بسم الله الرحمن الرحيم

King Saud University
College of Science
Department of Biochemistry

General Biochemistry-I (BCH 302)

Chapter 5
Nucleic Acids

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http://faculty.ksu.edu.sa/75112

Topic	No of Weeks	Lectures
• **Nucleic acids:** **Structure of a nucleotide,** - types of nitrogen bases, - structure of nucleosides - nomenclature of nucleosides and nucleotides, - phosphodiester bonds, - properties of nitrogen bases, - Roles of functional nucleotides	0.66	30-31
- Nucleotides derivatives (NAD, NADP, FAD, FMN, c AMP, c GMP)	0.66	32-33
• **Over view of DNA and RNA.** - DNA primary structure: Description and orientation of bonds. - RNA: Types, role and structure. - Secondary structure of DNA (double helix) - Double helix properties, base pairing, reading, stabilizing forces. - Tertiary structure of DNA (relaxed, coiled and associated proteins; histones, protamines). - DNA denaturation : significance and factors **Genetic code, exon and introns: Gene, genome and chromosome.** **Introduction to replication, transcription and translation and important enzymes**	1.66	34-38

Nucleic acids

Nucleic acids are macromolecules, like proteins, carbohydrates and lipids.
It is essential for all known forms of life. They are found in abundance in all living things, where they function in encoding, transmitting and expressing genetic information.
Nucleic acids are divided into:

- DNA (deoxyribonucleic acid) and
- RNA (ribonucleic acid)

Nucleic acids consist of polymers nucleotides monomers.
Each nucleotide has three components:

- nitrogenous base,
- 5-carbon sugar, and
- phosphate group.

Nucleic acids *carry the genetic and hereditary information* of the cell and all the necessary information needed for cell to perform all the life processes.
The ability to store and transmit genetic information from one generation to the next is a fundamental condition for life.

Structure of Nucleotides

Nucleotides are the building blocks of Nucleic Acids

- Nucleotides have three characteristic components:
  - a nitrogenous base,
  - a pentose, and
  - a phosphate.
- The molecule without the phosphate group is called a nucleoside.

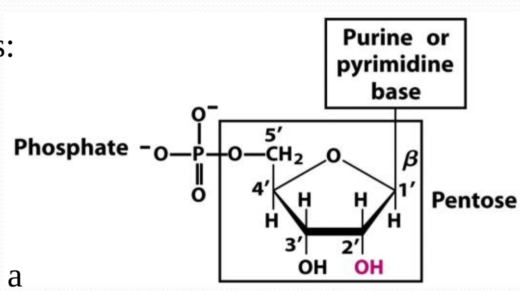

Figure 8-1a
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The nitrogenous bases

There two categories of nitrogenous bases in the nucleic acids:

- Purines (two ring structure and contain Adenine (A) and Guanine (G)
- Pyrimidines (one ring and contain Thymine (T), Cytosine (C) and Uracil (U)

- **DNA** contains Adenine, Guanine, **Thymine** and Cytosine
- **RNA** contains Adenine, Guanine, **Uracil** and Cytosine

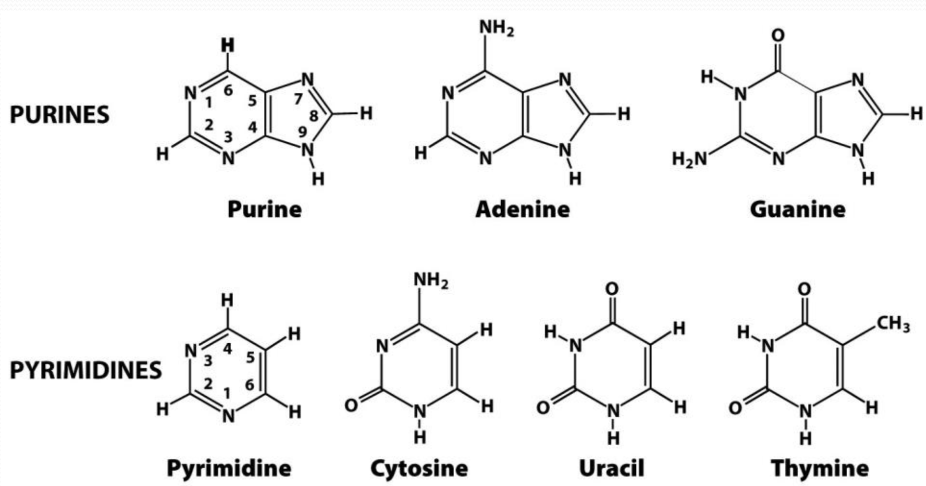

Figure 32.5
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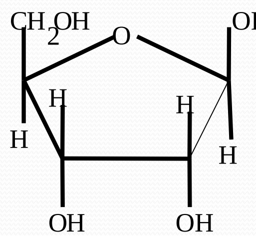
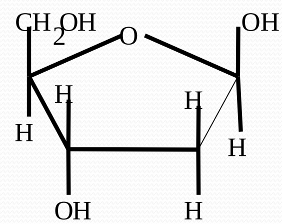
The pentose sugar

Nucleic acids have two kinds of pentoses.

- The RNA contains ribose
- The DNA contains deoxyribose (ribose missing oxygen at position C2).



Ribose, in RNA



Deoxyribose, in DNA

Nomenclature

nucleoside = sugar +base
nucleotide = sugar + base + phosphate

TABLE 8–1 Nucleotide and Nucleic Acid Nomenclature

Base	Nucleoside	Nucleotide	Nucleic acid
Purines			
Adenine	Adenosine	Adenylate	RNA
	Deoxyadenosine	Deoxyadenylate	DNA
Guanine	Guanosine	Guanylate	RNA
	Deoxyguanosine	Deoxyguanylate	DNA
Pyrimidines			
Cytosine	Cytidine	Cytidylate	RNA
	Deoxycytidine	Deoxycytidylate	DNA
Thymine	Thymidine or deoxythymidine	Thymidylate or deoxythymidylate	DNA
Uracil	Uridine	Uridylate	RNA

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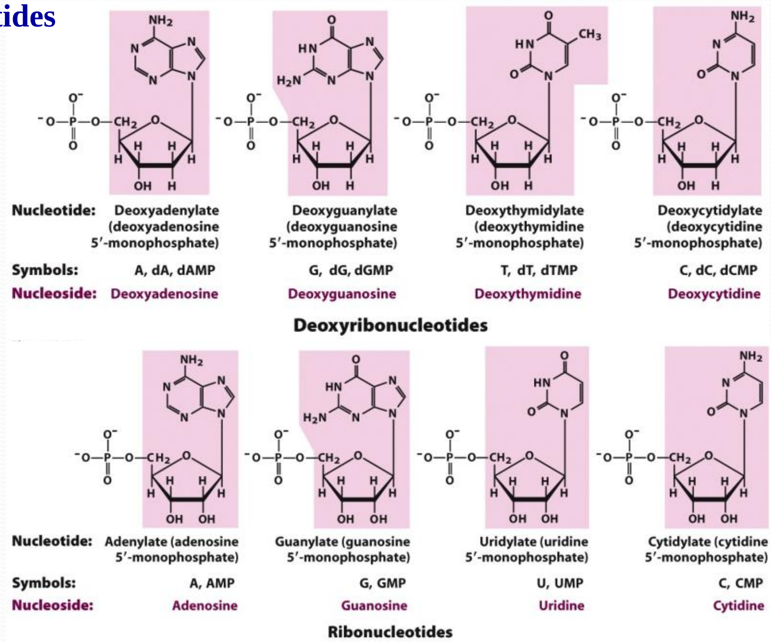
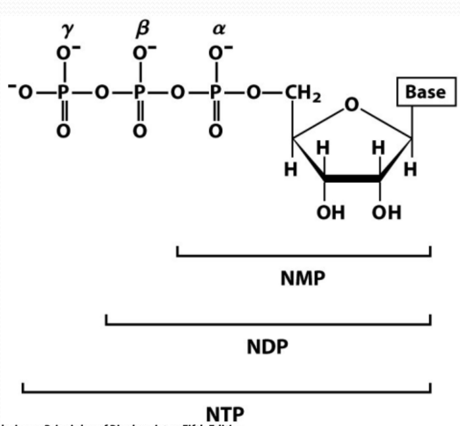
Nucleotides



Abbreviations of nucleoside phosphates



Abbreviations of ribonucleoside 5′-phosphates

Base	Mono-	Di-	Tri-
Adenine	AMP	ADP	ATP
Guanine	GMP	GDP	GTP
Cytosine	CMP	CDP	CTP
Uracil	UMP	UDP	UTP

Abbreviations of deoxyribonucleoside 5′-phosphates

Base	Mono-	Di-	Tri-
Adenine	dAMP	dADP	dATP
Guanine	dGMP	dGDP	dGTP
Cytosine	dCMP	dCDP	dCTP
Thymine	dTMP	dTDP	dTTP

Roles of functional nucleotides

Nucleotides have a variety of roles in cellular metabolism.

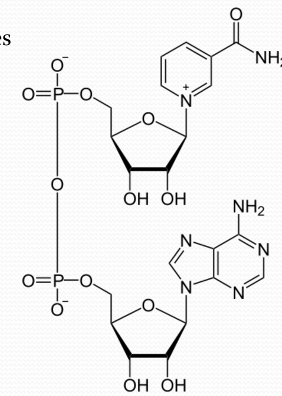
- They are the *constituents of DNA and RNA*.
- The structure of every protein, and ultimately of every biomolecule and cellular component, is a product of *informational codes* programmed into the nucleotide sequence of a cell's nucleic acids.
- They are the energy currency in metabolic transactions (mostly **ATP**)
- They are essential chemical links in the *response of cells to hormones* and other *extracellular stimuli*, and
- They are structural components of an array of *enzyme cofactors* and metabolic intermediates.

Nucleotides derivatives

- **NAD^+/ NADH**
- **$NADP^+$/ NADPH**
- **FAD/ $FADH_2$**
- **FMN/ $FMNH_2$**
- **c AMP,**
- **c GMP**

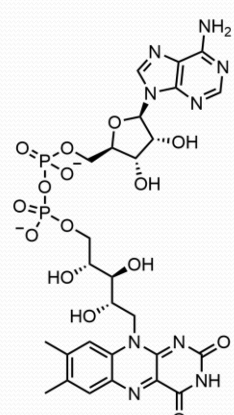
Nucleotides derivatives; NAD$^+$ & NADP$^+$

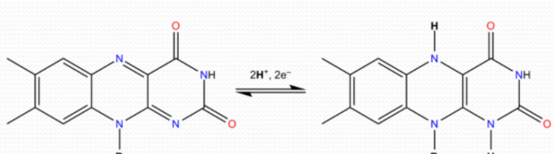
- Nicotinamide adenine dinucleotide (NAD$^+$) has many roles in the cell:
  - It is a coenzyme for many oxidoreductases. So, it carries electrons from one reactant to another.
  - It acts as a precursor of the second messenger molecule cyclic ADP-ribose,
  - It acts as a substrate for bacterial ligase.
- It consists of two nucleotides joined through their phosphate groups.
- One nucleotide contains an adenine base and the other nicotinamide (vitamin B3).
- NADH is the reduced form of NAD$^+$.
- NADP$^+$ has many functions in cell (search for its functions)



Nucleotides derivatives; FAD & FMN

- Flavin adenine dinucleotide (FAD) is a redox cofactor, for many oxidoreductases.
- FAD can be converted between three redox states by accepting or donating electrons.
- It is composed of adenine and flavin (Vitamin B2)
- Flavin mononucleotide is other cofactor as FAD

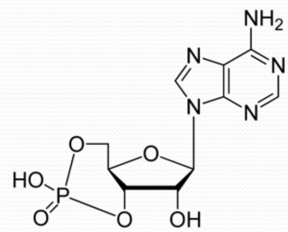




FAD **$FADH_2$**

Nucleotides derivatives; cAMP, cGMP

- Cyclic adenosine monophosphate (cAMP) is a second messenger important in many biological processes.
- It is a derivative of adenosine triphosphate (ATP)
- **Function**:
  - It is used for intracellular signal transduction in many different organisms such as transferring the effects of hormones that cannot pass through the plasma membrane into the inside of the cell (like glucagon and adrenaline).
  - It is also involved in the activation of protein kinases.
  - It binds to and regulates the function of some ion channels.



DNA

Primary structure:

Description of Phosphodiester Bond

DNA primary structure

Phosphodiester Bonds Link Successive Nucleotides in Nucleic Acids

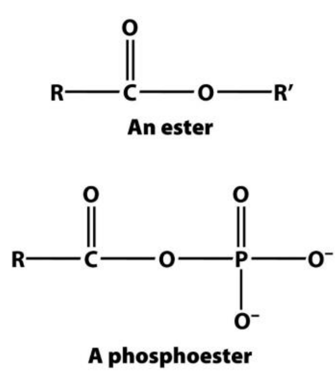
- The successive nucleotides of both DNA and RNA are ***covalently*** linked through phosphate-group “bridges,” in which the 5-phosphate group of one nucleotide unit is joined to the 3-hydroxyl group of the next nucleotide, creating a **phosphodiester linkage.**
- The backbones of both DNA and RNA are:
  - - *hydrophilic* due to the –OH, NH, phosphate and C=O groups
  - - *negatively charged* in neutral pH due to the phosphate groups.

DNA primary structure: (cont.)

Phosphodiester linkage

The DNA or RNA strands have two ends:

- 5- phosphate end
- 3’ OH end



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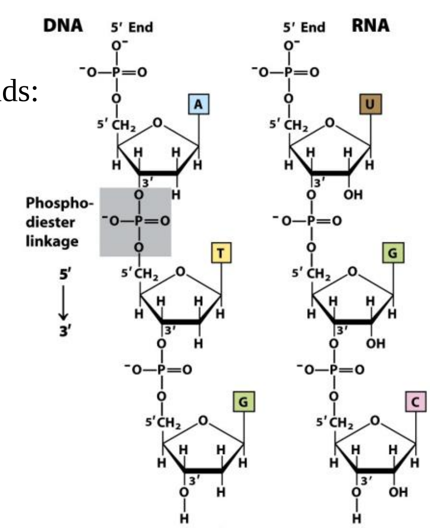

Figure 8-7
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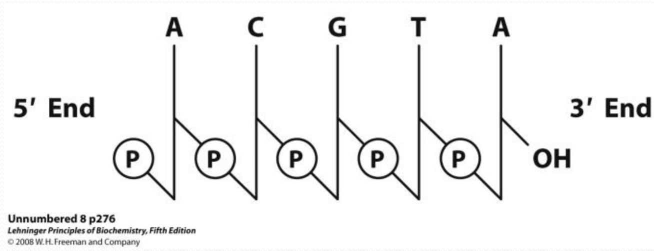
DNA primary structure: (cont.)

The covalent backbones of nucleic acids consist of alternating phosphate and pentose residues, and the nitrogenous bases may be regarded as side groups joined to the backbone at regular intervals.



Secondary structure of DNA (double helix)

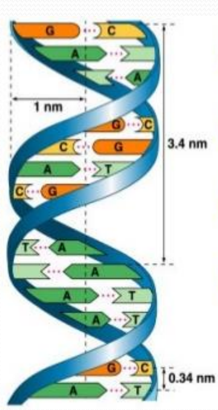
- DNA is the macromolecular that carries the genetic instructions for all biological processes.
- Most DNA molecules consist of *two strands* of deoxyribonucleotides forming a *double helix*.
- These two strands wound around the same axis in a *right handed manner*
- The bases forming the DNA are A, G, T and C
- All nucleotides in each strand are bound by phosphodiester bond (type of covalent bond).
- The two strands bind together by hydrogen bonds.
  - Adenine (A) in one strand binds Thymine (T) in the other by two hydrogen bonds.
  - Guanine (G) in one strand binds Cytosin (C) in the other by three hydrogen bonds.
- The two strands run in an antiparallel manner.

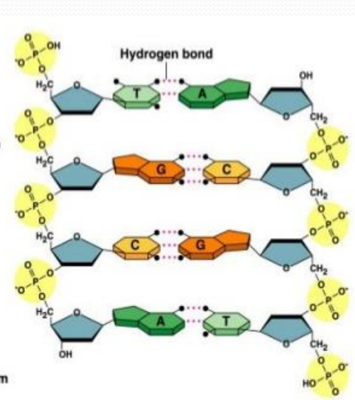
DNA secondary structure: (cont.)

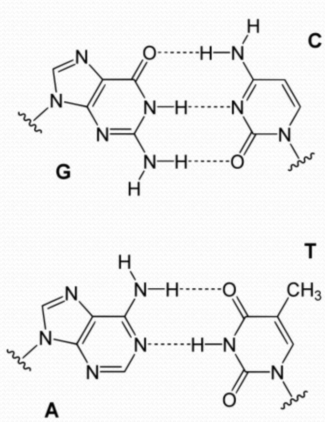
Double helix properties, base pairing, stabilizing forces

- The DNA double helix, or duplex, is held together by two forces,
  - - hydrogen bonding between complementary base pairs and
  - - base-stacking interactions.
- The complementarity between the two strands of DNA is attributed to the hydrogen bonding between base pairs.
- The base-stacking interactions, which are largely nonspecific with respect to the identity of the stacked bases, make the major contribution to the stability of the double helix.

DNA secondary structure: (cont.)







In DNA
The number of purines (A+G) = The number of pyrimidines (T+C)

Chargaff's rule
- The number of Guanine (G) = The number of Cytosine (C)
- The number of Adenine (A) = The number of Thymine (T)

So, We can calculate the ratio of three bases depending on the known ratio of any one base

Tertiary structure of DNA

The detailed structure of the double helix, the shapes of DNA, the major and minor groove triplexes and other forms

- Tertiary structure refers to the locations of the atoms in three-dimensional space, taking into consideration geometrical and steric constraints.
- The two antiparallel polynucleotide chains of double-helical DNA are **complementary** to each other.
  - Wherever adenine occurs in one chain, thymine is found in the other;
  - Wherever guanine occurs in one chain, cytosine is found in the other.
- The hydrophilic backbones of alternating deoxyribose and phosphate groups are on the *outside of the double helix*, facing the surrounding water.
- The furanose ring of each deoxyribose is in the C-2 endo conformation.
- The purine and pyrimidine bases of both strands are *stacked inside the double helix*, with their hydrophobic and nearly planar ring structures very close together and perpendicular to the long axis.
- The two strands creates a **major groove** and **minor groove**.

DNA

Base pairing (A ≡ G, T = C)

Anti-parallel strands (5’→3’ , 3’→ 5’)

Major groove is 22A wide.

Minor groove is 13A wide

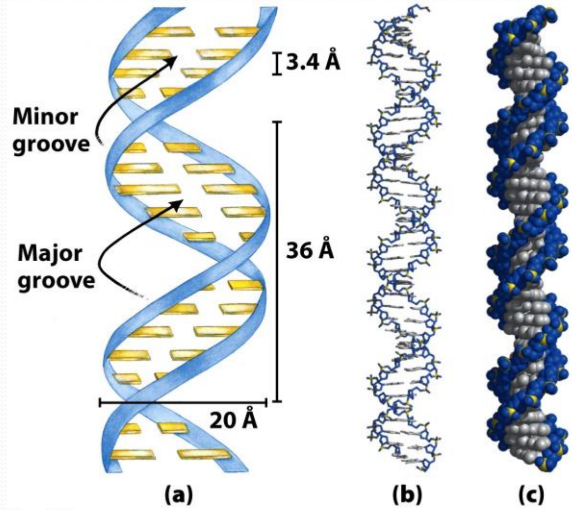


Figure 8-13
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Tertiary structure of DNA (cont.)

There three forms of DNA (A, B and Z)

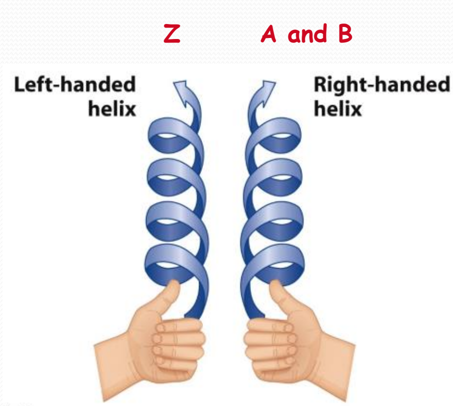
- **The B form DNA** is the classical resolved Watson-Crick structure**.** It occurs at high water concentrations; and it is the most common form of DNA *in vivo.*
  - Its structure is a more narrow with elongated right-handed double helix.
  - Its wide major groove makes it more accessible to proteins.
  - B-DNA base pairs are nearly perpendicular to the helix axis.
- **The A form** is favored in many solutions that are relatively devoid of water.
  - The DNA is still arranged in a right-handed double helix, but the helix is wider and the number of base pairs per helical turn is 11, rather than 10.5 as in B-DNA.
  - The plane of the base pairs in A-DNA is tilted about 20 with respect to the helix axis.
- **The Z form** is left-handed helix.
  - There are 12 base pairs per helical turn,
  - The structure appears more slender and elongated.
  - The DNA backbone takes on a zigzag appearance.
  - It is prominent when pyrimidines alternate with purines, especially alternating C and G.

Types of DNA

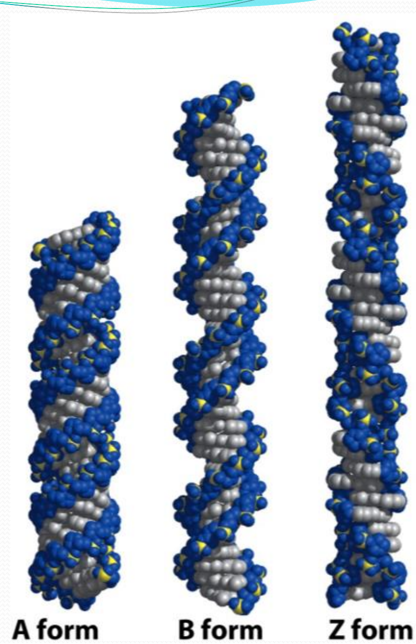
There three types of DNA, as shown in the table:

	A form	B form	Z form
Helical sense	Right handed	Right handed	Left handed
Diameter	~26 Å	~20 Å	~18 Å
Base pairs per helical turn	11	10.5	12
Helix rise per base pair	2.6 Å	3.4 Å	3.7 Å
Base tilt normal to the helix axis	20°	6°	7°
Sugar pucker conformation	C-3′ endo	C-2′ endo	C-2′ endo for pyrimidines; C-3′ endo for purines
Glycosyl bond conformation	Anti	Anti	Anti for pyrimidines; syn for purines

Figure 8-17 part 2



Box 4-1



Nucleic acid quaternary structure

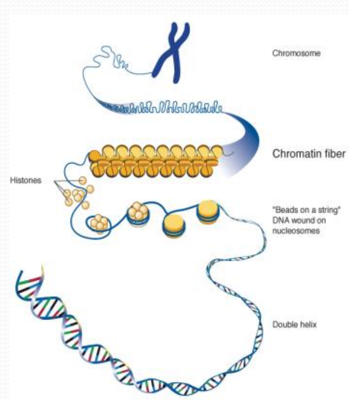
It refers to the interactions between separate nucleic acid molecules, or between nucleic acid molecules and proteins (like histones and protamines) to form **chromatin**.

Chromatin

- **Chromatin** is a complex of macromolecules found in eukaryotic cells, consisting of DNA, protein, and RNA.
- **The primary functions of chromatin are:**
  - to package DNA into a smaller volume,
  - to reinforce the DNA to allow mitosis,
  - to prevent DNA damage, and
  - to control gene expression and replication.

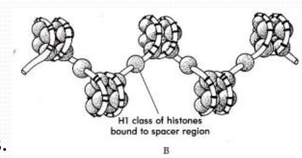
Chromatin is organized on three basic levels:

- primary (nucleosome)
- secondary (solenoid)
- tertiary/quaternary (final folding into chromosome shape)



Chromatin (cont.)

- A typical eukaryotic chromosome contains 1 to 20 cm of DNA.
- During metaphase of meiosis/mitosis, this DNA is package into a chromosome with a length of only 1 to 10 µm (10^4-fold smaller than the naked DNA).
- DNA is wrapped around some basic proteins to be packed into this small structure.
  - **Histones** are the primary protein components of chromatin in eukaryotes.
  - There are 5 types of histones namely **H1, H2A, H2B, H3 and H4.**
  - **Protamines** replace histones late in the haploid phase of spermatogenesis
  - There are other ***Non-histone protein*** in the chromatin like:
    - Scaffold proteins,
    - DNA polymerase,
    - Heterochromatin Protein 1
    - Polycomb and
    - numerous other structural, regulatory, and motor proteins.



Which type of protein belong histone and protamine?

DNA denaturation: significance and factors

- DNA is a remarkably flexible molecule.
- Considerable rotation is possible around a number of bonds in the sugar–phosphate (phosphodeoxyribose) backbone.
- Thermal fluctuation can produce bending, stretching, and unpairing (melting) of the strands.
- Solutions of carefully isolated, native DNA are highly viscous at pH 7.0 and room temperature (25 °C).
- When such a solution is subjected to extremes of pH or to temperatures above 80°C, its viscosity decreases sharply, indicating that the DNA has undergone a physical change.
- Heat and extremes pH cause denaturation, or melting, of double-helical DNA due to disruption of the hydrogen bonds between paired bases of the double helix to form unwind molecules.
- The covalent bonds in the DNA are NOT broken because they are strong.
- Slow cooling or neutralization of pH cause renaturation of the denatured DNA molecule.
- i.e. the unwound segments of the two strands spontaneously rewind, or **anneal,** to yield the intact duplex.

Types of RNA

RNAs have a broader range of functions, and several classes are found in cells.

Ribosomal RNAs (rRNAs) are components of ribosomes, the complexes that carry out the synthesis of proteins.

Messenger RNAs (mRNAs) are intermediaries, carrying genetic information from one or a few genes to a ribosome, where the corresponding proteins can be synthesized.

Transfer RNAs (tRNAs) are adapter molecules that carriy the amino acids needed for protein synthesis in order determined by the sequence of codons in mRNA and the collaboration of r-RNA.

In RNA
In contrary to DNA,
There is no relation between the number of purines and the number of pyrimidines (T&C)

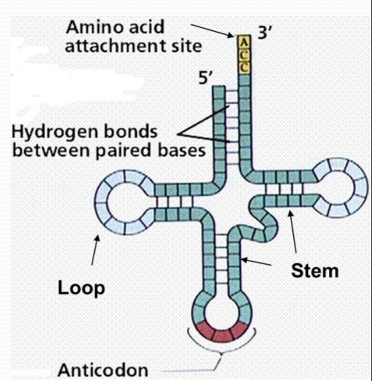
So, We can NOT predict the ratio or the number of any base if we know the ratio of any other base(s)

m-RNA

- It represents 5-10% of the total RNA%.
- It is formed in the nucleus from DNA by the process called *transcription*.
- It carries the genetic codes.
- Each three nucleotide is called *codon*.
- Each codon is translated into amino acid in the *translation* process.
- There are three untranslated codons called *"Stop Codons"*
- The number of nucleotide in mRNA is at least three times the number of amino acids to formed in translation (Why?)
- The length of the mRNA varies according to the gene it encodes.
- mRNA has a short life span (WHY?)

t-RNA

- It is the smallest type of RNA
- It is formed from 75-90 nucleotides
- It represents 10-20% from the total RNA
- There is at least one t-RNA for each amino acid.
- Some amino acids have more than one t-RNA and hence more than one codon.
- Each t-RNA has two important region:
  - 3' amino acid attachment site that has the same sequence of codon.
  - a region complementary to the codon known as "anticodon"
- t-RNA serves as the physical link between the mRNA and the amino acid sequence of proteins.
- Each t-RNA carries the amino acids to the protein synthetic machinery of a cell (ribosome) as directed by the sequence of codons in the m-RNA.
- t-RNA are not degraded after translation.



r-RNA

- It is the largest type of RNA
- It represents 50-65% of the total RNA
- It is located in the ribosome bound to nuclear proteins.
- r-RNA forms approximately 60% of the ribosome weight (the rest 40% is protein).
- Ribosome is the factory of protein synthesis.
- The ribosome forms two subunits:
  - the large subunit (LSU) which catalyze the peptide bond formation (ribozyme)
  - The small subunit (SSU).
- During translation, m-RNA is sandwiched between the small and large ribosome subunits, and the LSU catalyzes the formation of a peptide bond between the two amino acids that are localized in the ribosome.
- r-RNA is not degraded after translation.

Important definitions:

Genetic code, exon and introns, Gene, genome and chromosome

- **Gene** is a region of DNA which is made up of nucleotides and is the molecular unit of heredity.
- **Exon** is any part of a gene that will encode a part of the final mature RNA and is translated into proteins.
- **Introns** are untranslated segments in DNA or pre-mRNA flanking the coding regions (exons) and is removed by RNA splicing.
- **Genetic code,** is the set of rules by which information encoded within genetic material (DNA or mRNA sequences) is translated into proteins.
- **Chromosome** is a packaged and organized structure containing most of the DNA of a living organism.
- **Genome** is the complete set of **genetic material** of an organism.
  - It consists of DNA (or RNA in RNA viruses).
  - The genome includes both the genes, (the coding regions), the noncoding DNA and the genomes of the mitochondria and chloroplasts.

Genetic code

The ***codons*** are three nucleotide codes, that is translated into amino acid in the protein synthesis steps.
Each amino acid has one or more codons.
Some codons are not translated and they called "Stop codons"
There is only one start codon.
There are three stop codons.

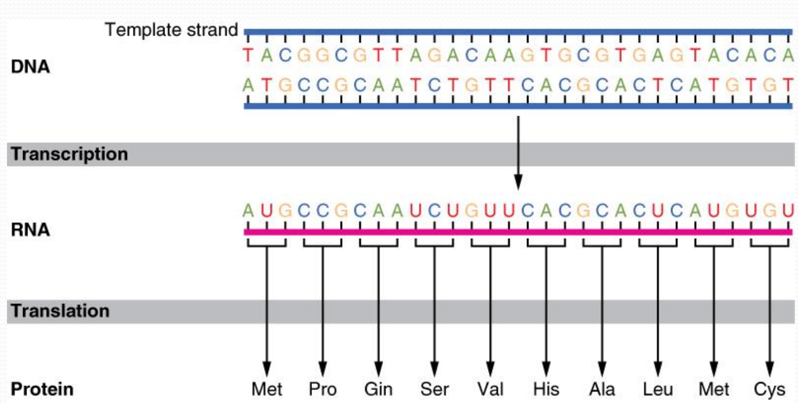
Name the start codon?

Name the stop codons?

Second Base

First Base	U		C		A		G		Third Base
U	UUU UUC	phe	UCU UCC UCA UCG	ser	UAU UAC	tyr	UGU UGC	cys	U C
	UUA UUG	leu			UAA UAG	stop stop	UGA UGG	stop trp	A G
C	CUU CUC CUA CUG	leu	CCU CCC CCA CCG	pro	CAU CAC	his	CGU CGC CGA CGG	arg	U C
					CAA CAG	gln			A G
A	AUU AUC AUA	ile	ACU ACC ACA ACG	thr	AAU AAC	asn	AGU AGC	ser	U C
	AUG	met (start)			AAA AAG	lys	AGA AGG	arg	A G
G	GUU GUC GUA GUG	val	GCU GCC GCA GCG	ala	GAU GAC	asp	GGU GGC GGA GGG	gly	U C
					GAA GAG	glu			A G

The relation between DNA, RNA & Proteins
Central Dogma of Molecular Genetics



Introduction to replication, transcription and translation and important enzymes

- **Replication,** making a copy to the DNA
- **Transcription,** formation of mRNA from DNA
- **Translation**, forming protein by the translation of the genetic code in the mRNA to amino acids in the ribosome

DNA replication

- **Definition**
- DNA replication is the biological process of producing two identical replicas of DNA from one original DNA molecule.
- This process occurs in all living organisms when the cell needs to divide into two identical cells.
- During replication, the two strands are separated by breaking the week hydrogen bonds between them.

- There are three types of DNA replication
  - Semiconservative Replication
  - Bidirectional Replication
  - Semidiscontinuous replication

Semiconservative Replication

- Each strand of the original DNA molecule then serves as a template for the production of its counterpart.
- Cellular proofreading and error-checking mechanisms ensure near perfect fidelity for DNA replication.
- Synthesis of DNA chain ONLY occurs in 5’ to 3’ direction

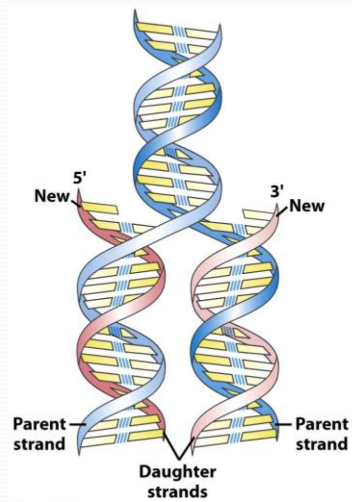


Figure 8-15
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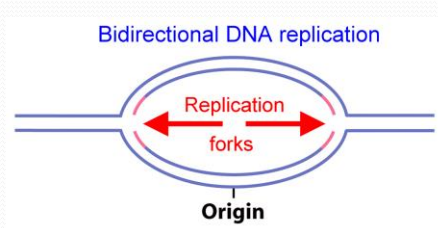
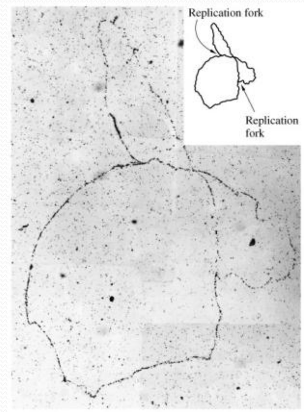
Bidirectional Replication in Eukaryotes

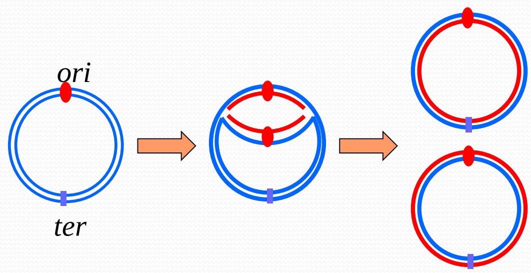
- Replication starts at site called origin of replication by the help of a protein at a point in DNA rich in adenine and thymine bases (Why not G & C?)
- Once the origin has been located, some other initiators are recruited and form the pre-replication complex, unzips the double-stranded DNA forming Replication Fork.
- Replication continues and the DNA pulp enlarges in two directions.

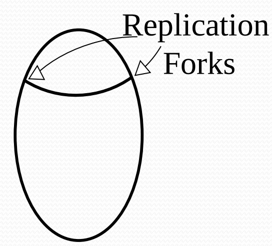


Bidirectional Replication in Prokaryotes



- Most bacterial chromosomes contain a circular DNA molecule – there are no free ends to the DNA.
- The replication occur in two direction forming what is called ***Bidirectional Replication and it forms a shape like the Greek letter Theta***

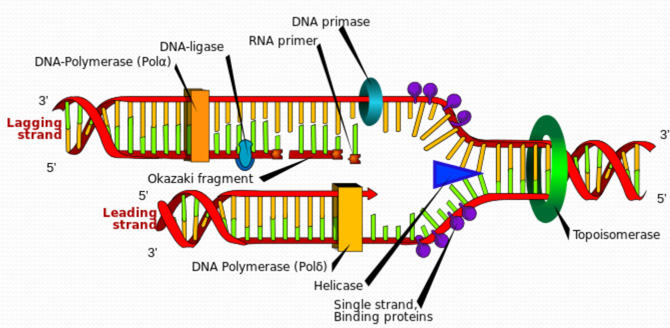


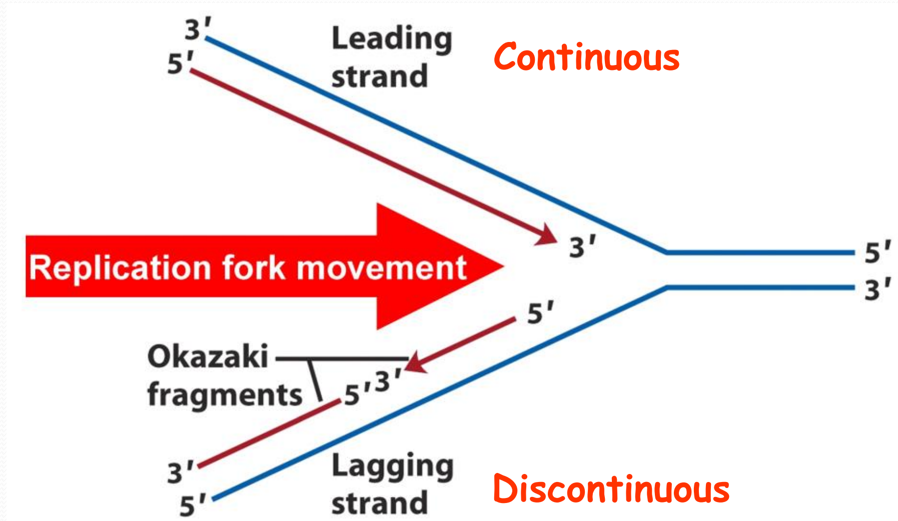


Semidiscontinuous replication

The replication occurs in the two strands.
It starts in one end and propagate in two ways:

- **Leading strand** continuously from 5' to 3' and the new stand is rapidly synthesized.
- **Lagging strand,** it is slow and gives short segments called ***Okazaki Fragments***. These fragments are joined together by ligase enzyme.

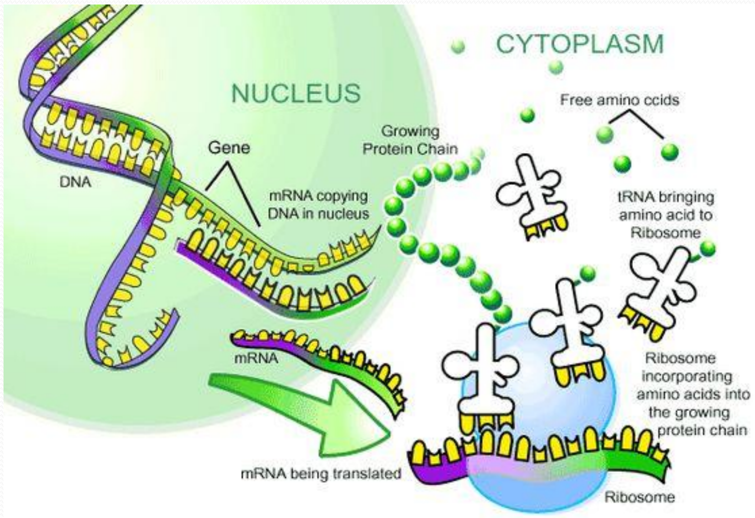




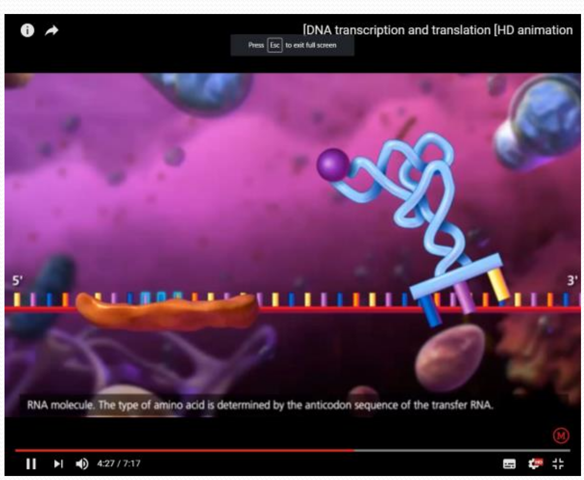
Enzymes used in DNA replication

Enzyme	Function in DNA replication
Topoisomerase	Relaxes the DNA from its super-coiled structure.
DNA Helicase	It separates the two strands of DNA at the Replication Fork behind the topoisomerase.
DNA Gyrase	Relieves strain of unwinding by DNA helicase; this is a specific type of topoisomerase
Primase	Provides a starting point of RNA (or DNA) for DNA polymerase to begin synthesis of the new DNA strand.
DNA Polymerase	catalyzes the addition of nucleotide substrates to DNA in the 5' to 3' direction. It needs a piece of RNA as primer to start replication (from primase). Also performs proof-reading and error correction. There are three types of DNA polymerases: DNA polymerase I, II and III
DNA Ligase	It joins Okazaki Fragments of the lagging strand.
Telomerase	Lengthens telomeric DNA by adding repetitive nucleotide sequences to the ends of eukaryotic chromosomes.

Transcription and Translation



https://www.youtube.com/watch?v=-K8Y0ATkkAI



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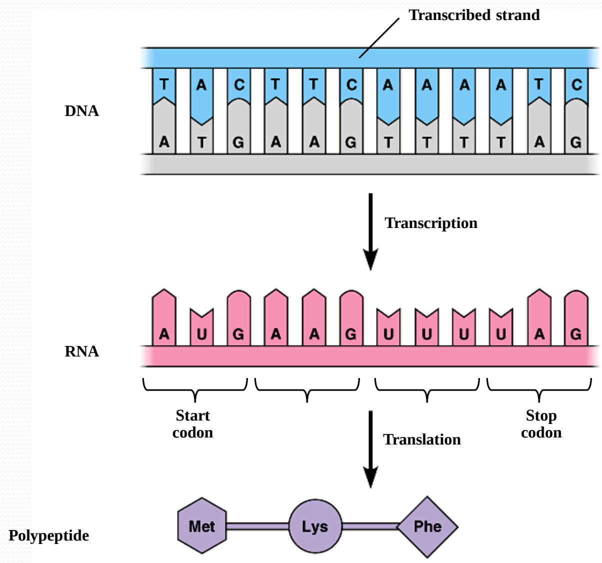

Figure 10.8B

ادخل الموقع

https://www.youtube.com/watch?v=jLyi2K-29xU

Nucleic acids-1

https://www.youtube.com/watch?v=TonMp6X-iVM

Nucleic acids-2

https://www.youtube.com/watch?v=2O4MtcenMmo

Nucleic acids-3