BME 2901-BIOCHEMISTRY

Nucleic Acids - I

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Nucleotides

- Nucleotides have a variety of roles in cellular metabolism. They are the energy currency in metabolic transactions, the essential chemical links in the response of cells to hormones and other extracellular stimuli, and the structural components of an array of enzyme cofactors and metabolic intermediates.
- But most importantly, they are the constituents of nucleic acids: deoxyribonucleic acid (DNA) and ribonucleic acid (RNA), the molecular repositories of genetic information.
- The structure of every protein, and ultimately of every biomolecule and cellular component, is a product of information programmed into the nucleotide sequence of a cell's nucleic acids.
- The ability to store and transmit genetic information from one generation to the next is a fundamental condition for life.

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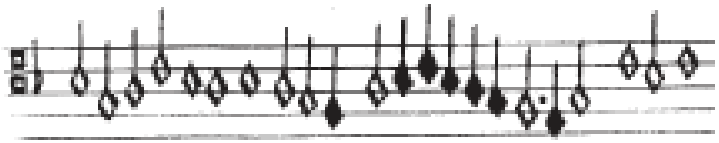
(B) 

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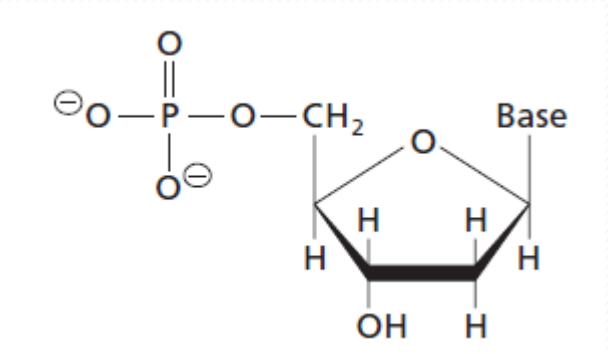
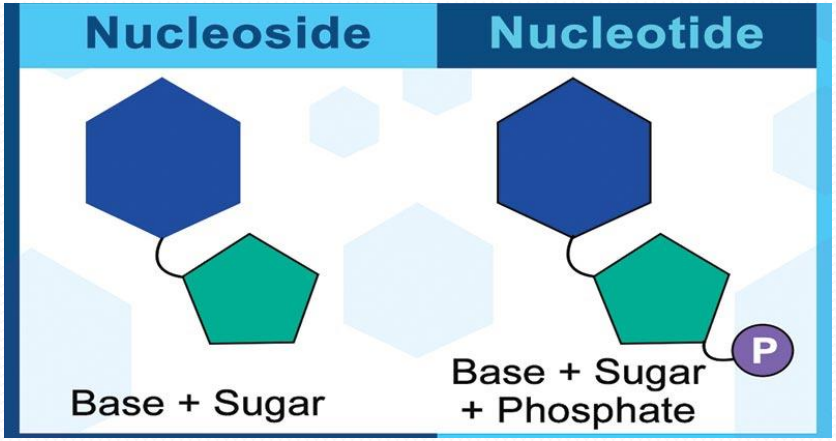
- The amino acid sequence of every protein in a cell, and the nucleotide sequence of every RNA, is specified by a nucleotide sequence in the cell's DNA.
- A segment of a DNA molecule that contains the information required for the synthesis of a functional biological product, whether protein or RNA, is referred to as a **gene.**
- A cell typically has many thousands of genes, and DNA molecules, not surprisingly, tend to be very large. The storage and transmission of biological information are the only known functions of DNA.
- RNAs have a broader range of functions, and several classes are found in cells:
  - **Ribosomal RNAs (rRNAs)** are components of ribosomes, the complexes that carry out the synthesis of proteins.
  - **Messenger RNAs (mRNAs)** are intermediaries, carrying genetic information from one or a few genes to a ribosome, where the corresponding proteins can be synthesized.
  - **Transfer RNAs (tRNAs)** are adapter molecules that faithfully translate the information in mRNA into a specific sequence of amino acids.
  - In addition to these major classes there is a wide variety of RNAs with special functions.

Nucleotide Structure

- Nucleic acids are polymers of nucleotides.
- Nucleotides have three components:
  - a five-carbon sugar (pentose),
  - one or more phosphate groups,
  - and a weakly basic nitrogenous compound called a base.

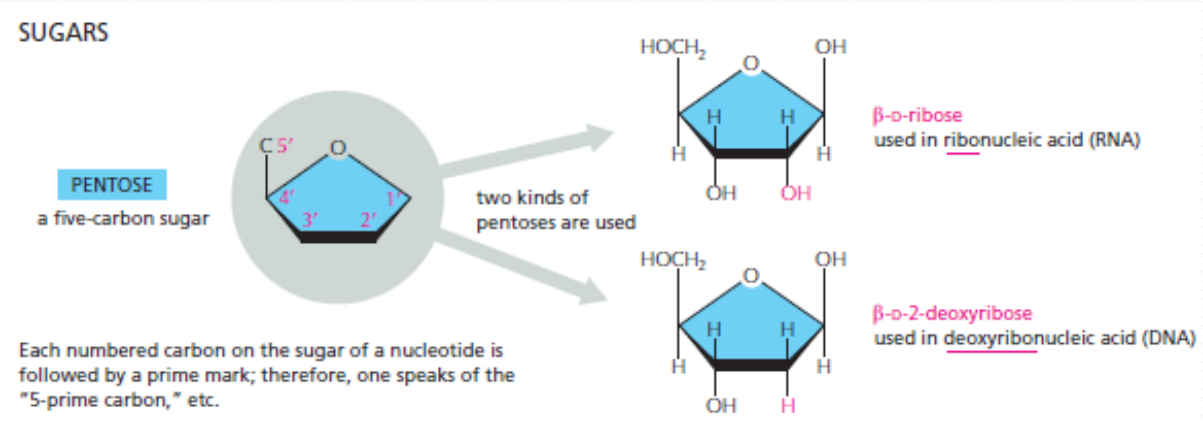


- The bases found in nucleotides are substituted pyrimidines and purines.
- The pentose is either ribose or 2-deoxyribose.
- The pyrimidine or purine *N*-glycosides of these sugars are called **nucleosides** (Sugar + Base).
- Nucleotides are the phosphate esters of nucleosides—the common **nucleotides** contain from one to three phosphoryl groups (Phosphate + Sugar + Base).
- Nucleotides containing ribose are called **ribonucleotides** and nucleotides containing deoxyribose are called **deoxyribonucleotides.**



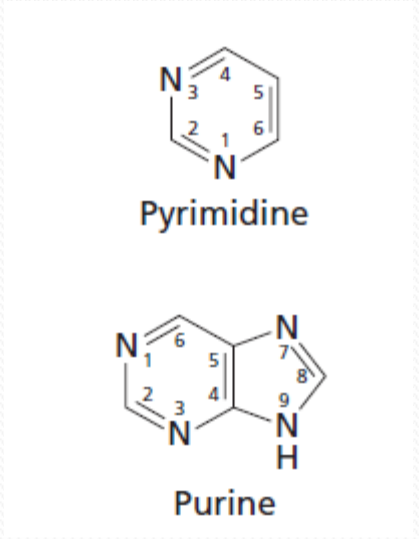
Pentose sugar

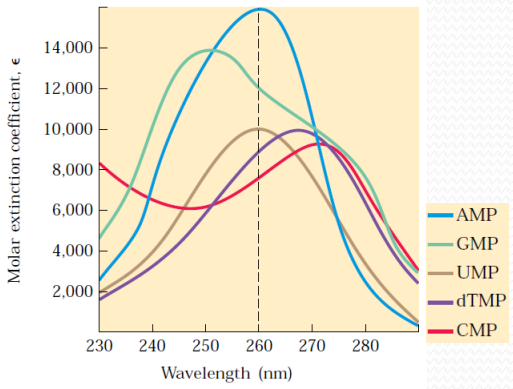
- Nucleic acids contain a 5 carbon sugar (pentose) in their structure.
- RNA contains ribose and DNA contains deoxyribose.
- Deoxyribose has a H atom at 2' instead OH group in ribose (lacks an O atom thus it is called deoxy-)



Purines and Pyrimidines

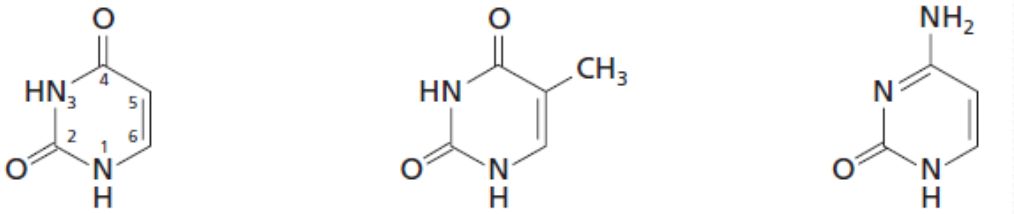
- The bases found in nucleotides are derivatives of either pyrimidine or purine.
- Pyrimidine has a single ring containing four carbon and two nitrogen atoms. Purine has a fused pyrimidine imidazole ring system.
- Both types of bases are unsaturated, with conjugated double bonds. This feature makes the rings planar and resonance among atoms in the ring gives most of the bonds partial double-bond character that accounts for their ability to absorb ultraviolet light.
- As a result of resonance, all nucleotide bases absorb UV light, and nucleic acids are characterized by a strong absorption at wavelengths near 260 nm.



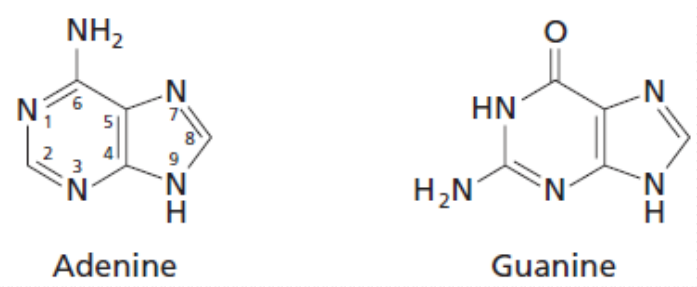


- The purine and pyrimidine bases are hydrophobic and relatively insoluble in water at the near-neutral pH of the cell. At acidic or alkaline pH the bases become charged and their solubility in water increases.
- Hydrophobic stacking interactions in which two or more bases are positioned with the planes of their rings parallel (like a stack of coins) are one of two important modes of interaction between bases in nucleic acids.
- The stacking also involves a combination of van der Waals and dipole-dipole interactions between the bases.
- Base stacking helps to minimize contact of the bases with water, and base-stacking interactions are very important in stabilizing the three-dimensional structure of nucleic acids.
- Within cells, however, most pyrimidine and purine bases occur as constituents of nucleotides and polynucleotides and these compounds are highly soluble.

- The major pyrimidines that occur in nucleotides are uracil (U), thymine (T), and cytosine (C).

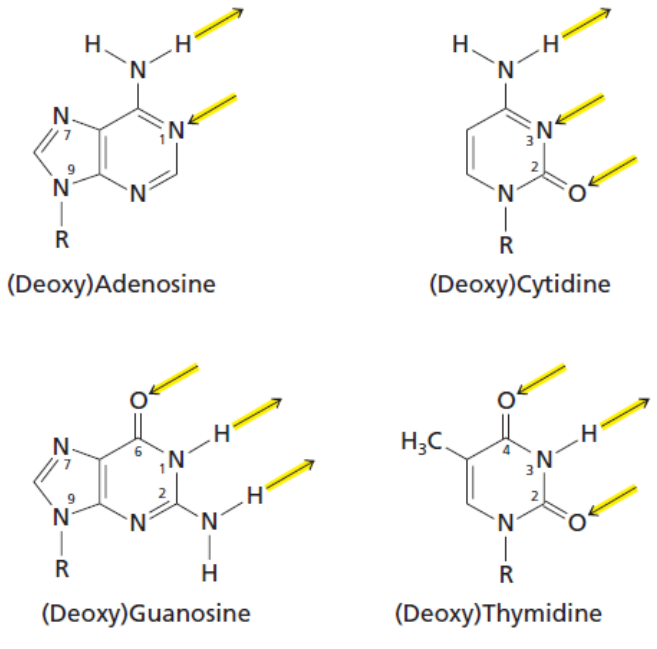


- The major purines are adenine (A) and guanine (G).

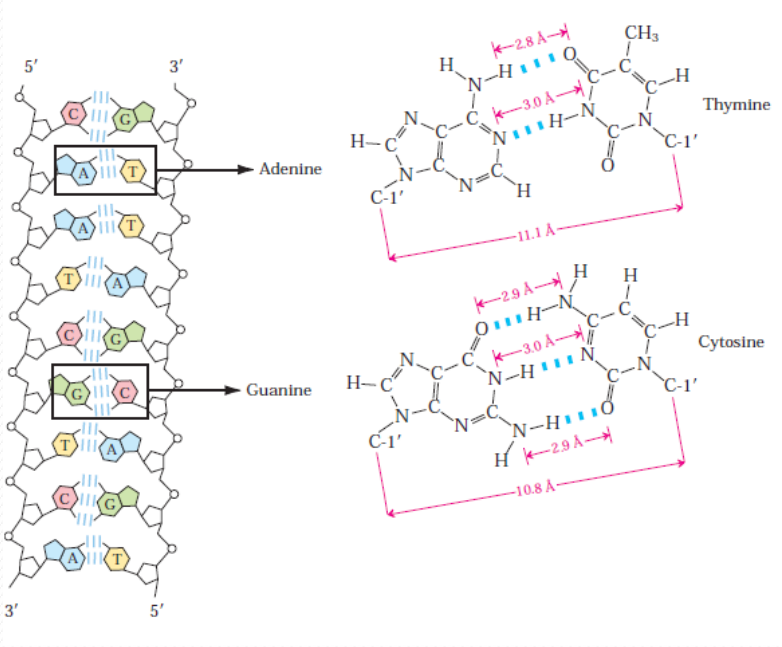


- Adenine, guanine, and cytosine are found in both ribonucleotides and deoxyribonucleotides.
- Uracil is found mainly in ribonucleotides and thymine is found mainly in deoxyribonucleotides.

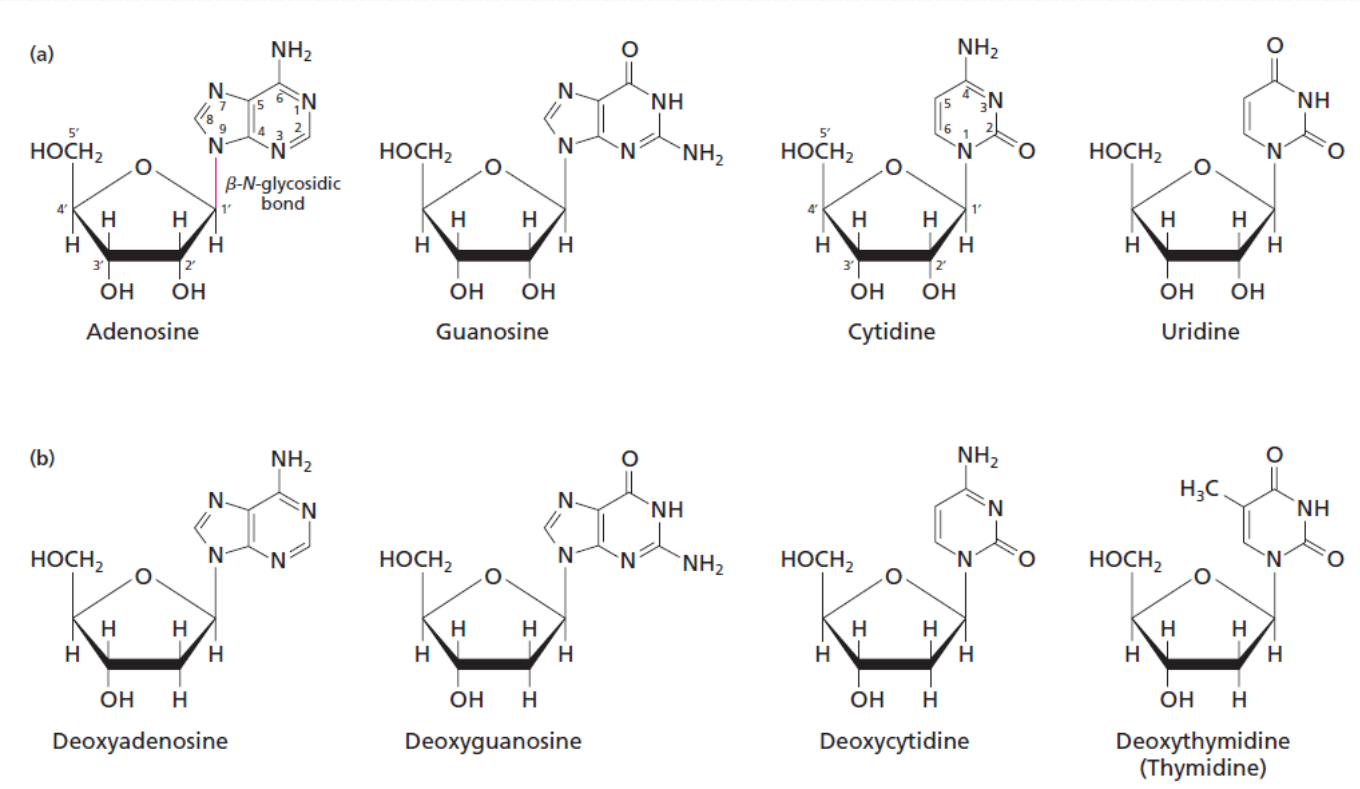
- All of the bases in the common nucleotides can participate in hydrogen bonding.
- The hydrogen-bonding patterns of bases have important consequences for the three-dimensional structure of nucleic acids.



- The most important functional groups of pyrimidines and purines are ring nitrogens, carbonyl groups, and exocyclic amino groups.
- Hydrogen bonds involve the amino and carbonyl groups and ring nitrogens in the bases of nucleic acid molecules.
- Hydrogen bonds between bases permit a complementary association of two strands of nucleic acid.
- Hydrogen-bonding patterns which predominate in DNA or RNA are defined by Watson and Crick in 1953, in which A bonds specifically to T (or U) and G bonds to C.



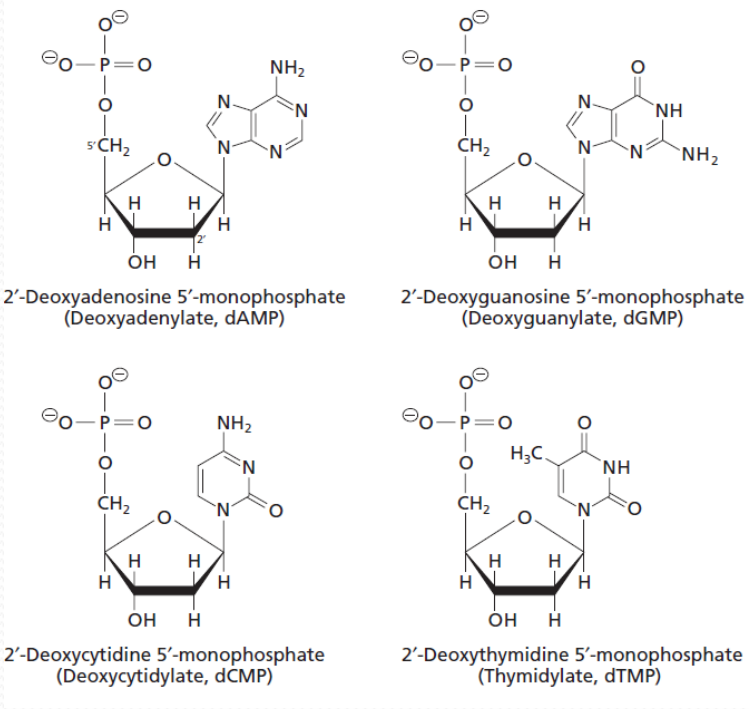
Nucleosides



- Consist of a pentose sugar and a base bonded by **glycosidic bond**.
- Ribonucleosides: A, G, C, and U
- Deoxyribonucleosides: dA, dG, dC, and dT (when it is necessary to distinguish them from ribonucleosides)

Nucleotides

- Nucleotides are phosphorylated derivatives of nucleosides.
- The phosphoryl groups in naturally occurring nucleotides are usually attached to the oxygen atom of the 5 –hydroxyl group by **ester bond**.
- Nucleoside monophosphates can be further phosphorylated to form nucleoside diphosphates (for example ADP) and nucleoside triphosphates (ATP).

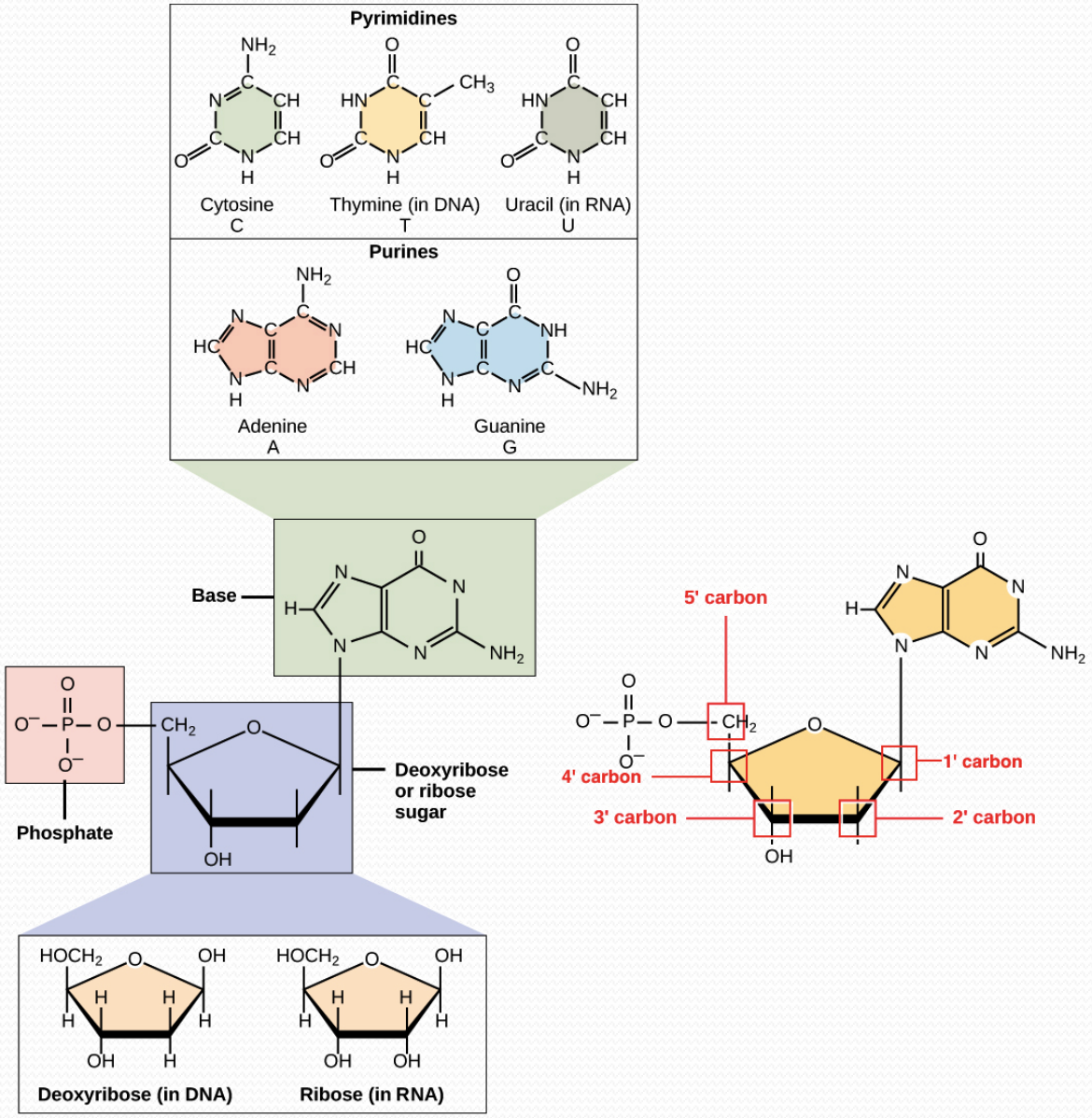


2′-Deoxyadenosine 5′-monophosphate (Deoxyadenylate, dAMP)

2′-Deoxyguanosine 5′-monophosphate (Deoxyguanylate, dGMP)

2′-Deoxycytidine 5′-monophosphate (Deoxycytidylate, dCMP)

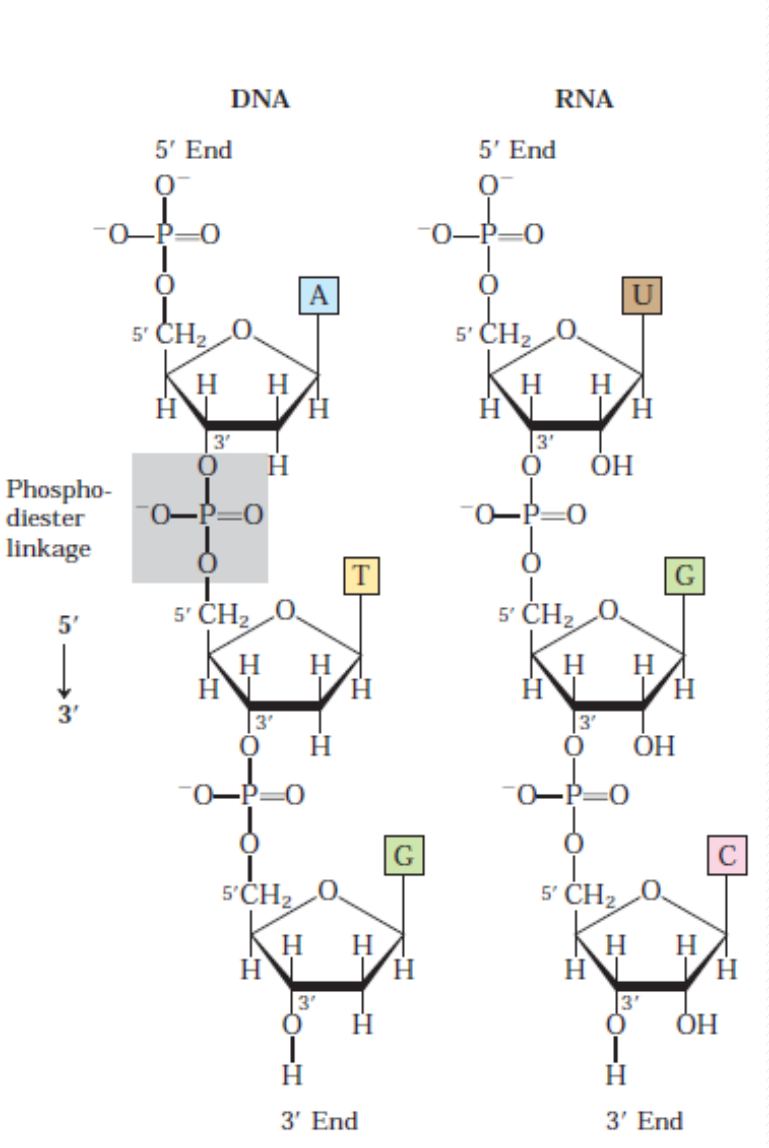
2′-Deoxythymidine 5′-monophosphate (Thymidylate, dTMP)


Pyrimidines
Cytosine
C
Thymine (in DNA)
T
Uracil (in RNA)
U
Purines
Adenine
A
Guanine
G
Base
Phosphate
Deoxyribose
or ribose
sugar
5' carbon
4' carbon
1' carbon
3' carbon
2' carbon
Deoxyribose (in DNA)
Ribose (in RNA)

Nucleic Acid Polymerization

- The successive nucleotides of both DNA and RNA are covalently linked through phosphate-group "bridges," in which the 5'-phosphate group of one nucleotide unit is joined to the 3'-hydroxyl group of the next nucleotide, creating a sugar phosphate backbone with a **phosphodiester linkage.**
- Thus the covalent backbones of nucleic acids consist of alternating phosphate and pentose residues, and the nitrogenous bases may be regarded as side groups joined to the backbone at regular intervals.

- All the phosphodiester linkages have the same orientation along the chain, giving each linear nucleic acid strand a specific **polarity** and distinct 5’ and 3’ ends.
- The nucleotide with a free 5’-phosphoryl group is called the 5’ end, and the nucleotide with a free 3’ -hydroxyl group is called the 3’ end.
- By convention, the structure of a single strand of nucleic acid is always written with the 5’ end at the left and the 3’ end at the right—that is, in the 5’→3’ direction.



- A short nucleic acid is referred to as an **oligonucleotide** and a nucleic acid with more than 50 nucleotides is referred to as a **polynuclotide.**
- The covalent backbone of DNA and RNA is subject to slow, nonenzymatic hydrolysis of the phosphodiester bonds.
- In the test tube, RNA is hydrolyzed rapidly under alkaline conditions, but DNA is not; the 2-hydroxyl groups in RNA (absent in DNA) are directly involved in the process.

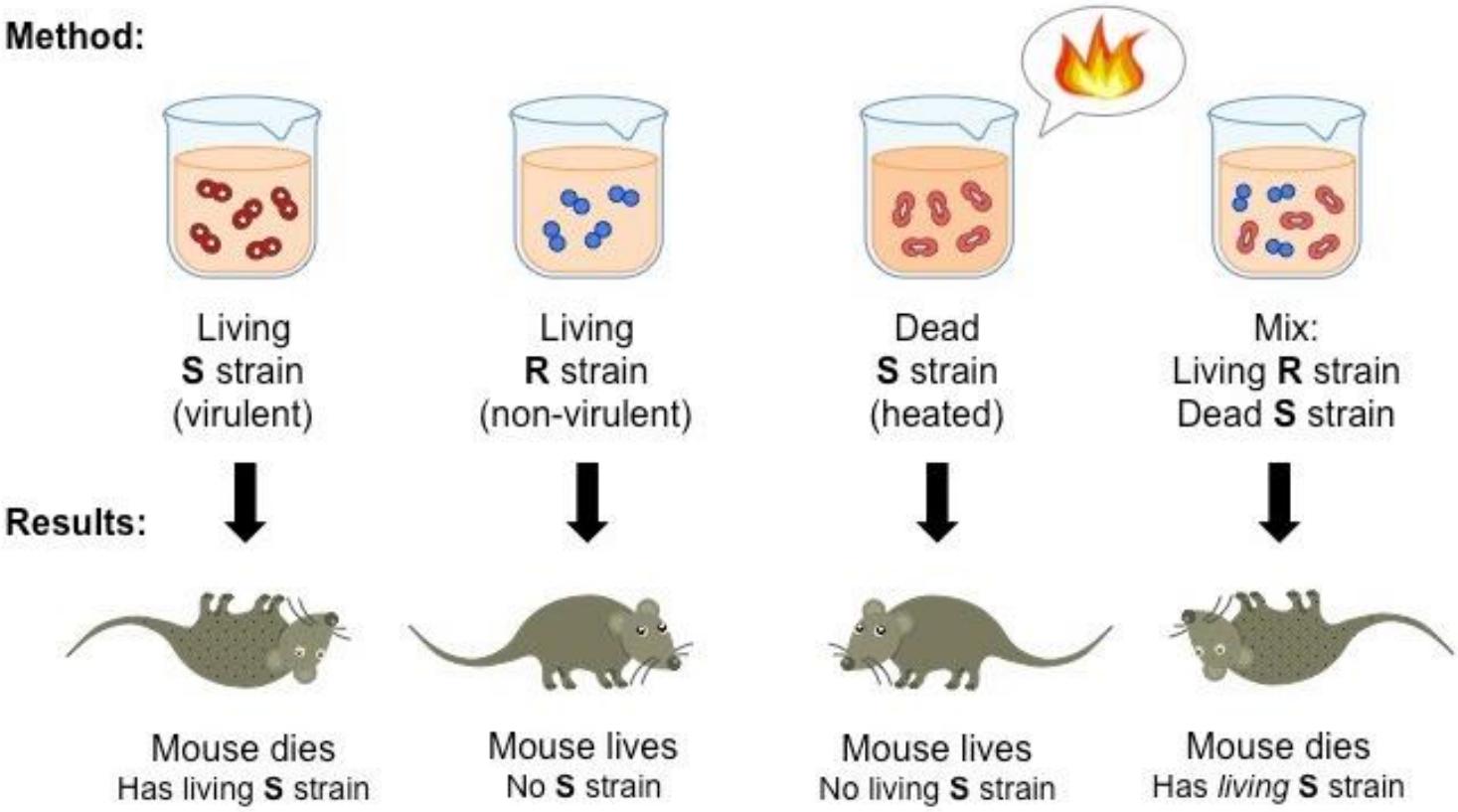
Discovery of DNA as the Source of Genetic Information

- The biochemical investigation of DNA began with Friedrich Miescher, who carried out the first systematic chemical studies of cell nuclei. In 1868 Miescher isolated a phosphorus-containing substance, which he called "nuclein," from the nuclei of pus cells (leukocytes) obtained from discarded surgical bandages.
- He found nuclein to consist of an acidic portion, which we know today as DNA, and a basic portion, protein.
- Miescher later found a similar acidic substance in the heads of sperm cells from salmon. Although he partially purified nuclein and studied its properties, the covalent (primary) structure of DNA) was not known with certainty until the late 1940s.

Griffith Experiment

- Miescher and many others suspected that nuclein (nucleic acid) was associated in some way with cell inheritance.
- In 1928, British bacteriologist Frederick Griffith conducted a series of experiments using *Streptococcus pneumoniae* bacteria and mice.
- Griffith found that nuclein from a virulent (disease-causing) strain of the bacterium *Streptococcus pneumoniae,* also known asn pneumococcus, genetically transformed a nonvirulent strain of this organism into a virulent form.

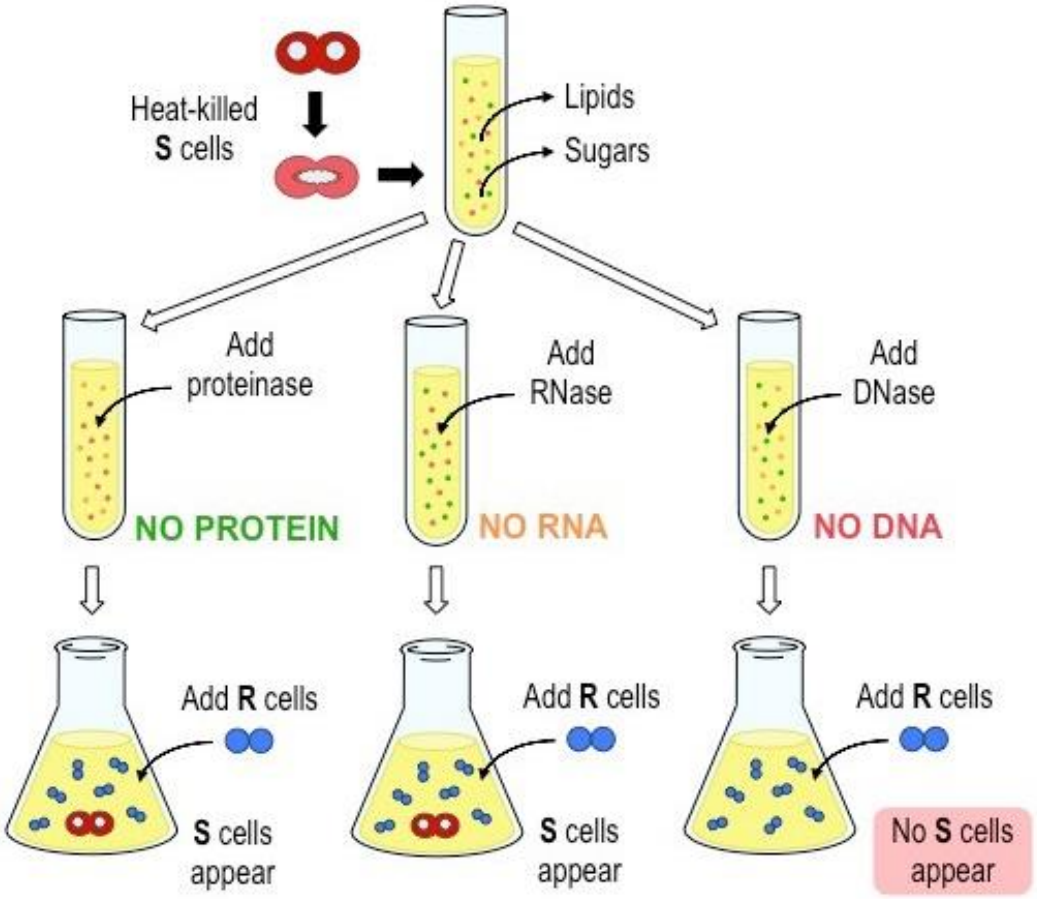
Griffith Experiment



Conclusion: A chemical substance from one cell is genetically transforming another cell

Avery-Macload-McCarty Experiment

- The first direct evidence that DNA is the bearer of genetic information came in 1944 through a discovery made by Oswald T. Avery, Colin MacLeod, and Maclyn McCarty.
- These investigators expanded upon the findings of Griffith and found that **DNA** extracted from a virulent strain of the bacterium *Streptococcus pneumoniae* genetically transformed a nonvirulent strain of this organism into a virulent form.



Remove lipids and sugars from a solution of heat-killed **S** cells.

Proteins, RNA and DNA remain

Treat solutions with enzymes to destroy protein, RNA or DNA

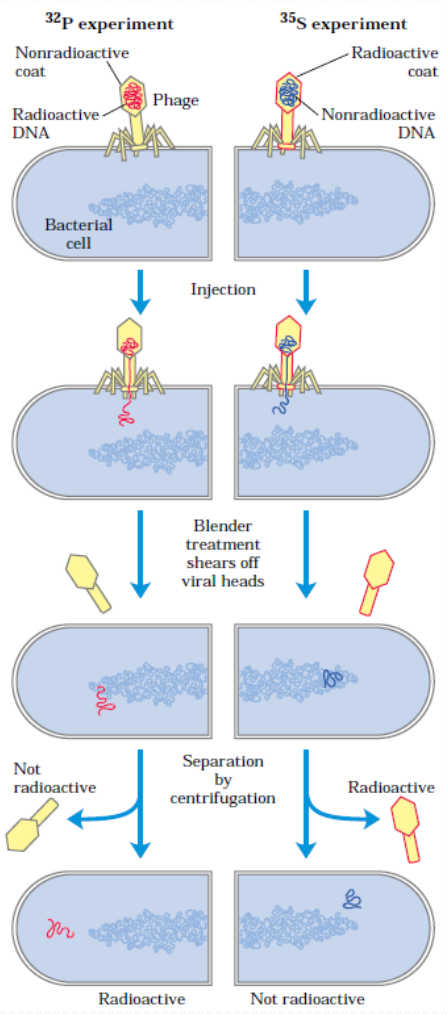
Add to culture containing living **R** cells.

Observe for transformation by testing for the presence of virulent **S** cells

Conclusion: Transformation requires DNA, therefore it is the genetic material of the cell

Hershey and Chase Experiment

- A second important experiment provided independent evidence that DNA carries genetic information.
- In 1952 Alfred D. Hershey and Martha Chase used radioactive phosphorus (^{32}P) and radioactive sulfur (^{35}S) tracers to show that when the bacterial virus (bacteriophage) T2 infects its host cell, *Escherichia coli*, it is the phosphorus-containing DNA of the viral particle, not the sulfur-containing protein of the viral coat, that enters the host cell and furnishes the genetic information for viral replication.



DNA Structure

- A most important clue to the structure of DNA came from the work of Erwin Chargaff and his colleagues in the late 1940s. They found that the four nucleotide bases of DNA occur in different ratios in the DNAs of different organisms and that the amounts of certain bases are closely related.
- These data, collected from DNAs of a great many different species, led Chargaff to the following conclusions:

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 number of adenosine residues is equal to the number of thymidine residues (that is, A = T), and the number of guanosine residues is equal to the number of cytidine residues (G = C). From these relationships it follows that the sum of the purine residues equals the sum of the pyrimidine residues; that is, A + G = T + C.

- These "Chargaff's rules," were a key to establishing the 3D structure of DNA and yielded clues to how genetic information is encoded in DNA and passed from one generation to the next.

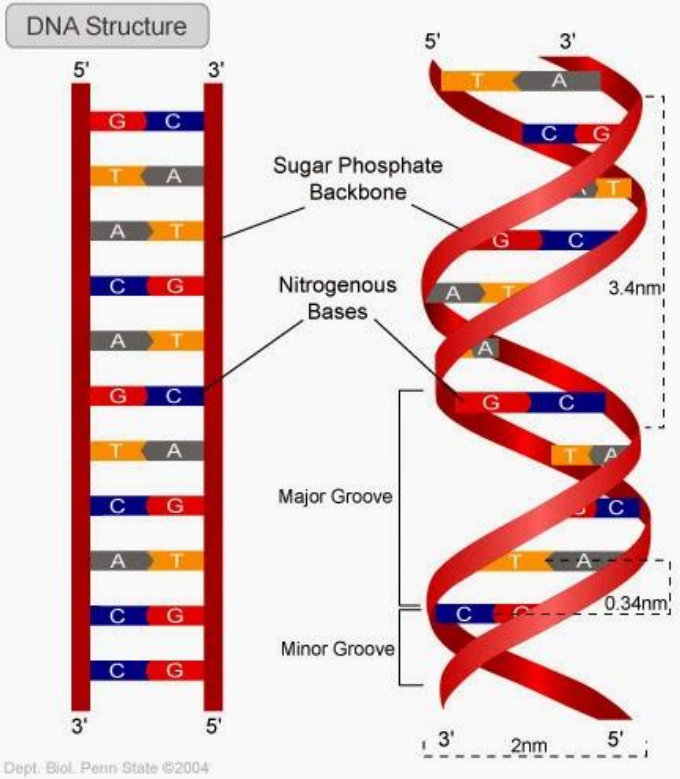
Double Helical Structure of DNA

- To shed more light on the structure of DNA, Rosalind Franklin and Maurice Wilkins used the powerful method of x-ray diffraction to analyze DNA fibers.
- They showed in the early 1950s that DNA produces a characteristic x-ray diffraction pattern.
- From this pattern it was deduced 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.
- The problem then was to formulate a 3D model of the DNA molecule that could account not only for the x-ray diffraction data but also for the specific A = T and G = C base equivalences discovered by Chargaff and for the other chemical properties of DNA.
- In 1953 Watson and Crick postulated a 3D model of DNA structure that accounted for all the available data.

1962 Nobel Prize in Physiology and Medicine

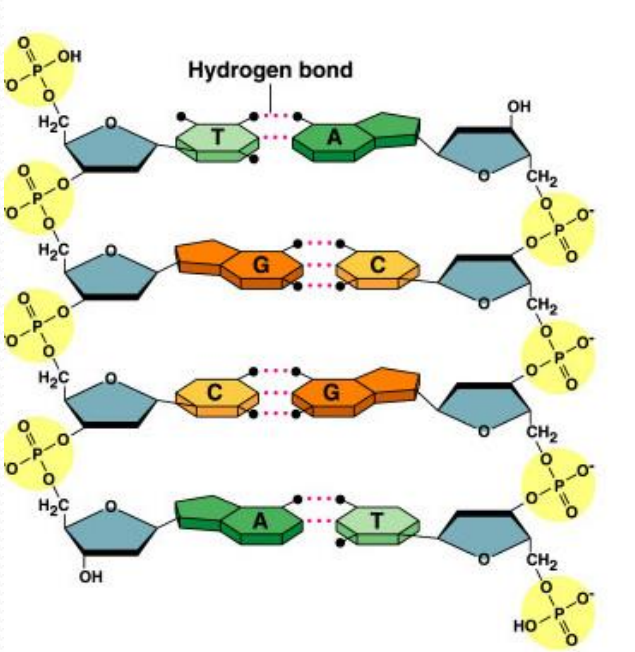
Watson-Crick Model of DNA

- It consists of two helical DNA chains wound around the same axis to form a right handed double helix.
- The hydrophilic backbones of alternating deoxyribose and phosphate groups are on the outside of the double helix, facing the surrounding water.
- The purine and pyrimidine bases of both strands are stacked inside the double helix, with their hydrophobic and nearly planar ring structures very close together and perpendicular to the long axis.
- Vertically stacked bases inside the double helix are 3.4 Å apart; the secondary repeat distance of about 34 Å accounts for the presence of 10 base pairs in each complete turn of the double helix.
- The offset pairing of the two strands creates a **major groove** and **minor groove** on the surface of the duplex.

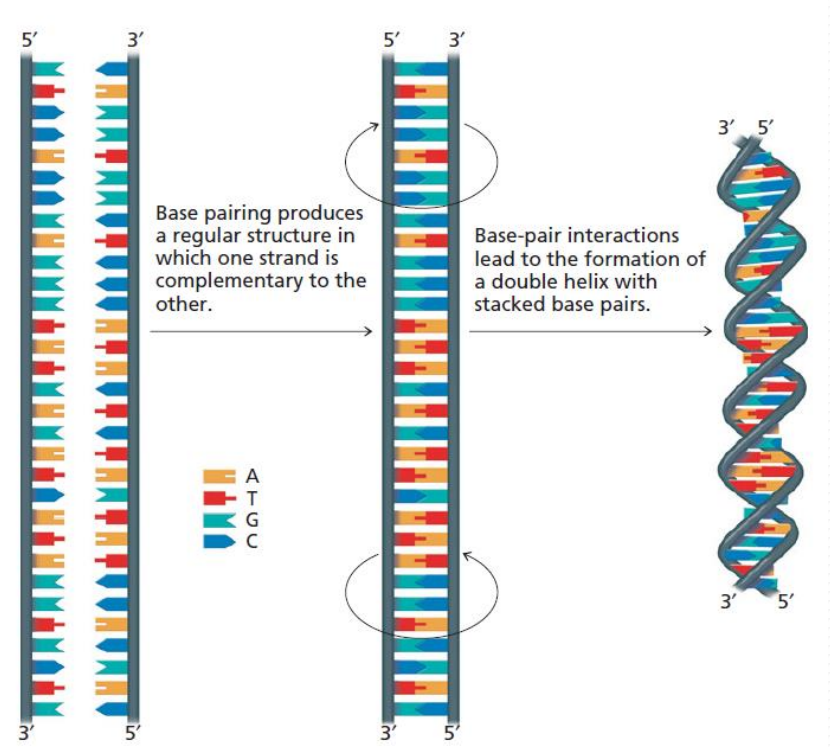


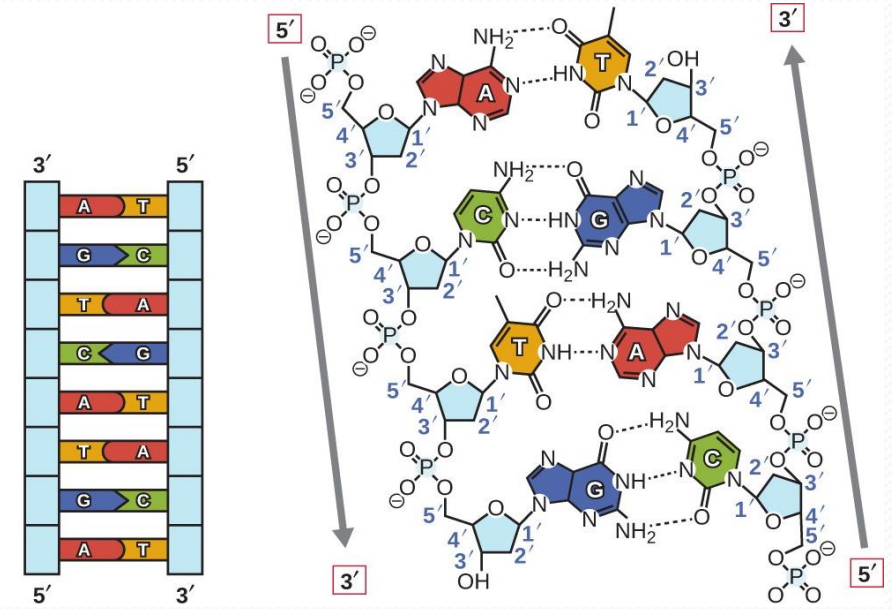
Watson-Crick Model of DNA

- Each nucleotide base of one strand is paired in the same plane with a base of the other strand. Watson and Crick found that the hydrogen-bonded base pairs, G with C and A with T, are those that fit best within the structure, providing a rationale for Chargaff's rule that in any DNA, G = C and A = T.
- Hydrogen bond pairing occurs in a manner that maximizes hydrogen bonding between potential sites to stabilize the structure.
- Each purine–pyrimidine pair is called a base pair, and this *complementary base-pairing* enables the base pairs to be packed in the energetically most favorable arrangement in the interior of the double helix.
- In this arrangement, each base pair has a similar width, thus holding the sugar–phosphate backbones an equal distance apart along the DNA molecule.
- Three hydrogen bonds can form between G and C but only two can form between A and T.
- This is one reason for the finding that separation of paired DNA strands is more difficult the higher the ratio of G-C to A-T base pairs.
- Other pairings of bases tend (to varying degrees) to destabilize the double-helical structure.

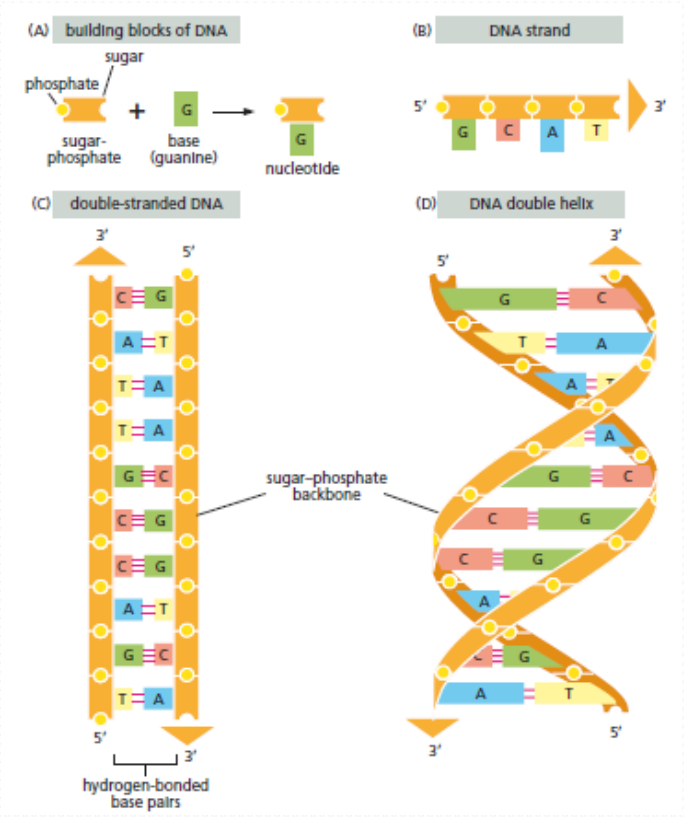


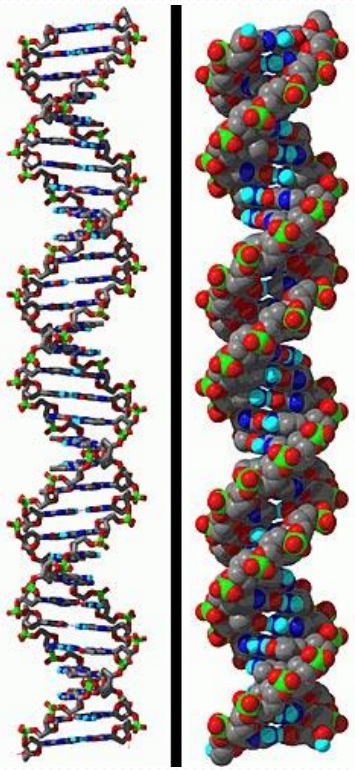
- The members of each base pair can fit together within the double helix because the two strands of the helix run *antiparallel* to each other—that is, they are oriented with opposite polarities.
- The antiparallel sugar–phosphate strands then twist around each other to form a double helix containing 10 base pairs per helical turn.
- This twisting also contributes to the energetically favorable conformation of the DNA double helix.





- Each end of double-stranded DNA is made up of the 5’ end of one strand and the 3’ end of another. The distance between the two sugar–phosphate backbones is the same for each base pair. Consequently, all DNA molecules have the same regular structure in spite of the fact that their nucleotide sequences may be quite different.





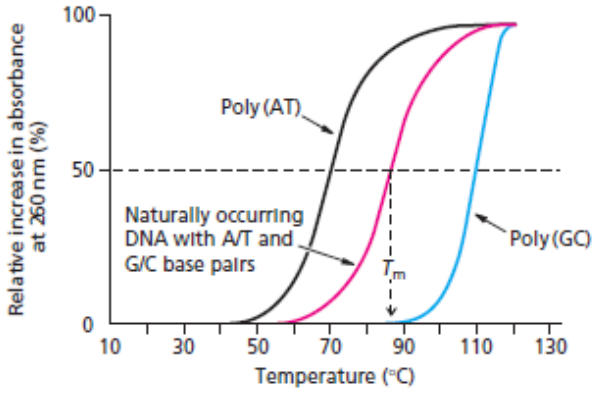
- Four types of interactions affect the conformation of double stranded DNA:

1. ***Stacking interactions.*** The stacked base pairs form van der Waals contacts. Although the forces between individual stacked base pairs are weak, they are additive so in large DNA molecules the van der Waals contacts are an important source of stability.
2. ***Hydrogen bonds.*** Hydrogen bonding between base pairs is a significant stabilizing force.
3. ***Hydrophobic effects.*** Burying hydrophobic purine and pyrimidine rings in the interior of the double helix increases the stability of the helix.
4. ***Charge–charge interactions.*** Electrostatic repulsion of the negatively charged phosphate groups of the backbone is a potential source of instability in the DNA helix. However, repulsion is minimized by the presence of cations such as Mg^{2+} and cationic proteins (proteins that contain an abundance of the basic residues arginine and lysine).

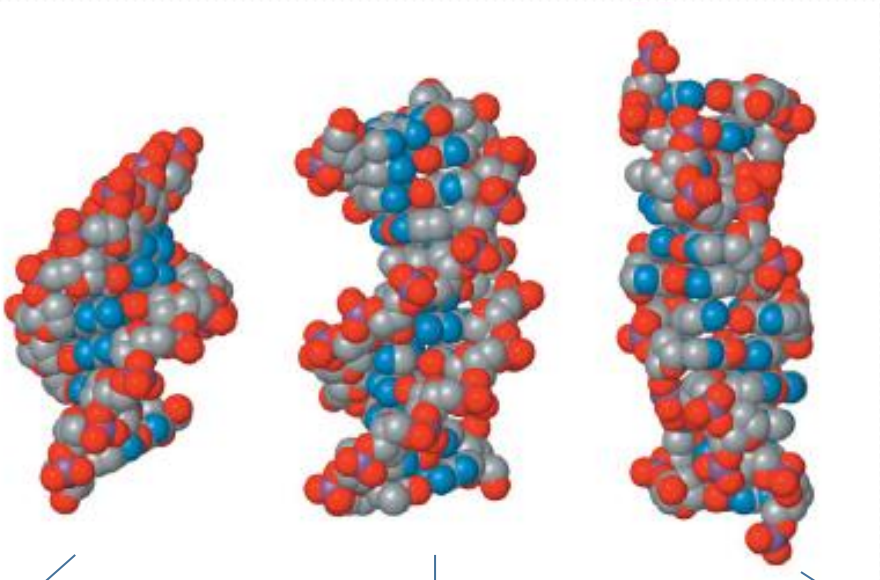
Denaturation of DNA structure

- Under physiological conditions, double-stranded DNA is thermodynamically much more stable than the separated strands and that explains why the double stranded form predominates *in vivo*.
- However, the structure of localized regions of the double helix can sometimes be disrupted by unwinding. Such disruption occurs during DNA replication, repair, recombination, and transcription.
- Complete unwinding and separation of the complementary single strands is called **denaturation**. Denaturation occurs only *in vitro*.
- Double-stranded DNA can be denatured by heat. The temperature of a solution of DNA is slowly increased. As the temperature is raised, more and more of the bases become unstacked and hydrogen bonds between base pairs are broken. Eventually, the two strands separate completely.
- The temperature at which half the DNA has become single stranded is known as the **melting point** (*T*m).

- Poly (GC) denatures at a much higher temperature than poly (AT).
- It is easier to melt A/T-rich DNA than G/C-rich DNA because A/T base pairs have weaker stacking interactions and hydrogen bonds.
- Stacking interactions are the first interactions to be disrupted by higher temperature.
- Once this process begins the hydrogen bonds—although collectively stronger in stacked DNA—become much weaker because they are exposed to water and the DNA is rapidly destabilized.
- Naturally occurring DNA is a mixture of regions with varying base compositions but A/T-rich regions are more easily unwound than G/C-rich regions.
- Thats why the initiation sites for transcription are often A/T-rich.



Conformations of DNA molecule

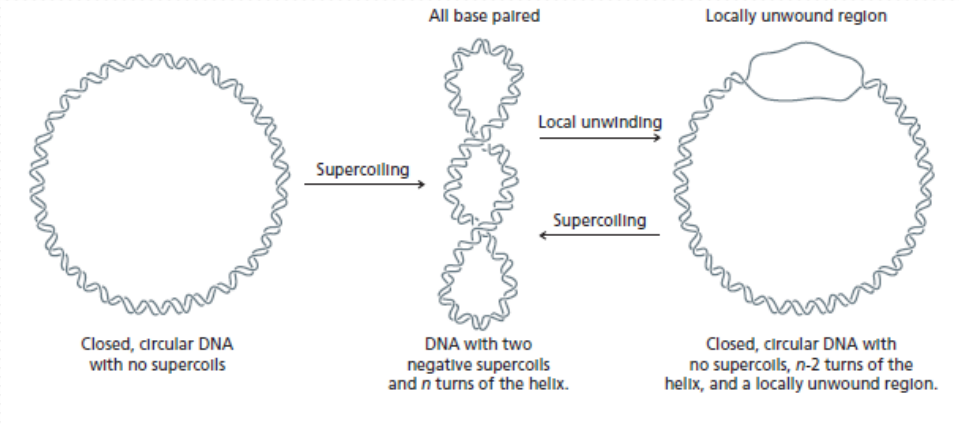


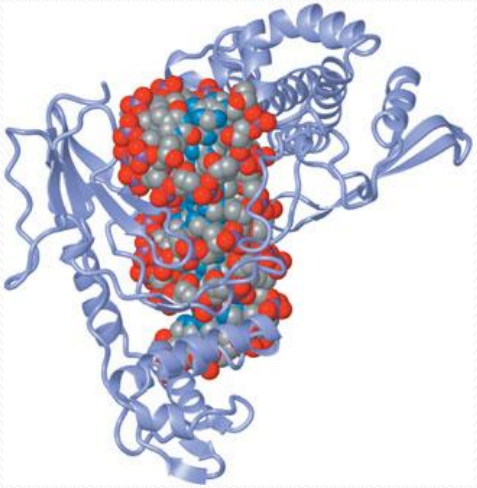
A-DNA
Dehydrated DNA

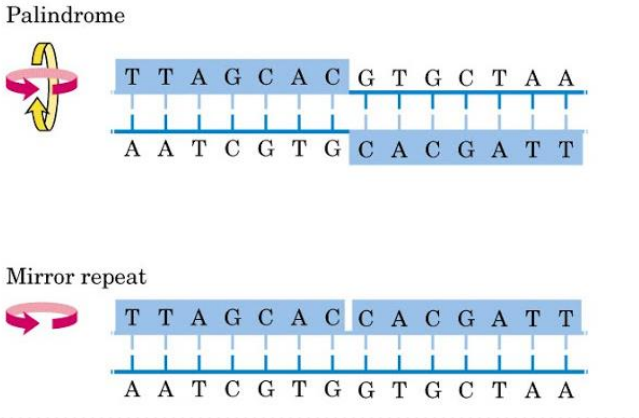
B-DNA
Normally occuring one

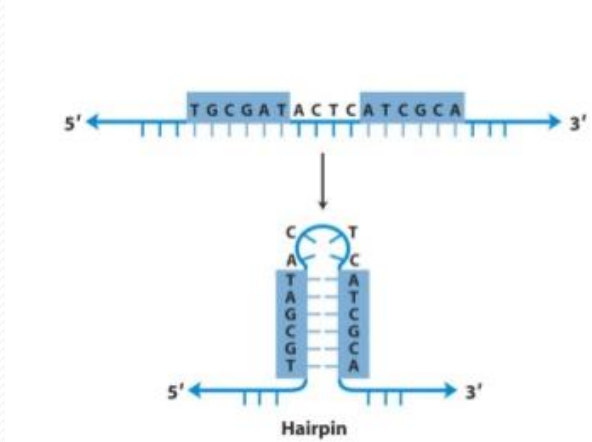
Z-DNA
GC rich regions

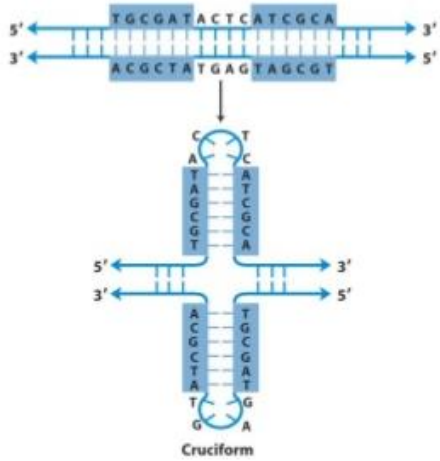
- All organisms have enzymes that can break DNA, unwind or overwind the double helix, and rejoin the strands to alter the topology. These enzymes, called topoisomerases, are responsible for adding and removing supercoils.
- Localized unwinding is an essential step in the initiation of DNA replication, recombination, repair, and transcription. Thus, negative supercoiling plays an important biological role in these processes by storing the energy needed for local unwinding.

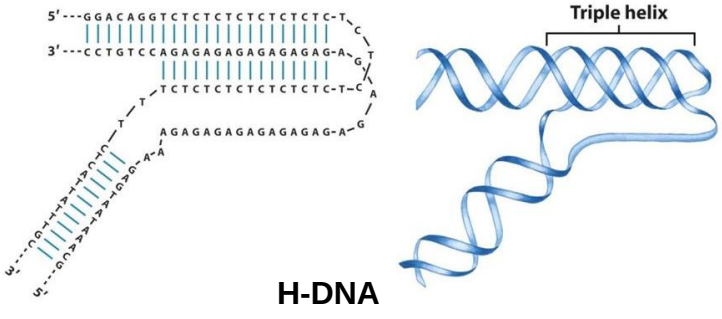





Palindrome
T T A G C A C G T G C T A A
A A T C G T G C A C G A T T
Mirror repeat
T T A G C A C C A C G A T T
A A T C G T G G T G C T A A


5′
TGCGATACTCATCGCA
3′
Hairpin


TGCGATACTCATCGCA
ACGCTATGAGTAGCGT
Cruciform


Triple helix
H-DNA

Higher Level Structures of DNA

- Large amounts of DNA are required to encode all the information needed to make even a single-celled bacterium, and far more DNA is needed to encode the information to make a multicellular organism.
- The entire genetic complement of an organism is called its **genome**.
- Each human cell contains about 2 meters (m) of DNA; yet the cell nucleus is only 5–8 μm in diameter.
- Tucking all this material into such a small space is the equivalent of trying to fold 40 km of extremely fine thread into a tennis ball.
- The complex task of packaging DNA is accomplished by specialized proteins that bind to and fold the DNA, generating a series of coils and loops that provide increasingly higher levels of organization.

Higher Level Structures of DNA

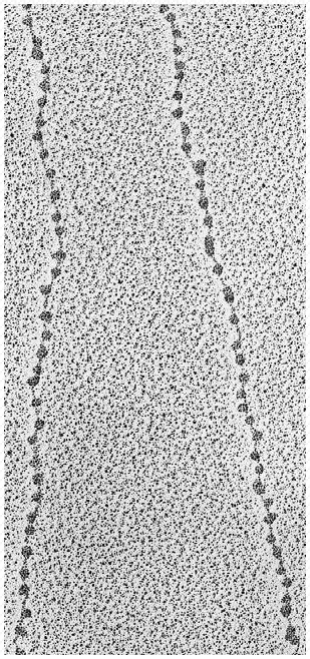
- In 1879, ten years after Miescher's discovery of nuclein, Walter Flemming observed banded objects in the nuclei of stained eukaryotic cells.He called the material **chromatin**.
- **Chromatin is now known to consist of DNA plus various proteins that package the DNA in a more compact form**.

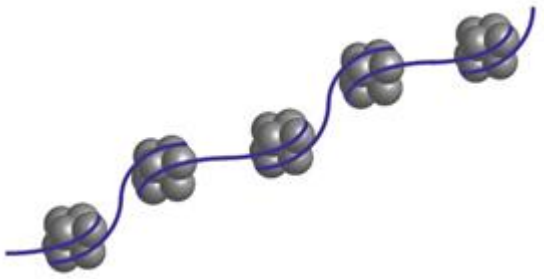
Chromatin Packing

- In a normal resting cell, chromatin exists as 30 nm fiber.
- In humans, the nucleus must accommodate 46 such chromatin fibers, or chromosomes.
- The largest human chromosome is about 2.4×10^8 bp; it would be about 8 cm long if it were stretched out in the B conformation.
- During metaphase (when chromosomes are most condensed) the largest chromosome is about 10 μm long.
- Bacteria typically carry their genes on a single, circular DNA molecule.
- Prokaryotic DNA is also associated with protein to form condensed structures inside the cell. These structures differ from those observed in eukaryotes and are usually not called chromatin.

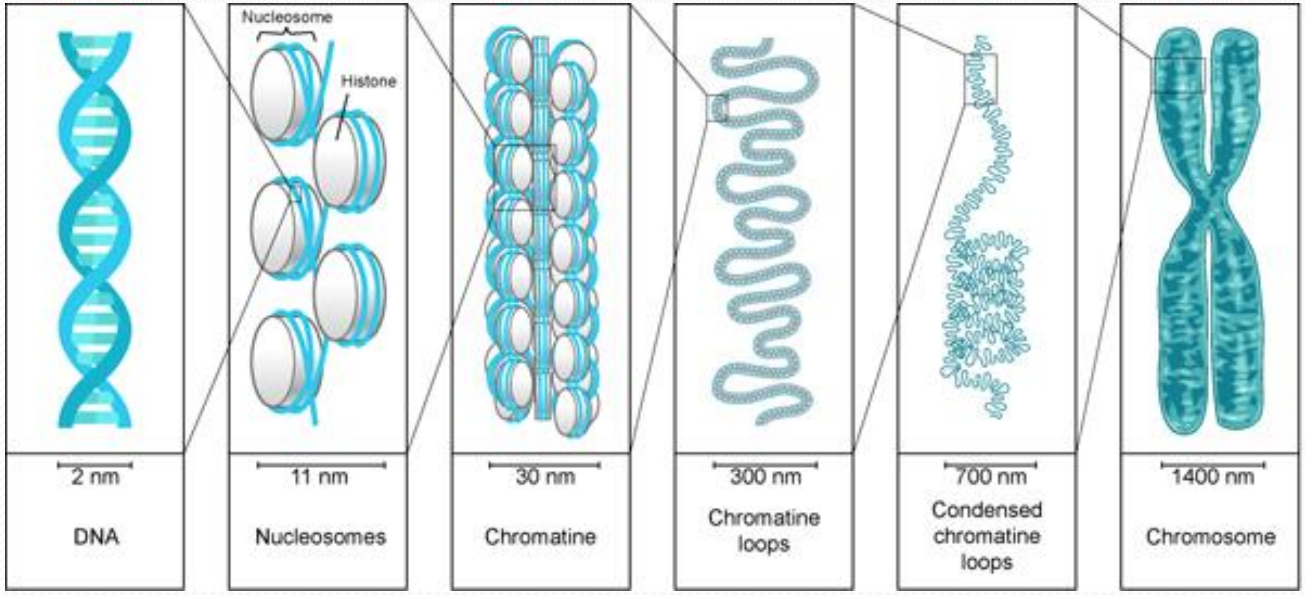
Histone Proteins

- Histone proteins are involved in the packing of DNA.
- Histones are small, basic proteins containing numerous lysine and arginine residues whose positive charges allow the proteins to bind to the negatively charged sugar–phosphate backbone of DNA.
- DNA is wrapped around the histone proteins.
- DNA-histone complex, which is chromatin fiber, looks like beads on a string in an electron micrograph.
- The “beads” are DNA–histone complexes called **nucleosomes** and the “string” is double-stranded DNA.



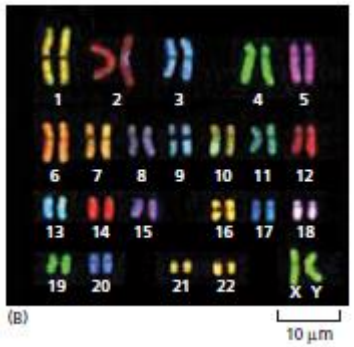


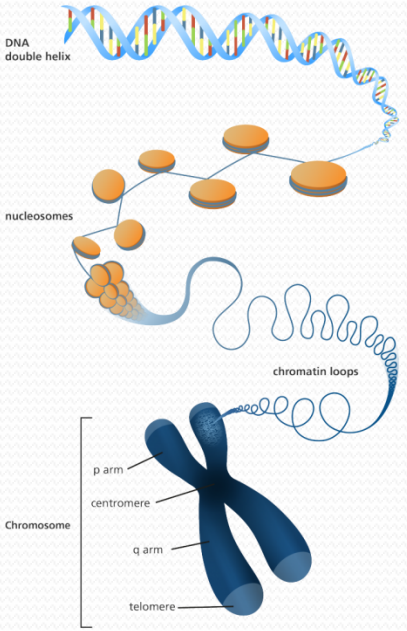
- The packaging of DNA into nucleosomes reduces the length of a DNA molecule about ten fold.
- The beads-on-a-string structure is itself coiled to yield the 30 nm fiber. Histones bind to each other cooperatively bringing the nucleosomes together into a more compact and stable form of chromatin. This condensation achieves a further four fold reduction in chromatin length.
- Finally, 30 nm fibers form loops that further condesate the chromatin structure which gives another 200 fold condensation.
- Chromosomes appear only after DNA replication in cell division during metaphase.

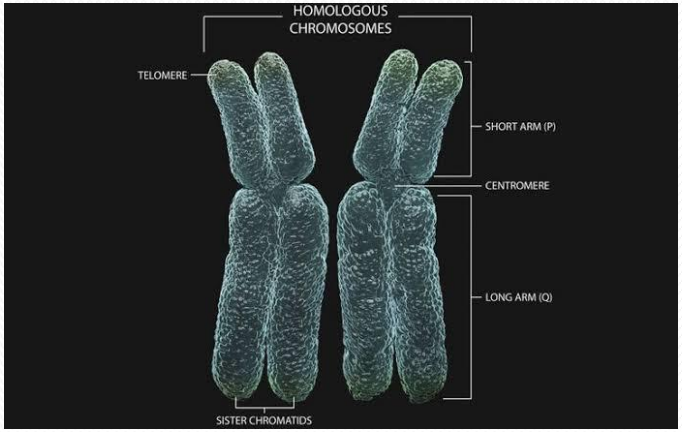


Chromosomes

- With the exception of the germ cells (sperm and eggs) and highly specialized cells that lack DNA entirely (such as mature red blood cells), human cells each contain two copies of each chromosome, one inherited from the mother and one from the father. The maternal and paternal chromosomes of a pair are called *homologous chromosomes* (*homologs*).
- The only nonhomologous chromosome pairs are the sex chromosomes in males, where a *Y chromosome* is inherited from the father and an *X chromosome* from the mother. (Females inherit one X chromosome from each parent and have no Y chromosome.)







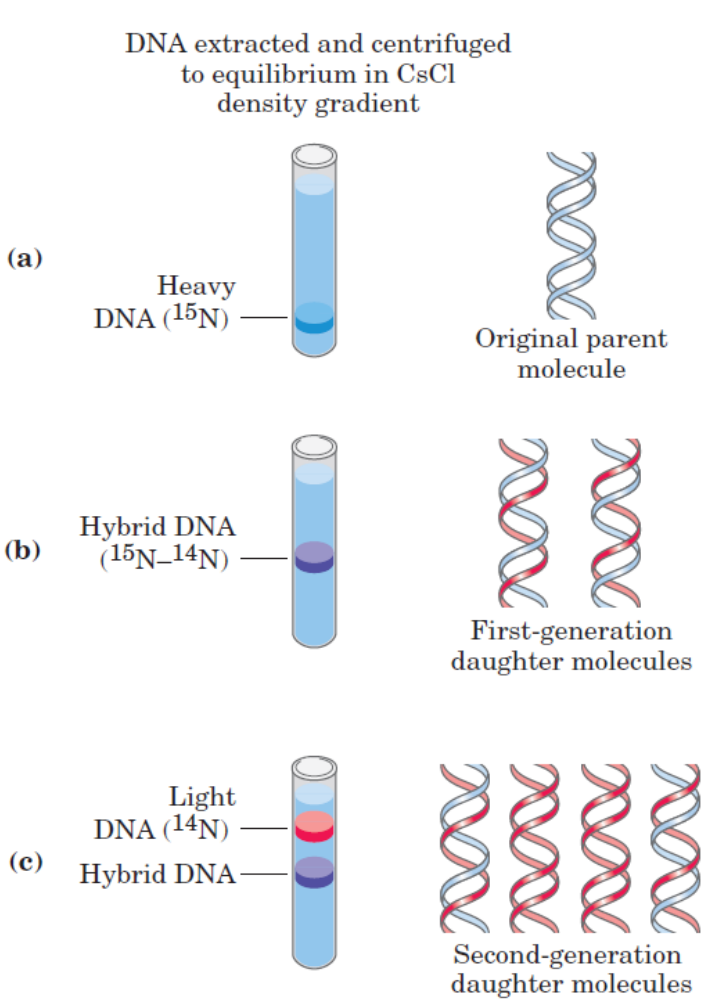
- Amazingly, the DNA is compacted in a way that allows it to remain accessible to all of the enzymes and other proteins that replicate it, repair it, and control the expression of its genes.
- **Histone acetylation** and **chromatin remodeling complexes** expose underlying DNA sequences to polymerases and other enzymes.
- These processes are reversible, so modified or remodeled chromatin can be returned to its compact state after transcription and/or replication are complete.

DNA Replication

- Since genetic information is carried in DNA, it follows that the transfer of information from a parental cell to two daughter cells requires exact duplication of DNA, a process known as DNA replication.
- The DNA structure proposed by Watson and Crick in 1953 immediately suggested a method of replication: The nucleotide sequence of one strand automatically specifies the sequence of the other since the two strands of double-helical DNA are complementary.
- Watson and Crick proposed that the two strands of the helix unwind during DNA replication and that each strand of DNA acts as a template for the synthesis of a complementary strand. In this way, DNA replication produces two double-stranded daughter molecules, each containing one parental strand and one newly synthesized strand.
- This mode of replication is termed semiconservative replication because one strand of the parental DNA is conserved in each daughter molecule.

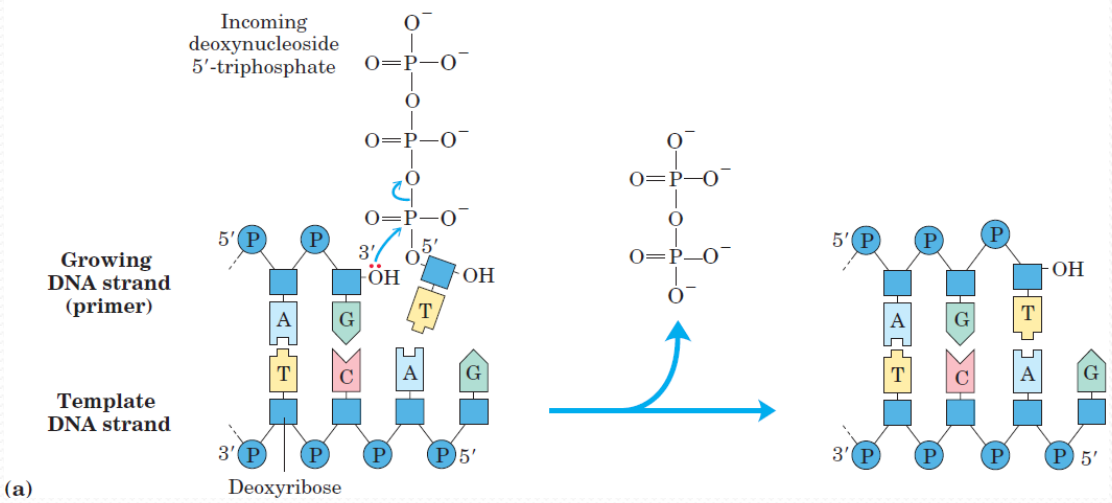
The Meselson-Stahl Experiment

- Although Watson and Crick proposed the hypothesis of semiconservative replication in 1953, the hypothesis was proved by ingeniously designed experiments carried out by Matthew Meselson and Franklin Stahl in 1957.

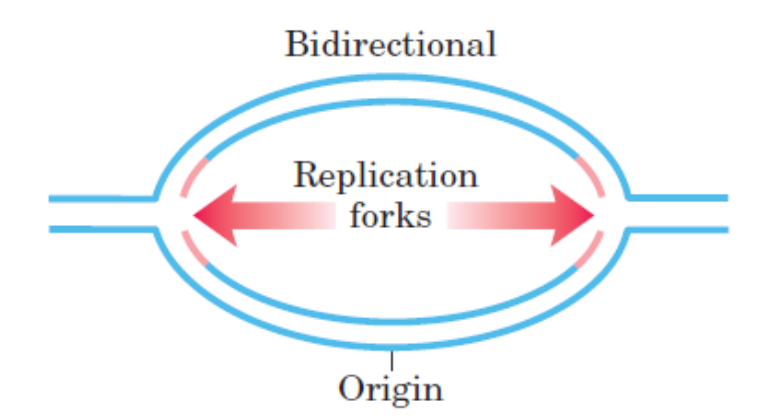


Cells were grown for many generations in a medium containing only heavy nitrogen, 15N, so that all the nitrogen in their DNA was 15N, as shown by a single band (blue) when centrifuged in a CsCl density gradient. **(b)** Once the cells had been transferred to a medium containing only light nitrogen, 14N, cellular DNA isolated after one generation equilibrated at a higher position in the density gradient (purple band). **(c)** Continuation of replication for a second generation yielded two hybrid DNA and two light DNAs (red), confirming semiconservative replication.

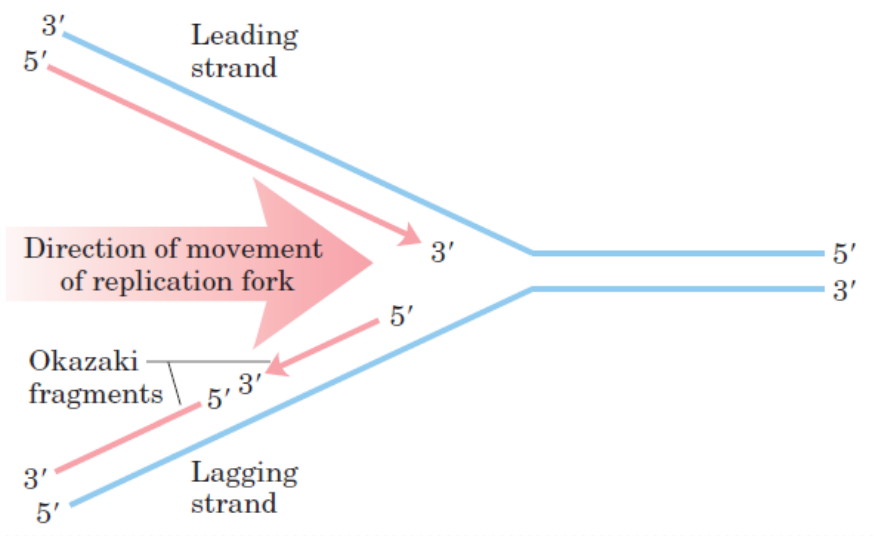
- The synthesis of a new strand of DNA is achieved by the successive addition of nucleotides to the 3' end of a growing chain.
- This polymerization is catalyzed by enzymes known as DNA polymerases.
- The nucleotide substrate is a deoxyribonucleoside-triphosphate (dNTP).
- DNA polymerase catalyzes the formation of a phosphodiester linkage between the incoming dNTP and the growing chain.
- The direction of polymerization (chain growth) is 5' → 3' with the free 3' OH as the point at which the DNA is elongated.



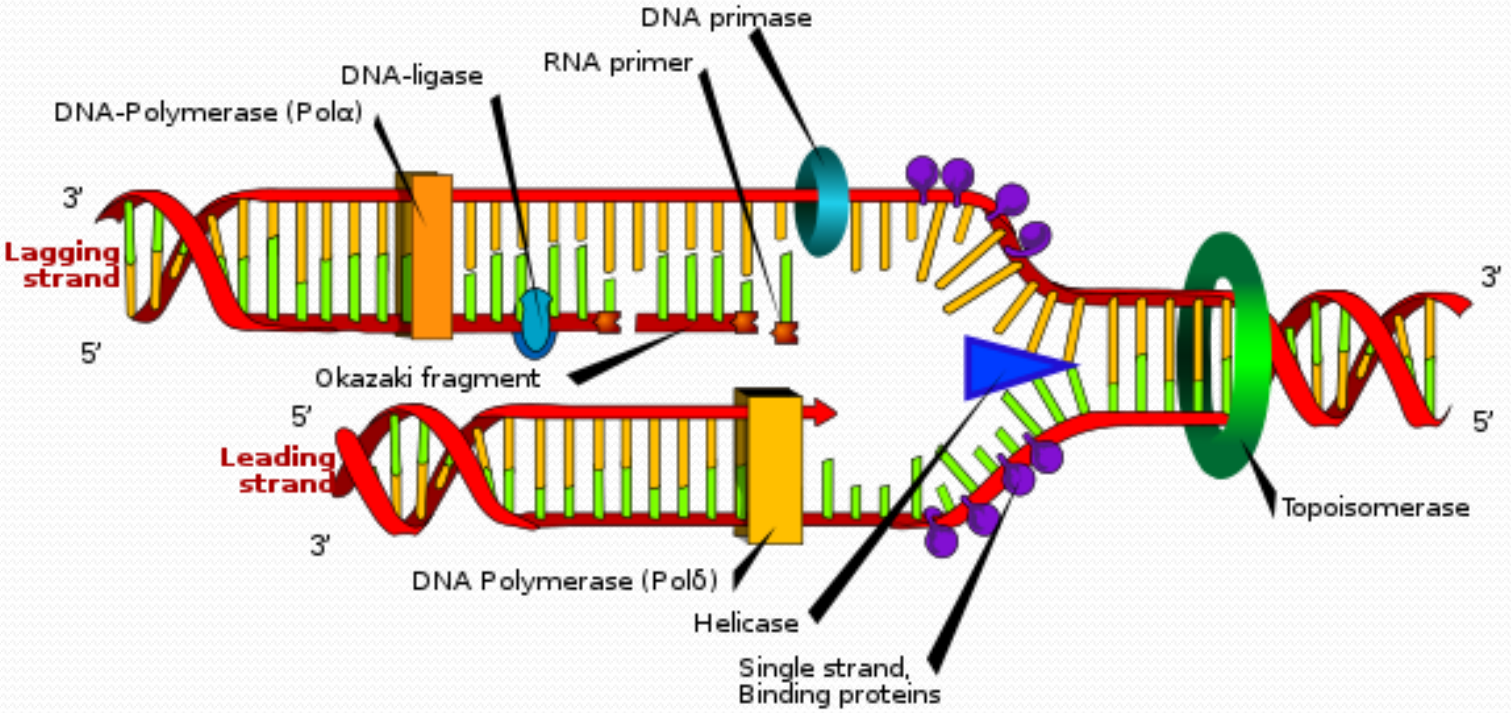
- DNA polymerases replicate both strands of the DNA simultanously and the replication is bidirectional.
- Replication starts at a particular sequence in a genome which is called origin of replication.
- Replication fork is formed to expose template strands.



- Since the two strands of DNA are antiparallel, synthesis using one of the strand as template occurs in the same direction as fork movement but synthesis using the other strand as template occurs in the direction opposite to the fork movement.
- Thus, one strand is synthesized continuously and the other discontinuously.
- The new strand formed by polymerization in the same direction as fork movement is called the **leading strand**.
- The new strand formed by polymerization in the opposite direction is called the **lagging strand**. In this strand DNA is synthesized in short pieces called **Okazaki fragments** which are then joined by **DNA ligase**.



- In order to synthesize DNA, DNA polymerize requires:
  - **a template**
    - according to the semiconservative DNA synthesis model)
  - **a primer sequence**
    - DNA polymerases can only add nucleotides to a preexisting strand.
    - Thus, primer is a strand segment (complementary to the template) with a free 3-hydroxyl group to which a nucleotide can be added.
    - Most primers are oligonucleotides of RNA rather than DNA, and specialized enzymes called **DNA primase** synthesize primers when and where they are required.


DNA primase
RNA primer
DNA-ligase
DNA-Polymerase (Polα)
3′
Lagging strand
5′
Okazaki fragment
5′
Leading strand
3′
DNA Polymerase (Polδ)
Helicase
Single strand Binding proteins
3′
5′
Topoisomerase

- As well as addition of nuclotides in 5'→3' direction, DNA polymerase also have 3'→5' proofreading and exonuclease activity.
- For proofreading, DNA polymerase double-checks each nucleotide after it is added.
- If the polymerase has added the wrong nucleotide, translocation of the enzyme to the position where the next nucleotide is to be added is inhibited.
- This kinetic pause provides the opportunity for a correction. The 3'→5' exonuclease activity removes the mispaired nucleotide, and the polymerase begins again.
- So that replication is very accurate.