Nucleic Acid Chemistry

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• NUCLEIC ACIDS

Are the largest and the most complex organic molecules. Friedrich Miescher who discovered nucleic acids in 1871

- Nucleic Acids are present in all living organisms.
- Nucleic Acids are those substances which are present in the nucleus and showing acidic properties.
- Genetic information is present in most of the organisms in DNA and in some virus in RNA.
- Nucleic Acid store information and transmit information from a parent cell to daughter cells. This information is used to synthesis proteins.
- Nucleic Acids are polymers of specific sequence of subunits or monomers are called nucleotides.

Nucleic Acids

- **Nucleic acids** are molecules that store information for cellular growth and reproduction

- There are two types of nucleic acids:
  - **deoxyribonucleic acid (DNA)** and **ribonucleic acid (RNA)**

- These are polymers consisting of long chains of monomers called nucleotides

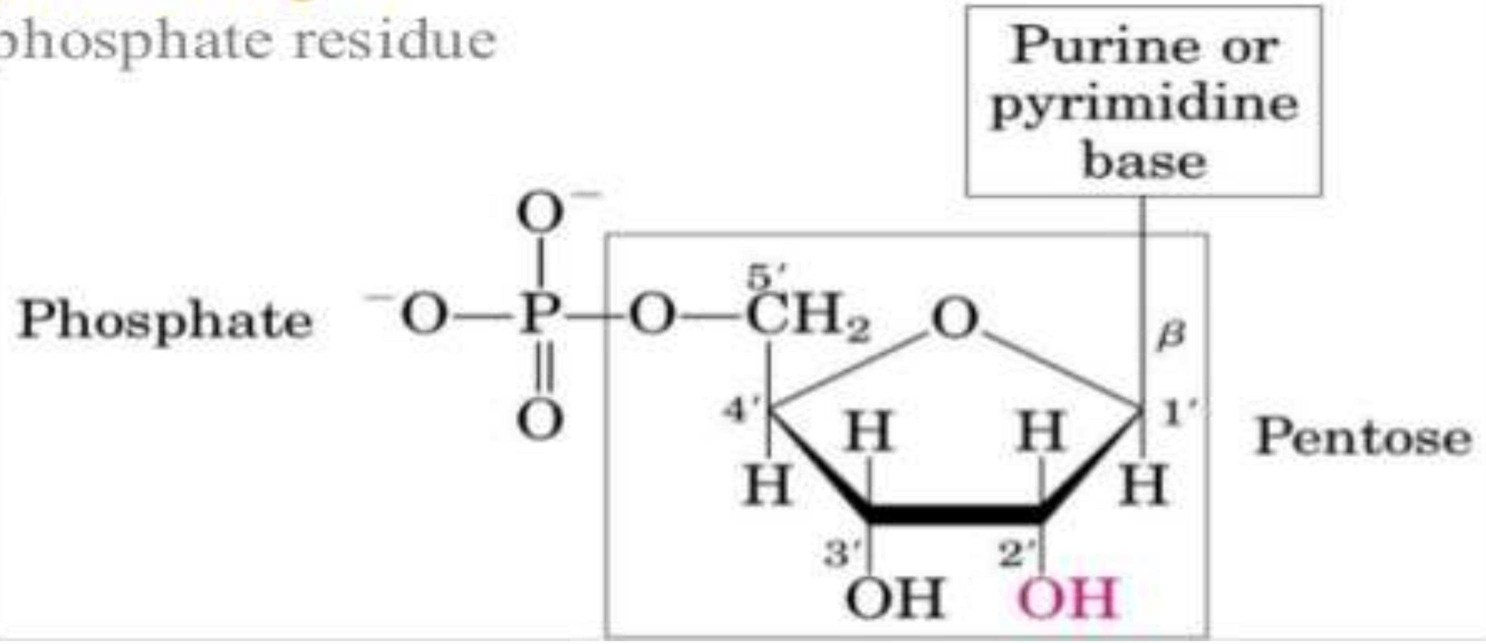
- A **nucleotide** consists of a nitrogenous base, pentose sugar and a phosphate group.

Nucleic Acids

DNA and RNA are nucleic acids, long, thread-like polymers made up of a linear array of monomers called nucleotides

All nucleotides contain three components:

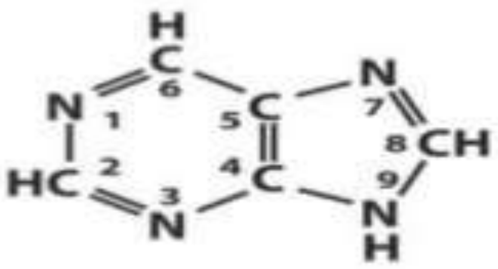
1. A nitrogen heterocyclic base
2. A pentose sugar
3. A phosphate residue



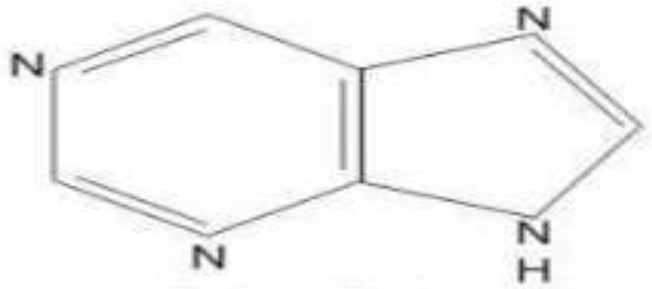
Nitrogenous base (N base):-

- N bases are of two types i.e. Purines and Pyrimidine.
- All nitrogenous base of nucleic acids are derived from two heterocyclic bases purine and pyrimidine.
- Purines are Adenine and Guanine.
- Pyrimidine are cytosine, uracil and thymine.

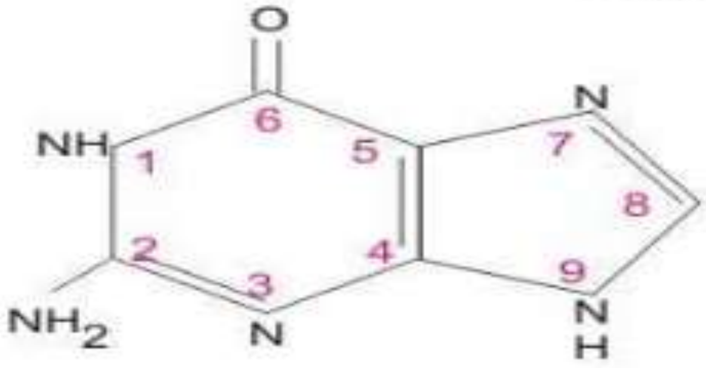
Purine Nucleotides



Purine

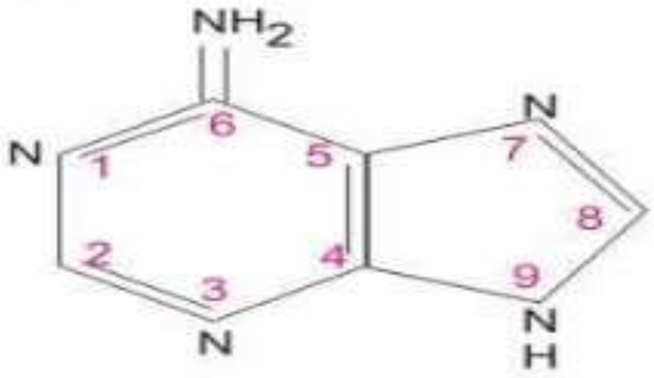


Purine Molecule



Guanine

2-amino-6-oxypurine



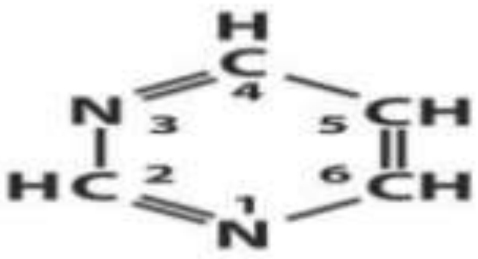
Adenine

6-aminopurine

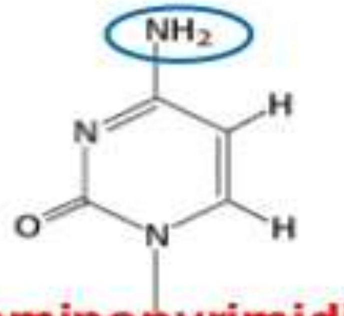
Purines

- Purine bases are nine membered ring structures consisting of pyrimidine ring fused to imidazole ring.
- The atoms of purine ring are numbered in the anticlockwise manner.
- Major bases in nucleic acids: Adenine & Guanine
- Adenine (6-amino purine):
- It contains amino group at 6^{th} position
- Guanine (2-amino 6-oxypurine):
- It contains amino group at 2^{nd} position & oxygen at 6^{th} position.

Pyrimidines: Cytosine, Uracil & thymine (CUT)

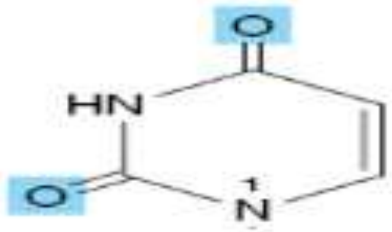


Pyrimidine



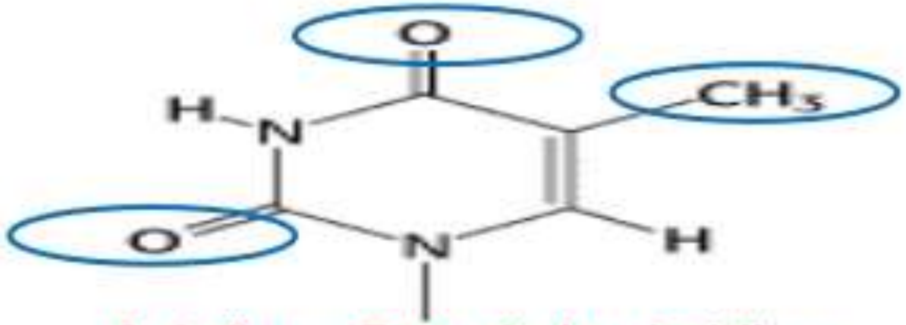
2-oxy-4-aminopyrimidine

Cytosine (C)



2,4-dioxy pyrimidine

Uracil (U)

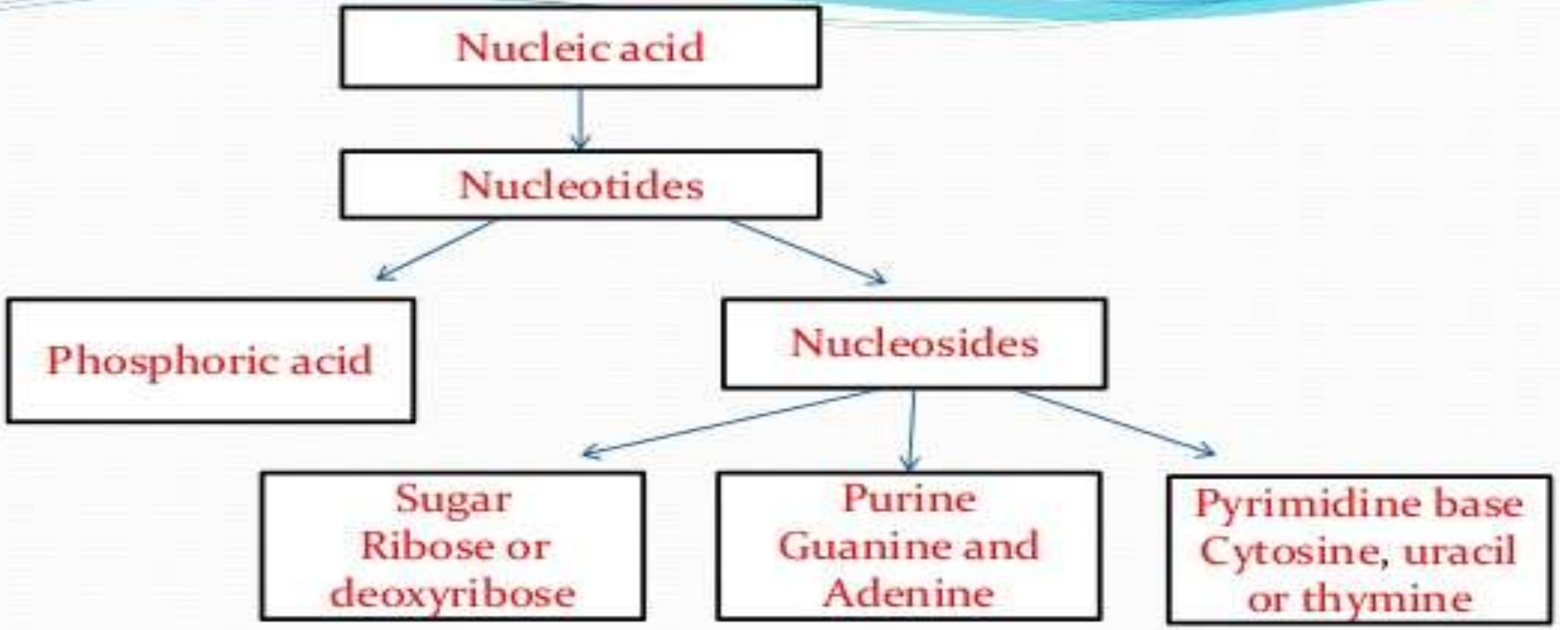


2,4-dioxy-5-methylpyrimidine

Thymine (T)

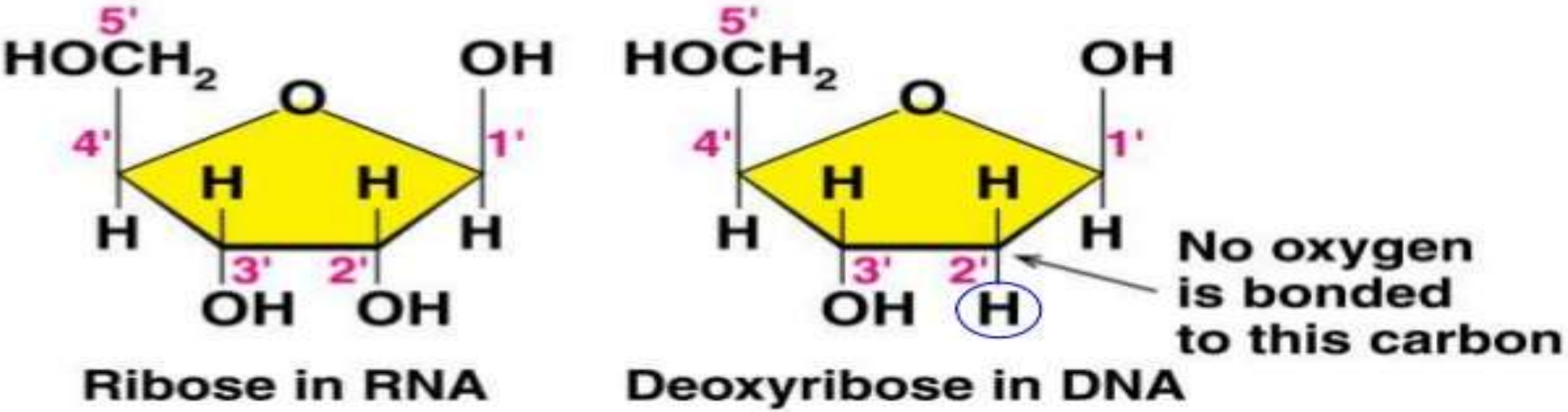
Pyrimidines

- Cytosine: Cytosine is found in both RNA & DNA.
- Cytosine (2-oxy,4-amino pyrimidine) has oxygen at position 2 & amino group at position 4.
- Uracil: Uracil is found only in RNA.
- Uracil (2,6-dioxy-pyrimidine) has oxygens at position 2 & 4
- Thymine:
- Thymine found in DNA and thymine (methyluracil) has oxygen at position 2 & 4, methyl group at position 5

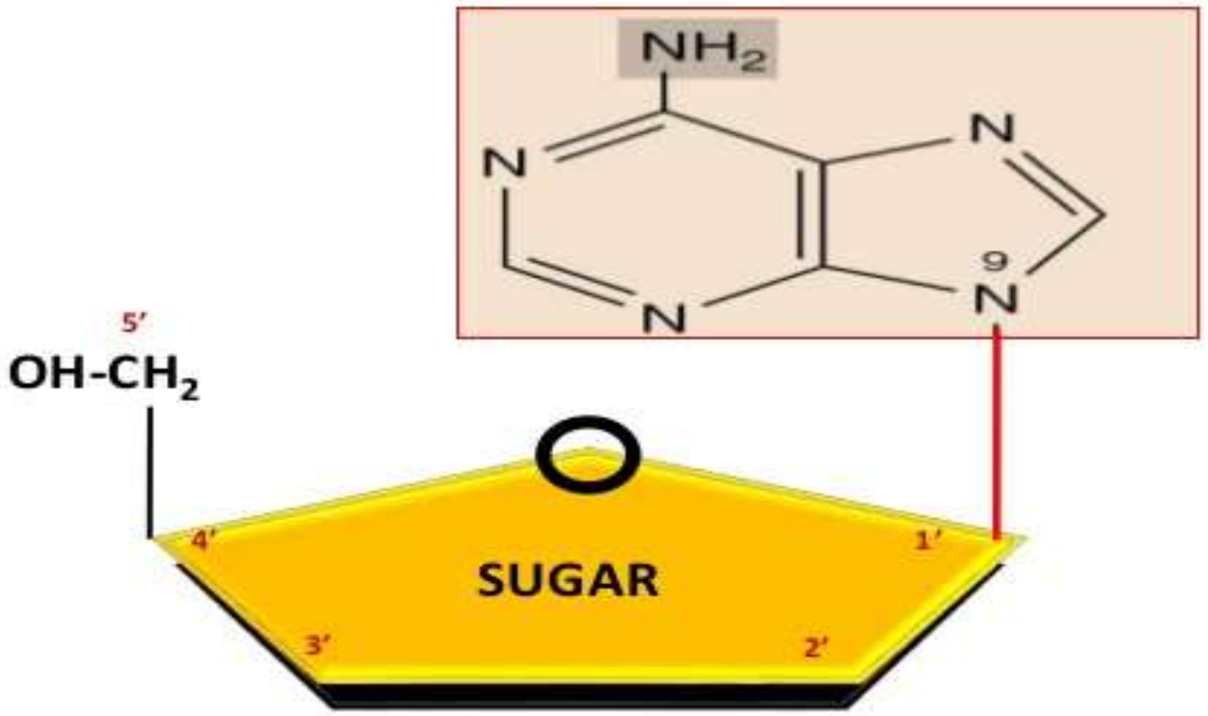

Nucleic acid
Nucleotides
Phosphoric acid
Nucleosides
Sugar
Ribose or
deoxyribose
Purine
Guanine and
Adenine
Pyrimidine base
Cytosine, uracil
or thymine

Sugars Present in DNA & RNA

- DNA & RNA are distinguished on the basis of the pentose sugar present.
- DNA contains β-D-2-deoxyribose
- RNA contains β-D-ribose.



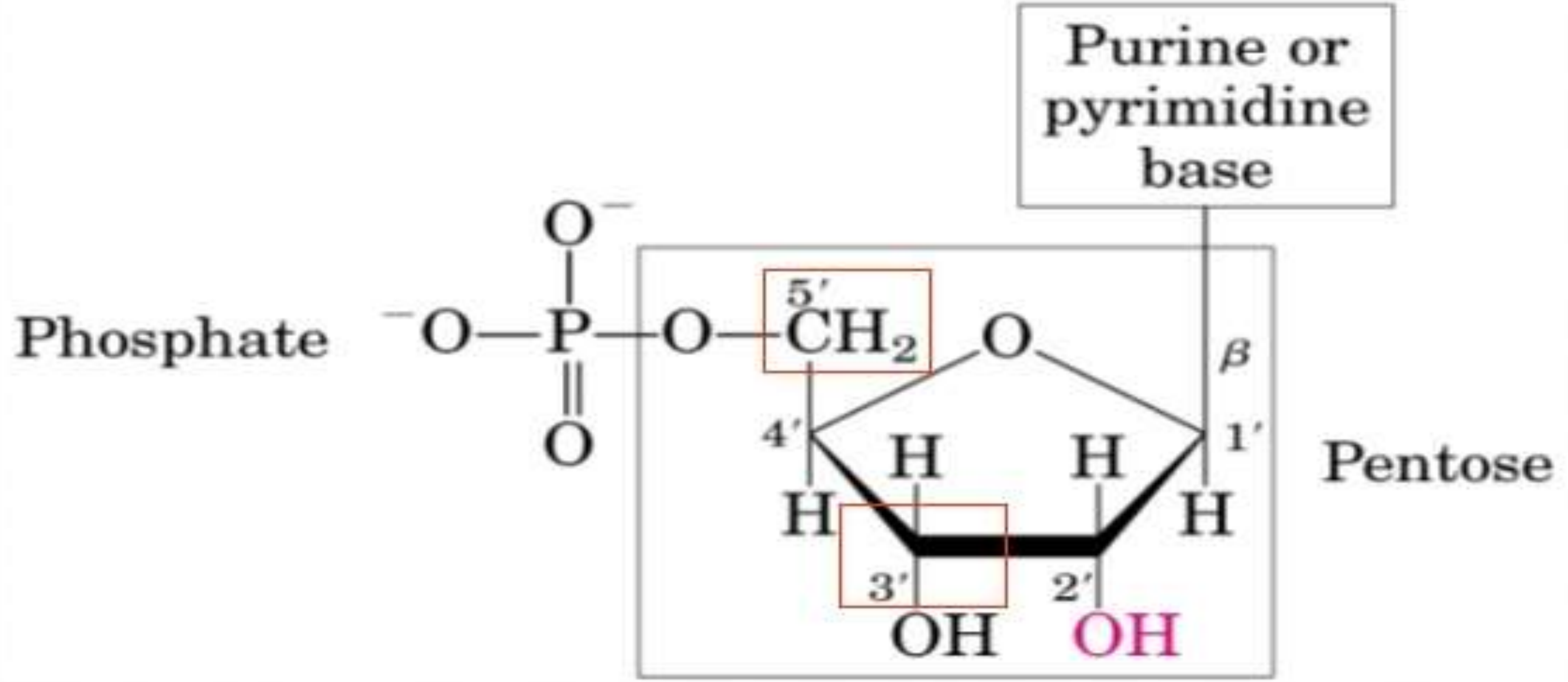
Nucleoside



Chemical Structure of DNA vs RNA

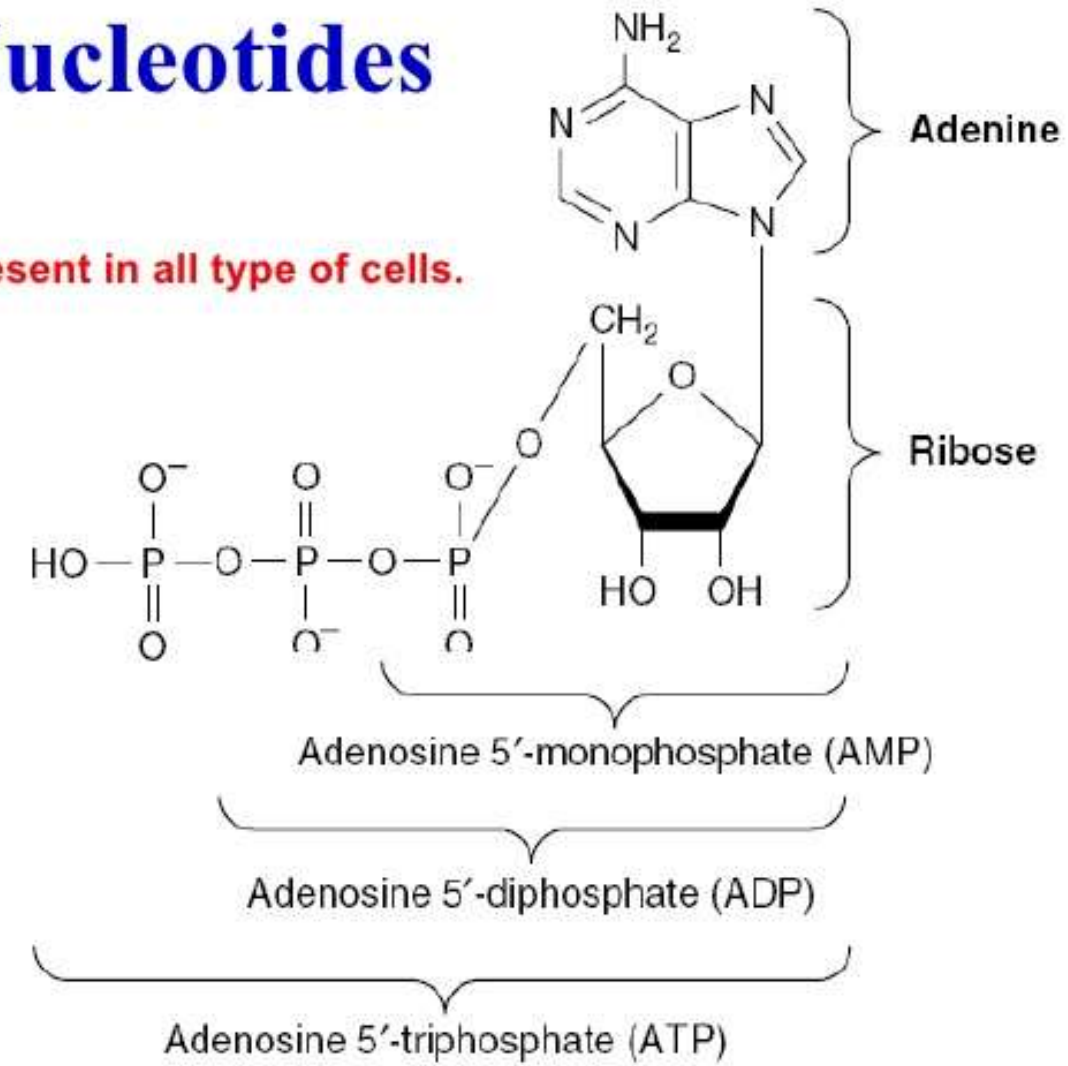
Ribonucleotides have a 2'-OH

Deoxyribonucleotides have a 2'-H

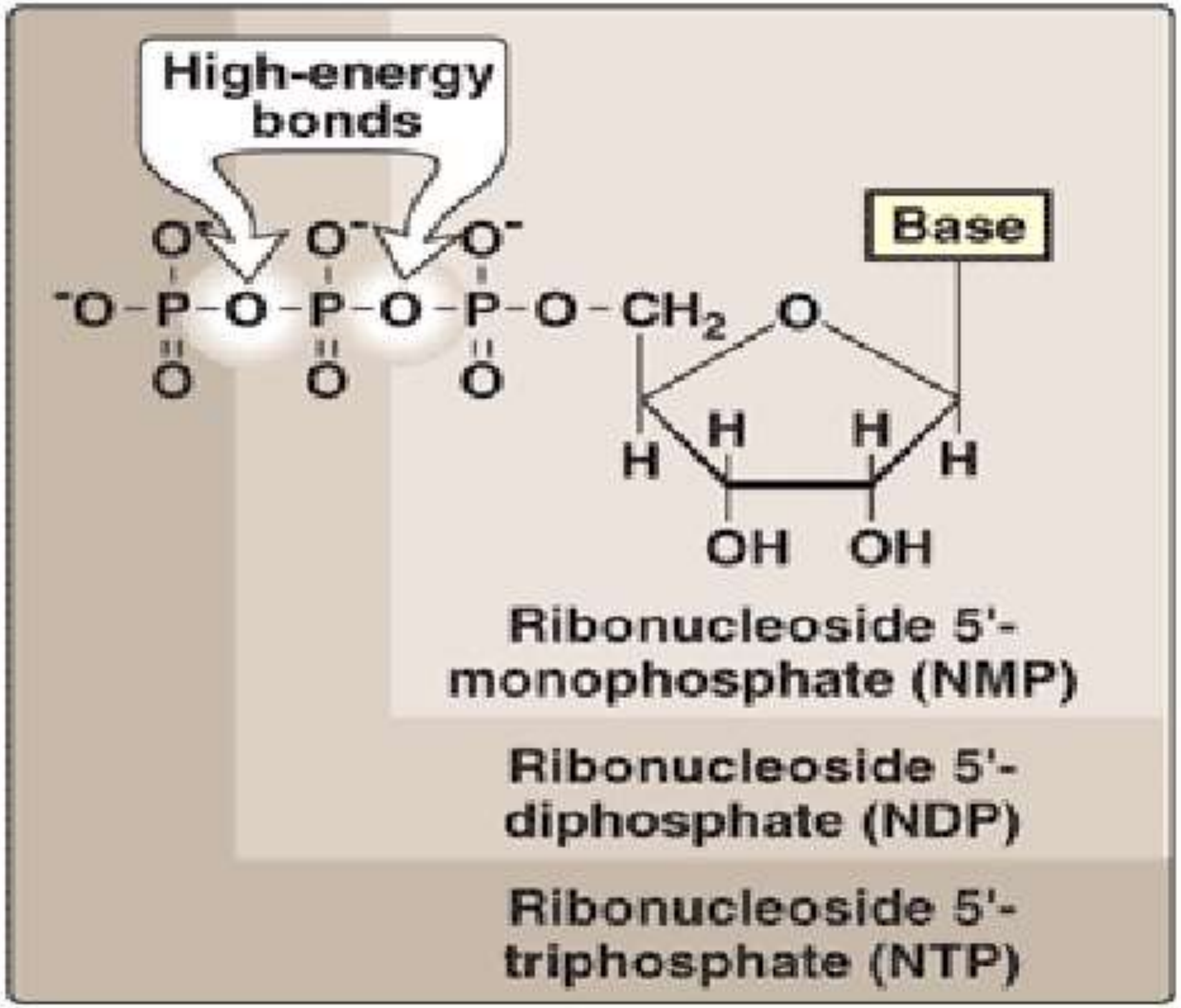


Nucleotides

Nucleostides are present in all type of cells.



Mononucleotides are nucleosides with a phosphoryl group esterfied to a hydrosyl group of the sugar. 3'- and 5' –nucleotides are nucleosides with a phosphoryl group on the 3' – or 5' –hydroxyl group of the Sugar.



Ribonucleoside monophosphate, diphosphate, and triphosphate.

Principal Nucleotides

- Ribonucleotides are named as
- Adenine = Adenosine monophosphate (AMP)
- Guanine = Guanosine monophosphate (GMP)
- Cytosine = Cytidine monophosphate (CMP)
- Thymine = Thymidine monophosphate (TMP)
- Uracil = Uridine monophosphate (UMP)
- Deoxyribonucleotides are named as
- Adenine = Deoxyadenosine monophosphate (dAMP)
- Guanine = Deoxyguanosine monophosphate (dGMP)
- Cytosine = Deoxycytidine monophosphate (dCMP)
- Thymine = Deoxythymidine monophosphate (dTMP)
- Uracil = Deoxyuridine monophosphate (dUMP)

Nucleotides

Base	Nucleosides	Nucleotides
RNA		
Adenine (A)	Adenosine (A)	Adenosine 5'-monophosphate (AMP)
Guanine (G)	Guanosine (G)	Guanosine 5'-monophosphate (GMP)
Cytosine (C)	Cytidine (C)	Cytidine 5'-monophosphate (CMP)
Uracil (U)	Uridine (U)	Uridine 5'-monophosphate (UMP)
DNA		
Adenine (A)	Deoxyadenosine (A)	Deoxyadenosine 5'-monophosphate (dAMP)
Guanine (G)	Deoxyguanosine (G)	Deoxyguanosine 5'-monophosphate (dGMP)
Cytosine (C)	Deoxycytidine (C)	Deoxycytidine 5'-monophosphate (dCMP)
Thymine (T)	Deoxythymidine (T)	Deoxythymidine 5'-monophosphate (dTMP)

Composition of Nucleotides

- Phosphate esters of nucleosides.
- Nucleotides are composed of nitrogenous base, a pentose sugar and phosphate
- Nucleotide = nucleoside + phosphate
- The esterification occurs at 5^{th} or 3^{rd} hydroxyl group of the pentose sugar
- *Most of the nucleoside phosphates involved in biological function are 5' phosphates.*
- 5' AMP is abbreviated as AMP, but 3' variety is written as 3'-AMP.

Minor purines present nucleic acids

- Several minor & unusual bases are often found in DNA & RNA
- These include 5-methylcytosine, N^4-acetylcytosine, N^6 methyl adenine, N^6 dimethyl adenine & N^7 methylguanine
- Importance:
- The unusual bases in nucleic acids help in the recognition of specific enzymes.

Minor (unusual) pyrimidines found in nucleic acids

- Methylcytosine present in DNA & dihydrouracil present in tRNA.
- Pyrimidine analogs:
- These have structural similarities to pyrimidines.
- They act either as inhibitors of enzymes in the metabolism of pyrimidines or interact with nucleic acids.
- 5-fluorouracil:
- It inhibits the enzyme thymidylate synthase.
- It is used in the treatment of cancer.

Minor/Unusual base

- Modification of Adenine:

  N-methyladenine,

  N_6N_6- dimethyladenine

- Modification of Guanine:

  7-methylguanine

- Modification of Cytosine:

  5-methylcytosine

  5-hydroxymethylcytosine

- Modification of Uracil:

  Dihydroxyuracil

- **Special Bases:**

  Hypoxanthine (6-oxopurine)

  Xanthine (2,6-dioxopurine)

  Uric acid (2,6,8-trioxopurine)

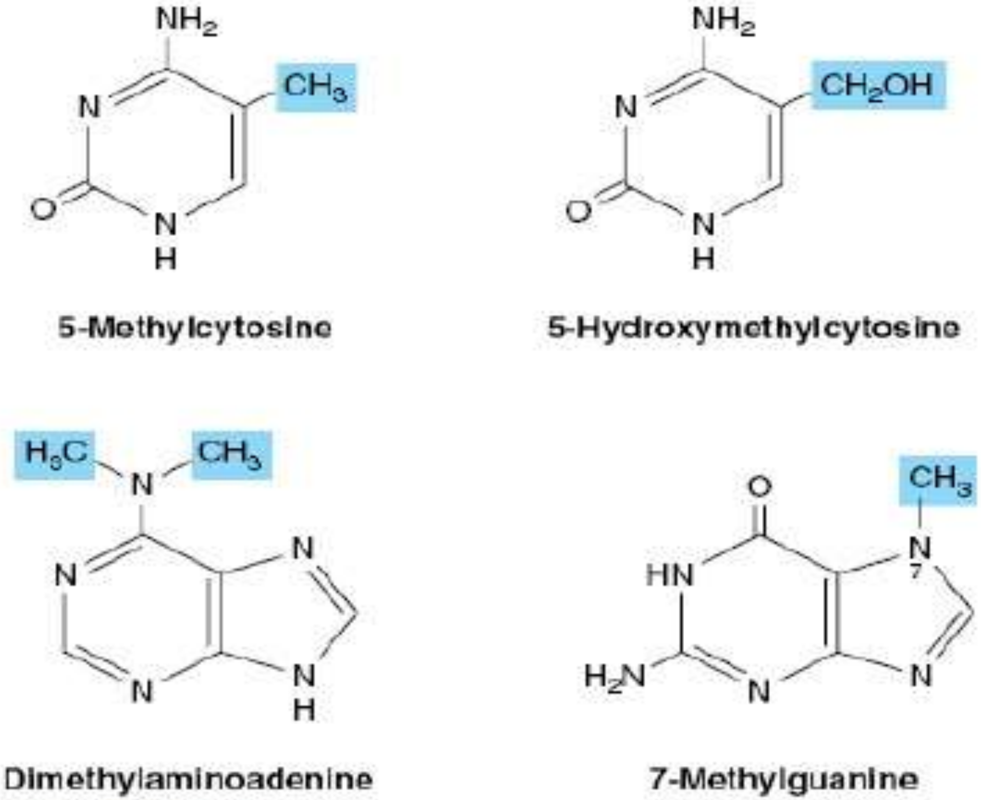


Figure 33–7. Four uncommon naturally occurring pyrimidines and purines.

Functions of Nucleotides

Functions of Nucleotides

- Activated precursors of DNA & RNA.
- ATP – Universal currency of energy.
- Required for activation of intermediates in many biosynthetic pathway.
- Carrier of methyl group in the form of SAM
- GTP-involved in protein biosynthesis as source of energy.
- Components of coenzymes: NAD, FAD & CoA.
- Metabolic regulators, e.g. cAMP, cGMP.

FUNCTION OF NUCLEIC ACIDS:

- **Functions of DNA (deoxyribonucleic acid):**
  -DNA is a permanent storage place for genetic information.
  -DNA controls the synthesis of RNA (ribonucleic acid).
  -The sequence of nitrogenous bases in DNA determines the protein development in new cells.

Some Important Nucleotides

- Adenine derivatives
- Guanine derivatives
- Cytosine derivatives
- Uracil derivatives
- Hypoxanthine derivatives
- Vitamin derivatives
- Synthetic derivatives

Physiological Important Nucleotides

- Nucleotides of Adenine
- Adenosine triphosphate (ATP)
- Adenosine diphosphate (ADP)
- Adenosine monophosphate (AMP)
- Cyclic adenosine monophosphate (cAMP)
- Phospho adenosine phospho sulfate (PAPS)
- S-adenosyl methionine (SAM)

Adenine nucleotides and their functions

1. ATP is energy currency of cell. In mammalian cells, its concentration is about 1 mM/L.
2. Oxidative phosphorylation of respiratory chain requires ADP. ADP is a high energy compound.
3. ATP, ADP and AMP are allosteric effectors of several enzymes.
4. Several hormones exerts their action through cyclic AMP or cAMP
5. Phosphoadenosine phosphosulfate (PAPS) is the donor of sulfate groups in many biosynthetic reactions
6. Adenine nucleotides are constituents of FAD and NAD^+, $NADP^+$ co enzyme A and vitamin B_{12} co-enzyme.
7. Diadenosine triphosphate and diadenosine poly phosphate are neurotransmitters and affect platelet aggregation and blood pressure.
8. Oligoadenylate is mediator for interferon action.
9. ATP is required for protein biosynthesis.

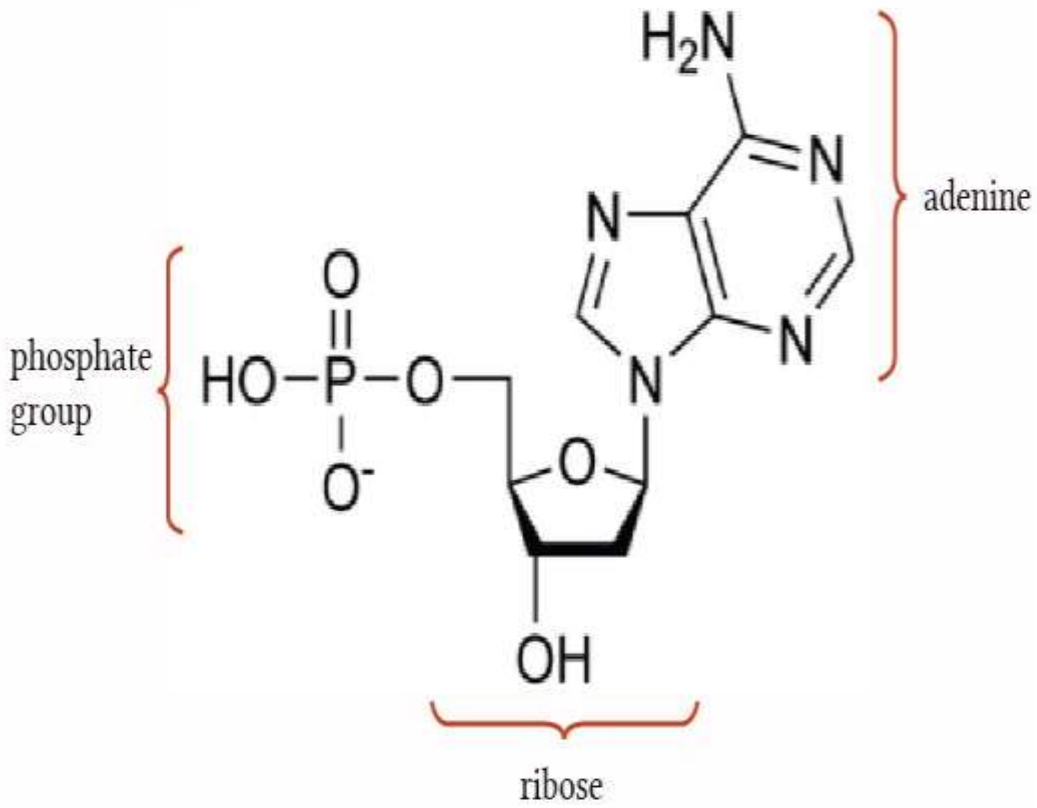
Cyclic AMP (cAMP)

- It is a major metabolic regulator.
- It mediates the action of several hormones at the cellular level.
- Adenylate cyclase of cell membrane being activated by the hormone epinephrine, norepinephrine and glucagon from cAMP from ATP.
- Thyroid hormones may increases the synthesis of adenylates cyclase.
- It is destroyed in tissues by its conversions to AMP by the enzyme phosphodiesterase.
- The intracellular cAMP concentrations are near 1 micromole.

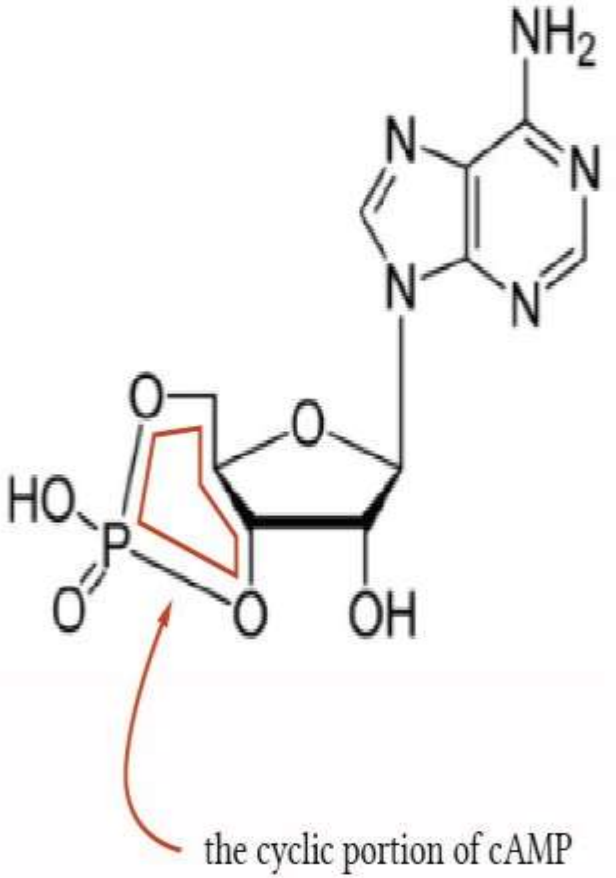
Functions of cAMP

- In the lungs it is involved in the regulation of many functions related to inflammatory cells.
- It supresses immune and inflammatory cell activity.

AMP



Cyclic AMP



Cyclic AMP and GMP

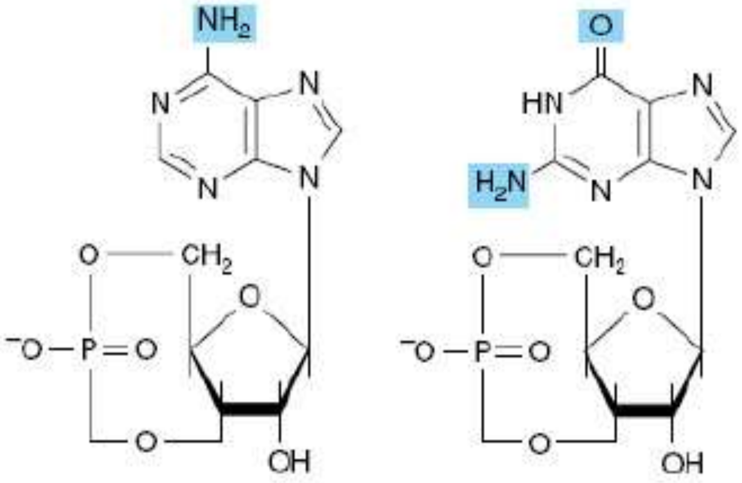
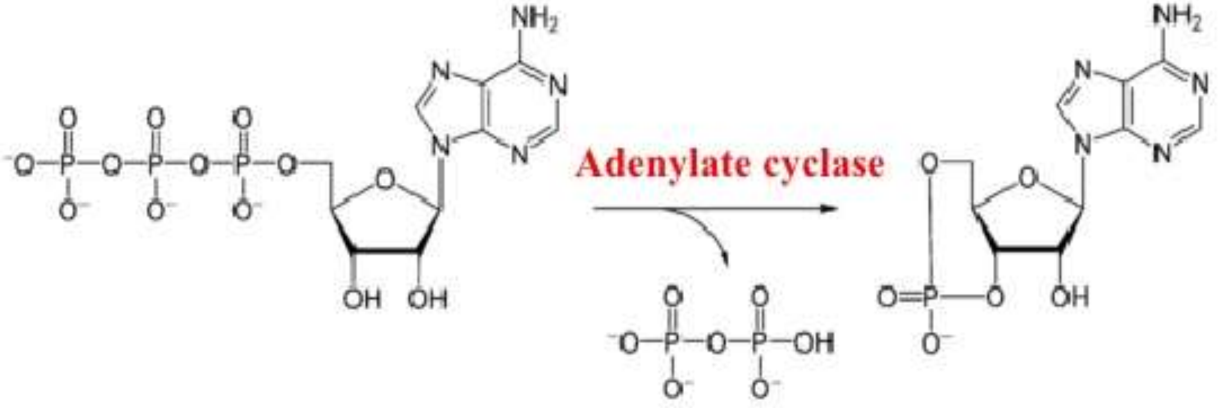


Figure 33–9. cAMP, 3′,5′-cyclic AMP, and cGMP.



Guanine nucleotides and their functions

1. GTP and GDP are high energy compounds. They participate in energy-dependent reactions.
2. GTP is required for protein biosynthesis.
3. Many hormones mediate their action though cyclic GMP or cGMP. cGMP is involved in vasodilation and smooth muscle relaxation.
4. G-proteins, which requires GTP and GDP are involved in signal transduction of several biological processes like vision, taste, metabolic regulation, olfaction, and cancer.
5. RNA is catalytically active in presence of GMP or Ribozyme action depends on GMP.
6. GDP is carrier of activated sugars in biosynthesis of mucopolysaccharides.

Hypoxanthine nucleotides

1. IDP and IMP are high energy compounds.
2. IMP is intermediate in purine ribonucleotide synthesis.

Uracil nucleotides

1. UTP and UDP are high energy compounds.
2. UDP is carrier of activated sugars and amino sugars needed for the synthesis of glycogen, glycoportein, gangliosides etc.
3. UDP-glucuronate serve as donor of glucuronide in conjugation reactions. For example, formation of bilirubin diglucuronide and detoxication reactions.

Cytosine nucleotides

1. CTP and CDP are high energy compounds.
2. CDP-choline serve as donor of choline in biosynthesis of phospholipid.
3. CMP-NANA is donor of NANA in biosynthesis of gangliosides.
4. Cyclic CMP also exist in cells.

Adenine nucleoside

S-adenosyl methionine is a adenine nucleoside. It is the donor of methyl groups in biosynthesis reactions.

Purine and pyrimidine analogs

Several synthetic analogs of purines and pyrimidines are used as anti-cancer agents. Their actions are detailed in next chapter.

Purine analogs

1. Mercaptopurine
2. Thioguanine
3. 2-Aminopurine
4. Allopurinol
5. Azathiopurine. A modified mercaptopurine. It is an immune suppressive agent.

Pyrimidine analogs

1. 5-Flurouracil

5-Fluorouracil

Nucleoside analogs containing modified bases or sugars are used as anti-cancer agents, anti-viral agents and mutagens.

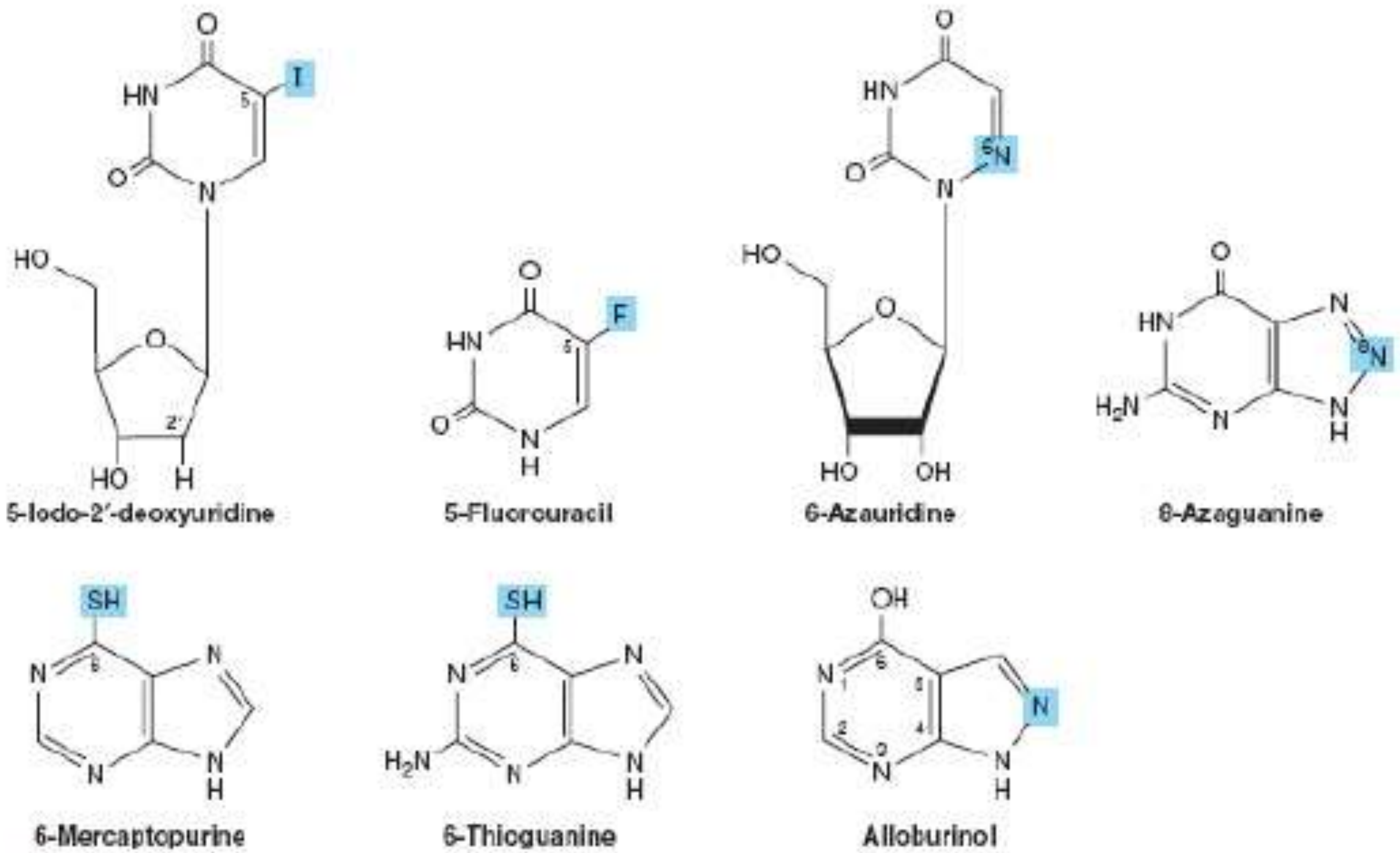


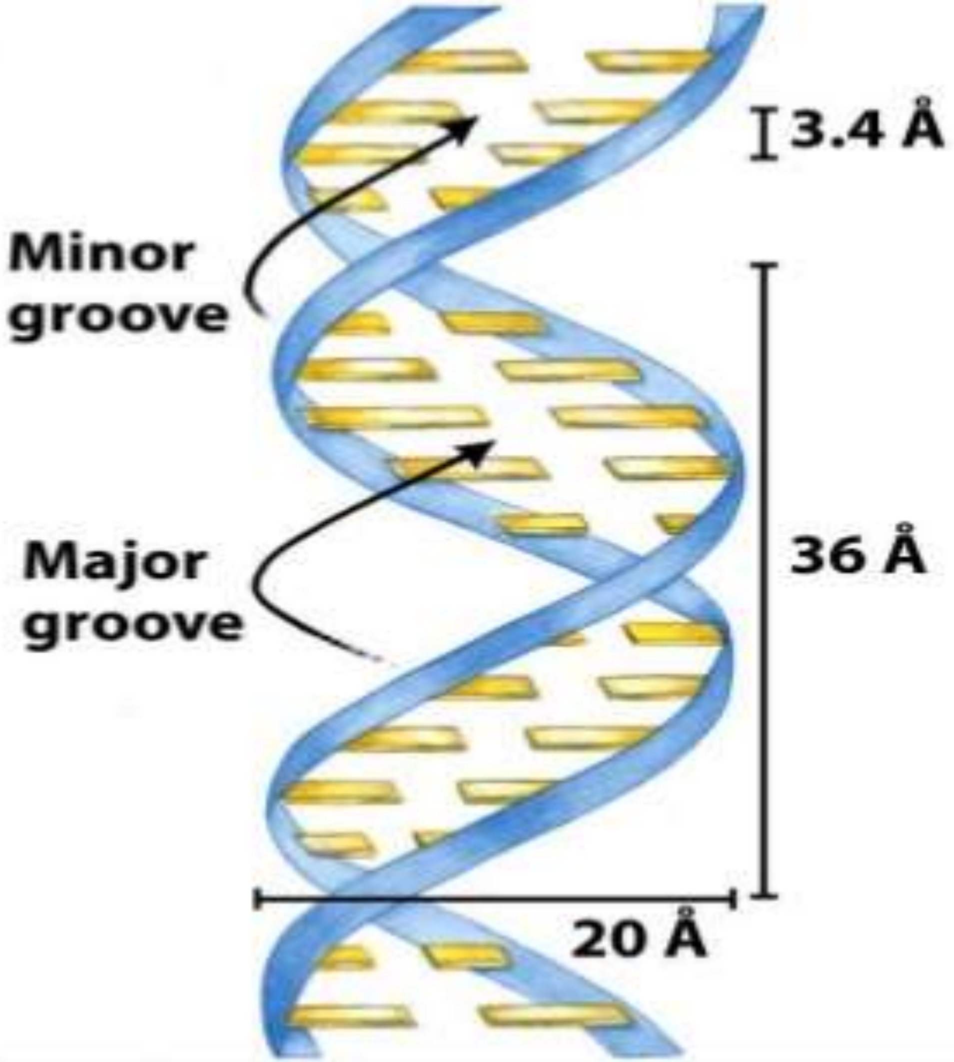
Figure 33–12. Selected synthetic pyrimidine and purine analogs.

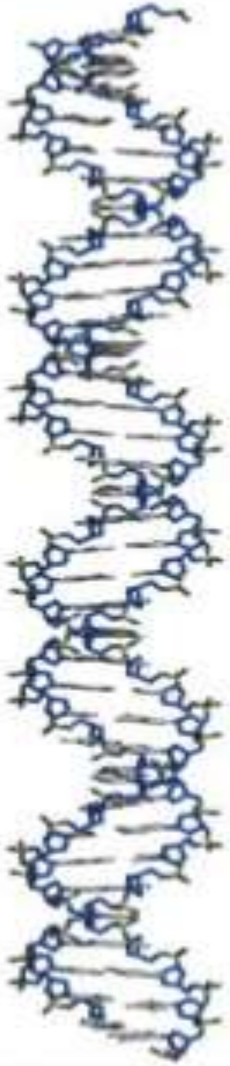
Deoxyribo Nucleic Acid
(DNA)

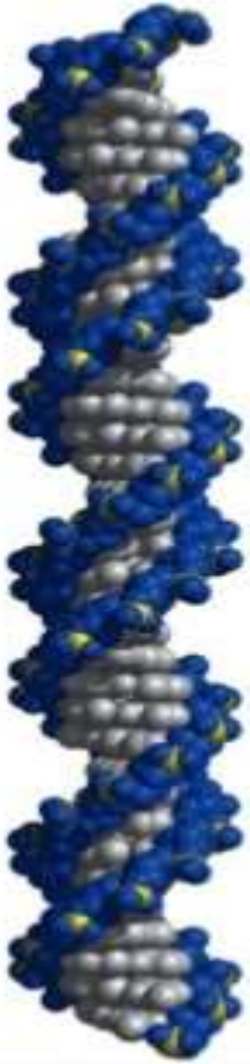
Structure of DNA

- DNA is a polymer of deoxyribonucleotides
- Composed of monomeric units namely
- Deoxyadenylate (dAMP)
- Deoxyguanylate (dGMP)
- Deoxycytidylate (dCMP)
- Deoxythymidylate (dTMP)
- The monomeric units held together by 3'5'-phosphodiester bonds as back bone.

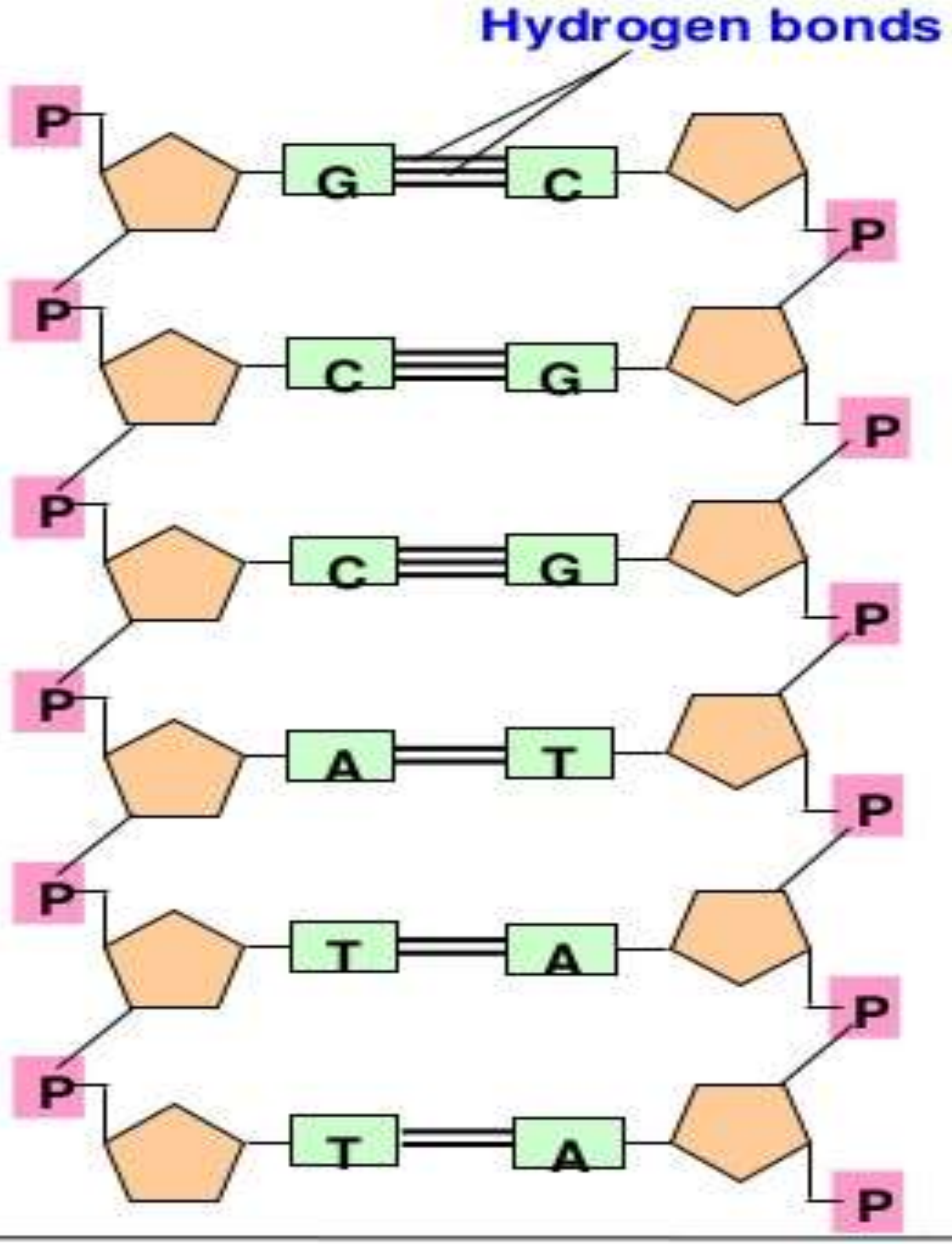
DNA is a Double-Helix

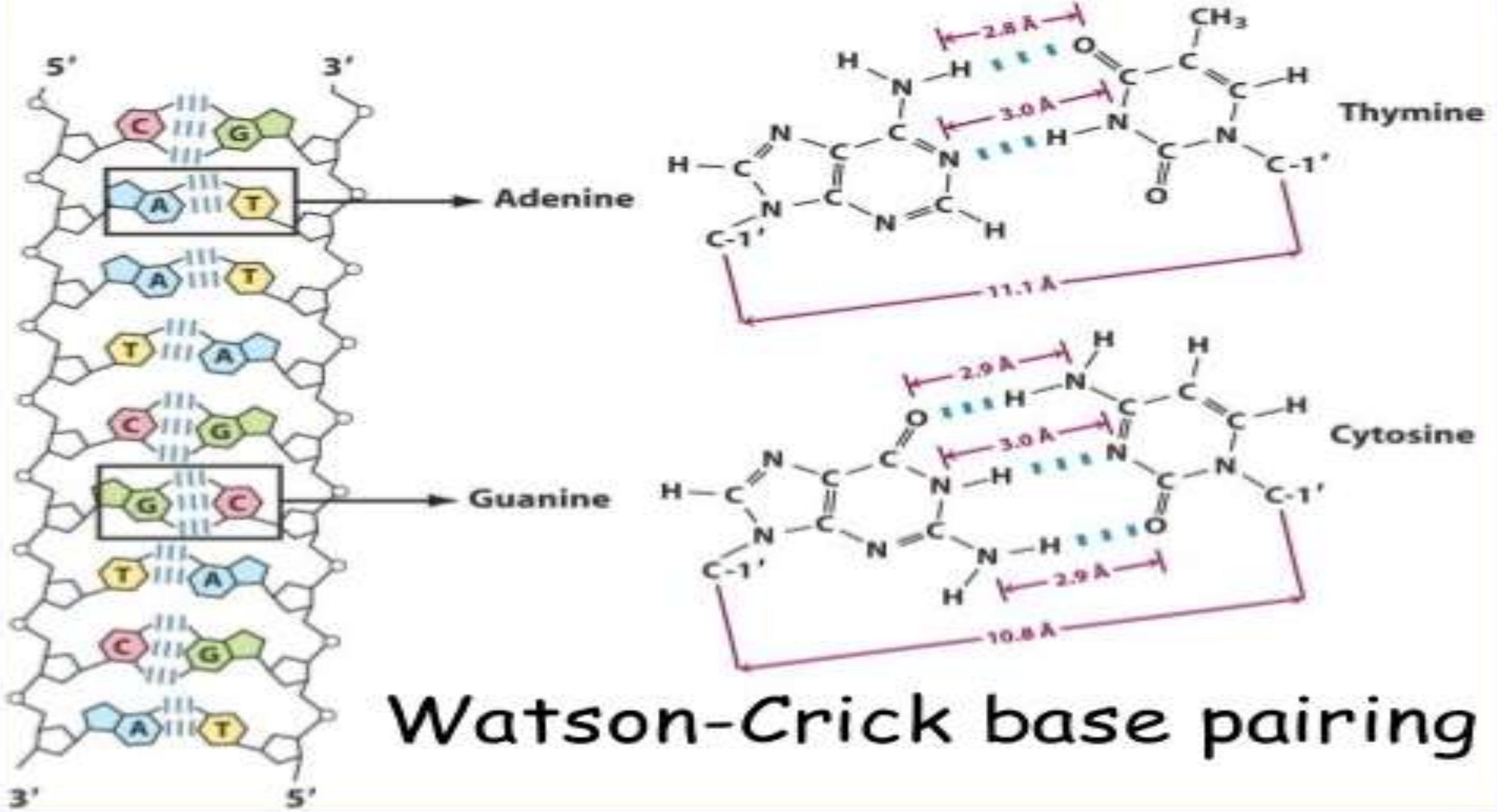






DNA IS MADE OF TWO STRANDS OF POLYNUCLEOTIDE





Watson-Crick base pairing

DNA structure determination

Rosalind Franklin, 1920–1958

Maurice Wilkins

James Watson

Francis Crick

- Franklin collected x-ray diffraction data (early 1950s) that indicated 2 periodicities for DNA: 3.4 Å and 34 Å.
- Watson and Crick proposed a 3-D model accounting for the data.

DNA double helix

- Double helical structure was proposed by Watson & Crick in 1953.
- The DNA is a right handed double helix.
- It consists of two polydeoxyribonucleotide chains twisted around each other on a common axis of symmetry.
- The chains are paired in an antiparallel manner, that is, the 5'-end of one strand is paired with the 3'-end of the other strand

- The two strands are antiparallel, i.e., one strand runs in the 5 ' to 3 ' direction while the other runs in 3' to 5 ' direction.
- The width (or diameter) of a double helix is $20A^0$ (2nm)
- Each turn of helix is 34 A^0 (3.4nm) with 10 pairs of nucleotides, each pair placed at a distance of about 3.4 A^0
- The DNA helix, the hydrophilic deoxyribose-phosphate backbone of each chain is on the outside of the molecule, whereas the hydrophobic bases are stacked inside.

- The polynucleotide chains are not identical but complementary to each other due to base pairing.
- The two strands are held together by hydrogen bonds.

$A = T, G \equiv C$

- The hydrogen bonds are formed between a purine & pyrimidine.
- The spatial relationship between the two strands in the helix creates a major (wide) groove and a minor (narrow) groove.

- These grooves provide access for the binding of regulatory proteins to their specific recognition sequences along the DNA chain.
- DNA helix proves Chargaff's.
- The genetic information resides on one of the two strands known as template strand or sense strand.
- The opposite strand is antisense strand.

Complementary strands

- The two strands of DNA are not identical but two strands are complementary to each other.
- The complementary results from base pairing.
- Adenine pairs with thymine through two hydrogen bonds.
- Guanine pairs with cytosine through three hydrogen bonds.
- G-C base pairs are more stable than A-T base pairs.
- Complementary base sequence accounts for chargaff's rule.
- It also accounts for each DNA strand acting as a template for the synthesis of its complementary strand during DNA replication.

Chargaff's rule

- DNA had equal numbers of adenine & thymine residues (A=T) and equal number of guanine & cytosine residues(G=C).
- This is called as *Chargaff's rule of molar equivalence of between purines & pyramidines in DNA structure.*
- RNAs which are usually single stranded, do not obey Chargaff's rule.

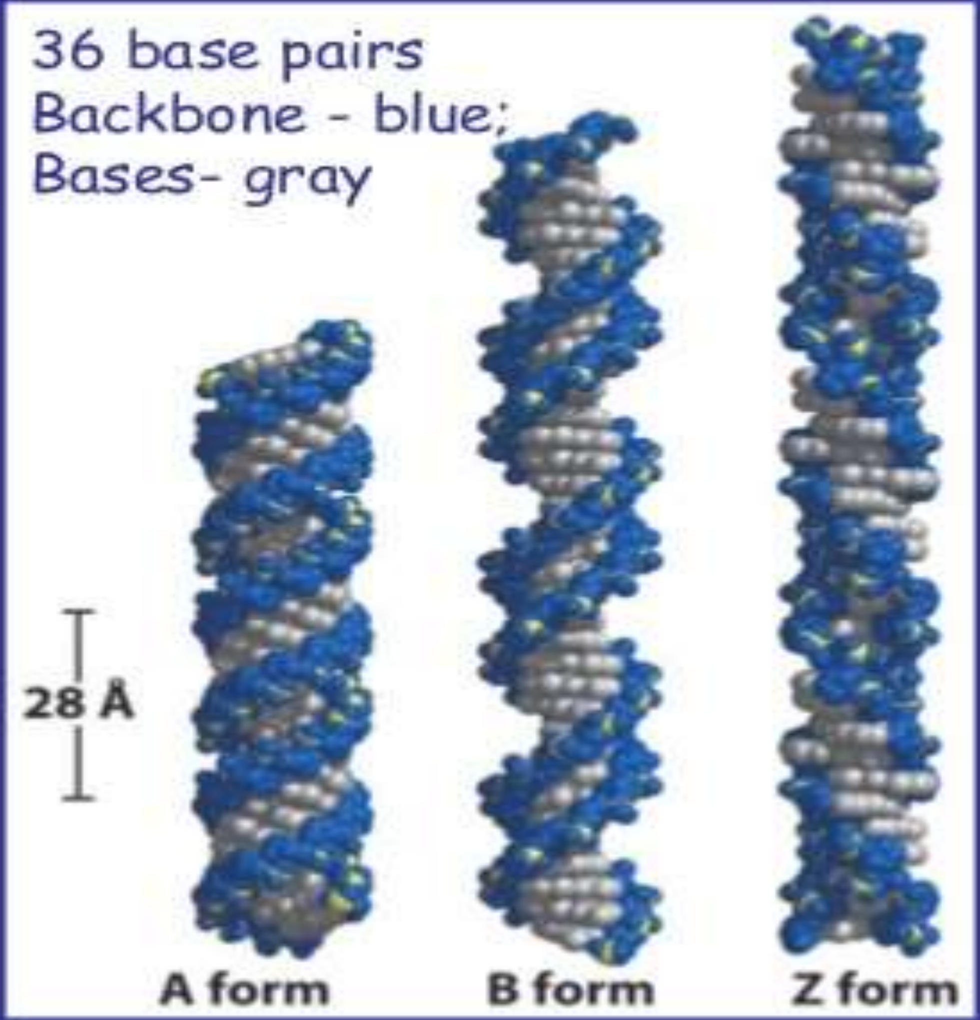
Conformations of DNA double helix

- The double helical structure of DNA exists in 6 forms A,B,C,D,E and Z form.
- Among these, B, A & Z forms are important.
- B-form is most predominant form under physiological conditions.
- A-from is also right-handed helix.
- Contains 11 base pairs.
- There is a tilting of the base pairs by 20^0 away from the central axis.
- Z-form is a left –handed helix and contains 12 base pairs per turn.

- The polynucleotide strands of DNA move in a somewhat zig-zag fashion, hence called as Z-DNA.
- Other types of DNA:
- DNA also exists in certain unusual structures.
- These structure are important for molecular recognition of DNA by proteins & enzymes.

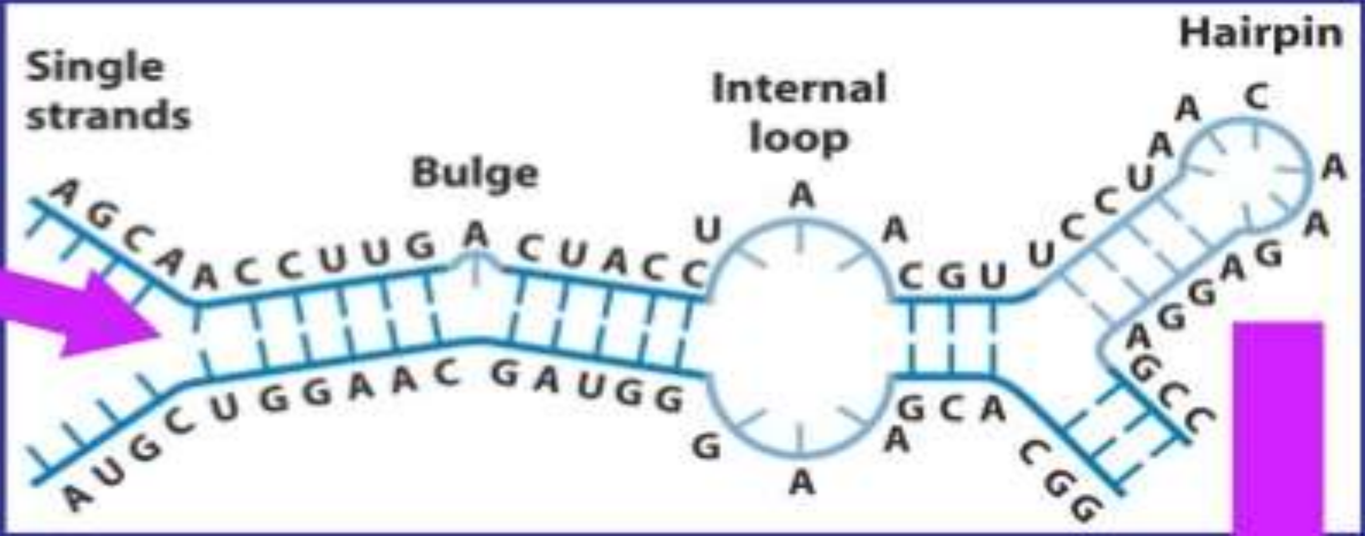
B,A and Z DNA

- B form - The most common conformation for DNA.
- A form - common for RNA because of different sugar pucker. Deeper minor groove, shallow major groove
- A form is favored in conditions of low water.
- Z form - narrow, deep minor groove. Major groove hardly existent. Can form for some DNA sequences; requires alternating syn and anti base configurations.

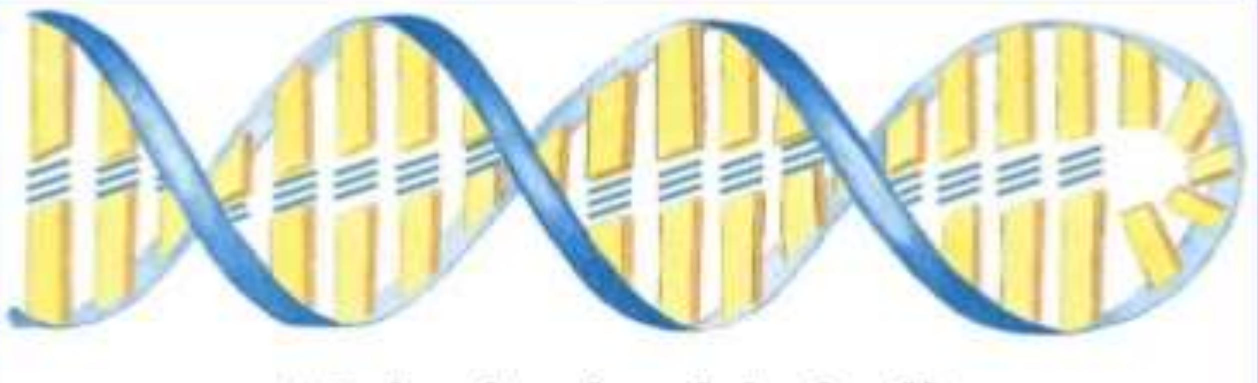


	A form	B form	Z form
Helical sense	Right handed	Right handed	Left handed
Diameter	~26 Å	~20 Å	~18 Å
Base pairs per helical turn	11	10.5	12
Helix rise per base pair	2.6 Å	3.4 Å	3.7 Å
Base tilt normal to the helix axis	20°	6°	7°
Sugar pucker conformation	C-3′ endo	C-2′ endo	C-2′ endo for pyrimidines; C-3′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

Watson-
Crick base pairs
(helical segments;
Usually A-form).
Helix is secondary
structure.
Note A-U pairs in
RNA.



DNA can
form
structures
like this as
well.

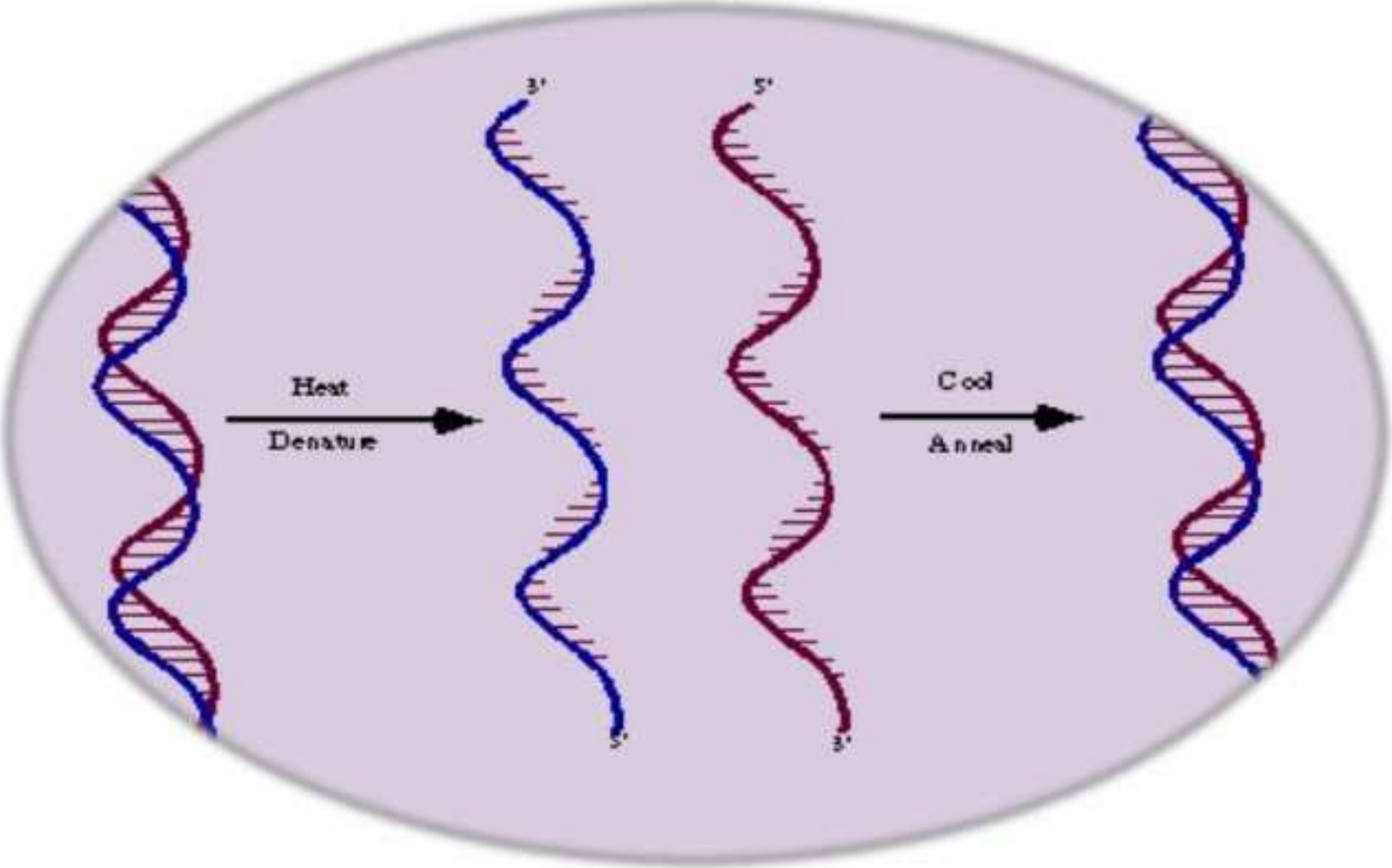


Hairpin double helix

Denaturation of DNA

- The two strands of DNA are held together by hydrogen bonds
- Disruption of hydrogen bonds (by change in pH or increase in temperature) results in separation of strands
- The phenomenon of loss of helical structure of DNA is known as denaturation
- Phosphodiester bonds are not broken by denaturation.
- It is measured by absorbance at 260nm.

Denaturation of DNA



Melting temperature (Tm)

- It is defined as the temperature at which half of the helical structure of DNA is lost.
- G-C base pairs are more stable than A-Tbp.
- Tm is greater for DNAs with high content of GC.
- Formamide destabilizes hydrogen bonds of base pairs.
- This is used in rDNA technology.
- **Renaturation (reannealing):**
- It is a process in which the separated complementary DNA strands can form a double helix.

Ribonucleic acid (RNA)

- RNA is also a polymer of mononucleotides.
- The bonds between the mononucleotides are similar to those in DNA, are polynucleotides, linked by phoshodiester bonds.

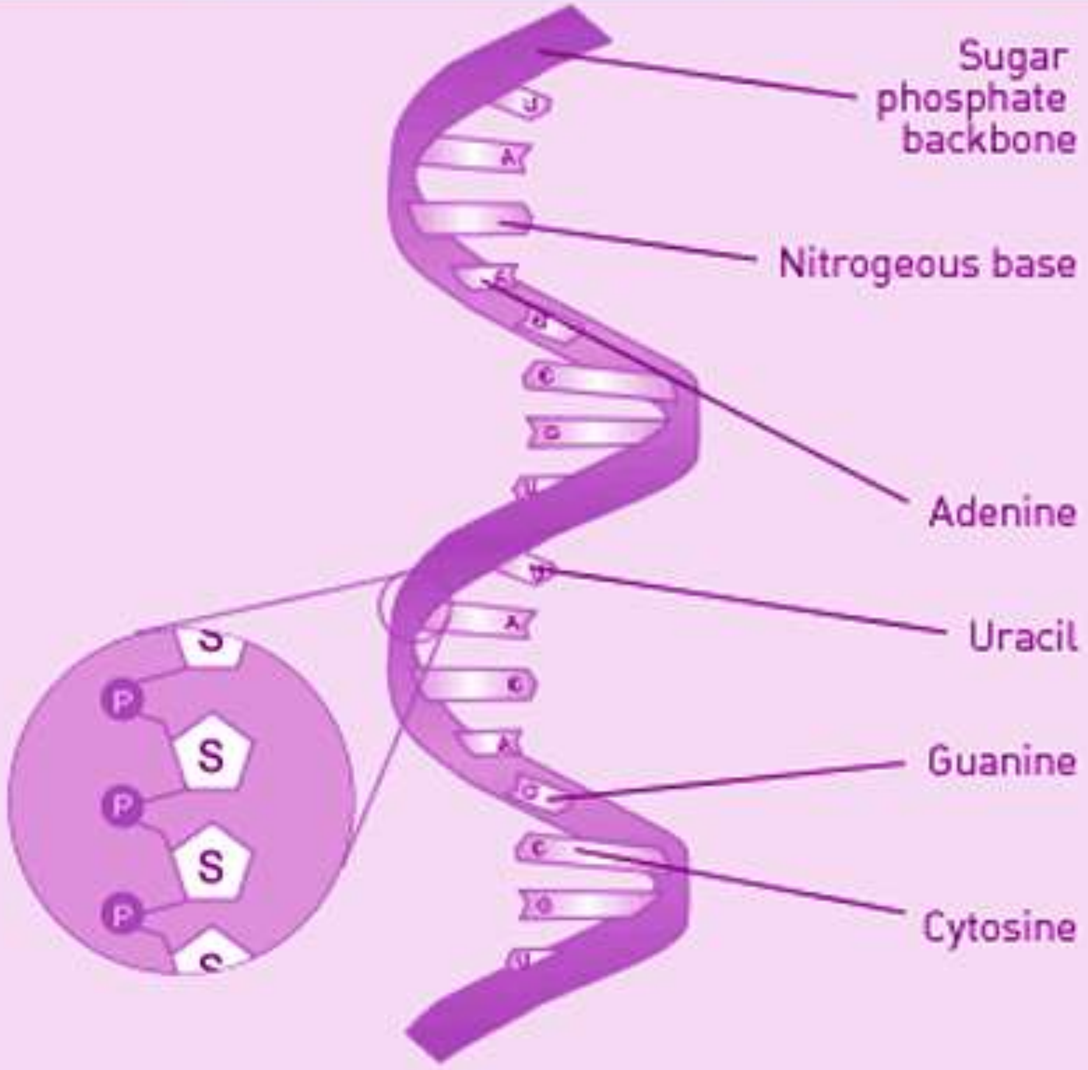
However, RNA differ from DNA in some ways.

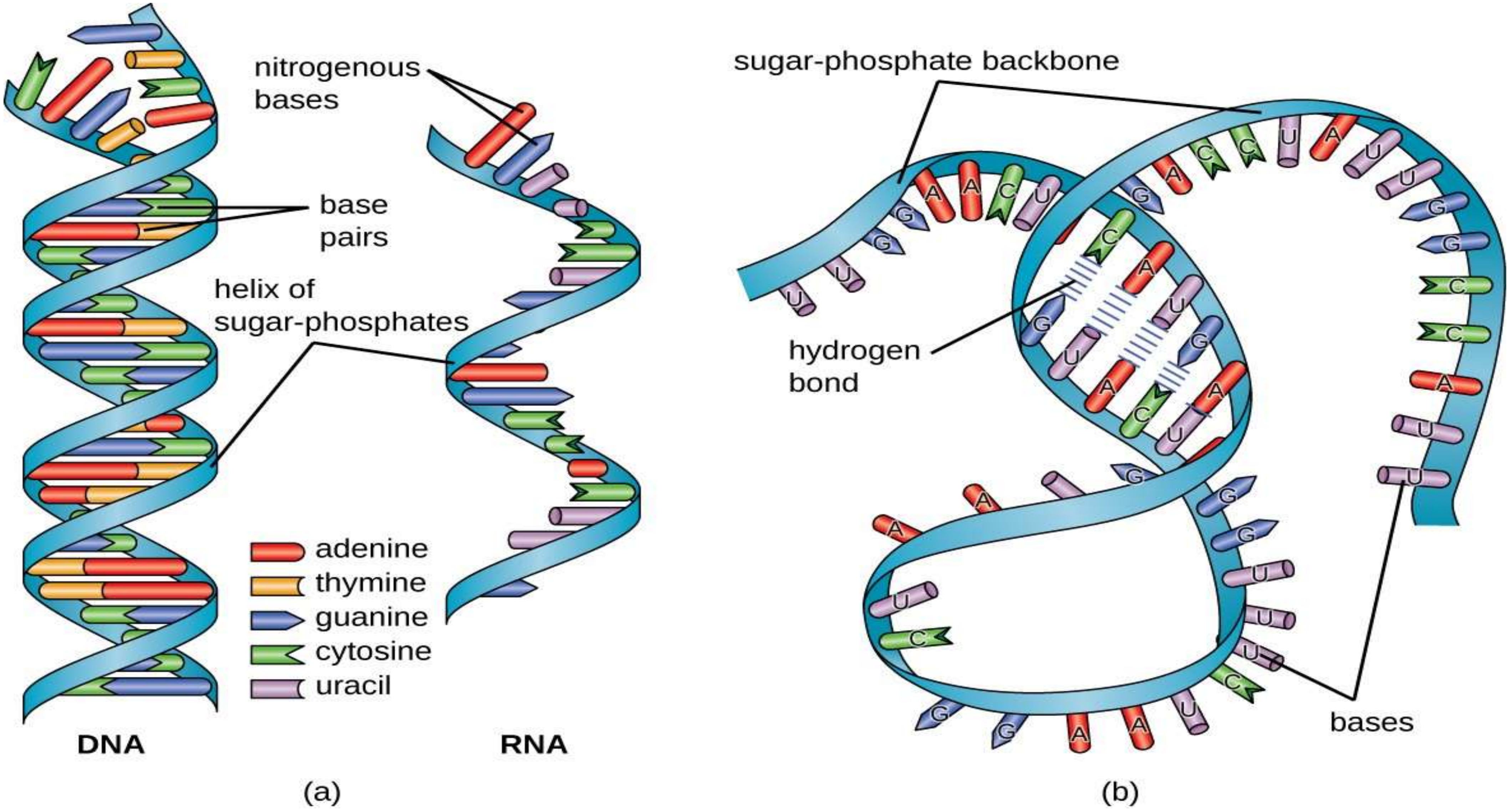
- They are much smaller in size and are mostly single stranded.
- The sugar they contain is ribose instead of $2^{\prime}$-deoxyribose of DNA.
- They contain a pyrimidine base; Uracil, instead of thymine of DNA.
- Their major role in gene expression, in contrast to DNA which stores genetic information.

Role of RNA:-

- RNA plays multiple roles.
- It serves as genetic material for some viruses (e.g. tobacco mosaic virus, poliovirus and influenza virus).
- It carries genetic information to the site of protein synthesis via mRNA.
- tRNA forms the link between mRNA and amino acids which help in protein synthesis.
- It is an essential component of ribosomes (rRNA).
- Some RNAs also have enzymatic activity and act as ribozymes.

Structure of RNA



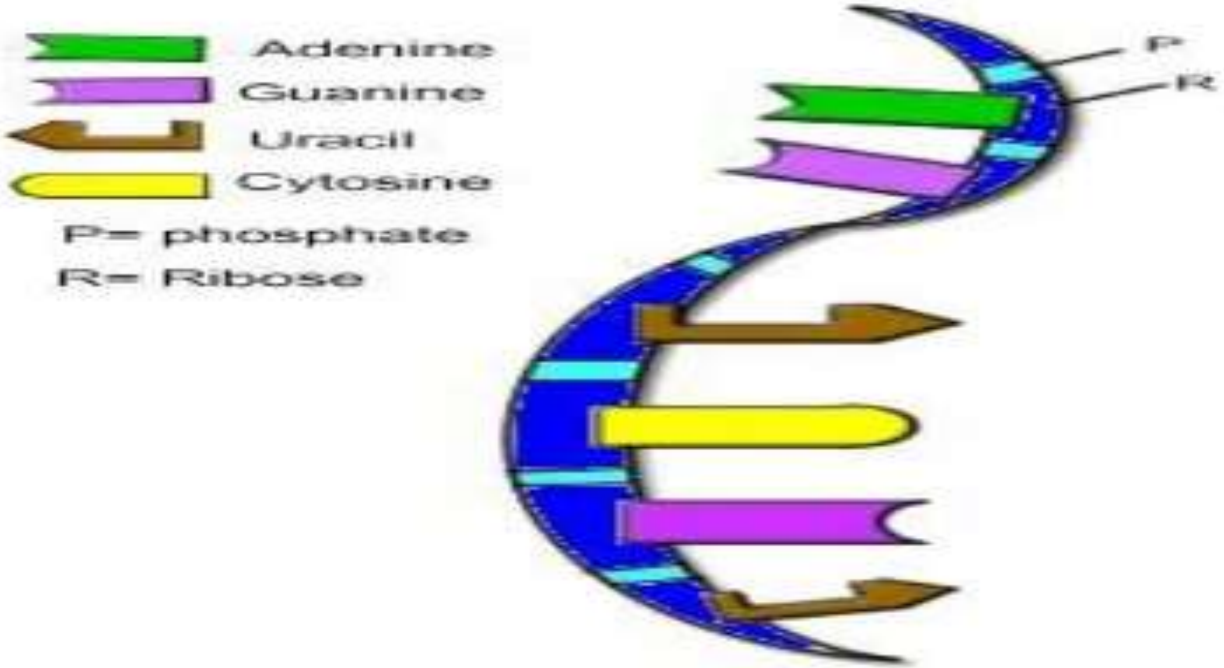

nitrogenous bases
base pairs
helix of sugar-phosphates
adenine
thymine
guanine
cytosine
uracil
DNA
RNA
(a)
sugar-phosphate backbone
hydrogen bond
bases
(b)

Types of RNA

- Three major types:
- Messenger RNA:5-10%
- Transfer RNA:10-20%
- Ribosomal RNA:50-80%
- RNAs are synthesized from DNA
- Involved in protein synthesis.
- Messenger RNA:
- ***mRNA:***
- It carries genetic information from DNA for protein synthesis.
- Precursor form is heterogeneous nuclear RNA(hnRNA).

- ***Structure:*** It contains
- Cap: is an inverted 7-methyl GTP attached to 5'end.
- 5'UTR: (5'untranslated region) is at the 5'end.
- Coding region contains 3 types of codons:
- Initiating codon-is always for AUG for methionine.
- Specific codon-for different amino acids
- Terminating codons-which are UGA, UAA & UAG.

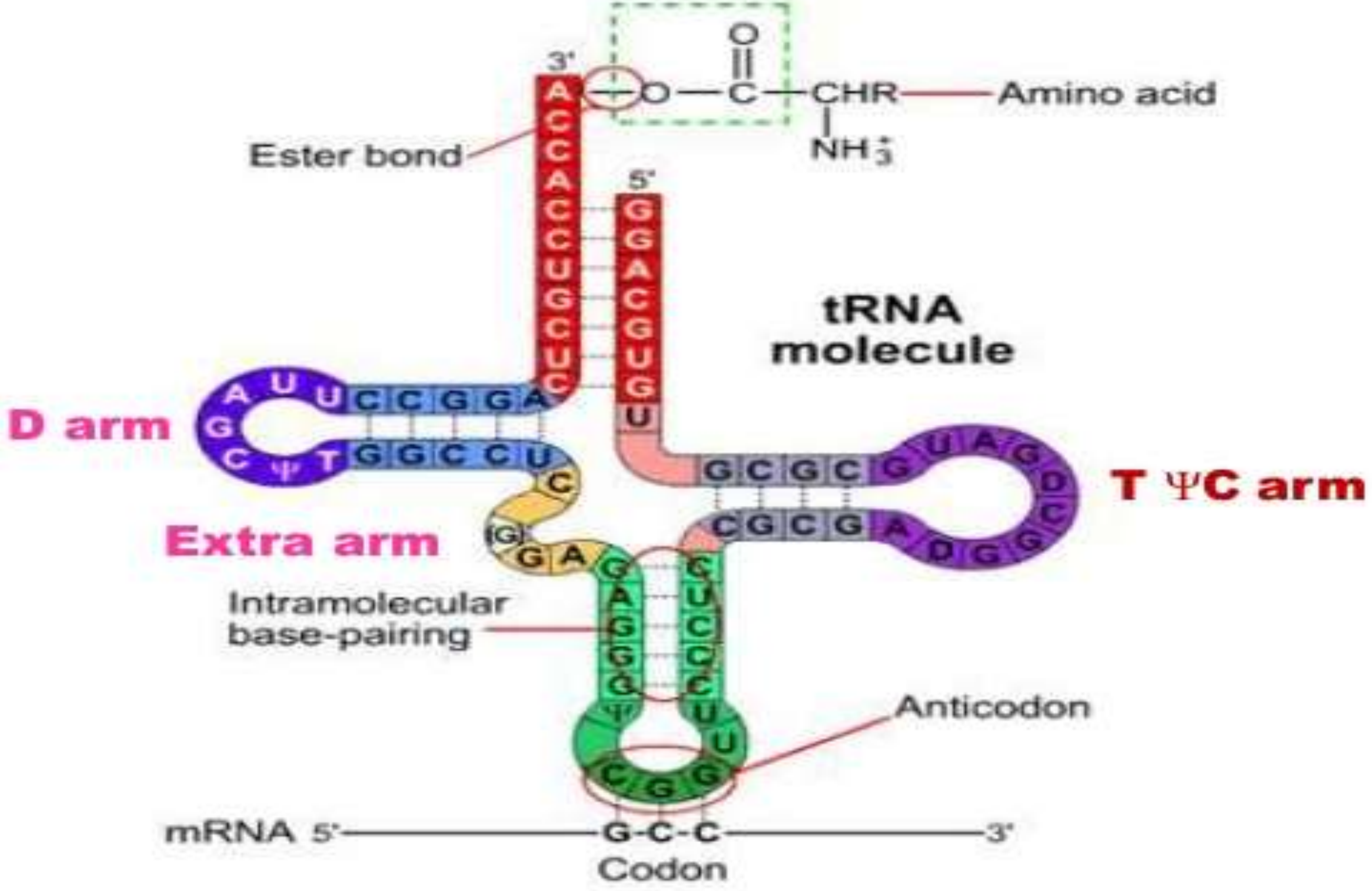
- 3'UTR (3'untranslated region) at 3' end.
- Polyadenylate tail (poly A tail): Consists of 200-300 adenylate residues at the 3' end.
- mRNA accounts for 5 to 10% of total RNA.
- Function: It is required for protein biosynthesis.



Transfer RNA

- Transfer RNA (soluble RNA) contains 71-80 nucleotides.
- Molecular weight-25,000.
- At least 20 species of tRNAs, corresponding to 20 Amino acids.
- Required for protein biosynthesis.
- It contains many unusual bases & nucleosides.
- *Unusual bases present in t RNA are thymine, dihydrouracil, hypoxanthine,1-methyladenine & 2-N dimethyl guanine*

Structure of tRNA



- Unusual nucleosides are formed from the unusual bases,
- Pseudouridine is an unusual nucleoside found in t-RNA.
- ***Structure:***
- Clover leaf structure & it has five arms.
- ***CCA arm:*** cytosine-cytosine-adenine (CCA-arm) present at 3'end. It is an acceptor arm for the attachment of amino acids to form amino acyl tRNA.
- ***D arm:*** contains dihydrouracil.

- T¥C arm: (thymidine-pseudouridine-cytosine arm) contains pseudouridine.
- Anticodon arm: contains of sequence of three bases that are complementary to codon mRNA.
- tRNA is also *called adapter tRNA because it carries specific amino acids on its 3' end corresponding to anticodon at its anticodon arm.*
- Extra arm: also called variable arm.
- Based on length of extra arm-tRNA is classified into
- Class-1 tRNA: Contain short arm (3-5 base pairs)
- Class-2t RNA: Contain long arm (13-20base pairs)

- tRNA accounts for 15-30% of total cellular RNAs.
- tRNA is smaller in size.
- tRNA is synthesized as precursor tRNA.
- Mature form is formed by post transcriptional modifications.
- Functions:
- tRNA is required fro protein synthesis.
- It is required for the transfer of specific amino acids to the site of protein synthesis.
- Also required for incorporation of specific amino acids to the growing polypeptide chain.

Ribosomal RNA

- r-RNA is found in ribosomes.
- Eukaryotic ribosomes are factories of protein synthesis.
- Composed of two major nucleoprotein complexes-60s subunit & 40s subunit
- 60s subunit contains-28s rRNA, 5s rRNA & 5.8s rRNA
- 40s subunit contains-18s rRNA
- Main function is protein biosynthesis.

What is Ribozyme ?

A **ribozyme** (ribonucleic acid enzyme) is an RNA molecule that is capable of performing specific biochemical reactions, similar to the action of protein enzymes.

Characteristic features of RNA molecule are:

- An enzyme that uses RNA as a substrate
- AN RNA with enzymatic activity
- An enzyme that catalyzes the association between the large and small ribosomal subunits
- An enzyme that synthesizes RNA as part of the transcription process
- An enzyme that synthesizes RNA primers during DNA replication

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- Investigators studying the origin of life have produced ribozymes in the laboratory that are capable of catalyzing their own synthesis under very specific conditions, such as an RNA polymerase ribozyme.

- Some ribozymes may play an important role as therapeutic agents, as enzymes which target defined RNA sequences for cleavage, as biosensors, and for applications in functional genomics and gene discovery.

Types of ribozyme

- Group I and group II intron splicing ribozymes
- RNase P
- Hammerhead Ribozyme
- Hairpin ribozyme
- Ribozome

Thank you