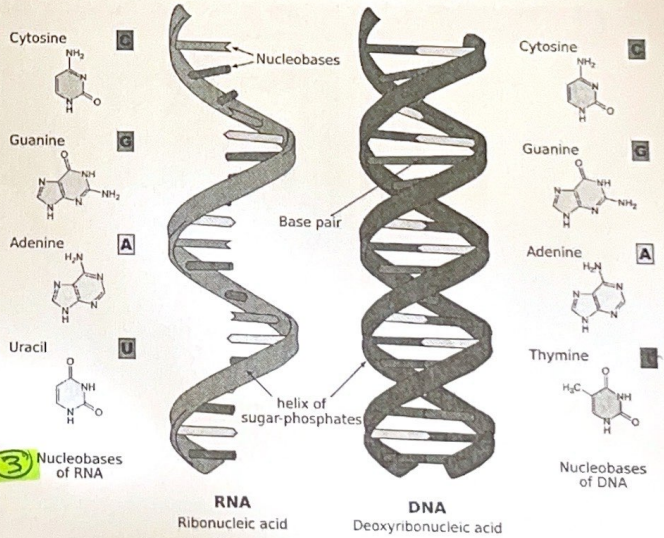
① RNA → nucleic acid (very important macromolecule / biomolecule)
ما بقدر نحصر أهميته بنقطة وحدة لانه في منه كتير أنواع وإلها
وظائف مختلفة بس أكتر نوع معروف هو mRNA

② transcription ← نسخ DNA على شكل mRNA (لانك mRNA هو bridge بين DNA و البروتين)

translation ← تحويل mRNA إلى بروتين

RIBONUCLEIC ACID (RNA)

- The bridge between DNA and protein synthesis is RNA.
  RNA is chemically similar to DNA, except that it contains ribose as its sugar and substitutes the nitrogenous base uracil for thymine.
- It is single-stranded rather than a double helix.
- To get from DNA, written in one chemical language, to protein, written in another, requires two major stages, **transcription** and **translation**.
- The process from DNA to RNA is known as **Transcription**. During transcription, a DNA strand provides a template for the synthesis of a complementary RNA strand. Transcription of a gene produces a messenger RNA (mRNA) molecule.



③ من أهم التطبيقات على mRNA هو الـ Vaccines مثل:
Corona (Pfizer) vaccine

DIFFERENT TYPES OF **RNA** & THEIR ROLES

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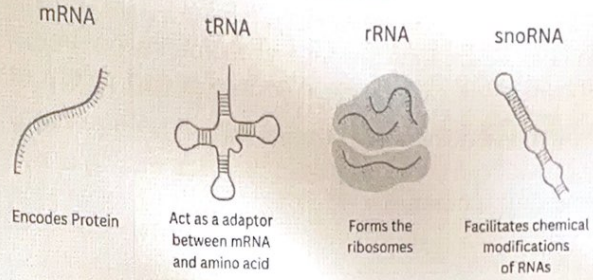
TYPES OF RNA



Messenger RNA (mRNA)

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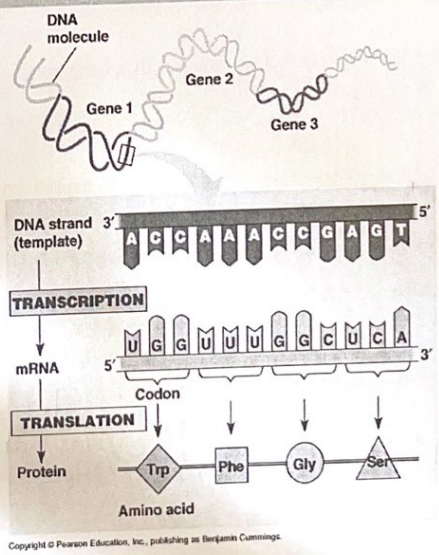
- During **translation**, the information contained in mRNA is used to determine the amino acid sequence of a polypeptide. Translation occurs at ribosomes.
- Unlike DNA, which stores genetic information, RNA helps **read** and **execute** that information!
- mRNA vaccines (e.g., Pfizer and Moderna COVID-19 vaccines)

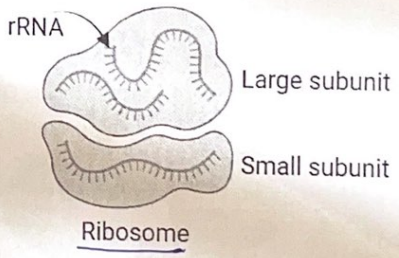


Messenger RNA (mRNA)

- **mRNA Vaccines**: The development of mRNA-based vaccines, like the Pfizer-BioNTech and Moderna COVID-19 vaccines, has revolutionized the field. These vaccines work by delivering mRNA instructions to cells, which then produce viral proteins that trigger an immune response. This approach has shown immense promise in terms of speed, safety, and effectiveness.
- **RNA Therapeutics**: Beyond vaccines, mRNA can be used in other therapeutic contexts, such as treating diseases by providing the instructions to produce missing or defective proteins in conditions like enzyme deficiencies.

Ribosomal RNA (rRNA)

- Ribosomal RNA (rRNA): The major structural and functional component of ribosomes.
- It helps catalyze the reaction that links amino acids together.
- The ribosome is made up of 40% ribosomal protein and 60% rRNA. Ribosomal DNA transcribes rRNA, which then attaches to the ribosomal proteins to form small and large ribosomal subunits



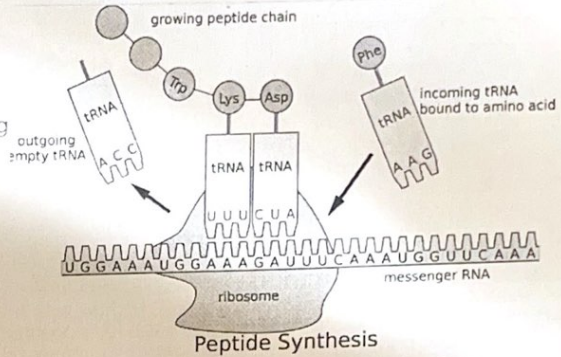
protein منه ٪40 ، two subunit لـ يتكون من

RNA ٪60

silencing بستخدمه في هاد المجال بإنه ممكن نعمل (miRNA) ③
using miRNA for harmful genes that causes certain
diagnostic ← مثلاً إذا في دراسات تثبت + disease
marker
معينة في الجسم miRNA مرتبط بنسبة إنه المرض هذا الفلاني

Transfer RNA (tRNA)

- Transfer RNA (tRNA): The 'adapter' molecules that deliver specific amino acids to ribosomes during protein synthesis.
- tRNA molecules have an anticodon that pairs with the corresponding codon on the mRNA, ensuring that the correct amino acid is added to the growing peptide chain.
- **Specificity of Aminoacyl-tRNA Synthetase:**
- Each amino acid has a specific enzyme called **aminoacyl-tRNA synthetase**, which ensures the correct tRNA is loaded with the correct amino acid.
- This enzyme binds both the amino acid and the corresponding tRNA and catalyzes the attachment of the amino acid to the tRNA. This process ensures that the correct amino acid will be delivered to the ribosome based on the anticodon.



Peptide Synthesis

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+RNA ① ← Ribosomes برضو إله علاقة بال
(مثل فكرة فيش الكهرباء ما بيجي تحط فيه إبريز بس مش
مناسب إله فبتروح بتجيب القطعة يلي بتخلي مناسب (العظمة
هاي القطعة بنسميها ال adaptor: هو بجيب الـ amino acid
by that matches the anti Codones
يلي موجودين بال mRNA

Micro RNA (miRNA):

to be translated

- Micro RNA (miRNA): Tiny RNAs that regulate gene expression by silencing specific mRNA molecules.
- miRNAs primarily function by binding to specific messenger RNAs (mRNAs), which are the templates for protein production.
- They either block the translation of these mRNAs or degrade them, reducing the amount of protein produced. This ensures precise control over protein levels in cells.
- miRNAs play crucial roles in **gene regulation**, making them potential targets for **gene therapy**, **cancer treatment**, and **diagnostic markers**.
- **Therapeutics**: miRNA-based drugs can be designed to either **mimic** or **inhibit** specific miRNAs, offering new treatments for diseases like cancer, cardiovascular disorders, and viral infections.
- This production process makes miRNAs valuable both in **biotechnology** and **pharmaceutical research** for controlling gene expression in disease contexts

موجودين أصلاً بجسمنا بتراكيز معينة

miRNA ②
متى بتكون هاي العملية مهمة؟ في حال الخلية بدها تعطّل
عنها أو مثلاً بدها تعمل downregulation ل function
أو مثلاً ارتفع نسبة بروتين معين بروح feedback بدي
مناهاد البروتين، بتصير الخلية تصنع miRNA اكسول عن
certain genes الخلية انو ما بدنا كمان
silencing of certain mRNA

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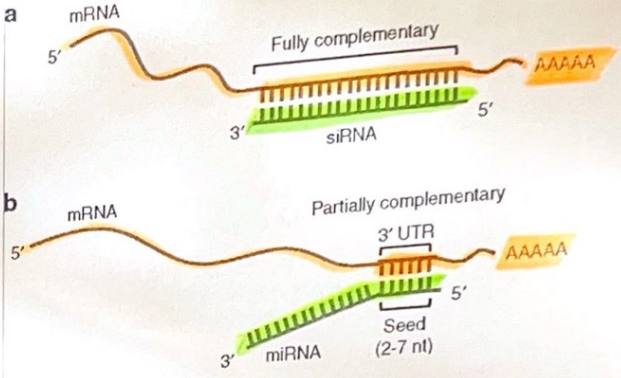
Small Interfering RNA (siRNA):

- Small interfering RNA (siRNA): Short RNA molecules that silence gene expression by degrading complementary mRNA targets.
- miRNA is less specific compared to siRNA. It typically binds to **multiple mRNAs**, usually with imperfect complementarity, and regulates their expression by blocking translation or promoting mRNA degradation. Because miRNA can target **many genes at once**, its effect can be more subtle and **widespread**, regulating various biological processes rather than focusing on a single target.
  - **siRNA** is more effective when the goal is precise and potent gene silencing for a specific gene.
  - **miRNA** is more effective when the goal is to modulate complex gene networks and physiological processes, though its action is less specific and may affect multiple genes simultaneously.



siRNA تشبه ← لكنه أطول وهادا اللي بنعكس miRNA

specificity على ال Oct-24 24

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6 أكثر specific يكون أكيد الأكبر راح يكون أكثر specificity

لانه هادا التسلسل أو sequence ممكن تتكرر بأكثر من مكان، كلما كان أطول احتمالية انه تلاقي نفس هادا sequence في مكان تاني أصعب

Long Non-coding RNA (lncRNA):

لا لانه ما بنتج عنه بروتين does not Code a protein

- Long non-coding RNA (lncRNA): Long RNAs with regulatory functions that can influence gene expression in various ways.

- Regulates gene expression at various levels, including chromatin modification, transcription, and post-transcriptional processing.
- lncRNAs can act as **scaffolds**, **guides**, or **decoys** for proteins involved in gene regulation.
- lncRNAs are versatile regulators of gene expression (i.e. upregulation and down3esx3regulation), modulating transcription, RNA splicing, and translation depending on the needs of the cell

Complex of protein بجمع البروتين في ← ممكن يشتغل كـ guide for other type of RNA → مسكان يروح لمكان معين

damage or silence for certain genes

المهم regulatory function

lncRNA ← له وظائف كثيرة منها انه إله دور كبير بالتحكم بالgene expression

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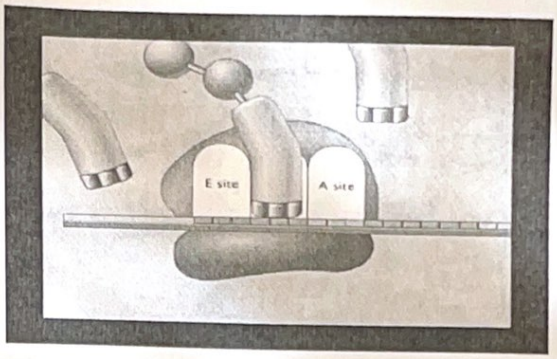
RIBOSOMES – Protein Synthesis

- Found in both the cytoplasm and attached to the endoplasmic reticulum (rough ER).
- Composed of ribosomal RNA (rRNA) and proteins
- Ribosomes translate mRNA into proteins.
- Protein Factories – Use the instructions from the nucleus to synthesize proteins
- In biotechnology, they are essential for producing therapeutic proteins (e.g., insulin, monoclonal antibodies).



https://www.youtube.com/watch?v=C4QiMqBSDe4

CONT'D

- Proteins synthesized by ribosomes are critical for every cellular function, including **enzymes**, **structural proteins**, and **signaling molecules**
- Key for the production of recombinant proteins in biotechnology.
- Ribosomes in bacterial cells synthesize human insulin using genetically engineered mRNA.
- Protein-based Diagnostic Kits
  - Ribosomes play a role in producing diagnostic proteins.
  - **Example**: Enzyme-linked immunosorbent assay (ELISA) kits use ribosome-produced antibodies or antigens to detect specific diseases (e.g., HIV)

THE CELL MEMBRANE – A PROTECTIVE BARRIER AND GATEWAY

- The **cell membrane** is a **phospholipid bilayer** that surrounds the cell, providing a **selective barrier** between the inside of the cell and its environment.
- Contains **proteins**, **lipids**, and **carbohydrates** that control the passage of substances in and out of the cell.
- **Selective Permeability**: Allows essential nutrients, ions, and molecules to enter the cell while keeping harmful substances out.
- **Membrane Proteins**: Key targets for many pharmaceuticals, such as **G-protein coupled receptors (GPCRs)**, which are involved in a variety of disease pathways.
- **Liposomal Drug Delivery**: Drugs like **AmBisome** (liposomal amphotericin B) are encapsulated in liposomes that merge with the cell membrane to deliver antifungal medication more effectively.
- **mRNA Vaccines: Lipid nanoparticles** protect and deliver mRNA into cells, where it crosses the cell membrane to instruct ribosomes to make proteins that trigger an immune response (e.g., **Pfizer-BioNTech** and **Moderna** COVID-19 vaccines).
- **Targeted Cancer Therapy: Monoclonal antibodies** that bind to specific proteins on the cell membrane can be used to deliver chemotherapy directly to cancer cells (i.e. Trastuzumab binds to the HER2 receptors)

THE CELL MEMBRANE – KEY POINTS TO CONSIDER

- Many drugs, especially biologics like antibodies and nucleic acids, use receptor-mediated endocytosis to enter cells. Understanding specific receptors can enhance targeting strategies.
- Liposomes and nanoparticles can be engineered to target specific cells by attaching ligands that bind to receptors on the cell membrane, improving therapeutic efficacy and reducing side effects.
- Lipid-based delivery systems can fuse with the cell membrane, allowing direct release of therapeutic agents into the cytoplasm (e.g., mRNA vaccines).

RELEVANCE OF CELL MEMBRANE IN RNA-BASED LIPOSOMES AND BIOPHARMACEUTICALS

- The interaction between RNA-based liposomes and the cell membrane is fundamental to their therapeutic efficacy.

Aspect	Description	Examples
Membrane Fusion	RNA-based liposomes can fuse with the cell membrane, allowing the release of their RNA payload into the cytoplasm.	**mRNA Vaccines: Lipid nanoparticles in vaccines merge with the cell membrane to deliver mRNA for protein expression (e.g., Pfizer-BioNTech and Moderna COVID-19 vaccines).**
Endocytosis	Cells can uptake liposomes via endocytosis, a process where the membrane engulfs the liposome, allowing entry of RNA into the cell.	**Patisiran: Liposomal delivery of siRNA silences the TTR gene through endosomal uptake for treating hereditary transthyretin amyloidosis.**
Targeting Specific Cells	Liposomes can be designed with surface modifications (e.g., ligands) that recognize specific receptors on the target cell membrane, enhancing selective delivery.	**Cancer Therapies: Liposomes engineered to target specific cancer cell receptors (e.g., trastuzumab-conjugated liposomes for HER2-positive breast cancer).**

RELEVANCE OF CELL MEMBRANE IN RNA-BASED LIPOSOMES AND BIOPHARMACEUTICALS

Aspect	Description	Examples
Stability and Protection	The lipid bilayer of liposomes protects the RNA from degradation by nucleases in the bloodstream, enhancing stability until reaching the target cell.	**Liposomal siRNA: Protects siRNA from degradation, allowing successful delivery into target cells (e.g., liposomal formulations for hepatitis B treatment).**
Cell Membrane Dynamics	Understanding the fluidity and composition of the cell membrane aids in optimizing liposomal formulations to enhance cellular uptake and therapeutic efficacy.	**Designing Liposomes: Tailoring lipid composition to improve fusion and endocytosis rates based on target cell membrane characteristics.**
Different Types of Biopharmaceuticals	The role of liposomes extends to various biopharmaceuticals, including proteins, antibodies, and nucleic acids, improving their delivery and efficacy.	**Monoclonal Antibodies: Liposomal formulations can enhance the delivery of antibody therapies (e.g., liposomal doxorubicin).** **Enzyme Replacement Therapies: Liposomes can encapsulate enzymes to target specific tissues (e.g., liposomal glucocerebrosidase for Gaucher disease).** **Gene Therapies: RNA-based liposomes delivering genes or gene-editing tools like CRISPR/Cas9 to target cells (e.g., liposomal delivery of CRISPR components for genetic disorders).**

MITOCHONDRIA – Energy Production For Cell Processes

- The energy powerhouse of the cell.
- Provide energy for processes like protein synthesis and cell functioning.
- In biotechnology, mitochondrial functions are sometimes targeted for therapies or used in energy-dependent biotechnological processes.
- Mitochondrial-Targeted Drugs: Compounds like mitochondrial-penetrating peptides can be designed to specifically enter cancerous cells and induce mitochondrial-mediated apoptosis, enhancing the efficacy of cancer treatments.

في كثير دراسات تربط بين levels of metabolites مثل different amino acids مع أمراض مختلفة

- **Disease Biomarkers**: Mitochondrial health serves as a diagnostic tool and offers a **personalized medicine** approach in many therapeutic areas.

mitochondria ← بصير فيها معظم metabolic prosses related to energy production like respiration (التنفس الخلوي)

وكثير من الأمراض بكون سببها خلل في إحدى pathways الموجودة بال mitochondria، لذلك علمنا بوظائف الميتوكوندريا وهاي ال pathways كثير مهم عشان نقدر نفعل targeting، مثلاً تعفيز أو تثبيط إنزيمات معينة

SUMMARY EXAMPLES – KEY CELLULAR ORGANELLES OR COMPONENT RELEVANT TO PHARMACEUTICAL BIOTECHNOLOGY

Organelle/Component	Function	Relevance to Pharmaceutical Biotechnology	Example Applications
Nucleus	Contains genetic material (DNA)	Target for gene therapies and genetic modifications	- CRISPR-Cas9 gene editing for genetic disorders (e.g., cystic fibrosis) - Gene therapy for hemophilia (AAV-based gene delivery)
Mitochondria	Energy production (ATP)	Mitochondrial-targeted therapies, role in energy metabolism	- Mitochondrial ELISA kits for measuring oxidative stress markers - Mitochondrial gene therapy for mitochondrial diseases
Ribosomes	Protein synthesis	Essential for recombinant protein production (e.g., therapeutic proteins)	- Recombinant insulin production in E. coli - Human growth hormone (HGH) produced via recombinant DNA technology
Endoplasmic Reticulum (ER)	Protein folding and modification	Site for post-translational modifications in biopharmaceutical production	- Monoclonal antibody production (e.g., Rituximab for cancer) - Production of clotting factors for hemophilia treatment
Golgi Apparatus	Protein processing and packaging	Involved in packaging proteins for secretion, essential for biopharma	- Glycosylation of Erythropoietin (EPO) for anemia treatment - Production of glycoproteins for use in vaccines

SUMMARY EXAMPLES – KEY CELLULAR ORGANELLES OR COMPONENT RELEVANT TO PHARMACEUTICAL BIOTECHNOLOGY

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Organelle/Component	Function	Relevance to Pharmaceutical Biotechnology	Example Applications
Lysosomes	Waste breakdown and recycling	Target for lysosomal storage disease therapies	- Enzyme replacement therapy for Gaucher disease - Targeting lysosomal pathways for cancer drug delivery
Plasma Membrane	Regulates cell entry/exit of substances	Targeted drug delivery and cell signaling pathways	- Liposomal drug delivery systems (e.g., Doxil for cancer treatment) - Monoclonal antibodies targeting surface receptors (e.g., Herceptin for HER2 breast cancer)
Vesicles	Transport of materials within cells	Delivery of biopharmaceuticals or therapeutic molecules	- Exosome-based drug delivery systems - Nanoparticles for targeted drug delivery in cancer therapy
RNA (mRNA, rRNA, tRNA, miRNA)	Various roles in protein synthesis and gene regulation	Used in gene expression control, therapeutic RNA molecules, mRNA vaccines	- mRNA vaccines (e.g., Pfizer and Moderna COVID-19 vaccines) - siRNA therapies for gene silencing (e.g., Onpattro for amyloidosis)

THE END ...

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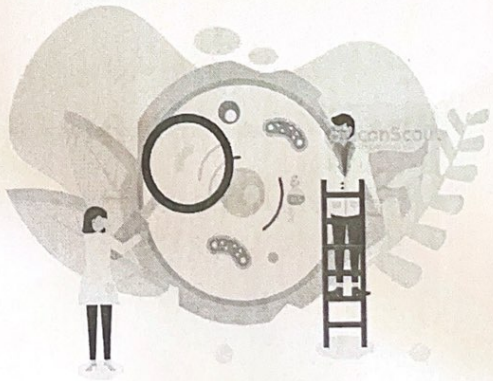
Any questions? ☺



Done by Sara Jammain

Artery academy

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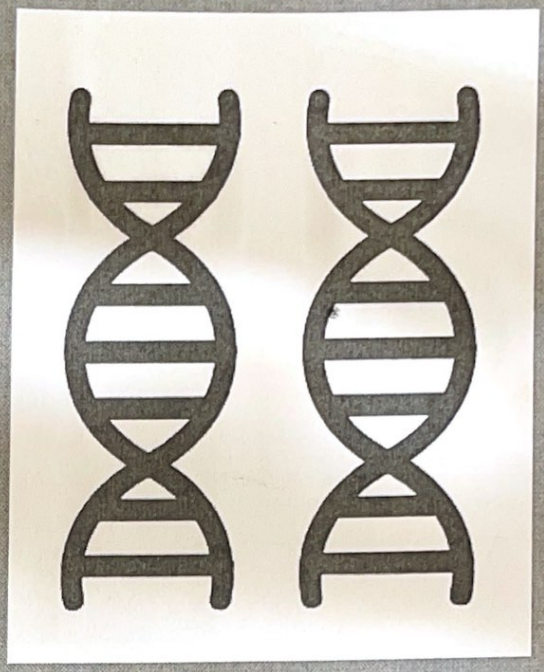
MOLECULAR BIOLOGY – DNA REPLICATION AND REPAIR



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AUTHORS DISCLOSURE

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The authors have no actual or potential financial conflicts of interest associated with this presentation.

This presentation is directed to the students registered on the 'Pharmaceutical Biotechnology' course at the School of Pharmaceutical Sciences, the Hashemite University, Jordan. Academic year 2024/2025.

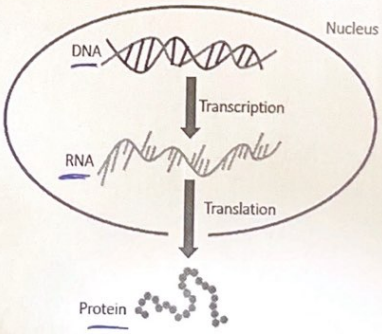
LEARNING OBJECTIVES

- By the end of this presentation, students will be able to:

1. Describe the mechanism of DNA replication.
2. Identify the key enzymes involved in the replication process,
3. Explain the directionality of DNA synthesis and the leading and lagging strand synthesis.
4. Identify the various sources of DNA damage (e.g., UV light, chemicals, replication errors) and the consequences of uncorrected DNA damage.
5. Explain how defects in DNA repair pathways can lead to mutations and contribute to diseases like cancer.
6. Identify how DNA replication and repair mechanisms can serve as targets for therapeutic drugs, such as anticancer agents

Deoxyribonucleic Acid (DNA)

- Nucleic acids are the biomolecules responsible for the transmission of gene information in cells. There are two types of nucleic acids: deoxyribonucleic acid (DNA) and ribonucleic acid (RNA)
- Definition of DNA: The molecule that carries genetic instructions for the development, functioning, and reproduction of all living organisms.
- Central Dogma: Flow of genetic information from DNA to RNA to protein. It was postulated by Francis Crick in 1958 and revised by the same author in 1970.



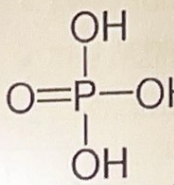
← هل وظيفته يحمل معلومات (جينات) دائماً تترجم إلى بروتينات ؟ غالباً اه لكن مو دائماً ممكن يوقف لعند RNA وممكن يكمل للحصول على بروتين

Deoxyribonucleic Acid (DNA)

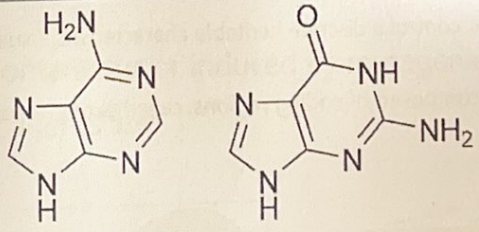
- Deoxyribonucleic acid (DNA) forms an unbranched polymer composed of monomers, called deoxyribonucleotides, connected by phosphodiester linkages.

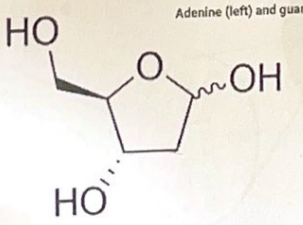
- Deoxyribonucleotides (dNTPs) are composed of:
  1. Phosphate group (H_3PO_4).
  2. Deoxyribose ($C_5H_{10}O_4$): is a pentose derived from ribose.
  3. Nitrogenous base: these are heterocyclic and aromatic compounds with two or more nitrogen atoms and classified into two groups: purine and pyrimidine bases. The purine bases, derived from purine, are composed of two rings joined together: Adenine (A) and Guanine (G). Pyrimidine bases, derived from pyrimidine and consisting of a single ring, are Thymine (T) and Cytosine (C).

dNTPs COMPONENTS

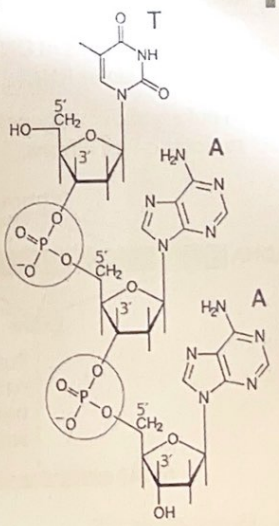


Phosphate group


Adenine (left) and guanine (right)



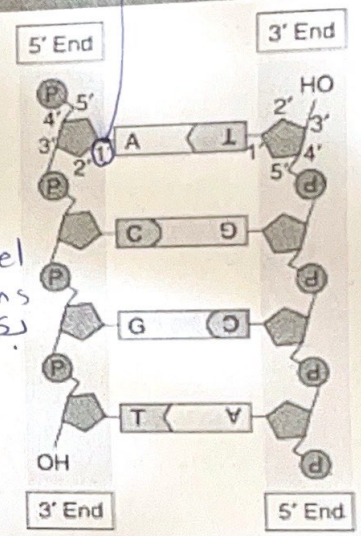
Deoxyribose



Phosphodiester bond

nitrogens مع Sugar ② الترقيم ببدش من (1) مكان اللقاء
base riboses
بعدين بنصير نعد clockwise لحد (5) ، هاد الترقيم مهم
3 and 5 prime end of DNA strand عشان نعرف وين

DNA PRIMARY STRUCTURE



- The primary structure of DNA consists of the sequence of deoxyribonucleotides. Since the first deoxyribonucleotide has a free 5′ carbon and the next deoxyribonucleotide has a free 3′ carbon, the sequence of nucleotides is ordered from 5′ to 3′ (5′ → 3′).

جزء من DNA ←

5'ATGACGTGGGGA3'
3'TACTGCACCCCT5'

anti parallel chains بكونو شابكين بالعكس

كل حرف يمثل nucleotide

- On the other hand, the number of base pairs (bp) that make the DNA sequence is used to indicate the size of the DNA

و بالتعريف nitrogenus base

(polymers) two chains ① هيا DNA عبارة عن

لازم يشبكو مع بعض من نقطة معينة attatchment point

و هي ال nitrogenous bases

transcription لازم نميزهم لانه ② (بالتابع) 3 and 5 prime end

3' ← 5' غالباً بصير باتجاه واحد من

هيك الإنزيمات مصممة بالجسم