7

Microbiology

Doctor 2017 | Medicine | JU

Sheet Slides

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DOCTOR

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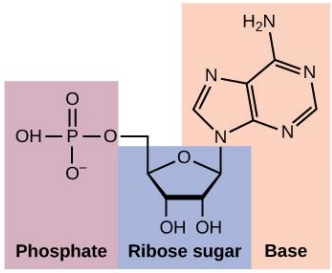
Some Information in this lecture may not be mentioned by the Dr. as thoroughly as this sheet. But they cannot be overlooked for a better understanding, so bear with this lecture :) and sorry for any inconveniences.

DNA Structure

The DNA structure of **Prokaryotes** is similar to that of **Eukaryotes**. The building blocks of DNA are **nucleotides**, which are made up of three parts: a **deoxyribose** (5-carbon sugar), a **phosphate group**, and a **nitrogenous base** .

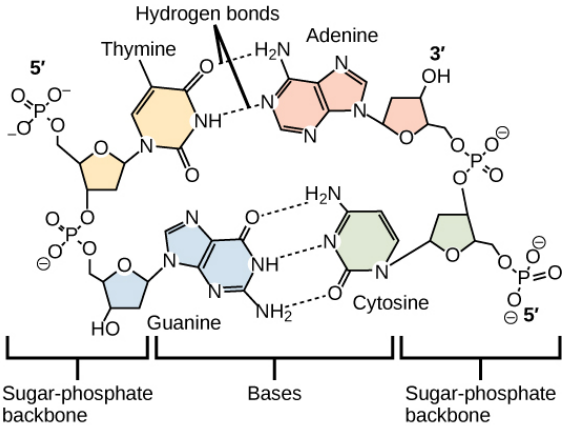


There are four types of nitrogenous bases in DNA :

- **Adenine** *(A)* and **thymine** *(T)* are connected by **2** hydrogen bonds.
  Cytosine *(C)* and **guanine** *(G)* are connected by **3** hydrogen bonds.

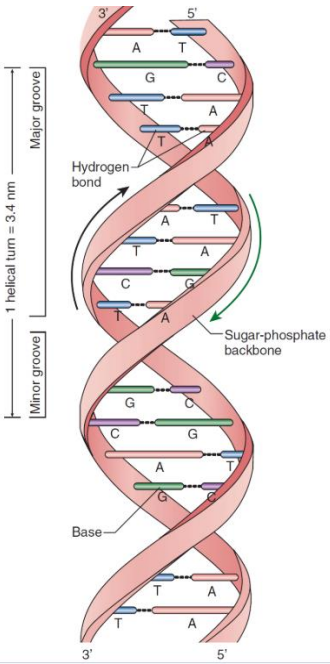
A and G are purines
C and T are pyrimidines

- The **phosphate group** of one **nucleotide** bonds covalently with the **sugar molecule** of the **next** nucleotide, and so on, forming a **long polymer** of nucleotide monomers.
- The **sugar–phosphate groups** line up in a ***"backbone"*** for each single strand of DNA, and the nucleotide bases stick out from this backbone.
- Naturally, each DNA molecule is composed of **two single strands** held together with **hydrogen bonds between the bases**.



Note: *When DNA is **heated**, the hydrogen bonds holding the 2 strands together **break**, and the 2 strands **fall apart.***
*Since G-C base pair are connected by **3 H-bonds**, A DNA with more G-C base pairs would need **more heat** to be broken.*

- The 2 **complementary strands** of a DNA double helix run in **opposite directions** alongside each other.
- The **antiparallel orientation** allows for the base pairs to complement one another. Antiparallel DNA is also **more structurally stable** than parallel DNA.



Note: *This orientation affects the **DNA replication**, since certain enzymes only functions in the **3'- 5' direction**. (discussed more later in this sheet)*

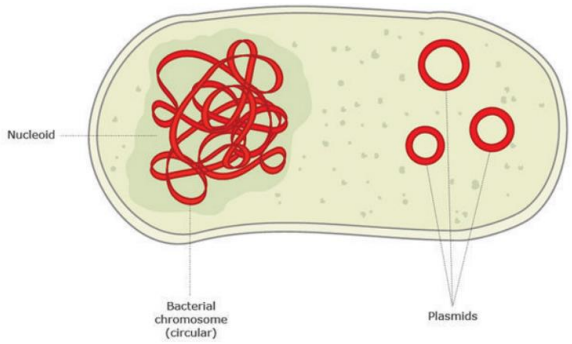
Measuring DNA Size

We can **measure the size** of the DNA molecule in **kilobase pair** (Kbp). In bacteria, a single DNA molecular size can be from **100s to 1000s Kilobase pairs**.

Bacterial Chromosome

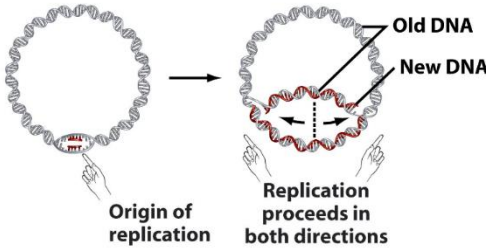
- A **haploid**, **double stranded circular** DNA with **no free ends** are characteristics of most **bacterial** chromosomes. Unlike the **linear** DNA of most **eukaryotes**.

- Many bacteria also contain **extra looped bits** of DNA known as **plasmids**.



- *The genetic information of the plasmid is usually **not essential** for the survival of the host bacteria, however it carries multiple **antibiotic resistance genes.***
  (discussed later in the next lecture)

- A **Replicon** is any genetic material that replicates itself in a bidirectional manner from a single origin. For **most** prokaryotes, the replicon is the **entire chromosome** and the **plasmids**.



- Bacterial chromosome if **untangled** is almost **1mm long**, while the **bacterium** is about a **micrometer** in size. Thus, **supercoiling** of the bacterial chromosome is **needed** for **packaging** and **storing** purposes inside the bacterium.

  In **Eukaryotes**, the chromosomes are tangled using **histones** which are not present in bacteria. **Bacteria** uses other compounds to promote supercoiling *(e.g. Polyamines).*

- **Haploid** Bacteria, with only **one copy** of each vital gene, are always **threatened** by mutations more than **diploid** Eukaryotes.

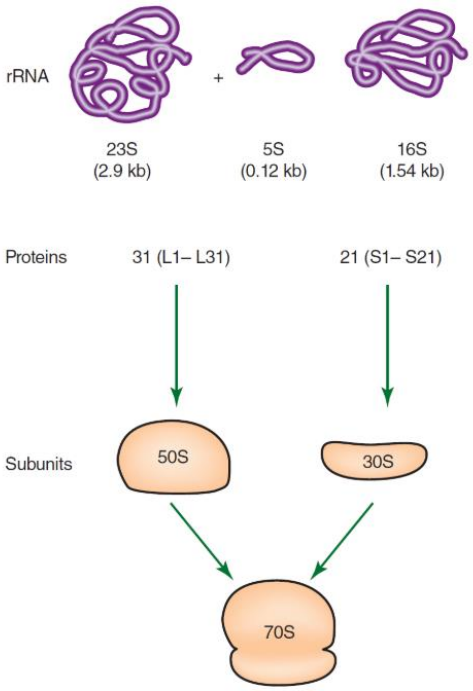
  In Eukaryotes, the **complementation** in **diploids** compensate the **deficiencies** of **mutated genes** by other normal genes.

DNA Replication Process

Important terms for further understanding before carrying on, also study thoroughly the following table of enzymes used in DNA replication:

1) ***Sedimentation Rate (S):*** *is a measurement of* ***the rate of sedimentation*** *in* ***centrifugation****, used to* ***describe*** *the ribosomal subunits and the rRNA fragments -but not the size of them- that's why they* ***don't add up*** *since it's a measurement of* ***rate*** *not size.*

2) ***Ribosomes:*** *Are complexes found within all living cells, that serves as the* ***site of biological protein synthesis*** *(translation) in the* ***cytoplasm****, where tRNA carries AAs from the cytoplasm into the ribosome. Ribosomes then link amino acids together in the order specified by the* ***mRNA molecules****.*



Ribosomes in prokaryotes consist of two major components:

a- *A* ***small ribosomal subunit (30S),*** *which reads the mRNA.*

b- *A* ***large subunit (50S),*** *which join amino acids to form a polypeptide chain.*

Each subunit is made up ***of rRNA*** *molecules and a variety of* ***ribosomal proteins.***

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

Step 1: Replication Fork Formation

- Before DNA can be replicated, the double stranded molecule must be **"untangled"** by an enzyme called **Topoisomerase**, then it is **"unzipped"** into two single strands by an enzyme known as **DNA helicase**. DNA helicase **disrupts the hydrogen bonding** between base pairs to separate the strands into a Y shape known as the **replication fork**. This area will be the template for **replication to begin**.

> ***Note:*** *One strand is oriented in the **3' - 5' direction (leading strand)** while the other is oriented **5' - 3' (lagging strand)**. The two sides are therefore replicated by **two different processes** to accommodate the **directional difference**, each discussed below.*

Step 2: Primer Binding

- Once the DNA strands have been **separated**, a short piece of RNA called a **primer** generated by the **DNA primase enzyme** binds to the **3' end** of the strand on the **leading strand.**

Step 3: Elongation

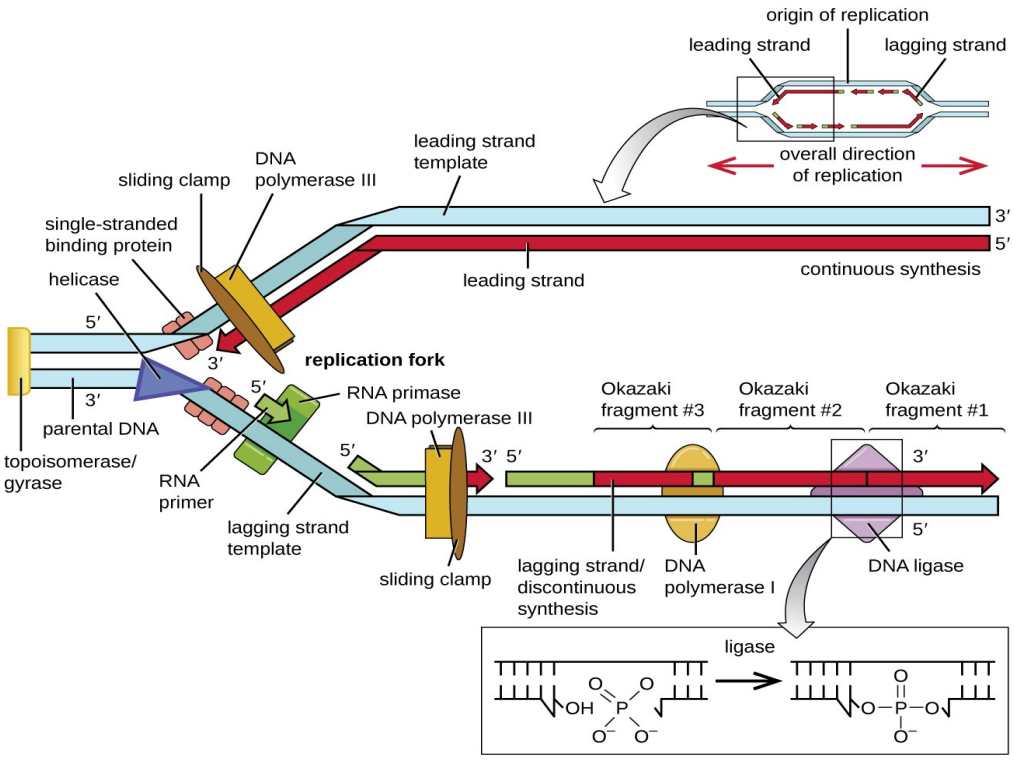
- **DNA polymerase III** binds at the **site of the primer** and begins adding new base pairs **complementary** to the **template strand**.
  Note that the template strand is read in the 3'-5' direction, while the new strand is added in the 5'-3' direction.

- The **lagging strand** begins replication by **binding with multiple primers**. Each primer is only several bases apart. DNA polymerase then **adds pieces of DNA**, called **Okazaki fragments**, to the strand **between primers**.

  *This process of replication is **discontinuous** and **slower** than the leading strand thus calling it a lagging strand.*

Step 4: Termination

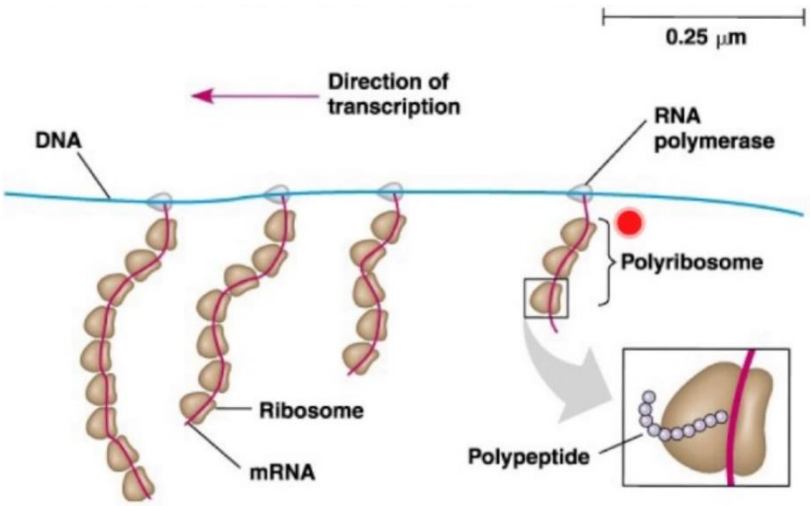
- Once both the continuous and discontinuous strands are **formed**, an enzyme called **DNA pol I** *(Exonuclease)* removes **all RNA primers** from the original strands. These primers are then **replaced** with **appropriate bases.**
- Another enzyme called **DNA ligase** joins **Okazaki fragments** together forming a **single unified strand.**
- In the end, replication produces **two DNA molecules**, each with one strand from the parent molecule and one new strand.



For additional information on this topic: https://www.khanacademy.org/science/biology/gene-expression-central-dogma/translation-polypeptides/v/rna-transcription-and-translation

> ***Note:*** *Antibiotics can target* ***cell walls***, ***topoisomerase enzymes*** *and* ***30S ribosome's subunit****. Antibiotics targeting topoisomerase are made especially for the* ***bacteria's topoisomerase****, due to the* ***slight similarities*** *in structure between the bacteria's and eukaryotic cells in the human body topoisomerases;* ***toxicity*** *from* ***antibiotics*** *can occur.*

- **Transcription** in Eukaryotes occurs in the **nucleus** while the **translation** occurs in the **cytoplasm**. However, transcription and translation of Prokaryotes are **coupled in the cytoplasm**, showing **higher efficiency** in the number of proteins made.



Regulation of Transcription in Prokaryotes

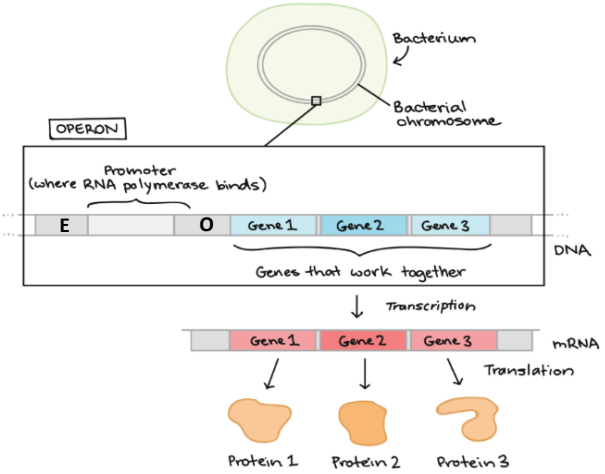
There are various forms of **gene regulation**, that is, **mechanisms for controlling** which **genes get expressed** and at what levels *(e.g. Attenuation)*. Bacteria have specific regulatory molecules that control whether a particular gene will be **transcribed into mRNA** or not.

Operons

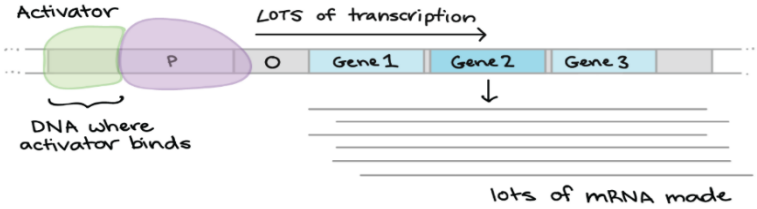
- In bacteria, genes are **often** found in **operons**. An operon is a cluster of genes under control of a **single promoter**.

- Operons are **common** in **bacteria**, but they are **rare** in **eukaryotes**.

  They have **3** regulatory regions: **Promoter**, **operator** *(O)* and the **enhancer** *(E)*.

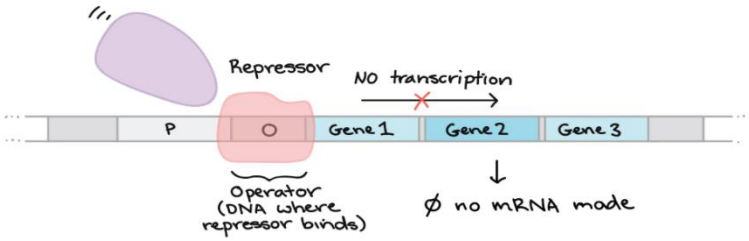


- Operons contain **regulatory DNA sequences** *(Promoter where RNA polymerase binds)* that **control transcription** of the operon's genes.

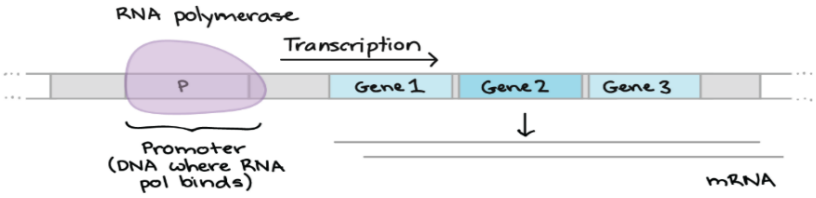


- Also, **most operons** have **regulatory DNA sequences** in addition to the promoter. These sequences *(called operators)* are **binding sites** for **regulatory proteins** that either **induces** or **reduces transcription**. *Types of regulatory proteins:*

1) **Repressor** binds on an **operator**. When bound to its operator, it **reduces transcription** *(e.g. By blocking RNA polymerase from moving forward on the DNA).*



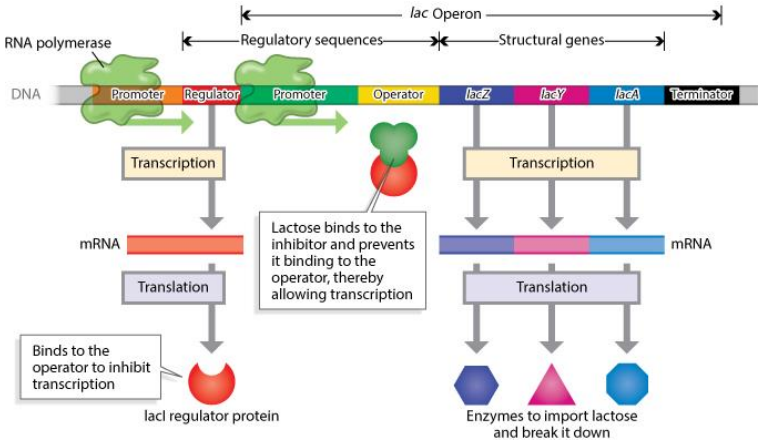
2) **Activator**, when bound to its **DNA binding site** *(enhancer)*, it **increases transcription** of the operon *(e.g. By helping RNA polymerase bind to the promoter).*



- Operons can either be **inducible** or **repressible**.

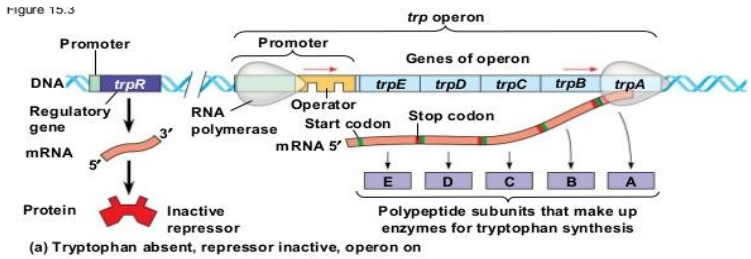
1) **Inducible operon** means that they are **usually Off** and doesn't turn on unless its **induced** by **small molecules** *(inducer).*

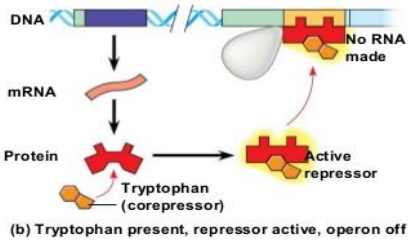
***Example**: Lac Operon which encodes enzymes for the metabolism of lactose, is usually off repressed by a lac regulatory protein. It's On only when lactose (inducer) is present which inhibits the repressor. So, its activated to break down that lactose.*



2) **Repressible operon** means that they are **usually On** unless it gets turned Off by a **small molecule** *(corepressor).*

***Example**: Trp Operon which encodes enzymes to synthesize the amino acid tryptophan. This operon is On by default, but can be repressed when high levels of the amino acid tryptophan (corepressor) are present to stop any further synthesis of Trp.*

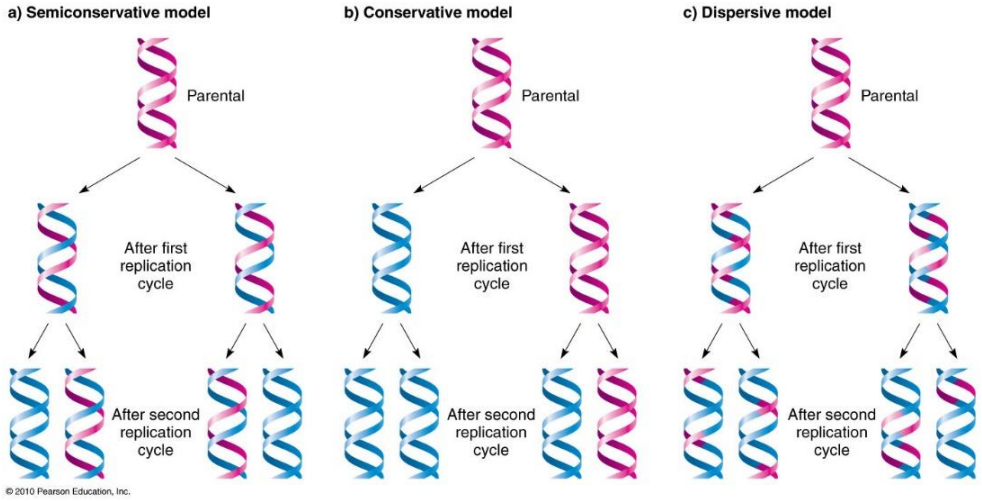




Note: *These gene regulations allows bacteria to* ***respond to environmental changes****, by altering* ***gene expression*** *and thus, changing the set of proteins present in the cell.*

I strongly recommend this site for any further understanding of operons, but it may not be required: https://www.khanacademy.org/science/biology/gene-regulation/gene-regulation-in-bacteria/v/operons-and-gene-regulation-in-bacteria

Models of DNA replication



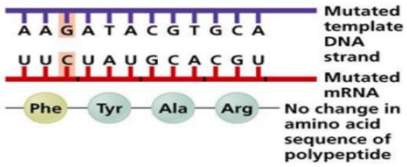
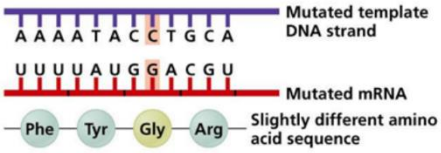
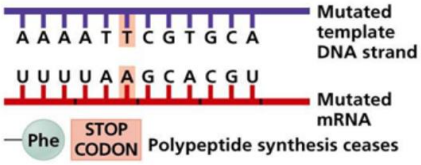
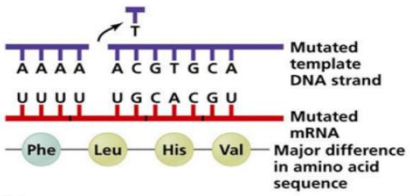
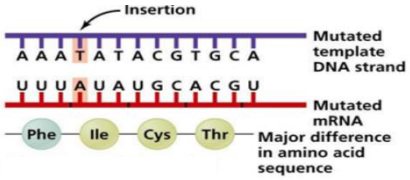
a- Semi-conservative model: The two parental strands **separate** and each makes a **copy of itself**. After one round of replication, the two daughter molecules each have **one old and one new strand.**

b- Conservative model: The parental molecule directs synthesis of **an entirely new double-stranded molecule,** such that after one round of replication, one molecule is conserved as two old strands. This is repeated in the second replication.

c- Dispersive model: The material in the two parental strands is **distributed randomly** between two daughter molecules.

➔ *The* ***semi-conservative model*** *is the correct/most appealing model.*

Types of Mutations

Substitution	**Silent**	If a **single base substitution** occurs and the resulting codon is a **synonymous codon**. Thus, the amino acid sequence encoded by the gene is **not changed** and the mutation is said to be **silent**. 
	Missense	When a **single base substitution** results in the generation of a **codon that gives a different amino acid** and hence leads to a **different protein**. *It can be:* **a- Conservative** If the structure and properties of the substituted amino acid is **very similar** to the original amino. **b- Nonconservative** If the substitution leads to an amino acid with **very different** structure and properties. 
	Nonsense	When a **single base substitution** results in a **stop codon** ultimately cutting off translation and most likely leading to **a nonfunctional protein.** 
Frameshift	**Deletion**	A **base or more** is **deleted** changing the AAs sequence from that point forward. 
	Insertion	A **base or more** is **inserted,** changing the AAs sequence from that point forward. 

Note: *Mutations can be a result of* ***many causes****, for example:* ***A defect in DNA polymerase****,* ***UV radiations****, etc.*

DNA Repair Mechanisms

1) Direct DNA repair

It is used for **reversal cell damages** (e.g. pyrimidine dimers and alkylated bases), the damaged area is repaired directly by **specialized proteins** in our body.
It is the **simplest** form of DNA repair and it does not require a **reference template** unlike the other mechanism.

2) Excision repair

Damaged bases are **removed/cut** out and replaced directly *(By DNA poly. III)* using the other **undamaged** strand as a **template**, and finally it gets **ligated**.

3) Post replication recombinational repair

If a DNA strand contain **lesions** *(sites of damage),* it will **prevent** base pairing thus **creating a gap** in the daughter strand during replication.
In this repair mechanism, the gap is **filled** by a sequence of information from a **parent strand** of a **sister chromosome** *(not by DNA poly. III).*

4) SOS response

SOS response is induced when DNA is **damaged** or when replication of DNA stops and single stranded DNA accumulates. When induced, a group of genes are **transcribed** to **repair the damage.**

5) Error prone repair

Is the **last resort** of a bacterial cell before it dies. An error prone polymerase is used to fill in gaps with a **random sequence** when a DNA template is **not available** for directing an accurate repair. However, a **higher risk** of **mutations** will arise due to the randomly mismatched base pairs.

The following table is only an additional revision of some points

Eukaryotic Replication	Prokaryotic Replication
Differences	
The rate of replication is **faster in Prokaryotes**.	
The DNA polymerase and Topoisomerase enzymes are **slightly different in structure**.	
Eukaryote's have **multiple, linear, double stranded diploid chromosomes.** *(two copies of each gene)*	Bacteria have a **single circular, double stranded, haploid chromosome.** *(one copy of each gene)*
Eukaryotes often have **multiple origins of replication** on each chromosome that initiate at different times.	Most bacteria have only **a single origin of replication** per circular chromosome.
Transcriptional modification of the mRNA is **required** *(e.g. Slicing, polyadenylation, etc.)*	Transcription and translation are almost simultaneous in a prokaryotic cell as it **doesn't require** post transcriptional modification
80s ribosomes	**70s** ribosomes
Similarities	
The DNA strand is **read** from **the 3'-5' direction** while the new strand is **synthesized** from the **5'- 3' direction.**	
Continues synthesis of the **leading strand** and **discontinuous synthesis** of the **lagging strand.**	

Good luck ☺ and don't hesitate to discuss anything