4. The single stranded F plasmid DNA **circularizes** in **F^-** cell and is used as a **template** to synthesize a **complementary strand** for a double-stranded plasmid DNA. The **F^-** recipient cell is now a **F^+** cell
5. Replication of the plasmid occurs via **rolling circle DNA replication** occurs

a) One strand of ds F plasmid is nicked by a nuclease ➔ free **3'OH end** is then used as a primer for strand elongation by DNA polymerase using the unnicked/intact strand as a **template ➔** elongation process is facilitated by the displacement of the 5' end of the nicked strand and is transferred across the mating bridge to the recipient bacterium ➔ Upon completion of a unit length of the plasmid DNA (after 1 round), **another nick** occurs to release the original strand

b) 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** to form a double stranded circular DNA.

Transduction

Definition:
Transduction is the process by which bacterial DNA from one host cell is introduced into another bacterial host cell by a bacteriophage due to aberrations in the phage reproductive cycle

Generalised Transduction
1. A phage infects a bacterium, injecting its **viral genome(DNA)** into the host cell
2. The bacterial DNA is degraded into **small fragments**, one of which may be **randomly packaged into a capsid head** during the spontaneous assembly of new viruses
3. Upon cell lysis, the **defective phage** will infect another bacterium and **inject bacterial DNA** from the previous host cell into the new bacterium
4. Foreign bacterial DNA can replace the homologous region in the recipient cell's chromosome through **homologous recombination**, allowing the expression of a **different allele** from the previous host

Specialised Transduction
1. **Temperate phage** infects a bacterium, injecting its viral genome into the host cell
2. The viral DNA is integrated into bacterial chromosome forming a **prophage**
3. which may be **improperly excised** to include **adjacent segment of bacterial DNA** and not the entire phage DNA during an **induction** event
4. Hence phage-bacterium hybrid DNA may be packaged into a capsid head during the spontaneous assembly of new viruses
5. Upon cell lysis, the **defective phage** will infect another bacterium and inject bacterial DNA from the previous host cell into the new bacterium
6. New alleles from the previous bacterial cell can be incorporated into the genome of the new host by **homologous recombination** or **integration** of phage-bacterium hybrid DNA as defective phage enters the lysogenic cycle

Compare the similarities and differences between the mechanisms of transformation, generalized, specialised transduction and conjugation

Point of Comparison	Transformation	Generalised Transduction	Specialised Transduction	Conjugation
Type of donor cell / source of DNA	Broken down DNA from lysed bacterial cells	Bacteria cell infected by virulent phage	Bacteria cell infected by virulent template phage	F^+ cell containing F plasmid
Agent mediating DNA transfer	Cell surface proteins or $CaCl_2$ artificially, which make cells competent	Bacteriophage e.g. T4 phage	Template bacteriophage e.g lambda phage	F factor on F plasmid, which codes for proteins involved in formation of sex pili and cytoplasmic mating bridge
Type of DNA transferred to recipient cell	Random fragments of the bacterial genome; usually from closely related species	Random fragments of the bacterial genome small enough to fit into phage capsid; usually from closely related species (infected by same type of phage)	DNA transferred is restricted to bacterial genes adjacent to the integrated prophage and part of the viral genome	F plasmid
Homologous recombination needed for permanent expression of foreign genes? (Yes/No)	Yes	Yes	Yes	No

Regulation of gene expression in bacteria

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.
➔It includes a common promoter, an operator, and one or more structural genes that are controlled as a unit to produce a single polycistronic mRNA.

Promoter: RNA polymerase binding site, upstream of structural genes

Operator: repressor protein binding site to prevent RNA polymerase from binding to the promoter and intiating transcription

Polycistronic mRNA: A messenger RNA that contains the base sequence coding for the amino acids sequence of several proteins.
- A single mRNA contains multiple start codons (AUG) and stop codons (UAG, UAA, UGA) (one per polypeptide e.g. 3 sets for lac operon, 5 sets for trp operon)
- Gives rise to a total of ___ different polypeptides which can be translated from a single mRNA, illustrating the polycistronic nature of the mRNA

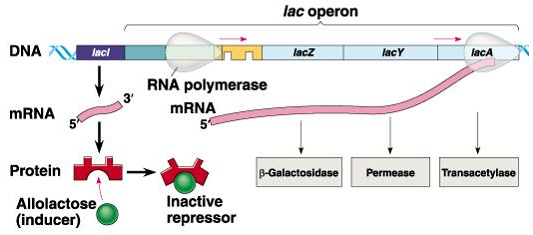
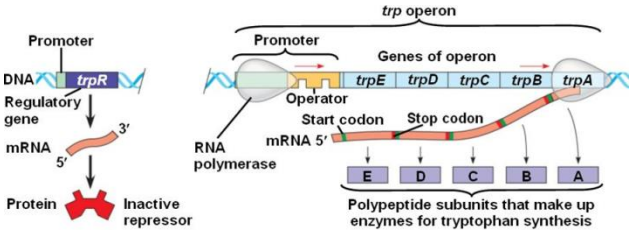
Structural gene : Any gene that codes for a protein product that forms part of a structure or has an enzymatic function

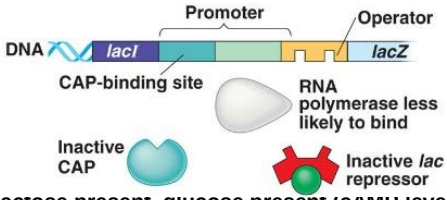
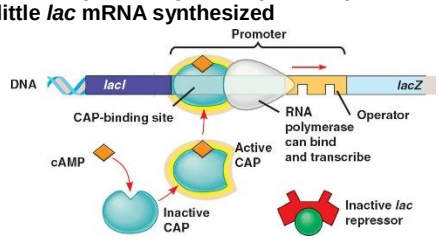
Regulator gene: Any of several kinds of nucleotide sequences involved in the control of the expression of structural genes
- Codes for a protein involved in regulating the expression of other genes e.g. repressor, CAP
- Has its own promoter and terminator sequences
- Not within operon, usually far away, but gene products that control the expression are diffusible

Effector: a small molecule that binds to a specific protein, causing a conformational change and hence regulating its biological activity.
In this context, includes inducer (allolactose in lac operon) and corepressor (trptophan in trp operon)

Purpose/Advantages of Regulation

- Allows the bacteria to make **economical use of energy and resources** – **prevents wastage/conserve resources** i.e. relevant genes are expressed only when necessary
  - Especially since bacteria are able to use a variety of metabolites e.g. glucose is metabolized preferentially over lactose, thus it is not economical to produce lac genes in the presence of glucose
  - No need to synthesize a metabolite when it can be taken in from the surroundings
- Operons
  - Can be turned 'on' or 'off' according to changes/ conditions of the environment
  - Allows for functionally related proteins to be synthesized as a unit
- Enable bacteria to **respond rapidly and appropriately to changes in environment**
- All this **provides a selective advantage** to such bacteria, who can respond to and survive when there are changes in the environment

	Lac operon	Trp operon
Structure	A cluster of **3 structural genes** ▪ **lacZ** codes for **beta-galactosidase**: enzyme that hydrolyses lactose into glucose and galactose ▪ **lacY** codes for **permease**: facilitates movement of lactose from outside of cell to inside of cell ▪ **lacA** codes for **transacetylase**: function remains unknown ▪ **Promoter** ➔ RNA polymerase binding site ▪ **Operator** ➔ lac repressor binding site Operator overlaps with promoter Catabolite Activator Protein (CAP) binding site within promoter	A cluster of **5 structural genes**: **trpE**, **trpD**, **trpC**, **trpB** and **trp A** ▪ Code for enzymes in tryptophan synthesis ▪ 5 genes ➔ 5 polypeptides ➔ 3 enzymes (2 of the enzymes are dimers, trpA+trpB & trpD+trpE & trpC on its own) ▪ Promoter ➔ RNA polymerase binding site ▪ Operator ➔ binding site for trp repressor complex with trp Operator within promoter
Regulatory gene	***Lac I*** gene that codes for **lac repressor**	***Trp R*** gene that codes for **trp repressor**
Purpose	It regulates the production of **inducible enzymes** such as beta-galactosidase and other proteins involved in the **breakdown of lactose**	It regulates the production of **repressible enzymes** for the **synthesis of the amino acid tryptophan**
		
Type of operon	**Inducible operon**	**Repressible operon**
Type of regulation	**Dual regulation** - negative regulation by the *lac* repressor and positive regulation by CAP	negative regulation by the *trp* repressor
Effector	**Inducer lactose**, the substrate	**Corepressor tryptophan**, an end product
Type of metabolic pathway	**Catabolic pathway:** breakdown metabolites	**Anabolic pathway:** synthesize metabolites
Default state of repressor	**Active**	**Inactive**
Effect of effector on operon	**Turns on** transcription of structural genes	**Turns off** transcription of structural genes
Default state of operon expression	**Off**	**On**

	Lac operon	Trp operon
Operon expression: How it works	1. In the absence of lactose, a basal level of beta galactosidase and permease is present in the cell: repression of lac operon by lac repressor is leaky 2. **Lactose enters** the cell by **permease** 3. And is converted to **allolactose** by **beta-galactosidase** 4. Allolactose acts as an **inducer** and **binds to allosteric site of lac repressor**. This causes a **change in confirmation** of lac repressor and lac repressor becomes **inactive** 5. The **inactive repressor** and **can no longer bind to operator** of lac operon (no longer complementary in shape and charge) 6. **Promoter** site available for **RNA polymerase to bind** 7. When glucose is absent ➔ high levels of cAMP is present ➔ cAMP binds to CAP and activates CAP ➔ activated CAP binds to promoter of lac operon which **increases the affinity of RNA polymerase to the promoter** 8. Transcription frequency of structural genes lacZ, lacY and lacA to produce beta-galactosidase, permease and transacetylase respectively to breakdown lactose thus increases. 9. (Give one example of a product and what it does) Note: there will be a time lag for lac operon genes to be expressed – time taken for transcription of genes and subsequent translation to form gene products  **Lactose present, glucose present (cAMP level low): little *lac* mRNA synthesized**  **Lactose present, glucose absent (cAMP level high): abundant *lac* mRNA synthesized**	1. When **tryptophan** is present in **high concentrations** in the cell 2. Tryptophan acts as a **corepressor** and binds to the **allosteric site of trp repressor** 3. This causes a **change in conformation** of trp repressor and trp repressor become **active** 4. The **active repressor** can **bind to the operator** 5. This **prevents binding of RNA polymerase** to **promoter** 6. And **prevents transcription of structural genes** & expression of operon 7. **Synthesis of tryptophan** is reduced/ **stopped**

Compare Viruses and Bacteria		
	Virus	**Bacteria**
Cellular organisation	Acellular	Is a cell
	Has no cell surface membrane, but may have a viral envelope	Always has a cell surface membrane
	Has no cell wall	Has a (peptidoglycan) cell wall
Genetic material	Genetic material is either DNA or RNA	Has both DNA & RNA
	Viral DNA or RNA may be single or double-stranded	Bacterial DNA is double-stranded & circular
Macromolecular machinery	Has no ribosomes	Has (70S) ribosomes
	Does not » respire » feed » excrete/ have metabolic wastes while outside host cell » grow	Carries out metabolism such as » respiration » acquisition of food » excretion of metabolic wastes Capable of cell growth on its own
Reproduction	Can only replicate & assemble within host cell	Reproduces either asexually or by binary fission
Size	Much smaller: 10-300nm	*E. coli* usually greater than 1µm
Pathogenicity	Both are capable of causing disease in humans	

Effect of Mutations *(using Lac operon as an example)*

Category	Observation	Location of Mutation	Details
Affects a single structural gene	Truncated/ non-functional/ absent beta-galactosidase (or transacetylase or permease)	*lacZ* (or *lacY* or *lacA*)	Permease and transacetylase coded by l*acY* and *lacA* will still be produced as the enzymes are translated independently because each has its own start & stop codon on the polycistronic mRNA
Affects all 3 structural genes equally	Enzymes not present, even when lactose is present ➔ indicates that operon is switched off/ not expressed	*lacI*	▪ Change in conformation of DNA binding site of repressor ➔ binds irreversibly to / cannot dissociate from operator ▪ Change in conformation of allosteric site of repressor ➔ cannot bind to the inducer and become inactivated ➔ remains bound to operator
		Promoter	RNA polymerase cannot bind to promoter ➔ no transcription
	Enzymes always present, even when lactose is absent ➔ genes in operon are constitutively transcribed	*lacI*	▪ Truncated/ non-functional/ absent repressor ▪ Conformational change in DNA binding site of repressor ➔ cannot bind to operator ▪ (for trp operon) Conformational change in allosteric site of repressor ➔ cannot bind to corepressor to form repressor complex ➔ cannot bind to operator
		Operator	Repressor unable to bind to operator ➔ RNA polymerase can continue to bind to promoter & transcribe structural genes