College of pharmacy

Biochemistry I third stage

Dr. Maytham Ahmed

Lecture 2

Nucleotides and Deoxyribonucleic acid

Nucleotides

Nucleotides are the monomer units of nucleic acids. Nucleotides are essential for all cells. Without them, neither RNA nor DNA can be produced and, therefore, proteins cannot be synthesized or cells proliferate. Nucleotides also serve as carriers of activated intermediates in the synthesis of some carbohydrates, lipids, and conjugated proteins and are structural components of several essential coenzymes, such as coenzyme A, FAD, NAD$^+$, and NADP$^+$.

Nucleotides, such as cyclic adenosine monophosphate (cAMP) and cyclic guanosine monophosphate (cGMP), serve as second messengers in signal transduction pathways. In addition, nucleotides play an important role as "energy currency" in the cell.

Nucleotide structure

Nucleotides are composed of a **nitrogenous base**, a pentose monosaccharide, and one, two, or three phosphate groups. The nitrogen containing bases are **purines** or **pyrimidines**.

Purine and pyrimidine structures
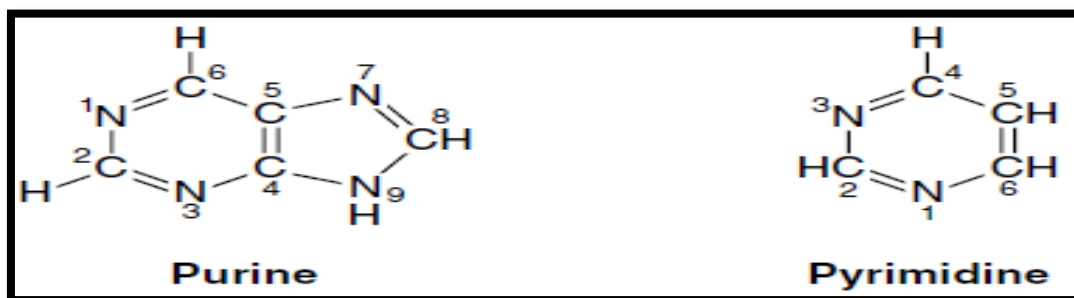

Both DNA and RNA contain the same purine bases: adenine (A) and guanine (G). Both DNA and RNA contain the pyrimidine cytosine (C), but they differ in their second pyrimidine base: DNA contains thymine (T), whereas RNA contains uracil (U).
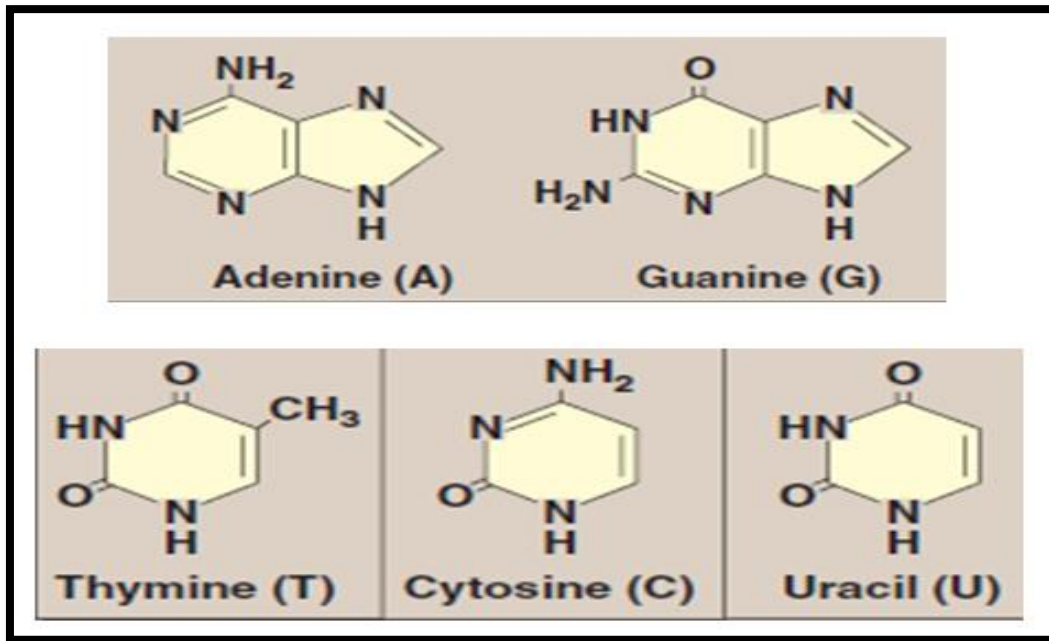
Nucleosides

Nucleosides are derivatives of purines and pyrimidines that have a sugar linked to a ring nitrogen. The sugar is linked to the heterocyclic base via a β-N-glycosidic bond, almost always to N-1 of a pyrimidine or to N-9 of a purine. If the sugar is ribose, a ribonucleoside is produced; if the sugar is 2-deoxyribose, a deoxyribonucleoside is produced. The ribonucleosides of A, G, C, and U are named adenosine, guanosine, cytidine, and uridine, respectively. The deoxyribonucleosides of A, G, C, and T have the added prefix, "deoxy-," for example, deoxythymidine.
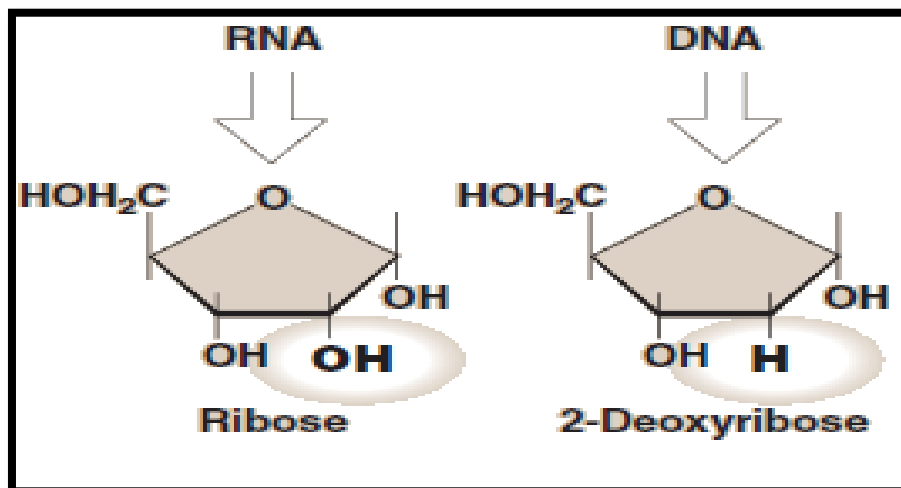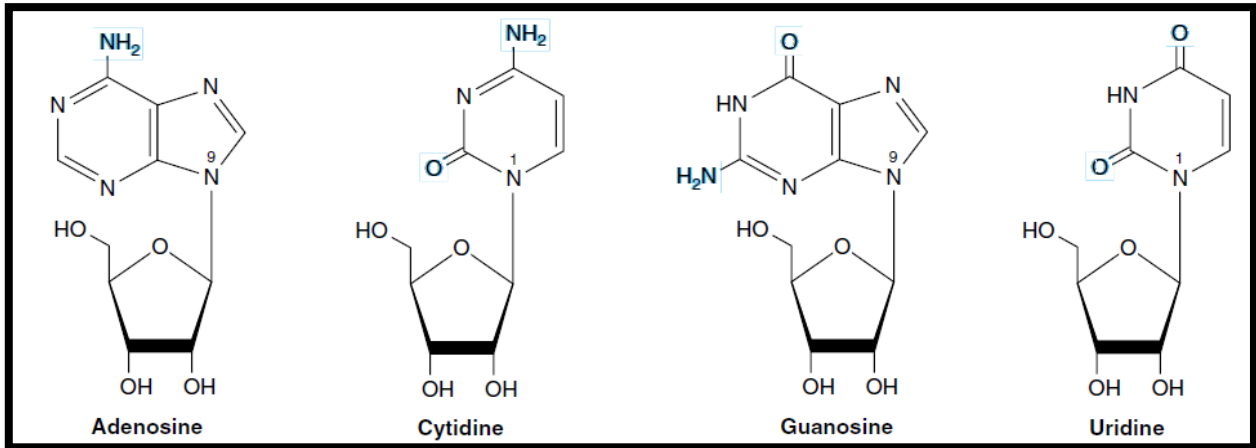
Ribonucleosides

Nucleotides

The addition of one or more phosphate groups to a nucleoside produces a nucleotide. The first phosphate group is attached by an ester linkage to the 5'-OH of the pentose. Such a compound is called a nucleoside 5' phosphate. If **one** phosphate group is attached to the 5'-carbon of the pentose, the structure is a **nucleoside monophosphate**, like adenosine monophosphate (AMP). If a **second** or **third** phosphate is added to the nucleoside, a **nucleoside diphosphate**, like adenosine diphosphate (ADP) or **nucleoside triphosphate**, like adenosine triphosphate (ATP) are produced.
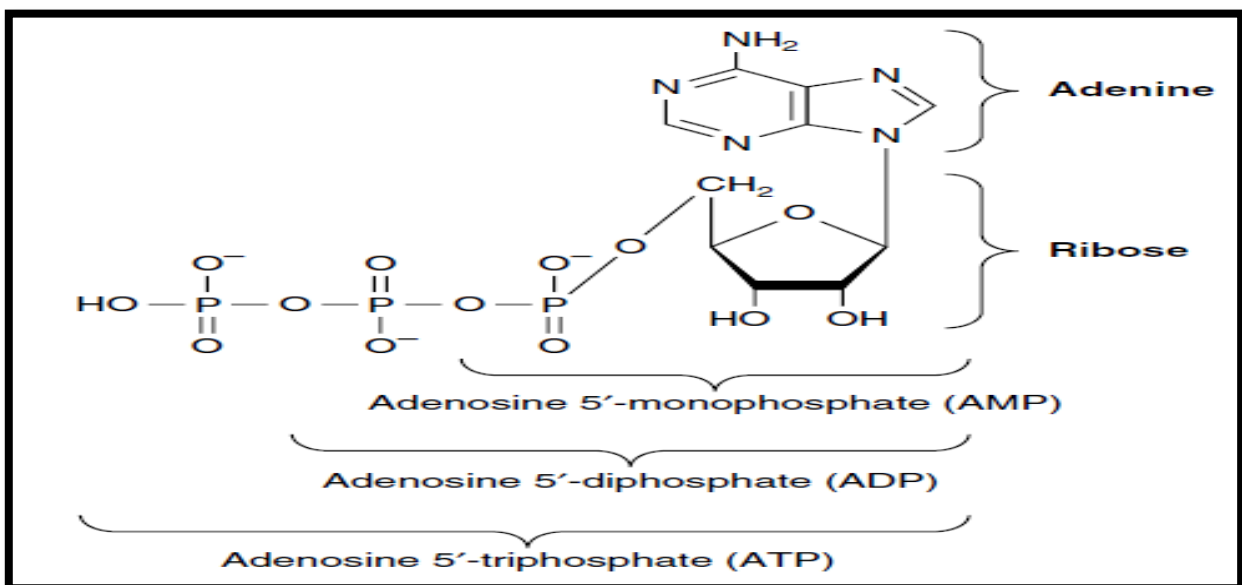

Adenosine 5'-mono, di, tri phosphate

DNA Replication

Nucleic acids are required for the storage and expression of genetic information. Deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) are chemically distinct types of nucleic acids. DNA, the source of genetic information, is present not only in chromosomes in the nucleus of eukaryotic organisms, but also in mitochondria. The genetic information found in DNA is copied and transmitted to daughter cells through DNA replication. DNA must be able to not only replicate precisely each time a cell divides, but also to have the information that it contains be selectively expressed. Transcription (RNA synthesis) is the first stage in the expression of genetic information. Next, the code contained in the nucleotide sequence of messenger RNA molecules is translated (protein synthesis), thus completing gene expression.

DNA Structure

DNA is a polymer of **deoxyribonucleoside monophosphates** covalently linked by 3'→5'–phosphodiester bonds. DNA exists as a **double stranded** molecule, in which the two strands wind around each other, forming a double helix. **Phosphodiester bonds** join the 3'-hydroxyl group of the deoxy pentose of one nucleotide to the 5'-hydroxyl group of the deoxy pentose of an adjacent nucleotide through a phosphate group. The resulting long, unbranched chain has polarity, with both a 5'-end (the end with the free phosphate) and a 3'-end (the end with the free hydroxyl) that are not attached to other nucleotides.

The bases written in sequence from the 5'-end of the chain to the 3'-end. For example, the sequence of bases in the DNA is read "thymine, adenine, cytosine, guanine" (5'-TACG-3'). Phosphodiester linkages between nucleotides (in DNA or RNA) can be cleaved by **chemicals** or **enzymes (nucleases)**: deoxyribonucleases for DNA and ribonucleases for RNA.
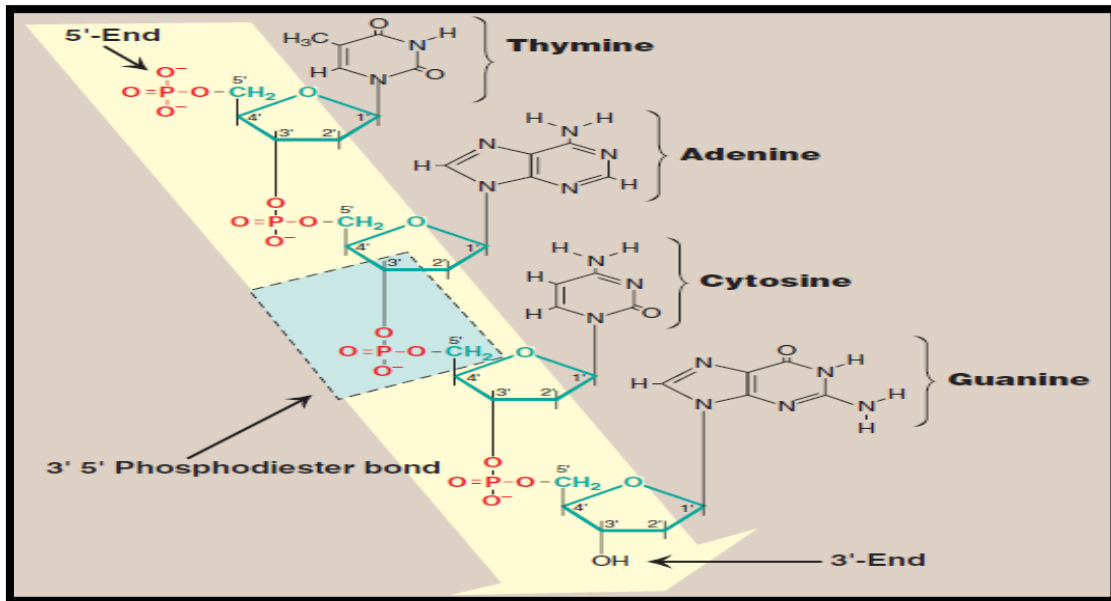
DNA chain with the nucleotide sequence (5'→ 3')
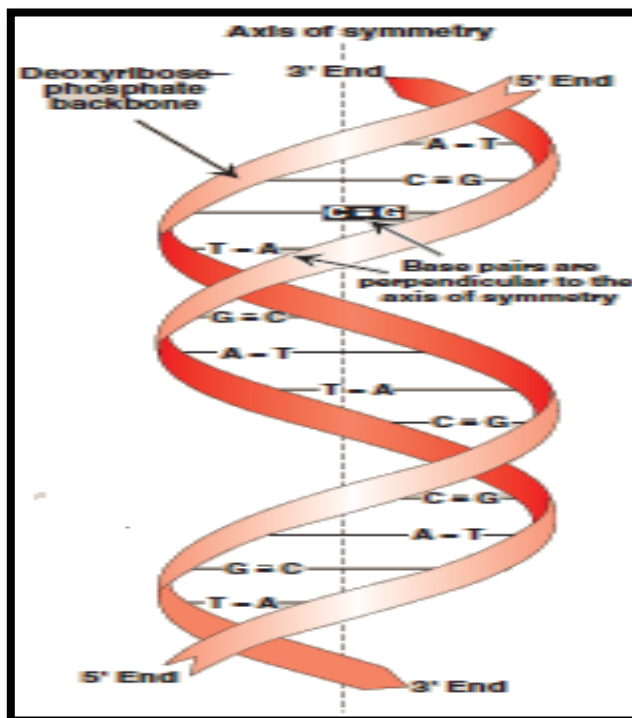
DNA double helix

In the **double helix**, the two chains are coiled around a common axis. The chains are paired in an **anti parallel manner**, that is, the 5'-end of one strand is paired with the 3'-end of the other strand. In the DNA helix, the hydrophilic deoxyribose–phosphate backbone of each chain is on the outside of the molecule, whereas the hydrophobic bases are stacked inside. The overall structure resembles a **twisted ladder**.

Base pairing

The bases of one strand of DNA are paired with the bases of the second strand, so that an adenine is always paired with a thymine and a cytosine is always paired with a guanine. The specific base pairing in DNA leads to the **Chargaff Rule**: In

any sample of double stranded DNA (dsDNA), the amount of adenine equals the amount of thymine, the amount of guanine equals the amount of cytosine, and the total amount of purines equals the total amount of pyrimidines. The base pairs are held together by hydrogen bonds: two between A and T and three between G and C. These hydrogen bonds stabilize the structure of the double helix.

Separation of the two DNA strands: The two strands of the double helix separate when hydrogen bonds between the paired bases are disrupted. Disruption can occur in the laboratory if the pH of the DNA solution is altered or if the solution is heated. Phosphodiester bonds are not broken by such treatment. When DNA is heated, the temperature at which one half of the helical structure is lost is defined as the **melting temperature** (Tm).

The loss of helical structure in DNA, called denaturation, can be monitored by measuring its absorbance at 260 nm. Because there are three hydrogen bonds between G and C but only two between A and T, DNA that contains high concentrations of A and T denatures at a **lower temperature** than G- and C-rich DNA. Under appropriate conditions, complementary DNA strands can reform the double helix by the process called renaturation (or reannealing).

Steps of DNA replication

When the two strands of the DNA double helix are separated, each can serve as a template for the replication of a new complementary strand. This produces two daughter molecules, each of which contains two DNA strands with an antiparallel orientation. This process is called semiconservative replication because, although the parental duplex is separated into two halves (and, therefore, is not "conserved" as an entity), each of the individual parental strands remains intact in one of the two new duplexes.

1. Separation of the two DNA strands

In order for the two strands of the parental double helical DNA to be replicated, they must first separate. In prokaryotic organisms, DNA replication begins at a single, unique nucleotide sequence a site called the origin of replication. This site includes a short sequence composed almost exclusively of AT base pairs that facilitate melting. In eukaryotes, replication begins at multiple sites along the DNA helix. Having multiple origins of replication provides a mechanism for rapidly replicating the great length of the eukaryotic DNA molecules.
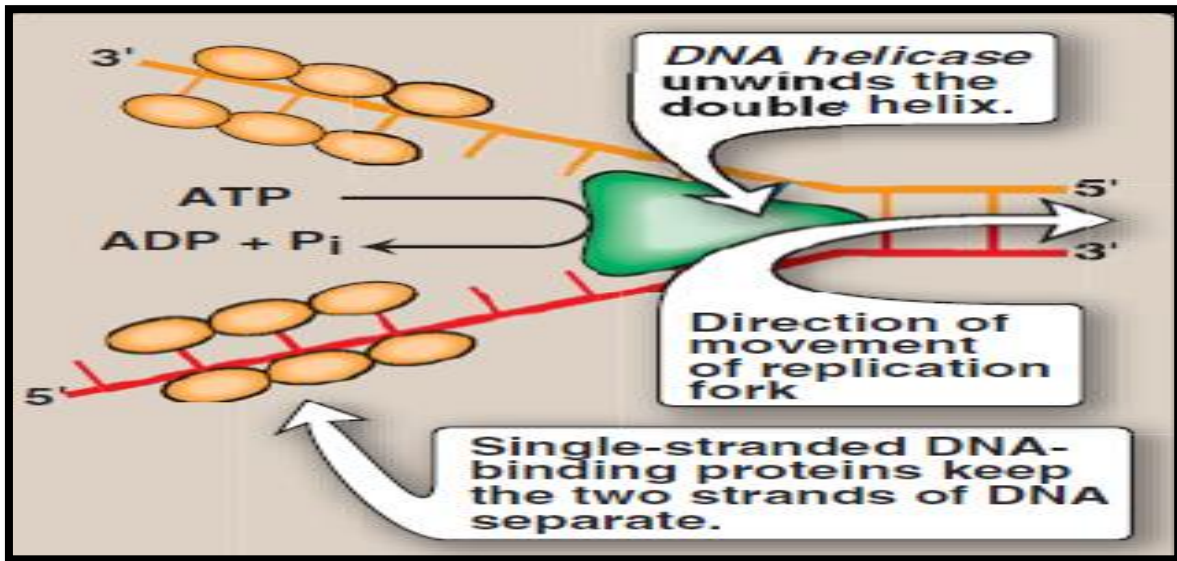
A. Formation of the replication fork

As the two strands unwind and separate, they form a "V" where active synthesis occurs. This region is called the **replication fork**. It moves along the DNA molecule as synthesis occurs. Proteins required for DNA strand separation:

1. DnaA protein: DnaA protein binds to specific nucleotide sequences at the origin of replication (AT-rich regions). Melting is ATP-dependent, and results in strand separation with the formation of localized regions of ssDNA.

2. DNA helicases: These enzymes bind to ssDNA near the replication fork, and then move into the neighboring double stranded region, unwinding the double helix. Helicases require energy provided by ATP.

3. Single-stranded DNA-binding (SSB) proteins: These proteins bind to the ssDNA generated by helicases. These proteins not only keep the two strands of DNA separated in the area of the replication origin, thus providing the single-stranded template required by polymerases, but also protect the DNA from nucleases that degrade ssDNA.

Formation of the replication fork

B. Solving the problem of supercoils:

As the two strands of the double helix are separated, a problem is encountered, namely, the appearance of supercoils in the region of DNA ahead of the replication fork. To solve this problem, there is a group of enzymes called DNA topoisomerases, which are responsible for removing supercoils in the helix.

2. Direction of DNA replication

The DNA polymerases responsible for copying the DNA templates are only able to "read" the parental nucleotide sequences in the 3'→5' direction, and they synthesize the new DNA strands only in the 5'→3' (antiparallel) direction. Therefore, beginning with one parental double helix, the two newly synthesized stretches of nucleotide chains must grow in opposite directions, one in the 5'→3' direction toward the replication fork and one in the 5'→3' direction away from the replication fork.

3. RNA primer

DNA polymerases cannot initiate synthesis of a complementary strand of DNA on a totally single-stranded template. Rather, they require an RNA primer that is, a short, double-stranded region consisting of RNA base paired to the DNA template,

with a **free hydroxyl group on the 3'-end** of the RNA strand. This hydroxyl group serves as the first acceptor of a deoxynucleotide by action of DNA polymerase. Primase (RNA polymerase) synthesizes the short stretches of RNA (approximately ten nucleotides long) that are complementary and antiparallel to the DNA template. In the resulting hybrid duplex, the U in RNA pairs with A in DNA.

4. Chain elongation

DNA polymerases elongate a new DNA strand by adding deoxyribonucleotides (dATP, dTTP, dCTP, and dGTP) to the **3'-hydroxyl group** of the RNA primer.
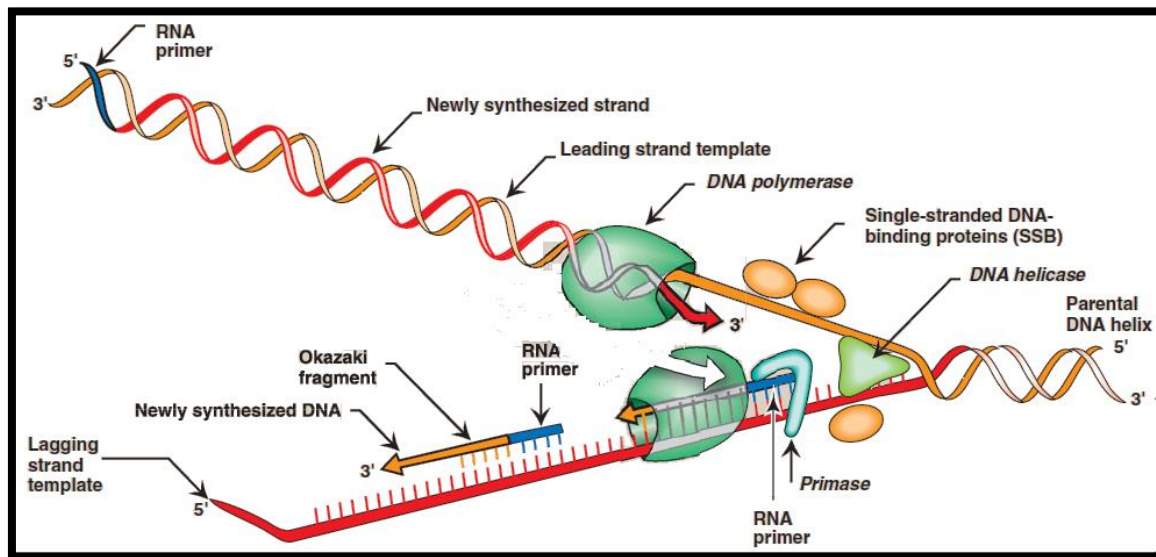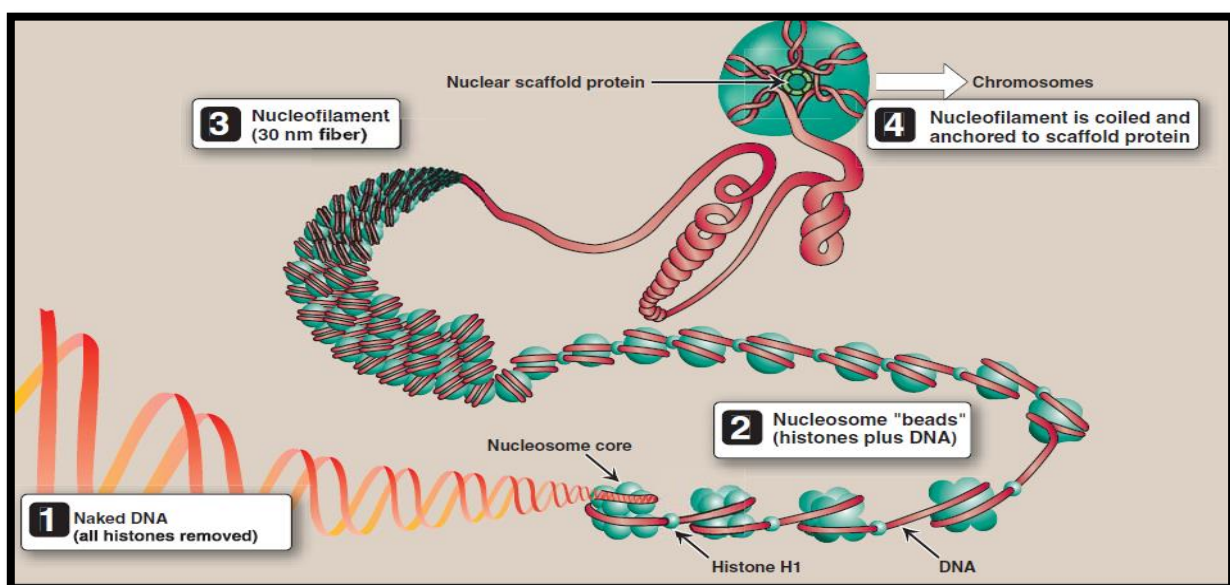

Elongation of the strands

Organization of eukaryotic DNA

A typical human cell contains 46 chromosomes, whose total DNA is approximately **1m** long. It is difficult to imagine how such a large amount of genetic material can be effectively packaged into a volume the size of a cell nucleus so that it can be efficiently replicated and its genetic information expressed. To do so requires the interaction of DNA with a large number of proteins, each of which performs a specific function in the ordered packaging of these long molecules of DNA.

Eukaryotic DNA is associated with tightly bound basic proteins, called histones. There are five classes of histones, designated H1, H2A, H2B, H3, and H4. These small proteins are positively charged at physiologic pH as a result of their high content of lysine and arginine. Because of their positive charge, they form ionic bonds with negatively charged DNA.

Two molecules each of H2A, H2B, H3, and H4 form the structural core of the individual nucleosome "beads." Around this core, a segment of the DNA double helix is wound nearly twice, forming a negatively super twisted helix. Neighboring nucleosomes are joined by "linker" DNA approximately 50 base pairs long. Histone H1, of which there are several related species, is not found in the nucleosome core, but instead binds to the linker DNA chain between the nucleosome beads.

Nucleosomes can be packed more tightly to form a polynucleosome (also called a nucleofilament). This structure assumes the shape of a coil, often referred to as a 30-nm fiber. The fiber is organized into loops that are anchored by a nuclear scaffold containing several proteins. Additional levels of organization lead to the final chromosomal structure.

Organization of eukaryotic DNA