

UNIVERSITY OF ZAMBIA
SCHOOL OF NATURAL SCIENCES
CHEMISTRY DEPARTMENT



NUCLEIC ACIDS

II

2020

What are nucleotides?

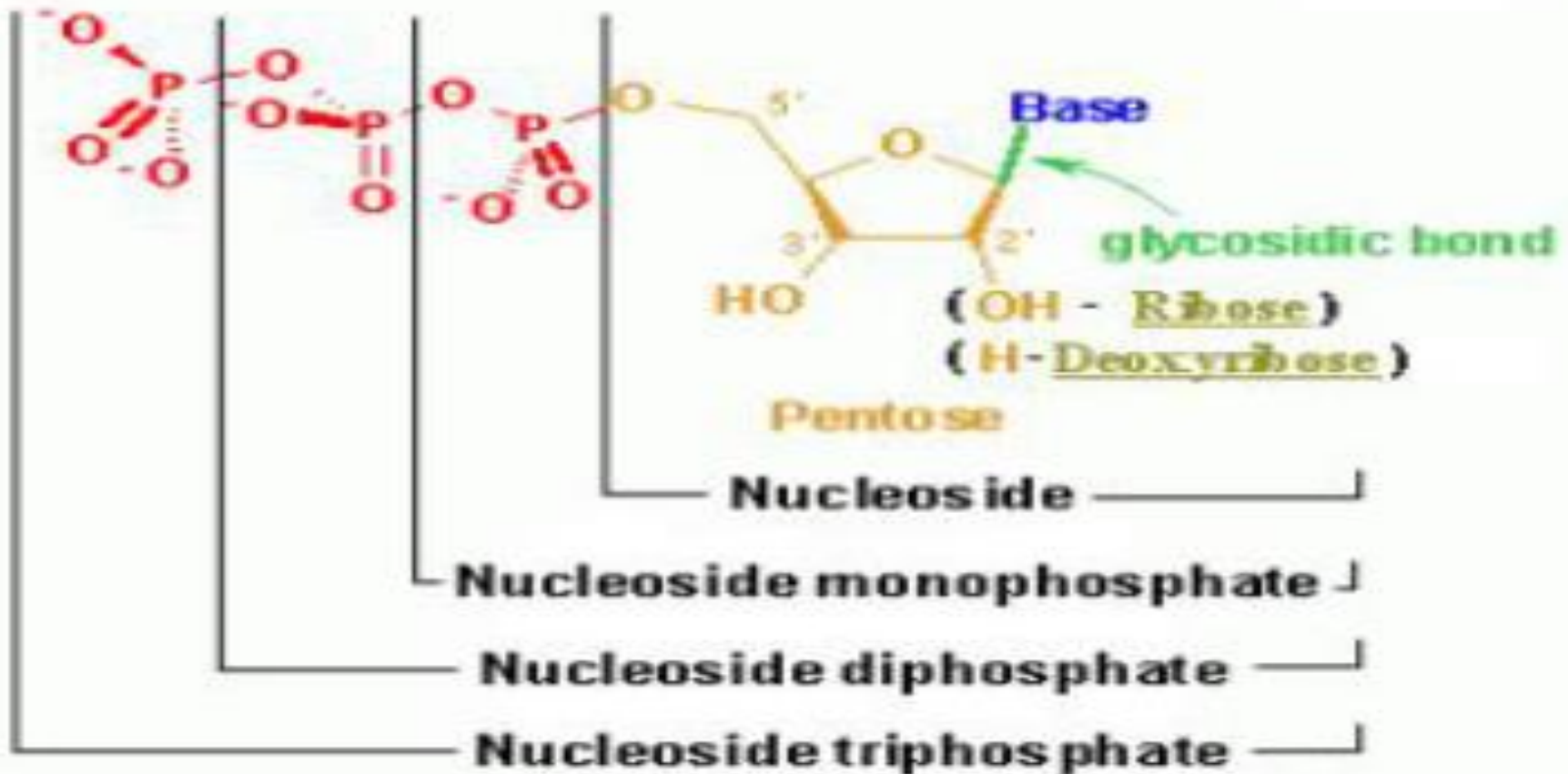


- A nucleotide is a phosphate salt of a nucleoside.
- Nucleotide can also be defined as phosphorous ester of a nucleoside.
- Nucleotide can exist as free form of a nucleoside or as components of nucleic acids or other compounds.
- Nucleotide is formed when a nucleosides is phosphorylated using a high energy compound such as ATP.
- A nucleosides may be monophosphorylated or dephosphorylated or triphosphorylated.
- Nucleotides may be classified as ribonucleotide and deoxyribonucleotide depending on the pentose sugar present.

What are nucleotides?



- Nucleotide is a phosphate salt of a nucleoside.

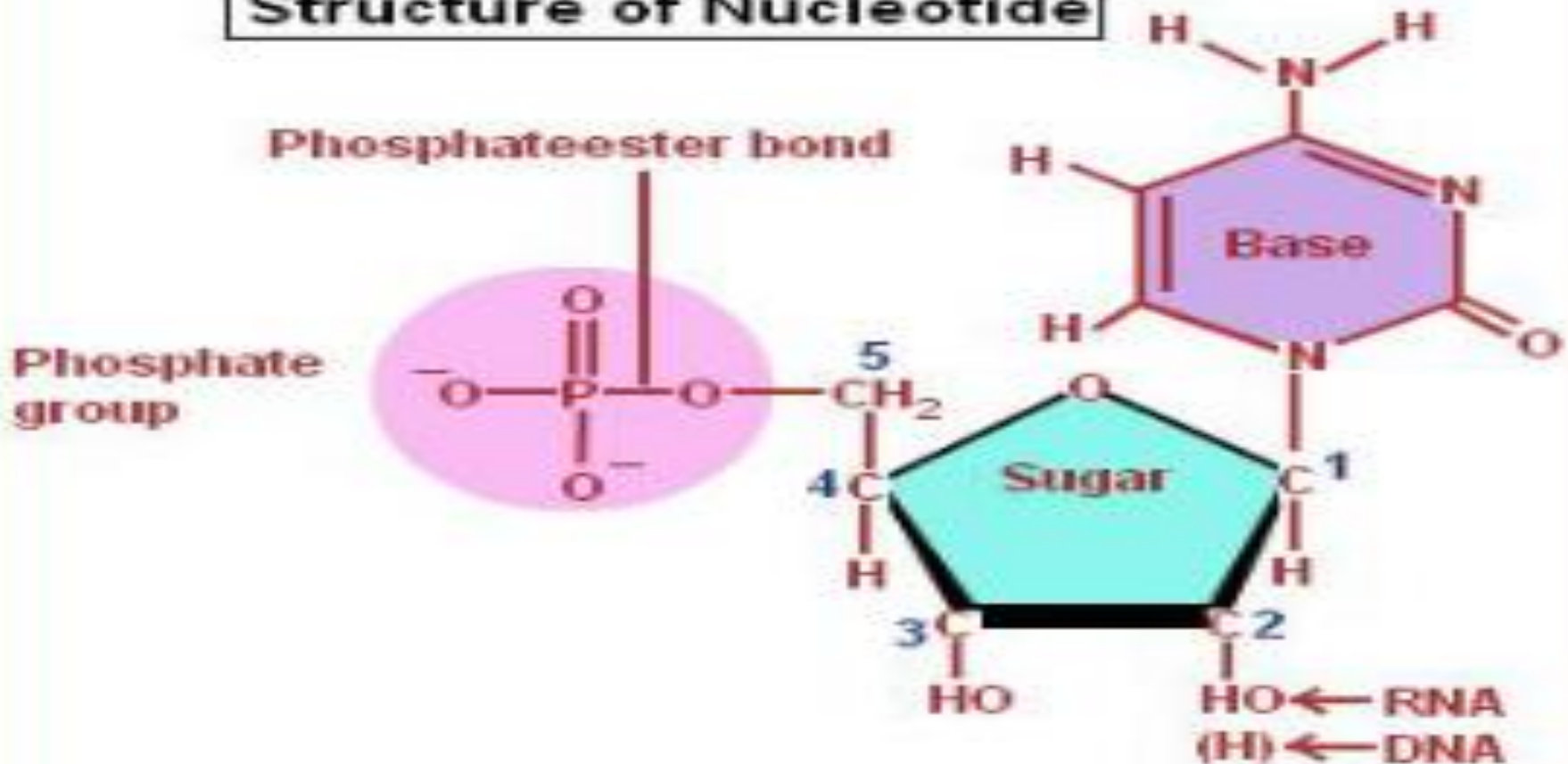


What are nucleotides?



- A monophosphate nucleotide.

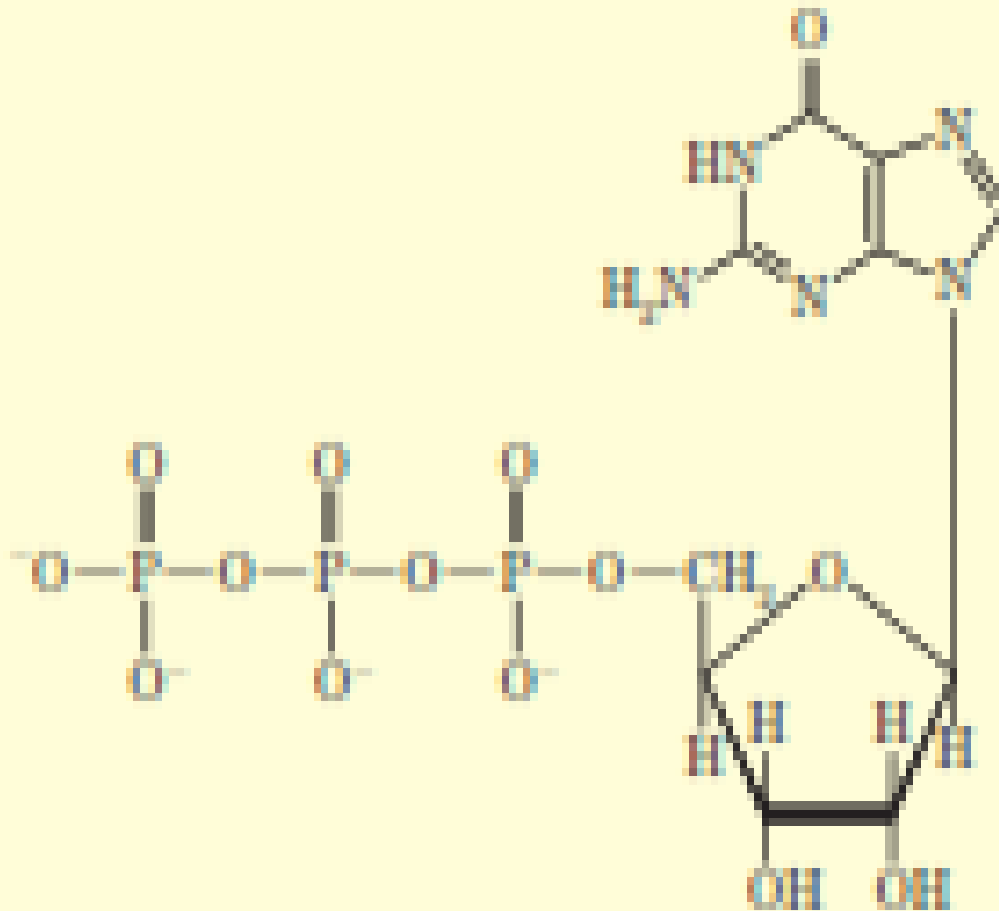
Structure of Nucleotide



What are nucleotides?



- A triphosphate nucleotide.



What are nucleotides?



- The hydroxide group of the ribose or deoxyribose of a nucleosides is the one that get phosphorylated to form a nucleotide.
- Specifically the hydroxide group of the ribose at carbon C2', C3' and C5' of the ribose in a nucleoside is phosphorylated.
- While the hydroxide group of the ribose at carbon C1' and C4' in a nucleosides are not phosphorylated because C1' is involved in the N-B-glycosidic bond and C4' is involved in the cyclization of the ribose.
- The hydroxide group of the deoxyribose at carbon C3' and C5' of the deoxyribose in a nucleoside is phosphorylated.
- In both cases it is the hydroxide group of the ribose or deoxyribose at carbon C5' that is commonly phosphorylated.

What is the nomenclature of nucleotide?



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

What are uses of nucleotides? (Chemical energy carriers in cells)



- Triphosphate nucleotides act as chemical energy carrier.
- By far the ATP is the commonly used nucleotide for this purpose.
- Others that used includes UTP,GTP and CTP.
- Hydrolysis triphosphate nucleotides release energy that can be used for biochemical processes in the cell.
- Also always the hydroxide group of the ribose or deoxyribose

What are uses of nucleotides? (Components of coenzymes)



- Some triphosphate nucleotides serve as components of coenzymes.
- Coenzymes are organic cofactors that activate enzymes.
- Adenine based triphosphate nucleotides are highly used as components of enzyme.
- Examples of coenzymes that are adenine based triphosphate nucleotides are:
 - NAD-Nicotinamide adenine dinucleotide
 - FAD-Flavin adenine dinucleotides
 - NADP--Nicotinamide adenine dinucleotide Phosphate
 - CoASH- Coenzyme A

What are uses of nucleotides? (Regulatory molecules)



- Some triphosphate nucleotides serves as regulatory molecules
- They regulate various activities in the cell
- Biochemical activities are facilitated and regulated by chemical signals.
- The extracellular chemical signal called first messengers interact with receptors on the cell membrane leading to production secondary messenger inside the cell which causes various changes and regulating biochemical reactions.
- Often the secondary messengers are nucleotides.
- Examples are cyclic monophosphate adenosine 3' 5'- cyclic monophosphate (cAMP).

What are uses of nucleotides? (intermediates of metabolism)



- Some nucleotides act as intermediates in metabolism.
- Examples are Uridine dinucleotide.
- UDP sugar participates in the biosynthesis of:
 - glycogen,
 - proteoglycan,
 - glycosyl disaccharides and
 - oligosaccharides of glycoproteins.
- UDP glucuronic is an important intermediate in drug metabolism and bilirubin metabolism.

What are uses of nucleotides? (Monomer of nucleic acids)



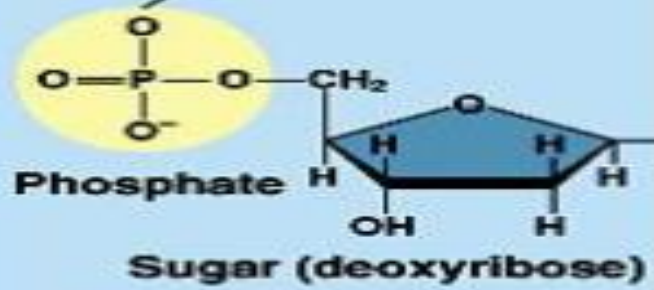
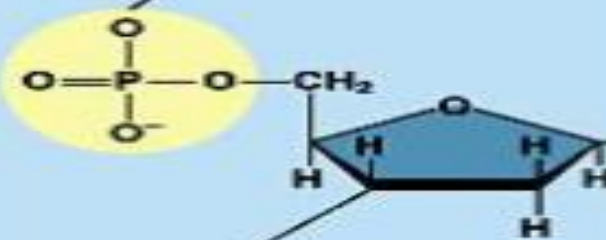
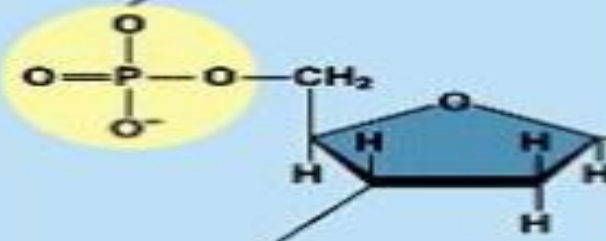
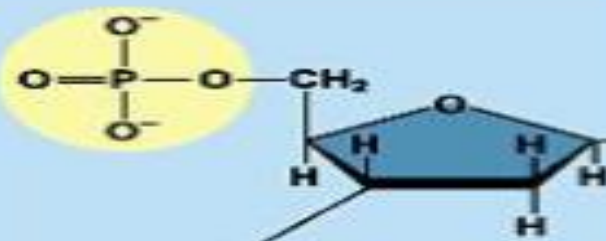
- Triphosphate nucleotides act as activated precursor of nucleic acid.
- Triphosphate ribonucleotides are used to synthesize ribonucleic acid (RNA).
- Triphosphate deoxyribonucleotides are used to synthesize deoxyribonucleic acid (DNA).
- Examples are RNA include mRNA, rRNA, tRNA, snRNA, miRNA.

What are nucleic acids?

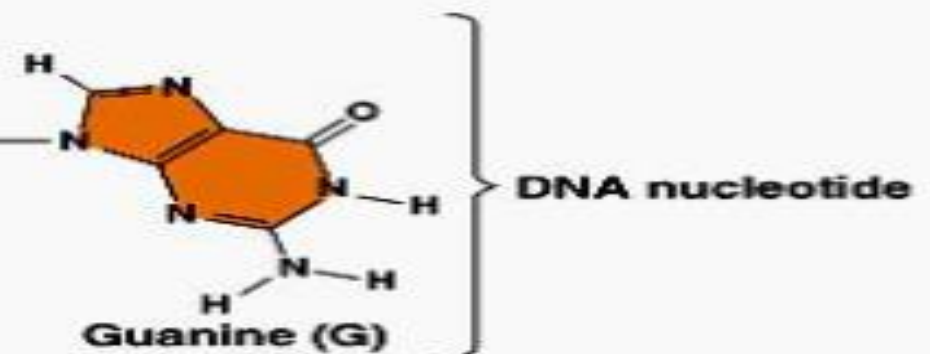
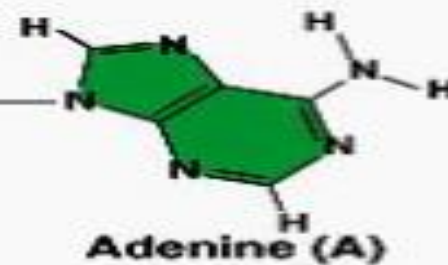


- Nucleic acids are polymers of nucleotides.
- The polymer of deoxyribonucleotides is DNA.
- It is formed when triphosphate deoxyribonucleotides are joined to each other to form a long strand of ribonucleotides.
- A long strand of deoxyribonucleotides is made of a backbone made up of phosphate alternating with deoxyribose sugar and branches or appendages of nitrogenous bases.
- The backbone is hydrophilic and the side chains are hydrophobic.
- The arrangement of deoxyribonucleotides is shown the next slide.

Sugar-phosphate backbone



Bases



What are features of DNA strand?

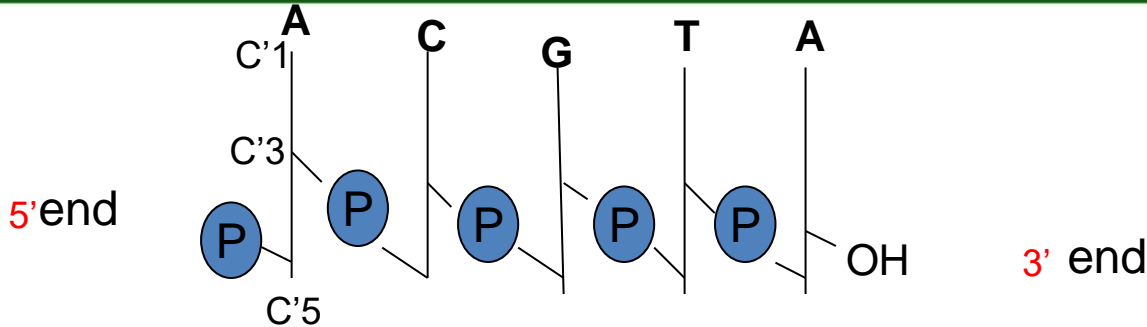


- DNA Strand has direction.
- It has the 5'-phosphate group of one end of polynucleotide and the 3'-hydroxyl group of the other end.
- The covalent backbones of nucleic acids consist of alternating phosphate and pentose residues and the nitrogenous bases may be regarded as side groups joined to the backbone at regular intervals.
- The nucleotides are joined by phosphodiester bond.
- The backbones of both RNA and DNA are hydrophilic.
- The hydroxyl groups of sugar residues can form hydrogen bonds with water.
- By definition the 5' end lacks a nucleotide at the 5' position and the 3' end lacks a nucleotide at the 3' position
- .

The sequence of DNA strand can be represented as shown below



As



- Or
- i. pA-C-G-T-AOH
 - ii. pApCpGpTpA
 - iii. pACGTA

- In general polymers with 50 or less nucleotides are oligonucleotides. A longer nucleic acid is called a polynucleotide

What is a gene?



- A gene is a stretch of DNA, containing a few hundred nucleotides, that carries one particular message.
- One DNA molecule may have between 1 million and 100 million bases.
- Therefore, many genes are present in one DNA molecule.
- In bacteria, this message is continuous; in higher organisms, it is not.
- That is, stretches of DNA that spell out (encode) the amino acid sequence to be assembled are interrupted by long stretches that seemingly do not code for anything.
- The coding sequences are called exons, short for “expressed sequences,” and the noncoding sequences are called introns, short for “intervening sequences.”

What is the three dimension structure of DNA?

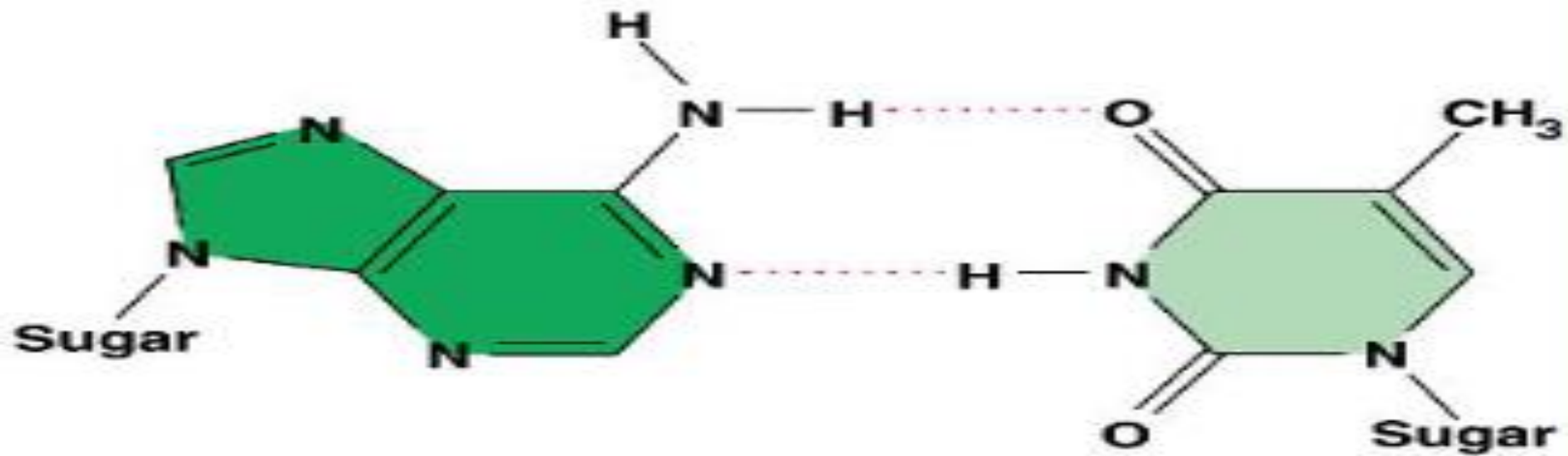


- DNA Strand tend wind over another DNA strand to form a double helix structure of DNA.
- Therefore the DNA form a double stranded DNA with 2nm diameter
- The nitrogenous bases on one strand interact with another nitrogenous base on another strand through hydrophobic interaction.
- This base pairing is the driving force for formation of double stranded DNA.
- The base pairing occurs in a specific sequence e.g
 - Adenine with thymine
 - Cytosine with guanine
- backbone is hydrophilic and the side chains are hydrophobic.
- The arrangement of deoxyribonucleotides is shown the next slide.

What is the three dimension structure of DNA?

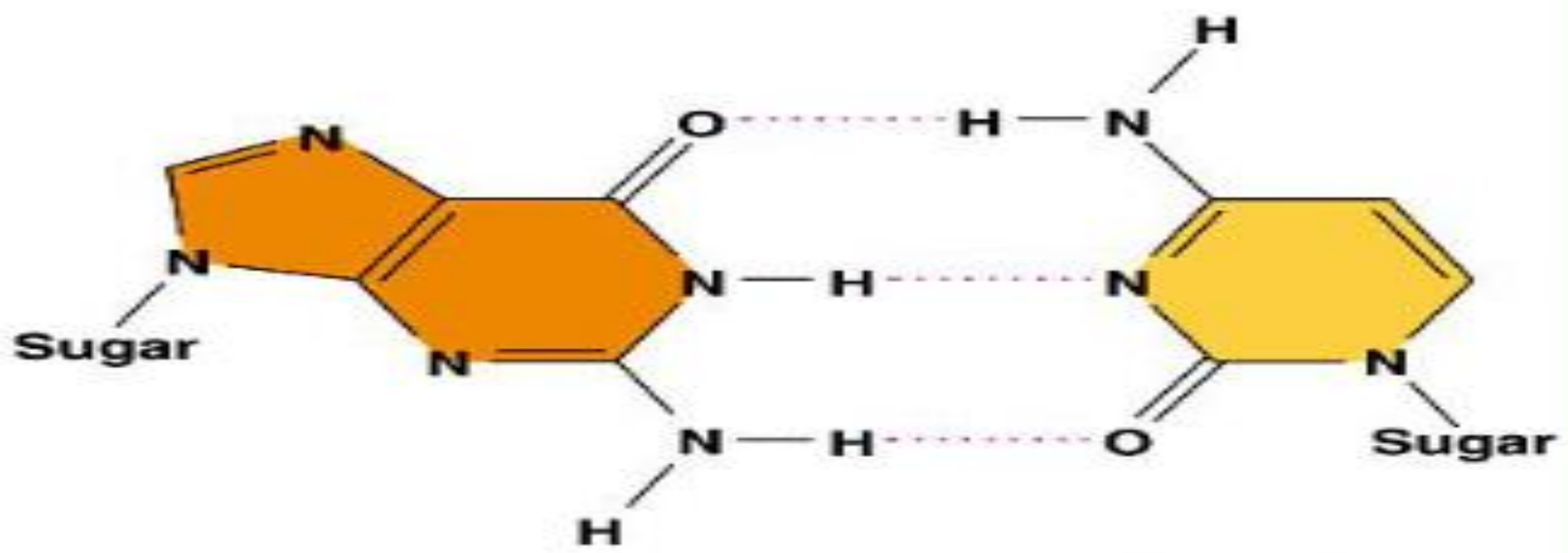


- The base pairing that forms a double helix with 2nm diameter is the purine-pyrimidine base pairing.
- A purine-purine base pairing forms a double helix with a distorted double stranded DNA because the diameter will be too big.
- A pyrimidine-pyrimidine base pairing forms a double helix with a distorted double stranded DNA because the diameter will be too small.
- Therefore in nature you find the purine-pyrimidine base pairing.
- Adenine forms two hydrogen bond with thymine.
- Guanine forms three hydrogen bond with cytosine
- The base pairing are stabilized by hydrogen bonding as shown in the next slide



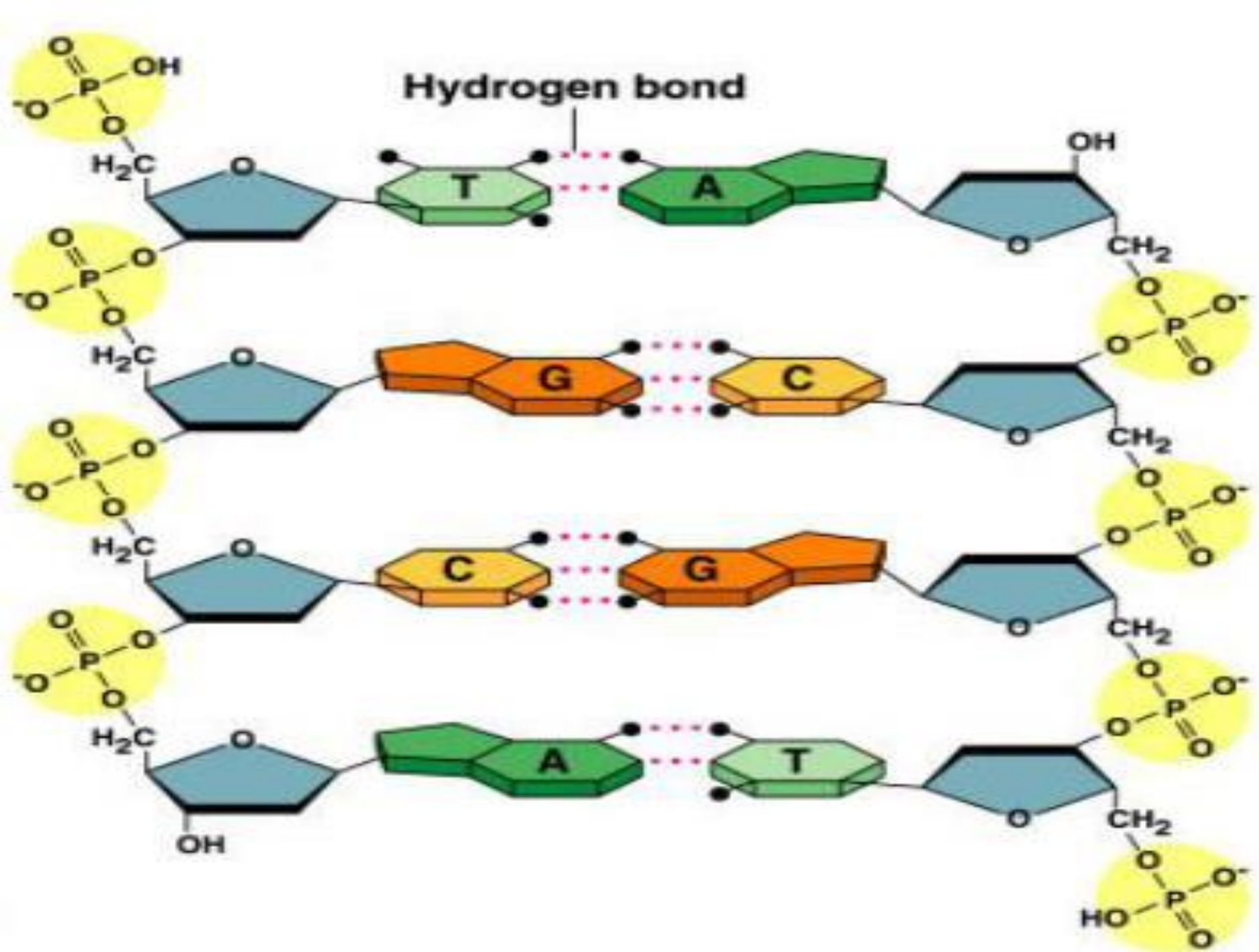
Adenine (A)

Thymine (T)



Guanine (G)

Cytosine (C)



What are Chargaff's rules?



- The base pairing mentioned was found through the work of Erwin Chargaff and his colleagues in the late 1940s.
- They found that the four nucleotide bases of DNA occur in different ratios in the DNAs of different organisms and that the amounts of certain.
- They led Chargaff to the following conclusions:
- The base composition of DNA generally varies from one species to another.
- DNA specimens isolated from different tissues of the same species have the same base composition.
- The base composition of DNA in a given species does not change with an organism's age, nutritional state, or changing environment.
- In all cellular DNAs, regardless of the species, the number of adenosine residues is equal to the number of thymidine residues (that is, $A = T$), and the number of guanosine residues is equal to the number of cytidine residues ($G = C$).

What are Chargaff rules?



- From these relationships it follows that the sum of the purine residues equals the sum of the pyrimidine residues; that is, $A + G = T + C$.
- These quantitative relationships, sometimes called “Chargaff’s rules,”
- **PROBLEM NO 1**
- A sample of a double DNA was found to have thymidate as 23% of the nucleotide residues. What should be the %GC of this DNA?
- **ANSWER**
- dsDNA has the following base ratios
- $A:T = 1:1$ and $G:C = 1:1$
- Since %T = 23% it implies that % A = 23% also
- Total A:T = $2 \times 23\% = 46\%$
- Therefore $G:C\% = 100 - 46 = 54\%$
-

What are Chargaff rules?



- **PROBLEM NO 2**
- A sample of a double stranded DNA was found to have Guanylate as 23% of the nucleotide residues. What should be the %A of this DNA?
- **ANSWER.**
- dsDNA has the following base ratios
- A:T = 1:1 and G:C = 1:1
- Since %G = 23% it implies that % C = 23% also
- Total G:C = $2 \times 23\% = 46\%$
- Therefore A:T = $100 - 46 = 54\%$
- Hence A% = $54/2 = 27\%$

What are the DNA conformations?



	B-DNA	A-DNA	Z-DNA
1	Diameter 20Å,	Diameter 26Å	Diameter 18Å
2	base pairs per helical turn 10.5	base pairs per helical turn 11	base pairs per helical turn 11 (12)
3	helix rise per base pair 3.4Å	helix rise per base pair 2.6Å	helix rise per base pair 3.7Å
4	sugar pucker conformation C-2' endo	sugar pucker conformation C-3' endo	(C-2 endo for pyrimidines; C-3 endo for Purines)
5	Planes of the bases are perpendicular to the helical axis (Planes of the bases are 7 degrees away from perpendicular the helical axis)	Planes of the bases are 7 degrees away from perpendicular the helical axis	Planes of the bases are 20 degrees away from perpendicular the helical axis

What is DNA denaturation?



- When a solution of duplex DNA is heated above a
- characteristic temperature, its native structure collapses and its two complementary strands separate and assume a flexible and rapidly fluctuating conformational state known as a random coil.
- This is called denaturation process.
- The denaturation of DNA may be described as the melting DNA.
- The temperature at which the DNA is half way separated is known as the melting temperature, T_m .
- Denaturation is accompanied by a qualitative change in the DNA's physical properties.
- For instance, the characteristic high viscosity of native DNA solutions drastically decreases when the duplex DNA decomposes (denatures) to two relatively freely jointed single strands.
- DNA Denaturation increases its absorption of UV light
-

What is DNA denaturation?



- Strand separation occurs over a temperature range.
- The midpoint is called the melting temperature (T_m)
- T_m is influenced by the base composition of the DNA and salt concentration of the solution.
- DNA rich in G:C pairs, with three hydrogen bond smelts at a higher temperature than that rich in A:T pairs, which have two hydrogen bonds.
- A tenfold increase in monovalent cation concentration increases the T_m by 16.6°C .
- Formamide destabilizes hydrogen bonding between bases, thereby lowering the T_m .
- For every 1% increase in formamide concentration, T_m reduces by 0.5°C
- Separated strands of DNA will renature when appropriate physiologic and salt conditions are achieved.

What is DNA denaturation?



- **PROBLEMS 3**

- Calculate the final melting temperature of a dsDNA molecule whose initial $T_m=78^\circ\text{C}$, If it is placed in a 90% of Formamide solution.

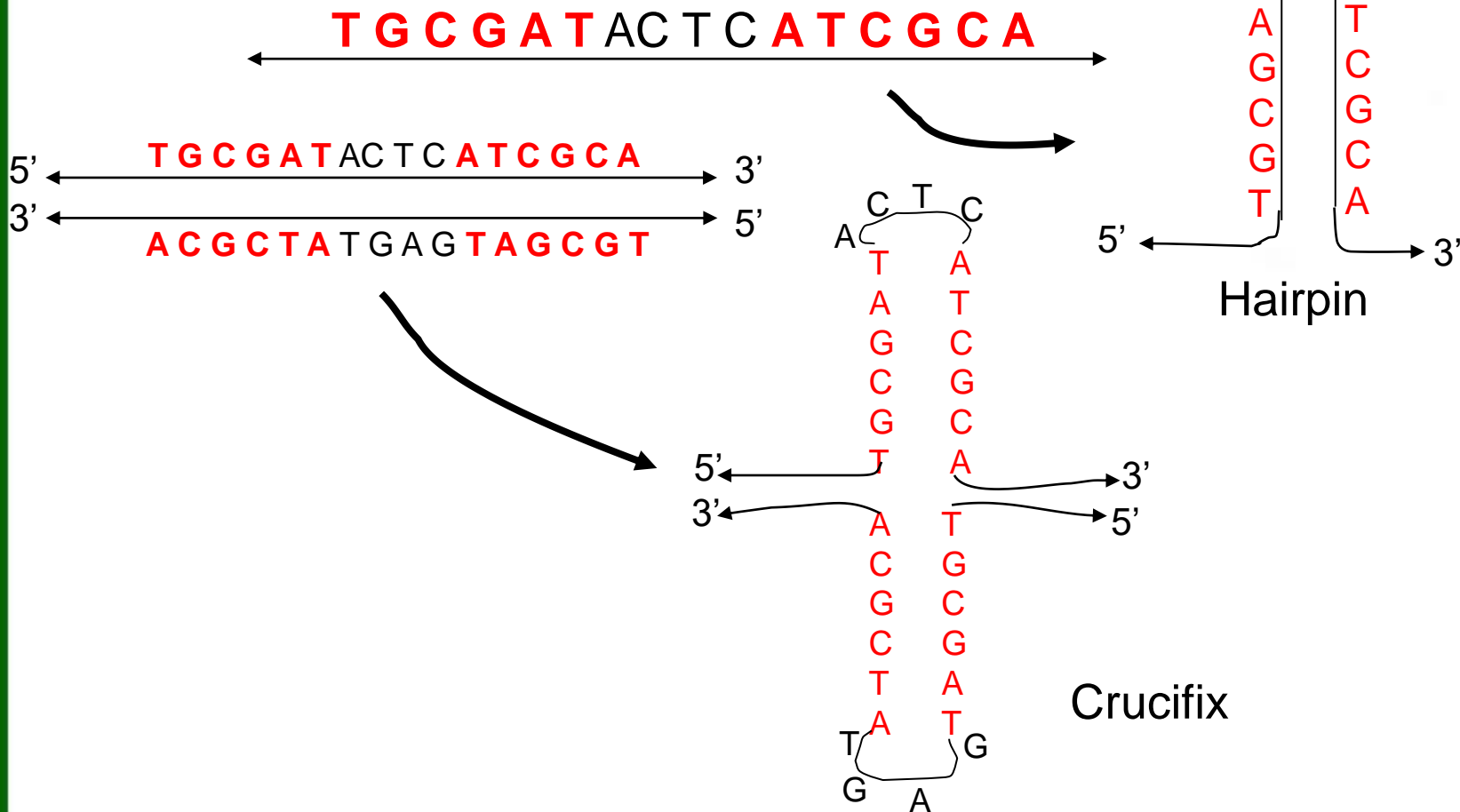
- **ANSWER**

- Initial $T_m= 78^\circ\text{C}$.
- For every 1% of formamide, T_m decreases by 0.5°C .
- 90% of formamide, will decrease T_m by x .
- Therefore $x = 90\% \times 0.5^\circ\text{C} / 1\% = 45^\circ\text{C}$.
- Hence final $T_m = 78 - 45 = 33^\circ\text{C}$

Unusual DNA structure



Certain DNA sequences adopt unusual structures



What is a palidrome?



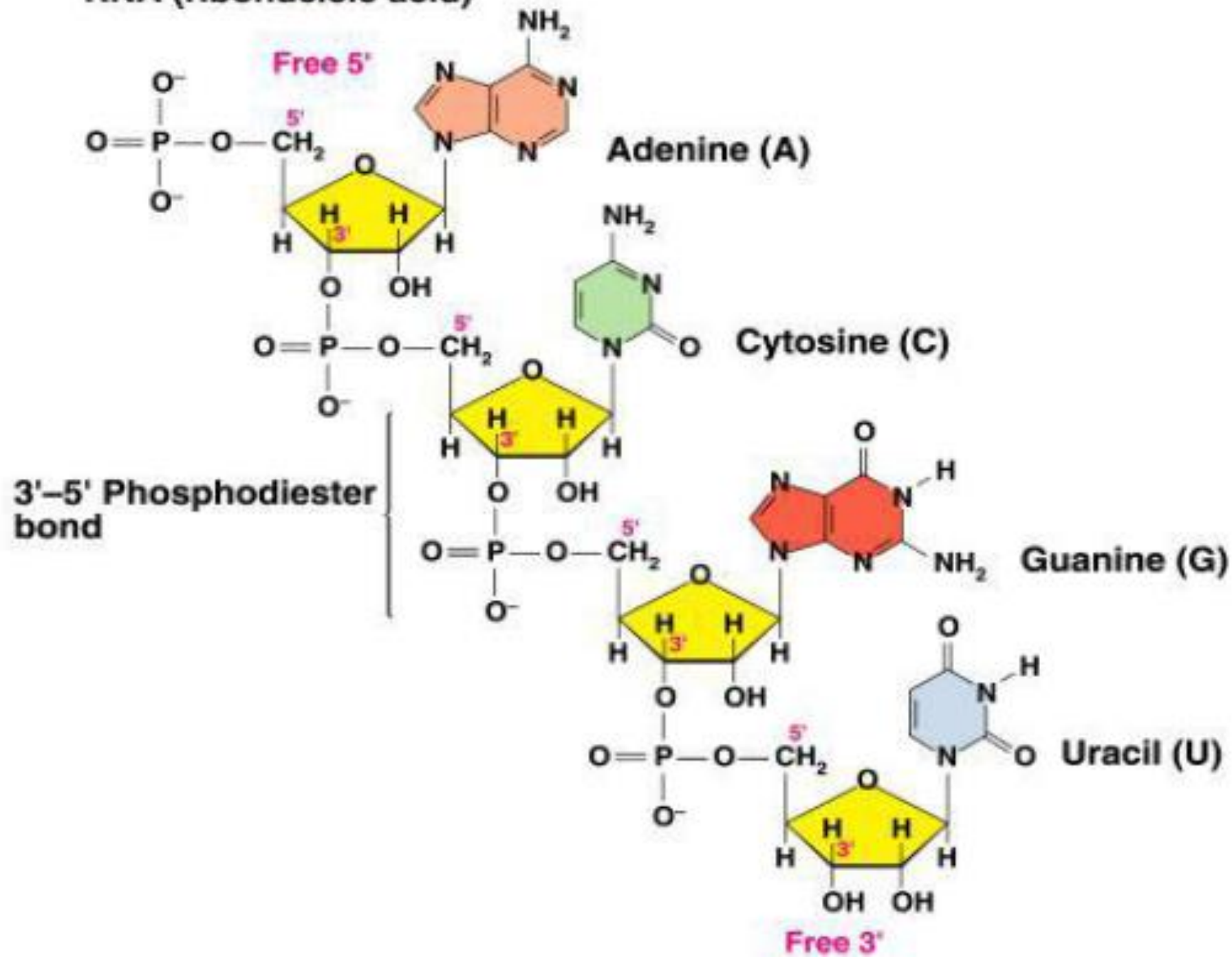
- Regions of DNA with inverted repeats of base sequence having two fold symmetry over the strands of DNA is called a palidrome.
- A palidrome has the potential to form hairpin or crucifix structures.
- Palidrome can be defined as a sequence or word or verse that read the same backward and forward e.g the following word can read the same in both direction.
 - Radar.
 - Repaper.
 - Madam.

What is a RNA?



- The polymer of ribonucleotides is RNA.
- It is formed when triphosphate ribonucleotides are joined to each other to form a long strand of ribonucleotides.
- A long strand of ribonucleotides like the deoxyribonucleotides is made
- of a backbone made up of phosphate alternating with ribose sugar and branches or appendages of nitrogenous bases.
- The backbone is hydrophilic and the side chains are hydrophobic.
- The arrangement of ribonucleotides is shown the next slide.

RNA (ribonucleic acid)



What is an RNA?

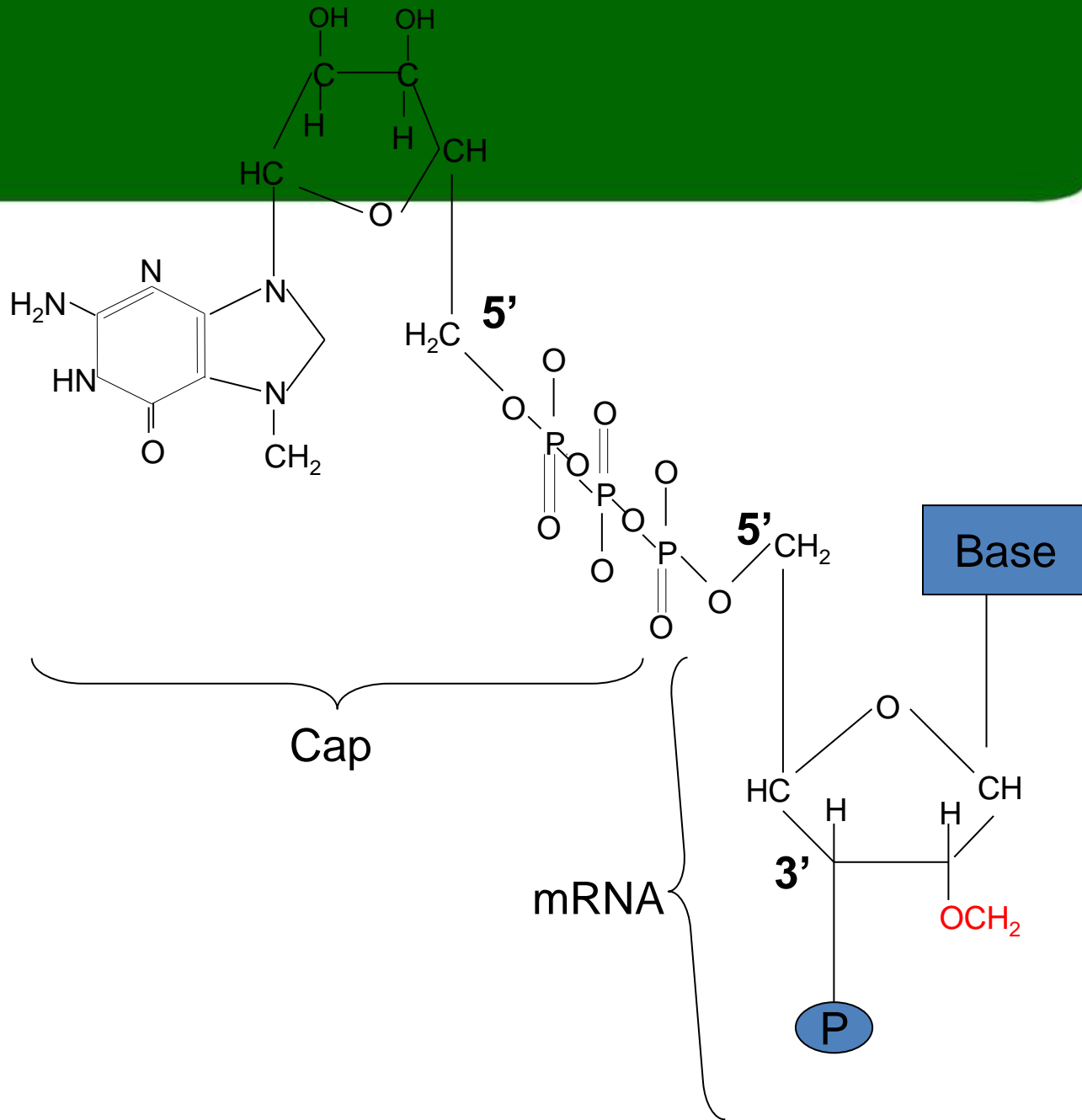


- Examples of RNA include:
 - Messenger RNA (mRNA).
 - Ribosomal RNA (rRNA).
 - Transfer RNA (tRNA)
 - Small nuclear RNA (snRNA)
- mRNA - transfer of genetic material from DNA to protein synthesizing machinery.
- rRNA – have structural roles and contribute to the formation and function of ribosomes (the organellar machinery for protein synthesis).
- tRNA – adaptor molecules for translation of RNA information into specific sequence of polymerized amino acids
- Small nuclear RNA (snRNA) are not directly involved in protein synthesis but play pivotal roles in RNA processing.

What is an mRNA?



- mRNA is the most heterogeneous in size and stability.
- Its 5'terminal of mRNA is 'capped' by a 7-methylguanosine triphosphate that is linked to the adjacent 2' O-methylribonucleoside at its 5' hydroxyl end through the three phosphates.
- The cap is involved in the recognition of mRNA by the translating machinery
- and prevents the mRNA from being attacked by 5'-exonucleases.
- The protein-synthesizing machinery begins translating the mRNA into proteins beginning down stream of the capped terminal



What is an mRNA?



- The 3' hydroxyl terminal has an attached polymer of adenylate residues of about 20-250 nucleotides in length, which is called the poly (A) 'tail'.
- The poly A tail probably maintains the stability of mRNAs by preventing the attack by 3'-exonucleases.
- Some mRNAs do not contain poly A tails.
- The poly A tail forms a base pair with oligodeoxythymidine polymers attached to a solid phase – can be used to separate mRNA from other species of RNA or mRNA that lacks this tail.
- In mammalian nuclei the immediate products of gene transcription are the heterogeneous nuclear RNA (hnRNA) molecules.
- These molecules are processed in the nuclei to mature mRNA which then enters the cytoplasm

What is a tRNA?

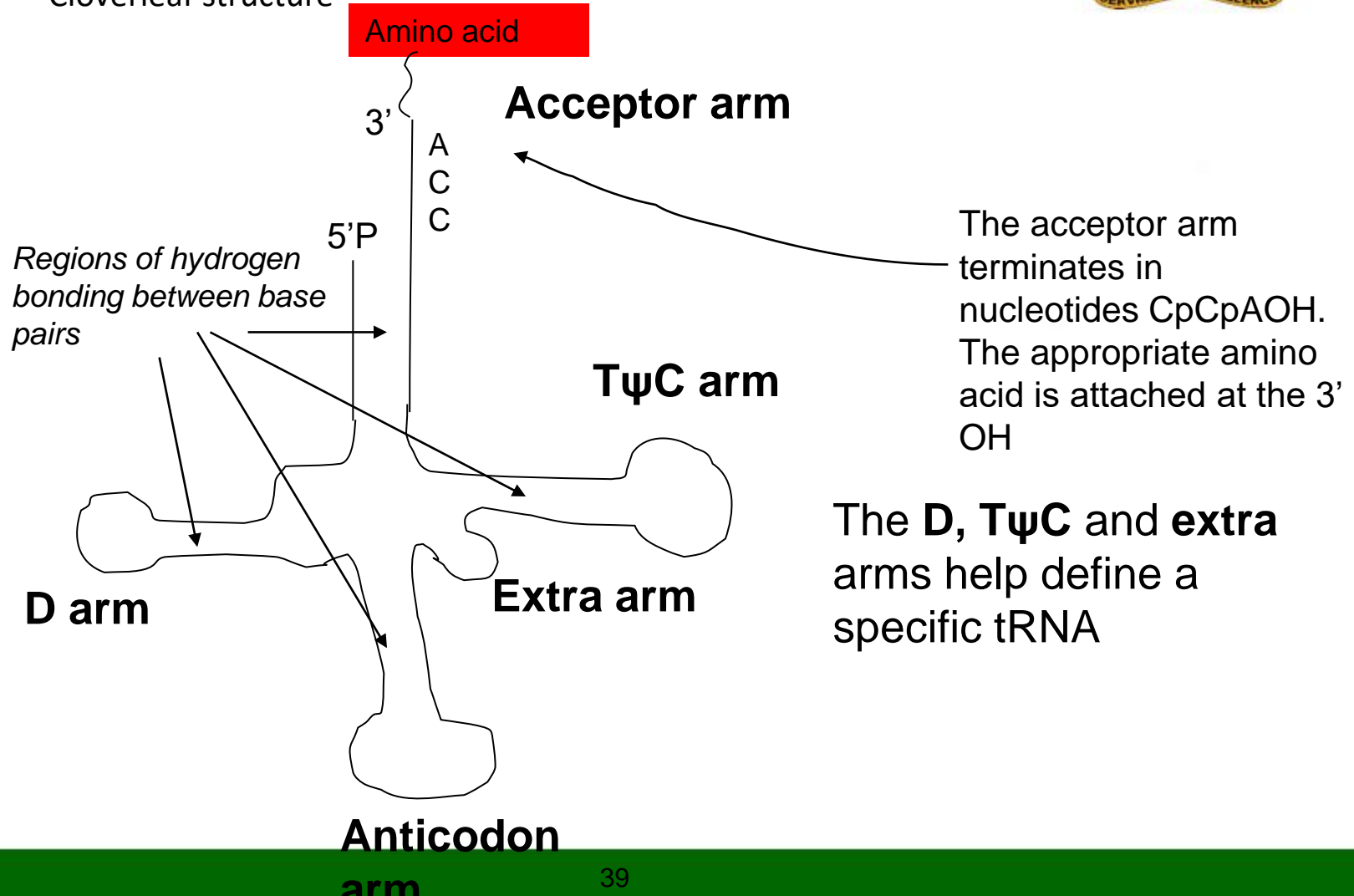


- The tRNA is an Adaptor molecules for the translation of information in the sequence of mRNA nucleotides into specific amino acids.
- The tRNA vary in length from 74 – 95 nucleotides.
- Also generated by nuclear processing of a precursor molecule.
- There are at least 20 species of tRNA molecules.
- At least one (often several) correspond to each of the 20 amino acids required for protein synthesis.
- Though tRNA molecules may differ in sequence of nucleotides, they have many feature in common.
- The primary structure, nucleotide sequence of all tRNA molecules allows extensive folding and intrastrand complimentarity to generate a secondary structure that appears like a cloverleaf or L shaped.
- tRNAs are less stable in eukaryotes and more stable in prokaryotes.



What is the structure of tRNA?

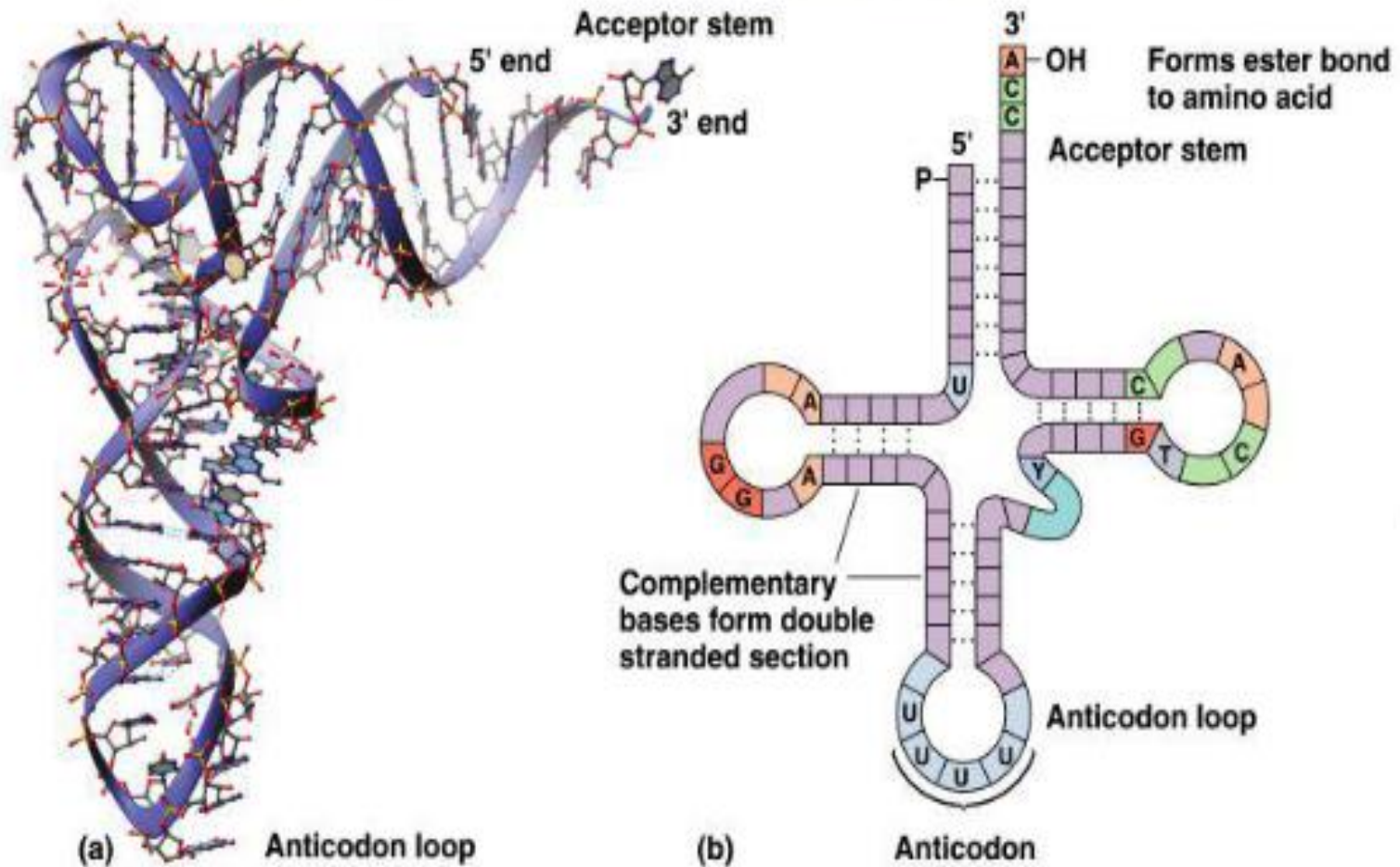
- Cloverleaf structure



What is an tRNA?



- A ribosome is a cytoplasmic nucleoprotein structure that



What is an rRNA?



- A ribosome is a cytoplasmic nucleoprotein structure that
- acts as the machinery for protein synthesis.
- On the ribosome the tRNA and mRNA interact to translate into a specific protein molecule information transcribed from the gene.
- In active protein synthesis many ribosomes are associated with a mRNA molecule in an assembly called polysome.
- Polysomes contains two major nucleoproteins in eukaryotes:
 - The 60S subunit (S is sedimentation rate in Svedberg units) contains 5S, 5.8S and 28S rRNA
 - The 40S subunit is smaller and contains a single 18S rRNA.
- All the rRNA molecules except the 5S rRNA are processed from a 45S precursor RNA molecule in the nucleolus.
- The highly methylated ribosomal RNA molecules are packaged in the nucleolus with the specific ribosomal proteins

What is a small RNA?



- Majority are complexed to protein to form ribonucleoprotein and are distributed in the nucleus, cytoplasm, or both.
- Small nuclear RNAs (snRNAs) are a subset involved in mRNA processing and gene regulation.
- U1, U2, U4, U5 and U6 are involved in intron removal and the processing of hnRNA.
- U7 may be involved in production of the correct 3' end of histone mRNA- which lacks the poly(A) tail.
- U4, and U6 may be required for poly(A) processing

What is a microRNA?



- Micro RNA (miRNA) are only 20–22 nucleotides long.
- They are important in the timing of an organism's development.
- They play important roles in cancer, stress responses, and viral infections.
- They inhibit translation of mRNA into protein and promote the degradation of mRNA.
- It was recently discovered, however, that these versatile RNAs can also stimulate protein production in cells when the cell cycle has been arrested.

What is a small interfering RNA?



- Small Interfering RNA (siRNA) are short stretches of RNA of about 20–30 nucleotides long.
- They have an enormous control over gene expression.
- This process serves as a protective mechanism in many species.
- The siRNAs being used to eliminate expression of an undesirable gene, such as one that causes uncontrolled cell growth or one that came from a virus.
- siRNAs lead to the degradation of specific mRNA molecules

What is a nuclease?



- A nuclease is an enzyme that digests nucleic acids by hydrolysing the phosphodiester bonds.
- Deoxyribonucleases digest deoxyribonucleic acids.
- Ribonucleases digest ribonucleic acids.
- Within the above classes, are enzymes capable of cleaving internal phosphodiester bonds to produce either 5'-phosphoryl and 3'-hydroxyl terminals or 5'-hydroxyl and 3'-phosphoryl terminals, these are known as endonucleases.
- Endonucleases that recognize specific sequences of DNA are referred to as restriction endonucleases.
- An exonuclease hydrolyses a nucleotide only when it is at the terminal of a molecule.
- Exonucleases act only in one direction ($3' \rightarrow 5'$ or $5' \rightarrow 3'$)

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What is the difference between RNA and DNA?



- **SUGAR CONTENT**

- Ribose is RNA and deoxyribose in DNA.
- The pyrimidine components-Uracil is not found in DNA and thymine is not found RNA.

- **NATURE OF STRAND.**

- RNA exists as a single strand.
- However given the proper complimentary base sequence with opposite polarity, the single strand of RNA is capable of folding back on it self like a hairpin and thus acquiring double stranded characteristics.
- DNA is a double stranded structure.

- **BASE CONTENT**

- Since RNA is single stranded the guanine content does not necessarily equal its cytosine content, nor does its adenine content necessarily equal to its uracil content as in in DNA

What is the difference between RNA and DNA?



- **STABILITY OF A NUCLEIC ACIDS**
- Because of the presence of 2' hydroxide group RNA can be hydrolysed by alkali to 2',3' cyclic diesters of the mononucleotides as shown in the next slide.
- This compounds that can not be formed from alkali-treated DNA because of the absence of the hydroxyl group at C-2' of the ribose.
- Therefore the DNA is more stable than RNA



What is the difference between RNA and DNA?

- **INSTABILITY OF A RNA**

