Microbiology 1

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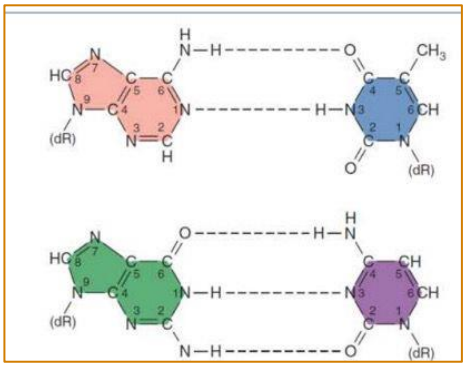
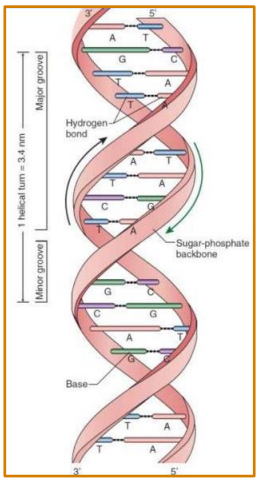
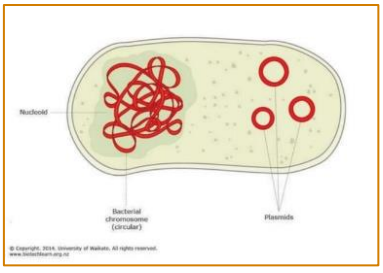
Bacteria genetic

- **Revision**
  - **DNA *(deoxyribonucleic acid)***
    - ✓ *Double Helix Structure:* two strands that coil around each other.
    - ✓ *Nucleotide Composition:* Each DNA strand is made up of nucleotides which consist of:
      - A phosphate group, a deoxyribose sugar, and a nitrogenous base (adenine, thymine, cytosine, or guanine).
    - ✓ *Base Pairing*: The nitrogenous bases on the two strands pair specifically (adenine with thymine and cytosine with guanine) through hydrogen bonds, ensuring accurate replication and transcription.
    - ✓ *Antiparallel Orientation*: The two strands of DNA run in opposite directions (5' to 3' and 3' to 5').
    - ✓ *Genetic Information Storage:* DNA carry genetic information to survival and growth.
    - ✓ *Exon* (coding region) and *Introne* (non-coding region)





- **Bacterial DNA**
  - Bacteria → Prokaryotes → have no nuclear membrane surround genetic material.
  - Bacteria genetic material:
    - ✓ *Bacterial chromosome*: is circular double helix supercoiled DNA (No histone or polyamines)
    - ✓ Extra genetic material (*plasmids*):
      - Small, circular, double-stranded DNA molecules, typically ranging from 1 to 200 kb in size, coding for at least one gene
      - Carry genes which is responsible for virulence factor and antibiotic resistance
    - ✓ ***Types*:**
      - Fertility (F) Plasmids: Contain genes for conjugation and enable the formation of sex pili.
      - Resistance (R) Plasmids: Carry genes that provide resistance to antibiotics or toxic substances.
      - Col Plasmids: Encode bacteriocins, which can kill other bacterial species.
      - Virulence Plasmids: Transform non-pathogenic bacteria into pathogenic forms.



- **Transcription and Translation**

  - Denaturation of DNA strands to provide a template for synthesizing complementary mRNA and translated to form polypeptides.

  - Transcription: process is to produce mRNA molecule from DNA

    1. ***Initiation***:
       - *RNA polymerase* binds to a specific region of the bacterial DNA called the *promoter*, which is located upstream of the gene to be transcribed.
       - Transcription factors may assist in this process by helping RNA polymerase recognize the promoter.

    2. ***Elongation***:
       - RNA polymerase unwinds the DNA helix and synthesizes a single-stranded RNA molecule by adding complementary RNA nucleotides (A, U, C, G) to the growing RNA chain.
       - This process occurs in the *5' to 3'* direction.

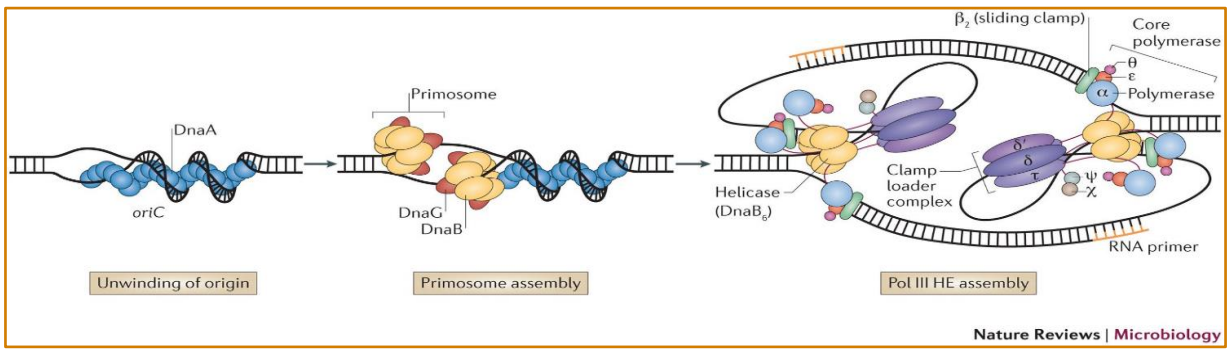
    3. ***Termination:***
       - Transcription continues until RNA polymerase encounters a *termination signal*. There are two main types of termination in bacteria:
         1) Rho-Independent: This involves a specific sequence in the RNA that forms a hairpin structure followed by a series of uracil residues. The hairpin causes RNA polymerase to pause, and the weak RNA-DNA hybrid (due to uracil-adenine pairing) causes the RNA molecule to detach.
         2) Rho-dependent Termination**:** This requires the Rho protein, which binds to the RNA and moves towards the RNA polymerase. Upon reaching the polymerase, Rho facilitates the dissociation of the RNA transcript from the DNA template.

- **Translation:** mRNA translated to certain sequence of amino acids that forms polypeptide chains

  1. ***Initiation***:
     - ✓ The first *tRNA*, carrying the amino acid methionine, binds to this *start codon* (usually AUG) of the mRNA

  2. ***Elongation***:
     - ✓ The ribosome moves along the mRNA, reading codons (three-nucleotide sequences).
     - ✓ *tRNAs* bring the corresponding amino acids to the ribosome, where peptide bonds form between them, elongating the polypeptide chain and faciliateing by *Peptidyltransferase*.

  3. ***Termination***:
     - ✓ Translation continues until the ribosome encounters a *stop codon* (UAA, UAG, UGA).
     - ✓ Release factors help detach the polypeptide chain from the ribosome, which then *disassembles*.



➢ ***Key Differences from Eukaryotes:***

- ✓ *Location*: In bacteria, transcription and translation can occur simultaneously in the cytoplasm, whereas in eukaryotes, transcription occurs in the nucleus and translation in the cytoplasm.
- ✓ *mRNA Processing*: Bacterial mRNA is usually not processed (no 5' cap or poly-A tail [poly adenylation]) and is often polycistronic (can encode multiple proteins).
- ✓ *Splicing procces*: in bacteria don't have intron (non-coding regions) no need splicing process as eukaryotic

➢ Aminoglycosides and tetracyclines are antibiotics that inhibit protein synthesis by binding to the 30S ribosomal subunit .

- **Regulation of transcription**

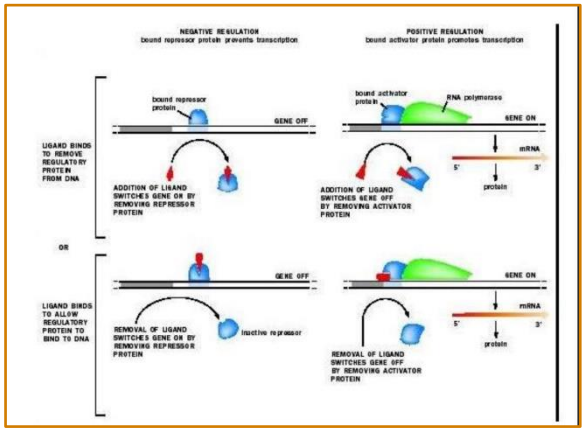
➢ Controlling gene expression in response to the needs of the cell and the availability of substrate.

➢ ***Transcription Factors:***

- ✓ *Activators*: (responsible for positive regulation) are proteins that increase the transcription of a gene in response to an external stimulus
- ✓ *Repressors*: (responsible for negative regulation) are proteins that suppress transcription of a gene in response to an external stimulus
- ✓ Both activators and repressors are regulated by inducers which are small molecules that either induce activators or repressors

➢ Regulatory genes are transcribed to produce regulatory proteins that *modulate* transcription and translation processes.

➢ Depending on the concentration of these regulators, gene expression can be positively or negatively *regulated*.



- **DNA replication**

➢ Steps of DNA Replication in Bacteria

- ✓ DNA replication is initiated from the origin of replication with a denaturation in the double strand DNA, each strand acts as a template to produce another complementary DNA strand.

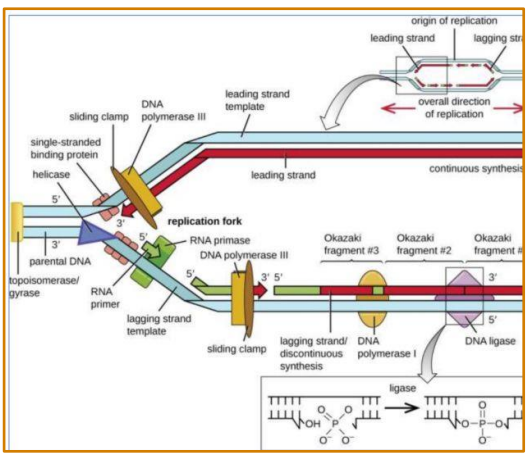
1. *Initiation*
   - The process starts with the binding of the DnaA protein to the DnaA boxes in at a specific location on the circular chromosome the oriC region, leading to the unwinding of the DNA.
2. *Unwinding the DNA*
   - ***Helicase*** (enzyme) unwinds the double-stranded DNA, creating single-stranded regions that will serve as templates for replication.
   - ***Single-Stranded Binding Proteins*** **(SSBs)**: These proteins bind to the single-stranded DNA to prevent it from re-annealing or forming secondary structures.

3. *Priming DNA Synthesis*
   - ***Primase*** synthesizes short RNA primers (approximately 10-12 nucleotides) complementary to the single-stranded DNA template. This provides a free 3'-OH group for DNA polymerase to begin synthesis.
4. *Elongation*
   - ***DNA Polymerase III****:* This is the main enzyme responsible for synthesizing the new DNA strand. It adds nucleotides to the growing DNA strand in the 5' to 3' direction, extending from the RNA primer forming leading strand.
   - ***Leading Strand****:* Synthesized continuously in the direction of the replication fork.
   - ***Lagging Strand****:* Synthesized in short segments known as **Okazaki fragments** because it runs in the opposite direction to the movement of the replication fork.
5. *Replacing RNA Primers*
   - ***DNA Polymerase I****:* This enzyme removes the RNA primers from the lagging strand and replaces them with DNA nucleotides.
   - ***5' to 3' Exonuclease Activity****:* DNA Polymerase I has exonuclease activity that allows it to remove the RNA primers.
6. *Joining Okazaki Fragments*
   - ***DNA Ligase:*** This enzyme seals the gaps between the Okazaki fragments by forming phosphodiester bonds, resulting in a continuous DNA strand.
7. *Termination*
   - DNA replication continues until the entire chromosome has been replicated, and specific termination sites help ensure that replication is completed accurately. The process ends when the replication forks meet.
   - The replication continues until it reaches the stop codon.
   - After the two DNA strands are ligated, they will coil into their 3D configuration, resulting in genetic material identical to that of the mother cell.
   - 1DNA→ 2 DNA

- **Summary of Key Enzymes**
  - DnaA: Initiator protein that binds to the origin of replication.
  - (Helicase): Unwinds the DNA double helix.
  - SSBs (Single-Stranded Binding Proteins): Stabilize unwound DNA strands.
  - (Primase): Synthesizes RNA primers.
  - DNA Polymerase III: Main enzyme for DNA synthesis.
  - DNA Polymerase I: Replaces RNA primers with DNA.
  - DNA Ligase: Joins Okazaki fragments.

Table 1. The Molecular Machinery Involved in Bacterial DNA Replication

Enzyme or Factor	Function
DNA pol I	Exonuclease activity removes RNA primer and replaces it with newly synthesized DNA
DNA pol III	Main enzyme that adds nucleotides in the 5' to 3' direction
Helicase	Opens the DNA helix by breaking hydrogen bonds between the nitrogenous bases
Ligase	Seals the gaps between the Okazaki fragments on the lagging strand to create one continuous DNA strand
Primase	Synthesizes RNA primers needed to start replication
Single-stranded binding proteins	Bind to single-stranded DNA to prevent hydrogen bonding between DNA strands, reforming double-stranded DNA
Sliding clamp	Helps hold DNA pol III in place when nucleotides are being added
Topoisomerase II (DNA gyrase)	Relaxes supercoiled chromosome to make DNA more accessible for the initiation of replication; helps relieve the stress on DNA when unwinding, by causing breaks and then resealing the DNA
Topoisomerase IV	Introduces single-stranded break into concatenated chromosomes to release them from each other, and then reseals the DNA



- The **regions** of genetic material classified into:
  1) *Conservative regions*; these regions are highly similar upon replication of the genetic material.
  2) *Semiconservative regions*; a new double stranded DNA molecule is constructed using one old strand of DNA as a template paired with one new strand which is exposed to mutations.
  3) *Dispersive regions*; a hybrid sequences upon replication results in high exposure for mutations.

- **The different between DNA replication and translation:**
  - DNA replication and translation are two fundamental processes in bacterial cells, each serving distinct purposes. Here are the main differences between them:

    ✓ *Purpose*
    - ***DNA Replication****:* The process of copying the bacterial DNA to produce two identical copies, ensuring that genetic information is passed on during cell division.
    - ***Translation****:* The synthesis of proteins from mRNA, where the information encoded in the mRNA is used to assemble amino acids into a polypeptide chain.

    ✓ *Enzymes Involved*
    - ***DNA Replication****:* Involves DNA polymerases, which synthesize new DNA strands by adding nucleotides complementary to the template strand.
    - ***Translation****:* Involves ribosomes, transfer RNA (tRNA), and various translation factors that facilitate the assembly of amino acids into proteins based on the mRNA sequence.

    ✓ *Templates Used*
    - ***DNA Replication****:* Uses the original DNA strand as a template to create a new complementary DNA strand.
    - ***Translation****:* Uses mRNA as a template to guide the sequence of amino acids in a protein.

    ✓ *Products*
    - ***DNA Replication****:* Produces two identical DNA molecules, each consisting of one original and one newly synthesized strand (semi-conservative replication).
    - ***Translation****:* Produces a polypeptide (protein) that may undergo further folding and modifications to become functional.

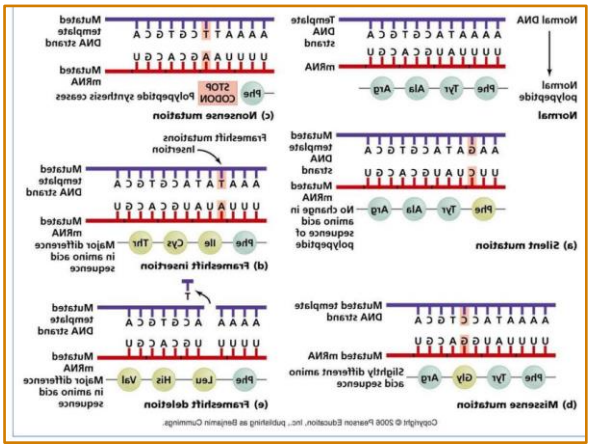
    ✓ *Location*
    - ***DNA Replication****:* Takes place in the cytoplasm in bacteria, where the circular DNA is located.
    - ***Translation****:* Also occurs in the cytoplasm, specifically at the ribosomes, where mRNA is translated into proteins.

    ✓ *Process Type*
    - ***DNA Replication****:* A semi-conservative process that involves unwinding the DNA double helix, synthesizing new strands, and proofreading for errors.
    - ***Translation****:* Involves initiation, elongation, and termination phases where ribosomes read the mRNA codons and tRNAs bring the corresponding amino acids.

- **DNA mutations**
  - Bacterial genetic material is susceptible to various factors **(Exogenous Triggers)**, such as UV radiation, chemicals, oxidative compounds, acids, organic mutagens, and certain antibiotics which can induce mutations in DNA. There are two main types of mutations:

1. Substitution Mutations
   - ✓ Involves the replacement of one nucleotide, leading to the formation of a different codon, which can result in:
     - ***Silent Mutation**:* The new codon still codes for the same amino acid.
     - ***Missense Mutation:*** The new codon codes for a different amino acid; this can be classified as a conservative mutation if the new amino acid has similar properties
     - ***Nonsense Mutation**:* The new codon is a stop codon, terminating translation prematurely and preventing the expected protein from being produced.
     - ***Null Mutations**:* These mutations result in non-functional genes.

2. Frameshift Mutation: Caused by the *insertion* or *deletion* of one nucleotide, which alters the reading frame of the mRNA. This results in a *completely different* amino acid sequence and can lead to the introduction of a stop codon, drastically affecting the resulting polypeptide.



- **DNA mutations repair**

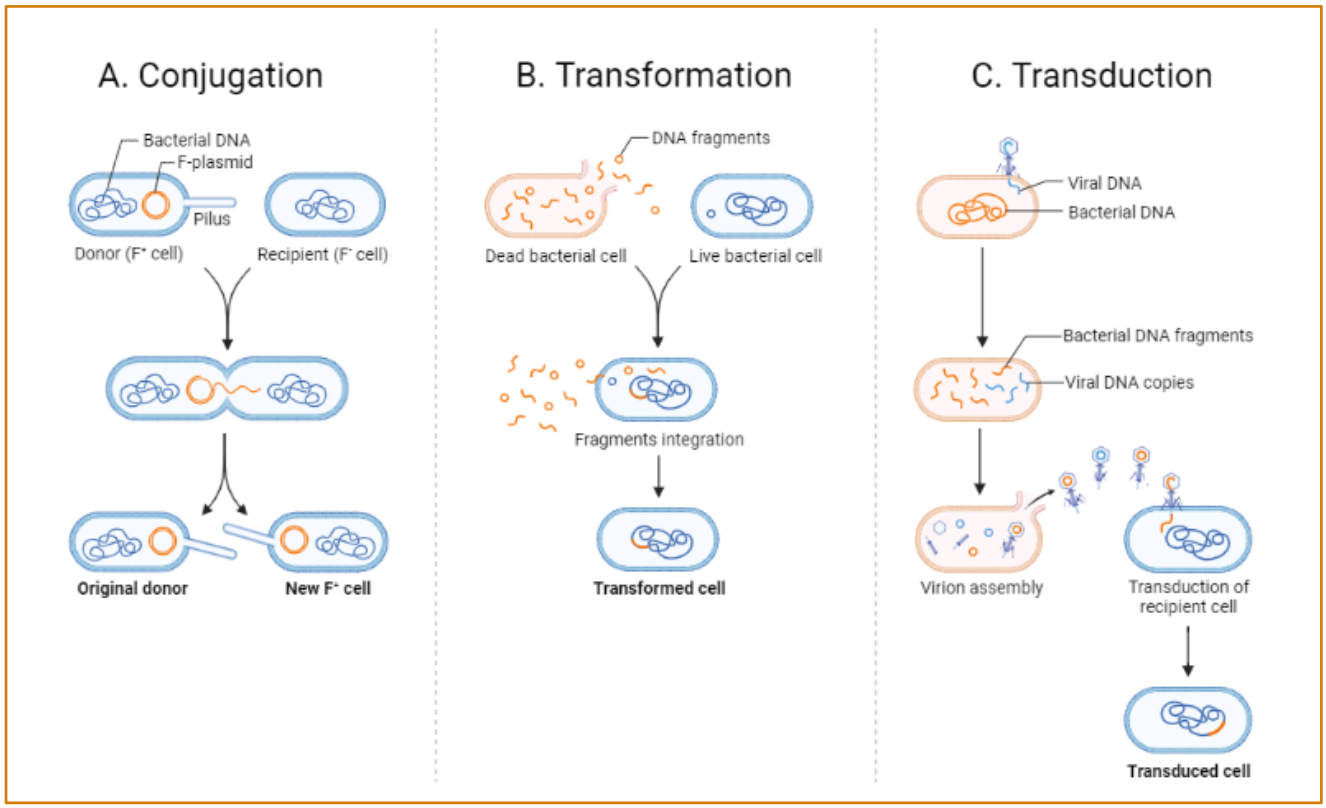
1. *Direct DNA repair* is the enzymatic removal of damage, such as pyrimidine dimers and alkylated bases.
2. *Excision repair* is the removal of a DNA segment containing the damage, followed by synthesis of a new DNA strand.
3. *Recombinational or postreplication repair* replaces a missing or damaged section of DNA with the same or similar sequences that may be present during replication or on extrachromosomal DNA.
4. *The SOS response* is the induction of many genes (≈15) after DNA damage or interruption of DNA replication to promote recombination or error-prone repair.
5. *Error-prone repair* is the last resort of a bacterial cell before it dies. It is used to fill in gaps with a random sequence when a DNA template is not available for directing an accurate repair.

- **Horizontal Gene transfer (HGT)**
  - The *transfer* of genetic material between *two bacterial* cells, as opposed to vertical transfer from parent to offspring. This process can occur through several mechanisms:

1) ***Conjugation***
   - *Definition*: This involves direct transfer of genetic material between two adjacent bacterial cells through a process called plasmogamy, where the cytoplasm of the two cells connects.
   - *Mechanism*: A pilus (sex pilus) forms a bridge between the two cells, allowing the transfer of plasmids or DNA fragments. The genetic material integrates into the recipient cell's genome and is replicated, potentially introducing new traits.

2) ***Transformation***
   - *Definition*: This process occurs when a bacterial cell dies and lyses, releasing its genetic material into the surrounding environment.
   - *Mechanism*: Another bacterial cell can uptake this free genetic material and integrate it into its own genome, acquiring new characteristics as a result.

3) ***Transduction***
   - *Definition*: This involves the transfer of genetic material via bacteriophages (viruses that infect bacteria).
   - *Mechanism*: When a phage infects a bacterial cell, it can integrate its genetic material with that of the bacterium. The phage uses the bacterial machinery to replicate and assemble itself, eventually causing lysis of the host cell and releasing new phage particles that can infect other bacteria. This process can introduce new traits to the recipient cells.



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