Genetics
& Cell biology

◉ Sheet ○ Slides

Number

1

Done by:

Zahra Muneer

Corrected by:

Leena Al Nsour

Doctor

Dr. Mamoun

Molecular Biology is the future of medicine. It is the branch of biology that deals with the structure and function of the macromolecules (e.g. proteins and nucleic acids) which are essential for life.

Most diagnosis of certain diseases are now based on molecular biology. Nowadays, most of the drugs are prescribed depending on the molecular profile of the disease and the patient's genetic makeup. This is called *personalized medicine.* For example, for a patient with breast cancer, we need to preform the **molecular profiling test** in order to know which drugs to give her. This test (depending on the type cancer) might include Her2 receptor, estrogen receptors and many more.

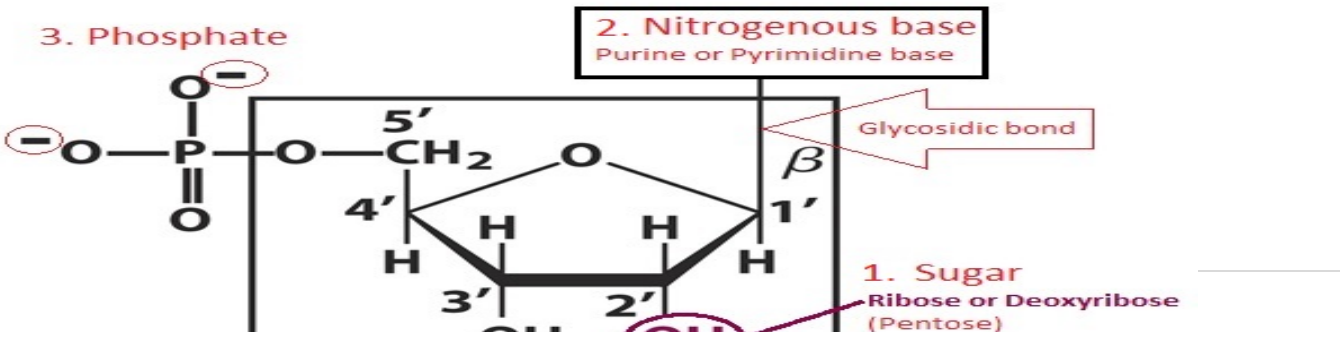
DNA is now used in computers as a part of the wires. This is due to the characteristics of DNA. Firstly, it is *flexible and versatile*. This enables the DNA, as a strand, to transmit electrons; electros can move along the strand of the DNA. Secondly, DNA is a *decision maker*; it can determine whether the signal should go left or right. Thirdly, gene activity depends on the amount of molecules that the gene codes for. For example, if the molecules that the gene codes for are abundant, he gene stops the transcription process. This shows how *sensitive* the DNA is; hence, making the DNA a good *sensor*.

Nucleic Acids

- DNA
- RNA

- **The primary structure** of nucleic acids is the order of bases in the polynucleotide sequence. Example: A-C-G-A-C …etc.
- **The secondary structure** is the three-dimensional conformation of the backbone. (Double helix)
- **The tertiary structure** is specifically the supercoiling of the molecule. It is how the DNA is packed and inserted in the nucleus. (We'll talk about them later)

The monomer of the nucleic acid is a nucleotide, and has 3 main components:



1. Sugar

- It is a pentose that is linked to different molecules as follows:

A. Carbon number 2

➔ If it is attached to a hydroxyl group (OH), it is a **Ribose** (like the one shown in the figure above).

➔ If the hydroxyl group (OH) is absent, it is a **Deoxyribose.**

Thus, what attaches to carbon 2 determines if the sugar is a ribose or a deoxyribose. This gives us two types of nucleic acids: Ribonucleic acids (RNA) and Deoxyribonucleic acid (DNA).

B. Carbon number 1 forms a *glycosidic bond*[1] with:

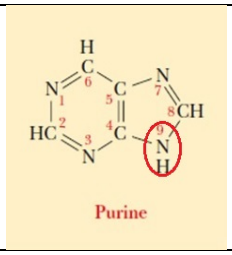
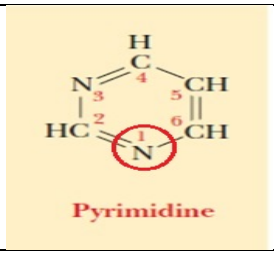
➔ Nitrogen #9 of purine

➔ Nitrogen #1 of Pyrimidine

C. Carbon number 5 attaches to phosphate.

2. Nucleotide Bases

We have two classed of bases: Purines and Pyrimidines.

Nucleotide base		
# of rings	Two rings	Single ring
Attachment to carbnon number 1	With nitrogen number **9**	With nitrogen number **1** (remember pyrimidine has 1 ring so it doesn't have 9 Nitrogens)
Example	Adenine Guanine	Cytosine (DNA & RNA) Thymine (DNA some RNA) Uracil (only RNA)

- Note that the long name refers to the smaller structure while the short name refers to the larger one.
- There are A LOT of different purines and pyrimidine, but only the mentioned above exist in our DNA and RNA. (Just like how there are MANY amino acids but only 20 exist in our body.)

[1] *Glycosidic bond* is a bond that joins the anomeric carbon of the sugar to another group (In this case it is either purine or pyrimidine).

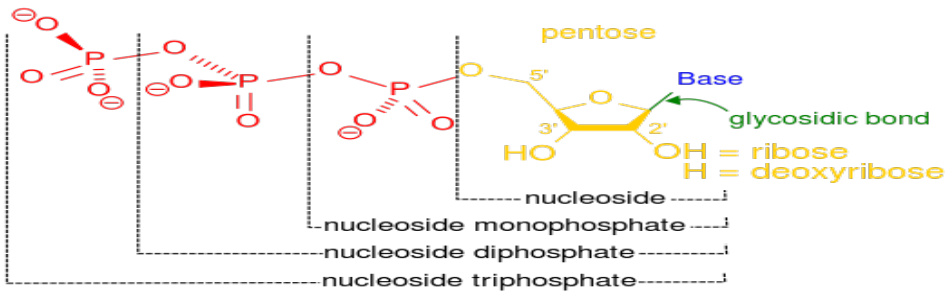
3. **Phosphate**

Phosphates are negatively charged. The presence of phosphate makes the ribonucleic acid an ACID. This is due to the presence of lots of phosphate molecules (negative part) along the ribonucleic acid strand. This has very important implications in the structure and even the uses and techniques that will be discussed later.

Now, if these negative charges left as they are, the molecule won't be stable due to the high repulsion forces between the negative charges. Thus, we need to add positively charged ions (Na+ or Mg2+) and peptides to stabilize the DNA since they can mask the negative charges and neutralize them.

Throughout the upcoming lectures, we'll be talking about eukaryotic system and prokaryotic system. It is important to know the prokaryotic one because we know more about it (simple to understand) than the eukaryotic one (more complex).

Nomenclature:



1. If the phosphate doesn't exist, we call it a *nucleoside* (sugar and base only).
2. If phosphate is present it is called a *nucleotide*.

However, we can't determine how many phosphate groups the molecule has just from the word "Nucleotide" alone. So, if we want to indicate the number of phosphate groups we simply use: "nucleoside" + (greek prefix for the number)phosphate.

Examples: if we have one phosphate group: nucleoside **mono**phosphate (which is a nucleotide with 1 phosphate)

If we have two ➔ nucleoside diphosphate… etc.

3. The letter "d" can be added to indicate a deoxyribonucleotide residue, examples:

With deoxy→ DNA (deoxyadenosine) ➔ dA or dAMP

Without deoxy → RNA (adenosine) ➔ A or AMP

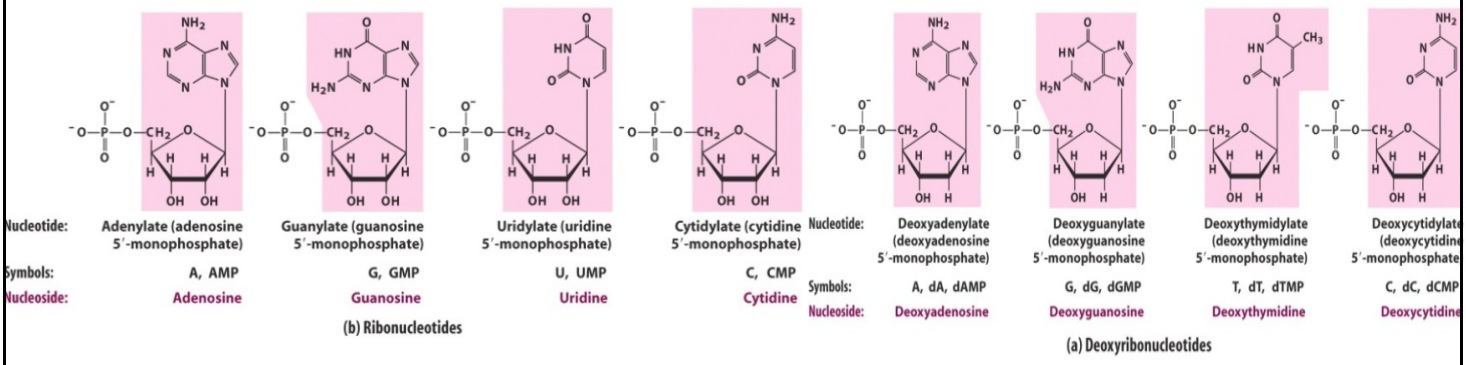
Thus, the deoxy analogue of a ribooligonucleotide would be: d(GACAT), or dG, dA,dC,dA,dT.

Please note that we are required to know the names of those nucleotides, so we should be able to say the following about each example:

A. Adenylate ➔ a nucleotide that contains adenine, but we can't determine the number of phosphate groups.

B. Adenosine monophosphate: We know that it is a nucleotide with 1 phosphate.

You need to know *HOW* to name the structure, distinguish it, but not how to draw it. You should be able to differentiate between a purine (2 rings) and a pyrimidine (1 ring), but you don't have to differentiate between adenine and guanine since both are purines.

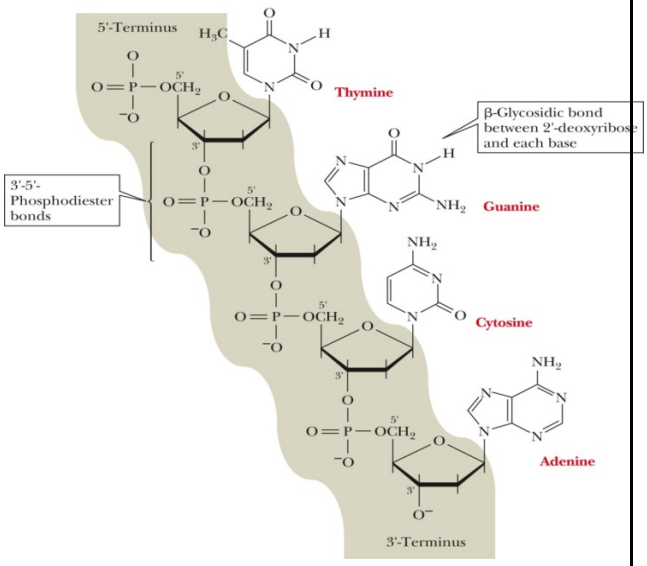


Nucleic acid polymer

- Nucleotides are connected to each other.
- Directionality of synthesis:

5' (5 prime end) → 3' (3 prime end)

- 5' refers to carbon number 5 of the sugar, and 3' refers to carbon number 3 of the sugar.
- Carbon 5 is attached to a phosphate group, so we can't add anything to it. Carbon 3, on the other hand, is free, so we add more nucleotides to the 3' side.



Example: What can you till about: d(**TGCA**)?

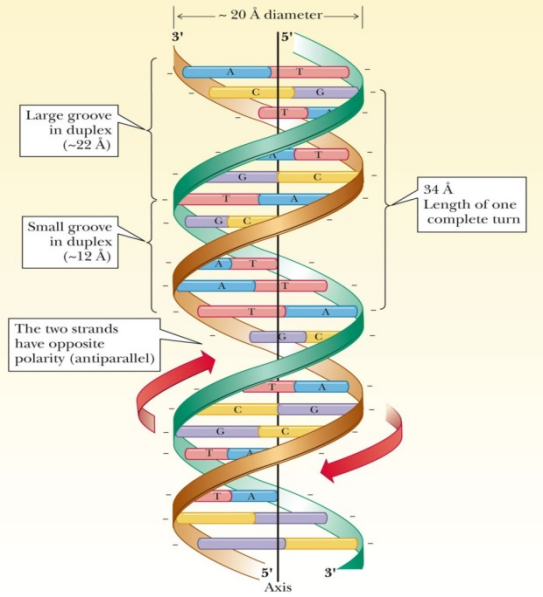
- d→ for deoxy (DNA)
- T is on the 5' end and A on the 3' end. Thus, the first base is thyamine and the last is Adenine.

Note: General steps for figuring the name of a given structure on the exam:

1. Look for the phosphate ➔ nucleotide or nucleoside.
2. Sugar ➔ hydroxyl group: (ribose), no hydroxyl group (deoxyribose)
3. Pyrimidine or purine.

[1]The names adenylate, guanylate… etc. don't indicate anything in particular.

DNA Structure



- Discovered by Watson and Crick.
- Specific base-pairing:
  - A - T; C - G; Pur - Pyr.
  - Purines always pair with pyrimidines.
  - Guanine and cytosine always pair together forming 3 hydrogen bonds.
  - Adenine and Thymine always pair together forming 2 hydrogen bonds.
- Complementary: important for the concept of hybridization.
- A double helix:

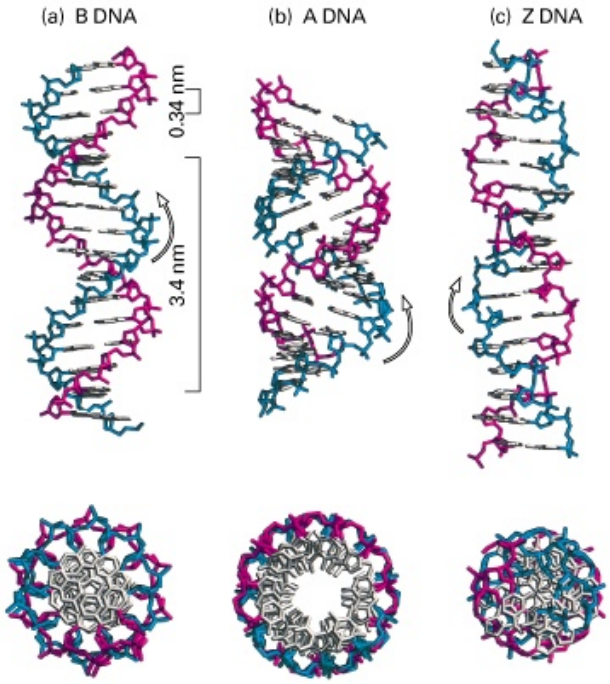
- Made of two helical strands.

- Backbone vs. side chains
  - Backbone: phosphate and sugar alternating; Phosphate, sugar, phosphate…etc.
  - Side chains: nitrogenous bases that form hydrogen bonds between them. Hydrogen bonds are weak, but due to the high number of hydrogen bonds, they are stable and cannot be separated or denatured easily. Also, The nitrogenous bases are perpendicular to the backbone.
- Antiparallel
  - One strand starts with 5’ on top and with 3’ on the bottom. The second one, which is complementary to the first, has the 3’ on top and the 5’ on the bottom.
- Stable because of the presence of high amount of hydrogen bonds.
- Flexible: can easily bend and rotate without breaking.
- Stability vs. flexibility
- Groovings
  - Unlike collagen, which can bend perfectly, the DNA is a helical structure and helical structures don’t rotate perfectly. Thus, they have major and minor grooves, and they are always opposite to each other: The major groove faces the minor from the opposite side. If we go vertically, the order will be: major, minor, major..etc. Meaning, they alternate vertically.
  - The grooves are important for the DNA- protein interactions. The proteins prefer to interact with the major grooves of DNA because they can fit there.

DNA forms:

DNA has different forms even in our cells

- **B-DNA**
  - **The principal form of DNA**. (The DNA explained by Watson and Crick)
  - Right-handed: ALWAYS going upward to the *right*.
  - 10 bp[1]/turn:
  - Base pairs are perpendicular.
- **A-DNA**
  - 11 bp/turn (compressed)
  - Wider than B-DNA: Due to the presence of more base pairs.
  - Base pairs lie at an angle
  - Right-handed.



- **Z-DNA**
  - Left-handed: ALWAYS going upward to the *left*.
  - Occurs when we have **alternating purine–pyrimidine** and sequences with methylated C.
  - Narrower/ slimmer than B-DNA

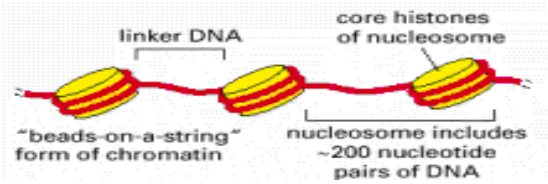
The bacterial DNA is small; therefore, it doesn't need any help to swim inside the bacterial cytosol. The eukaryotic DNA, on the other hand, is complex and large; therefore, it needs to be packed in a small nucleus. How do we pack the DNA?

- In eukaryotes, DNA is coiled to package the large DNA and regulate gene activity. Thus, Eukaryotic DNA is complexed with a number of proteins, principally histones[2], which are surrounded by DNA. Both the DNA and the proteins form what we call a chromatin.
- **Chromatin** = DNA molecule + proteins (histones or other proteins)**.**

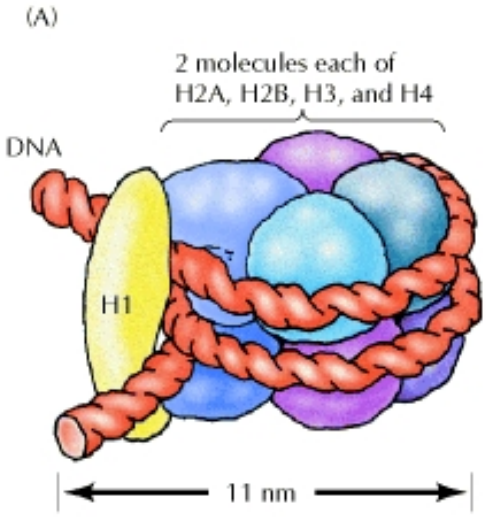
[1] bp = base pair; you need to know this abbreviation for the exam.

[2] Histones are positively charged proteins found in the nucleus, and they help in packing the DNA into the nucleus.

The DNA is packed in a structure known as a nucleosome.



- A **nucleosome** consists of DNA wrapped around a histone core (***DNA+ DNA linker+ histones***). This structure is also known as beads on a string, or a rope with nodes.
  - ***Linker DNA***: a free DNA)/spacer region that connects the octamer-DNA complexes to each other.)
  - The ***histone*** protein core is an *octamer* composed of: two molecules of histones each consists of H2A, H2B, H3, and H4.
  - **H1** acts as a lock that locks the DNA around the bead (as shown in the figure). Thus, it is bound to the octamer and wraps the DNA. This structure alone, without the linker DNA, is known as *chromatosome*.
  - Histones are **positively charged** while the DNA is negatively charged. Meaning, it facilitates DNA interaction and charge neutralization.



Light absorbance of nucleic acids:

DNA can absorb light, but we can't see the DNA because pyrimidines and purines absorb light at the UV range.
If we extract the DNA, however, we can see it as white strands. Why?
Because it's stabilized by Mg and Na ions (salts), and those salts are what gives the DNA its color.

We can calculate the concentration of DNA by measuring how much UV light it absorbs using *spectrophotometer*. The absorbance of nucleic acids at 260 nm ($A260^1$) is constant:

- **dsDNA: 1.0 unit = 50 ug/ml absorb 1 unit of light**
- **ssDNA: 1.0 unit = 30 ug/ml absorb 1 unit of light**
- **ssRNA: 1.0 unit =40 ug/ml absorb 1 unit of light**

Example: If we have a solution of dsDNA, with a light absorption of 0.5 units. What is the concentration of the DNA sample? 0.5 * 50 = 25ml

If we dilute this DNA to 1:10 and the absorbed light is at 0.5 units, what is the concentration of this solution? 10* 0.5 *50 = 250ml

[1] *The peak absorbance is at 260 nm wavelength*

1. *Which molecule absorbs more light?*

ssDNA (little of ssDNA absorb more light). This is because little part of DNA absorbs more light. The light absorbtion is done by the rings structure; the nitrogenous bases. Thus, when the DNA is in its double strand form, the bases are hidden → less absorption of light, but when exposes → more absorption.

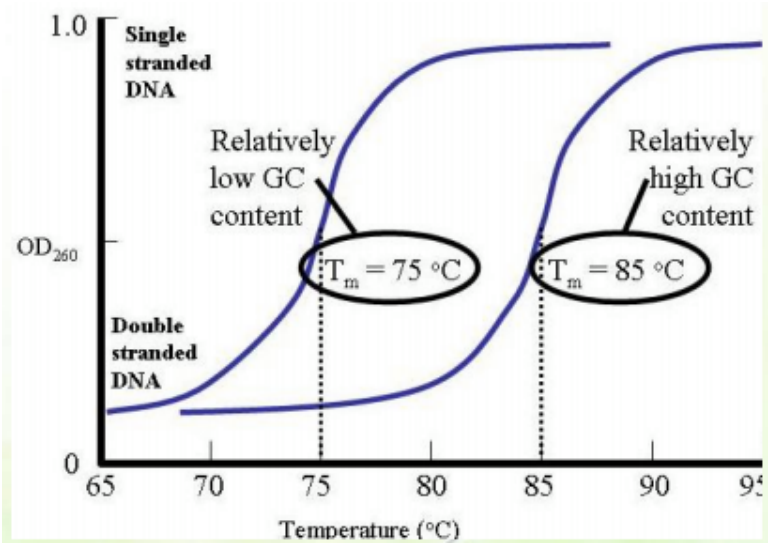
2. How can we separate or denaturant the two stranded (denature)?

By Heat (boiling the sample)

The transition temperature, also known as Melting point (Tm), is the temperature where 50% of DNA is denatured, and 50% of it is not. It is the middle point between the ssDNA and dsDNA. Different DNA fragments have different values for Tm.

Tm depends on:

- ↑G-C pairs→ ↑hydrogen bonds →↑Tm.
- pH.
- Length: longer DNA; ↑Tm because of the presence of high number of hydrogen bonds.
- ↑Salt and ion concentration (more stable)→ ↑Tm
- Destabilizing agents (alkaline solutions, formamide, urea) they break the hydrogen bonds → ↓Tm



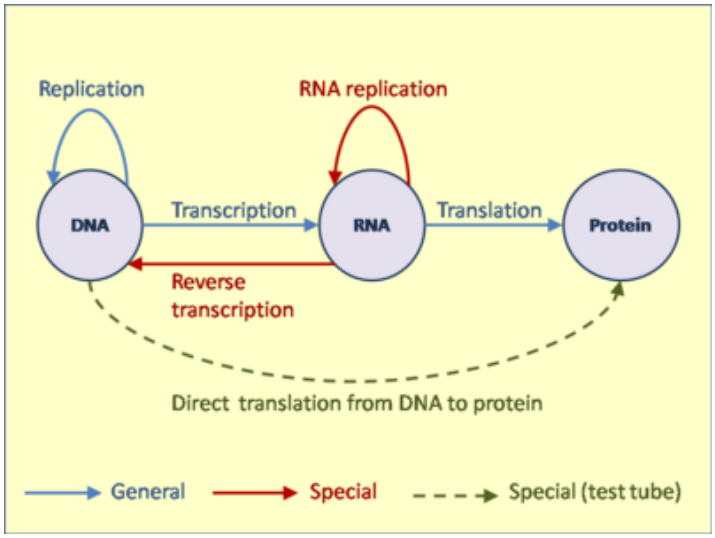
Central Dogma of Biology:

DNA is the source by which we can make RNA → RNA makes ➔ protein

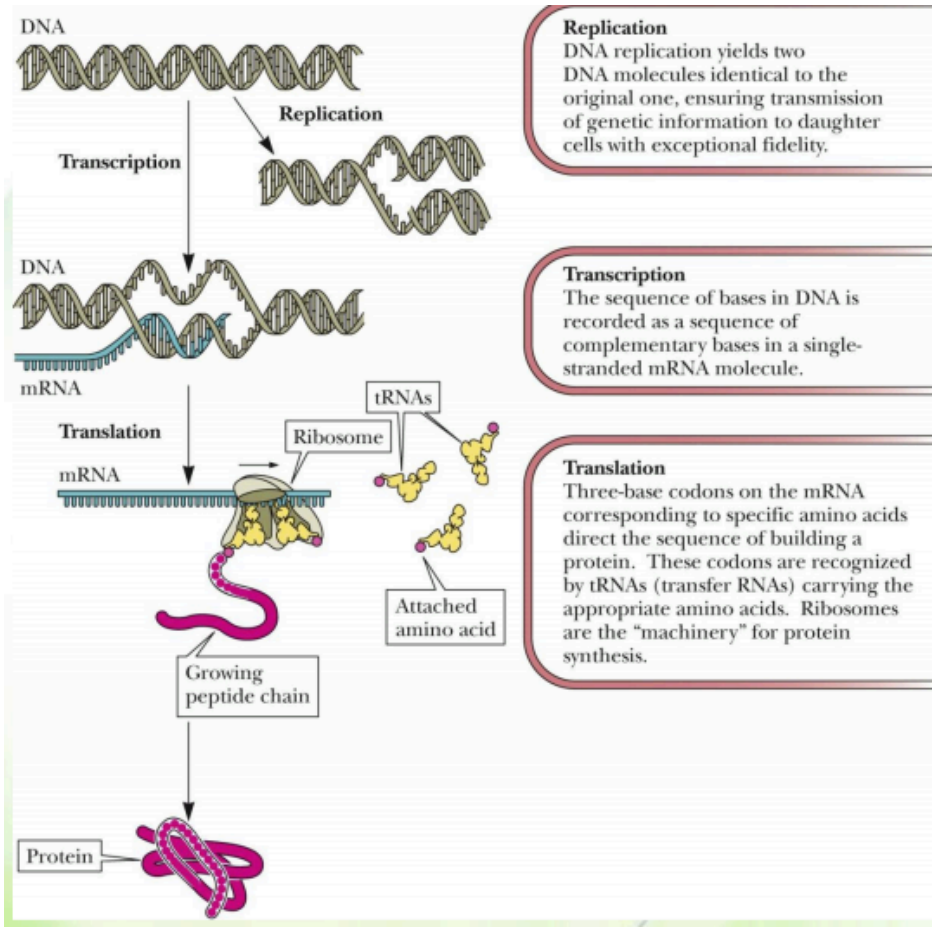
RNA → reverse transcription → DNA

RNA can make a copy of itself

DNA can make a copy of itself

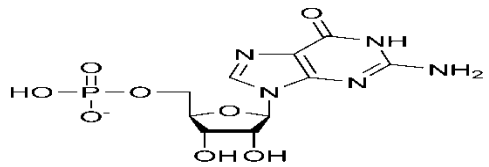


Thus→ Genetic information must be preserved via DNA replication. Information must be translated into action makers (proteins) via Transcription and translation. RNA Sequence is dictated by DNA sequence.

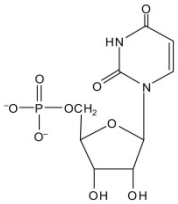


Questions that might come up on the exam:

1. Name the following structures:



   A. Deoxyriboguanosine
   B. Deoxyriboguanosine monophosphate
   C. Deoxyadenoasine monophosphate
   D. Deoxyuracil monophosphate
   E. Guanosine monophosphate
   F. Thymine monophosphate



   A. Guanosine monophosphate
   B. Deoxycytosine monophosphate
   C. Deoxyuracil monophosphate
   D. None of the above

2. What is the concentration of a double stranded DNA sample diluted at 1:10 and the A260 is 0.1?
3. What is the concentration of a double stranded DNA sample that is denatured into ssDNA diluted at 1:10 and the A260 is 0.5?
4. The glycosidic bond that exists in nucleosides is formed between:
   a. Number 3 carbon of sugar and N9 of adenine
   b. Number 1 carbon of sugar and N1 of guanine
   c. Number 5 carbon of sugar and N1 of cytosine
   d. Number 1 carbon of sugar and of N9 guanine
   e. Number 5 carbon of sugar and of N1 thymine

Answers:

1. E, D. 2. DNA concentration **= 0.1 x 10 x 50 µg/ml = 50 µg /ml.** 3. DNA concentration= **0.5 x 10 x 30 µg/ml = 150 µg/ml.** 4. D (**C 1 and N9 of a purine or N1 of pyrmidine)**