Nucleotide chemistry and Functions

Objectives

1. What are nucleotides
2. Why are bases named so
3. What are syn and anti conformers
4. What are different tautomers
5. Why mutation is common in genes
6. Biomedical Importance of nucleotides
7. Synthetic nucleotide
8. Clinical problem and MCQ

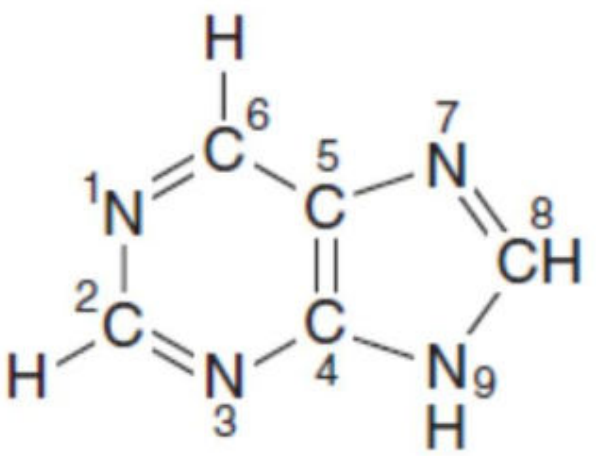
A 36 years old male patient is diagnosed with Gout. Doctor has decided to prescribe Allopurinol.
What modifications in the generic purine ring would help a pharmacist to prepare Allopurinol?

What are nucleotides

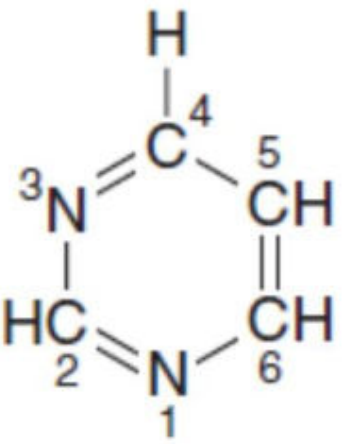
Why are bases named so

Circumstances of discovery
Guanine:
Isolated from Guano(bird manure)
Thymine: from thymus

Purine and pyrimidine. The atoms are numbered according to the international system.

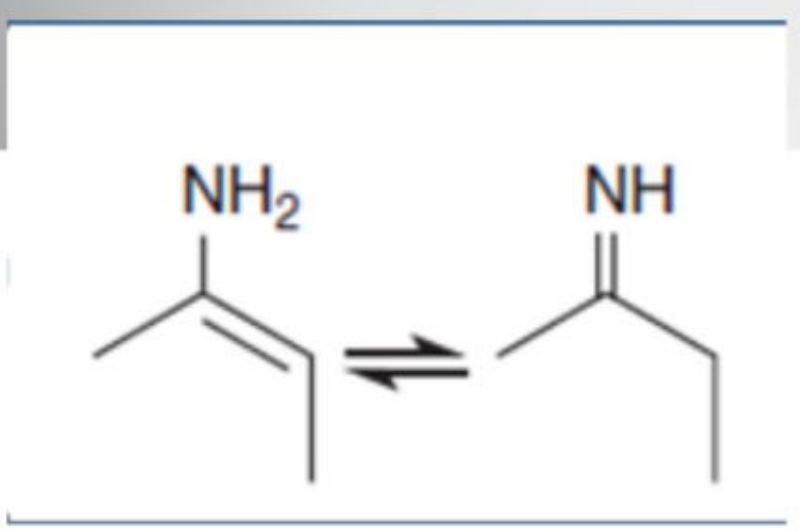


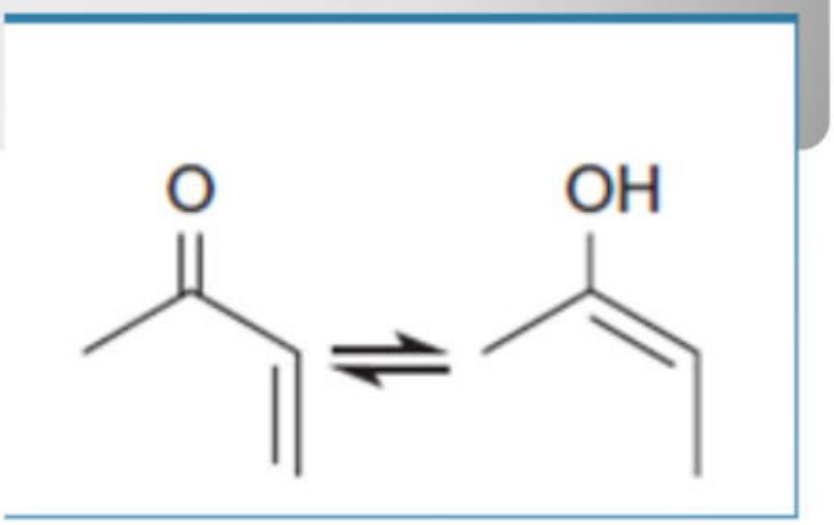
Purine



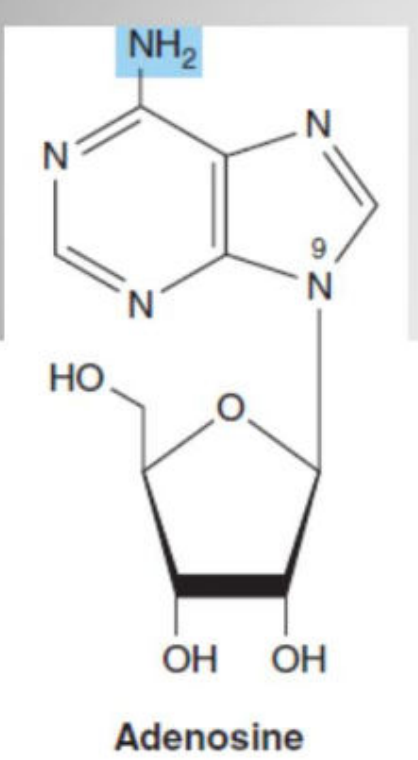
Pyrimidine

Tautomerism of the oxo and amino Functional groups of purines and pyrimidines

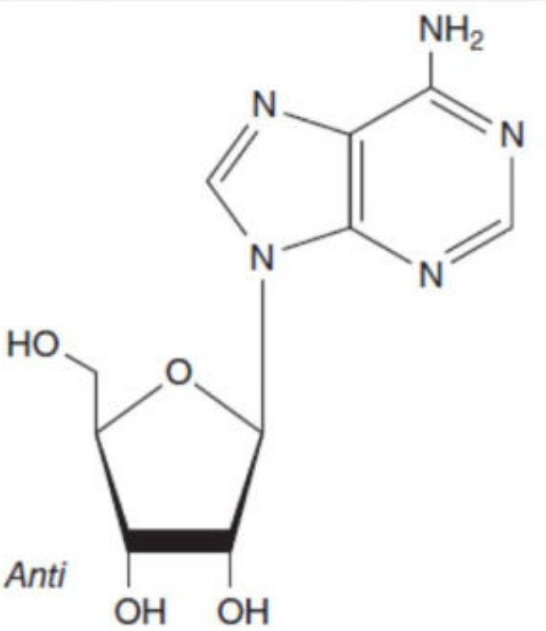


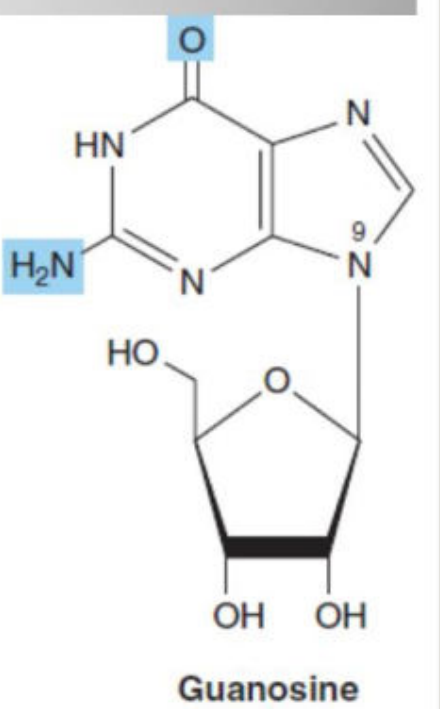


Ribonucleosides: Syn and Anti conformers



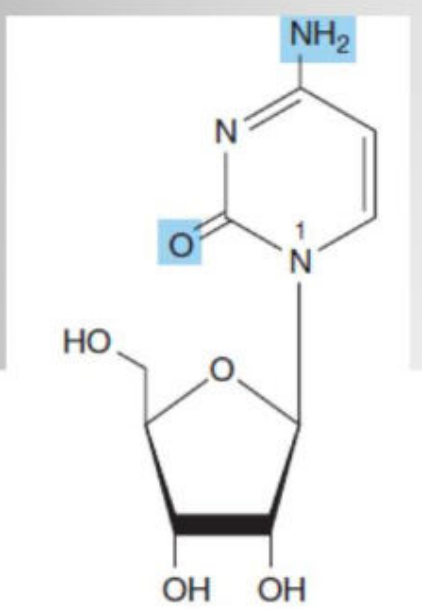
Adenosine



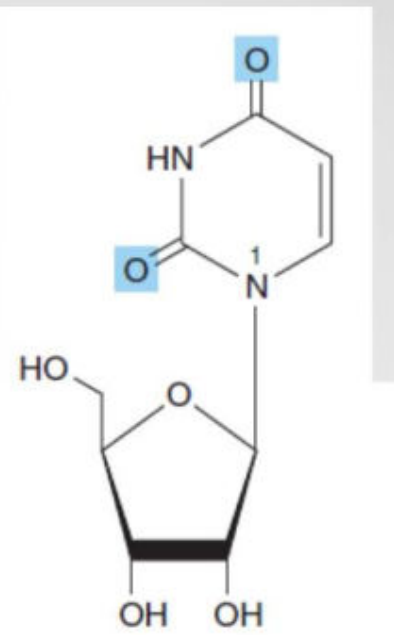


Guanosine

Ribonucleosides: Syn conformer

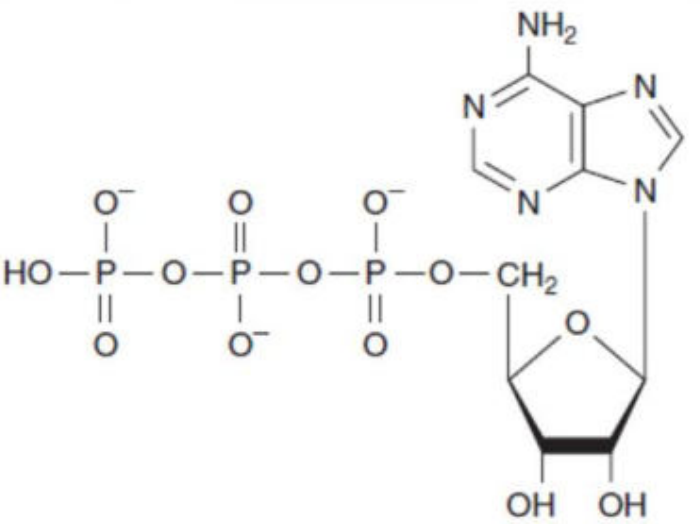


Cytidine



Uridine

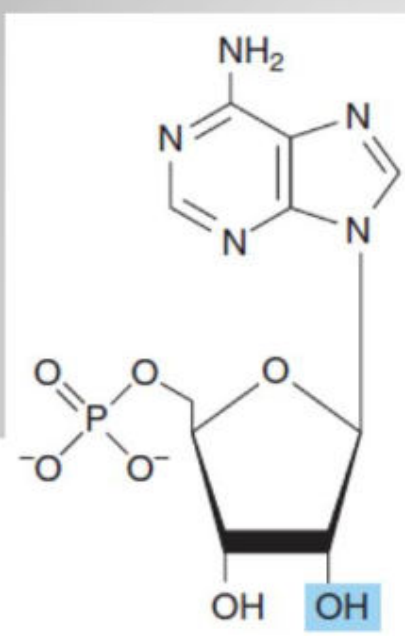
Ribonucleotides



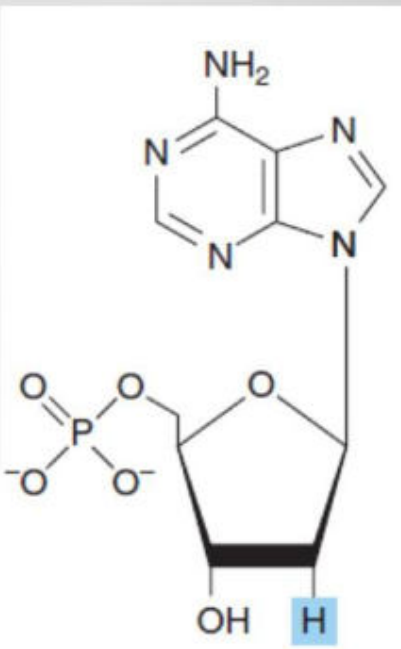
Adenosine 5′-monophosphate (AMP)

Adenosine 5′-diphosphate (ADP)

Adenosine 5′-triphosphate (ATP)



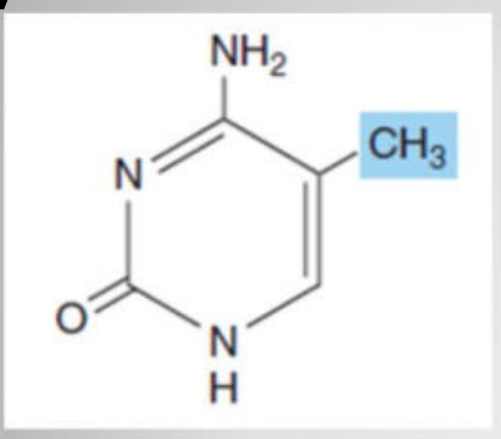
AMP



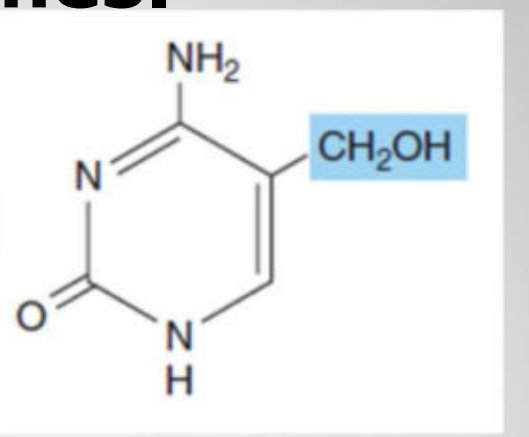
dAMP

Modification of Polynucleotides Can generate additional structure

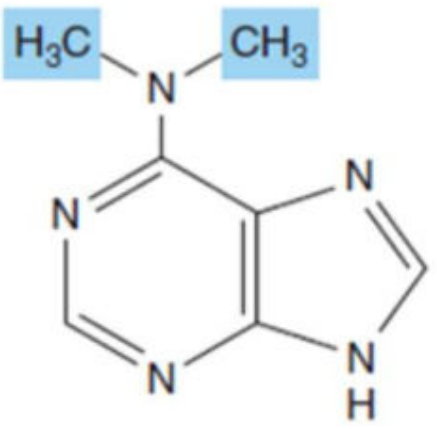
uncommon naturally occurring pyrimidines and purines.



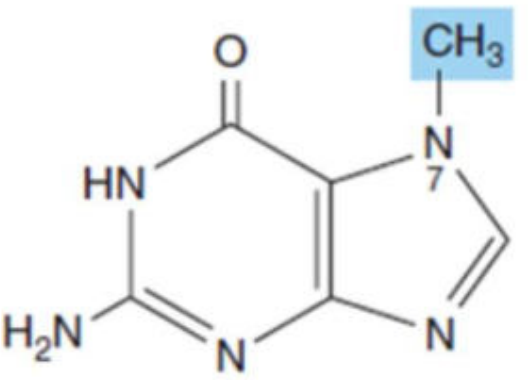
5-Methylcytosine



5-Hydroxymethylcytosine

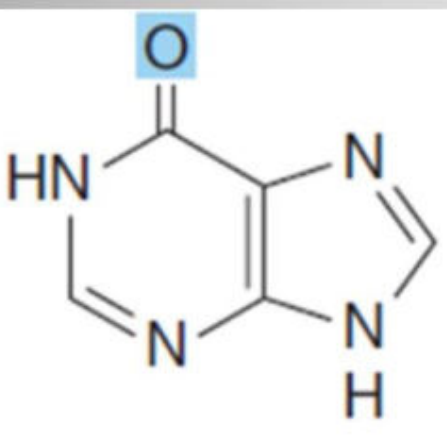


Dimethylaminoadenine

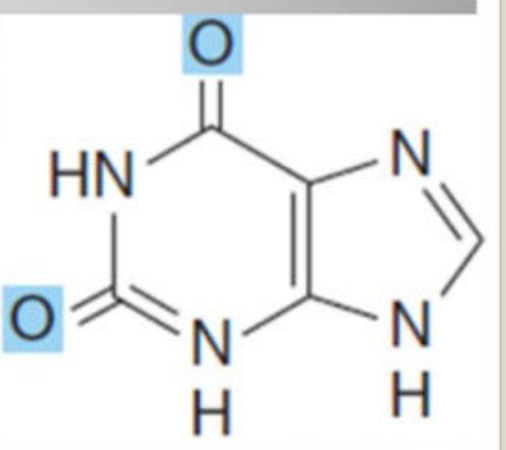


7-Methylguanine

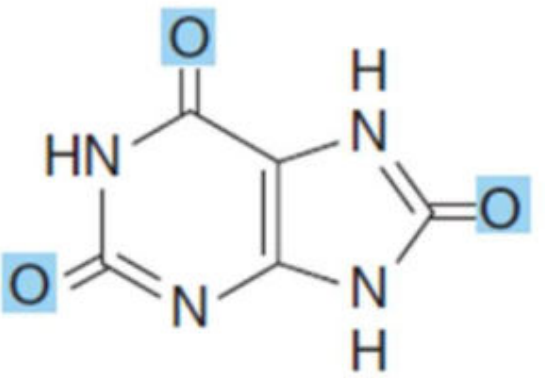
Intermediates in the catabolism of purine



Hypoxanthine
(6-oxopurine)

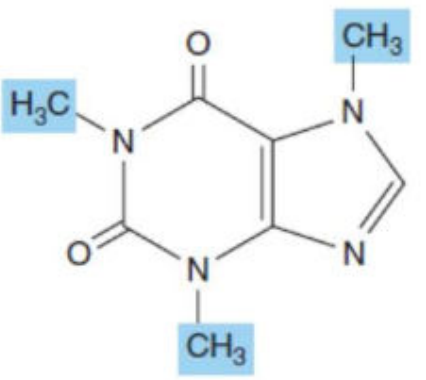


Xanthine
(2,6-dioxopurine)



Uric acid
(2,6,8-trioxypurine)

Caffeine, a trimethylxanthine.



Theobromine:
3,7 dimethyl xanthine
Theophyline:
1,3 dimethyl xanthine

Why UV light causes mutagenesis?

Conjugate double bond

How the concentration of nucleic acid and nucleotides are expressed and its implication as research tool

Absorbance at 260 nm

Purity of nucleic acid

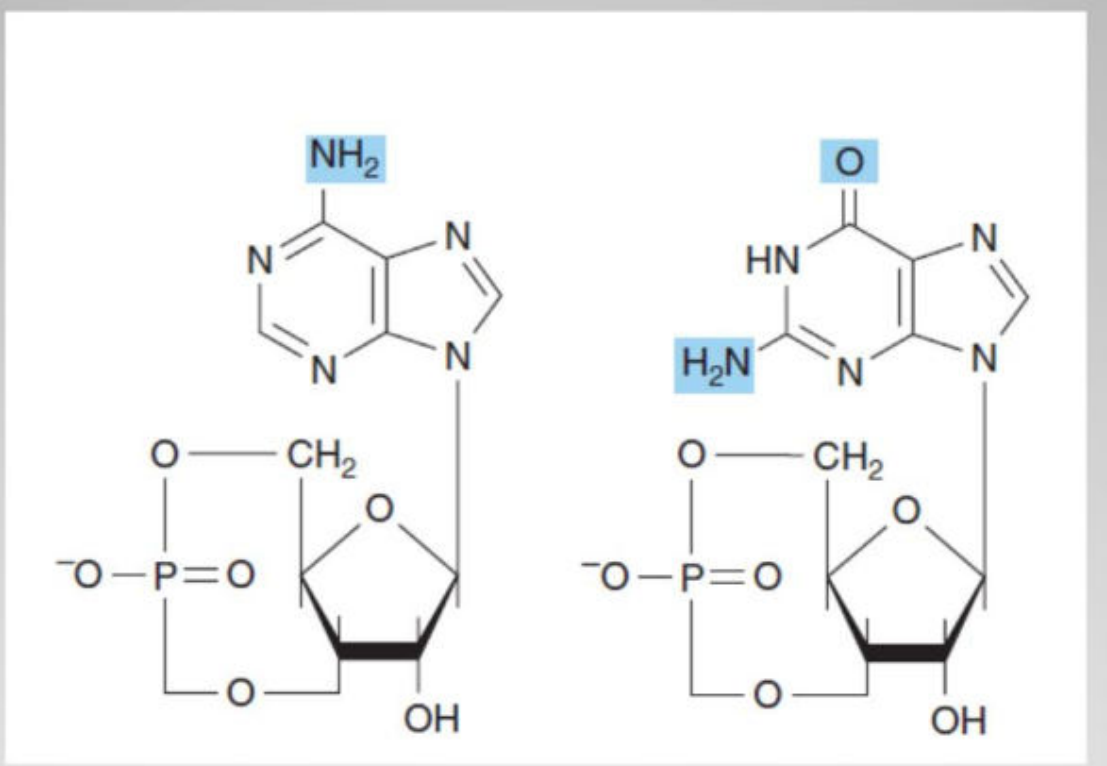
Biomedical importance

1. Serve as precursor of nucleic acid

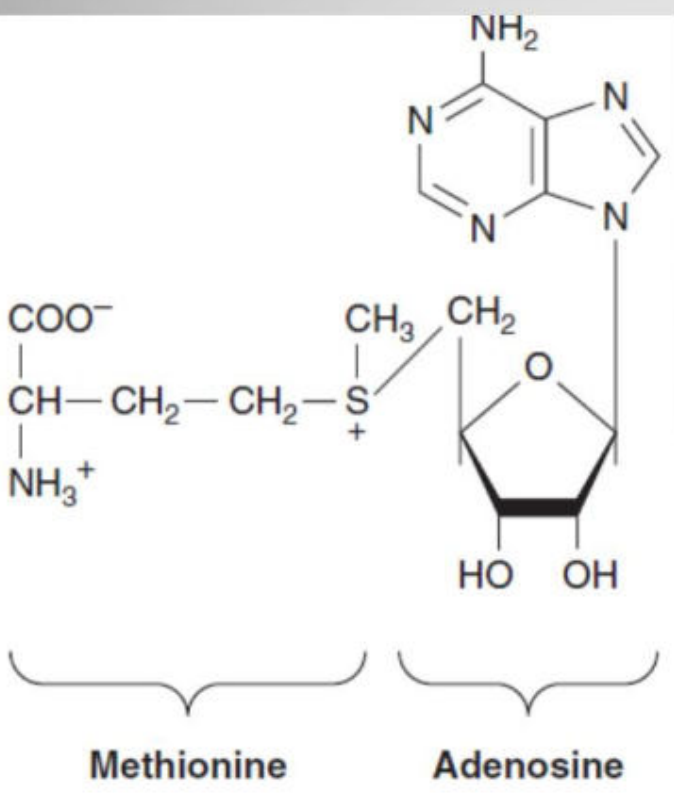
2. Principal biologic transducer of free energy
   e,g ATP, GTP

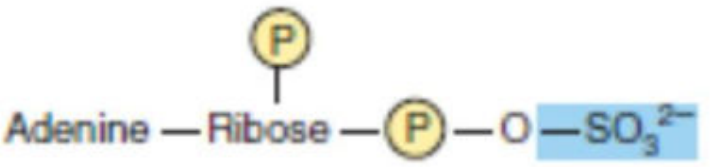
3. Second messanger
   cAMP, cGMP

cAMP and cGMP



4. Methyl group donor and sulfate donor





Adenosine 3′ phosphate-5′ phisphosulfonate

S-Adenosylmethionine

5. **Allosteric regulator:**

e,g ATP acts as allosteric inhibitor of phosphofructokinase

6. **Sugar derivatives**

a. UDP glucose and galactose: Biosynthesis of glycogen, glucosyl disaccharide, oligosaccharides of glycoprotein and proteoglycan

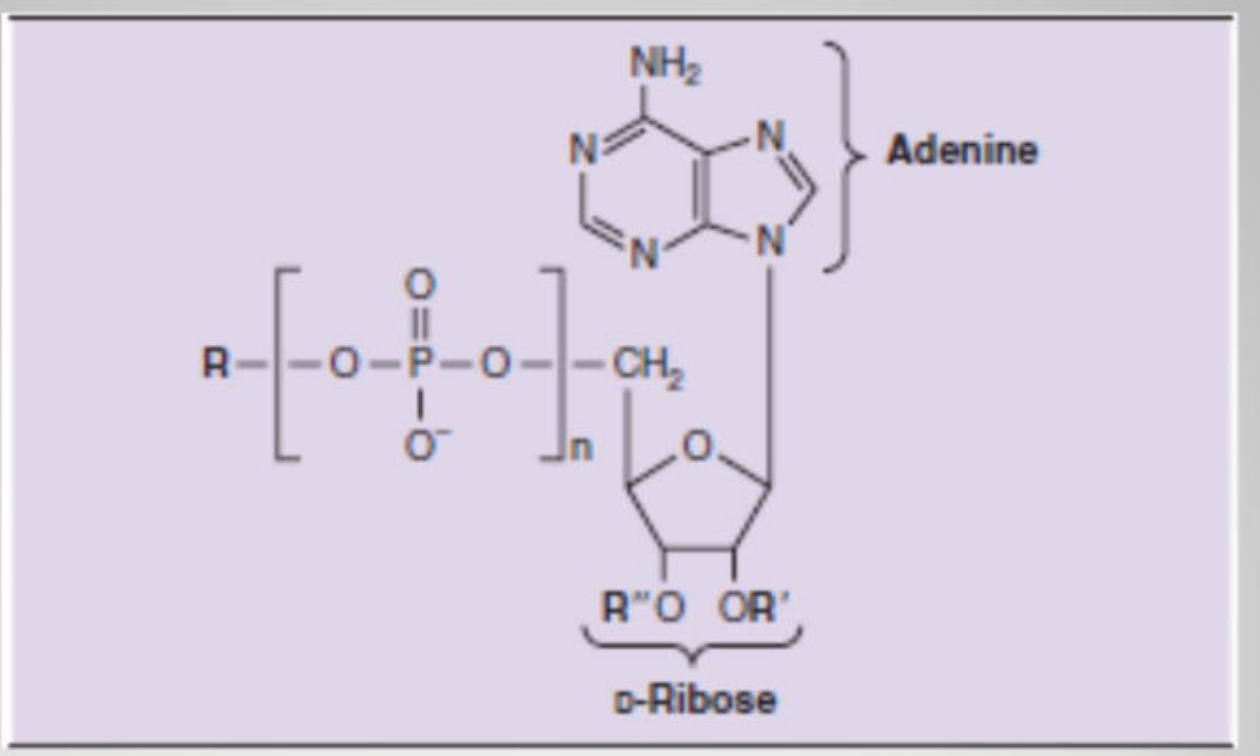
b. UDP glucuronic acid : Glucuronide conjugation of bilirubin, drugs

7. **Lipid derivatives**

CDP acyglycerol: participates in biosynthesis of phosphoglyceride, shingomyelin, sphingosine

8. **Vitamin derivatives: Nucleotides form a portion of Coenzymes**

Many coenzymes and related compounds are derivatives of Adenosine monophosphate



Example of coenzymes : Derivative of ribonucleoside

Coenzyme	R
Active methionine	Methionine[a]
Amino acid adenylates	Amino acid
Active sulfate	SO_3^{2-}
3′,5′-Cyclic AMP	
NAD[b]	Nicotinamide
NADP[b]	Nicotinamide
FAD	Riboflavin
Coenzyme A	Pantothenate

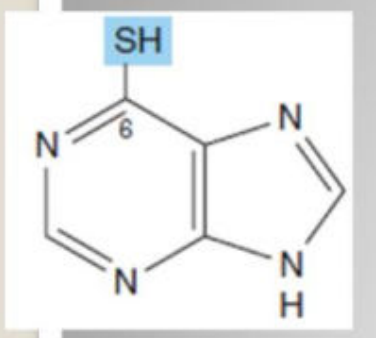
Clinical Application:

1. Chemotherapy of cancer and AIDS

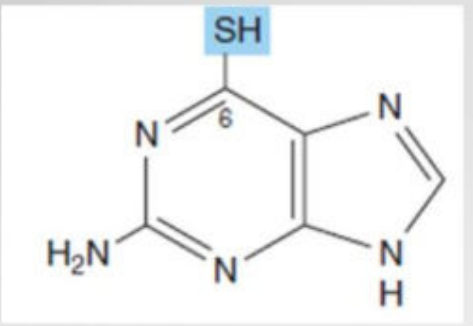
2. As suppressor of immune response During Organ transplantation

Synthetice Nucleotides and their clinical implications

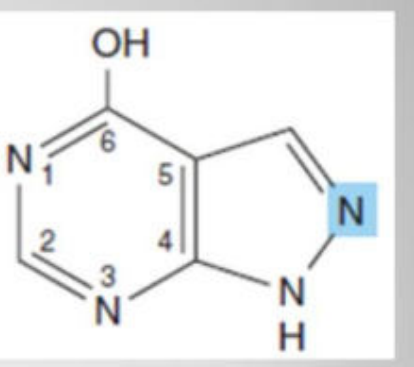
Synthetic purine analogs



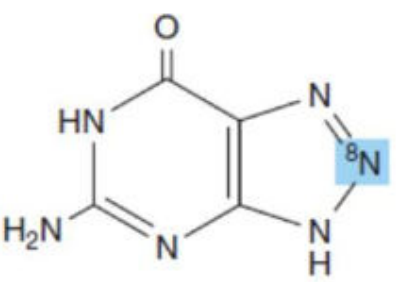
6-Mercaptopurine



6-Thioguanine



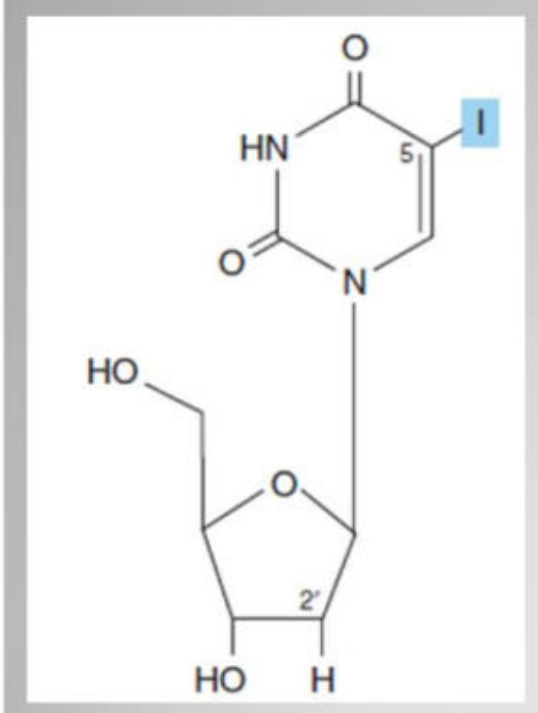
Allopurinol



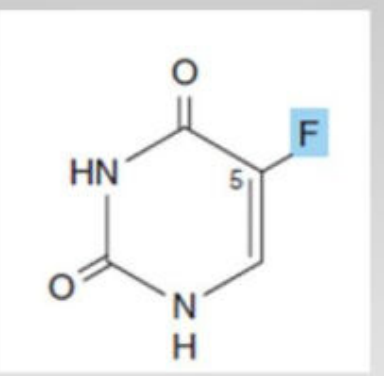
8-Azaguanine

25

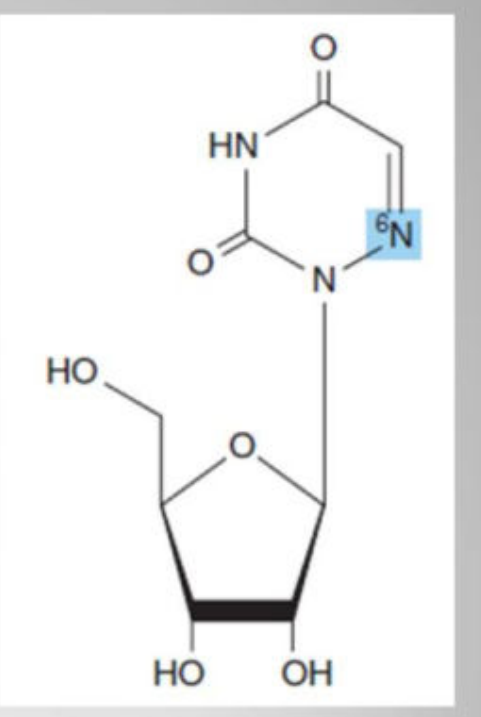
Synthetic pyrimidine analogs



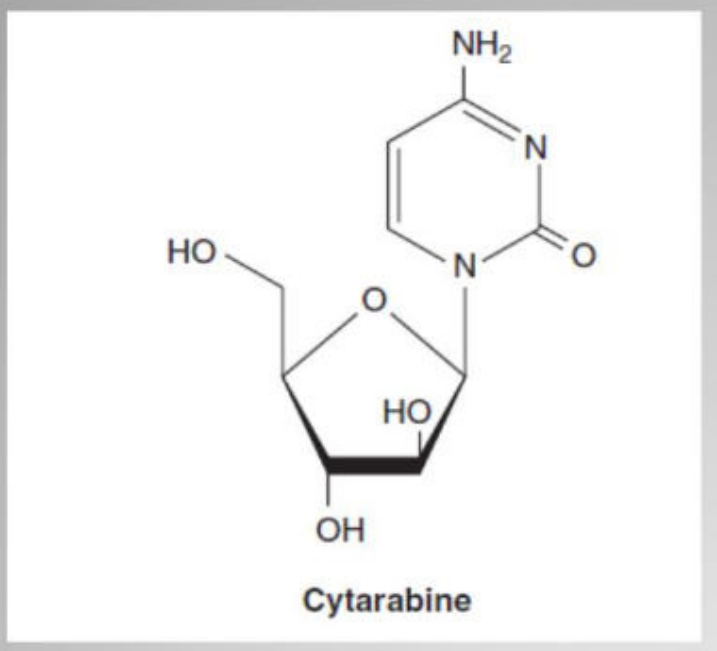
5-Iodo-2′-deoxyuridine



5-Fluorouracil

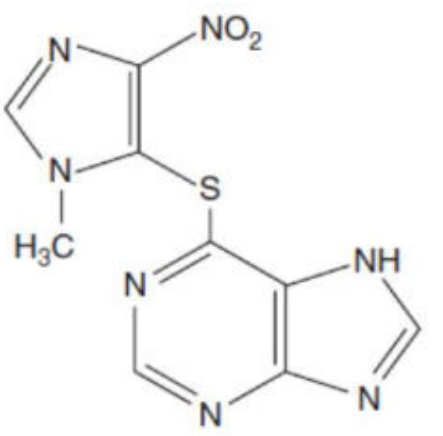


6-Azauridine



Cytarabine

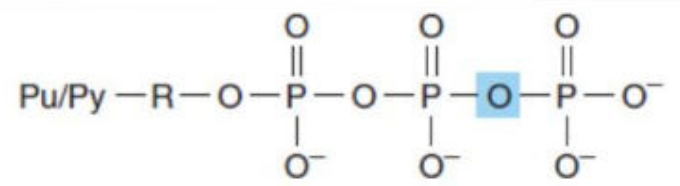
chemotherapy of cancer



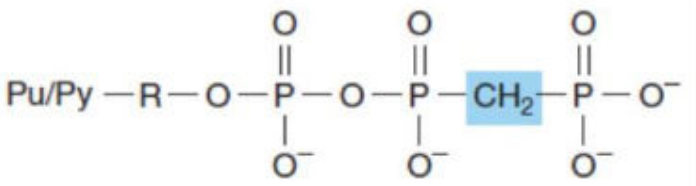
Azathioprine

organ transplantation to suppress immunologic rejection

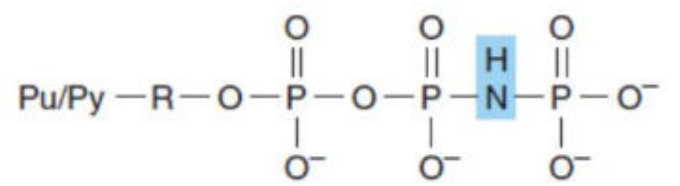
Synthetic derivatives of nucleoside triphosphates



Parent nucleoside triphosphate



β,γ-Methylene derivative



β,γ-Imino derivative

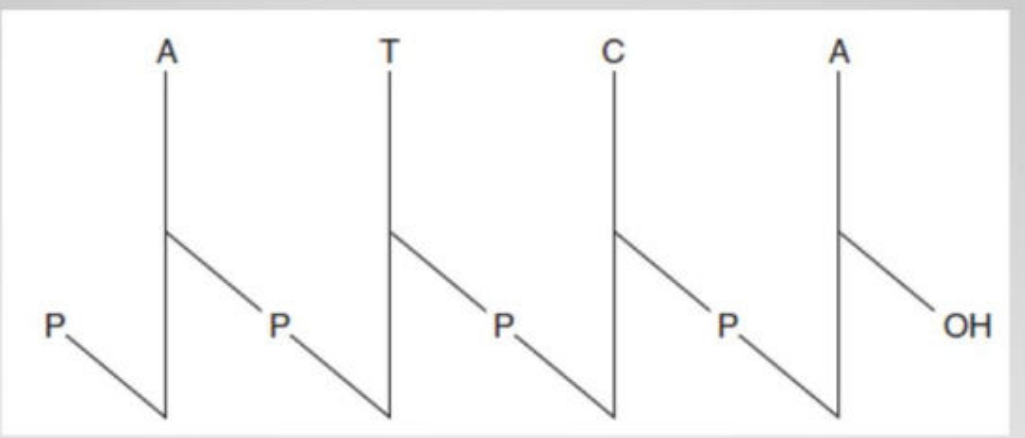
Incapable of release of Terminal phosphoryl group

Used as research tool

What is DNA and RNA?

Polynucleotide

How to represent The **primary structure of a polynucleotide**



Summary

1. Under physiologic conditions, the amino and oxo tautomers predominate

2. Nucleic acids contain traces of 5-methylcytosine, 5-hydroxymethylcytosine, pseudouridine (ψ), and *N-methylated heterocycles.*

3. D-ribose or 2-deoxy-D-ribose linked to *N-1 of a pyrimidine or to N-9 of a purine by a β-glycosidic* bond
anti conformers predominate

4. A primed numeral indicates the hydroxyl to which the phosphoryl group of the sugars of mononucleotides (eg, 3′-GMP,5′-dCMP) is attached

5. Additional phosphoryl groups linked to the first by acid anhydride bonds form nucleoside diphosphates and triphosphates.

6. Nucleoside triphosphates have high group transfer potential and participate in covalent bond syntheses

7. The cyclic phosphodiesters cAMP and cGMP function as intracellular second messengers.

8. Mononucleotides linked by 3′ → 5′-phosphodiester bonds

9. When represented as pTpGpT or TGCATCA, the 5′-end is at the left, and all phosphodiester bonds are 3′ → 5′.

Clinical Problems and Multiple choice questions

1. Which of the following statements about β,γ-methylene and β,γ-imino derivatives of purine and pyrimidine triphosphates Is CORRECT?

a. They are potential anticancer drugs

b. They are precursors of B vitamins.

C. They readily undergo hydrolytic removal of the terminal phosphate.

d. They can be used to implicate involvement of Nucleotide triphosphates by effects other than phosphoryl transfer

Ans d

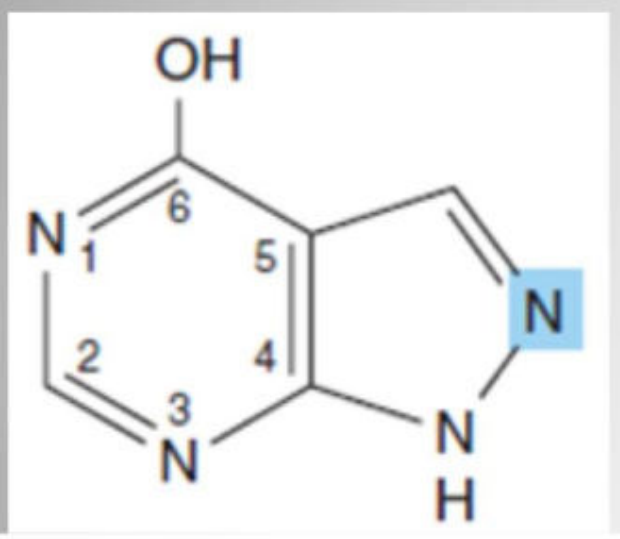
2. Which of the following statements about nucleotide structures is *NOT CORRECT*?

a. Nucleotides are polyfunctional acids.
b. Caffeine and theobromine differ structurally solely with respect to the number of methyl groups attached to their ring nitrogens.
c. The atoms of the purine ring portion of pyrimidines are numbered in the same direction as those of a pyrimidine.
d. NAD+, FMN, "active methionine" and coenzyme A all Are derivatives of ribonucleosides
e. 3',5'-Cyclic AMP and GMP (cAMP and cGMP) serve as second messengers in human biochemistry.

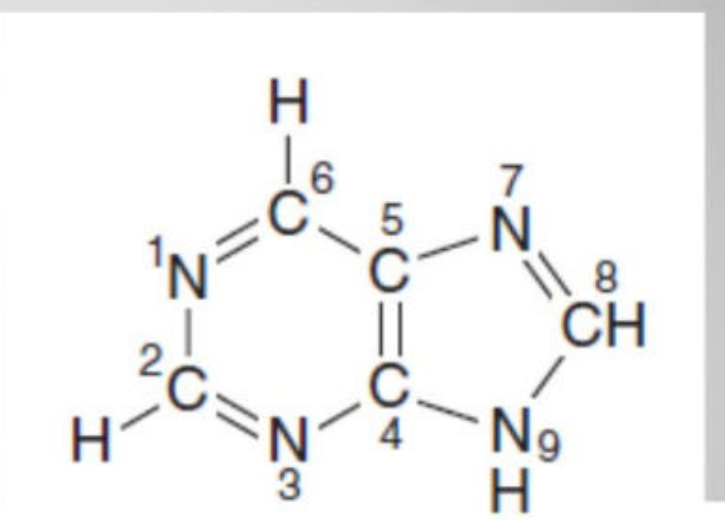
Ans c.

A 36 years old male patient is diagnosed with Gout. Doctor has decided to prescribe Allopurinol.
What modifications in the generic purine ring would help a pharmacist to prepare Allopurinol

Ans: Exchange of C and N at 7 and 8 positions



Allopurinol



Purine

- VIDEO for DNA structure

Nucliec acid stucture and Function

Objectives

1. Functions of nucleic acid
2. What are the different forms of DNA
3. What are complementary pairs and why
4. Why there are specific number of hydrogen bonds
5. Why DNA is more stable-
6. Chargaffs rule
7. Watson Crick model
8. Tm

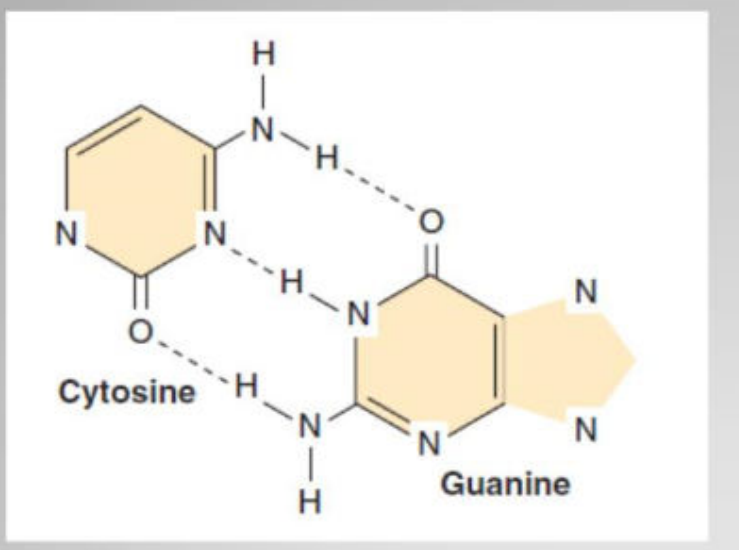
Nucleoprotein

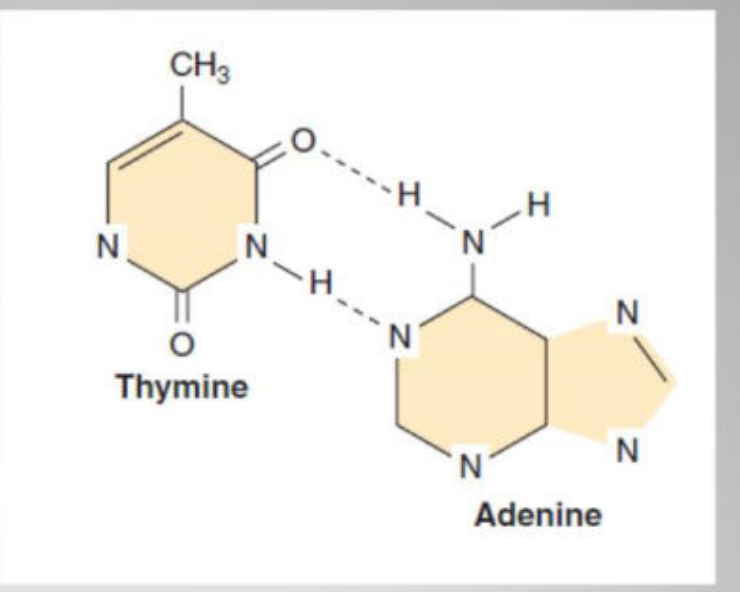
Functions of nucleic acid

- The main functions is to store and transfer genetic information.
- The deoxyribonucleic acid is the storage place for genetic information in the cell.
- The function of the nitrogenous base sequences in the DNA backbone determines the proteins being synthesized
- DNA controls the synthesis of RNA in the cell.
- The genetic information is transmitted from DNA to the protein synthesizers in the cell.
- The function of the double helix of the DNA is that no disorders occur in the genetic information if it is lost or damaged.

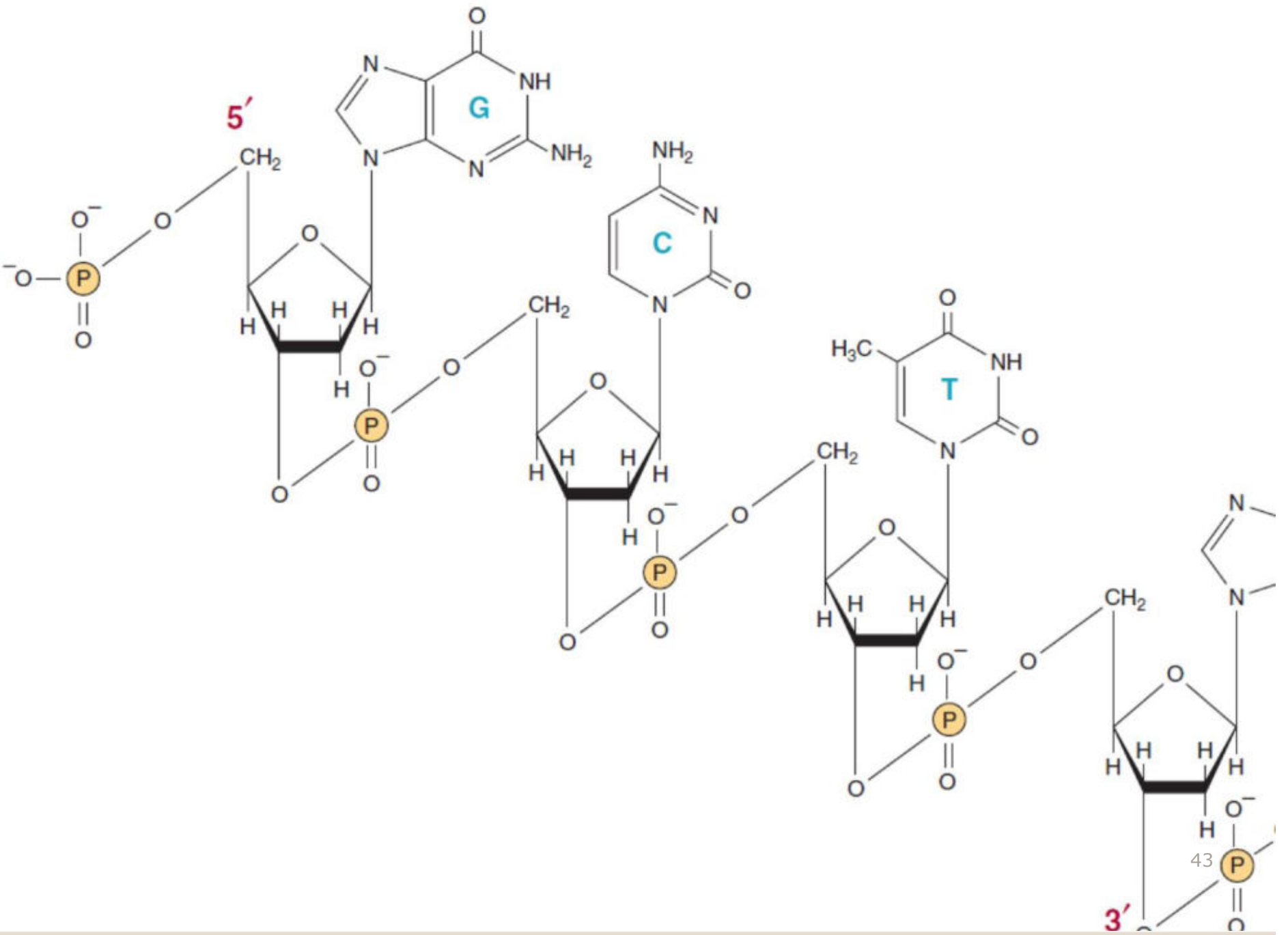
Functions of nucleic acid contd

- RNA directs the production of new protein by transmitting genetic information to the protein building structures
- m-RNA takes genetic message from RNA.
- t-RNA transfers activated amino acid, to the site of protein synthesis.
- r-RNA are mostly present in the ribosomes, and responsible for stability of m-RNA.

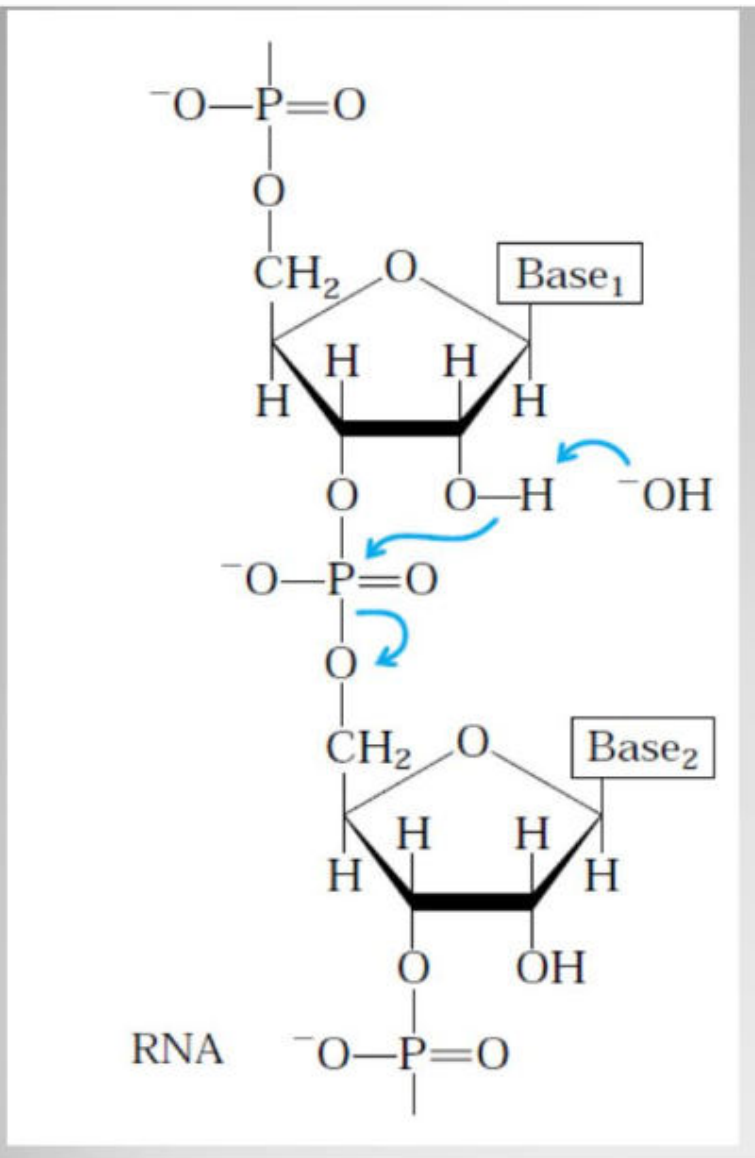




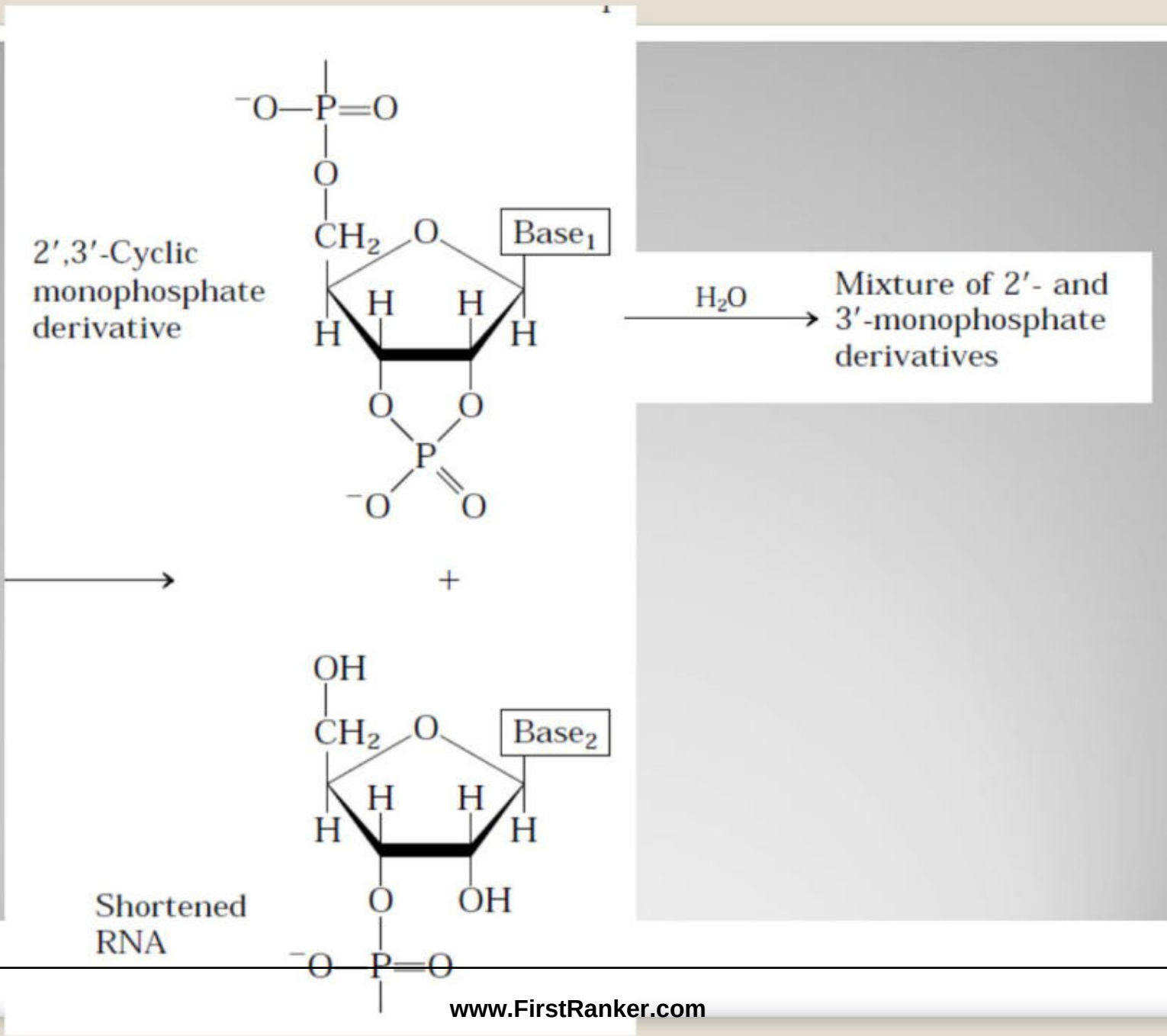
base pairing between complementary deoxynucleotides: formation of hydrogen bonds



- **Why RNA is more susceptible to degradation**
- **Hydrolysis of RNA under alkaline conditions**



Hydrolysis of RNA



Based on data collected from DNAs of a great many different species, **Chargaff concluded**:

1. The base composition of DNA generally varies from one species to another.

2. *DNA specimens isolated from different tissues of* the same species have the same base composition.

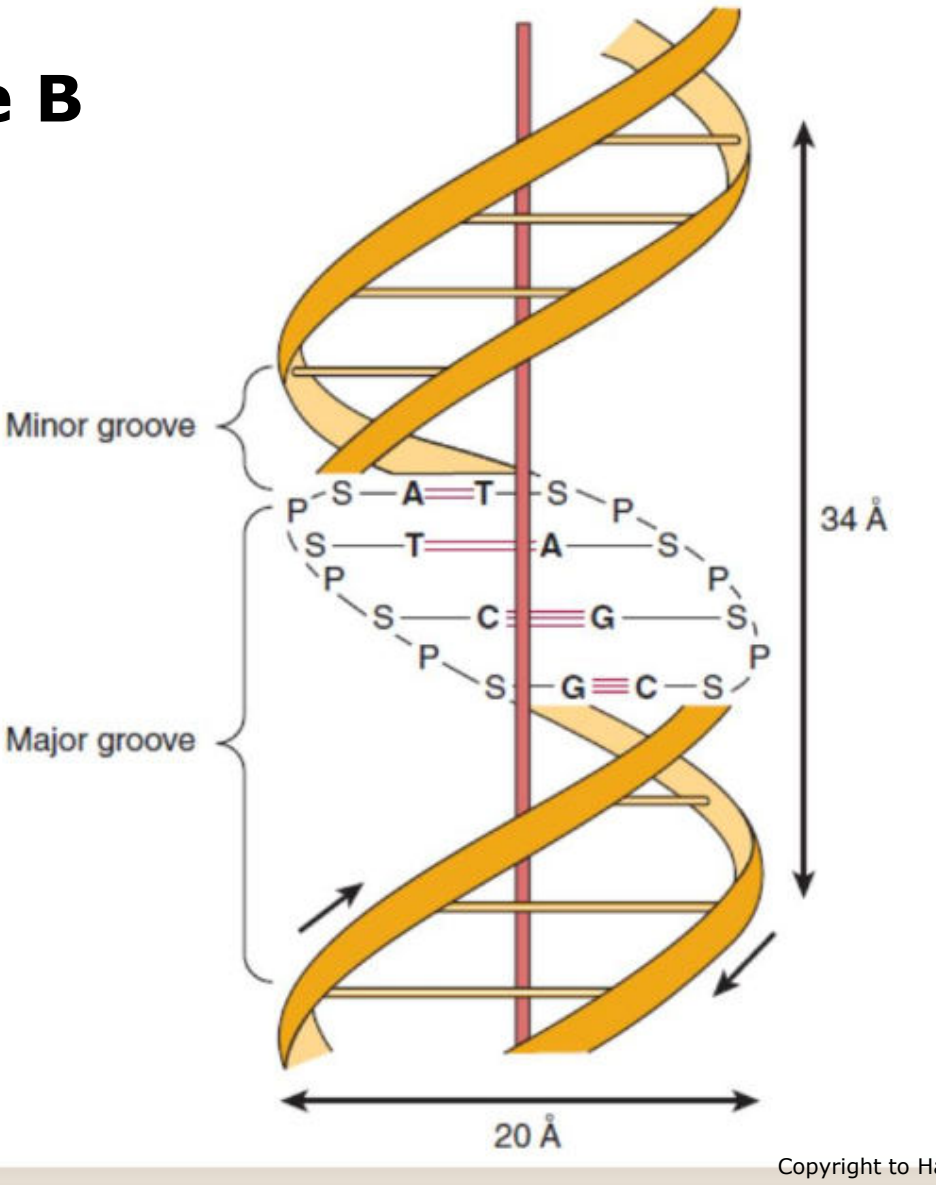
3. *The base composition of DNA in a given species* does not change with an organism's age, nutritional state, or changing environment.

4. *In all cellular DNAs, regardless of the species, the n*umber 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).

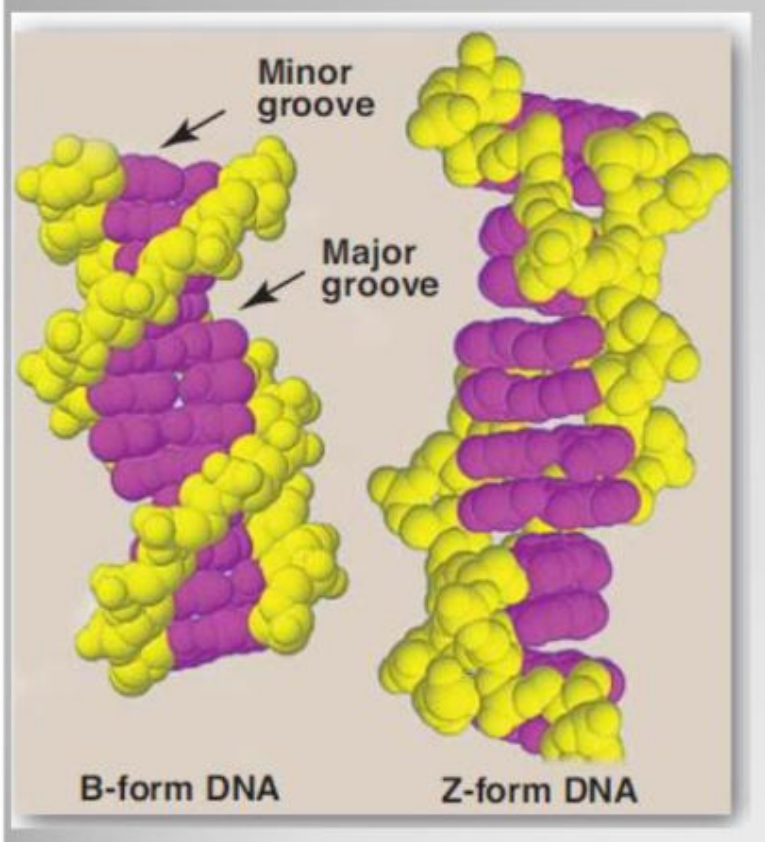
The sum of the purine residues equals the sum of the pyrimidine residues; that is, A+G=T+C

These quantitative relationships, sometimes called "**Chargaff's rules**,"

The Watson and Crick model of the double-helical structure of the B form of DNA



Structures of different forms of DNA

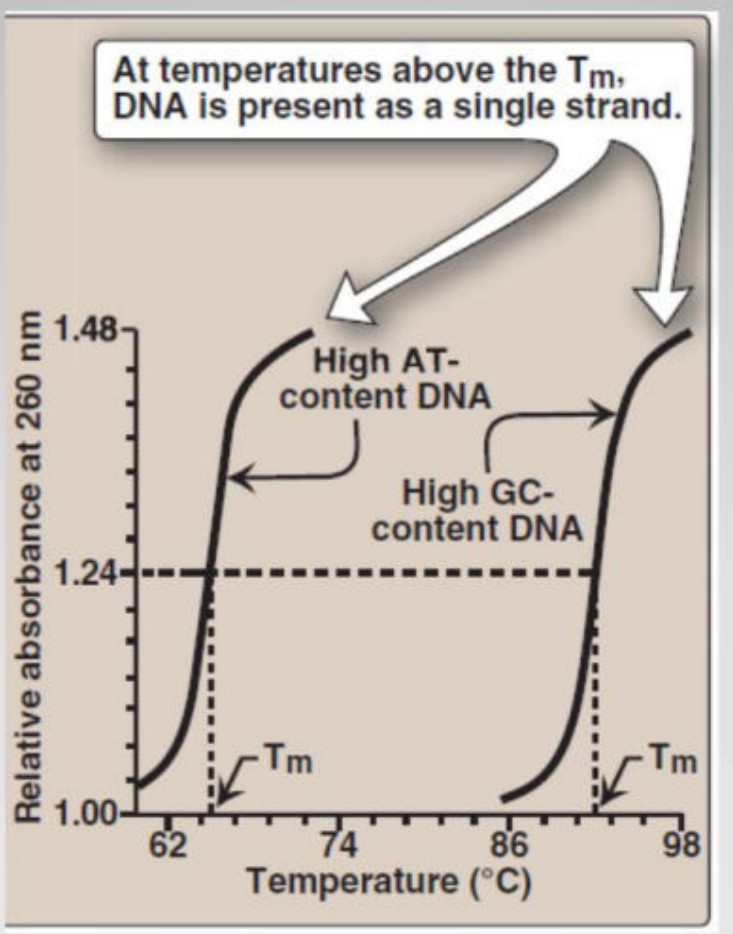


Different forms: A-E &Z

A form: DNA-RNA hybrids and RNA-RNA double stranded regions

Z form: alternating purines and pyrimidi

Melting temperature of DNA molecules with different nucleotide composition



Adopted from lippincott

For sequences less than 14 nucleotides the formula is:

Tm= (wA+xT) * 2 + (yG+zC) * 4

where w,x,y,z are the number of the bases A,T,G,C in the sequence, respectively.

For sequences longer than 13 nucleotides, the equation used is

Tm= 64.9 +41*(yG+zC-16.4)/(wA+xT+yG+zC)

Both equations assume that the annealing occurs under the standard conditions of 50 nM primer, 50 mM Na^+, and pH 7.0.

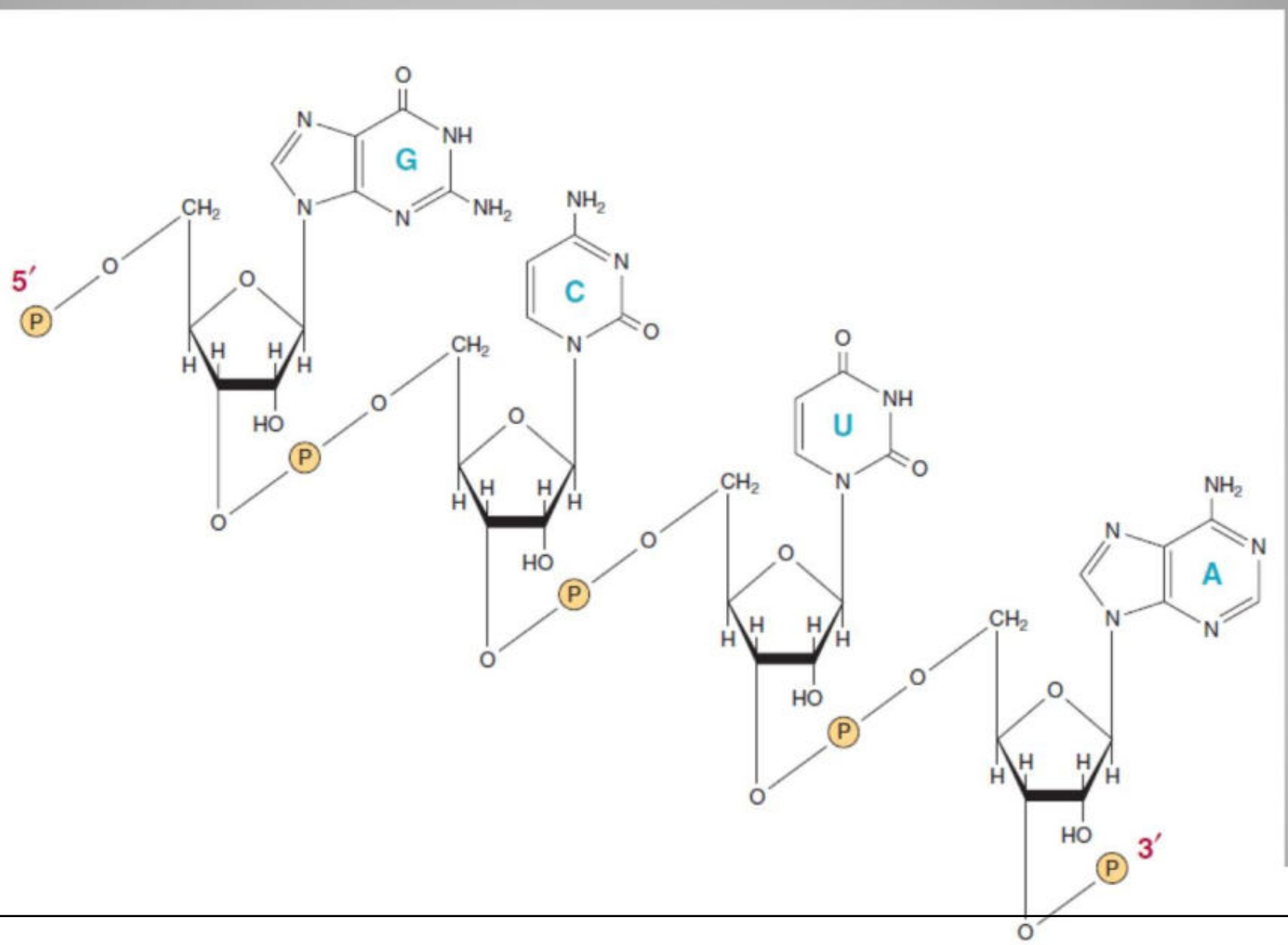
- Base composition
- Salt concentration:
  - 10 fold monovalent cation concentration increase=Tm?
- Organic solvent:
  - Fomamide addition= Tm ?

Factors influencing Tm

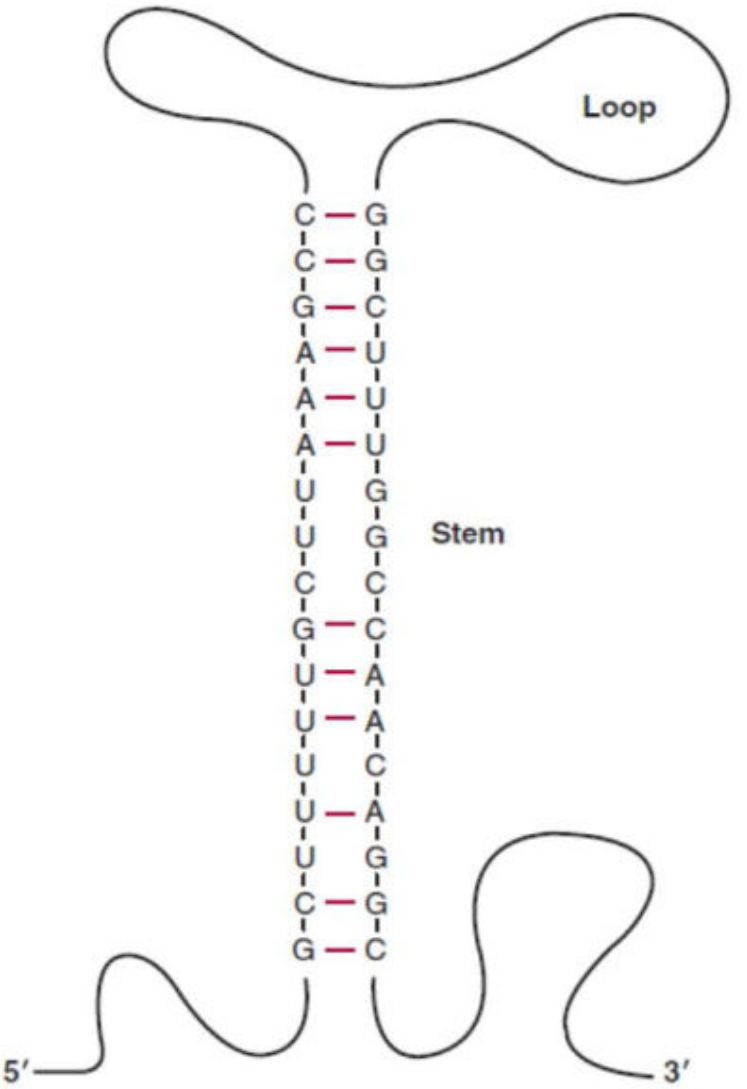
- Importance of melting temperature
- Video on PCR

Annealing temperature

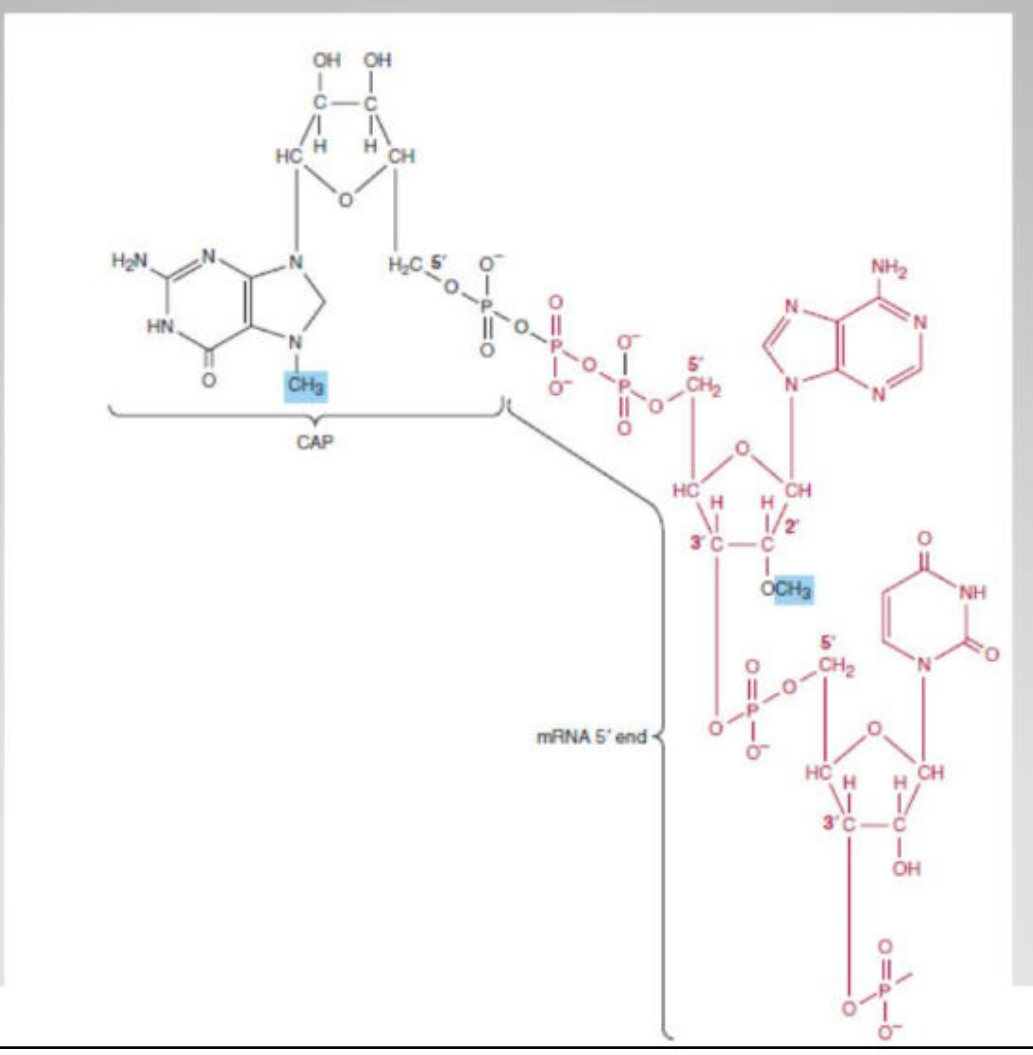
RNA



Secondary structure of RNA



Cap structure attached to eukaryotic mRNA



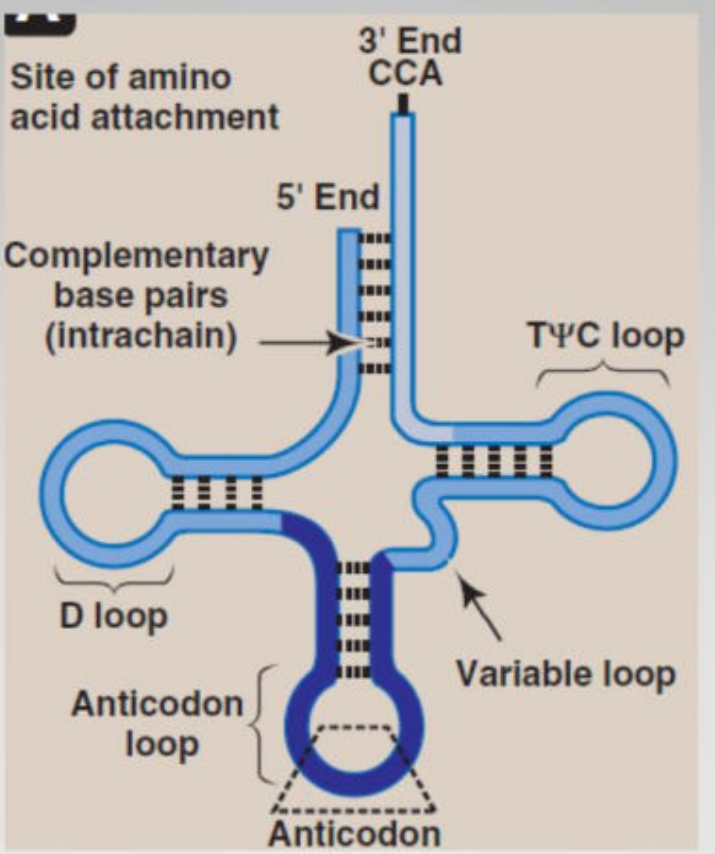
Components of mammalian ribosome

Component	Mass (MW)	Protein		RNA		
		Number	Mass	Size	Mass	Bases
40S subunit	1.4×10^6	33	7×10^5	18S	7×10^5	1900
60S subunit	2.8×10^6	50	1×10^6	5S	3.5×10^4	120
				5.8S	4.5×10^4	160
				28S	1.6×10^6	4700

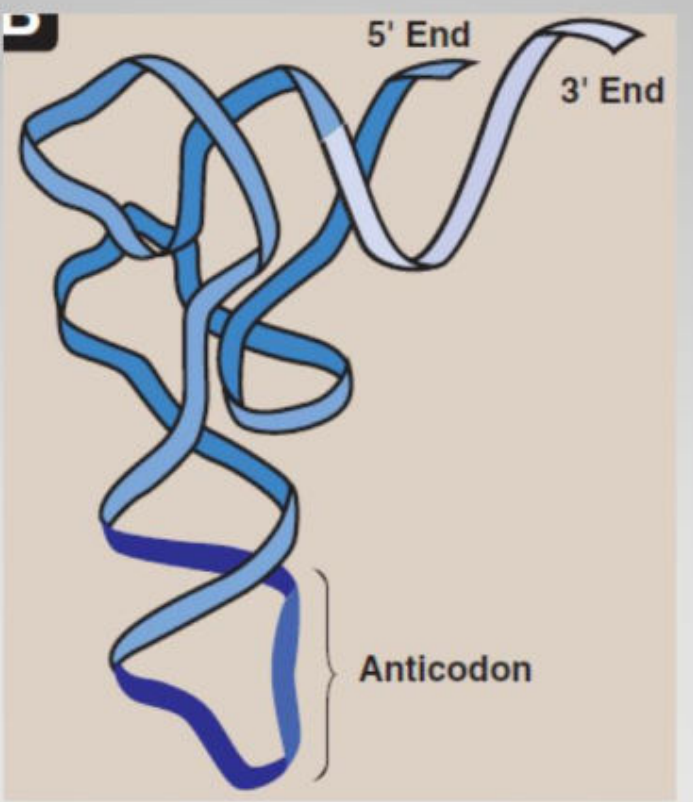
- A subset of small RNA (20-1000 nucleotides)
- Involved in rRNA and mRNA processing and gene regulation
- U1,U2,U4,U5,U6---involved in intron removal
- U7 ---in production of correct 3’ end of histone mRNA

Small Nuclear RNA (snRNA)

tRNA secondary structure



Tertiary structure of tRNA



- DNA consists of four bases—A, G, C, and T—that are held in linear array by phosphodiester bonds through the 3′ and 5′ positions of adjacent deoxyribose moieties.
- DNA is organized into two strands by the pairing of bases A to T and G to C on complementary strands. These strands form adouble helix around a central axis.
- The 3 × 109 bp of DNA in humans are organized into the haploid complement of 23 chromosomes. The exact sequence of these 3 billion nucleotides defines the uniqueness of each individual
- RNA exists in several different single-stranded structures, most of which are directly or indirectly involved in protein synthesis or its regulation. The linear array of nucleotides in RNA consists of A, G, C, and U, and the sugar moiety is ribose.
- The major forms of RNA include mRNA, rRNA, tRNA, and snRNAs and regulatory ncRNAs.
- Certain RNA molecules act as catalysts (ribozymes)—e.g,.the large rRNA component performs the peptidyl transferase activity.

Summary

- While studying the structure of a small gene that was recently sequenced during the Human GenomeProject, an investigator notices that one strand of the DNA molecule contains 20 As, 25 Gs, 30 Cs, and 22 Ts. How many of each base is found in the complete double-stranded molecule?

A. A = 40, G = 50, C = 60, T = 44.
B. A = 45, G = 45, C = 52, T = 52.
C. A = 50, G = 47, C = 50, T = 47.
D. A = 42, G = 55, C = 55, T = 42
E. A = 44, G = 60, C = 50, T = 40.

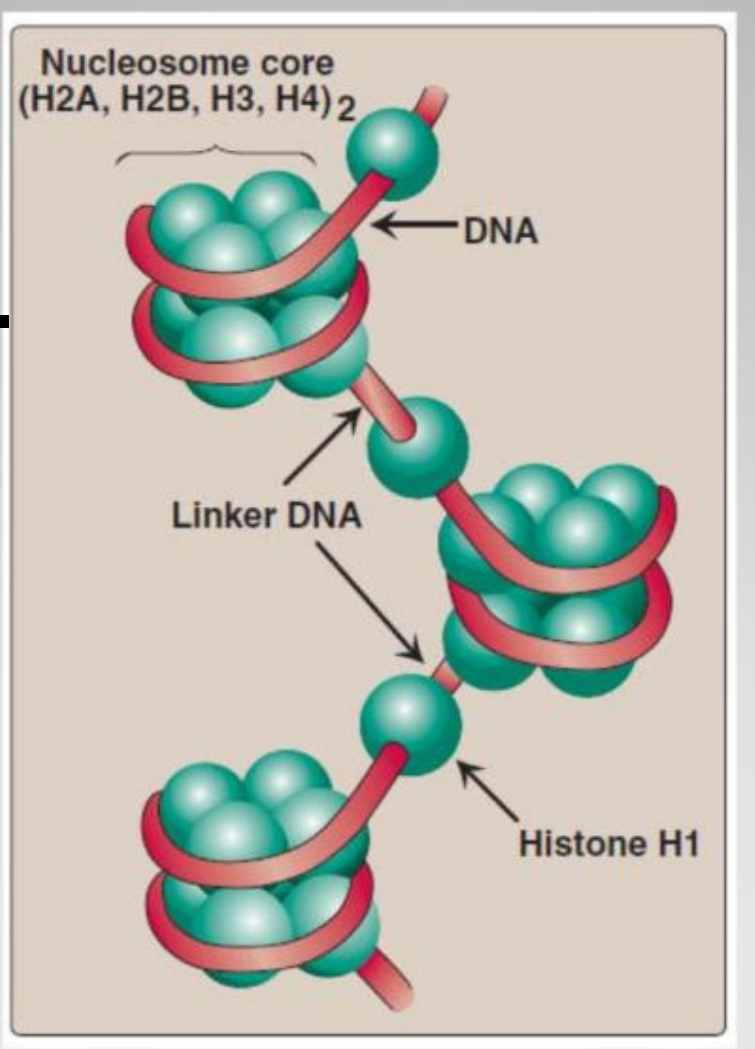
MCQ1

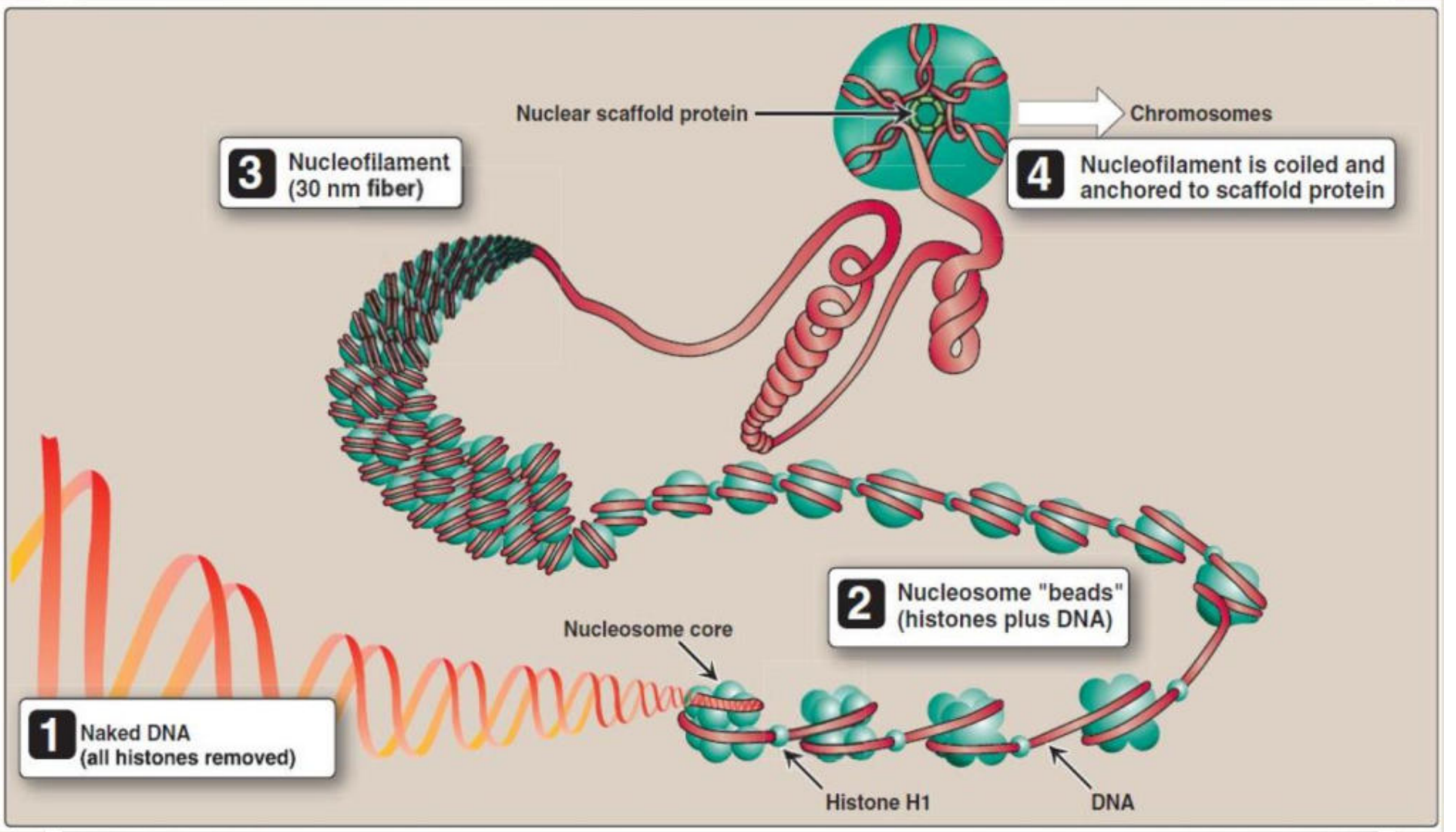
- Any proteins that are structurally associated with nucleic acids, either DNA or RNA
- Nucleoproteins tend to be positively charged, facilitating interaction with the negatively charged nucleic acid chains.
- A **deoxyribonucleoprotein** (DNP) is a complex of DNA and protein.
  - examples are nucleosomes-DNA is wrapped around clusters of eight histone proteins to form chromatin
  - Function : regulating DNA replication and ~~transcri~~ption.
    - involved in homologous recombination, a process for repairing DNA

Nucleoprotein

- **Ribonucleoproteins**: a complex of ribonucleic acid and RNA-binding protein
- Examples: ribosome, the enzyme telomerase, RNase P, hnRNP and small nuclear RNPs
- Functions: DNA replication, regulating gene expression and regulating the metabolism of RNA

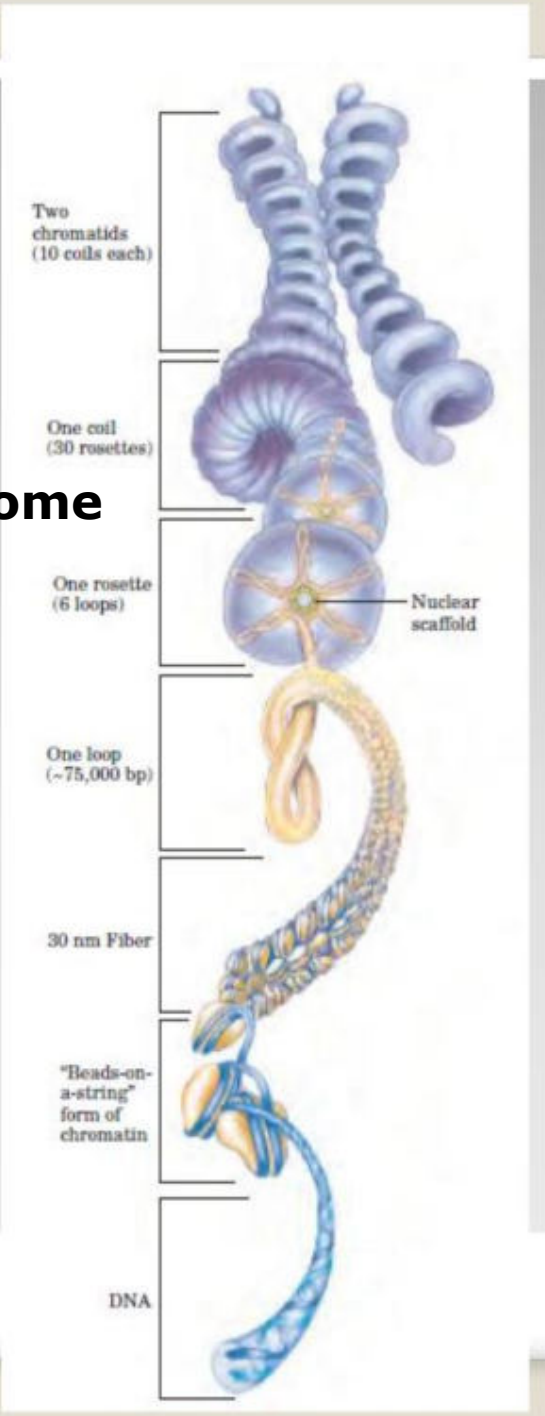
structure of nucleosomes.

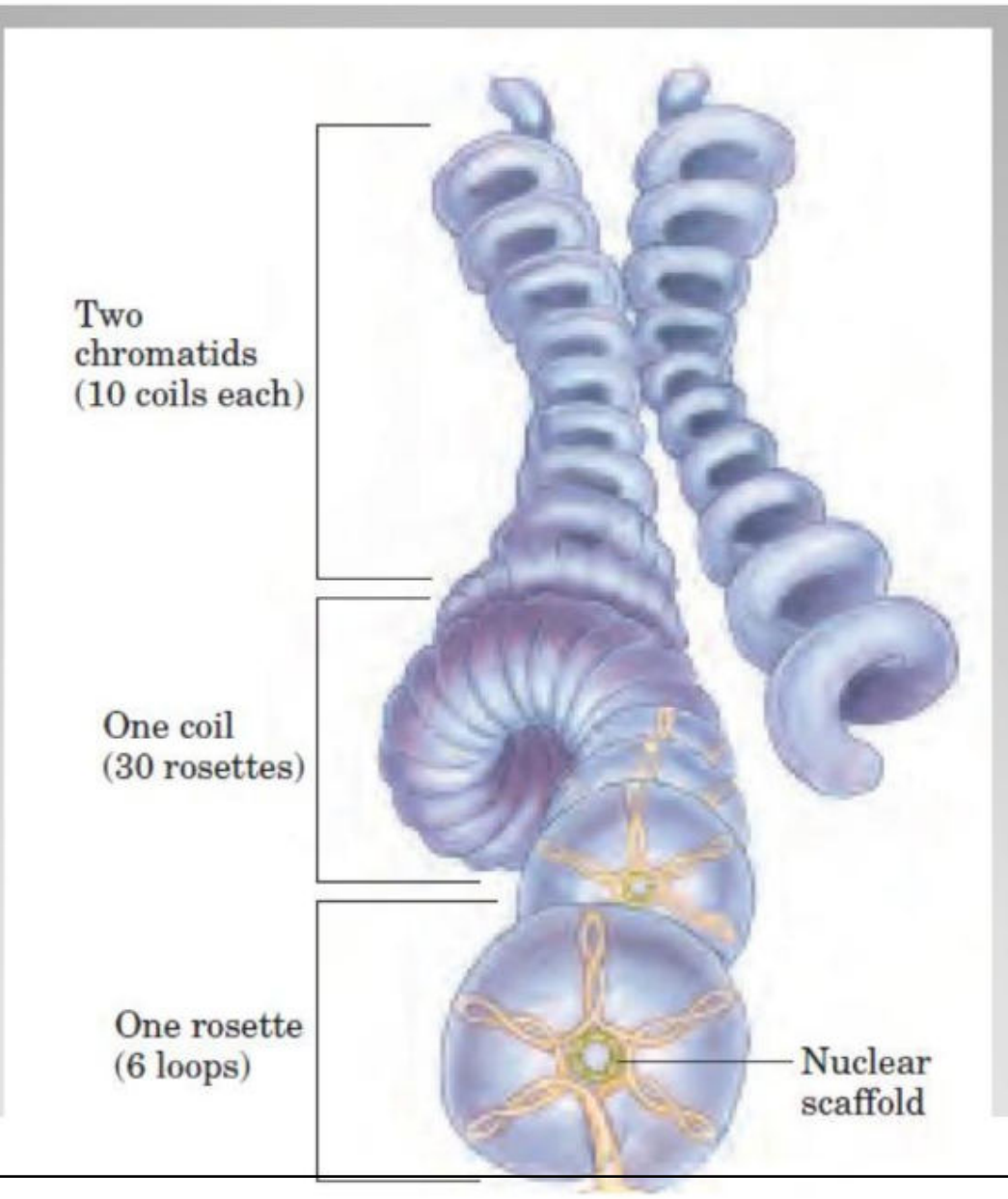


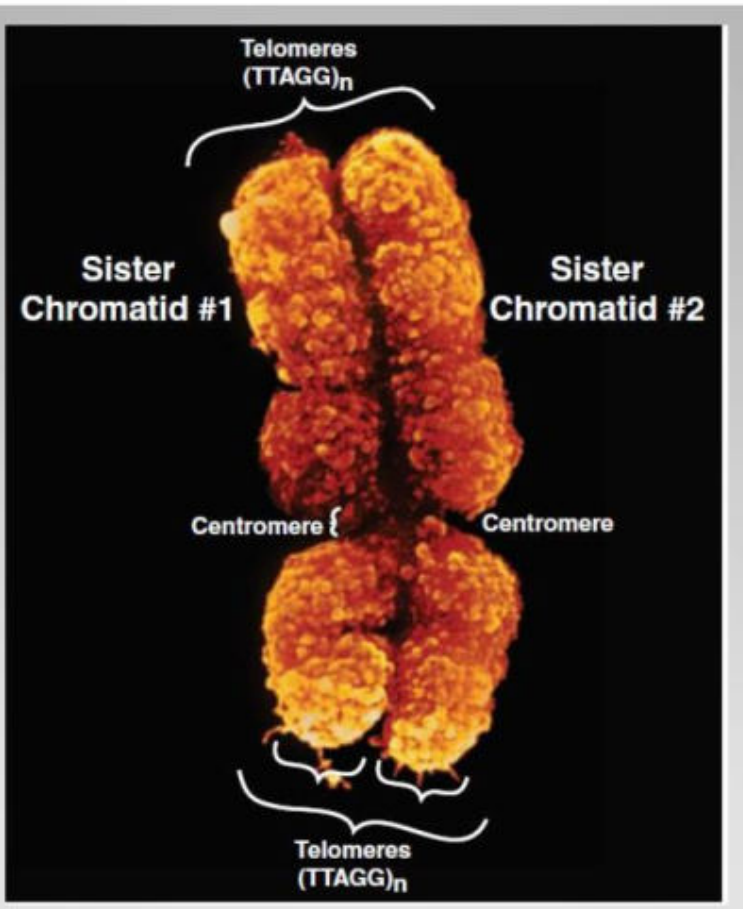


Structural organization of eukaryotic DNA

Compaction of DNA in a eukaryotic chromosome







- Video on DNA Packaging

- Euchromatin: Transcriptionally active chromatin, stains less densely
- Heterochromatin: Transcriptionally inactive chromatin, densely packed during interphase

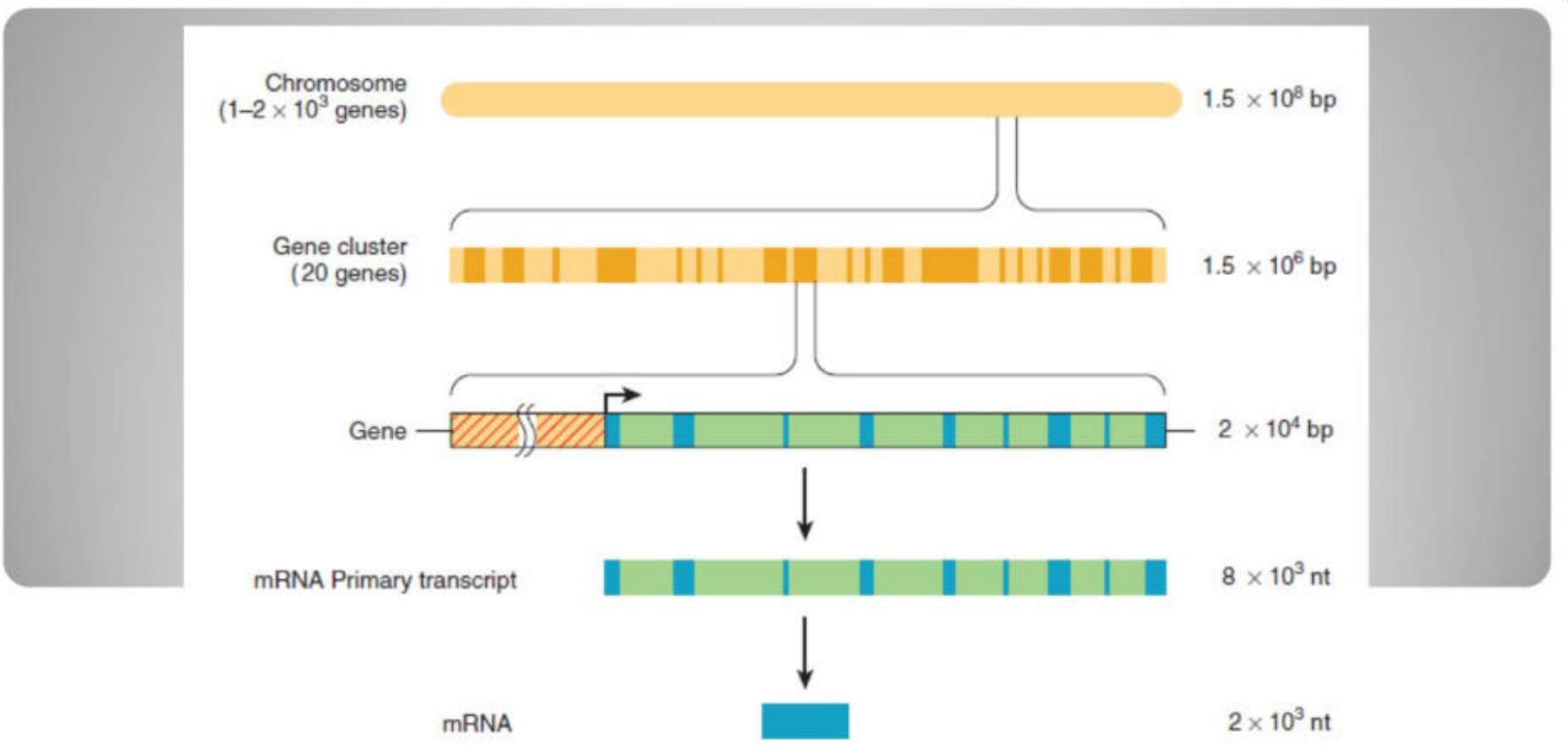
Euchromatin and Heterochromatin

- Constitutive : Found near centromere and telomere
- Facultative : One X chromosome in mammalian female

Hetero Chromatin:

Roles of modified histones

Modification	Effect
Acetylation of histones H3 and H4	**gene transcription**
Acetylation of core histones	Chromosomal assembly during DNA replication.
Phosphorylation of histone H1	condensation of chromosomes during the replication cycle
ADP-ribosylation of histones	DNA repair
Methylation of histones	activation and repression of gene transcription.
Monoubiquitylation	gene activation, repression, and heterochromatic gene silencing
Sumoylation of histones (SUMO; small ubiquitin-related modifier)	transcription repression



The relationship between chromosomal DNA and mRNA

Sequence classes of genome

Unique sequence DNA: single copy gene that code for protein
>50%

Repetitive sequence DNA: include sequences that vary in copy number from 2 to 10^7
30%

- Highly repetetive: 5-500 base pairs length repeated
  - 1-10 million copies per haploid
  - Clustered
  - Transcriptionally inactive
  - Structural role

- Moderately repetitive DNA sequences
  - < 1 million copies per haploid genome
  - Long/sh Interspersed repeats
  - Transcribed by RNA poymerase II

  - LINES (Long interspersed repeat sequences)
    - 20,000-50,000 copies of 6-7 kbp

  - SINES (Short interspersed repeat sequences)
    - 70-300 bp > 100,000 copies per genome
    - e,g Alu family

Both types appear to be **Retroposons**:

Disastrous consequence of transposition : Neurofibromatos is

Microsatellite repeat sequences

2-6 bp sequences repeated upto 50 times

Most common dinucleotide repeats

AC repeat sequences occur at 50,000-100,000 locations

Useful in constructing genetic map

Useful to screen large number of family members rapidly

Trinucleotide repeat sequences

CGG---Fragile X syndrome
CAG---Huntington' Chorea
CTG—myotonic dystrophy
CAG—Spinobulbar muscular atrophy
Kennedy disease

Major Features of Human Mitochondrial DNA

- Is circular, double-stranded,
- Contains 16,569 bp
- Encodes 13 protein subunits of the respiratory chain

Seven subunits of NADH dehydrogenase (complex I)
Cytochrome *b of complex III*
Three subunits of cytochrome oxidase (complex IV)
Two subunits of ATP synthase

- Encodes large (16S) and small (12S) mt ribosomal RNAs
- Encodes 22 mt tRNA molecules
- **Genetic code differs slightly from the standard code**

UGA (standard stop codon) is read as Trp
AGA and AGG (standard codons for Arg) are read as stop codons

- Contains very few untranslated sequences
- High mutation rate (5-10 times that of nuclear DNA)
- Comparisons of mtDNA sequences provide evidence about evolutionary origins of primates and other species

Transposon

Common in all types of organisms are short pieces of DNA (usually less than 10Kb in length) that can move from one position to another in the chromosome of a cell.

Retroposon:

Eukaryotic genomes contain a special type of Transposable elements, called retroposons, which use reverse transcriptase to transpose through an **RNA** intermediate.

- ■ DNA in eukaryotic cells is associated with a variety of proteins, resulting in a structure called chromatin.
- ■■ Much of the DNA is associated with histone proteins to form a structure called the nucleosome. Nucleosomes are composed of an octamer of histones around which about 150 bp of DNA is wrapped.
- ■■ Histones are subject to an extensive array of dynamic covalent modifications that have important regulatory consequences.
- ■■ Nucleosomes and higher-order structures formed from them serve to compact the DNA.

Summary

- ■■ DNA in transcriptionally active regions is relatively more sensitive to nuclease attack in vitro; some regions, so-called hypersensitive sites are exceptionally sensitive and are often found to contain transcription control sites.
- ■■ Highly transcriptionally active DNA (genes) is often clustered in regions of each chromosome. Within these regions, genes may be separated by inactive DNA in nucleosomal structures.
- ■■ After transcription, during RNA processing, introns are removed and the exons are ligated together to form the mature mRNA that appears in the cytoplasm; this process is termed RNA splicing.

Summary

Epigenetics

Information that is passed from one genetation to the next – to daughter cells at cell divisions or from parent to offspring –but is not encoded on DNA sequences is referred to as epigenetic information.

- 1. DNA Methylation
  - A covalent modification of nucleotide cytosine at 5’ position
    - By the enzyme DNA methytransferase 1
    - DNA methylation is the most useful epigenetic marker for human diseases studies because it is stable over a period of decades and is present in archival specimen including paraffin blcks

Epigenetic information takes 3 forms

- 2. Post translational modifications of nucleosomal histones
  - ATP independent process involving acetylation, phosphorylation, ubiquitylation
  - Post translational modifications act through recruitment of transcription factor, activation of transcriptional enhancers, recruitment of repressive proteins and interaction with DNA methylation machinery

Second form of epigenetic information

- Higher order Chromatin structure
  - Example of which include loop organization revealed by chromosome conformation capture method
  - Large organized chromatin lysin modifications
  - Nuclear lamina associated domain

Third form of Epigenetic information

- Diet
  - Deprivation of methionine and folate lead to liver and colon cancer
    - Folate deficieny--- impaired biosynthesis of active precursor for DNA methylation, S adenosyl methionine---Impairs synthesis of thymidylate
    - Exposure to nicotine causes substantial epigenetic changes in smokers

Modulation of Epigenetic information

- **Cancer**
  - DNA methyltransferases, TET demethylase, MBD (methyl CpG binding domain) family of methylation recognition gene are mutated in lymphoma and colon cancer
  - Variability in DNA methylation was markedly increased in the samples from women in whom cancer developed years later
- **Aging**
  - Genome wide hypomethylation in blood is associated with breast cancer years later

- The interconnecting bonds that connecting the nucleotides of RNA and DNA are termed:
  A. *N*-glycosidic bonds
  B. 3′-5′-phosphodiester linkages
  C. Phosphomonoesters
  D. -2′-phosphodiester linkages
  E. Peptide nucleic acid bonds

MCQ1

- Which of the forces or interactions listed below play the predominant role in driving RNA secondary and tertiary structure formation?

A. Hydrophilic repulsion
B. Formation of complementary base pair regions
C. Hydrophobic interaction
D. van der Waals interactions
E. Salt bridge formation

MCQ2

- Which entry below correctly describes the approximate number of bp of DNA_____, which is separated into _____chromosomes in atypical diploid human cell in a nonreplicating state?
-

A. 64 billion, 23
B. 6.4 trillion, 46
C. 23 billion, 64
D. 64 billion, 46
E. 6.4 billion, 46

MCQ3

- All but one of the following histones are found located within the superhelix formed between DNA and the histone octamer; this histone is
-
A. Histone H2B
B. Histone H3
C. Histone H1
D. Histone H3
E. Hisone H4

MCQ4

- Chromatin can be broadly defined as active and repressed; a subclass of chromatin that is specifically inactivated at certain times within an organism's life and/or in particular sets of differentiated cells is termed

- A. Constitutive euchromatin
B. Facultative heterochromatin
C. Euchromatin
D. Constitutive heterochromatin

MCQ5