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College of Science

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General Biochemistry (BCH 202)

Chapter 5 Nucleic Acids

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

- ☐ Nucleic acids consist of polymers of nucleotides monomers.
- 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:
 - Nitrogenous base,
 - Pentose sugar,
 - Phosphate.
- The molecule without the phosphate group is called a nucleoside.

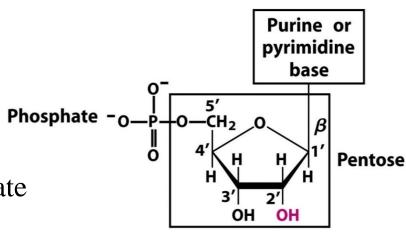


Figure 8-1a
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Structure of Nucleotides

The base of a nucleotide is joined covalently (at N-1 of pyrimidines and N-9 of purines) in an N-β-glycosyl bond to the C-1' of the pentose sugar, and the phosphate is esterified to the 5'carbon.

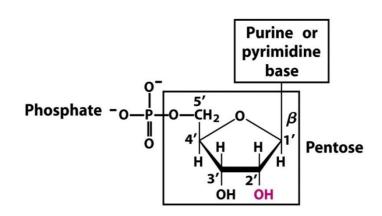


Figure 8-1a
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The nitrogenous bases

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

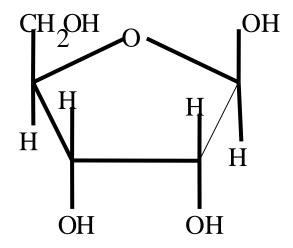
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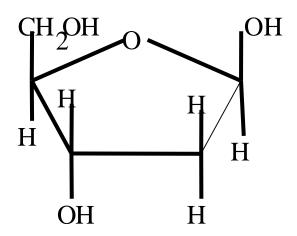
The pentose sugar

Nucleic acids have two kinds of pentoses.

- The RNA contains ribose
- The DNA contains deoxyribose (ribose missing oxygen at position C_2).



Ribose, in RNA



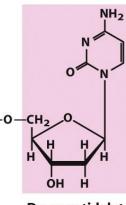
Deoxyribose, in DNA

Nomenclature

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

TABLE 8-1	Nucleotide and Nucleic Acid Nomenclature			
Base	Nucleoside	Nucleotide	Nucleic acid	
Purines				
Adenine	Adenosine Deoxyadenosine	Adenylate Deoxyadenylate	RNA DNA	
Guanine	Guanosine Deoxyguanosine	Guanylate Deoxyguanylate	RNA DNA	
Pyrimidines				
Cytosine	Cytidine Deoxycytidine	Cytidylate Deoxycytidylate	RNA DNA	
Thymine	Thymidine or deoxythymidine	Thymidylate or deoxythymidylate	DNA	
Uracil	Uridine	Uridylate	RNA	

Nucleotides



Ö

Nucleotide:

Deoxyadenylate (deoxyadenosine

5'-monophosphate)

Symbols:

A, dA, dAMP

Nucleoside: Deoxyadenosine

Deoxyguanylate (deoxyguanosine 5'-monophosphate)

G, dG, dGMP

Deoxyguanosine

Deoxythymidylate (deoxythymidine 5'-monophosphate)

T, dT, dTMP

HN

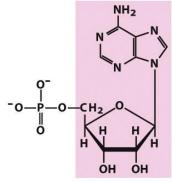
Deoxythymidine

Deoxycytidylate (deoxycytidine

C, dC, dCMP

Deoxycytidine

Deoxyribonucleotides



Nucleotide: Adenylate (adenosine 5'-monophosphate)

Symbols: A, AMP **Nucleoside:** Adenosine

HN H₂N 0-OH OH

Guanylate (guanosine

5'-monophosphate)

Uridylate (uridine 5'-monophosphate)

G, GMP Guanosine ö OH OH

> U, UMP Uridine

5'-monophosphate)

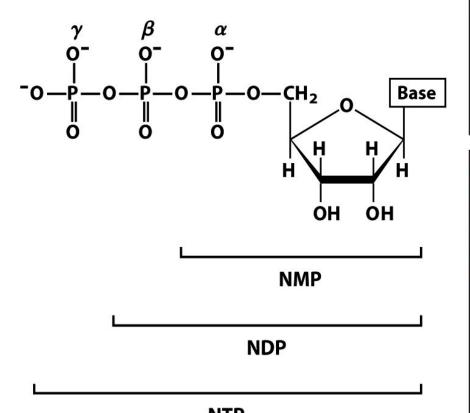
ö OH ÓН

> Cytidylate (cytidine 5'-monophosphate)

> > C, CMP Cytidine

Ribonucleotides

Nucleotides are mono-, di-, or triphosphate esters of nucleosides.
-The phosphate group is attached by an ester linkage to the 5`-OH of the pentose.



Abbreviations of ribonucleoside 5'-phosphates Base Mono-Di-Tri-Adenine **AMP ADP ATP GMP GTP** Guanine **GDP** 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 building blocks of DNA and RNA.
- 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*, ex. cyclic adenosine monophosphate (cAMP)
- They are structural components of an array of *enzyme cofactors* and metabolic intermediates (ex. NAD+, FAD, FMN)

Nucleotides derivatives

- NAD+/ NADH
- NADP+/ NADPH
- FAD/ FADH₂
- FMN/ FMNH₂
- c AMP
- c GMP

Nucleotides derivatives; NAD+

- 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 ADPribose.

Nucleotides derivatives; NAD⁺ (cont.)

- 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⁺.

Nucleotides derivatives; FAD

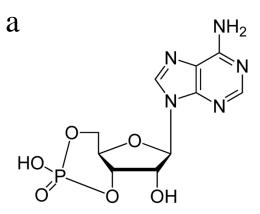
- Flavin adenine dinucleotide (FAD) is a redox cofactor, for many oxidoreductases.
- It is composed of adenine and flavin (Vitamin B₂)

HO, HO,, HO,

 NH_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)
- The phosphate group attached to C_5 form cyclic form with the –OH of C_3 , so the name cyclic AMP



Nucleotides derivatives; cAMP, cGMP (cont.)

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.

3', 5' -Cyclic AMP (cAMP) 3', 5' -Cyclic GMP (cGMP)

Nature Reviews | Molecular Cell Biology

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

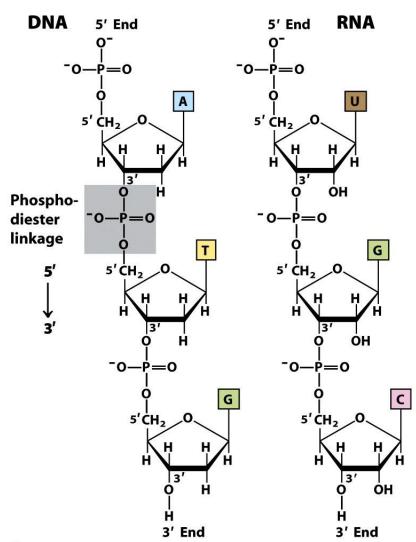
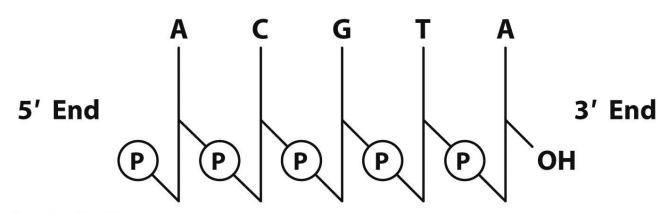


Figure 8-7
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DNA primary structure (cont.)

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.



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DNA secondary structure (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*

Right-handed

helix

manner

• The bases forming the DNA are A, G, T and C

DNA secondary structure (cont.)

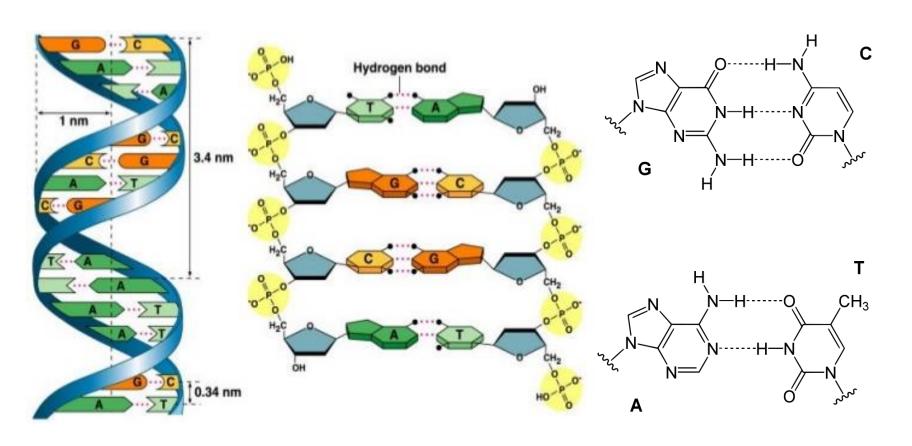
- All nucleotides in each strand are bound by phosphodiester bond (type of covalent bond).
- The two strands bind together by hydrogen bonds.
 - Adenine (A) in one strand binds Thymine (T) in the other by two hydrogen bonds.
 - Guanine (G) in one strand binds Cytosin (C) in the other by three hydrogen 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
 - 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.)



Chargaff's rule

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

Double helix properties, base pairing, stabilizing forces

- Tertiary structure refers to the locations of the atoms in threedimensional 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.

Tertiary structure of DNA

- The hydrophilic backbones of alternating deoxyribose and phosphate groups are on the *outside of the double helix*, facing the surrounding water.
- 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

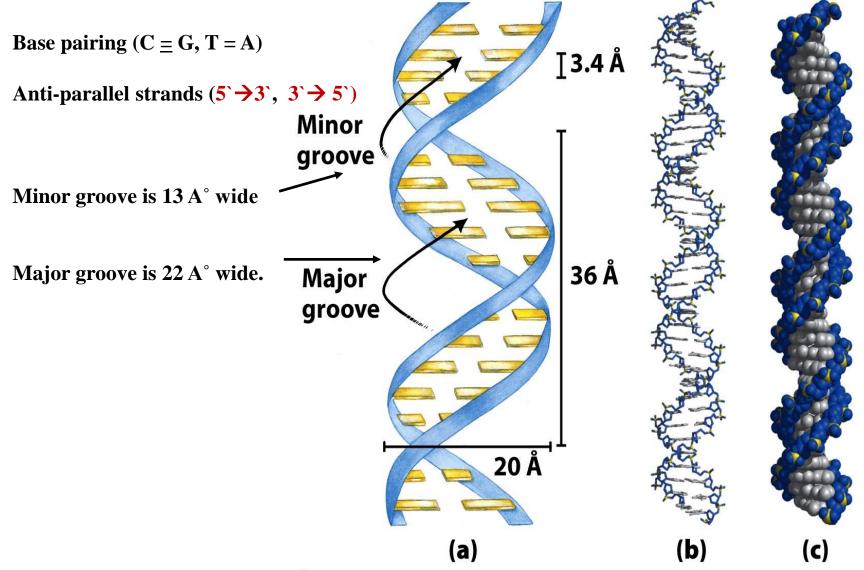
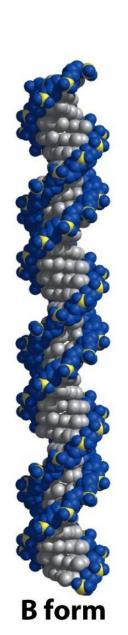


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Tertiary structure of DNA (cont.)

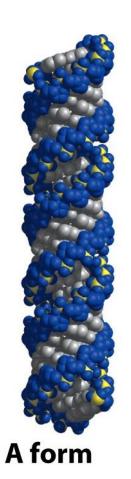
There are three forms of DNA (A, B and Z)

- 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 righthanded double helix.
- Its wide major groove makes it more accessible to proteins.
- The number of base pairs per helical turn is 10.5.
- B-DNA base pairs are nearly perpendicular to the helix axis.



Tertiary structure of DNA (cont.)

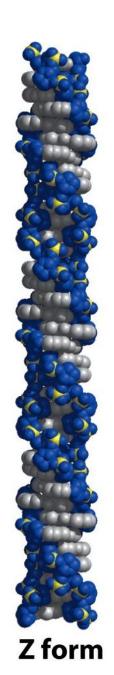
- **The A form** is favored in many solutions that are relatively devoid of water.
 - The DNA is still arranged in a right-handed double helix, but the helix is wider
 - The number of base pairs per helical turn is 11.
 - The plane of the base pairs in A-DNA is tilted about 20° with respect to the helix axis.

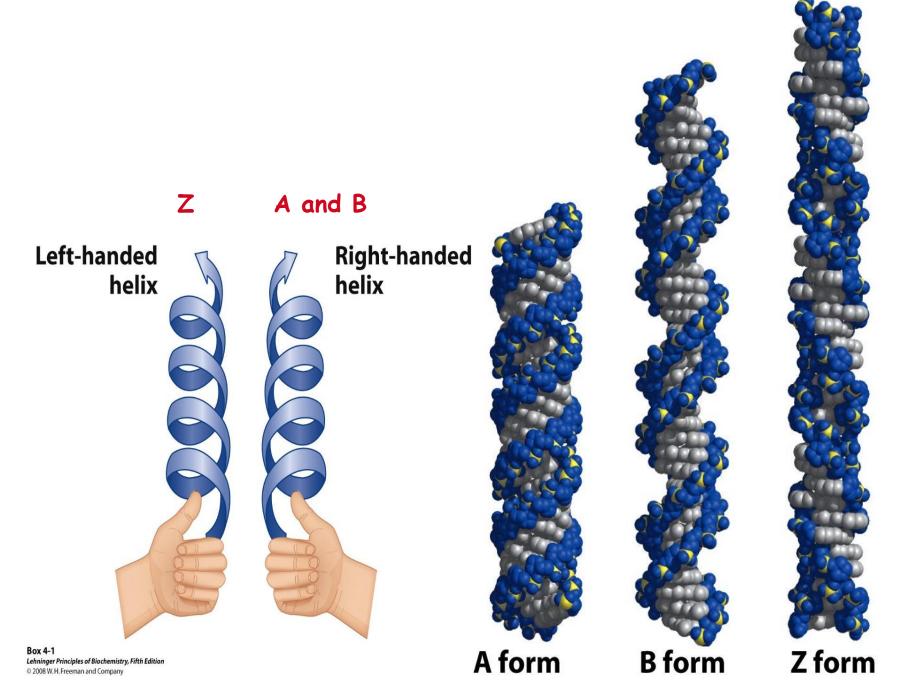


Tertiary structure of DNA (cont.)

The Z form is left-handed helix.

- The structure appears more slender and elongated.
- The DNA backbone takes on a zigzag appearance.
- The number of base pairs per helical turn is 12.
- It is prominent when pyrimidines alternate with purines, especially alternating C and G.





Quaternary structure of DNA

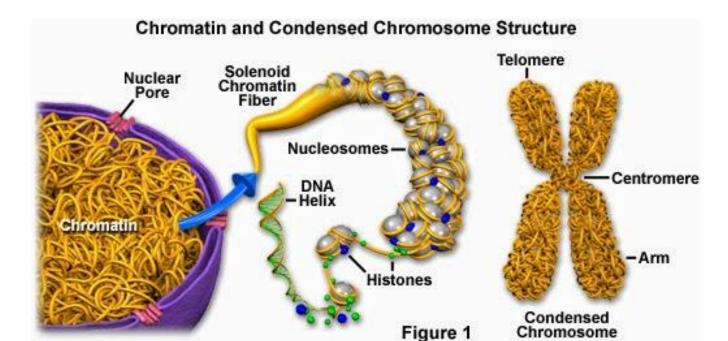
• It refers to the interactions between separate nucleic acid molecules, or between nucleic acid molecules and proteins (like histones and protamines) to form **chromatin**.

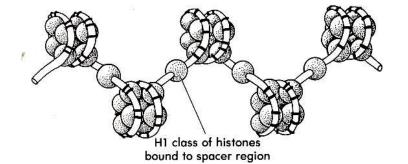
Chromatin

- Chromatin is a complex of macromolecules found in eukaryotic cells, consisting of DNA, protein, and RNA.
- The primary functions of chromatin are:
 - Package DNA into a smaller volume
 - Reinforce the DNA to allow mitosis
 - Prevent DNA damage
 - Control gene expression and replication

Chromatin (cont.)

- Chromatin is organized on three basic levels:
 - primary (nucloesome)
 - 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 μ m (10⁴-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.

Chromatin (cont.)

- 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
 - Numerous other structural, regulatory, and motor proteins

Chromosome

The genetic organization of prokaryotic and eukaryotic chromosomes are significantly different.

Prokaryotic chromosome:

✓ A prokaryotic organism contains a single, double stranded, supercoiled circular chromosome.

Eukaryotic chromosome:

- ✓ Each species of eukaryotic cell has a characteristic number of chromosomes; in human somatic cells there are 46.
- ✓ Each eukaryotic chromosome consists of a single linear DNA molecule complexed with histone proteins to form nucleohistone.

DNA denaturation:

- DNA is a remarkably flexible molecule.
- 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.

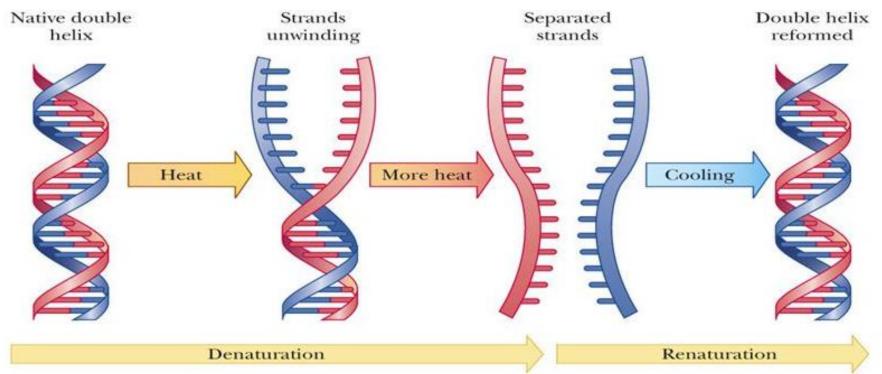
DNA denaturation (cont.)

- When DNA is heated, the temperature at which half of the helical structure is lost is defined as **the melting temperature** (T_m) .
- The loss of helical structure in DNA is called
 denaturation and can be monitored by measuring its
 absorbance at 260 nm.
- Denaturation of a double stranded DNA produce an increase in absorption called **the hyperchromic effect.**
- ✓ because single-stranded DNA has a higher relative absorbance at this wave length than does dsDNA.



DNA denaturation (cont.)

- DNAs rich in GC pairs have higher melting points than DNAs rich in AT pairs.
- Slow cooling or neutralization of pH causes renaturation of the denatured DNA molecule.
- ✓ i.e. the unwound segments of the two strands spontaneously rewind, or **anneal**, to yield the intact duplex.
- Under appropriate conditions, complementary DNA strands can reform the double helix by the process called **renaturation** (or reannealing).



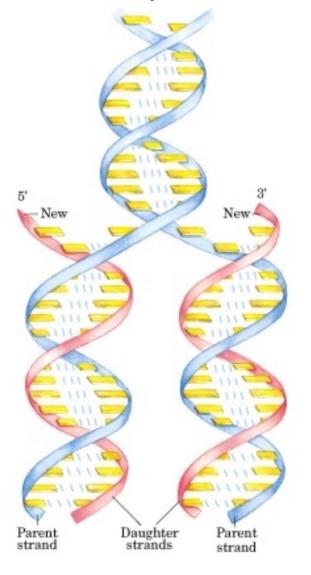
DNA replication

- 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 cells.
- During replication, the two strands are separated by breaking the week hydrogen bonds between them.
- There are three features of DNA replication
 - Semiconservative Replication
 - Bidirectional Replication
 - Semidiscontinuous replication

Semiconservative Replication

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

DNA Replications



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.

Replication

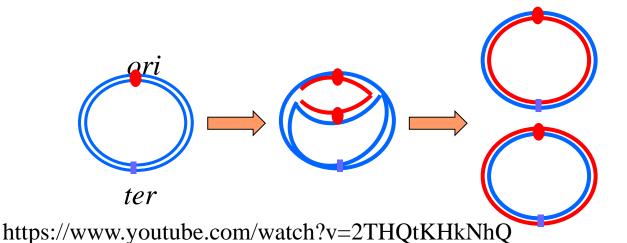
forks

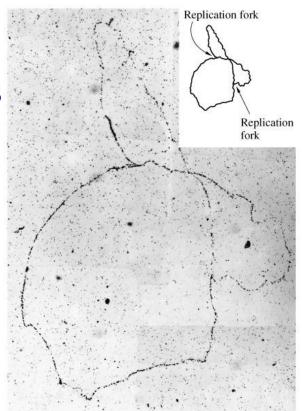
Origin

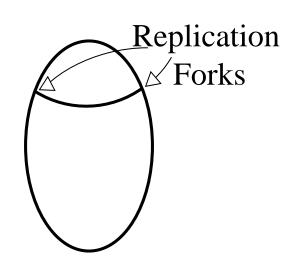
Replication continues and the
 DNA pulp enlarges in two directions.

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 θ





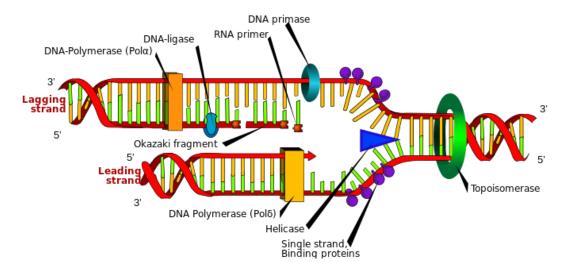


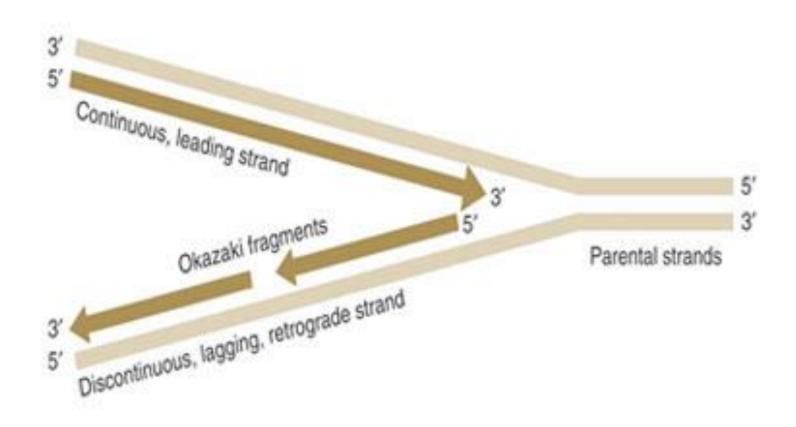
Semidiscontinuous replication

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.





https://www.youtube.com/watch?v=J3SV_2f1XBQ

Enzymes used in DNA replication

Enzyme	Function in DNA replication				
Topoisomerase	relaxes the DNA from its super-coiled structure.				
DNA Helicase	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	joins Okazaki Fragments of the lagging strand.				
Telomerase	lengthens telomeric DNA by adding repetitive nucleotide sequences to the ends of eukaryotic chromosomes.				

DNA replication

https://www.youtube.com/watch?v=TEQMeP9GG6M

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

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 carry 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)

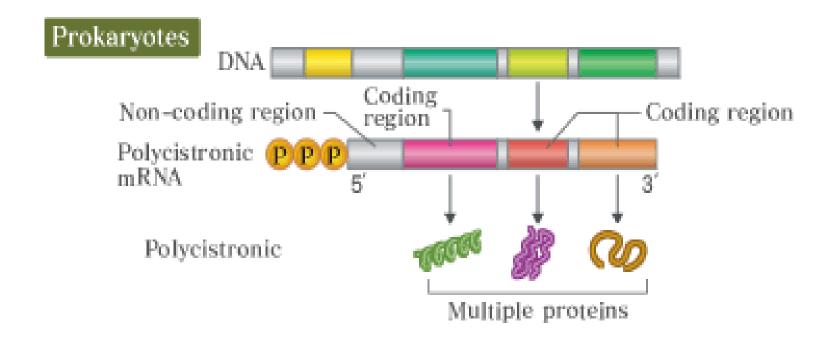
So, We can NOT predict the ratio or the number of any base if we know the ratio of any other base(s)

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
- The length of the mRNA varies according to the gene it encodes.
- mRNA has a short life span

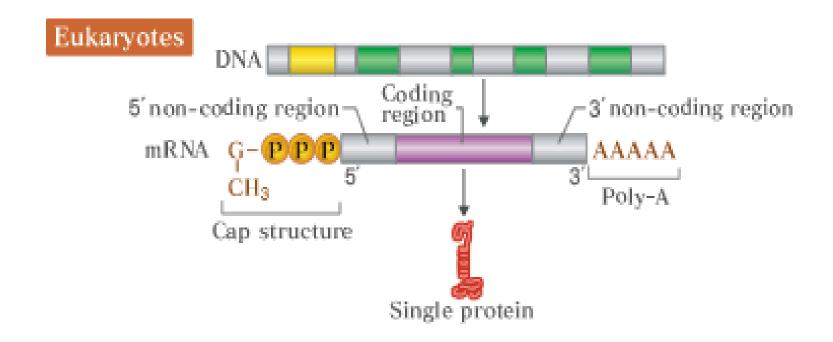
m-RNA (cont.)

 Prokaryotic mRNAs are polycistronic; that is, they contain coding information for several polypeptide chains.



m-RNA (cont.)

 Eukaryotic mRNA codes for a single polypeptide and is therefore referred to as monocistronic.



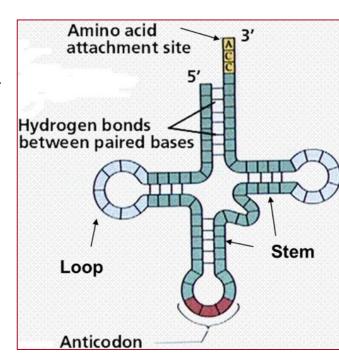
m-RNA (cont.)

Eukaryotic mRNA has special structural characteristics:

- Long sequence of adenine nucleotides (a"poly-A tail") on the 3` end of the RNA chain.
- ✓ This tail correlated with the stability of the mRNA molecule
- ❖ Cap on the 5`end consisting of a molecule of 7-methylguanosine attached "backward" through a triphosphate linkage.
- ✓ The 5`cap has a positive effect on the initiation of message translation as the cap structure is recognized by a single ribosomal protein.

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 (cont.)

- 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 the proteins.
- r-RNA forms approximately 60% of the ribosome weight (the rest 40% is protein).
- Ribosome is the factory of protein synthesis.

r-RNA (cont.)

- 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.

r-RNA (cont.)

- The ribosomes of prokaryotes and eukaryotes are similar in shape and function, although they differ in size and in their chemical composition.
- Both types of ribosome consist of two subunits of unequal size (large subunit and small subunit), which are usually referred to in terms of their S values.

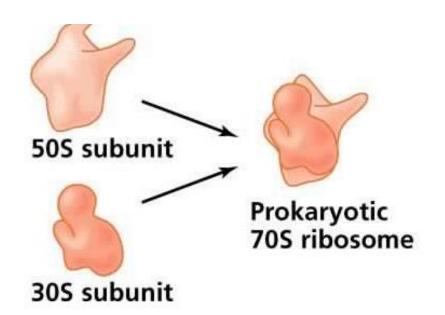
[note: the Svedberg (or sedimentation) unit, S, is a measure of sedimentation velocity in a centrifuge].

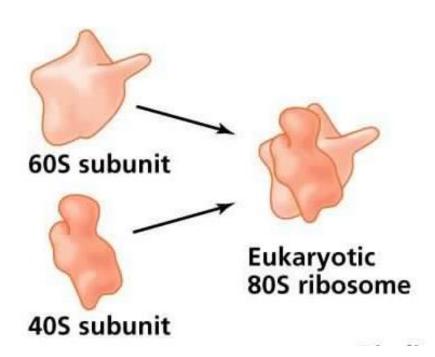
• Prokaryotic ribosomes (70S):

Composed of a 50 S subunit and a 30S subunit.

• Eukaryotic ribosomes (80S):

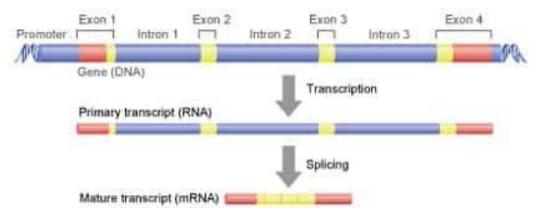
Contain 60S subunit and a 40S subunit.





Important definitions:

- 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.



Important definitions:

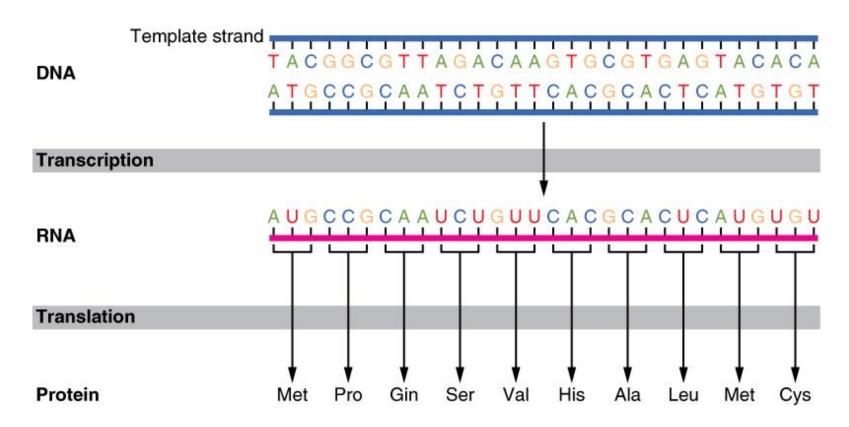
- Genetic code is the set of rules by which information encoded within genetic material (DNA or mRNA sequences) is translated into proteins.
- Chromosome is a packaged and organized structure containing most of the DNA of a living organism.
- Genome is the complete set of genetic material of an organism.
 - 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.

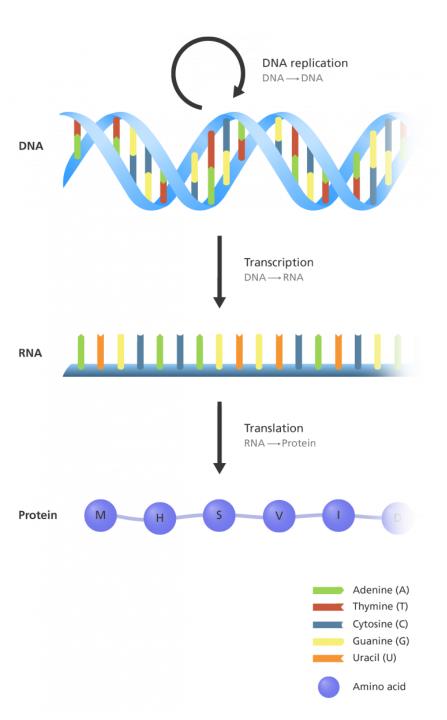
The relation between DNA, RNA & Proteins

- The flow of information from DNA to RNA to protein is termed the "central dogma".
- The genetic information within the nucleotide sequence of DNA is *transcribed* in the nucleus into the specific nucleotide sequence of an RNA molecule.
- The sequence of nucleotides in the RNA transcript is complementary to the nucleotide sequence of the template of its gene.
- Several different types of RNA, including mRNA, rRNA, and tRNA are involved in protein synthesis.

The relation between DNA, RNA & Proteins

Central Dogma of Molecular Genetics

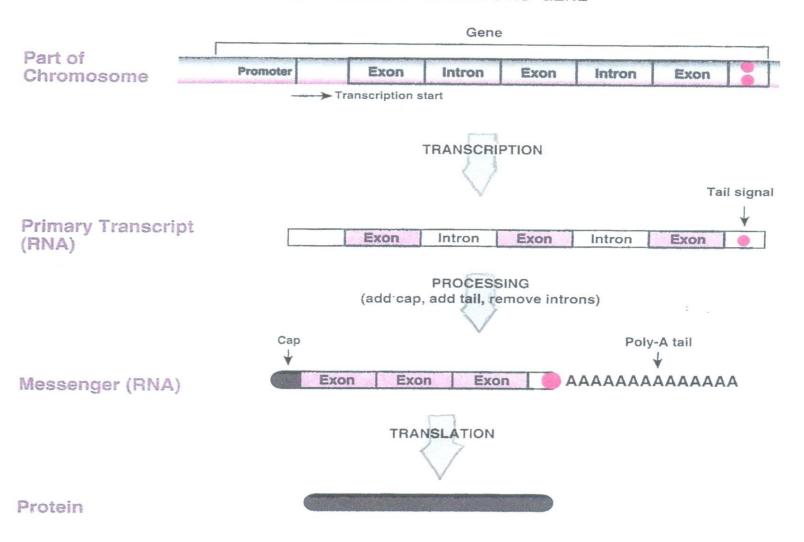




Transcription

- ❖It is the enzymatic process whereby the genetic information contained in one strand of DNA is used to specify a complementary sequence of bases in an mRNA chain.
- The primary transcript (the large mRNA precursor) which contain exons and introns is processed in the nucleus, (introns are removed).
- Then exons are spliced together to form mature mRNA, which is transported to cytoplasm, where it is translated into protein.

EXPRESSING A EUKARYOTIC GENE



Translation

- The process by which the genetic message (the genetic information) carried by mRNAs directs the synthesis of protein with the aid of ribosomes.
- The translation of the information in the mRNA nucleotide sequence into the amino acid sequence of a protein requires an adapter molecule (tRNA adapter molecule).
- The recognition of specific codon in the mRNA by the tRNA adapter molecules is dependent upon their anticodon region and specific base pairing rules.

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.

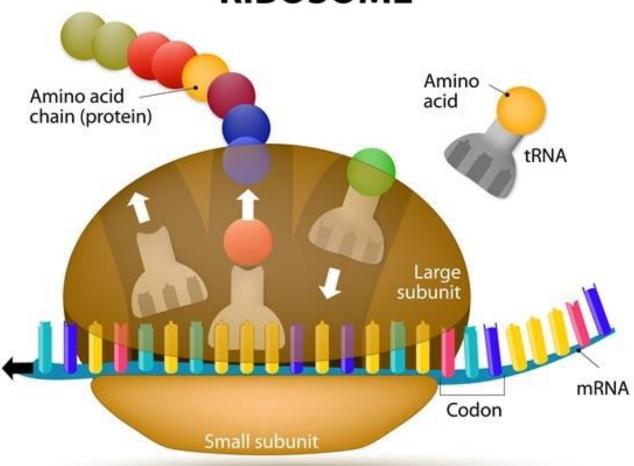
✓ There are three stop codons.

n.		U	С	A	G	
	U	UUU } phe UUA } leu	UCU UCC UCA UCG	UAU	UGU	U C A G
	С	CUU CUC CUA CUG	CCU CCC CCA CCG	CAU his CAC gln CAG	CGU CGC CGA CGG	U C A G
	A	AUC ile AUA AUG met (start)	ACU ACC ACA ACG	AAU	AGU ser AGC arg	U C A G
	G	GUU GUC GUA GUG	GCU GCC GCA GCG	GAU asp GAC glu GAG glu	GGU GGC GGA GGG	U C A G

Second Base

hird Base

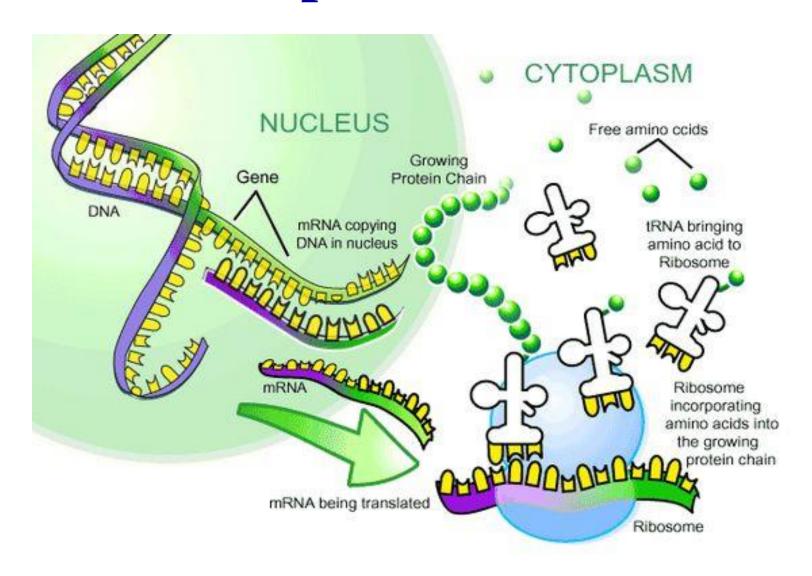
RIBOSOME



Replication, transcription and translation

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

Transcription and Translation



https://www.youtube.com/watch?v=gG7uCskUOrA



https://www.youtube.com/watch?v=-zb6r1MMTkc



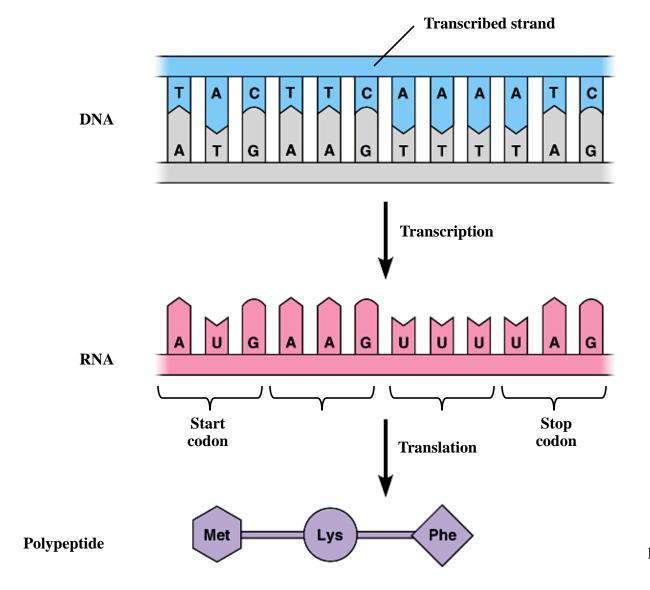


Figure 10.8B