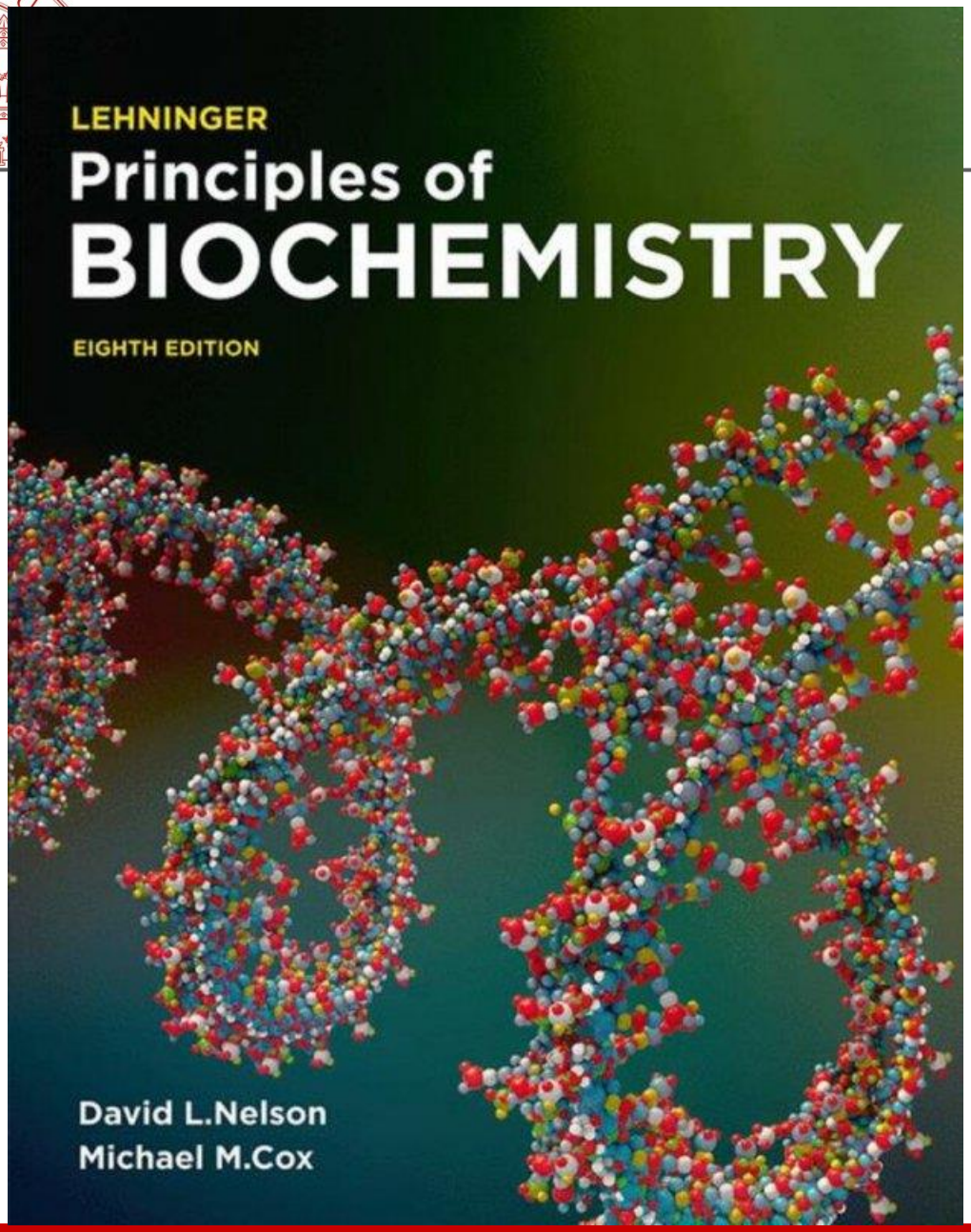
NUCLEIC ACIDS – BIOCHEMISTRY MODULE 2

- Structure and Function of Nucleic Acids
- Genes and Chromosomes (brief summary)
- DNA Replication
- DNA Transcription
- Protein Synthesis

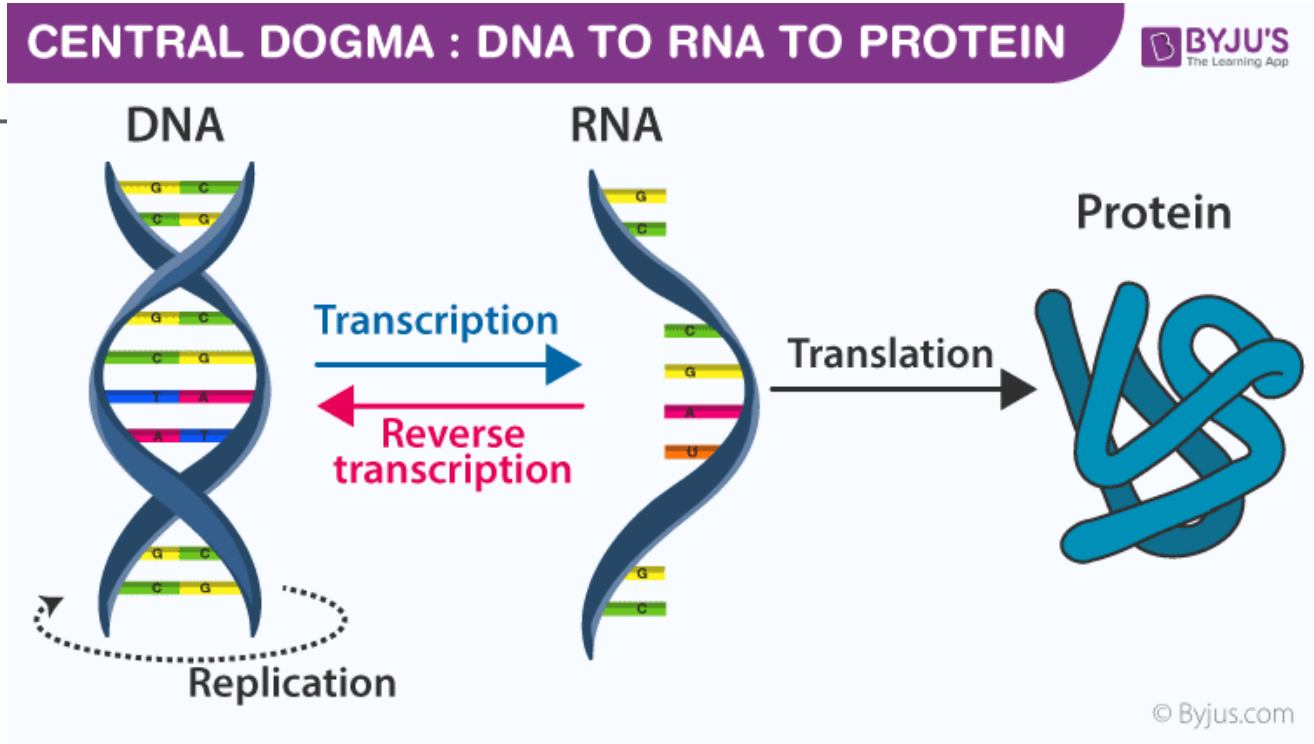
Textbook



Lehninger Principles of Biochemistry
Autori: David L. Nelson, Michael M. Cox

w.h.freeman
Macmillan Learning
New York

7th or 8th editions are both fine!


CENTRAL DOGMA : DNA TO RNA TO PROTEIN
BYJU'S
The Learning App
DNA
RNA
Protein
Transcription
Reverse transcription
Translation
Replication
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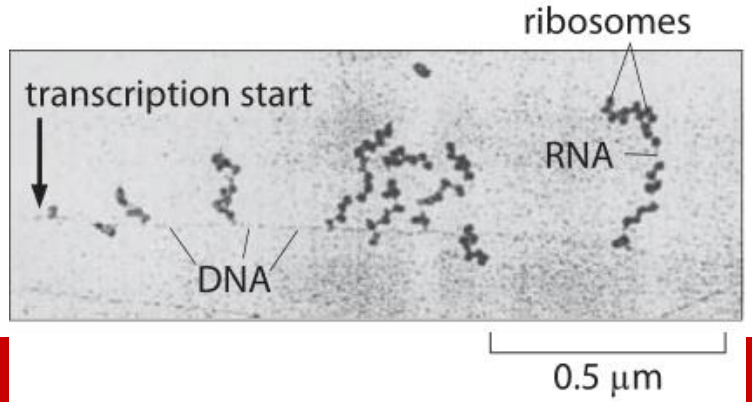
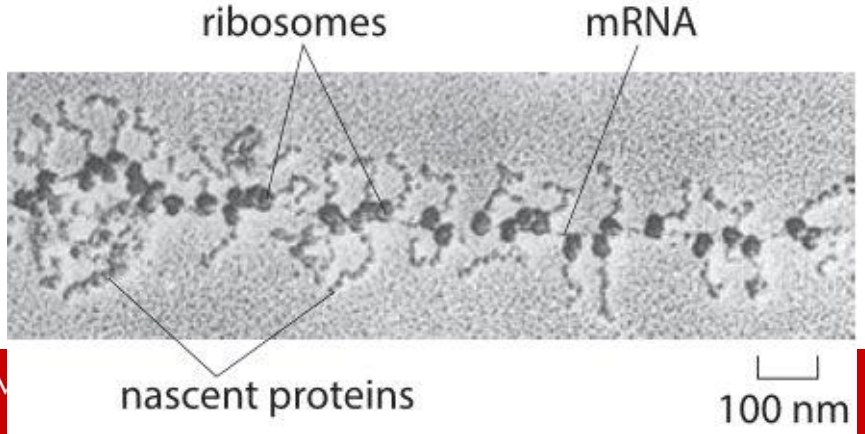

ribosomes
transcription start
RNA
DNA
0.5 µm


ribosomes
mRNA
nascent proteins
100 nm

Functions of Nucleotides

1. The energy currency in metabolic transactions

2. The chemical link in the response of cells to hormones and other extracellular stimuli

3. Structural components of enzyme cofactors and metabolic intermediates

4. The ***constituents of the NUCLEIC ACIDS***:

    - Deoxyribonucleic Acid (DNA)

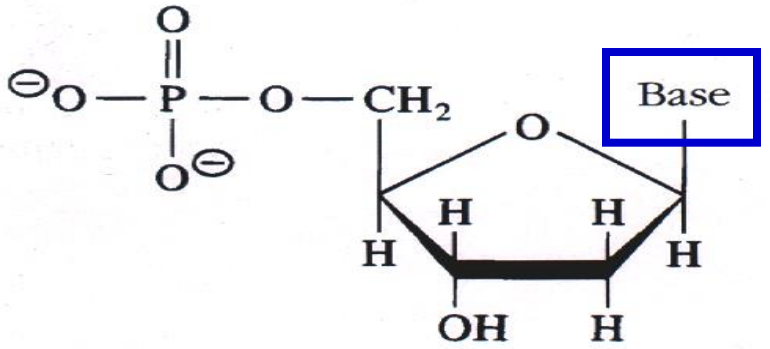
    - Ribonucleic Acid (RNA)

NUCLEOTIDES have 3 characteristic components:

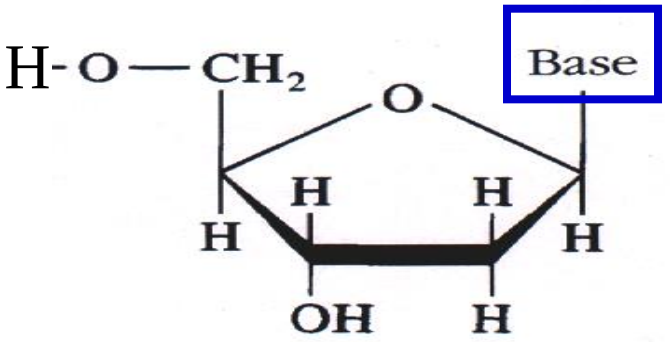
- **A nitrogenous Base**

- **A pentose (sugar with 5 C-atoms)**

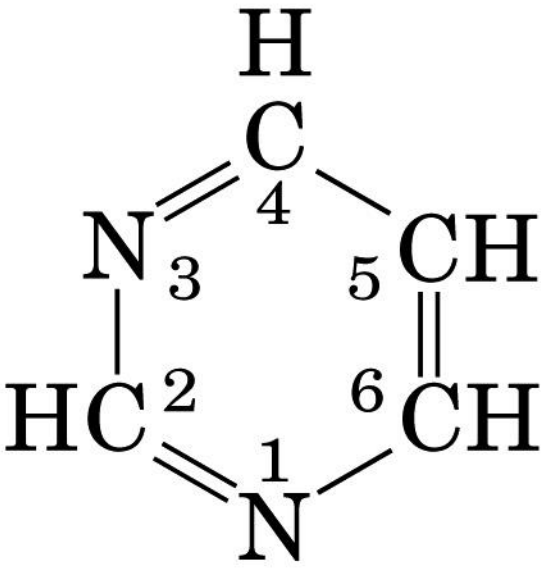
- **A phosphate group**



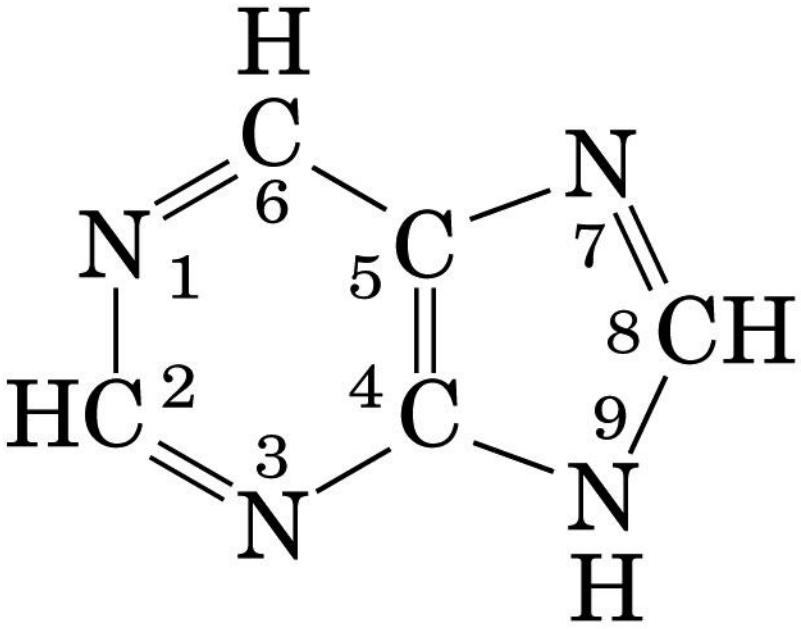
The molecule without the phosphate group is called a **NUCLEOSIDE**



The Nitrogenous Bases are derivatives of:



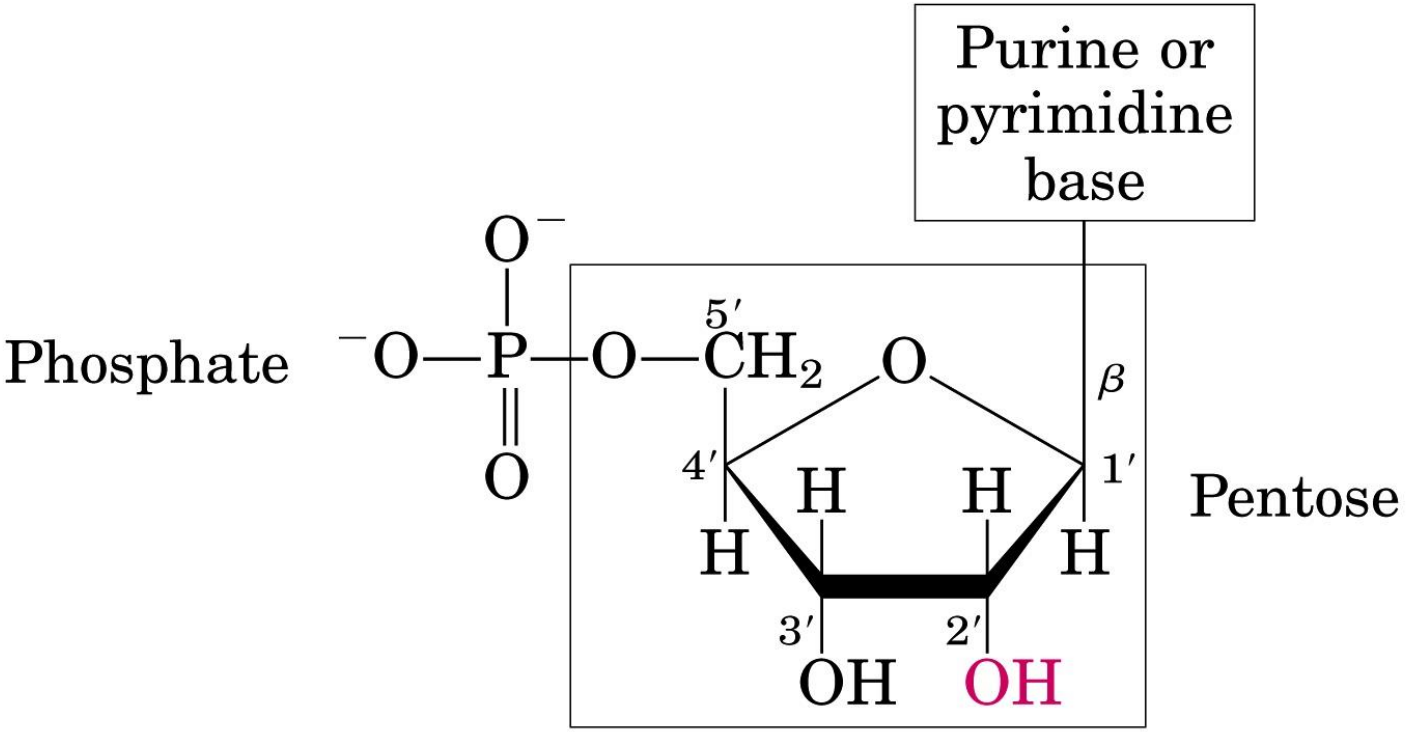
Pyrimidine



Purine

These are heterocyclic rings

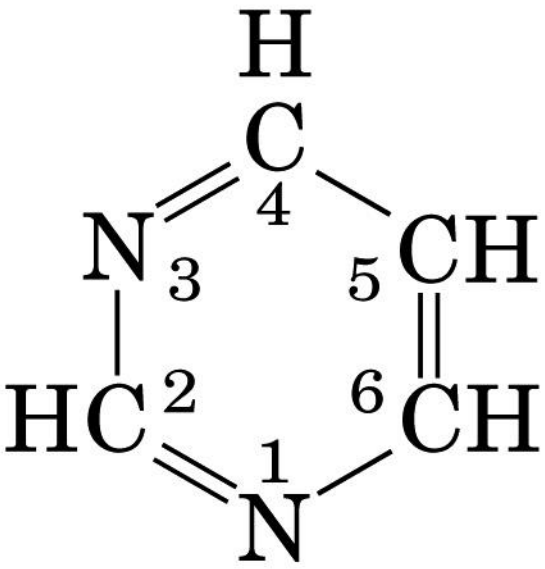
Structure of Nucleotides



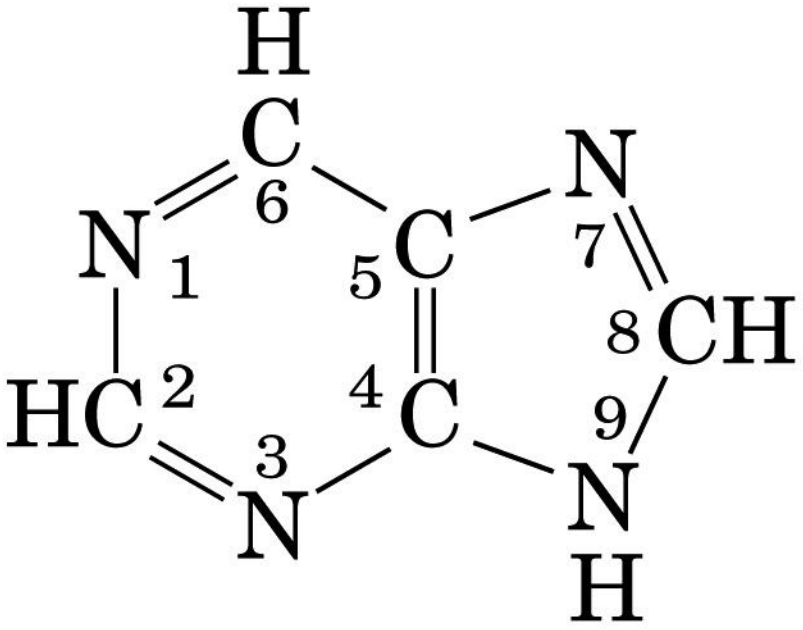
Phosphate Group:

sp^3 (regular tetrahedron)

Nitrogenous Bases of the Nucleic Acids



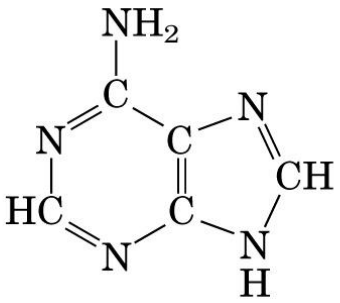
Pyrimidine



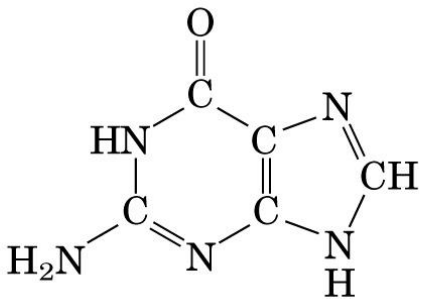
Purine

Molecules → Aromatic (Planar, Hydrophobic)
→ Basic

The Nitrogenous Bases

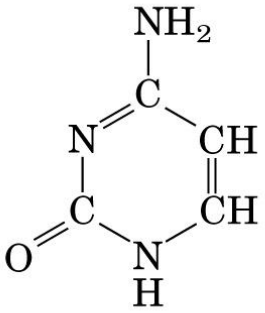


Adenine

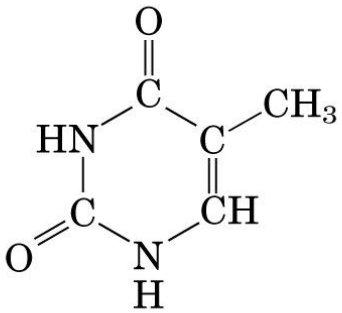


Guanine

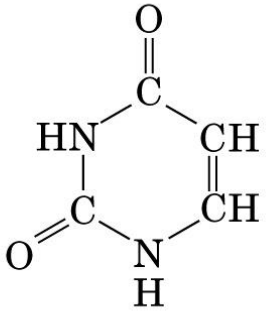
Purines



Cytosine



Thymine
(DNA)

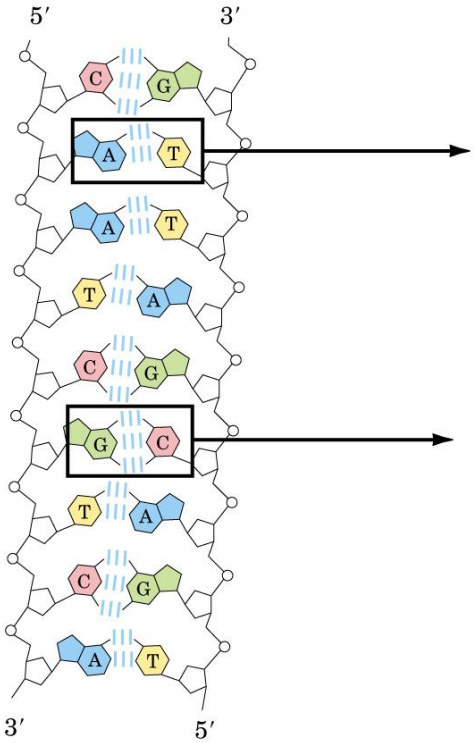


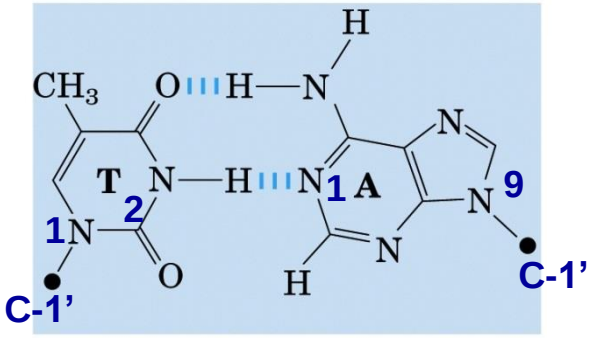
Uracil
(RNA)

Pyrimidines

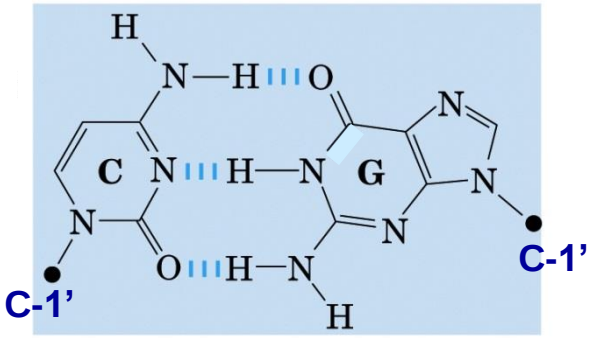
Nitrogenous Base Complementarity

Hydrogen bond





T=A

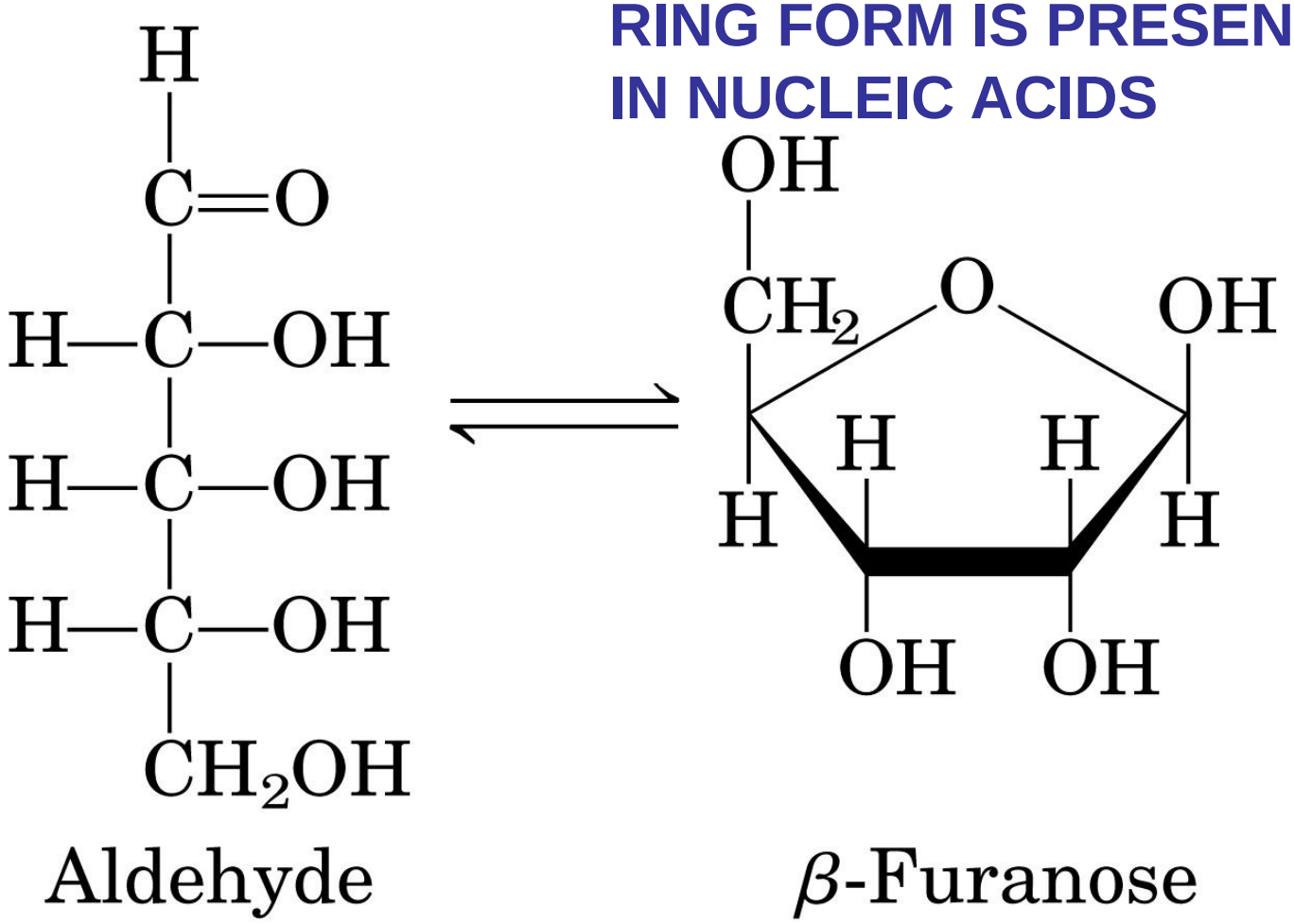


C≡G

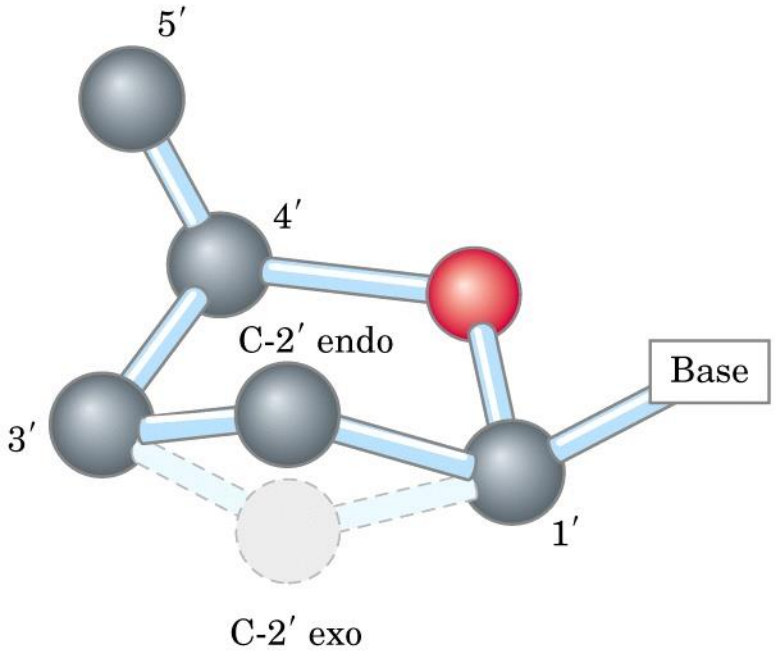
Same geometry!

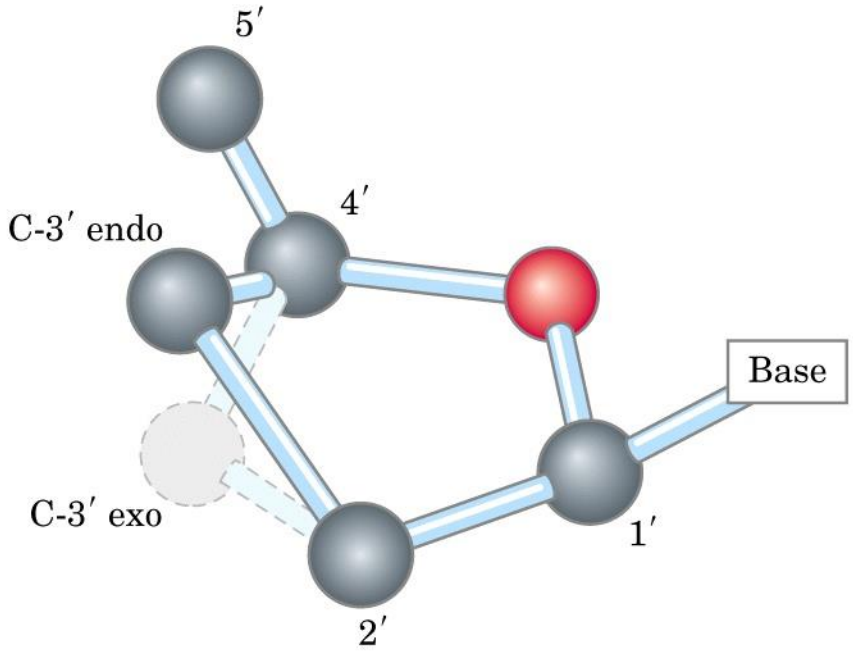
CONFORMATION OF RIBOSE

ONLY THE β-D-FURANOSE RING FORM IS PRESENT IN NUCLEIC ACIDS



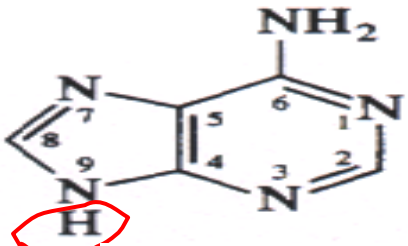
CONFORMATION OF RIBOSE
4 different puckered conformations



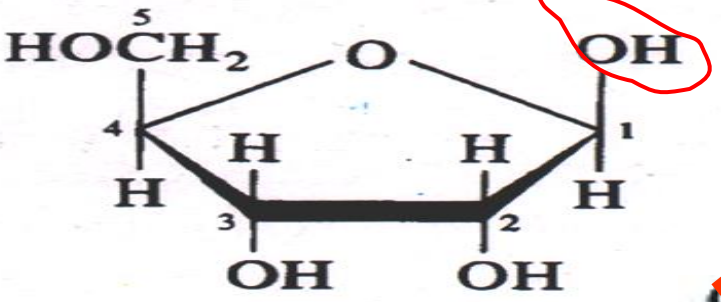


4 out of 5 atoms are nearly in a single plane.

C-2’ or C-3’ is on either the same (**ENDO**) or the opposite (**EXO**) side of the plane relative to the C-5’ atom.



Adenine

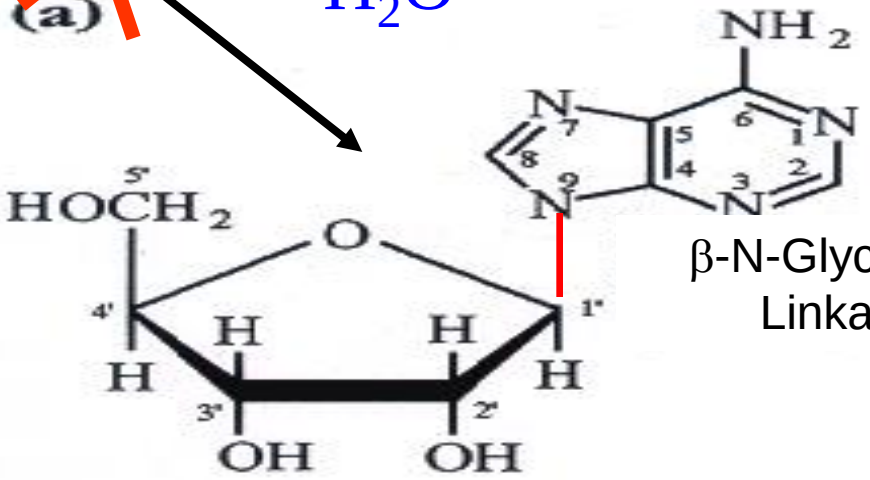


Ribose
(β-D-Ribofuranose)

The bond between the Nitrogenous Base and the pentose is called **N-β-glycosidic, but its condensation does not occur spontaneously due to thermodynamic reasons**

(a)

H_2O

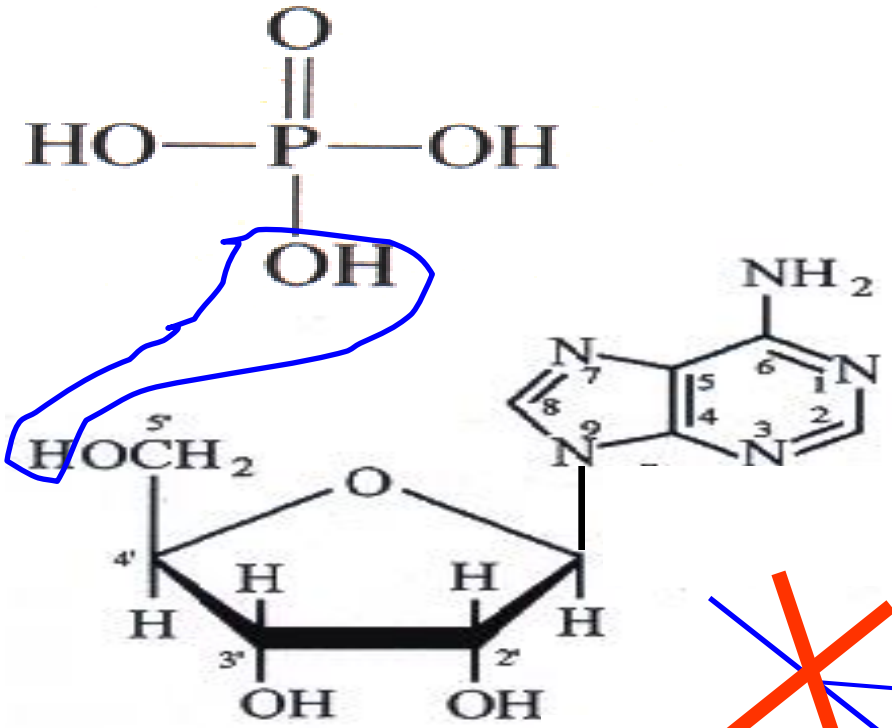


β-N-Glycosidic Linkage

NUCLEOSIDE

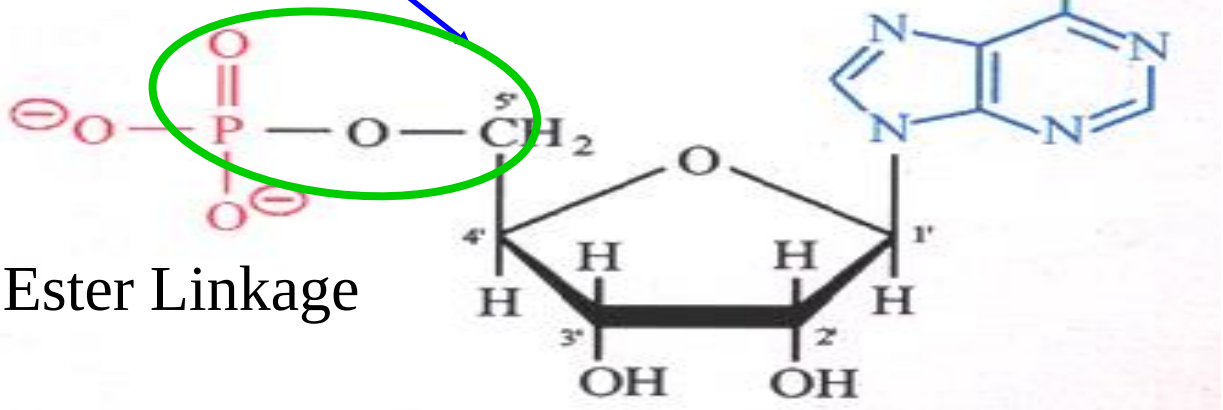
Adenosine

The phosphate group and the Nucleoside are linked by an **ESTER Bond**



Adenosine

H_2O

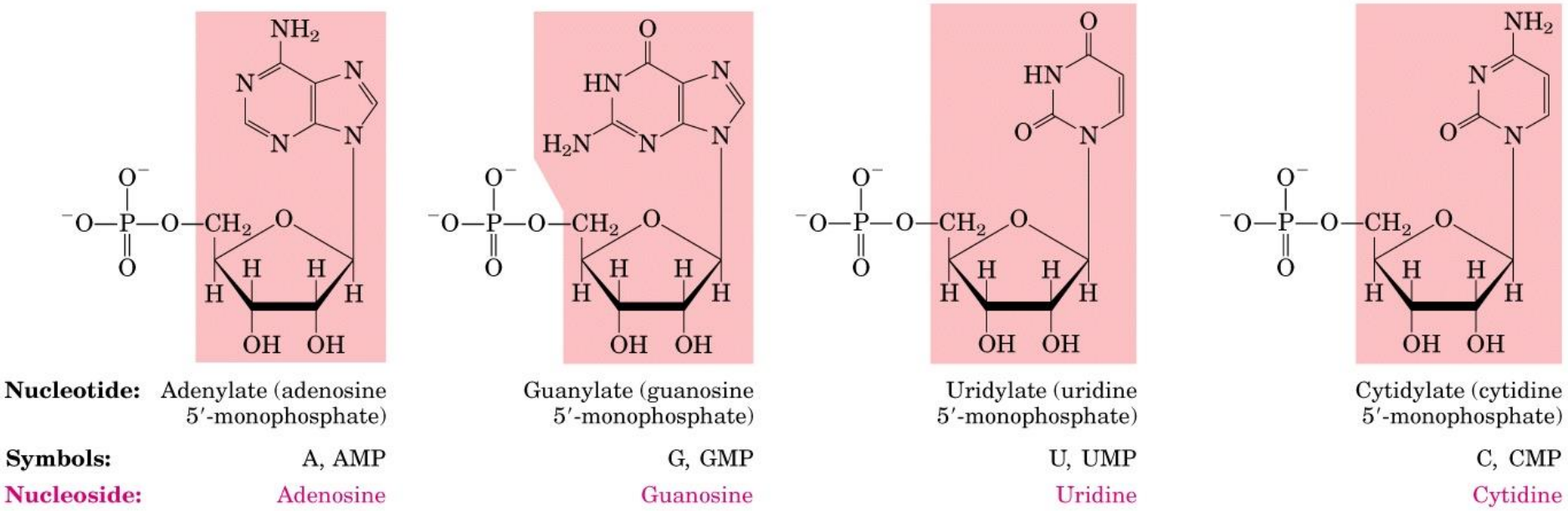


Ester Linkage

Adenosine 5'-monophosphate (AMP)

The Nucleotide contains a **Phosphoester Bond**

Nomenclature: RIBONUCLEOTIDES (RNA)

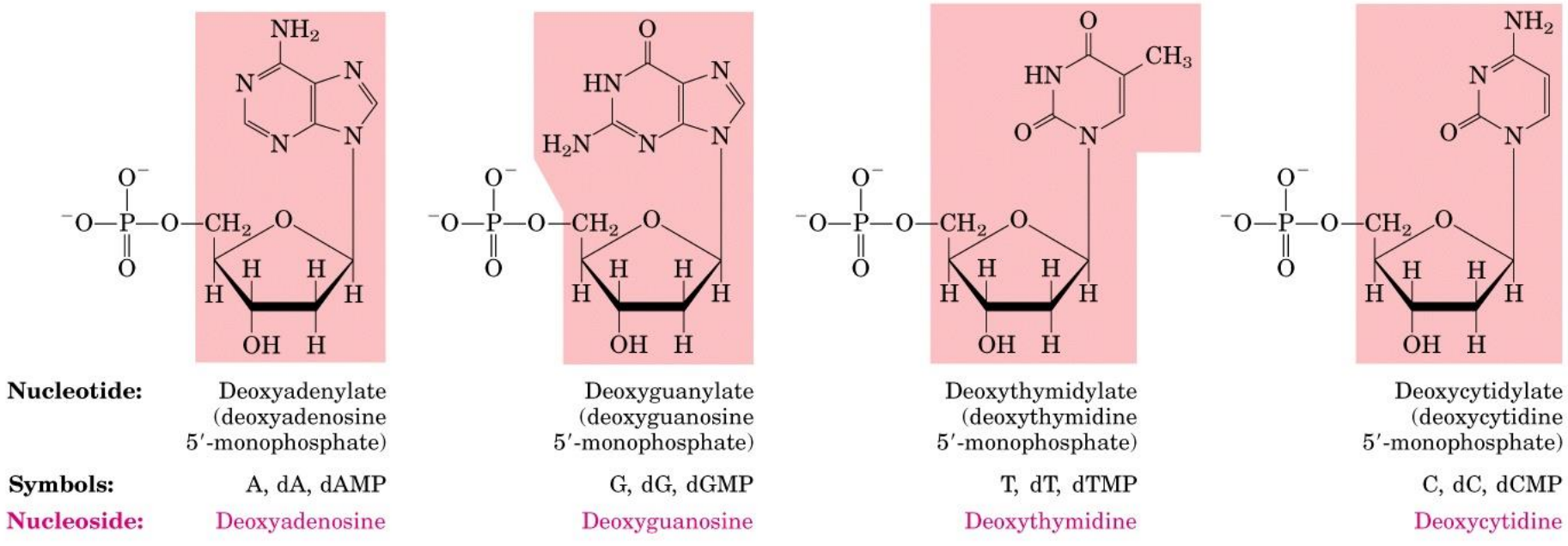


(b) Ribonucleotides

A **G** **U** **C**

AMP **GMP** **UMP** **CMP**

Nomenclature: DEOXYRIBONUCLEOTIDES (DNA)

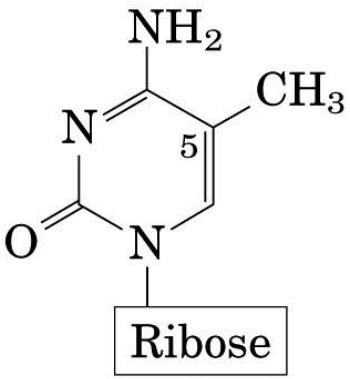


(a) Deoxyribonucleotides

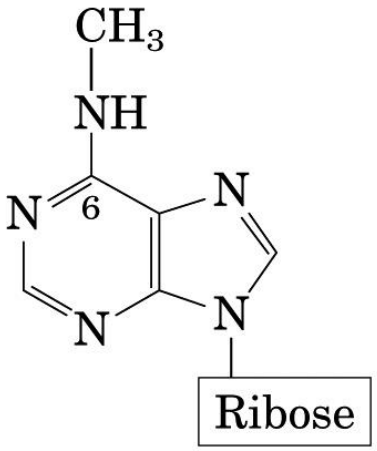
dA **dG** **dT** **dC**

dAMP **dGMP** **dTMP** **dCMP**

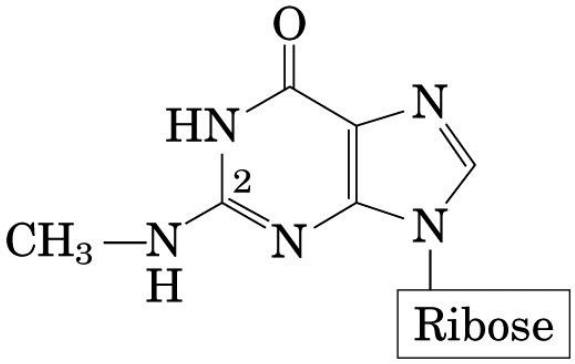
Nomenclature of some minor Purine and Pyrimidine Bases and nucleosides



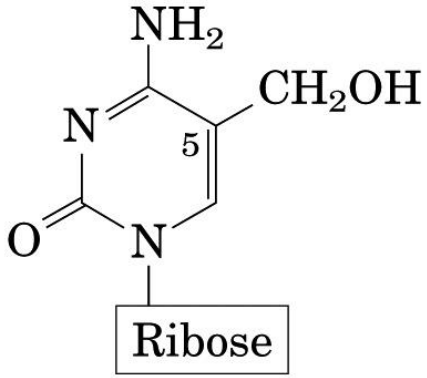
5-Methylcytidine



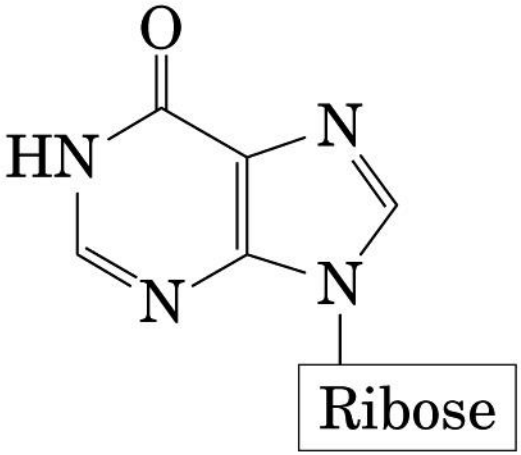
N^6-Methyladenosine



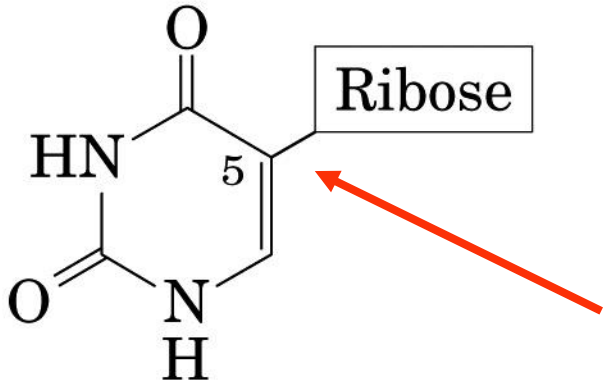
N^2-Methylguanosine



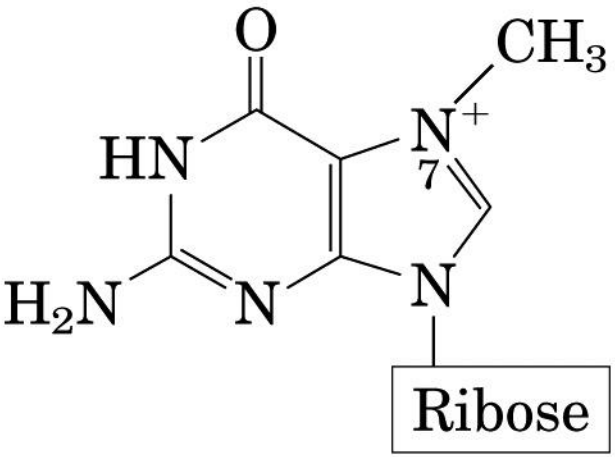
5-Hydroxymethylcytidine



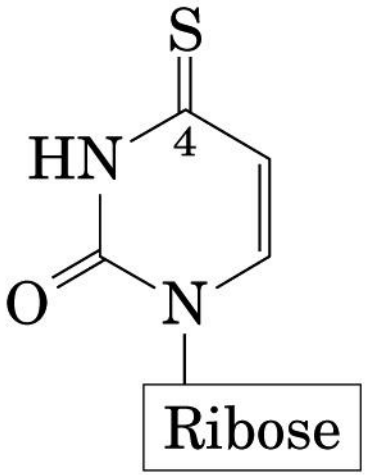
Inosine



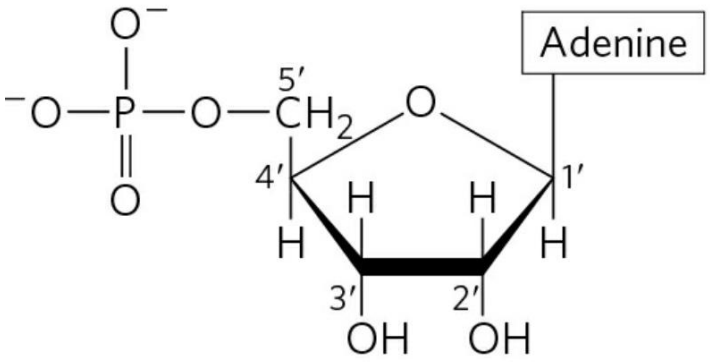
Pseudouridine



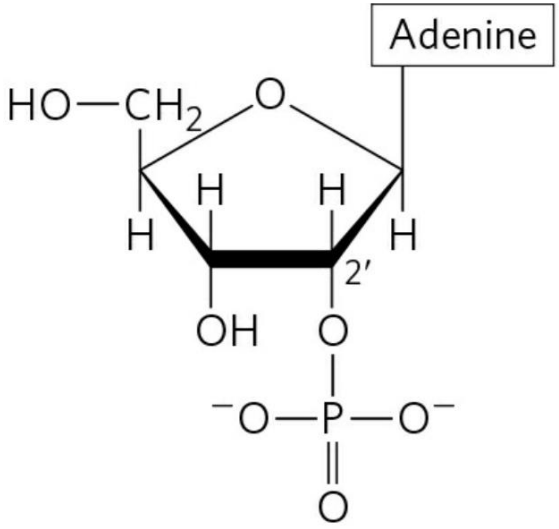
7-Methylguanosine



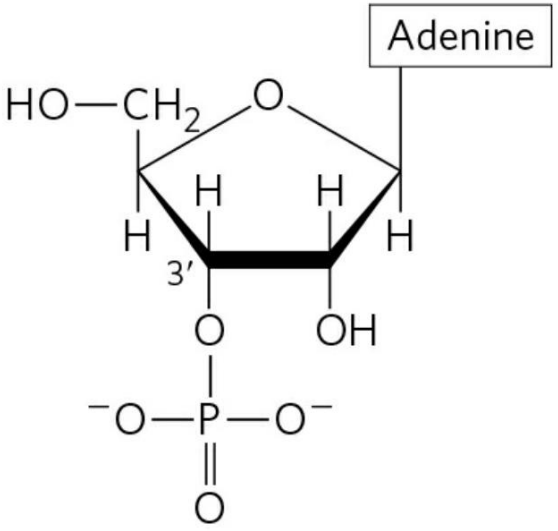
4-Thiouridine



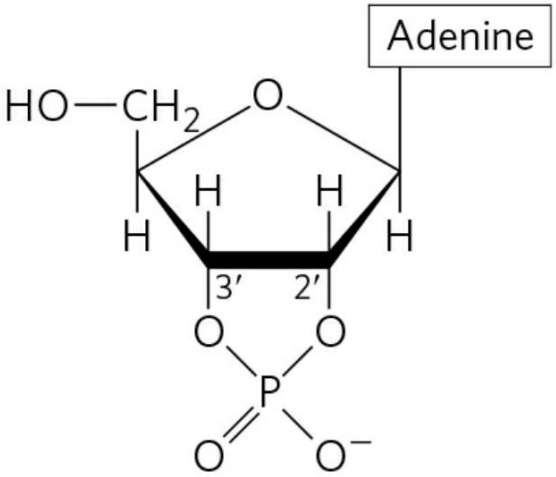
Adenosine 5′-monophosphate



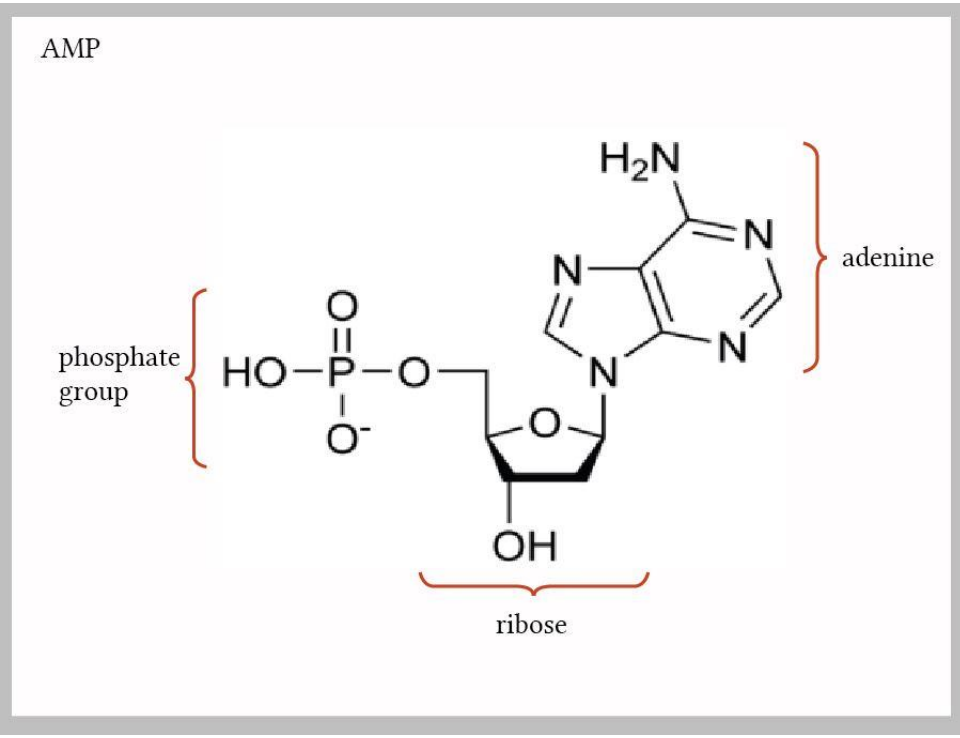
Adenosine 2′-monophosphate

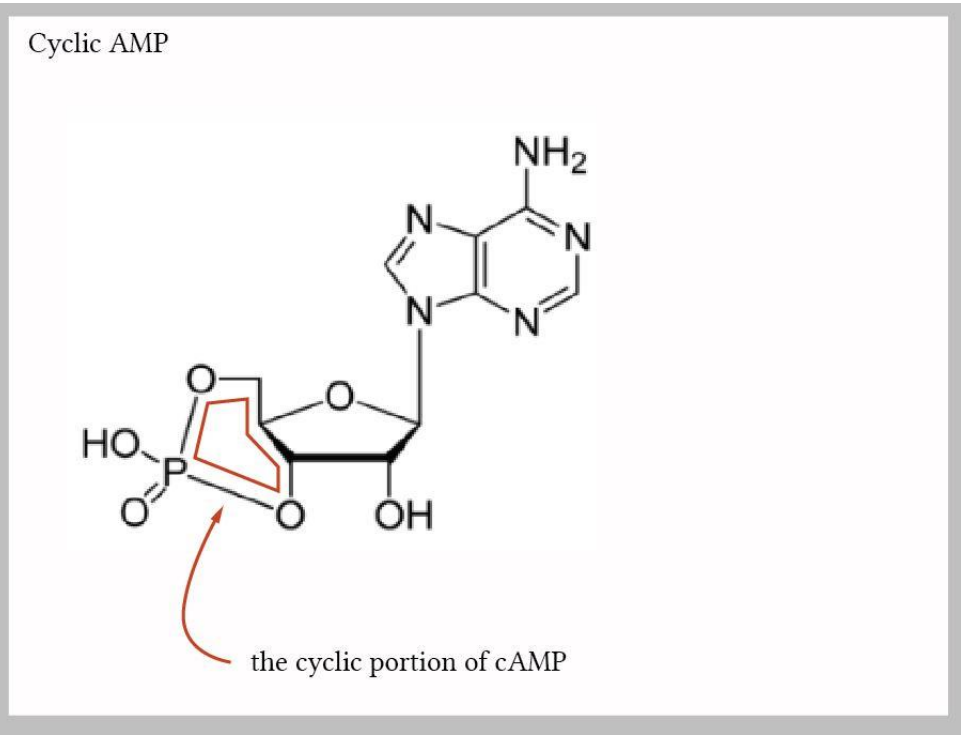


Adenosine 3′-monophosphate



Adenosine 2′,3′-cyclic monophosphate





Cyclic adenosine monophosphate (cAMP) is a crucial second messenger in cellular signaling, playing a significant role in various physiological processes. It is synthesized from adenosine triphosphate (ATP) by the enzyme adenylyl cyclase, which is activated by G protein-coupled receptors (GPCRs) responding to extracellular signals such as hormones and neurotransmitters.

How are Successive Nucleotides linked in Nucleic Acids?

Through the formation of Phosphodiester Bonds between C-3' and C-5' of the two nucleotides

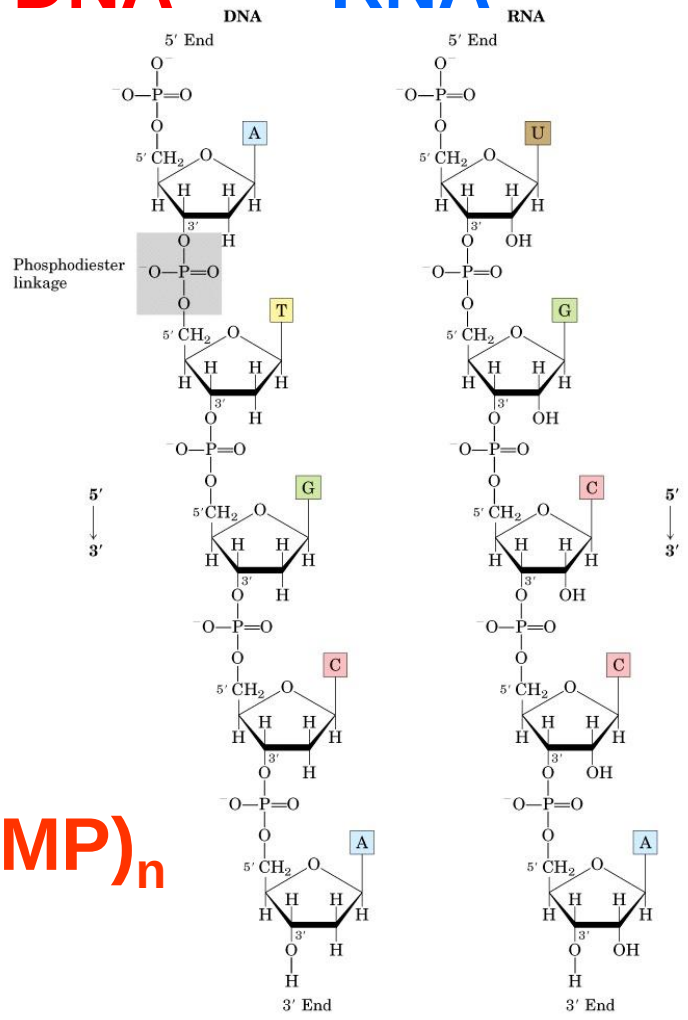
Oligo- and Poly-nucleotides

«note 3'- and 5'- ends»

Nucleotides are linked with Phosphodiester Bonds 3'-5'

Phosphodiester linkages are the covalent backbone of DNA and RNA

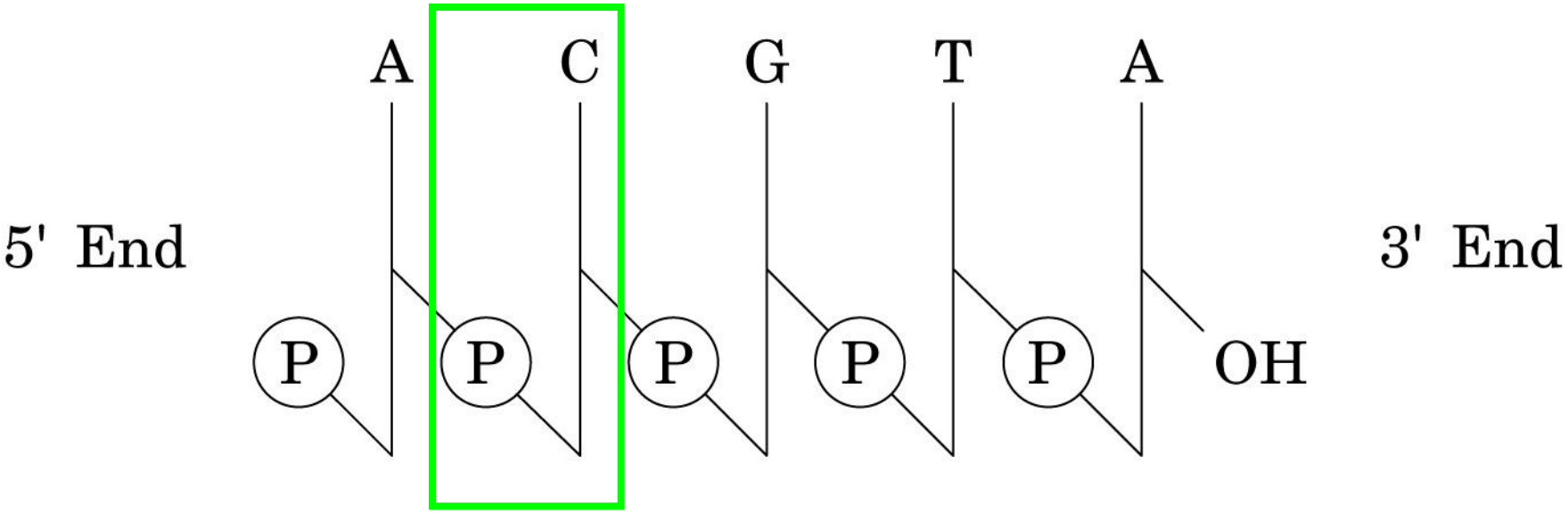
DNA RNA



$DNA\ (dNMP)_n$

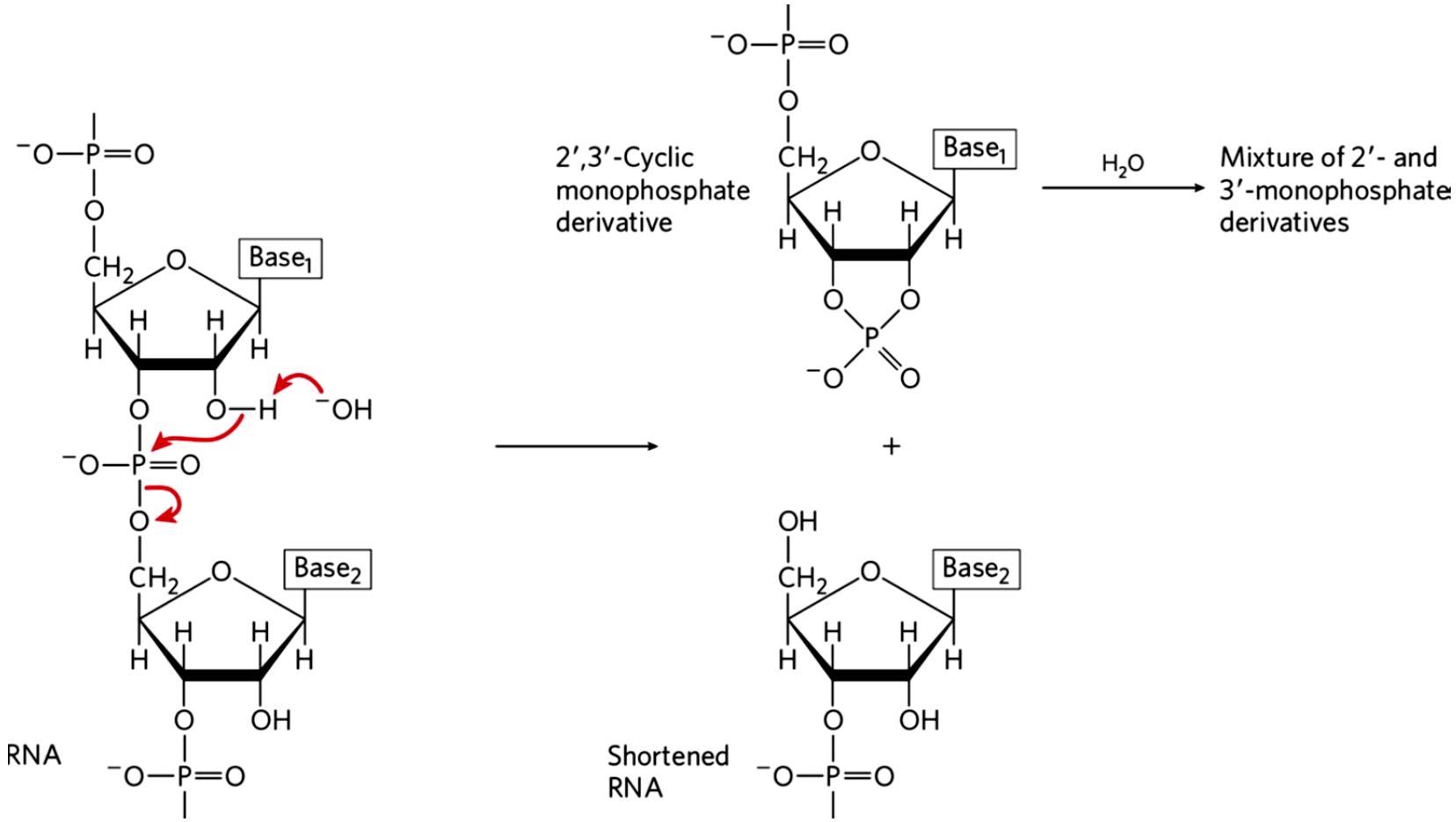
$RNA\ (NMP)_n$

Simplified Oligonucleotide Scheme



The phosphate groups are symbolized by **P** , and each deoxyribose is symbolized by a vertical line, from C-1′ at the top to C-5′ at the bottom (but keep in mind that the sugar is always in its closed-ring *β*-furanose form in nucleic acids). The connecting lines between nucleotides (which pass through **P**) are drawn diagonally from the middle (C-3′) of the deoxyribose of one nucleotide to the bottom (C-5′) of the next.

The covalent backbone of RNA is subject to nonenzymatic hydrolysis of the phosphodiester bonds in alkaline conditions



Nucleic Acid Structure

PRIMARY: The covalent structure and nucleotide sequence

SECONDARY: Any regular, stable structure taken up by some or all of the nucleotides

TERTIARY: The complex folding of large chromosomes or the elaborate folding of large tRNA or rRNA

DNA tridimensional Structure (Secondary structure)

A little bit of History

1869: Friedrich Miescher isolates DNA and calls it "*nucleolin*".

1940: Osvald Avery, Colin MacLeod, Maclyn McCarty → DNA is the genetic material. Non-virulent *S. pneumoniae* could become virulent in mice if DNA from virulent strain was transferred (Avery experiment).

1940: Erwin Chargaff et al. → Chargaff's rules

1952: Alfred Hershey, Martha Chase → labeling DNA of bacteriophage viruses to show transfer of genetic material into bacterial cells.

1950-3: Rosalind Franklin, Maurice Wilkins → X-ray diffraction patterns of DNA fibers

1953: James Watson, Francis Crick → The DNA double-helix model

DNA BASE COMPOSITION

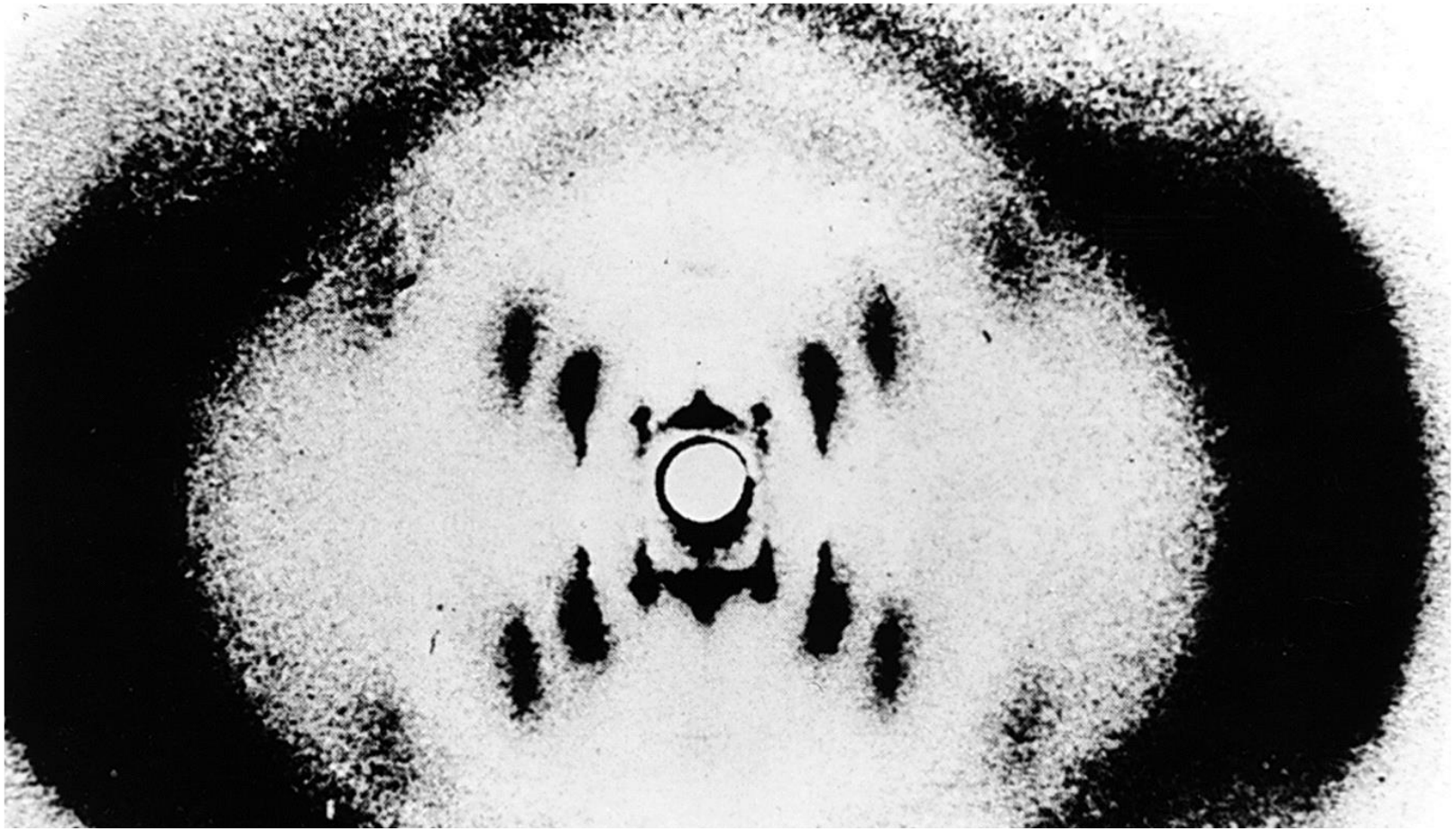
(Chargaff's rules)

purine pyrimidines

1. **A=T and G=C, therefore A+G = T+ C**
2. **Base composition varies from one species to another**
3. **Same composition of bases from different tissues of the same species**
4. No variations with age, nutritional state, or changing environment

X-ray diffraction pattern of DNA fibers (1950-3):

The spots forming a cross in the center denote a helical structure. The heavy bands at the periphery arise from recurrent bases

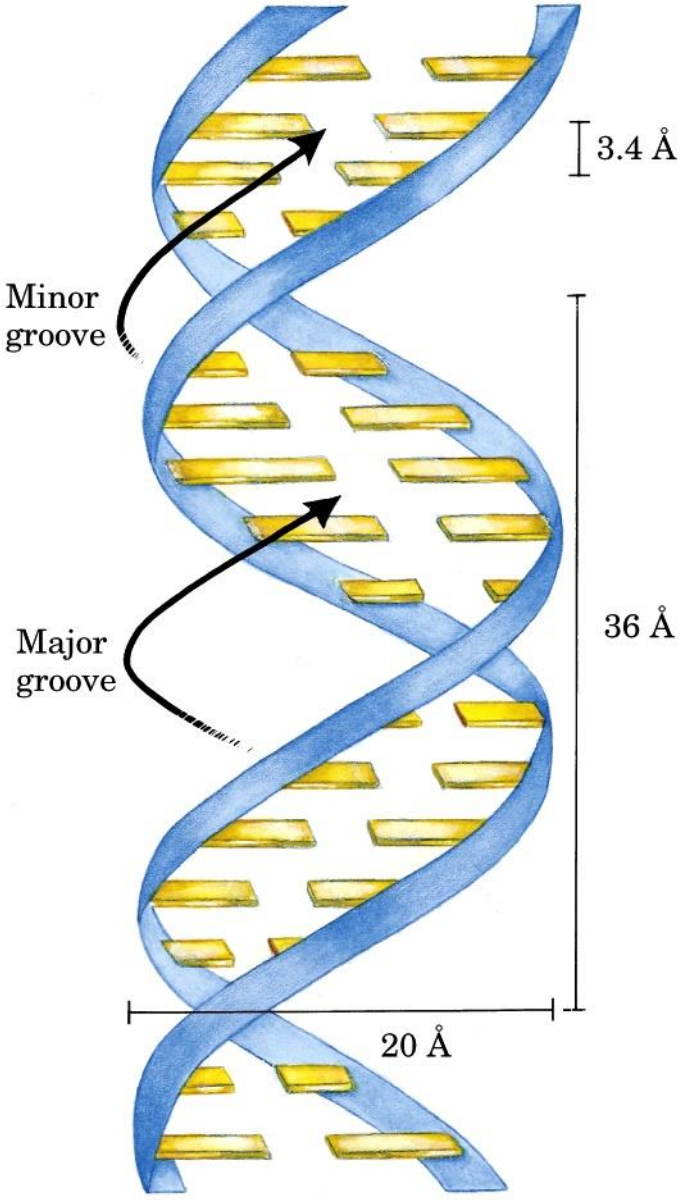


The pattern revealed that DNA molecules are helical, with two periodicities along their long axis, a primary one of 3.4 Å and a secondary one of 34 Å.

Watson & Crick model, 1953

(fulfills the thermodynamic requirements and the A+G=T+C base equivalence)

Vertically stacked bases inside the double helix would be 3.4 Å apart; secondary repeat distance of about 34-36 Å was accounted by 10 bases. The structure in aqueous solution differs slightly from that in fibers, having 10.5 bases pairs (36 Å or 3.6 nm) per helical turn.



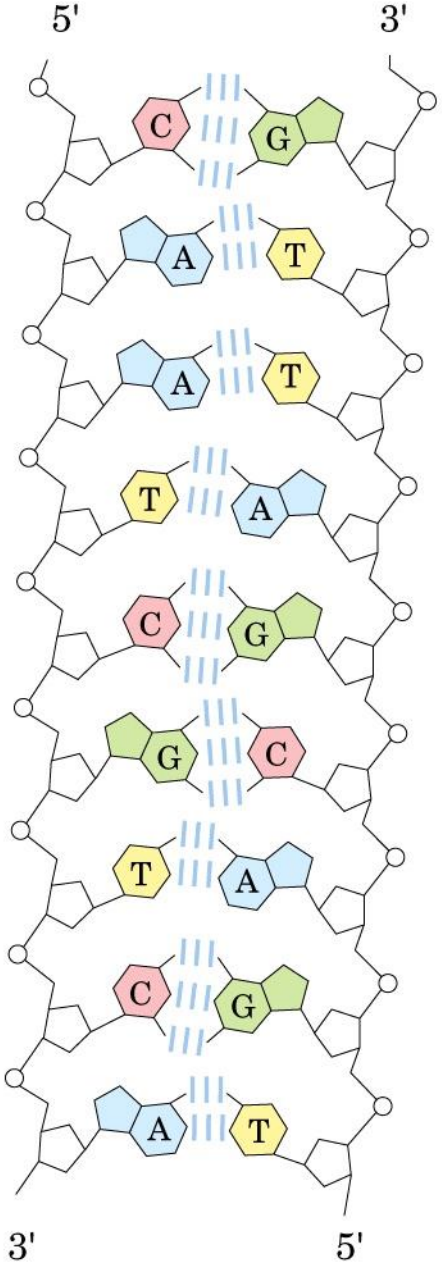
right-handed double helix

- **Thermodynamic Requirements** (hydrophylic groups exposed to H_2O, inner hydrophobic groups and H bonds)

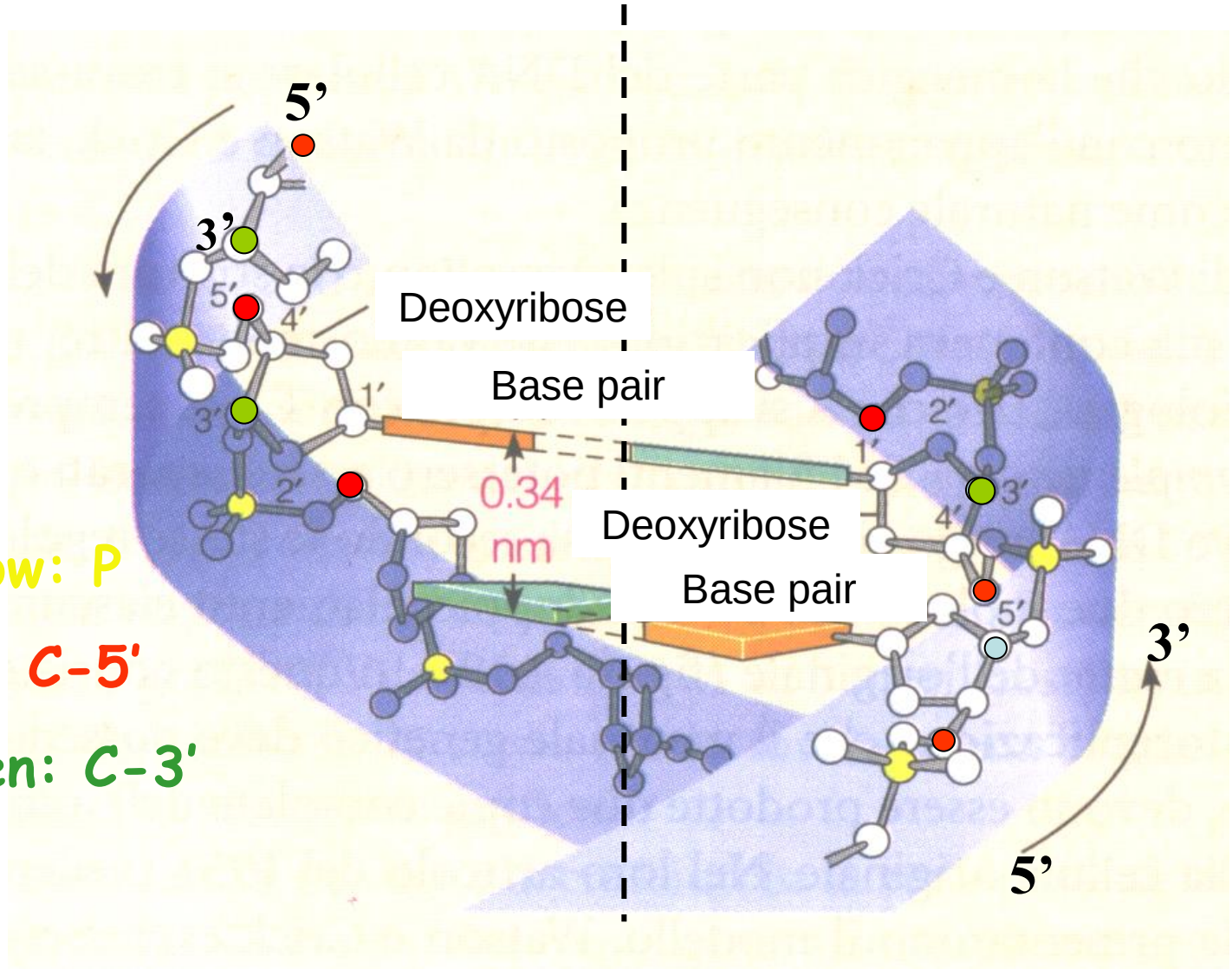
- **Base Complementarity**

  A/T and G/C: Watson & Crick pairing

- **ANTIPARALLEL strands**



1. The strands are ANTIPARALLEL
2. The atoms of complementary nitrogenous bases lie on the same plane (almost perpendicular to the helix axis)



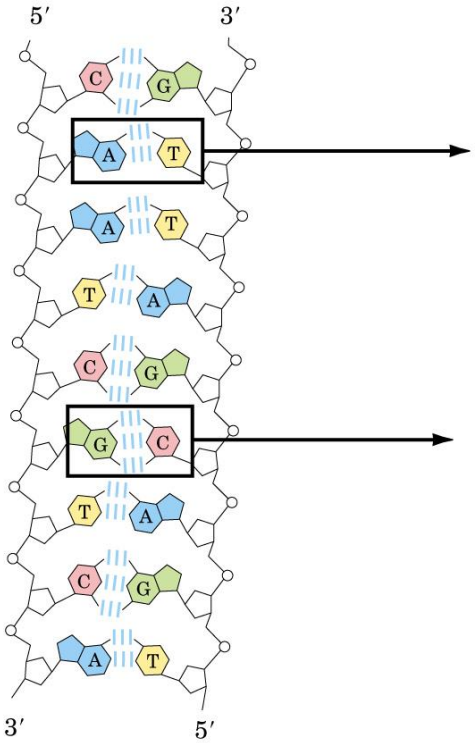
Yellow: P

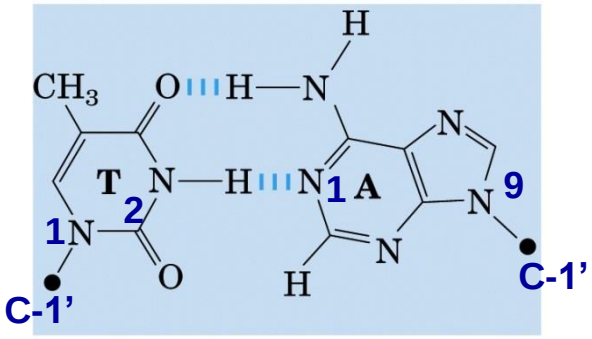
Red: C-5'

Green: C-3'

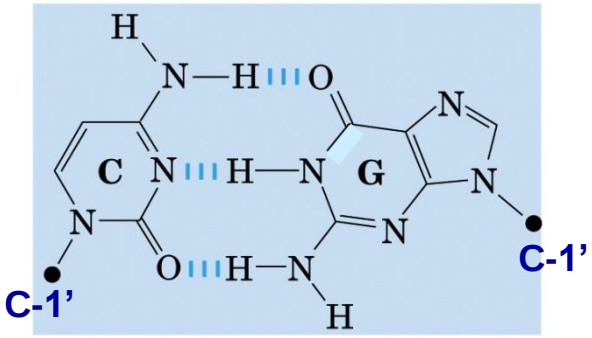
Nitrogenous Base Complementarity

Hydrogen bond





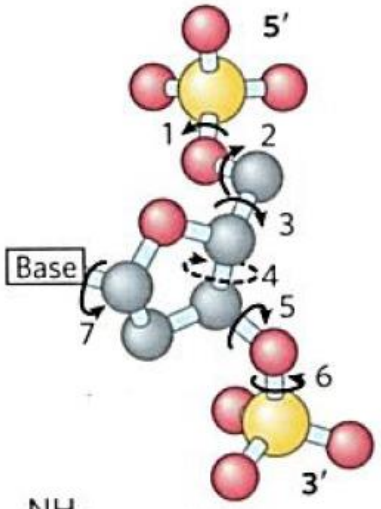
T=A

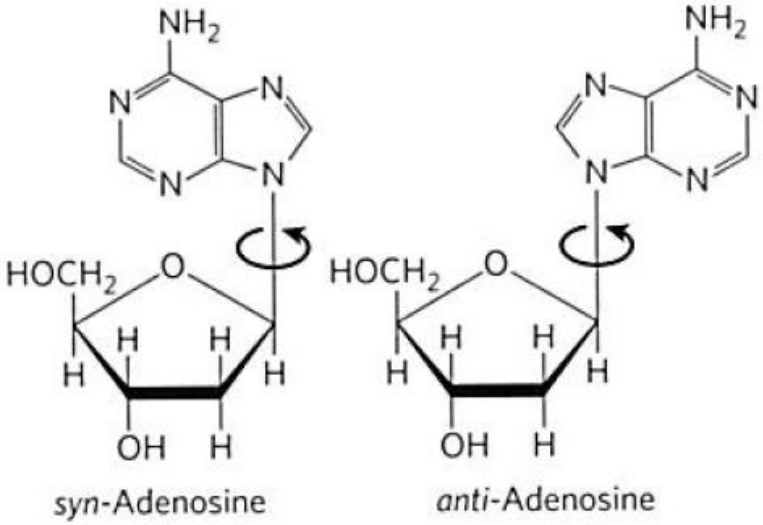


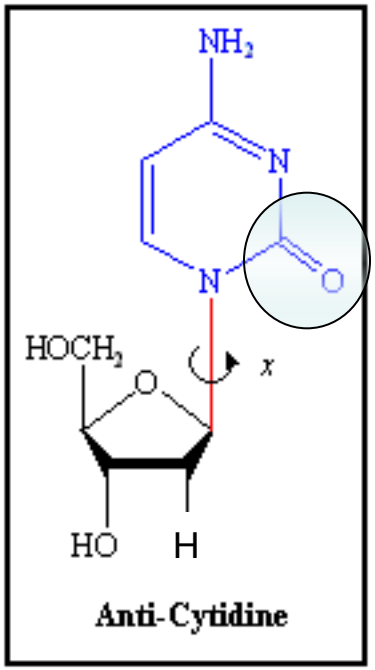
C≡G

Same geometry!

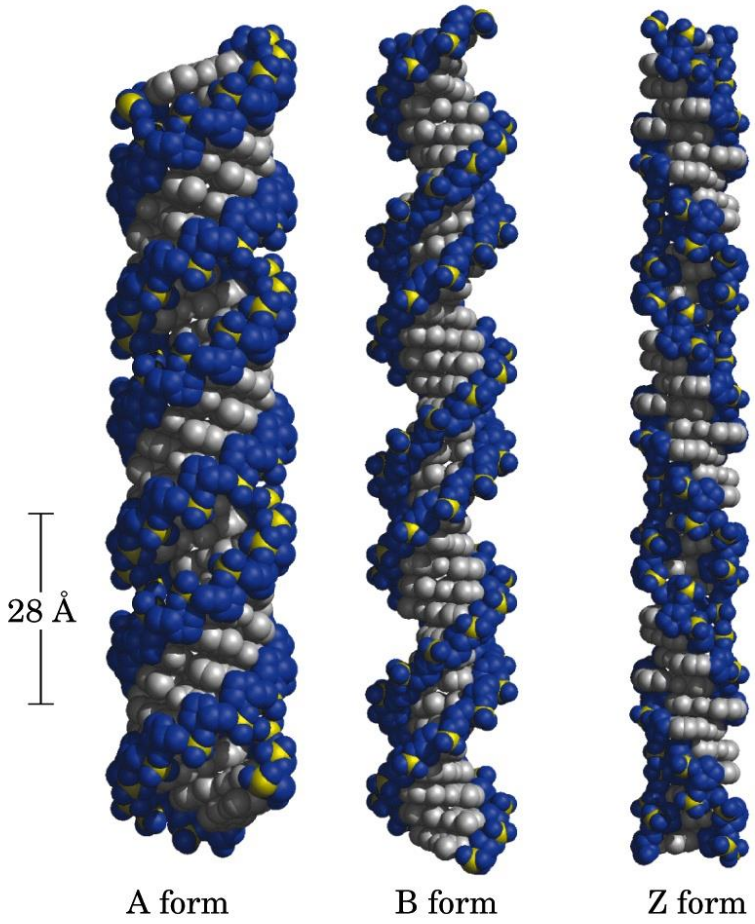
Structural Variation in DNA





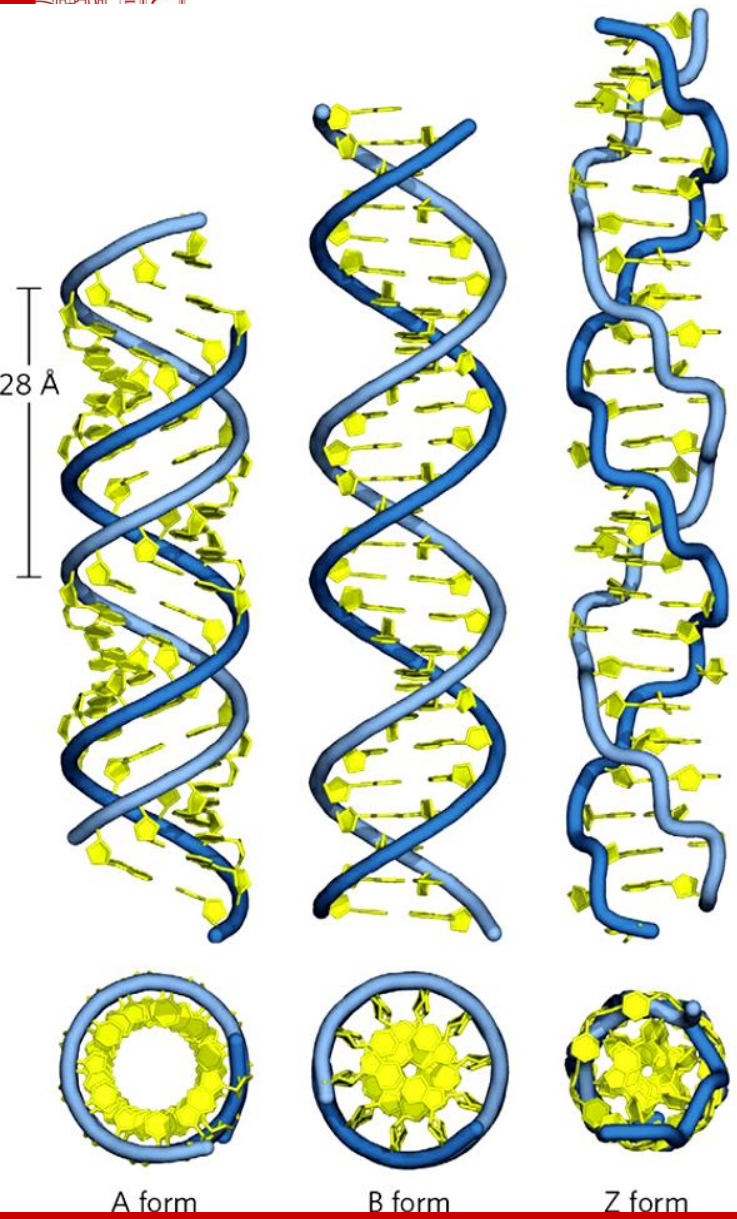


Different 3-Dimensional Forms of DNA



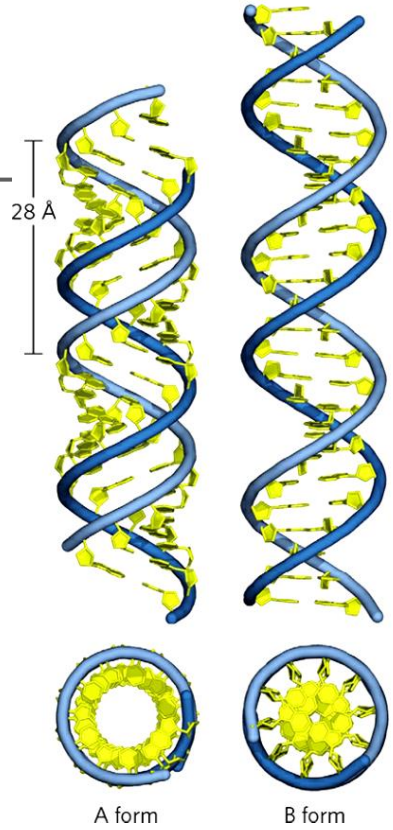
B form is the most stable structure under physiological conditions and is the point of reference in any study of the properties of DNA

Different 3-Dimensional Forms of DNA



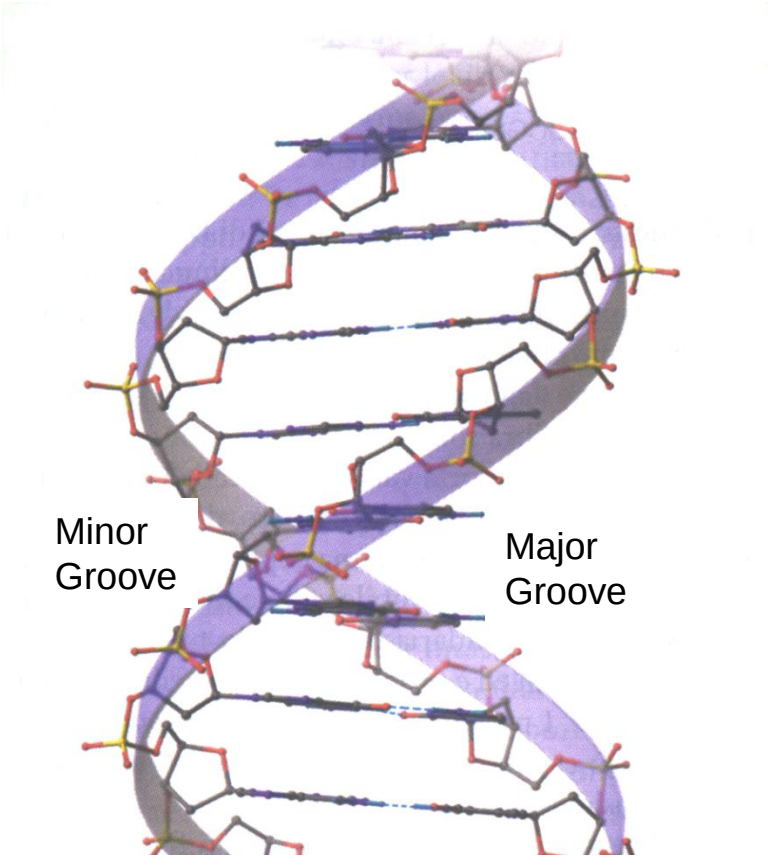
	A form	B form	Z form
Helical sense	Right handed	Right handed	Left handed
Diameter	~26 Å	~20 Å	~18 Å
Base pairs per helical turn	11	10.5	12
Helix rise per base pair	2.6 Å	3.4 Å	3.7 Å
Base tilt normal to the helix axis	20°	6°	7°
Sugar pucker conformation	C-3′ endo	C-2′ endo	C-2′ endo for pyrimidines; C-3′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

DNA A-form



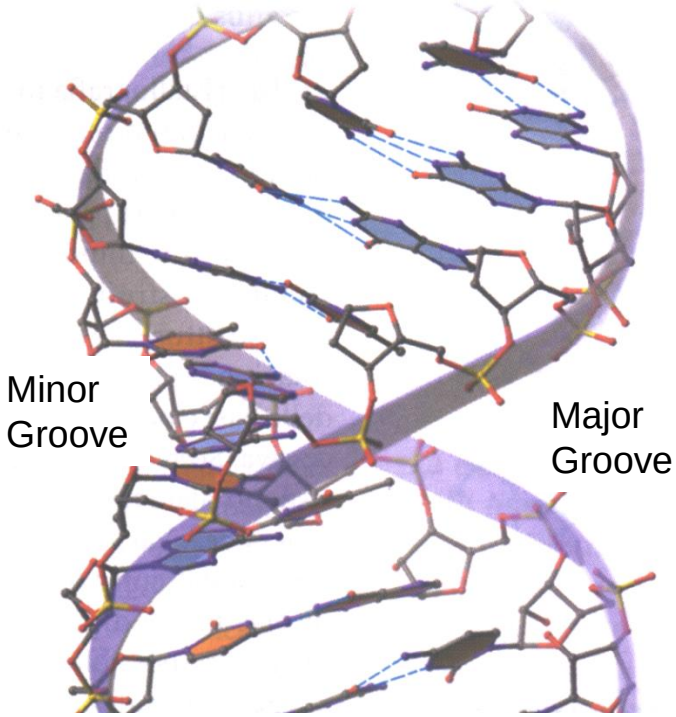
- Favored in solutions devoid of water
- Right-handed double helix
- Helix is wider than B-form (26 Å)
- The number of base pairs per helical turn is 11
- The plane of the base pairs is tilted about 20° relative to B-DNA base pairs (no perpendicularity to the helix axis)
- Deeper major groove, shallower minor groove
- The most represented form in DNA crystallization

B DNA

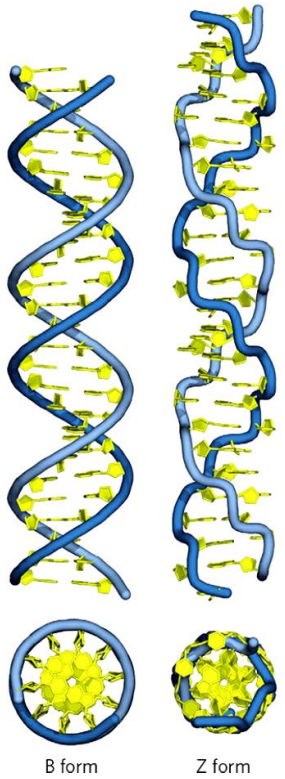


A DNA

(Bases non on the same plane and bigger angle to the helix axis)



DNA Z-form



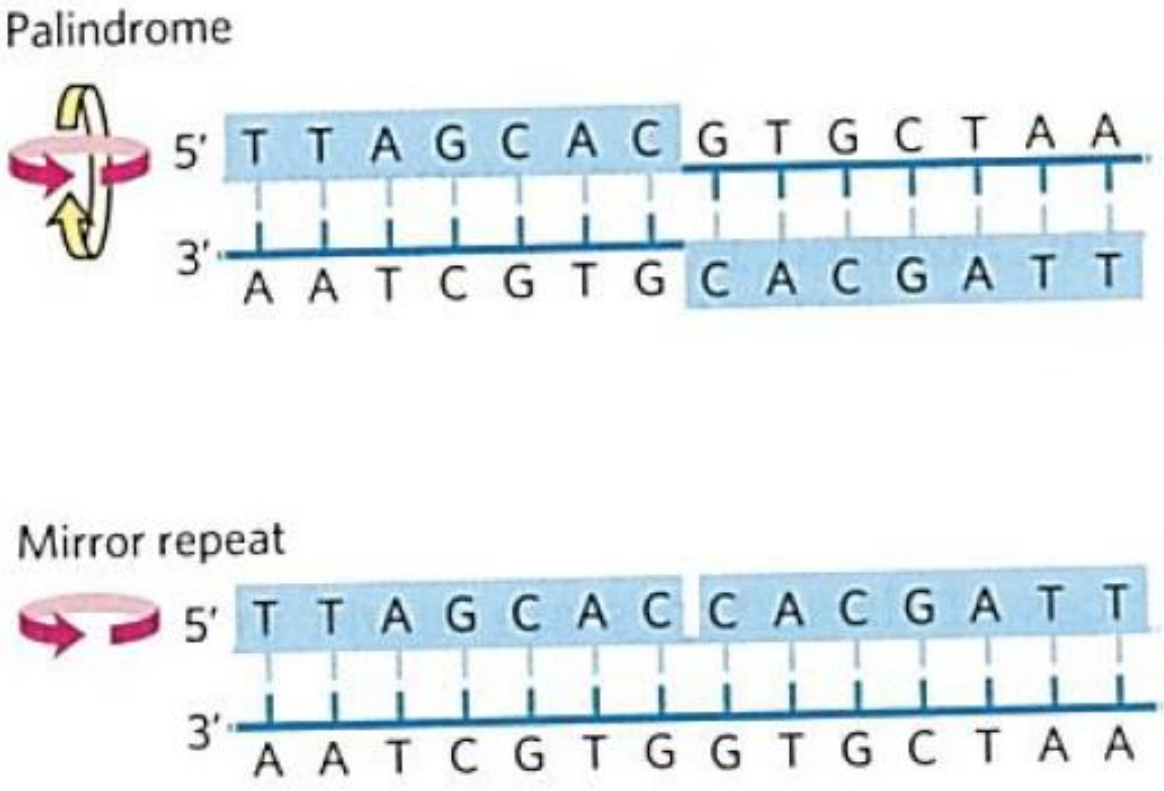
- Left-handed double helix
- The number of base pairs per helical turn is 12
- Structure appears more slender and elongated
- The DNA backbone takes on a zigzag appearance
- The major groove is barely apparent and the minor groove is narrow and deep
- Certain nucleotide sequences fold into Z-DNA more readily than others (e.g. pyrimidines alternating with purines C-G alternating G-C, 5-methyl-C and G residues, and purines flip to syn- alternating with pyrimidines in the anti-conformation).
- May play a yet unknown role in gene expression regulation and genetic recombination.

Comparison of A, B, and Z forms of DNA

	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°

Whether A-DNA occurs in cells is uncertain, but there is evidence for some short stretches (tracts) of Z-DNA in both bacteria and eukaryotes.
These Z-DNA tracts may play a role (as yet undefined) in regulating the expression of some genes or in genetic recombination.

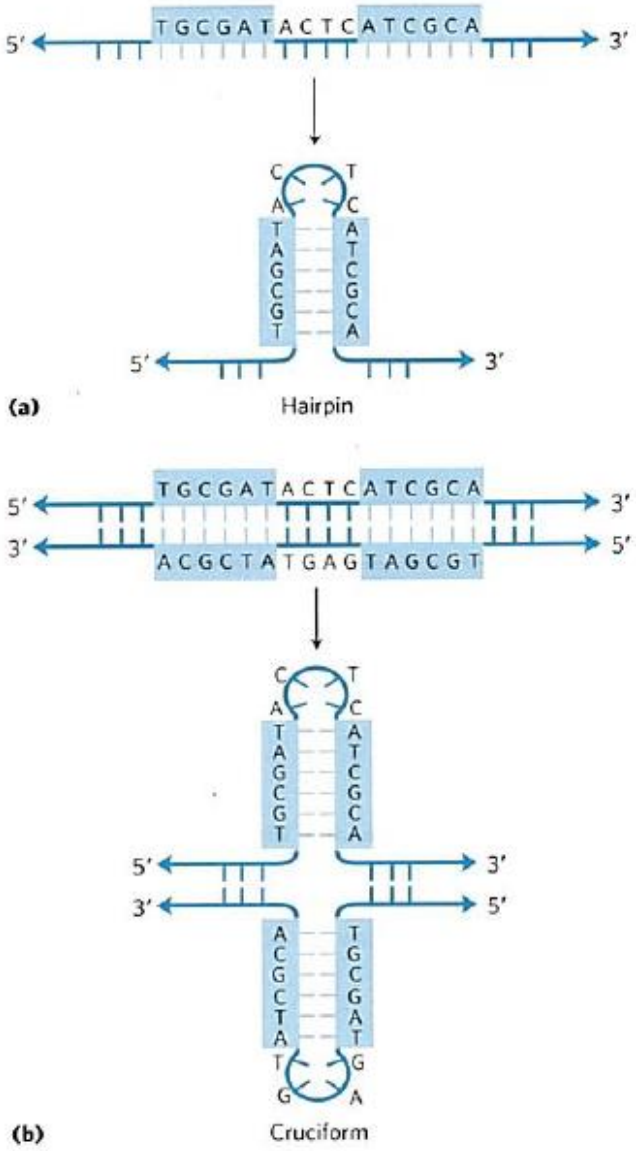
Unusual DNA/RNA structures



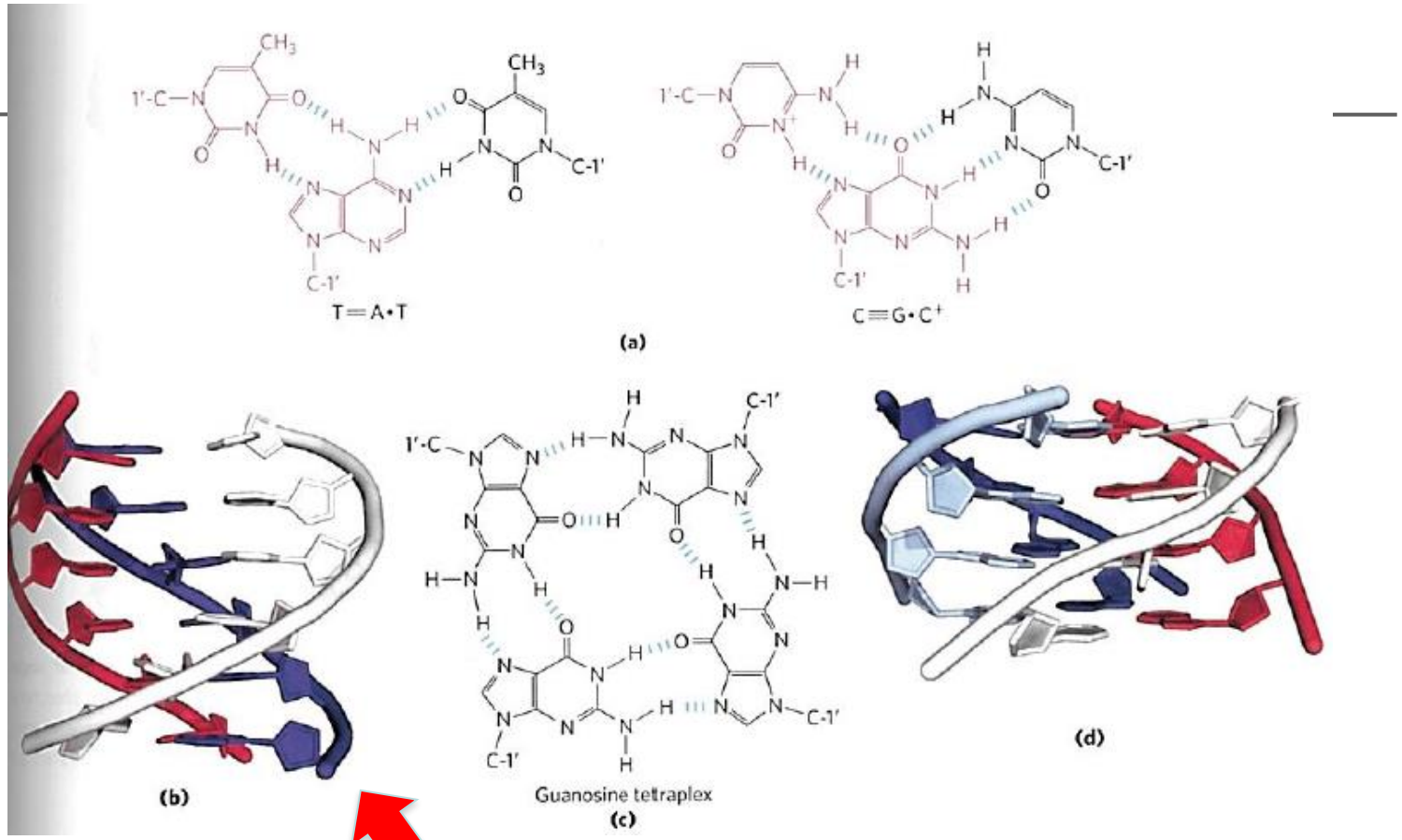
Hairpins and cruciforms from palindrome structures

Involvement of a single DNA/RNA strand

Involvement of a duplex DNA



Hoogsteen pairing: Triple DNA and Quadruplex



Purine strand= Blue
Pyrimidine strands= Red and White

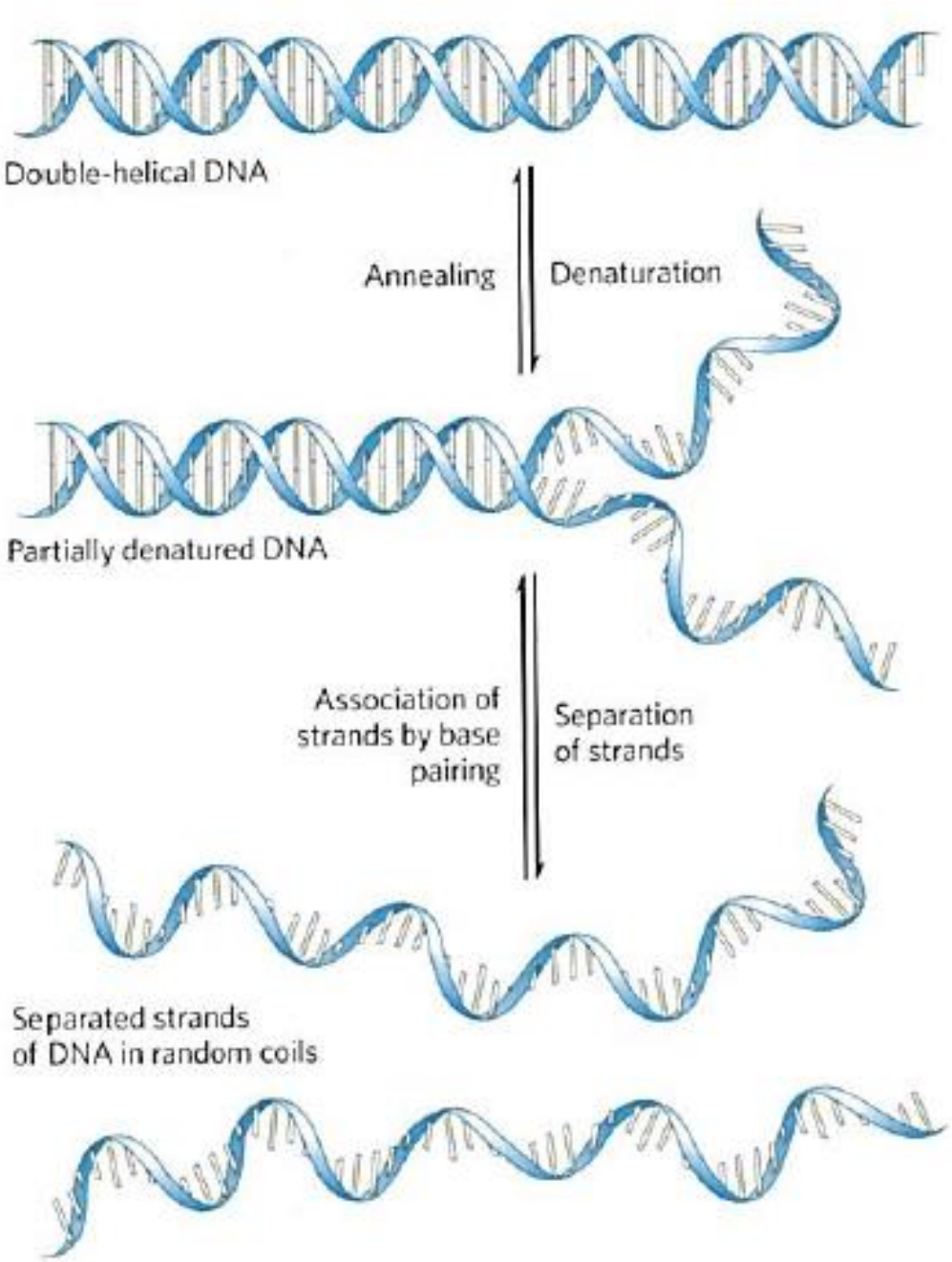
Blue and White = **ANTIPARALLEL**
Red = **Parallel to Blue** and **Antiparallel to White**

DNA denaturation

"Reversible disruption of the hydrogen bonds between paired bases and of base-stacking interactions causing unwinding of the double helix to form two single strands, completely separated from each other along the entire length or part of the length (partial denaturation) of the molecule."

NO COVALENT BONDS ARE BROKEN!

Reversible denaturation and annealing (renaturation) of DNA



Promoting Factors:

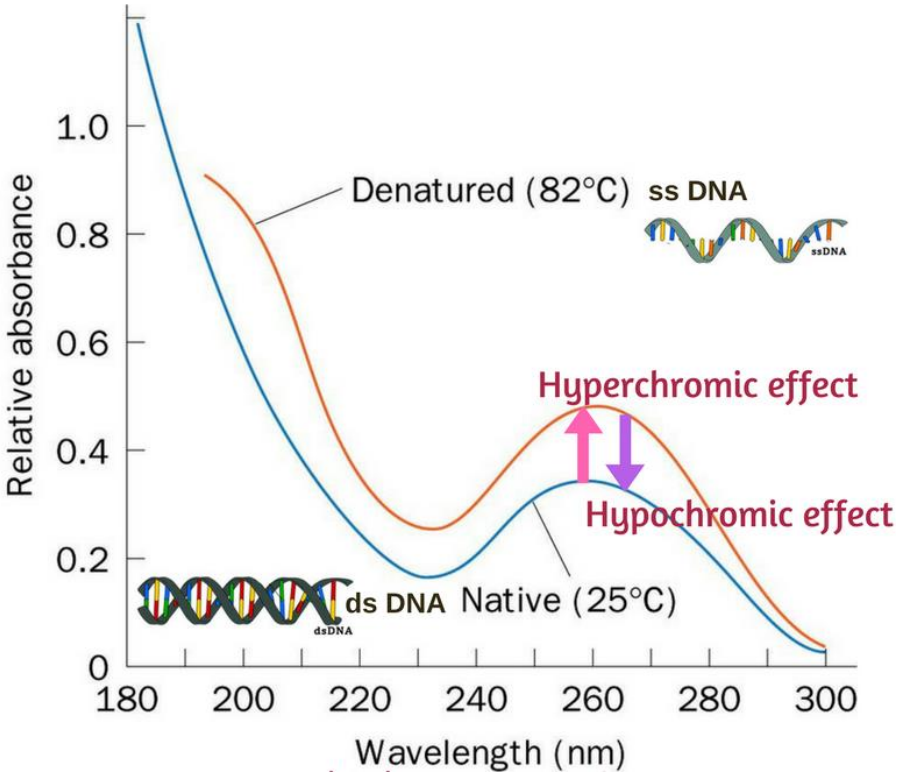
1. ***High Temperature***
2. ***pH***

Denaturation and UV light absorption

HYPOCHROMIC EFFECT: The close interaction between stacked bases DECREASES UV light absorption compared to a solution with the same concentration of FREE nucleotides. Absorption is further decreased when 2 DNA strands are paired.

HYPERCHROMIC EFFECT: The increased UV absorption occurring when a double-stranded nucleic acid is denatured.

The transition from dsDNA to denatured, ssDNA can be detected by monitoring **UV absorption at 260 nm**.

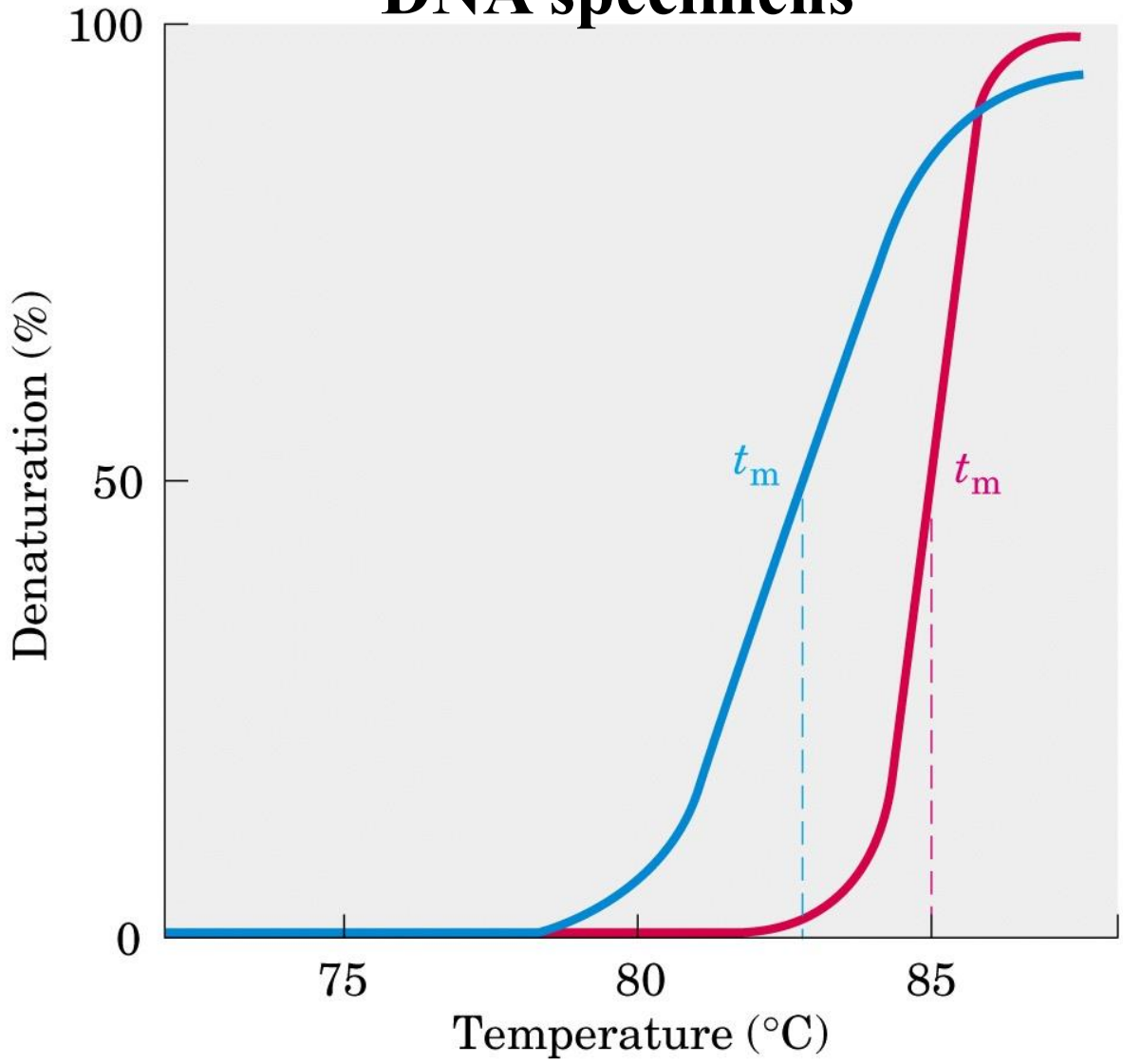


Melting Temperature (t_m)

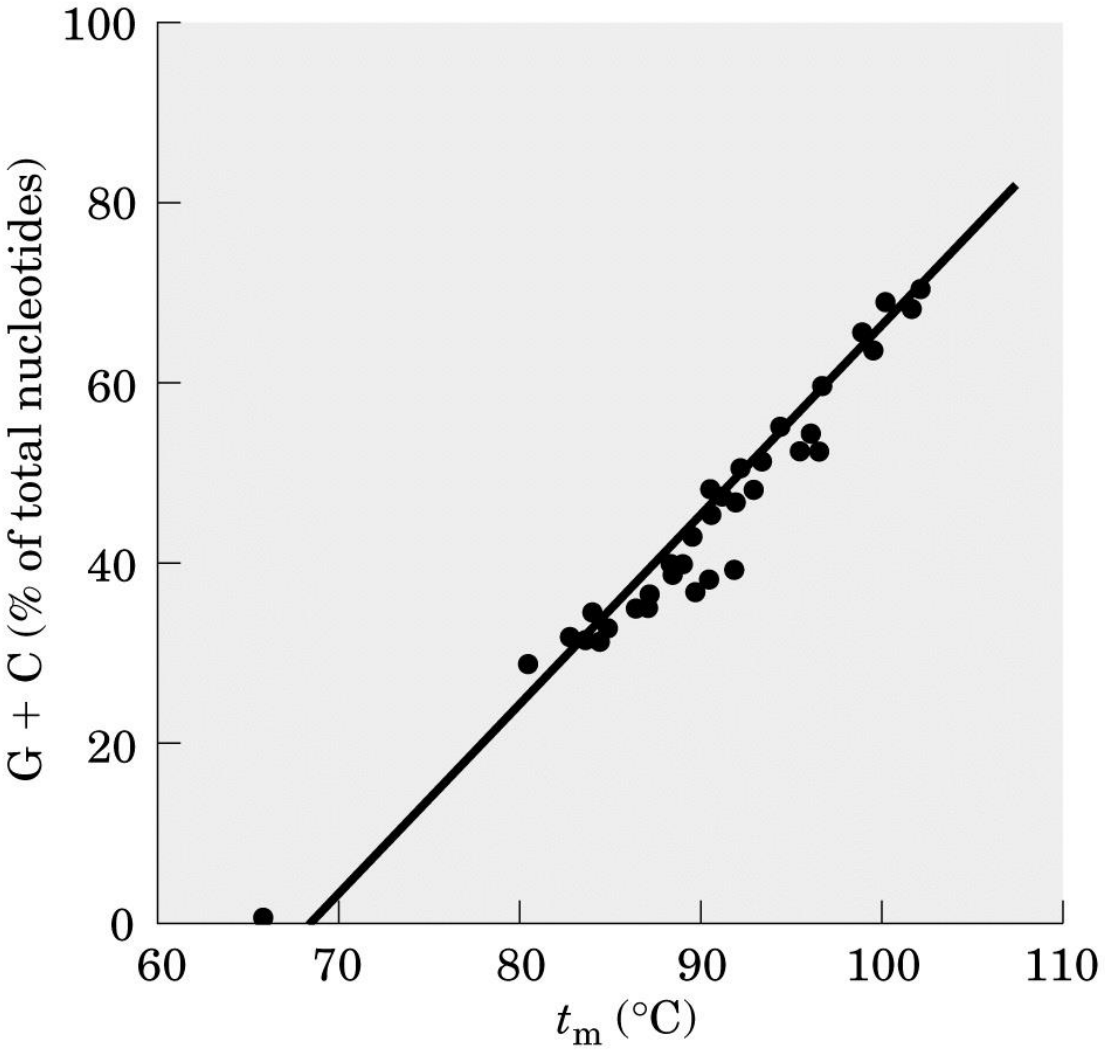
The temperature at which half of the DNA is present as separate single strands

- Each species of DNA has a characteristic t_m.
  - Increases with %GC content

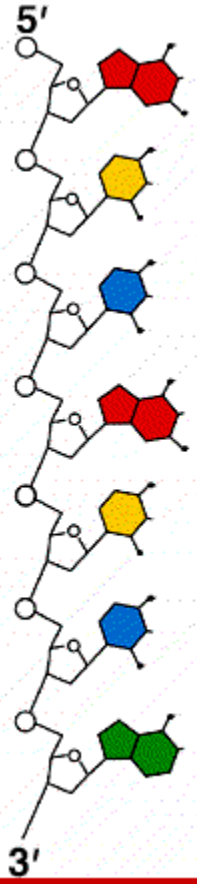
Denaturation (melting) curves of 2 DNA specimens

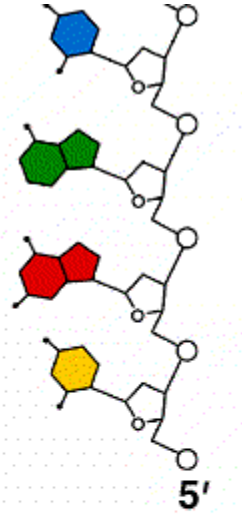


Relationship between G/C and t_m



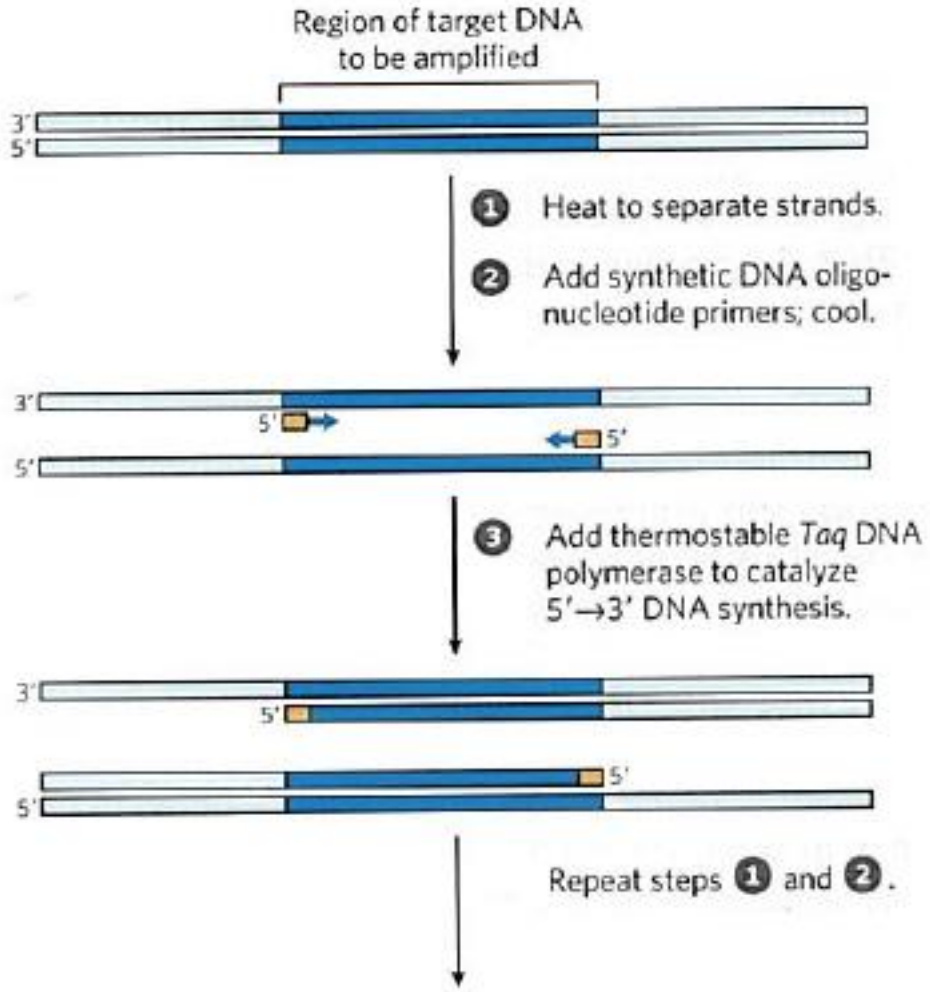
Nitrogen Base complementarity makes Denaturation a REVERSIBLE process through re-annealing



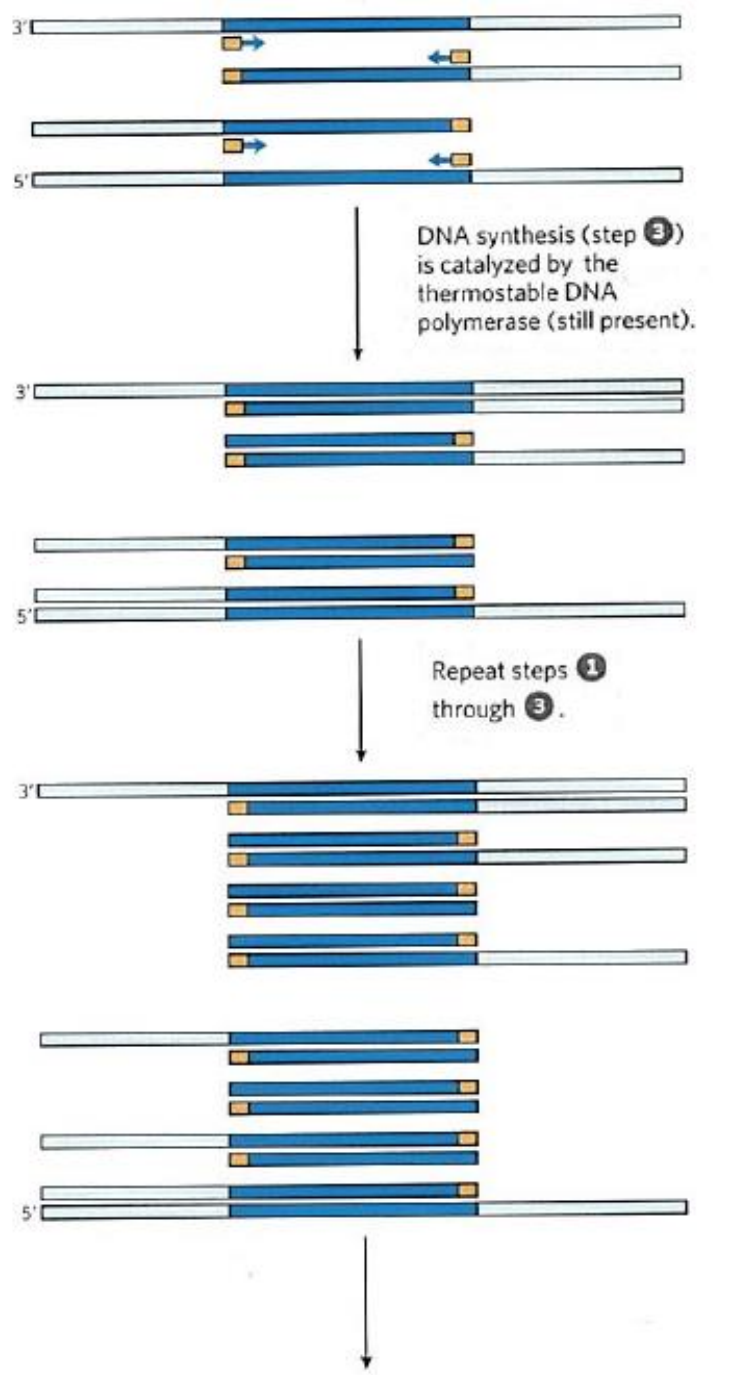


Nucleic acids base-pairing pattern creates a strong specificity in the interaction between polynucleotides: two complementary strands of DNA can “search” for each-other in solution, and then pair, leading to a stable double-helix

Polymerase Chain Reaction (PCR)
Kary Mullis (1983)

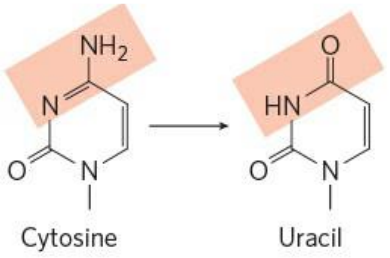


Polymerase Chain Reaction (PCR)- continued

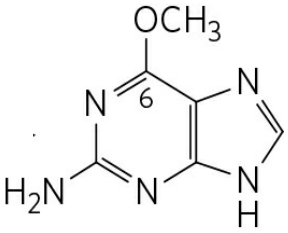


After 20 cycles, the target sequence has been amplified about 10^6-fold.

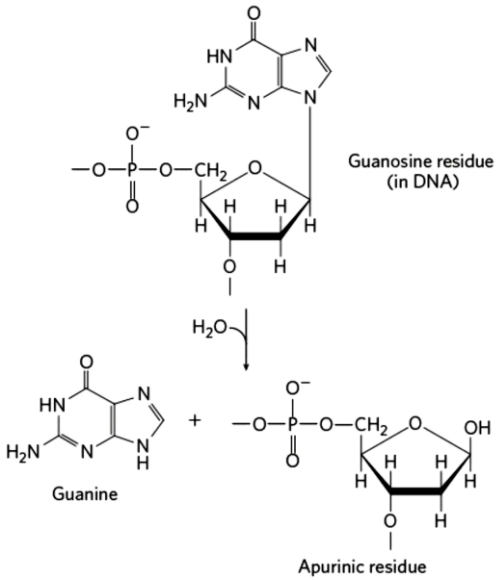
Nucleotides and Nucleic Acids Undergo Nonenzymatic Transformations



- ✓ DEAMINATION: Several nucleotide bases undergo spontaneous loss of their exocyclic amino groups
- ✓ Hydrolysis of the N-β-glycosyl bond between the base and the pentose, creating a DNA lesion called an AP (apurinic > apyrimidinic) or abasic site.
- ✓ DEAMINATING AGENTS (HNO_2)
- ✓ ALKYLATING AGENTS (Methylation)
- ✓ OXIDATIVE DAMAGE ($OH^{\cdot}$)



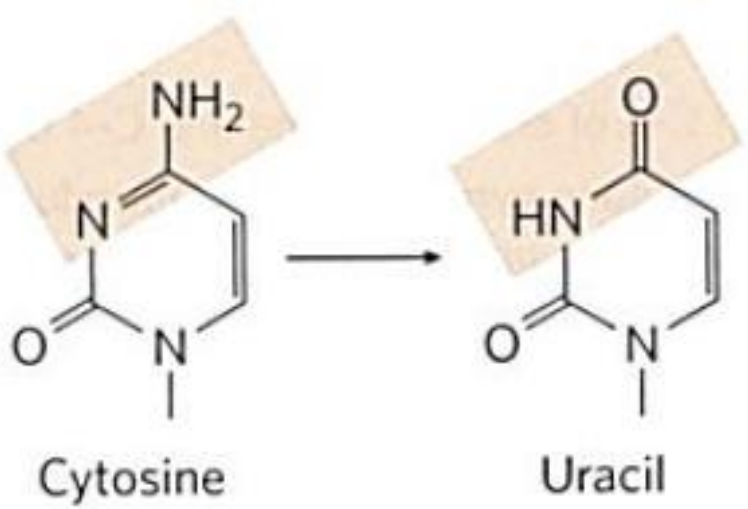
O^6-Methylguanine



Nonenzymatic Transformations of Nucleotides and Nucleic Acids

Occur VERY SLOWLY but they are significant since they can lead to **MUTATIONS**

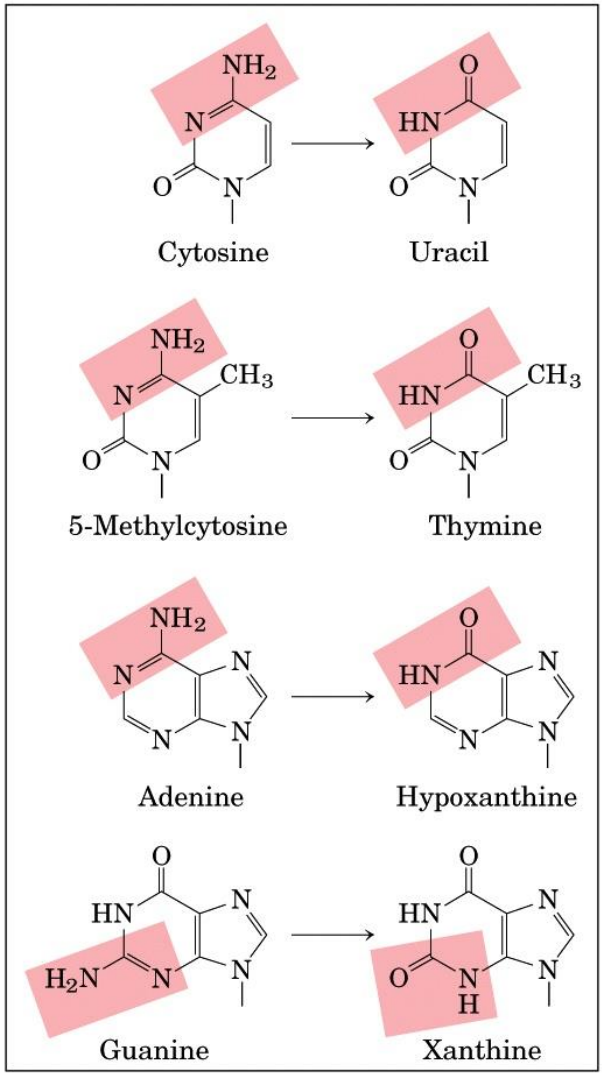
1. **DEAMINATION**: Spontaneous loss of their exocyclic amino group



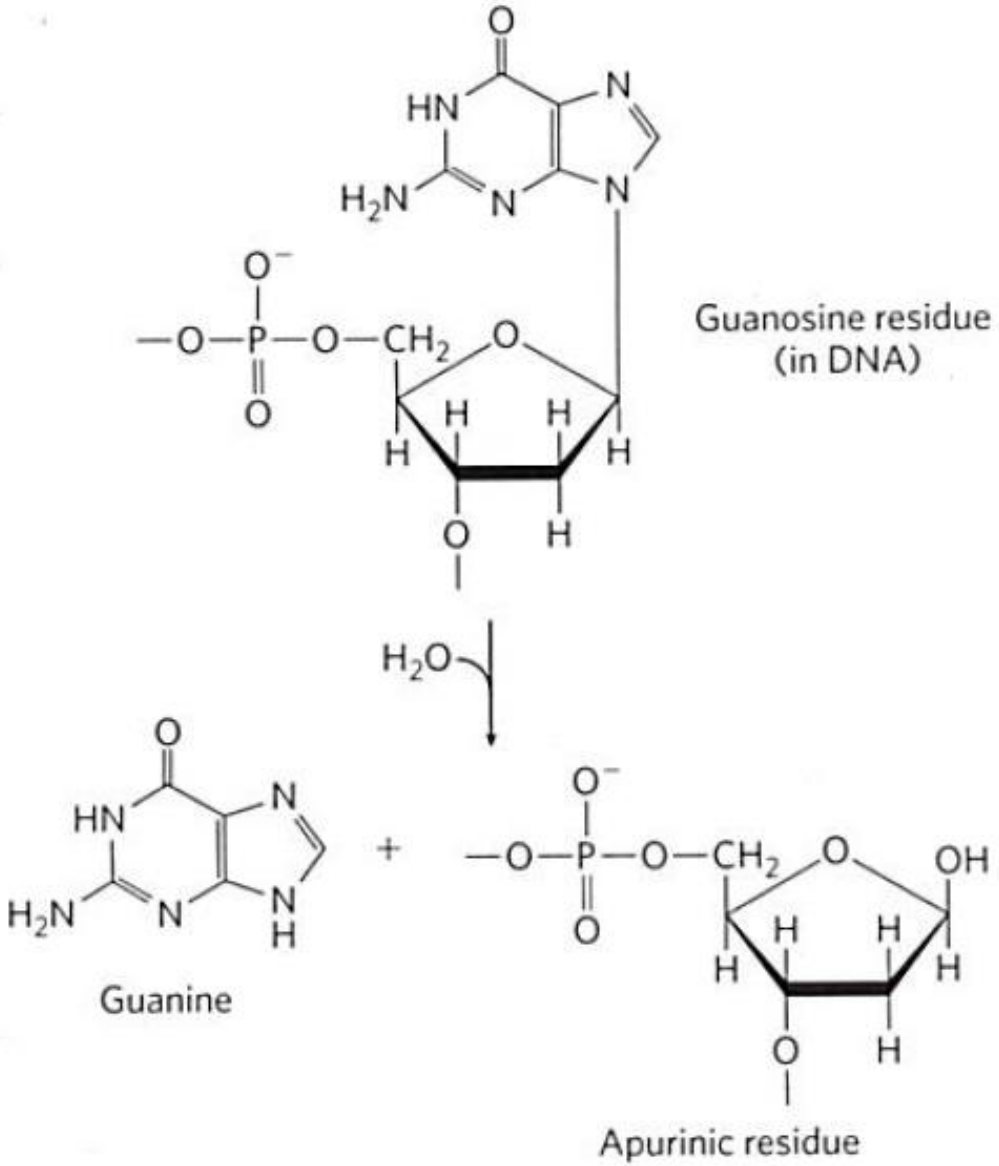
100 spontaneous events/day, average, in a mammalian cell

Almost certainly the reason why DNA contains THYMINE rather than URACIL!

Deamination (continued)



Nonenzymatic Transformations of Nucleotides and Nucleic Acids

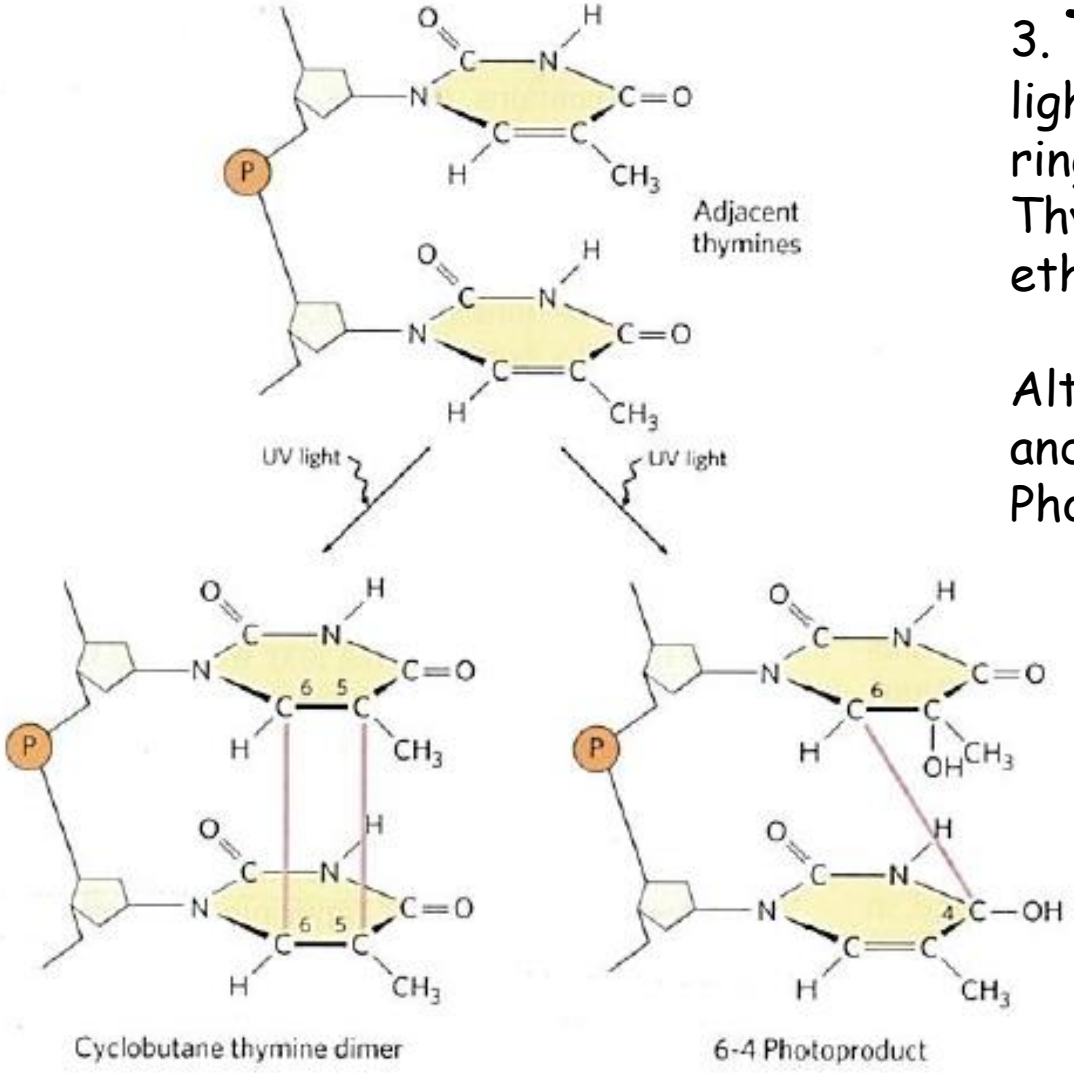


2. **DEPURINATION**:
Spontaneous hydrolysis of the N-β-glycosyl bond between the base (most frequently a purine, but possibly also with pyrimidines) and the pentose.

Base is LOST, creating a DNA lesion called AP (apurinic, apyrimidinic) site, or ABASIC Site

About 10,000 purines/day are lost in each mammalian cell, due to DEPURINATION.

Nonenzymatic Transformations of Nucleotides and Nucleic Acids



3. **THYMINE DIMERS**: UV light induces formation of Cyclobutane rings between two adjacent Thymidines by condensation of two ethylene groups, involving C-5 and C-6.

Alternatively, a linkage between C-6 and neighbor C-4 can create a 6-4 Photoproduct.

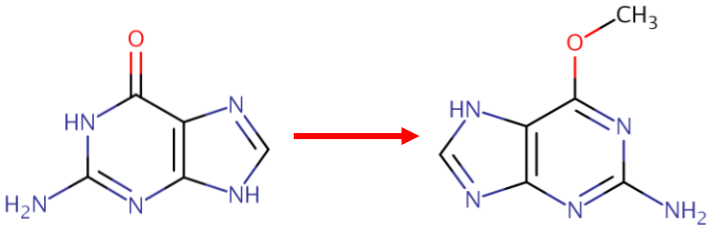
They create kinks or bends in the DNA secondary structure.

UV and ionizing radiation are responsible for about 10% of all DNA damage caused by environmental agents.

Nonenzymatic Transformations of Nucleotides and Nucleic Acids

4. **DEAMINATING AGENTS**: Nitrosamine is a precursor of Nitrous Acid (HNO_2) which promotes deamination reactions. Bisulfite has similar effects. Both agents are used as processed food preservatives.

5. **ALKYLATING AGENTS**: Some agents can alter certain bases of DNA. E.g. DIMETHYLSULFATE can methylate a G to yield an O6-methyl-guanine, which CANNOT PAIR WITH CYTOSINE!



6. **OXIDATIVE DAMAGE**: Reactive Oxygen Species (ROS): Hydrogen Peroxide, Hydroxyl Radicals, Superoxide Radicals---- formed during irradiations or as byproducts of AEROBIC metabolism. They cause oxidation of deoxyribose and base moieties to strand breaks. Cells have defensive mechanisms (catalase and superoxide dismutase) converting ROS into harmless products, however a fraction of ROS escape and cause DNA damage.

OXYDATIVE STRESS

Reactive Oxygen Species and Peroxides

$O_2^{\bullet -}$ Superoxide anion

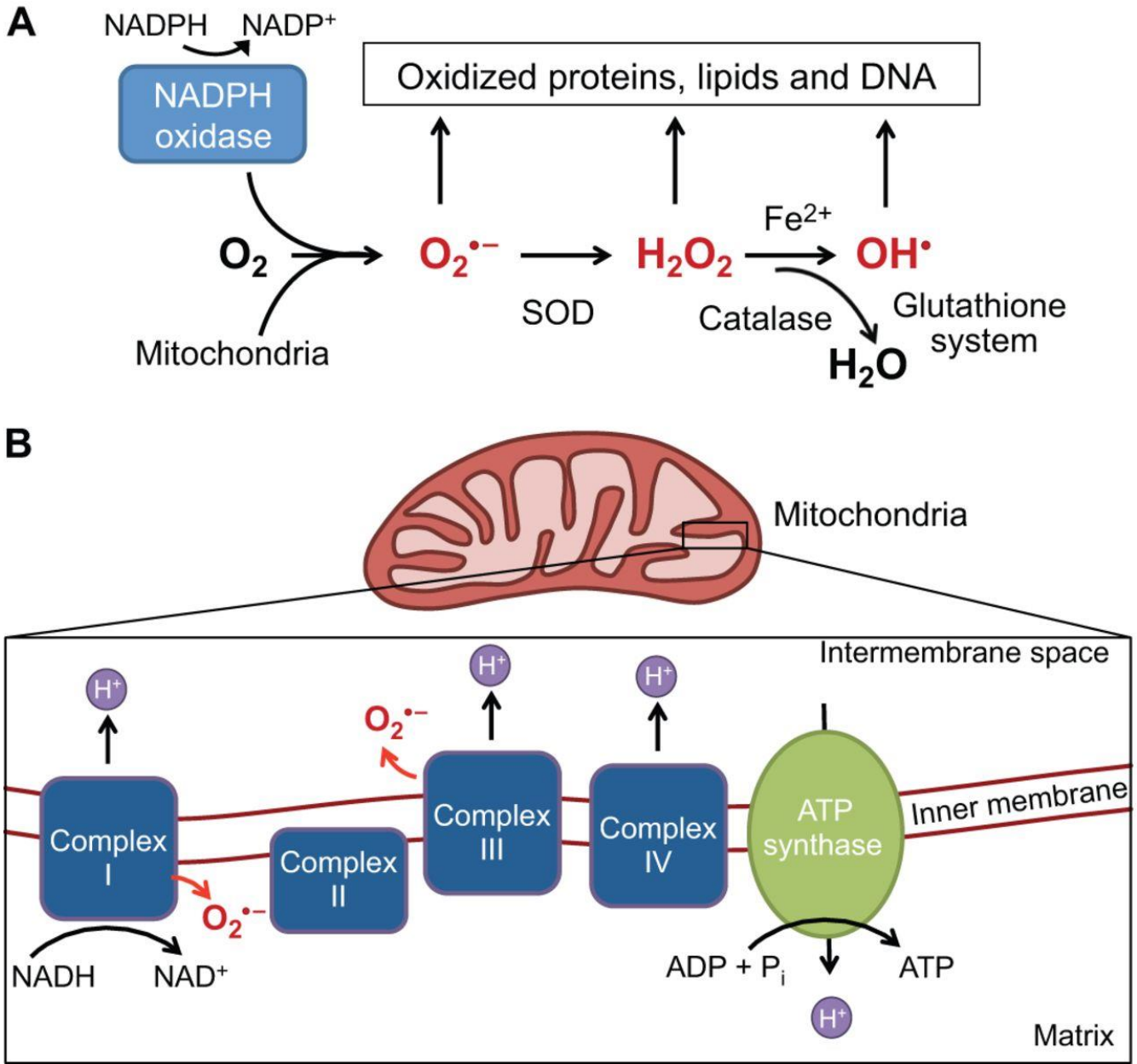
$OH^{\bullet}$ Hydroxyl radical

H_2O_2 Hydrogen peroxide

NO Nitric oxide

$OONO^{\bullet -}$ peroxynitrite

Reactive Oxygen Species (ROS)



Some DNA Bases are METHYLATED

1. Adenine and Cytosine are methylated MORE OFTEN than Guanine and Thymine

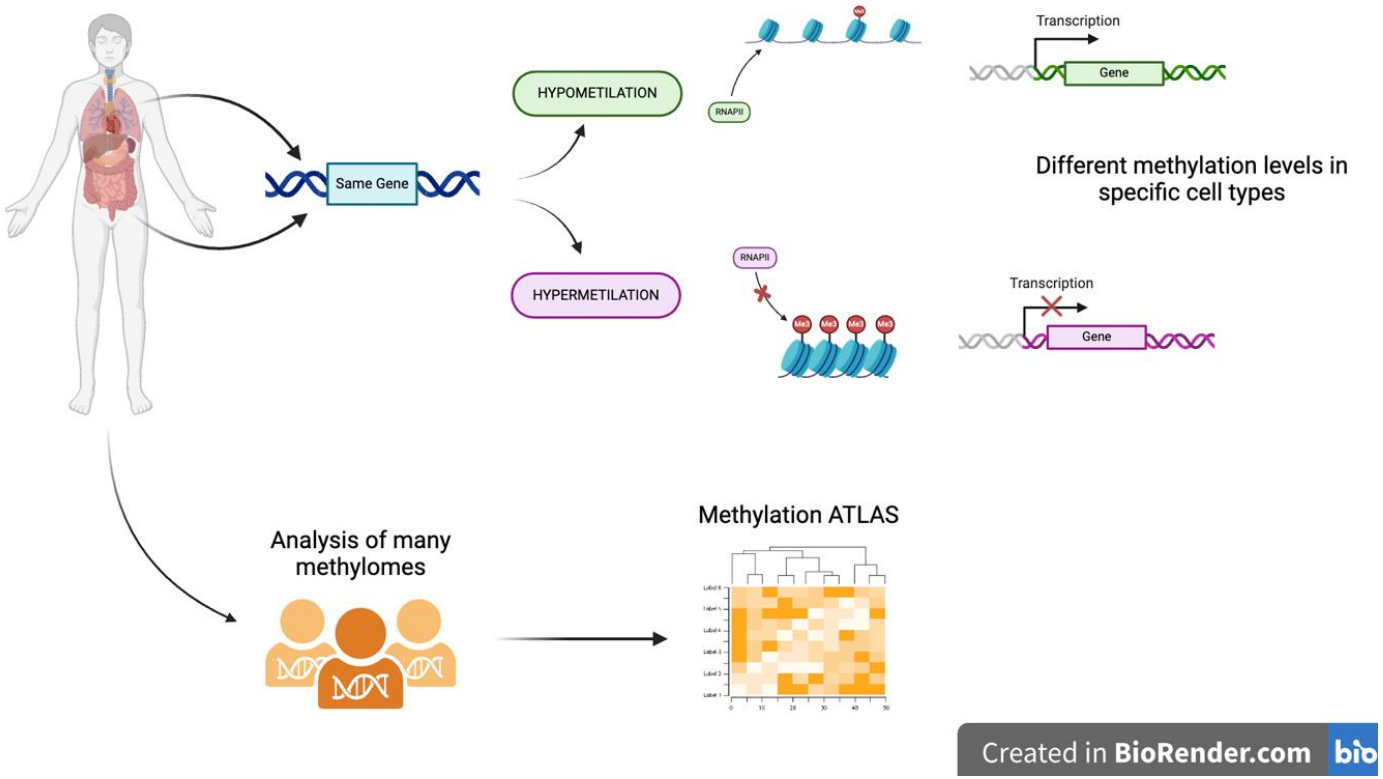
2. Methylation is confined to specific regions of DNA (CpG Islands)

3. All known DNA methyltransferases use S-adenosylmethionine as a methyl group donor

4. In eukaryotic cells, about 5% of Cytidine residues in DNA are methylated to 5-methylcytidine

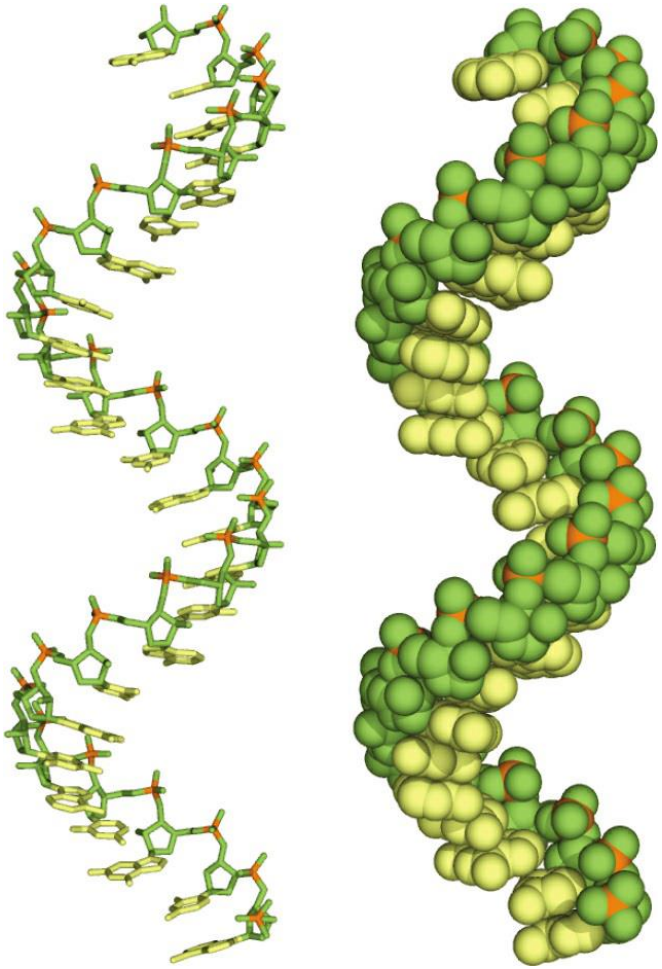
5. DNA methylation has relevance for Gene Expression Regulation

Methylation can affect gene expression without altering the underlying DNA sequence, typically repressing transcription when present in gene promoters



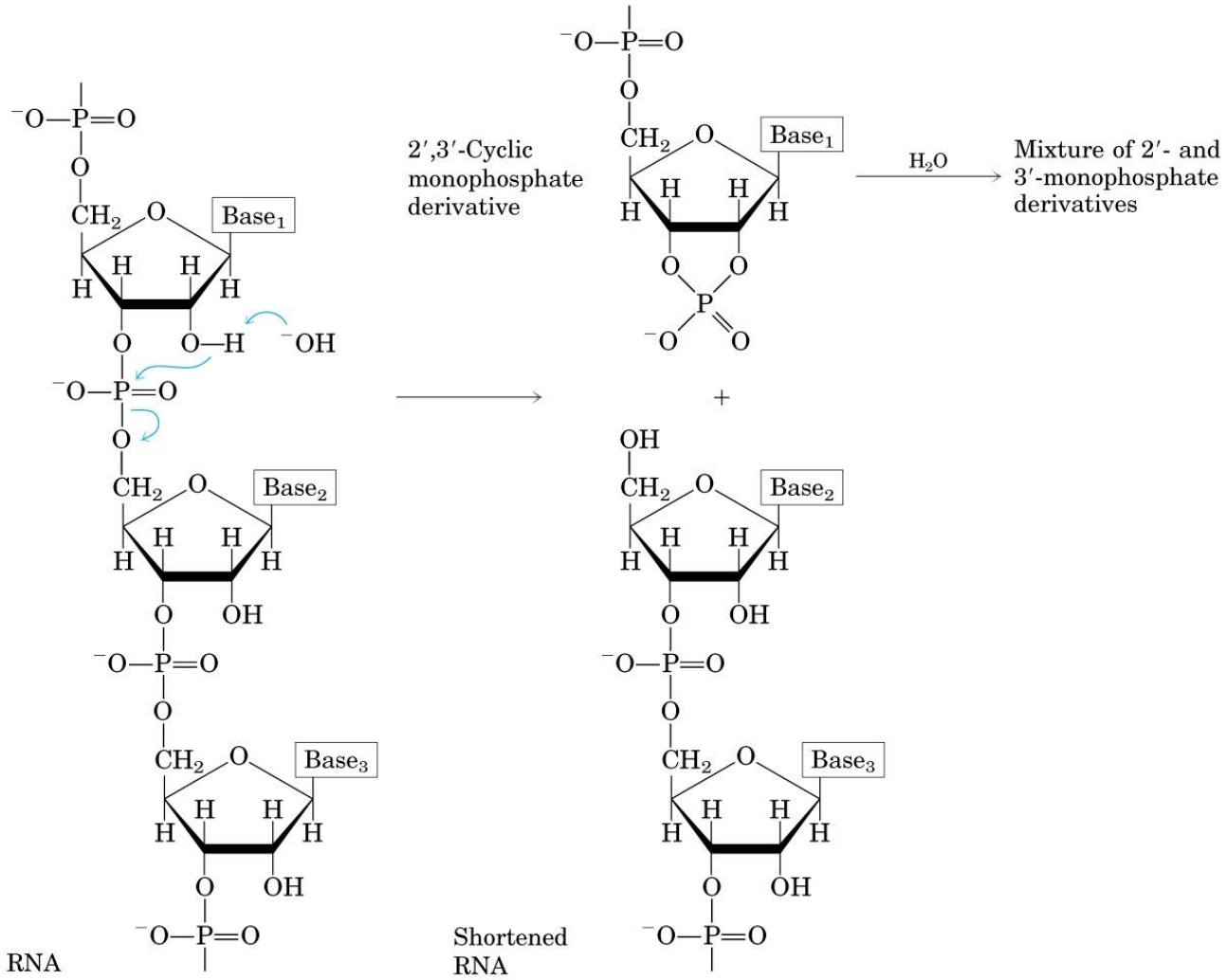
methylome analysis is a powerful tool in epigenetics that provides insights into how DNA methylation influences gene regulation and cellular processes, with significant implications for understanding health and disease.

RNAs

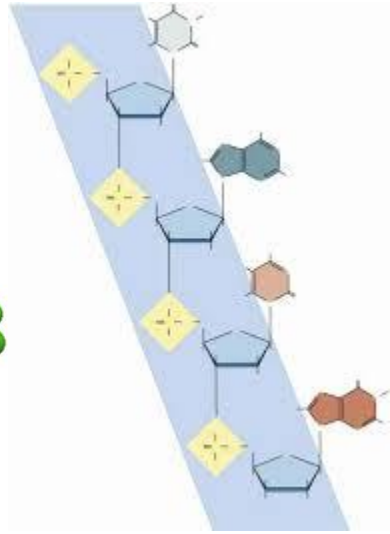


- **mRNAs:** formed on a DNA template by the process of transcription, provide the templates that specify amino acid sequences in polypeptide chains
- **tRNAs**: adapter molecules that act in protein synthesis; covalently linked to an amino acid at one end, each tRNA pairs with the mRNA in such a way that amino acids are joined to a growing polypeptide in the correct sequence.
- **rRNAs** are components of ribosomes.
- **Ribozymes** that have enzymatic activity

Slow and spontaneous hydrolysis of RNA (not DNA) in a basic environment can alter the covalent structure

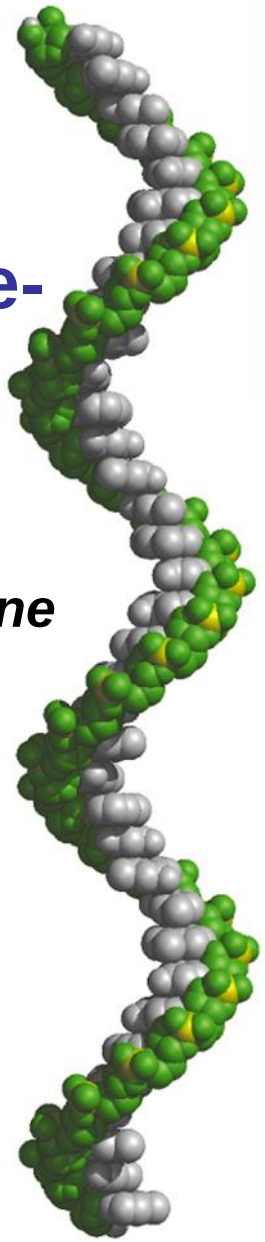


SINGLE-STRANDED RNA (ssRNA)



Right-handed helical conformation dominated by base-stacking interactions.

Purine-Purine stacking is the strongest. A pyrimidine separating them can be displaced from the stacking pattern to allow purine-purine interaction.



Gray= Bases

Green= Riboses and Oxygens in phosphate groups

Yellow= Phosphorus atoms

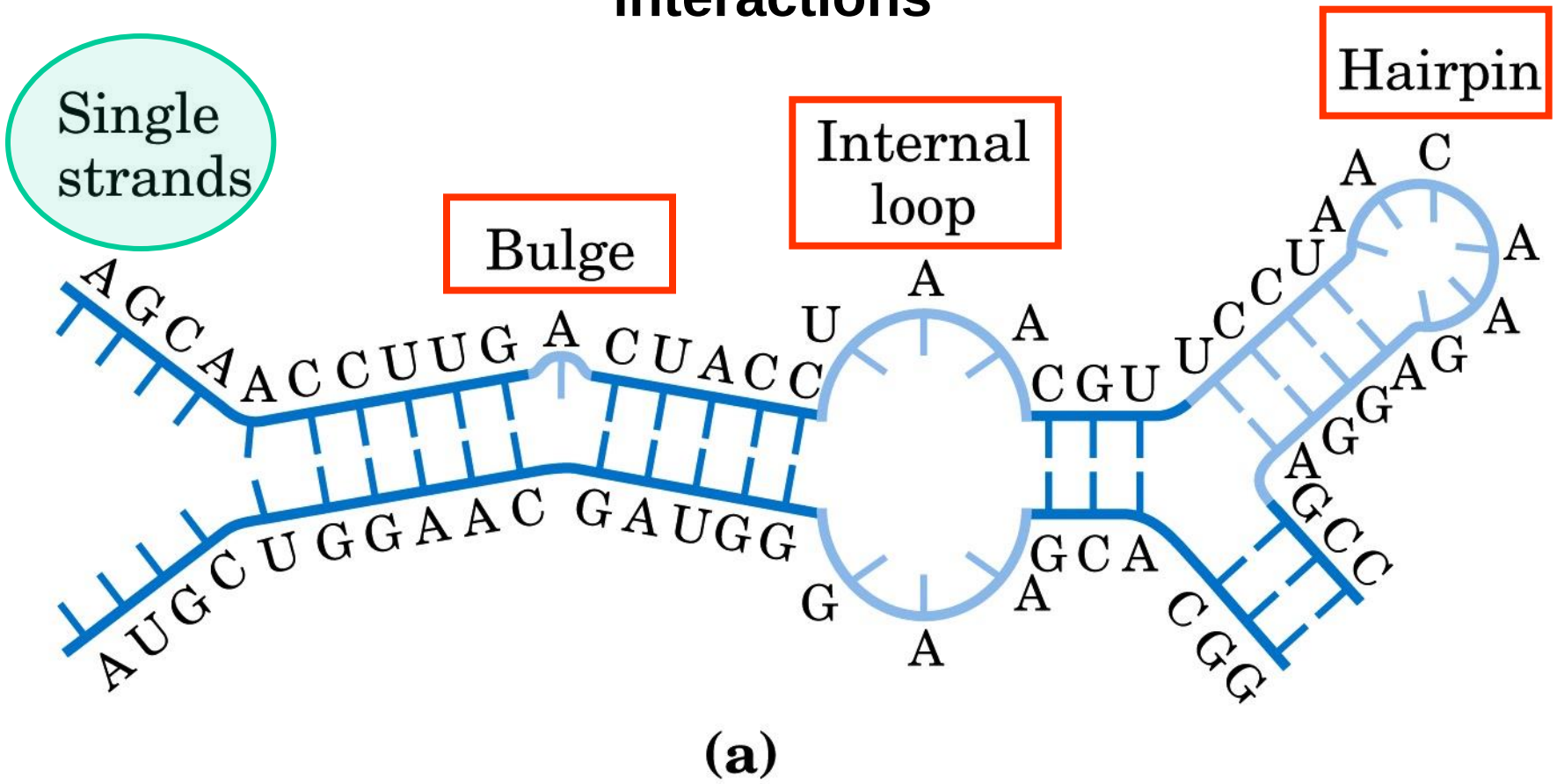
SECONDARY STRUCTURE of RNAs:

RNA can base-pair with complementary regions of either RNA or DNA forming complex secondary structures stabilized by hydrogen bonds between bases

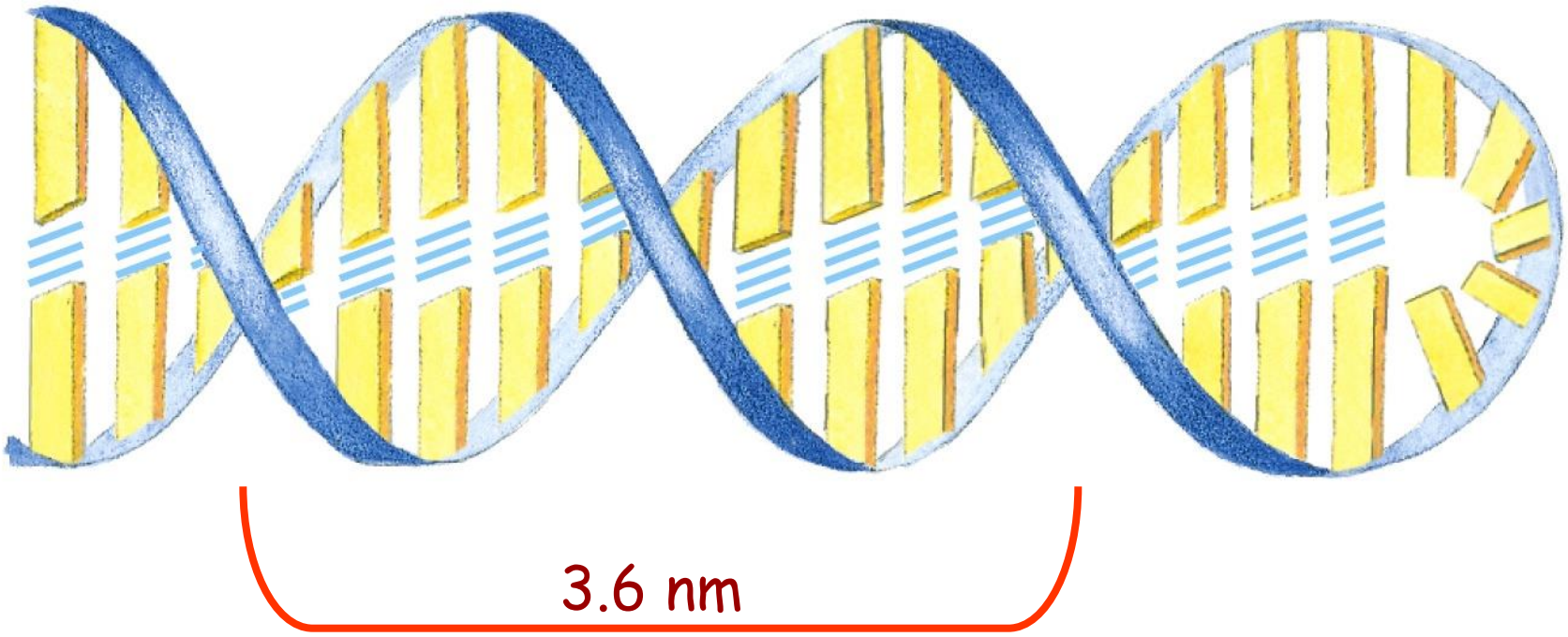
The paired strands in RNA or RNA-DNA duplexes are **ANTIPARALLEL**, as in DNA

SECONDARY STRUCTURE of RNAs:

The single strand spontaneously forms interactions and foldings (due to Hydrogen bonds within the strand), in order to maximize hydrophobic interactions



RNA: Hairpin double Helix



The paired regions generally have an A-form right-handed helix.

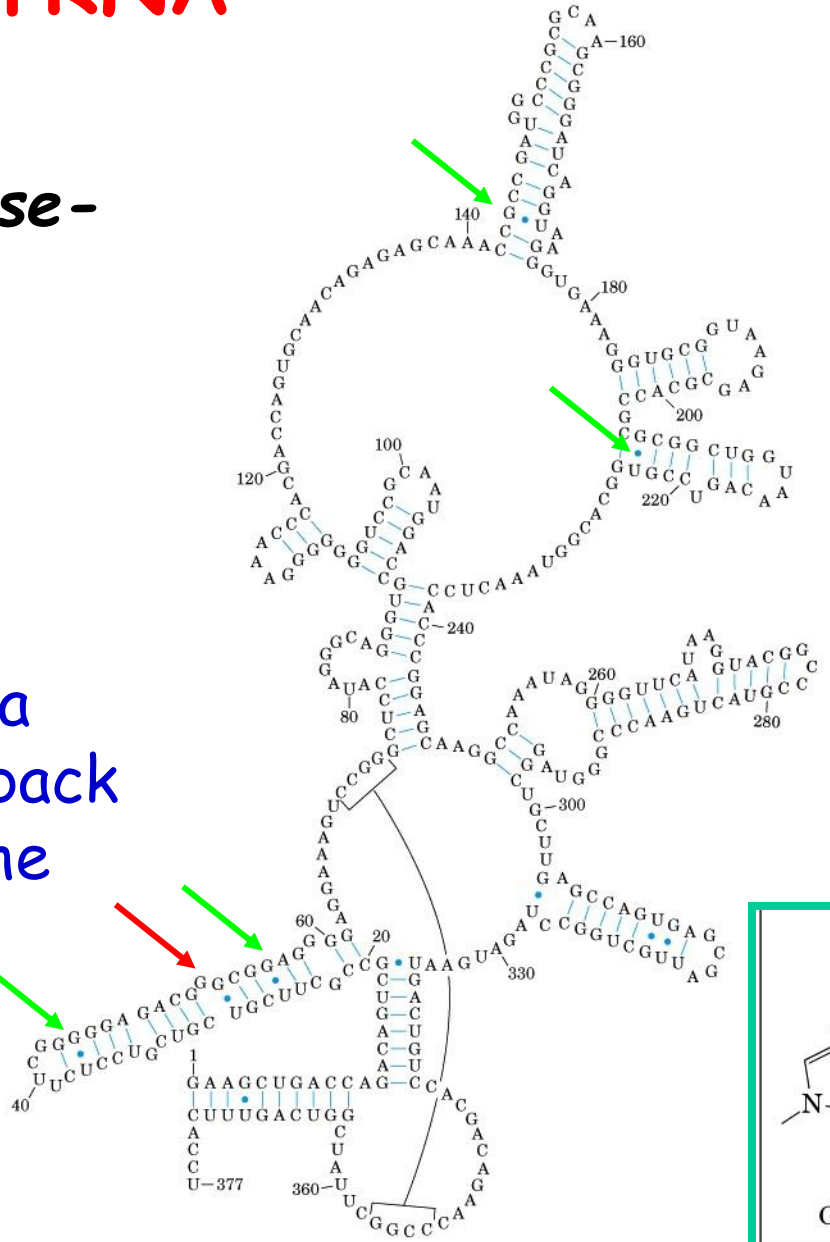
The B form of RNA **has not been observed**.

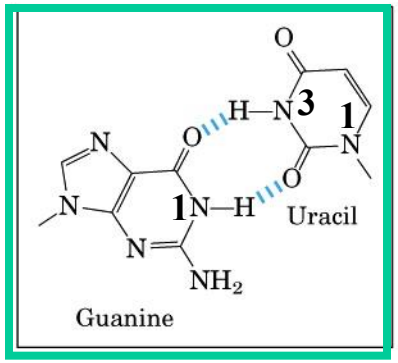
The Z form of RNA **has been made in the lab** (high-salt, high-temp conditions)

rRNA

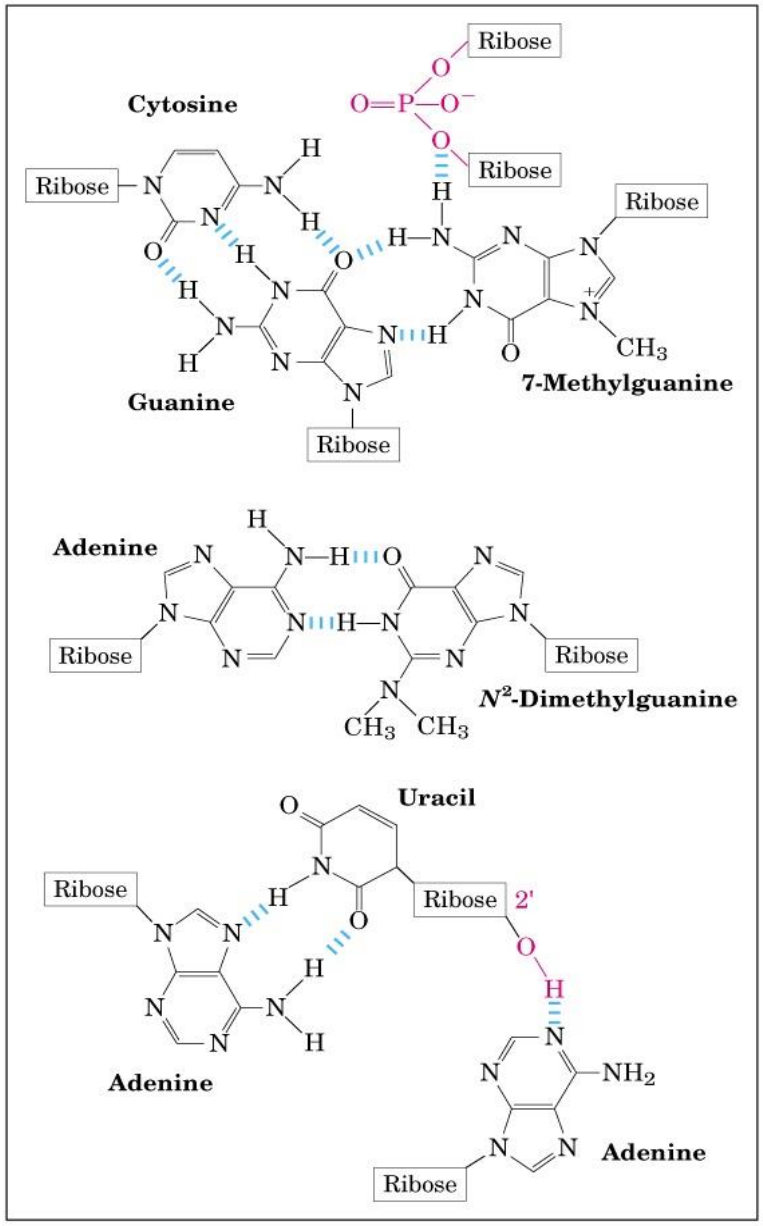
Unconventional Base-pair interaction is POSSIBLE:

G-U when complementary sequences in two ssRNAs (or within a ssRNA that folds back on itself to align the residues) pair with each other.





Other possible UNCONVENTIONAL base pairing in RNAs.



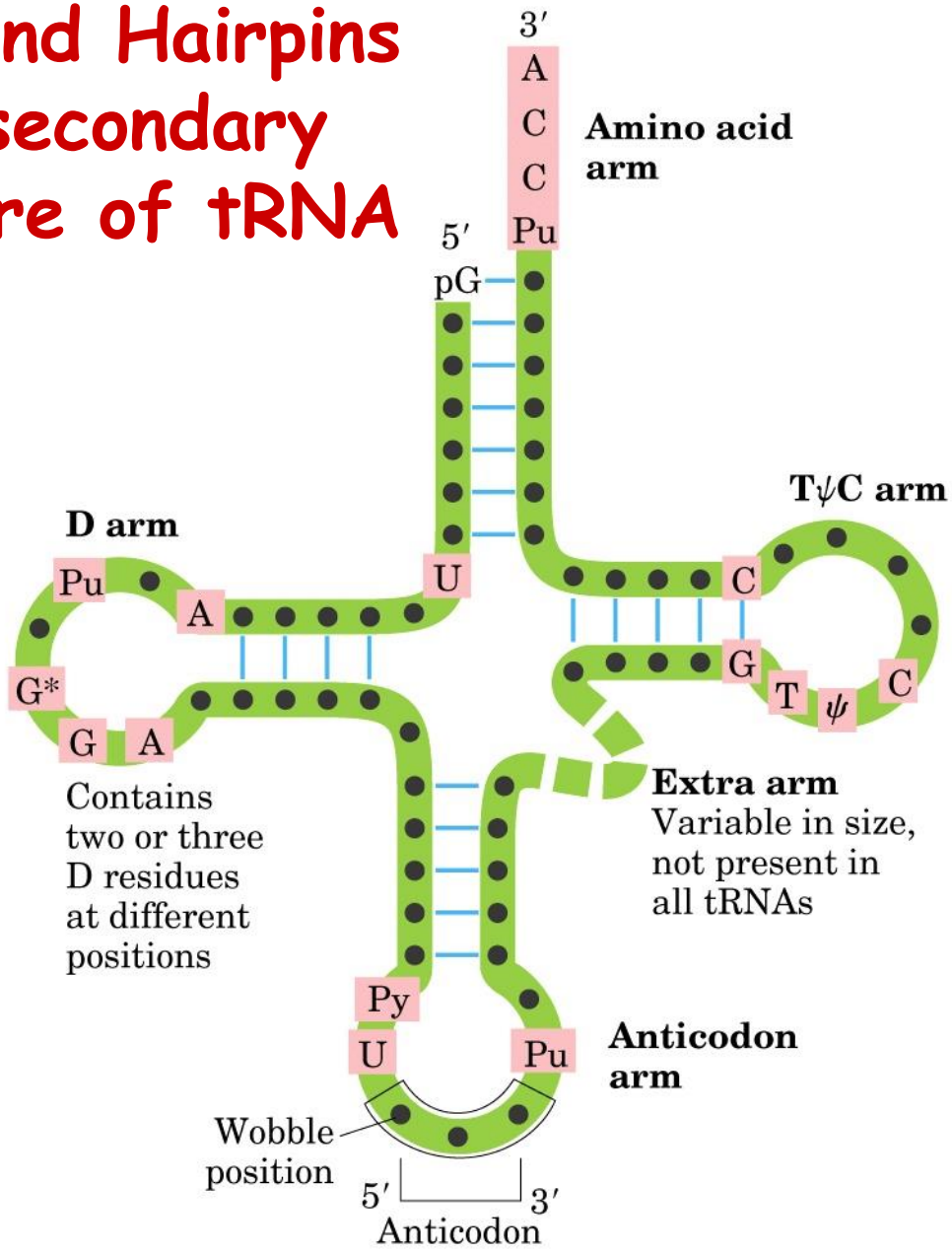
(b)

Interaction between 3 Bases + Pi

A = G

A = U = A

Loops and Hairpins in the secondary structure of tRNA



D=5,6-dihydrouridine

ψ =pseudouridine (base bound with C-5')

T = ribothymidine (thymine in RNA!)

Pu purine

Py pyrimidine

Can Nucleic Acids and oligonucleotides be degraded or eliminated by cells?

Yes, through a reaction catalyzed by the enzymes:

Deoxyribonucleases and Ribonucleases (NUCLEASES: DNases and RNases)

Their substrate is the <u>single</u> polynucleotidic strand

ENDONUCLEASES

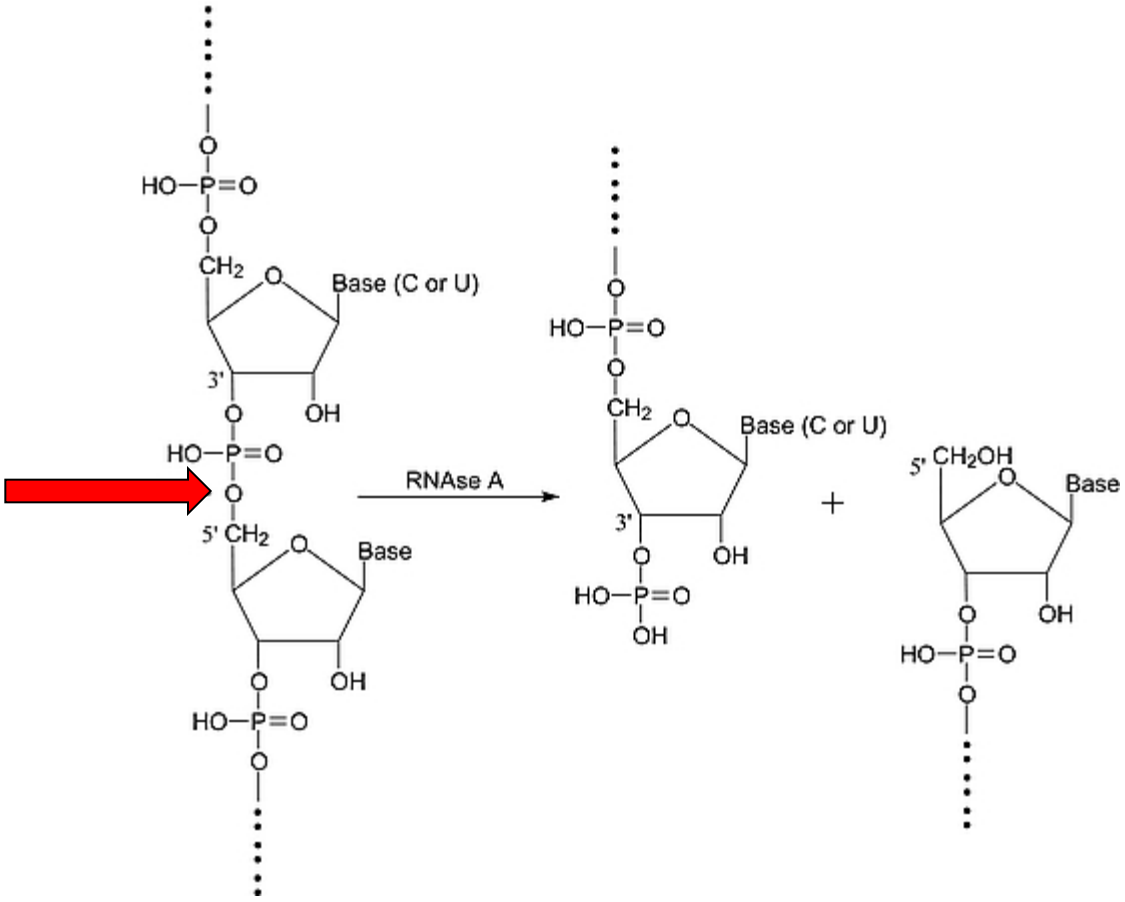
Degrade at specific INTERNAL sites in a nucleic acid strand or molecule, reducing it to smaller fragments (i.e. internal phosphodiester bonds)

EXONUCLEASES

Degrade nucleic acids (phosphodiester bonds) from ONE END of the molecule.
Many operate in only the 5'---3' or the 3'---5' direction, removing nucleotides only from the 5' or the 3' end, respectively, of one strand of a ds nucleic acid or of a ssDNA.

PANCREAS Ribonuclease

It catalyses the hydrolysis of phosphodiester bonds in which pyrimidine nucleotides participate with C-3', in RNA ONLY!!

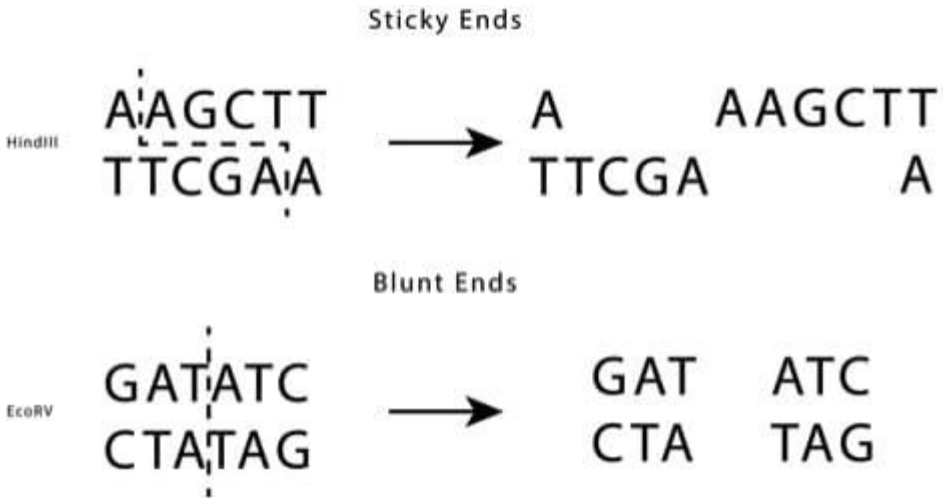


RESTRICTION ENDONUCLEASES
(BACTERIAL RESTRICTION ENZYMES)

Endonucleases able to recognize a specific (and usually palindromic) genomic sequence and operate a specific cut in both DNA strands.

They generate "sticky" or "blunt" ends and are very beneficial in Molecular Biology, especially for cloning purposes.

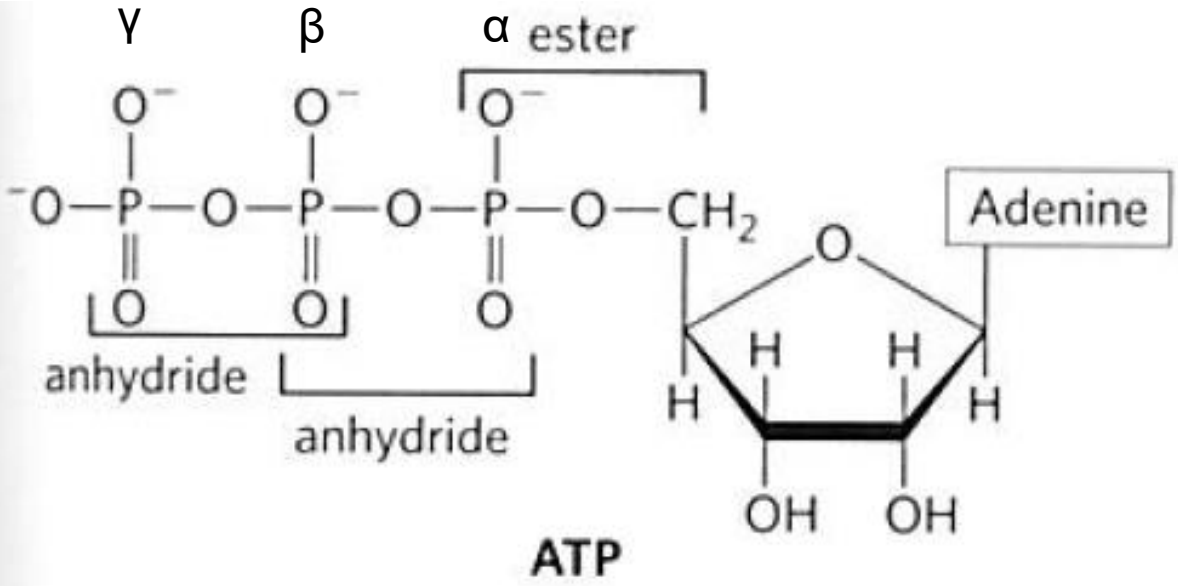
Fragments of DNA that end with unpaired nucleotides have cohesive «sticky» ends



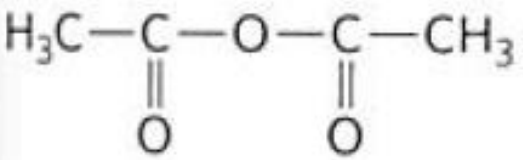
Fragments of DNA that end with complete base pairs have «blunt» ends

Other Functions of Nucleotides

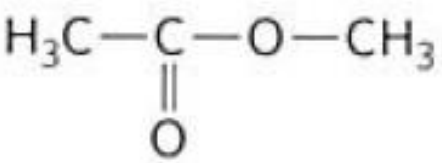
Nucleotides carry chemical energy in the Cells



ATP



Acetic anhydride, a carboxylic acid anhydride



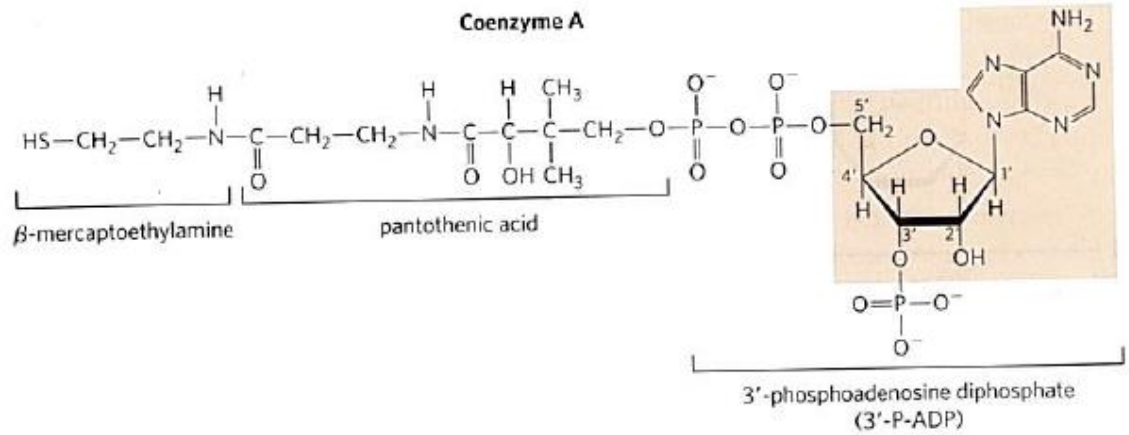
Methyl acetate, a carboxylic acid ester

Hydrolysis of:

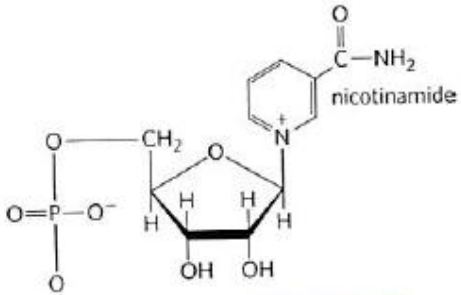
ESTER linkage= 14 KJ/mol

Each ***ANHYDRIDE linkage= 30.5 kJ/mol***

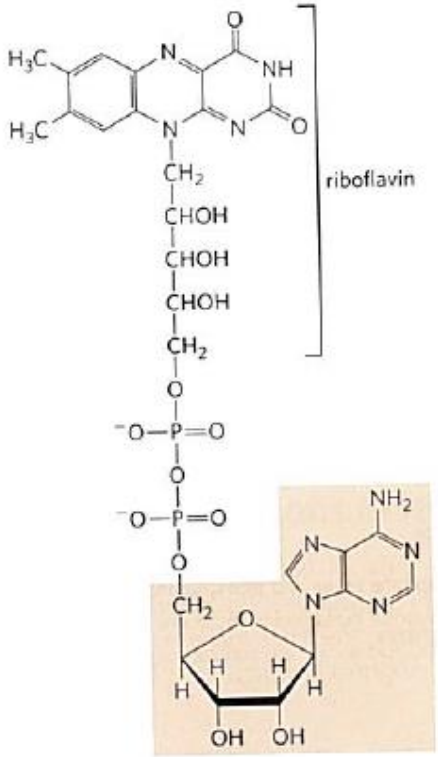
Other Functions of Nucleotides



Some coenzymes contain Adenosine

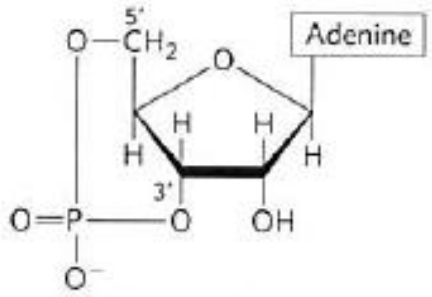


Nicotinamide adenine dinucleotide (NAD^+)

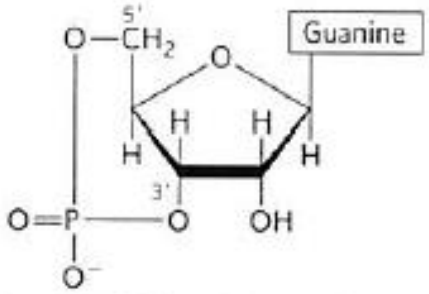


Flavin adenine dinucleotide (FAD)

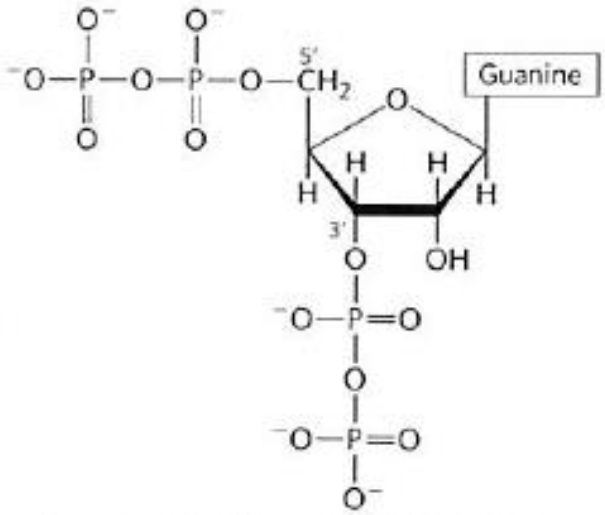
Other Functions of Nucleotides



Adenosine 3′,5′-cyclic monophosphate
(cyclic AMP; cAMP)



Guanosine 3′,5′-cyclic monophosphate
(cyclic GMP; cGMP)



Guanosine 5′-diphosphate,3′-diphosphate
(guanosine tetraphosphate; ppGpp)

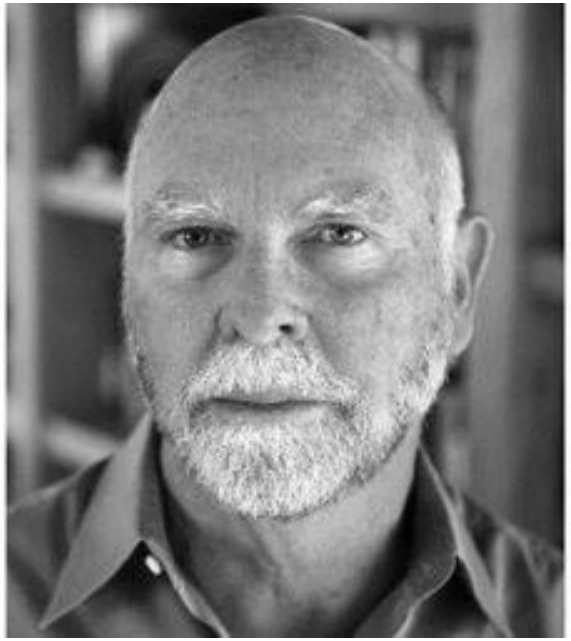
Some Nucleotides function as Second Messenger in the cells and work as Regulatory Molecules (cAMP, cGMP)

Nucleotides can also function as Neurotransmitters and Ligands:

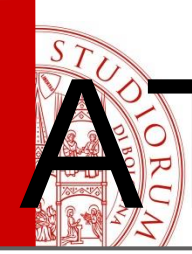
- *ATP binds to P2x receptors in post-synapsis → taste, inflammation, smooth muscle contraction*
- *ADP binds to P2γ receptors in platelets promoting clotting (clopidogrel interferes)*

Genome: our instruction manual

Riccardo Sabatini, an Italian scientist, has printed out Graig Venter genome in 175 volumes (a total of 262000 pages).



One haploid human genome consists of approximately 3 billion base pairs of DNA, which are distributed across 23 chromosomes (3,054,815,472 with a X, and 2,963,015,935 with a Y). Human chromosomes range in size from about 50 million to 300 million base pairs.



ATGCATGCCCATTA

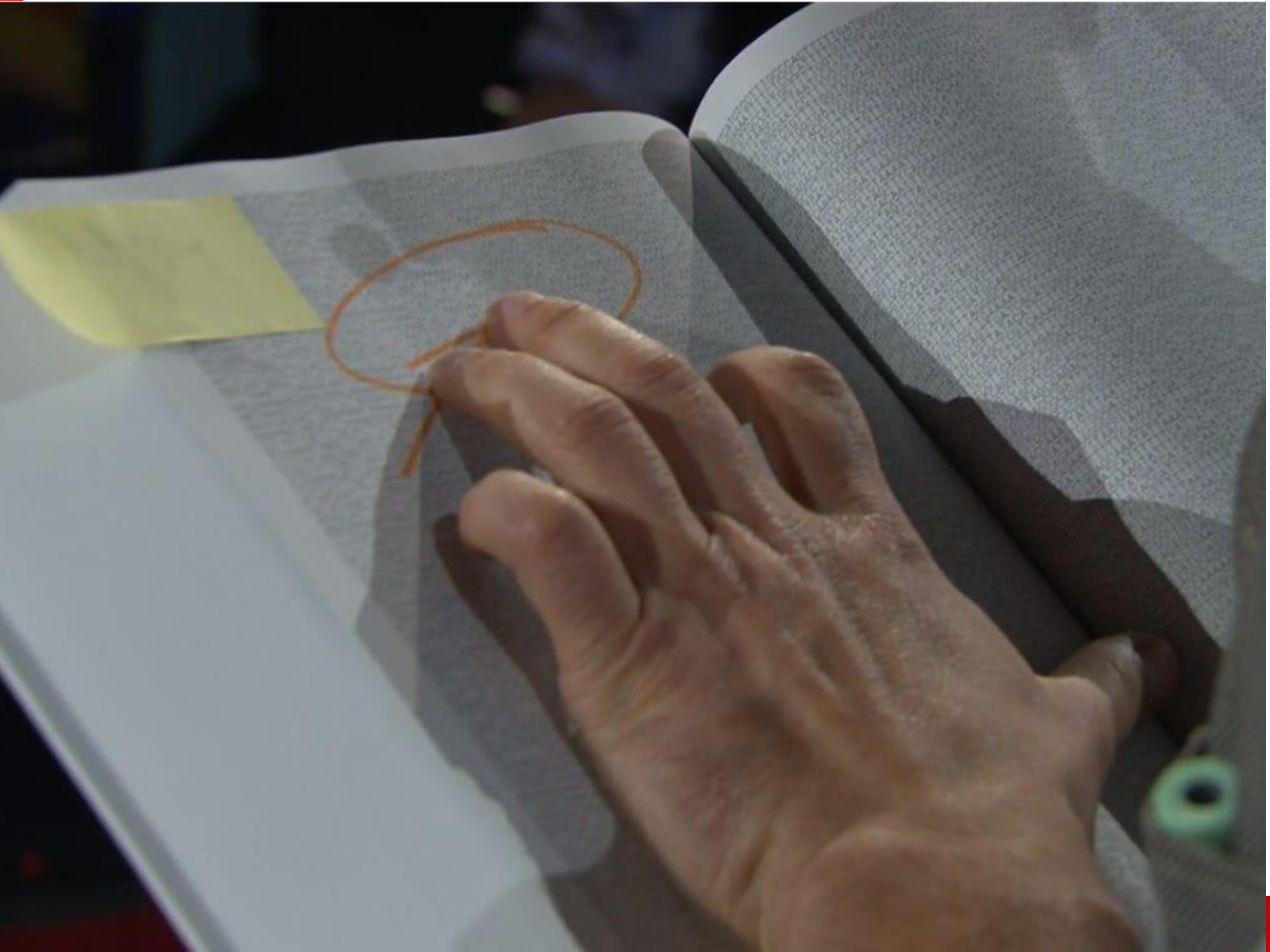
GCATTATCGACGGG

ATCCTTACGGCGGG

ATCGATCCATTATC

GACGGGATCCCATT

ATCGACGGGATCCC



The term **GENOME** refers to the entire nucleotide sequence, i.e. , an organism's coding and non-coding DNA sequences.

PROKARYOTIC GENOME:

- small;
- very compact;
- often contains plasmids.

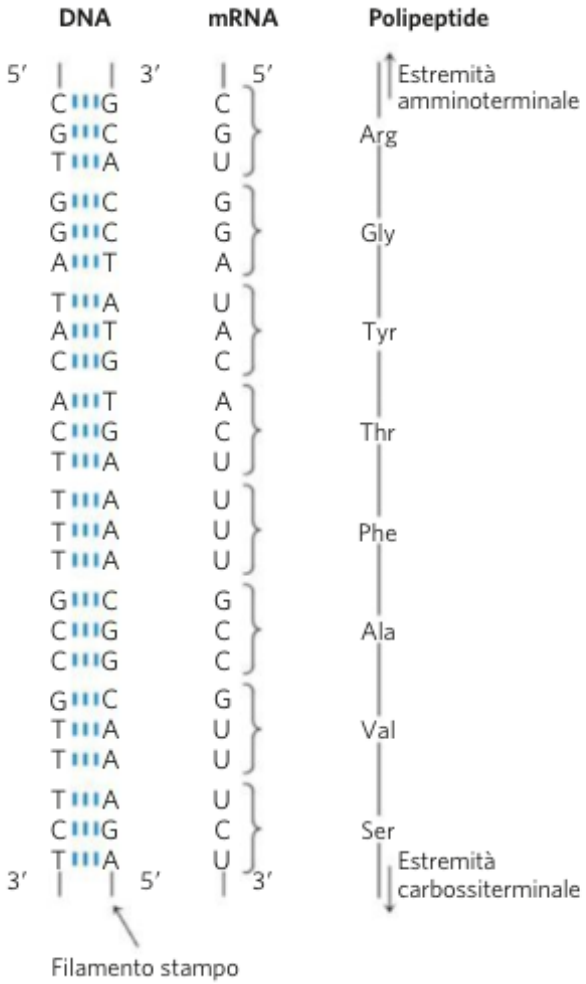
Bacteria have no delimited nucleus and metabolic activities take place in the cytoplasm.

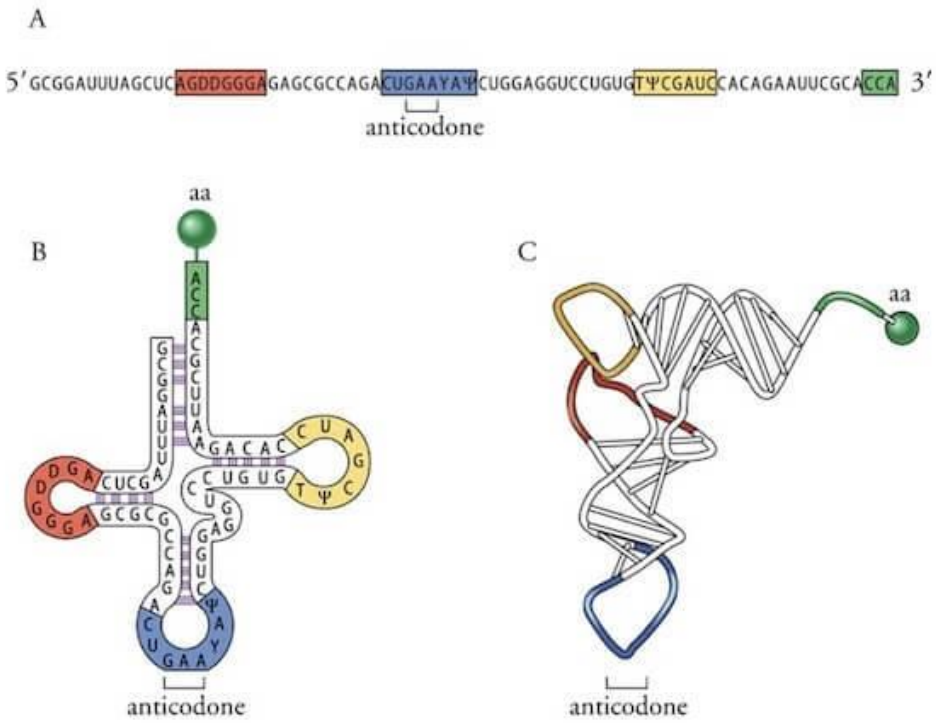
EUKARYOTIC GENOME:

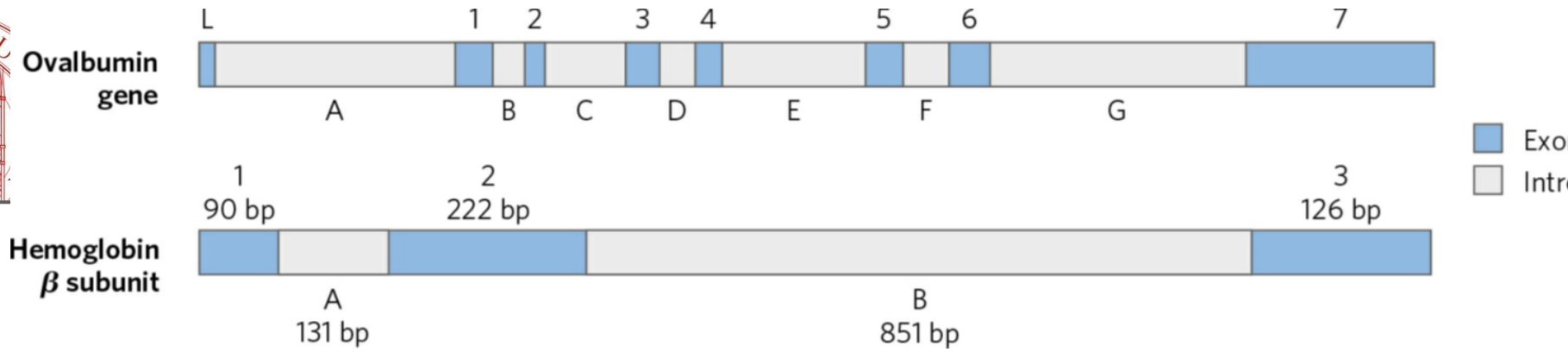
- larger than that of prokaryotes;
- organised in chromosomes;
- possesses telomeres;
- contains repetitive sequences;
- possesses many interrupted genes;
- contains regulatory sequences;
- transcription and translation take place in separate environments.

GENE

A portion of DNA encoding the primary sequence of a final gene product, which may be a polypeptide or an RNA with a specific structural or catalytic function

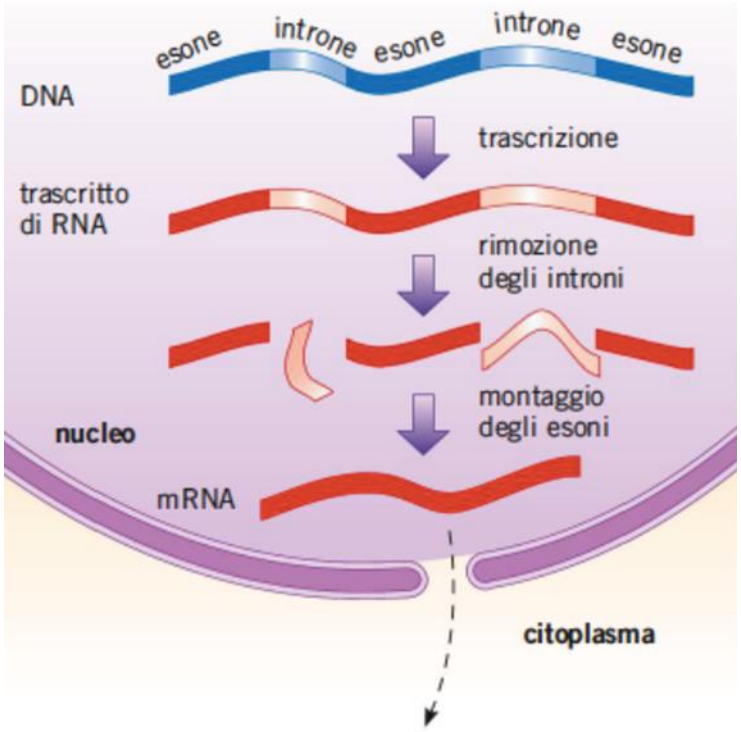


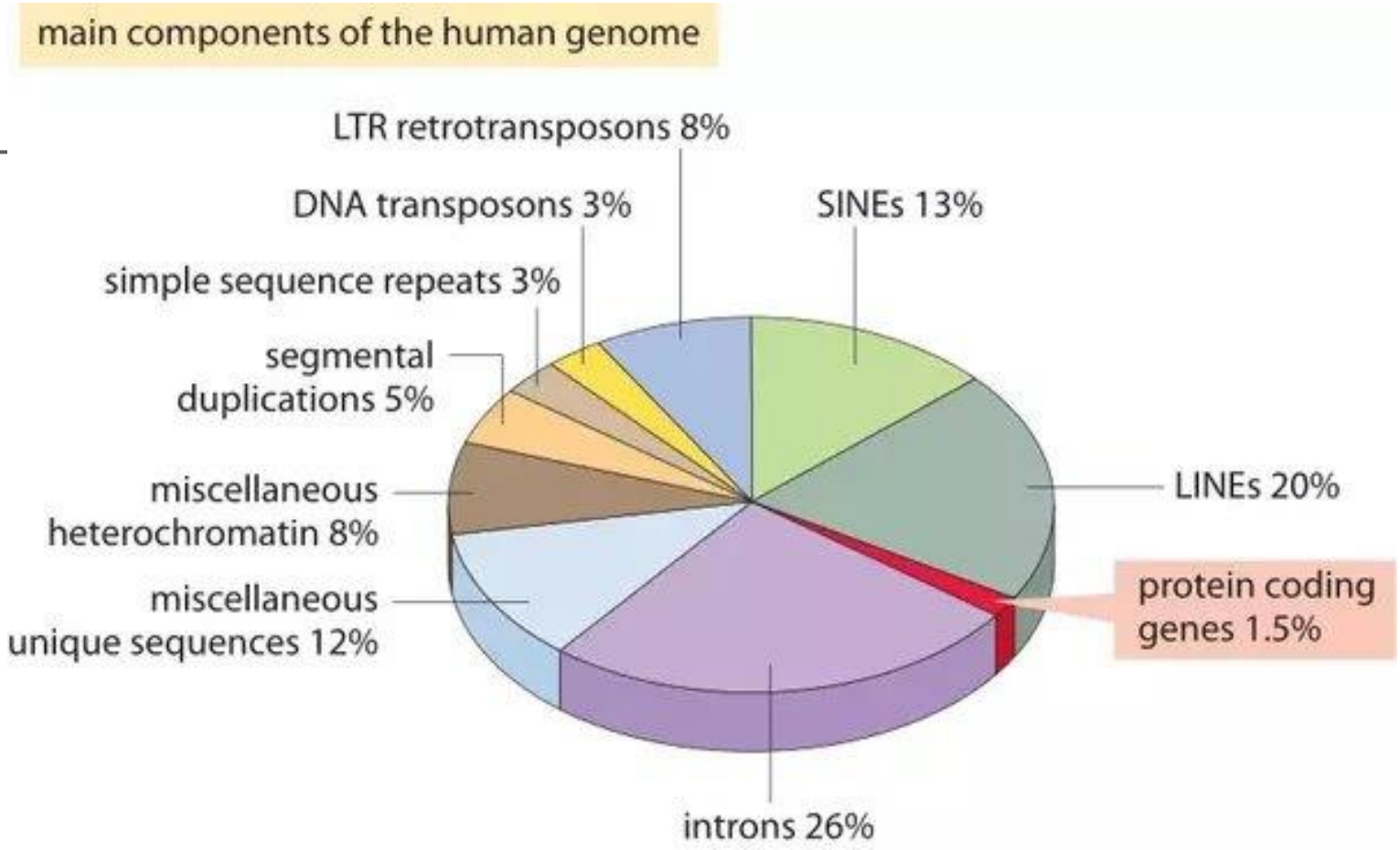




- Many genes in eukaryotic cells (but few in bacteria and archaea) are interrupted by noncoding sequences, or **introns**. The coding segments separated by introns are called **exons**.

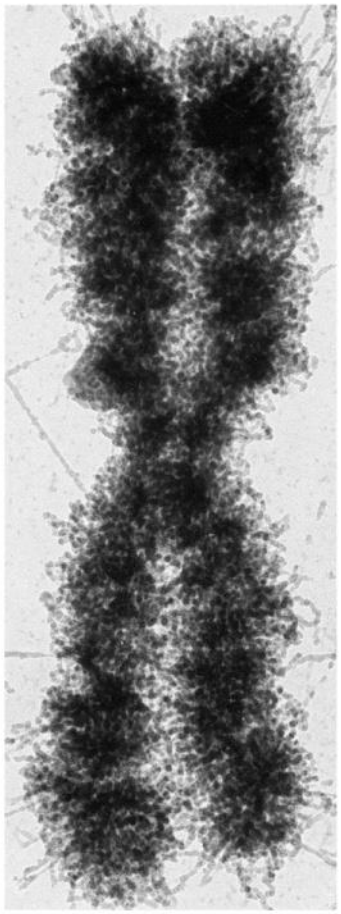
- The process of removing introns and joining exons is called **RNA splicing.**

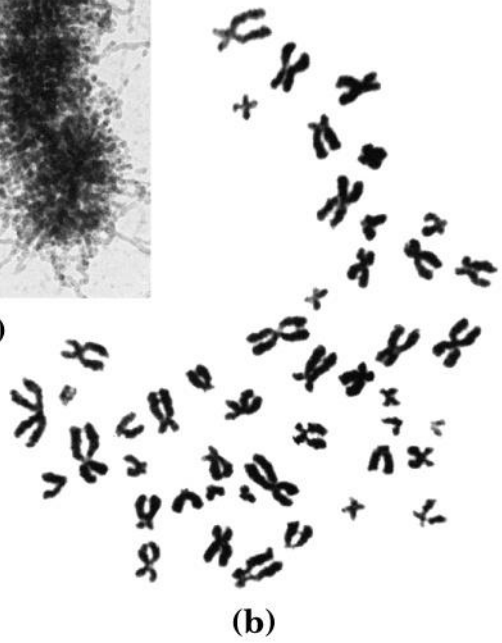




non-coding regions:

- introns (non-coding segments within genes),
- regulatory elements (such as promoters and enhancers that control gene expression),
- various types of RNA that are involved in regulation and structural roles, such as ribosomal RNA (rRNA), transfer RNA (tRNA) and miRNA





(a)

(b)

In the Eukaryotic Cell DNA is located in the nucleus, organized as CHROMATIN, meaning combined to proteins. 90% of these proteins are HISTONES.

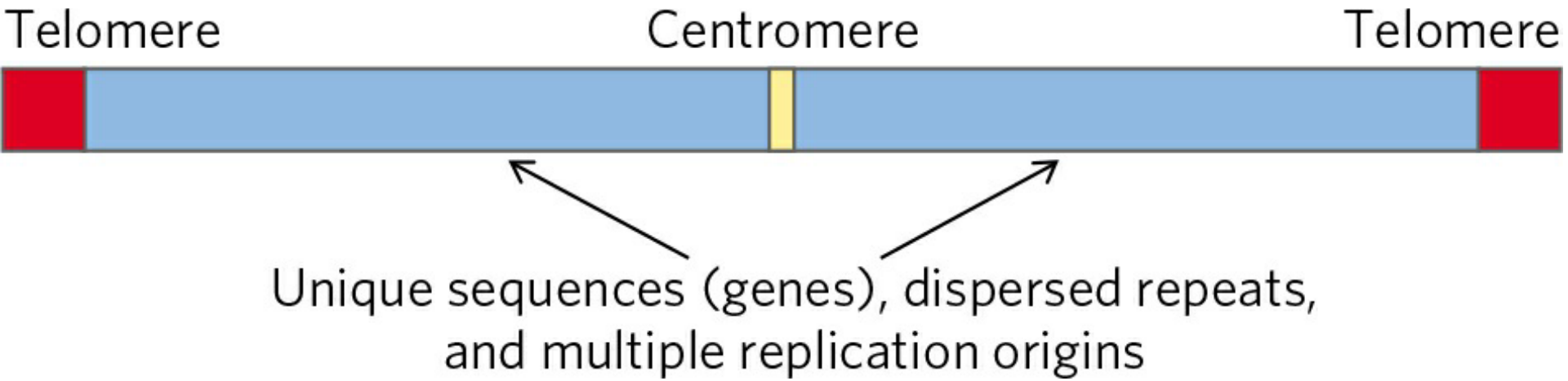
In the post-replicative phase of the cell cycle, chromatin is organized in CHROMOSOMES.

In a human somatic cell there are 46 chromosomes (22 pairs + XX or XY).

Each type of chromosome carries a characteristic set of genes.

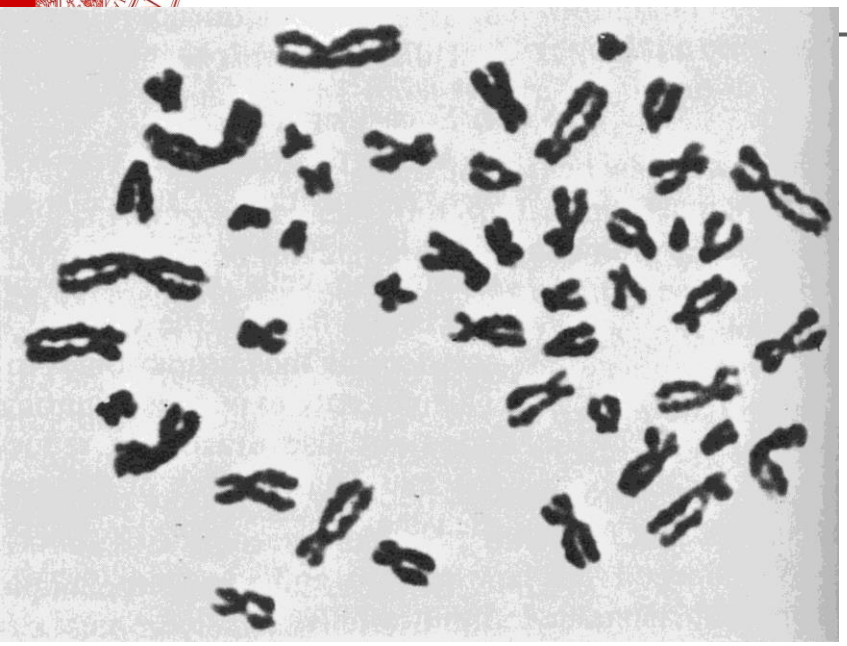
Each chromosome contains 1 molecule of linear DNA (Total human DNA length: 2m; 3 x 10^9 bp).

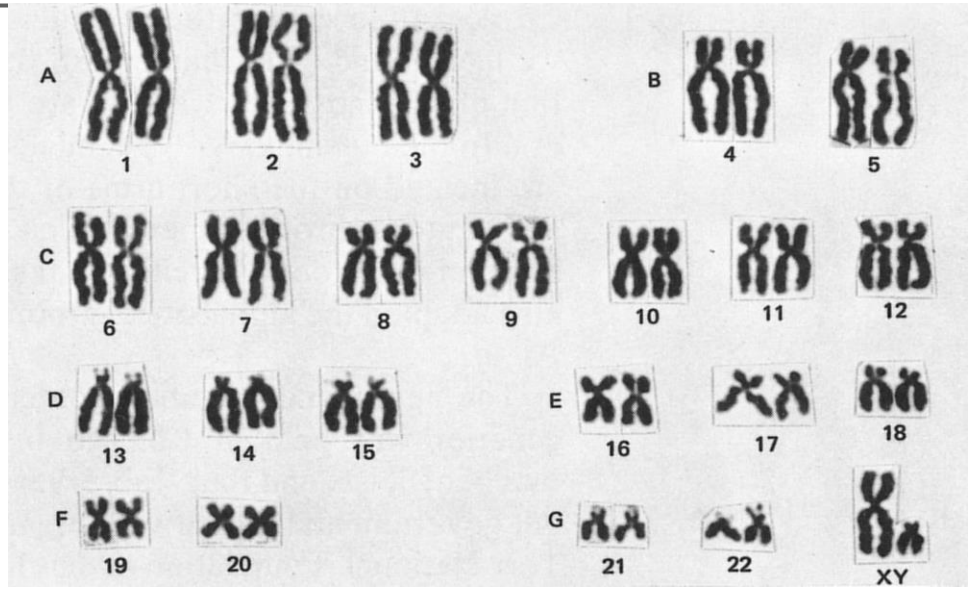
Chromosome



Humans are diploid organisms, meaning they have two sets of chromosomes—one inherited from each parent. This results in 23 pairs: 22 pairs of autosomes (non-sex chromosomes) and 1 pair of sex chromosomes.

Human Karyotype

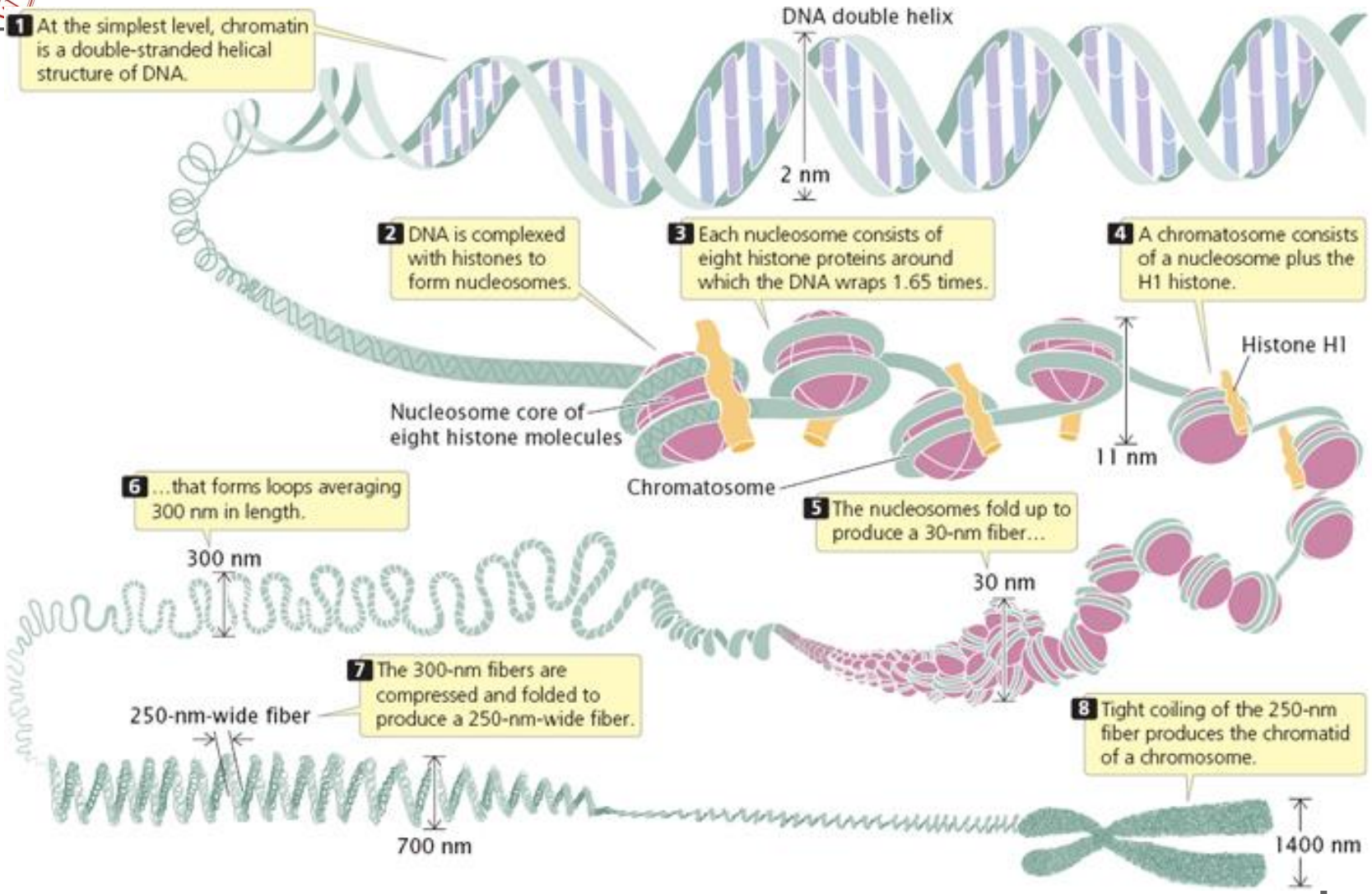




Karyotyping is often used in clinical genetics to detect chromosomal abnormalities. For instance:

- Down syndrome (Trisomy 21) is caused by an extra chromosome 21.
- Klinefelter syndrome involves an extra X chromosome in males (XXY).
- Turner syndrome occurs when females have only one X chromosome (X0).

An adult human body contains approximately 10^4 cells, so the total length of DNA is approximately 2×10^{11} km (Earth's circumference = 4×10^4 km Earth-Sun distance = 1.5×10^8 km)



CHROMATIN

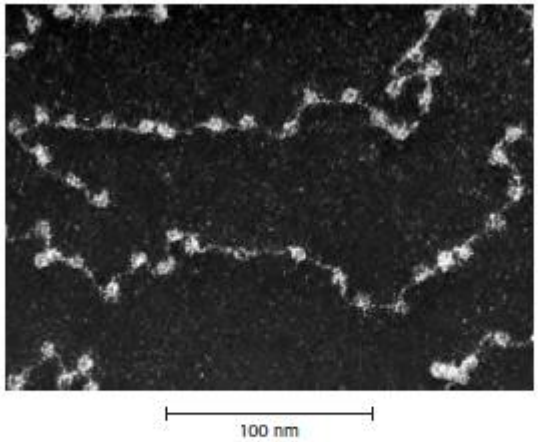


Figure 32.2 Chromatin structure. An electron micrograph of chromatin showing its "beads on a string" character. The beads correspond to DNA complexes with specific proteins. [Courtesy of Dr. Ada Olins and Dr. Donald Olins.]

Chromatin consists of **protein and DNA** in approximately equal amounts, plus a significant portion of associated **RNA**.

EUCHROMATIN: Lightly packed form of chromatin that is transcriptionally ACTIVE.
HETEROCHROMATIN: Tightly packed form of chromatin that is transcriptionally INACTIVE).

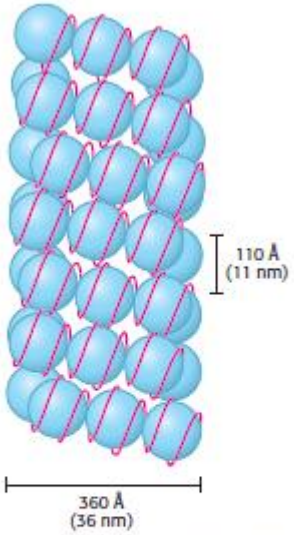
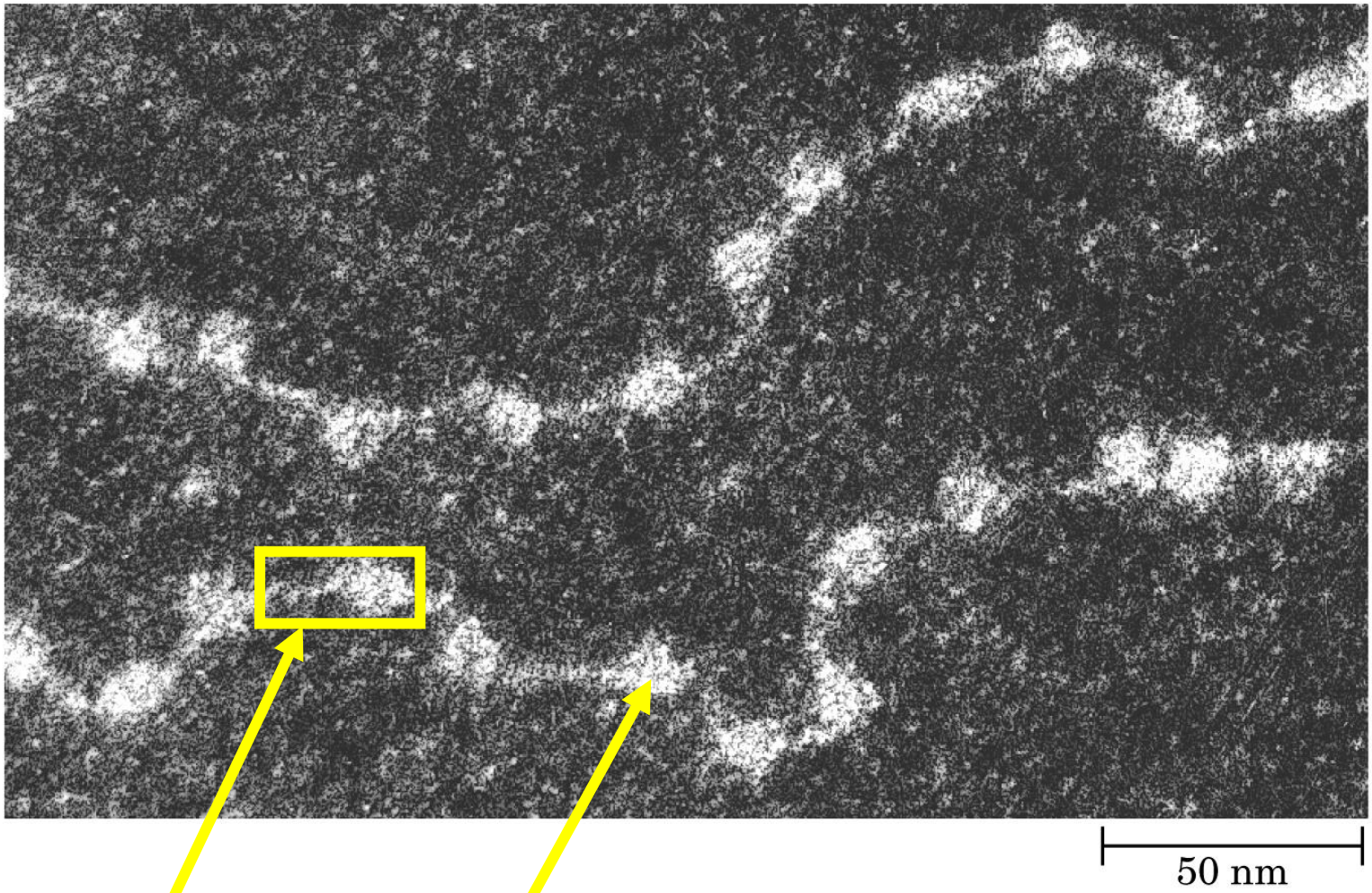


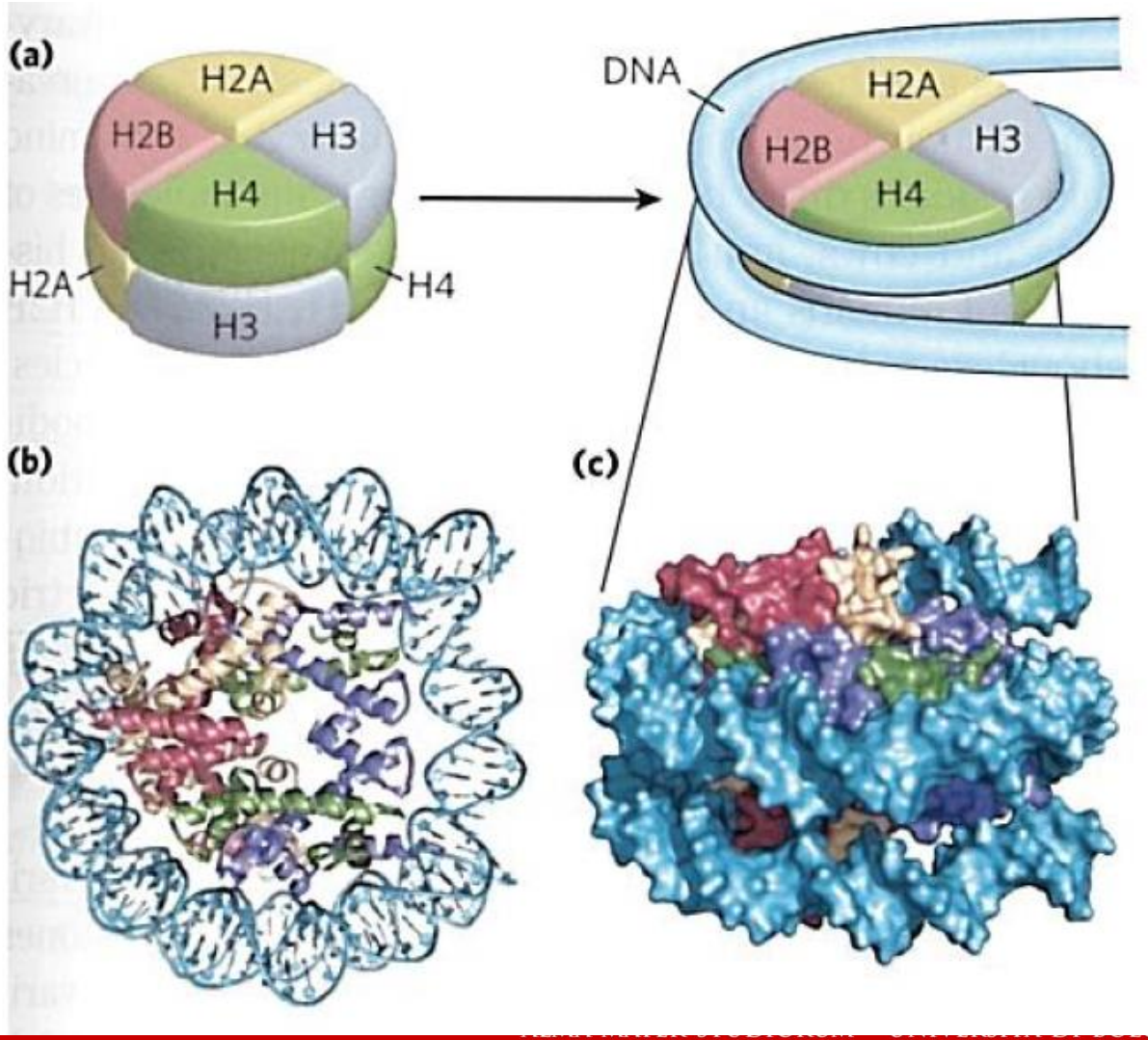
Figure 32.5 Higher-order chromatin structure. A proposed model for chromatin arranged in a helical array consisting of six nucleosomes per turn of helix. The DNA double helix (shown in red) is wound around each histone octamer (shown in blue). [After J. T. Finch and A. Klug. *Proc. Natl. Acad. Sci. U. S. A.* 73:1897–1901, 1976.]

Chromatin DNA interacts very closely with proteins called **HISTONES**, which condense and sort the DNA into structural units called **NUCLEOSOMES**

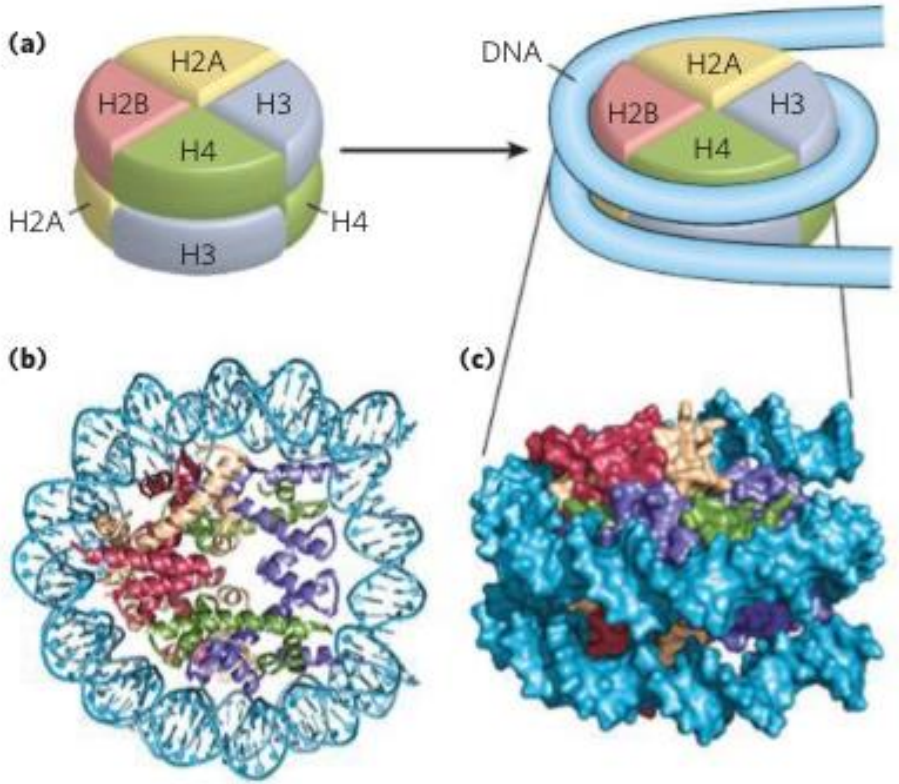
Image of a part of a partially disassembled chromosome



DNA is wrapped around an octamer Histone Core



HISTONES



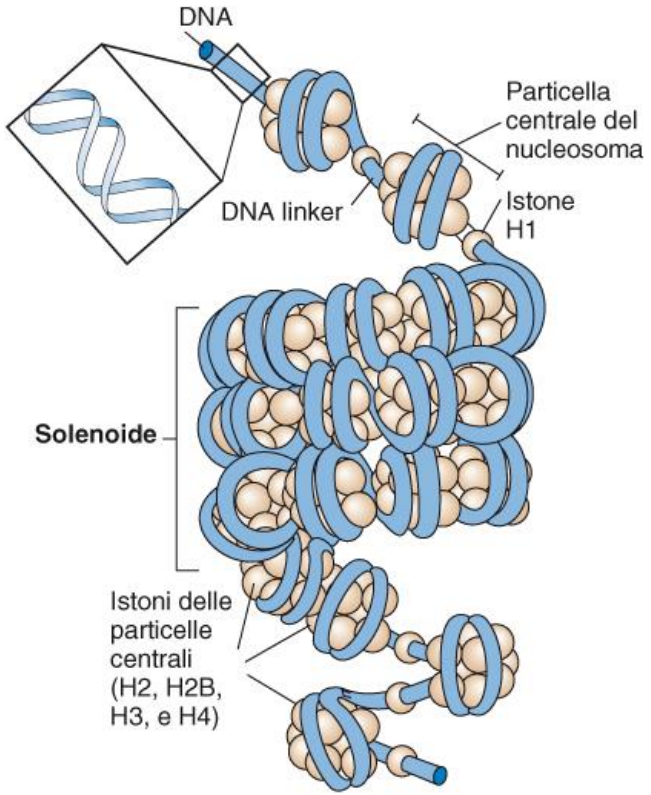
Histones are small proteins rich in the **BASIC AMINOACIDS** arginine and lysine
Five main classes of histones: **H3, H4, H2A, H2B, H1**

- Each nucleosome contains **eight histone molecules**: two copies each of histones H2A, H2B, H3 and H4.
- **The nucleosomes** constitute a repeating unit consisting of approximately 200 bp, of which 146 are tightly bound to the central histone nucleus, known as the core, and the remainder allow the connection with DNA between the nucleosomes. Histone H1 binds to the connecting DNA.

TABLE 24-5 Types and Properties of the Common Histones

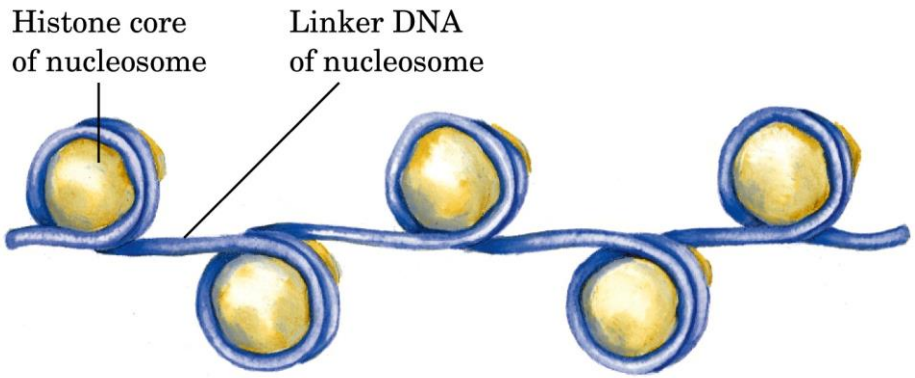
Histones	Molecular weight	Number of amino acid residues	Content of basic amino acids (% of total)	
			Lys	Arg
H1[a]	21,130	223	29.5	11.3
H2A[a]	13,960	129	10.9	19.3
H2B[a]	13,774	125	16.0	16.4
H3	15,273	135	19.6	13.3
H4	11,236	102	10.8	13.7

[a]The sizes of these histones vary somewhat from species to species. The numbers given here are for bovine histones.

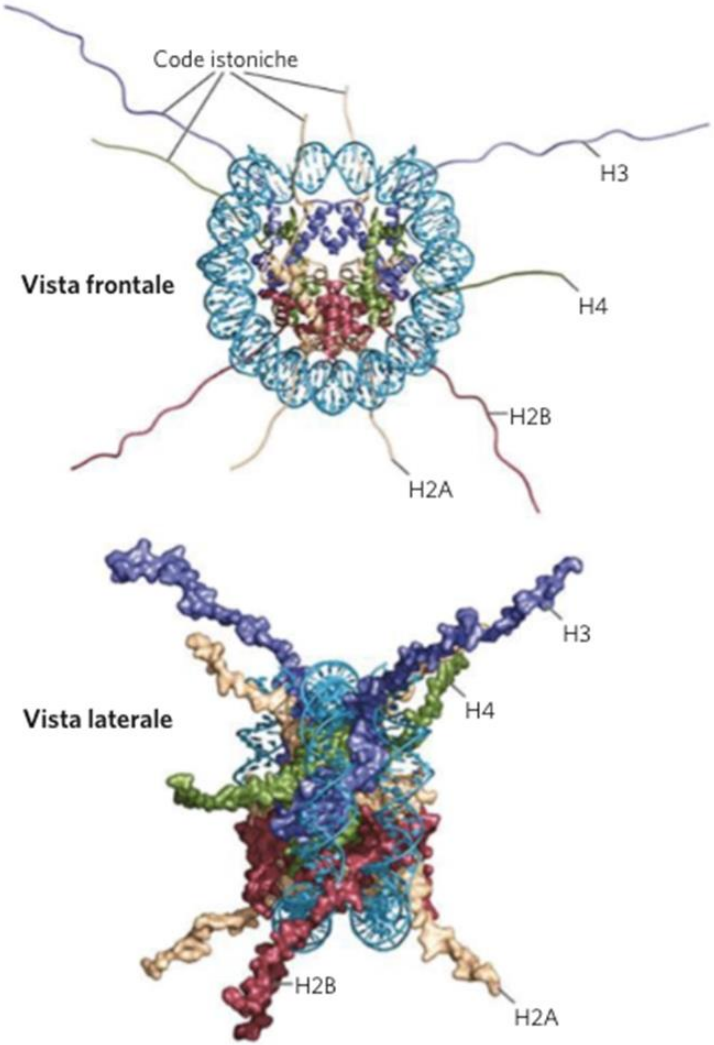


M. Lieberman **Marks Biochimica medica** Copyright 2010 C.E.A. Casa Editrice Ambrosiana

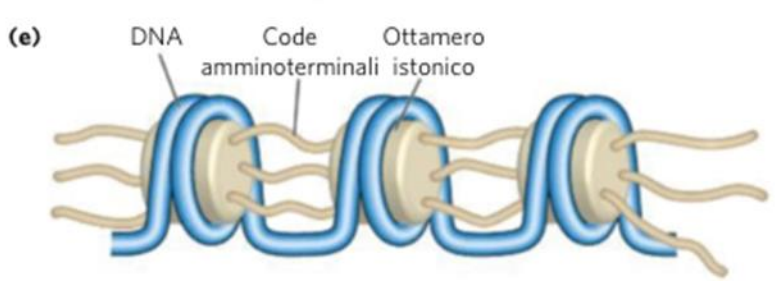
200 base-pairs: 146 wrapped around the **octamer** Histone Core + 54 as linker DNA

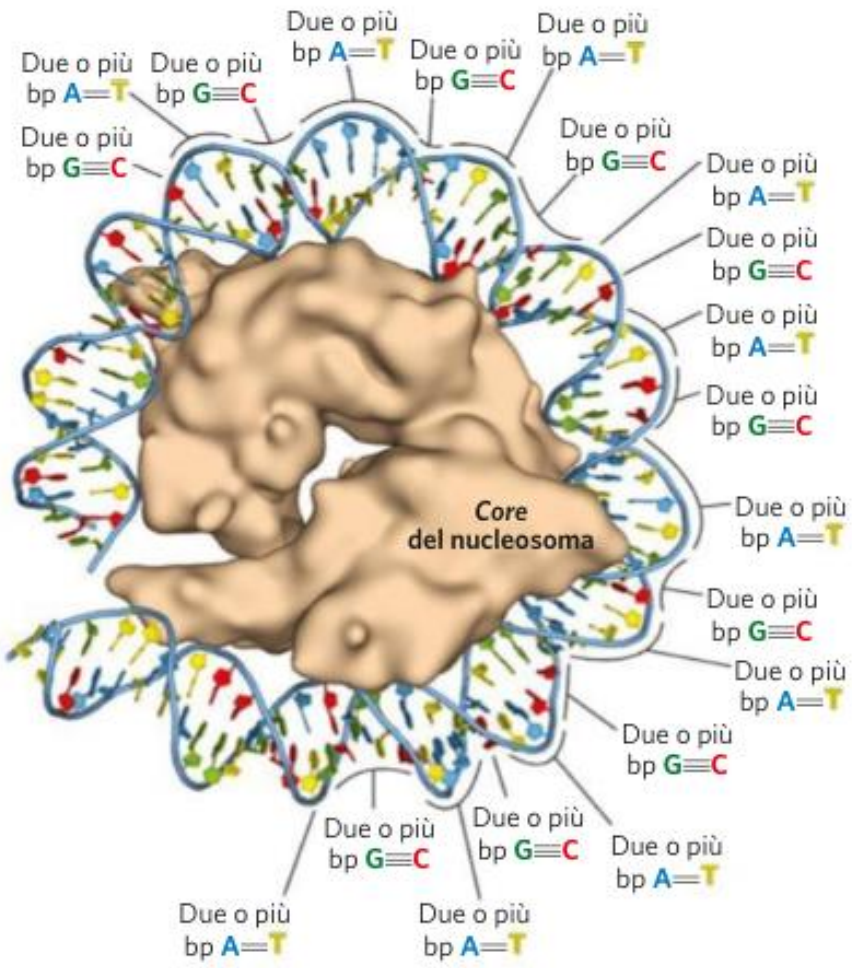


Proteic core of the Nucleosome (Histones have tails composed of aa that can be reversibly modified by methylation, acetylation, phosphorylation, etc…)



- These modifications change the net electrical charge, shape and other properties of the histone molecules and consequently also the structure and functional properties of chromatin;
- play an important role in the regulation of transcription and chromatin structure at different stages of the cell cycle





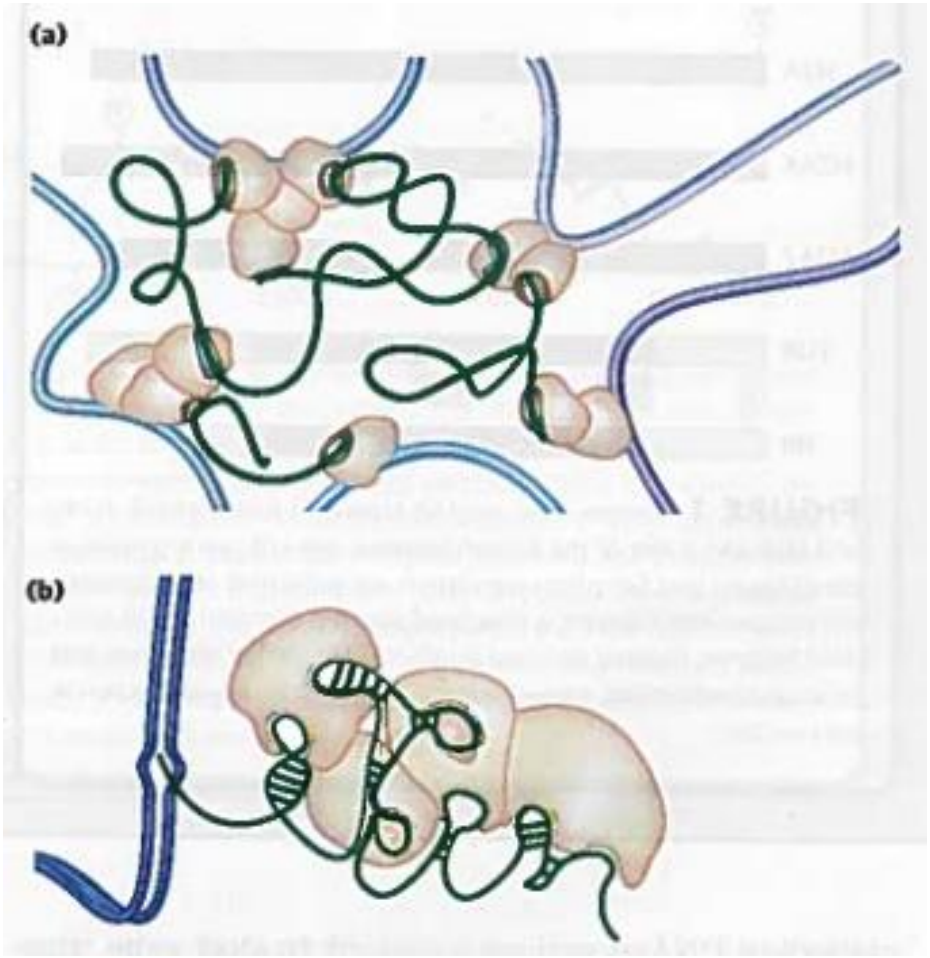
Another important factor controlling the **DNA binding** to the histone core of nucleosomes is the sequence of the bound DNA itself. The histone core does not bind to random positions on the DNA molecule.

DNA sites containing two or more bp A-T favour the curvature of the DNA molecule, while zones containing two or more bp G-C have the opposite effect.

Consecutive A-T bp, at intervals of about 10 bp, help the DNA molecule to fold in a circle.

The **consecutive G-C bp sequences, at 10 bp intervals and offset by 5 bp from the A-T bp sequences,** facilitated DNA binding to the nucleosome.

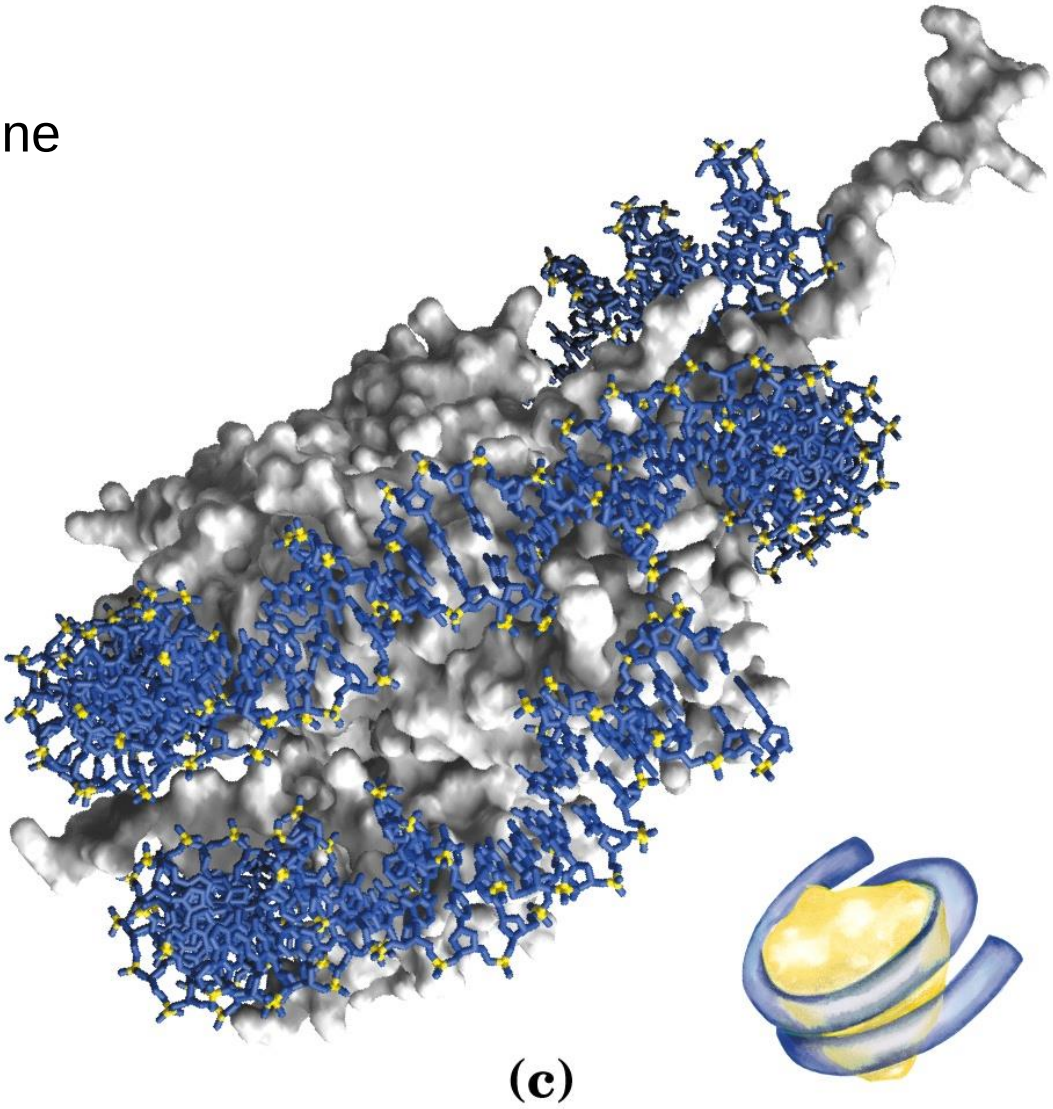
Another important component that defines the structure of chromosomes is a particular class of RNA molecules **called lncRNA (long non-coding RNA).**



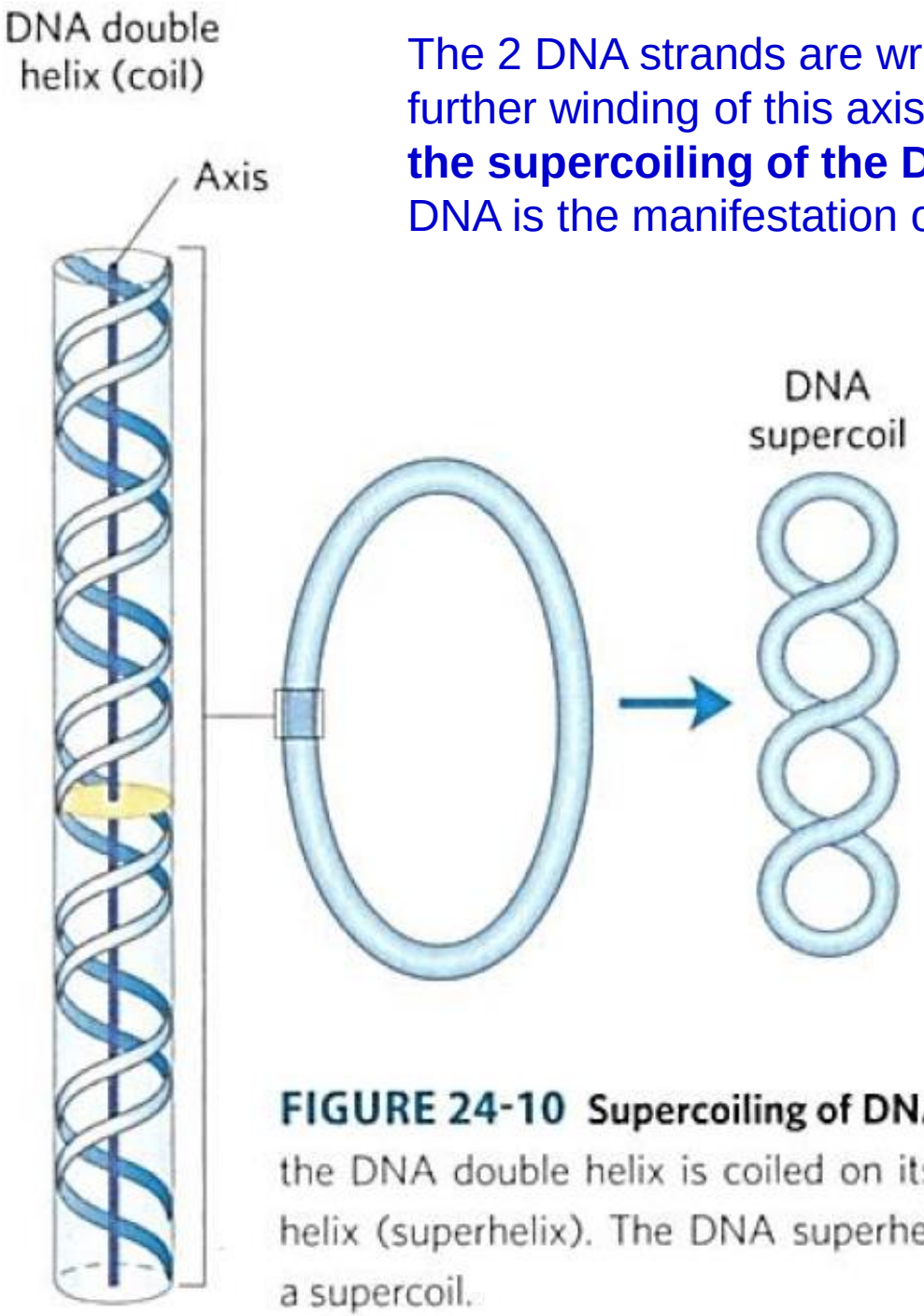
a) LncRNA (green) interacts with DNA-binding proteins to tether distant DNA segments;
b) LncRNA (green) interacts with DNA sequence and bring gene-regulatory proteins to the region, suppressing/activating transcription nearby.

Nucleosome in which the “van der Waals surface” (gray) represents Histones

Assembly of the DNA-Histone complex is promoted by specific proteins that are activated during DNA replication: TOPOISOMERASES



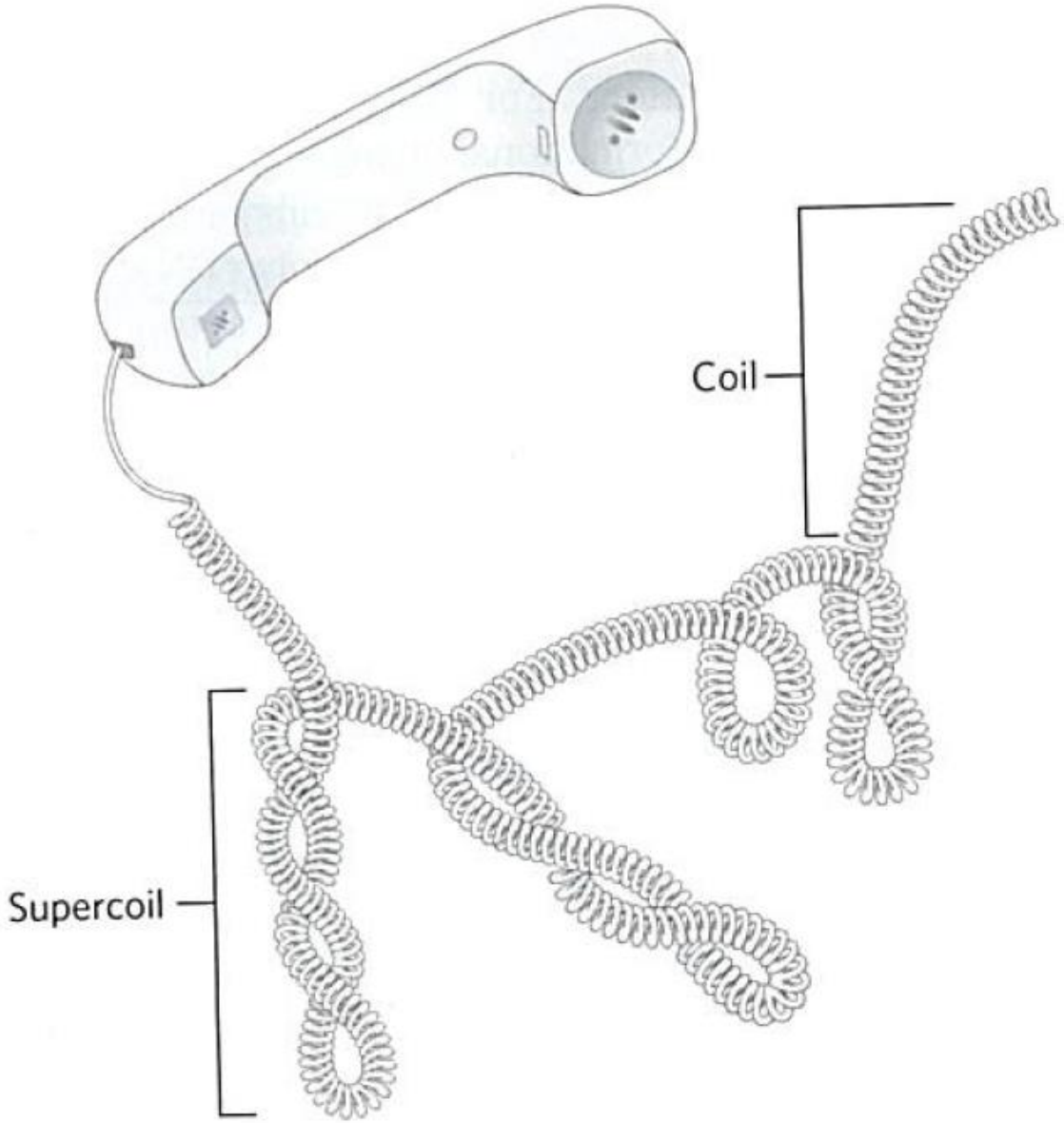
The 2 DNA strands are wrapped around an axis. A further winding of this axis around itself results **in the supercoiling of the DNA**. Supercoiling of the DNA is the manifestation of structural tension.



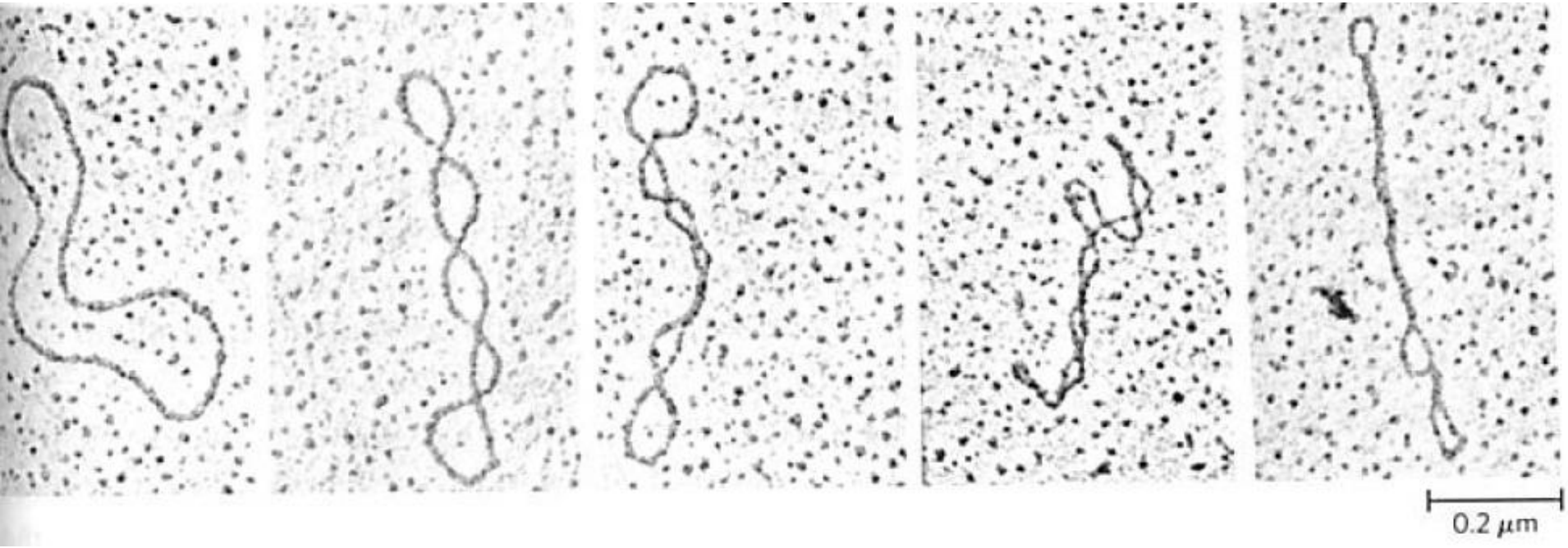
Supercoiling compacts the DNA and, because supercoiled
DNA is partly unwound, it is more accessible for interactions with other biomolecules.

FIGURE 24-10 Supercoiling of DNA. When the axis of the DNA double helix is coiled on itself, it forms a new helix (superhelix). The DNA superhelix is usually called a supercoil.

Coil and Supercoil



Relaxed and Supercoiled circular DNA



Coiling

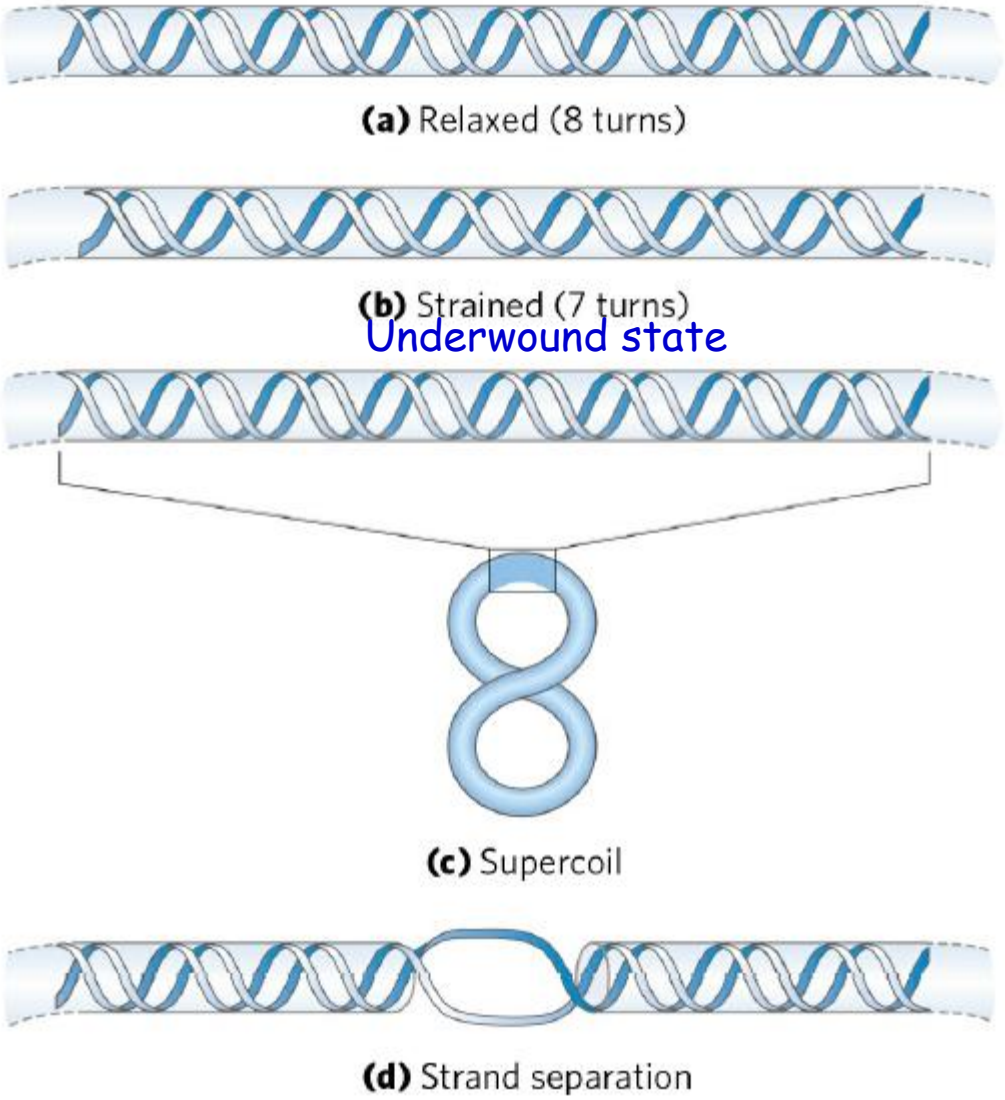
Narration Play

If we turn one end of the segment to the left, in the opposite direction from the twists in the DNA helix, the DNA strands unwind slightly.

The force of the turn causes the DNA to twist around itself, producing a negative supercoil.

Replay

Pause between scenes



In almost every instance, the strain is a result of the **underwinding** of the DNA double helix in the closed circle (strained state). In strained state, the DNA has *fewer* helical turns than would be expected for the more stable B-form structure.

The strain is generally accommodated by the formation of a supercoil.

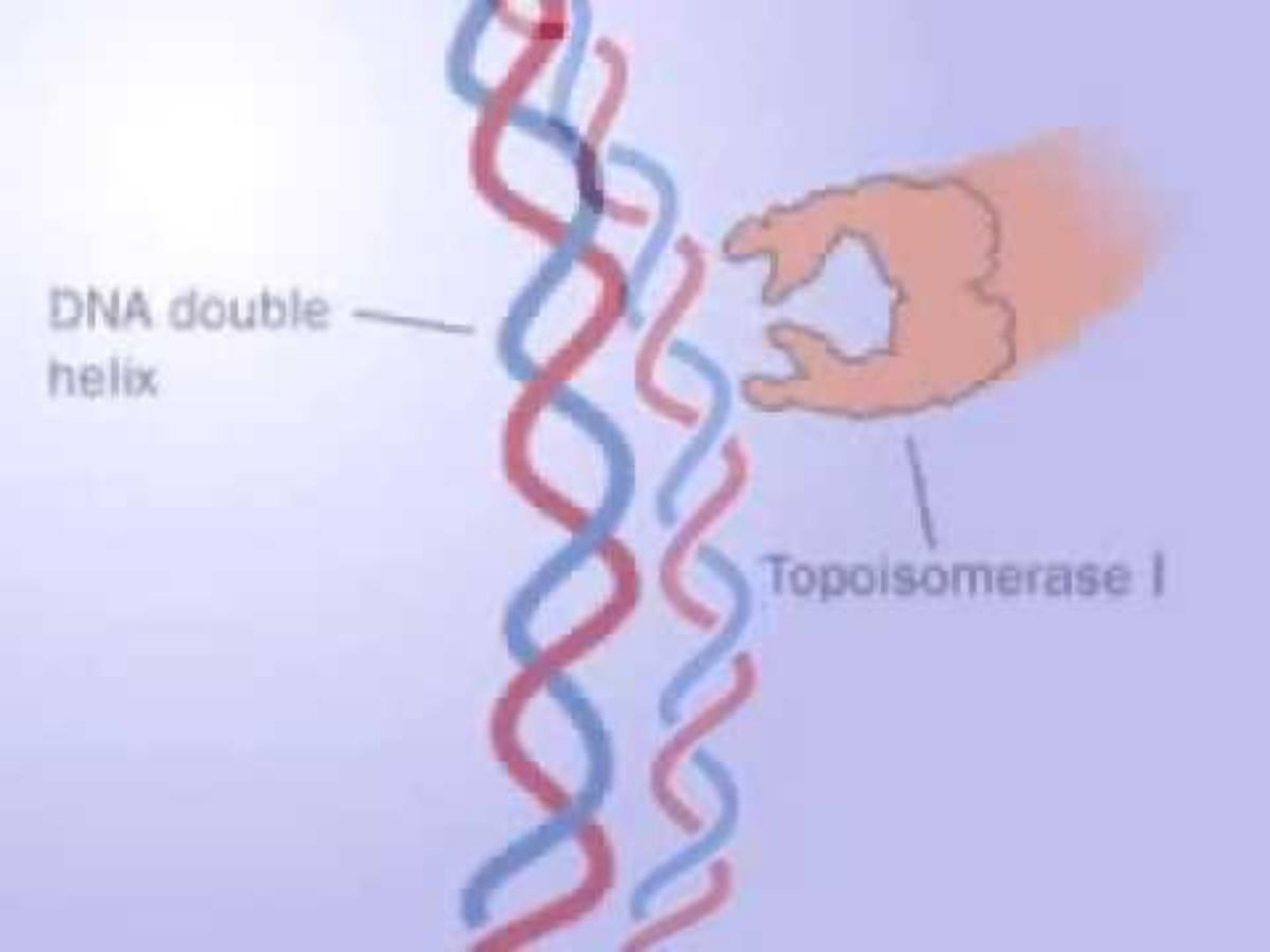
DNA underwinding makes the separation of strands somewhat easier. In principle, each turn of underwinding should facilitate strand separation over about 10 bp; however, the hydrogen-bonded base pairs would generally preclude strand separation over such a short distance, and the effect becomes important only for longer DNAs and higher levels of DNA underwinding.

Every cell actively underwinds its DNA with the aid of enzymatic processes, through Topoisimerases

Every cell has enzymes with the sole function of underwinding and/or relaxing DNA. These enzymes that increase or decrease the extent of DNA underwinding are topoisomerases, playing an important role in processes such as replication and DNA packaging.

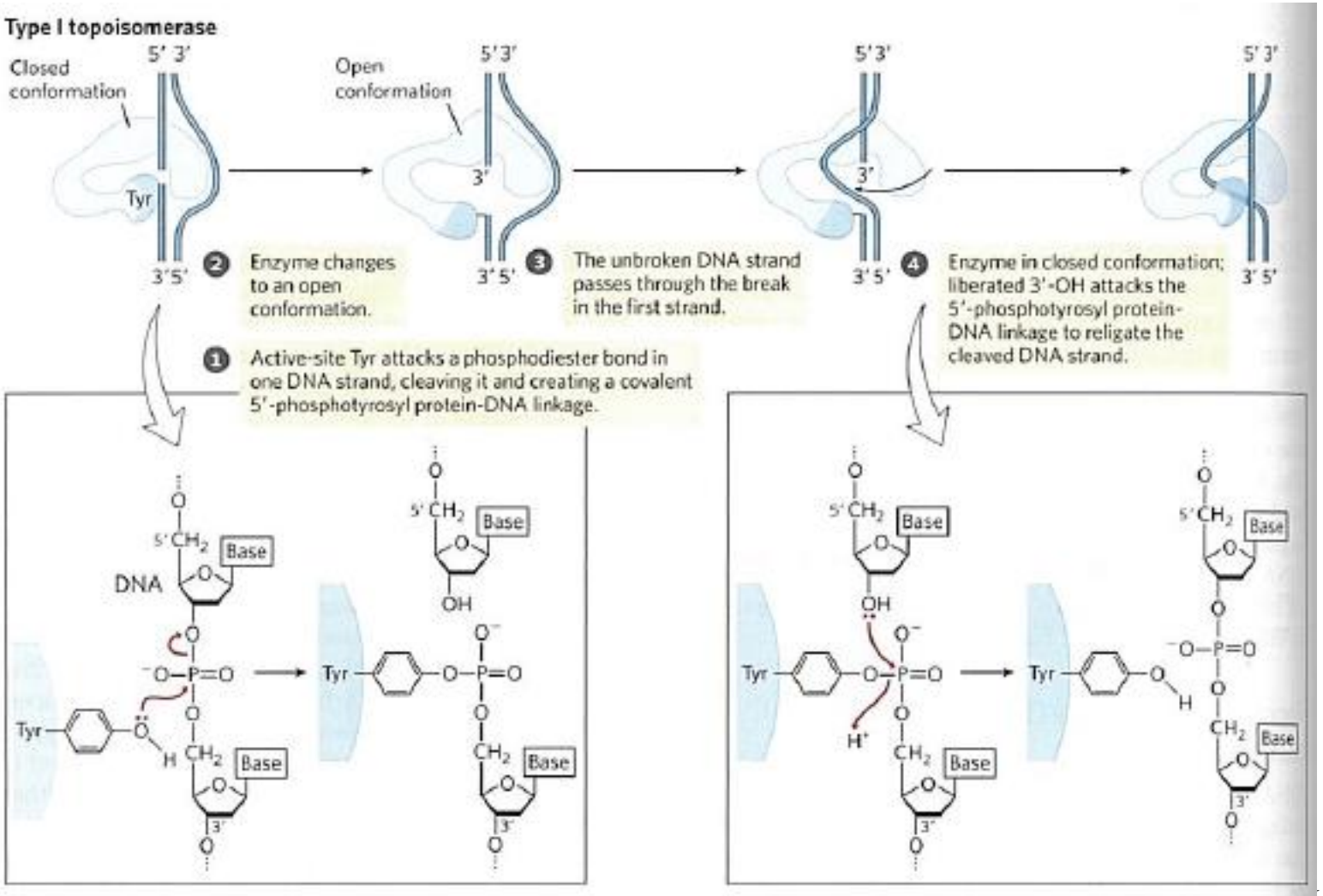
There are two classes of topoisomerases.

- ✓ **Type I topoisomerases:** act by transiently breaking one of the two DNA strands, passing the unbroken strand through the break and rejoining the broken ends;
- ✓ **Type II topoisomerases:** break both DNA strands.


DNA double helix
Topoisomerase I

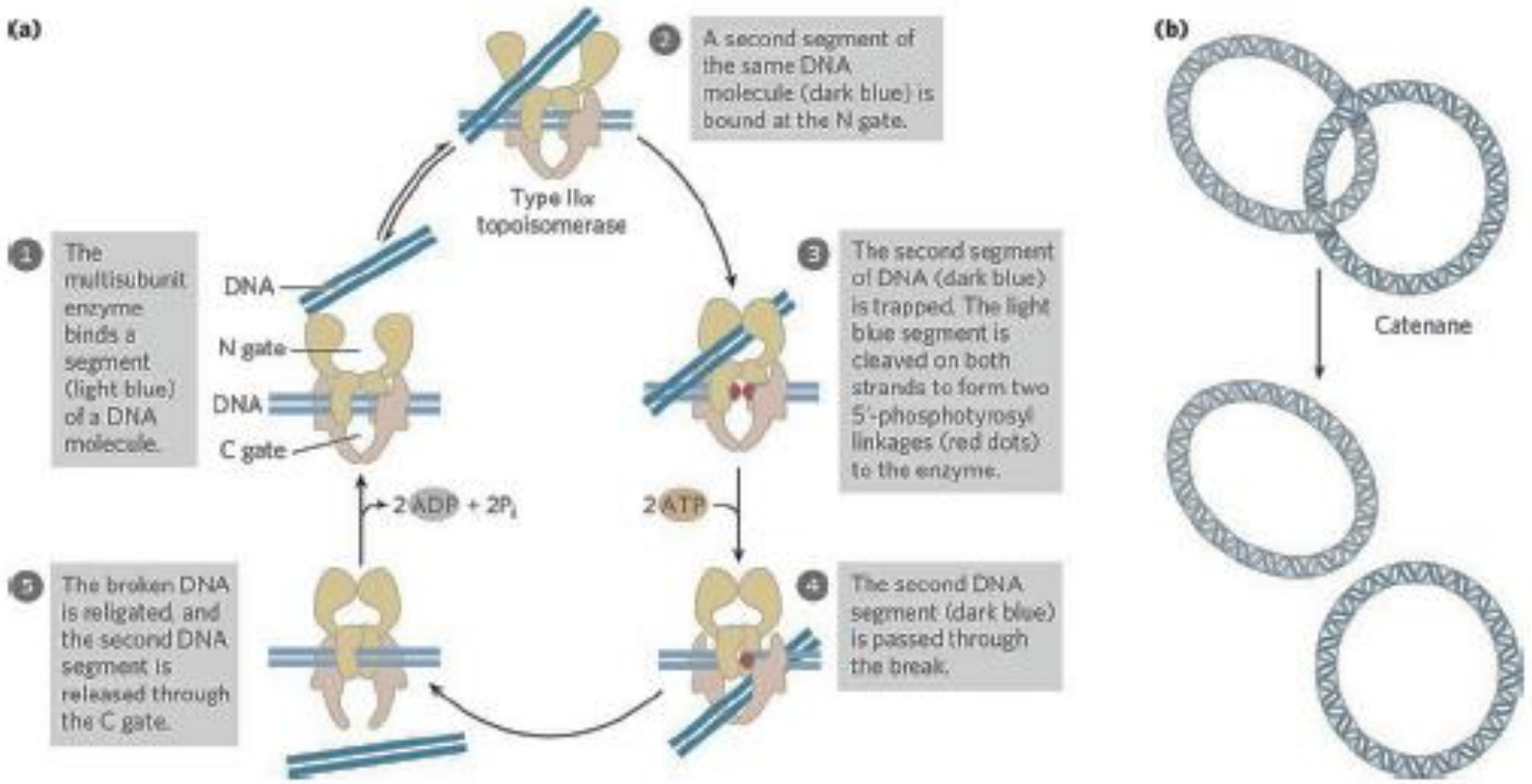
Type I Topoisomerases

Transiently break ONE of the two DNA strands, passing the unbroken strand through the break and rejoining the broken ends



Type II Topoisomerases

Transiently break BOTH DNA strands, allowing the passage of an intact duplex DNA segment through the break.



Different types of Topoisomerases

TABLE 24-4 Diversity in DNA Topoisomerases

Type	Mechanism	Family (defined by structural class)	Domain(s)	Notes
IA	Strand passage[a]	Topoisomerase I	Bacteria, eukaryotes	Relaxes (–)
		Topoisomerase III	Bacteria, eukaryotes	Relaxes (–)
		Reverse gyrase	Archaea, bacteria	Uses ATP to introduce positive supercoils; thermophilic bacteria and archaea only
IB	Swivelase[b]	Topoisomerase IB	Bacteria, eukaryotes	A few bacteria; all eukaryotes
IC	Swivelase	Topoisomerase V	Archaea	*Methanopyrus* only
IIA	Strand passage[c]	Topoisomerase II (DNA gyrase)	Archaea, bacteria	Introduces negative supercoils (ATPase)
		Topoisomerase IIα	Eukaryotes	Relaxes (+ or –)
		Topoisomerase IIβ	Eukaryotes	Relaxes (+ or –)
		Topoisomerase IV	Bacteria	Decatenase[d]
IIB	Strand passage	Topoisomerase VI	Archaea, bacteria, eukaryotes	Among eukaryotes, plants, algae, and protists only

Topoisomerases are excellent therapeutic targets of antibiotics and chemotherapic agents

Inhibitors of Topoisomerases

TOPO I Inhibitors:

CAMPTOTHECINS:

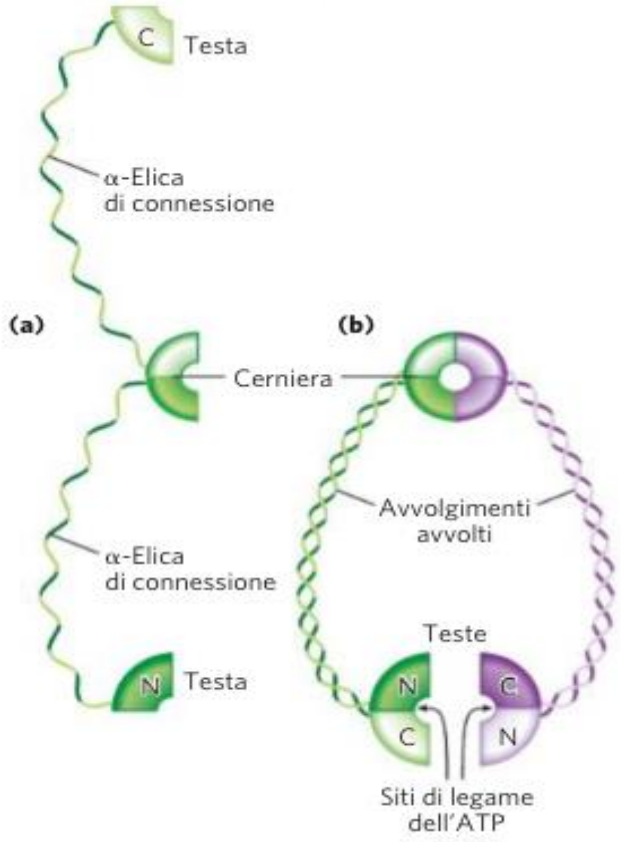
- Topotecan *(Ovarian, lung cancer)*
- Irinotecan *(Colorectal cancer)*

TOPO II Inhibitors:

- Etoposide (VP16) *(Lung cancer)*
- Doxorubicin *(Breast cancer)*

SMC proteins (Structural Maintenance of Chromosomes)

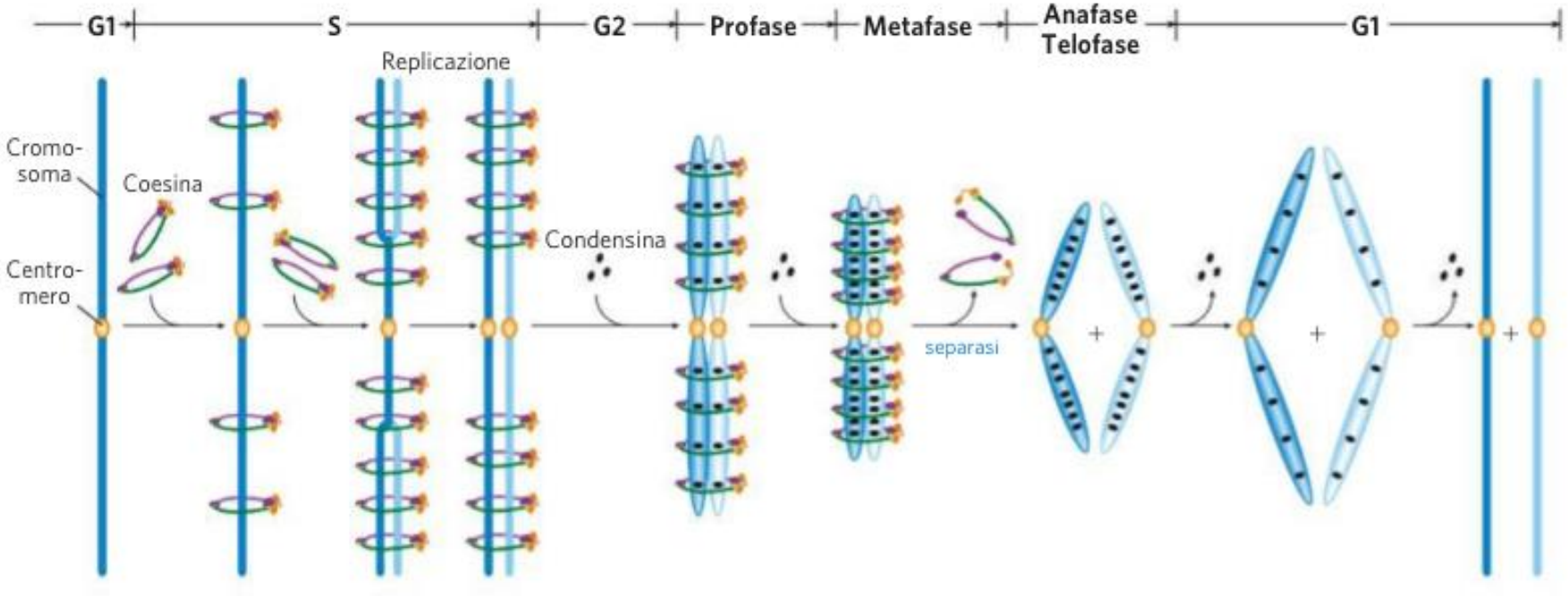
- Responsible for maintaining the structure and integrity of chromosomes following their replication.
- The primary structure of SMC proteins consists of five distinct domains. The globular amino- and carboxy-terminal domains, N and C, each of which possesses part of a hydrolytic site for ATP, are joined by two α-helical coiled-coil regions and connected by a hinge domain.
- The proteins are normally dimeric.



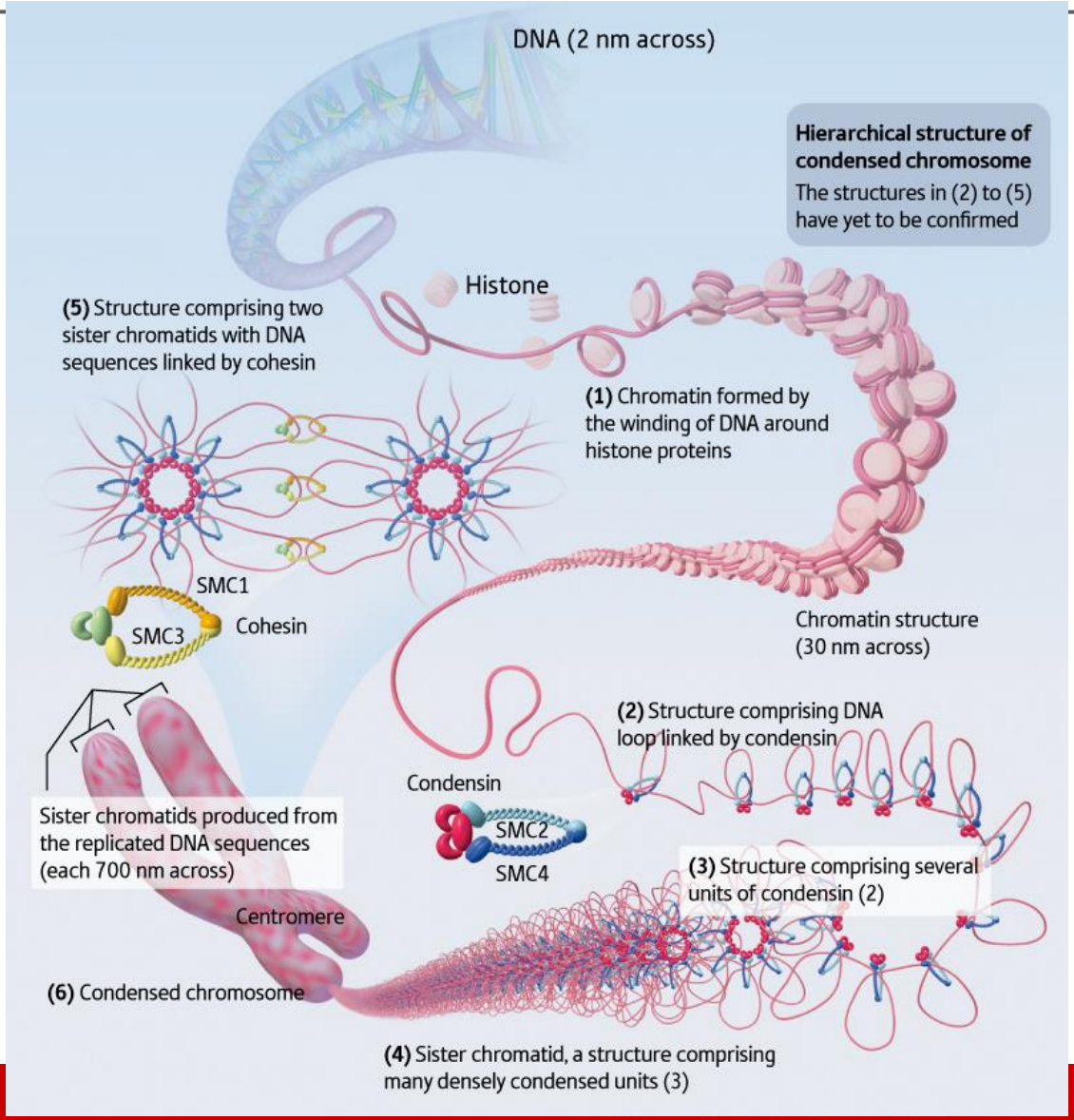
SMC proteins (Structural Maintenance of Chromosomes)

COHESINS: play an essential role in binding sister chromatids immediately after replication and holding them together when they condense in metaphase.

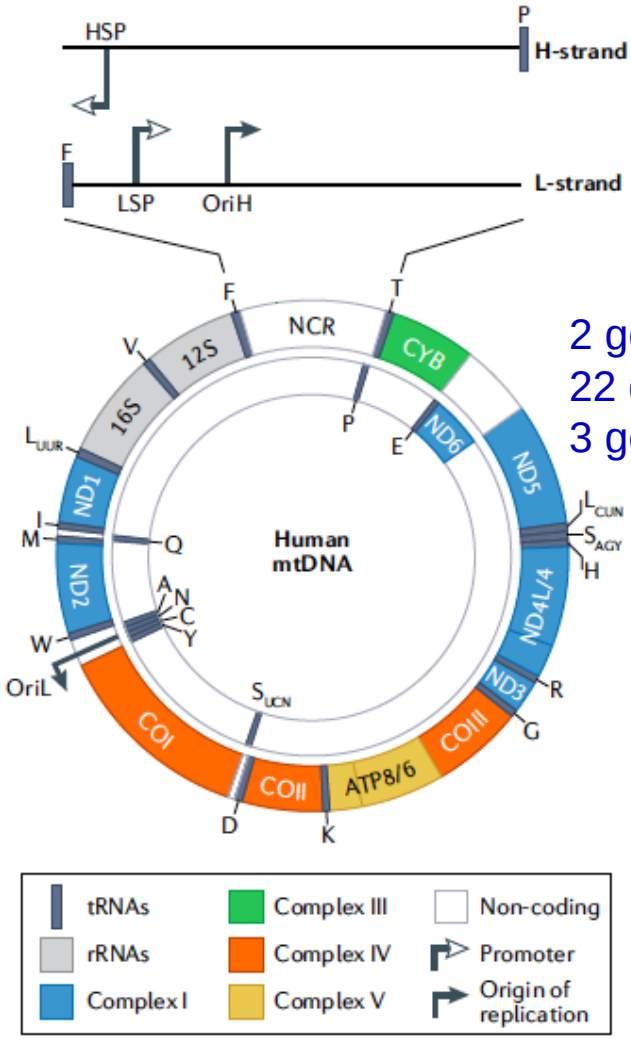
CONDENSINS: these are essential for the condensation of chromosomes when cells enter mitosis.



Chromosome condensation



MITOCHONDRIAL DNA



Circular double-stranded DNA.
Multiple copies (100 in leukocytes - 10000 in neurons).
Replication is independent of the cell cycle
Mother inheritance

2 genes coding for rRNA
22 genes coding for tRNA
3 genes coding for OXPHOS proteins

mtDNA repair mechanisms are present but less efficient than nuclear ones and, given the very high number of replications, the accumulation of variants is widespread.

Homoplasmy: mtDNA molecules with the same nucleotide sequence are in the same cell.
Heteroplasmy: co-presence in the same cell of mtDNA molecules with different sequence (mutation or polymorphism)

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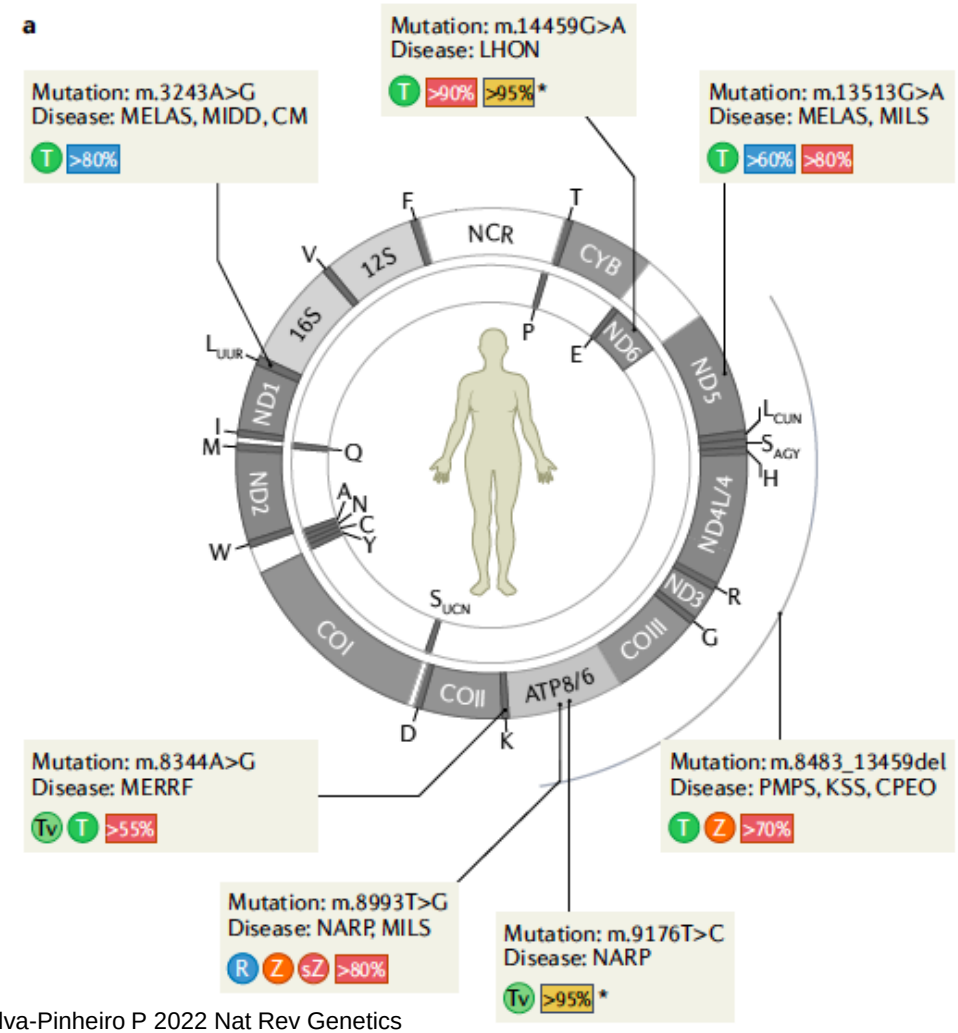
Clinical manifestation often requires heteroplasmic mutant mtDNA to be present above a certain pathogenicity threshold (typically ~60-90%).



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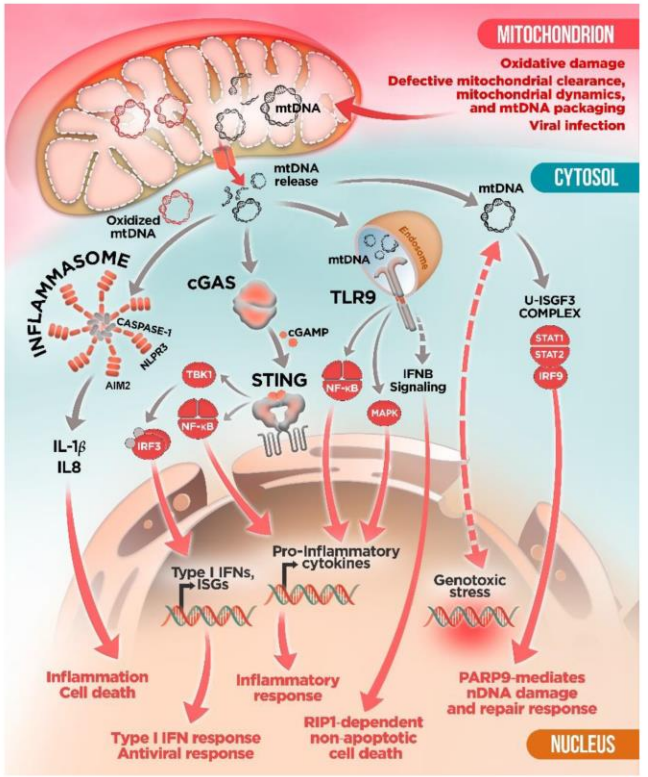
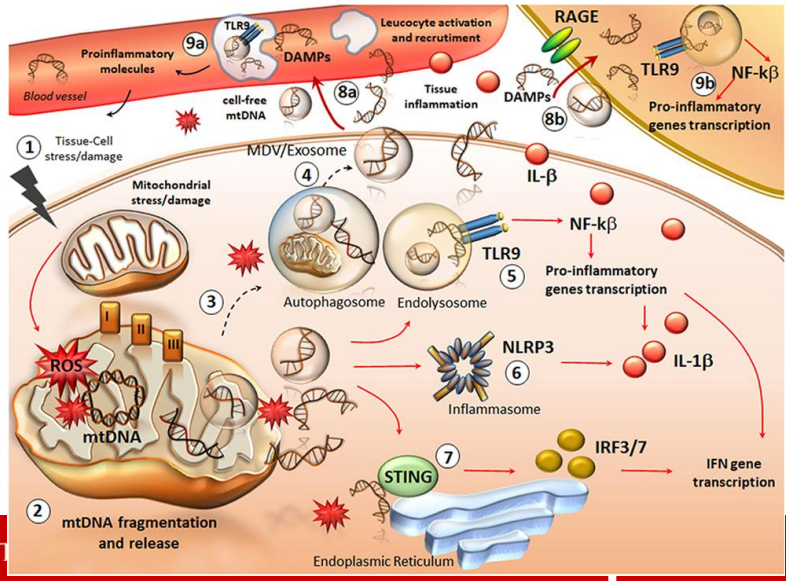
CM, cardiomyopathy;
CPEO, chronic progressive external ophthalmoplegia;
KSS, Kearns–Sayre syndrome;
LHON, Leber hereditary optic neuropathy;
MELAS, mitochondrial encephalomyopathy, lactic acidosis
and stroke-like episodes;
MERRF, myoclonic epilepsy with ragged red fibres;
MIDD, maternally inherited diabetes and deafness;
MILS, maternally inherited Leigh syndrome;
NARP, neurogenic muscle weakness, ataxia and retinitis pigmentosa;
PMPS, Pearson marrow and pancreas syndrome;

Where mitochondrial DNA is located inside cells?

- **Mitochondria**
- **Nuclear genome**
- **Cytoplasmic mtDNA:** mitochondrial insults (oxidative stress, infections, etc.) or impairment of mitochondrial quality control cause mtDNA release into the cytosol, activating the signalling pathways of inflammation and innate immunity.
- **circulating cell-free mtDNA (CCF-mtDNA):** mtDNA can be released from cells passively, through damage or physical injury to cells or actively, via extracellular vesicles (EVs). In addition, mtDNA can enter the circulatory system and be detected as so-called free circulating mtDNA (CCF-mtDNA)






Kahoot!