Nucleotides, Nucleic acids: General Information about Structure, Functions and Metabolism

A manual for independent work at home and in class for students of second year study of international faculty Speciality “Medicine”

Zaporizhzhia, 2016
The manual was approved on the Central Methodological Council of ZSMU on «_____» ___________2016, the protocol №_______

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This manual is recommended to use for students of International Faculty (the second year of study) for independent work at home and in class.

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INTRODUCTION

A study of questions for this manual is the basis for learning of all metabolic pathways for nucleotides and nucleic acids. The knowledge about replication, transcription and metabolism of nucleotides gives ability for any student to explain in a right way the mechanism of some somatic and genetic disorders development (a gout, some inherited disorders of nucleotide synthesis, etc.), to understand the influence of some regulation factors on nucleotide and nucleic acids metabolism, among them some preparations are discussed for treatment of these disorders.
Nucleoproteins are compounds containing nucleic acid and protein, especially, protamines and histones. These are usually the salt-like compounds of proteins since the two components have opposite charges and are bound to each other by electrostatic forces. They are present in nuclear substances as well as in the cytoplasm. These may be considered as the sites for the synthesis of proteins and enzymes.

There are two kinds of nucleoproteins: 1) Deoxyribonucleoproteins (DNP) – deoxyribonucleic acid (DNA) is prosthetic group; 2) Ribonucleoproteins (RNP) – ribonucleic acid (RNA) is prosthetic group.

Nucleoproteins are of central importance in the storage, transmission, and expression of genetic information.

Nucleotides and their derivatives are biologically ubiquitous substances that participate in nearly all biochemical processes:

1. They form the monomeric units of nucleic acids and thereby play central roles in both the storage and the expression of genetic information.

2. Nucleoside triphosphates, most conspicuously ATP, are the “energy-rich” end products of the majority of energy-releasing pathways and the substances whose utilization drives most energy-requiring processes.

3. Most metabolic pathways are regulated, at least in part, by the levels of nucleotides such as ATP and ADP. Moreover, certain nucleotides function as intracellular signals that regulate the activities of numerous metabolic processes.

4. Nucleotide derivatives, such as nicotinamide adenine dinucleotide, flavin adenine dinucleotide, and coenzyme A, are required participants in many enzymatic reactions.

5. As components of the enzymelike nucleic acids known as ribozymes, nucleotides have important catalytic activities themselves.

Nucleotides, Nucleosides, and Bases

Nucleotides are phosphate esters of a five-carbon sugar (which is known as a pentose) in which a nitrogenous base is covalently linked to C1′ of the sugar residue.
In ribonucleotides (Fig. 1), the monomeric units of RNA, the pentose is D-ribose, whereas in deoxyribonucleotides (or just deoxynucleotides; Fig. 1), the monomeric units of DNA, the pentose is 2′-deoxy-D-ribose (note that the “primed” numbers refer to the atoms of the ribose residue; “unprimed” numbers refer to atoms of the nitrogenous base). The phosphate group may be bonded to C5′ of the pentose to form a 5′-nucleotide (Fig. 1) or to its C3′ to form a 3′-nucleotide. If the phosphate group is absent, the compound is known as a nucleoside. A 5′-nucleotide, for example, may therefore be referred to as a nucleoside-5′-phosphate. In all naturally occurring nucleotides and nucleosides, the bond linking the nitrogenous base to the pentose C1′ atom (which is called a glycosidic bond) extends from the same side of the ribose ring as does the C4′-C5′ bond (the so-called β configuration) rather than from the opposite side (the α configuration). Note that nucleotide phosphate groups are doubly ionized at physiological pH’s; that is, nucleotides are moderately strong acids.

Figure 1. Chemical Structures of Ribonucleotides and Deoxyribonucleotides.

The nitrogenous bases are planar, aromatic, heterocyclic molecules which, for the most part, are derivatives of either purine or pyrimidine.

The structures, names, and abbreviations of the common bases, nucleosides, and nucleotides are given in Table 1. The major purine components of nucleic acids are adenine and guanine residues; the major pyrimidine residues are those of cytosine, uracil (which occurs mainly in RNA), and thymine (5-methyluracil, which occurs mainly in DNA). The purines form glycosidic bonds to ribose via their N9 atoms,
whereas pyrimidines do so through their N1 atoms (note that purines and pyrimidines have dissimilar atom numbering schemes).

Table 1. Names and Abbreviations of Nucleic Acid Bases, Nucleosides, and Nucleotides.

<table>
<thead>
<tr>
<th>Base Formula</th>
<th>Base (X = H)</th>
<th>Nucleoside (X = ribose*)</th>
<th>Nucleotide (X = ribose phosphate*)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Adenine</td>
<td>Adenosine</td>
<td>Adenylic acid</td>
</tr>
<tr>
<td></td>
<td>Ade</td>
<td>Ado</td>
<td>Adenosine monophosphate AMP</td>
</tr>
<tr>
<td></td>
<td>Gua</td>
<td>Guanosine</td>
<td>Guanylic acid</td>
</tr>
<tr>
<td></td>
<td>G</td>
<td>Guo</td>
<td>Guanosine monophosphate GMP</td>
</tr>
<tr>
<td></td>
<td>Cyt</td>
<td>Cytidine</td>
<td>Cytidylic acid</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>Cyd</td>
<td>Cytidine monophosphate CMP</td>
</tr>
<tr>
<td></td>
<td>Ura</td>
<td>Uridine</td>
<td>Uridylic acid</td>
</tr>
<tr>
<td></td>
<td>U</td>
<td>Urd</td>
<td>Uridine monophosphate UMP</td>
</tr>
<tr>
<td></td>
<td>Thymine</td>
<td>Deoxythymidine-</td>
<td>Deoxythymidyl acid</td>
</tr>
<tr>
<td></td>
<td>Thy</td>
<td>dThd</td>
<td>Deoxythymidine monophosphate dTMP</td>
</tr>
</tbody>
</table>

* The presence of a 2′-deoxyribose unit in place of ribose, as occurs in DNA, is implied by the prefixes “deoxy” or “d.” For example, the deoxynucleoside of adenine is deoxyadenosine or dA. However, for thymine-containing residues, which rarely occur in RNA, the prefix is redundant and may be dropped. The presence of a ribose unit may be explicitly implied by the prefixes “ribo” or “r.” Thus the ribonucleotide of thymine is ribothymidine or rT.

The Chemical Structures of DNA and RNA. The chemical structures of the nucleic acids were elucidated by the early 1950s largely through the efforts of Phoebus Levene, followed by the work of Alexander Todd. Nucleic acids are, with few exceptions, linear polymers of nucleotides whose phosphate groups bridge the 3′ and 5′ positions of successive sugar residues (e.g., Fig. 2). The phosphates of these polynucleotides, the phosphodiester groups, are acidic, so that, at physiological pH’s, nucleic acids are polyanions. Polynucleotides have directionality, that is, each
has a 3′ end (the end whose C3′ atom is not linked to a neighboring nucleotide) and a 5′ end (the end whose C5′ atom is not linked to a neighboring nucleotide).

Figure 2. The Tetranucleotide Adenyl-3′,5′-uridyl-3′,5′-cytidyl-3′,5′-guanylyl-3′-phosphate.

**DNA has equal numbers of adenine and thymine residues (A = T) and equal numbers of guanine and cytosine residues (G = C).** These relationships, known as **Chargaff’s rules**, were discovered in the late 1940s by Erwin Chargaff, who first devised reliable quantitative methods for the separation and analysis of DNA hydrolysates. Chargaff also found that the base composition of DNA from a given organism is characteristic of that organism; that is, it is independent of the tissue from which the DNA is taken as well as the organism’s age, its nutritional state, or any other environmental factor. The structural basis for Chargaff’s rules is that in double-
stranded DNA, G is always hydrogen bonded (forms a **base pair**) with C, whereas A always forms a base pair with T (Fig. 3).

**Figure 3.** Base Pairs in DNA Structure.

DNA’s base composition varies widely among different organisms. It ranges from ~25% to 75% G+C in different species of bacteria. It is, however, more or less constant among related species; for example, in mammals G+C ranges from 39% to 46%.

RNA, which usually occurs as single-stranded molecules, has no apparent constraints on its base composition. However, double-stranded RNA, which comprises the genetic material of certain viruses, also obeys Chargaff’s rules (here A base pairs with U in the same way it does with T in DNA). Conversely, single-stranded DNA, which occurs in certain viruses, does not obey Chargaff’s rules. On entering its host organism, however, such DNA is replicated to form a double-stranded molecule, which then obeys Chargaff’s rules.

Some DNAs contain bases that are chemical derivatives of the standard set. For example, dA and dC in the DNAs of many organisms are partially replaced by **N^6-methyl-dA** and **5-methyl-dC**, respectively.
The altered bases are generated by the sequence-specific enzymatic modification of normal DNA. The modified DNAs obey Chargaff’s rules if the derivatized bases are taken as equivalent to their parent bases. Likewise, many bases in RNAs and, in particular, those in transfer RNAs (tRNAs) are derivatized.

**Nucleic Acids**

DNA and RNA are long linear polymers, called nucleic acids, that carry information in a form that can be passed from one generation to the next. These macromolecules consist of a large number of linked nucleotides, each composed of a sugar, a phosphate, and a base. Sugars linked by phosphates form a common backbone, whereas the bases vary among four kinds. Genetic information is stored in the sequence of bases along a nucleic acid chain. The bases have an additional special property: they form specific pairs with one another that are stabilized by hydrogen bonds. The base pairing results in the formation of a double helix, a helical structure consisting of two strands. These base pairs provide a mechanism for copying the genetic information in an existing nucleic acid chain to form a new chain. Although RNA probably functioned as the genetic material very early in evolutionary history, the genes of all modern cells and many viruses are made of DNA. DNA is replicated by the action of DNA polymerase enzymes. These exquisitely specific enzymes copy sequences from nucleic acid templates with an error rate of less than 1 in 100 million nucleotides.

**DNA.** The determination of the structure of DNA by Watson and Crick in 1953 is often said to mark the birth of modern molecular biology. The **Watson-Crick structure** of DNA is of such importance because, in addition to providing the structure of what is arguably the central molecule of life, it suggested the molecular
mechanism of heredity. Watson and Crick’s accomplishment, which is ranked as one of science’s major intellectual achievements, tied together the less than universally accepted results of several diverse studies:

1. Chargaff’s rules. At the time, the relationships $A=T$ and $G=C$ were quite obscure because their significance was not apparent. In fact, even Chargaff did not emphasize them.

2. Correct tautomeric forms of the bases. X-ray, nuclear magnetic resonance (NMR), and spectroscopic investigations have firmly established that the nucleic acid bases are overwhelmingly in the keto tautomeric forms shown in Table 1. In 1953, however, this was not generally appreciated. Indeed, guanine and thymine were widely believed to be in their enol forms (Fig. 4) because it was thought that the resonance stability of these aromatic molecules would thereby be maximized. Knowledge of the dominant tautomeric forms, which was prerequisite for the prediction of the correct hydrogen bonding associations of the bases, was provided by Jerry Donohue, an office mate of Watson and Crick and an expert on the X-ray structures of small organic molecules.

![Figure 4. Some Possible Tautomeric Conversions for Bases.](image)

3. Information that DNA is a helical molecule. This was provided by an X-ray diffraction photograph of a DNA fiber taken by Rosalind Franklin. This photograph
enabled Crick, an X-ray crystallographer by training who had earlier derived the equations describing diffraction by helical molecules, to deduce (a) that DNA is a helical molecule and (b) that its planar aromatic bases form a stack of parallel rings which is parallel to the fiber axis.

This information only provided a few crude landmarks that guided the elucidation of the DNA structure. It mostly sprang from Watson and Crick’s imaginations through model building studies. Once the Watson-Crick model had been published, however, its basic simplicity combined with its obvious biological relevance led to its rapid acceptance. Later investigations have confirmed the essential correctness of the Watson-Crick model, although its details have been modified.

**The Watson-Crick Structure: B-DNA.** Fibers of DNA assume the so-called B conformation, as indicated by their X-ray diffraction patterns, when the counterion is an alkali metal such as Na\(^+\) and the relative humidity is >92%. B-DNA is regarded as the native (biologically functional) form of DNA because, for example, its X-ray pattern resembles that of the DNA in intact sperm heads.

The Watson-Crick structure of B-DNA has the following major features:

1. It consists of two polynucleotide strands that wind about a common axis with a right-handed twist to form an ~20-Å-diameter double helix (Fig. 5). The two strands are antiparallel (run in opposite directions) and wrap around each other such that they cannot be separated without unwinding the helix. The bases occupy the core of the helix and the sugar-phosphate chains are coiled about its periphery, thereby minimizing the repulsions between charged phosphate groups.

2. The planes of the bases are nearly perpendicular to the helix axis. Each base is hydrogen bonded to a base on the opposite strand to form a planar base pair (Fig. 5). It is these hydrogen bonding interactions, a phenomenon known as complementary base pairing, that result in the specific association of the two chains of the double helix.

3. The “ideal” B-DNA helix has 10 base pairs (bp) per turn (a helical twist of 36° per bp) and, since the aromatic bases have van der Waals thicknesses of 3.4 Å
and are partially stacked on each other (base stacking, Fig. 5), the helix has a pitch (rise per turn) of 34 Å.

![Figure 5. Three-dimensional Structure of B-DNA.](image)

The most remarkable feature of the Watson-Crick structure is that it can accommodate only two types of base pairs: Each adenine residue must pair with a thymine residue and vice versa, and each guanine residue must pair with a cytosine residue and vice versa. The geometries of these A-T and G-C base pairs, the so-called Watson-Crick base pairs, are shown in Figure 6. It can be seen that both of these base pairs are interchangeable in that they can replace each other in the double helix without altering the positions of the sugar-phosphate backbone’s C1’ atoms. Likewise, the double helix is undisturbed by exchanging the partners of a Watson-Crick base pair, that is, by changing a G-C to a C-G or an A-T to a T-A. In contrast, any other combination of bases (e.g., A-G or A-C) would significantly distort the double helix since the formation of a non-Watson-Crick base pair would require considerable reorientation of the sugar-phosphate chain.
B-DNA has two deep exterior grooves that wind between its sugar-phosphate chains as a consequence of the helix axis passing through the approximate center of each base pair. However, the grooves are of unequal size (Fig. 5) because (1) the top edge of each base pair, as drawn in Figure 6, is structurally distinct from the bottom edge; and (2) the deoxyribose residues are asymmetric. The minor groove exposes that edge of a base pair from which its C1’ atoms extend, whereas the major groove exposes the opposite edge of each base pair.

Although B-DNA is, by far, the most prevalent form of DNA in the cell, double helical DNAs and RNAs can assume several distinct structures.

**Figure 6.** Watson-Crick Base Pairs.

**Other Nucleic Acid Helices.** X-ray fiber diffraction studies, revealed that nucleic acids are conformationally variable molecules. Indeed, double helical DNA and RNA can assume several distinct structures that vary with such factors as the humidity and the identities of the cations present, as well as with base sequence. For example, fibers of B-DNA form in the presence of alkali metal ions such as Na⁺ when the relative humidity is 92%.
When the relative humidity is reduced to 75%, B-DNA undergoes a reversible conformational change to the so-called A form. Fiber X-ray studies indicate that A-DNA forms a wider and flatter right-handed helix than does B-DNA (Table 2). A-DNA has 11.6 bp per turn and a pitch of 34 Å, which gives A-DNA an axial hole (Fig. 7). A-DNA’s most striking feature, however, is that the planes of its base pairs are tilted 20° with respect to the helix axis. Since its helix axis passes “above” the major groove side of the base pairs rather than through them as in B-DNA, A-DNA has a deep major groove and a very shallow minor groove; it can be described as a flat ribbon wound around a 6-Å-diameter cylindrical hole. Most self-complementary oligonucleotides of <10 base pairs, for example, d(GGCCGGGCC) and d(GGTATAACC), crystallize in the A-DNA conformation. Like B-DNA, these molecules exhibit considerable sequence-specific conformational variation although the degree of variation is less than that in B-DNA.

<table>
<thead>
<tr>
<th></th>
<th>A-DNA</th>
<th>B-DNA</th>
<th>Z-DNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Helical sense</td>
<td>Right-handed</td>
<td>Right-handed</td>
<td>Left-handed</td>
</tr>
<tr>
<td>Diameter</td>
<td>~26 Å</td>
<td>~20 Å</td>
<td>~18 Å</td>
</tr>
<tr>
<td>Base pairs per helical turn</td>
<td>11.6</td>
<td>10</td>
<td>12 (6 dimers)</td>
</tr>
<tr>
<td>Helical twist per base pair</td>
<td>31°</td>
<td>36°</td>
<td>9° for pyrimidine-purine steps; 51° for purine-pyrimidine steps</td>
</tr>
<tr>
<td>Helix pitch (rise per turn)</td>
<td>34 Å</td>
<td>34 Å</td>
<td>44 Å</td>
</tr>
<tr>
<td>Helix rise per base pair</td>
<td>2.9 Å</td>
<td>3.4 Å</td>
<td>7.4 Å per dimer</td>
</tr>
<tr>
<td>Base tilt normal to the helix axis</td>
<td>20°</td>
<td>6°</td>
<td>7°</td>
</tr>
<tr>
<td>Major groove</td>
<td>Narrow and deep</td>
<td>Wide and deep</td>
<td>Flat</td>
</tr>
<tr>
<td>Minor groove</td>
<td>Wide and shallow</td>
<td>Narrow and deep</td>
<td>Narrow and deep</td>
</tr>
</tbody>
</table>

A-DNA has, so far, been observed in only three biological contexts: at the cleavage center of topoisomerase II, at the active site of DNA polymerase, and in certain Gram-positive bacteria that have undergone sporulation (the formation, under environmental stress, of resistant although dormant cell types known as spores; a sort of biological lifeboat). Such spores contain a high proportion (20%) of small acid-soluble spore proteins (SASPs). Some of these SASPs induce B-DNA to assume the A form, at least in vitro. The DNA in bacterial spores exhibits a resistance to UV-
induced damage that is abolished in mutants that lack these SASPs. This occurs because the B→A conformation change inhibits the UV-induced covalent cross-linking of pyrimidine bases, in part by increasing the distance between successive pyrimidines.

Figure 7. Structures of A-, B-, and Z-DNAs.

Occasionally, a seemingly well-understood or at least familiar system exhibits quite unexpected properties. Over 25 years after the discovery of the Watson-Crick structure, the crystal structure determination of the self-complementary hexanucleotide d(CGCGCG) by Andrew Wang and Alexander Rich revealed, quite surprisingly, a left-handed double helix (Fig. 7, Table 2). A similar helix is formed by d(CGCATGCG). This helix, which has been dubbed Z-DNA, has 12 Watson-Crick base pairs per turn, a pitch of 44 Å, and, in contrast to A-DNA, a deep minor groove and no discernible major groove (its helix axis passes “below” the minor groove side of its base pairs). Z-DNA therefore resembles a left-handed drill bit in appearance. The line joining successive phosphorus atoms on a polynucleotide strand of Z-DNA therefore follows a zigzag path around the helix (hence the name Z-DNA) rather than a smooth curve as it does in A- and B-DNAs.
A high salt concentration stabilizes Z-DNA relative to B-DNA by reducing the otherwise increased electrostatic repulsions between closest approaching phosphate groups on opposite strands (8 Å in Z-DNA vs 12 Å in B-DNA). The methylation of cytosine residues at C5, a common biological modification, also promotes Z-DNA formation since a hydrophobic methyl group in this position is less exposed to solvent in Z-DNA than it is in B-DNA.

Does Z-DNA have any biological function? Rich has proposed that the reversible conversion of specific segments of B-DNA to Z-DNA under appropriate circumstances acts as a kind of switch in regulating genetic expression, and there are indications that it transiently forms behind actively transcribing RNA polymerase. It was nevertheless surprisingly difficult to prove the in vivo existence of Z-DNA. A major difficulty was demonstrating that a particular probe for detecting Z-DNA, for example, a Z-DNA-specific antibody, does not in itself cause what would otherwise be B-DNA to assume the Z conformation – a kind of biological uncertainty principle (the act of measurement inevitably disturbs the system being measured). However, Rich has discovered several proteins that specifically bind Z-DNA, including a family of Z-DNA-binding protein domains named Zα. The existence of these proteins strongly suggests that Z-DNA does, in fact, exist in vivo.

The DNA molecules in human chromosomes are linear. However, electron microscopic and other studies have shown that intact DNA molecules from some other organisms are circular (Fig. 8). The term circular refers to the continuity of the DNA chains, not to their geometric form. DNA molecules inside cells necessarily have a very compact shape. Note that the E. coli chromosome, fully extended, would be about 1000 times as long as the greatest diameter of the bacterium.

A closed DNA molecule has a property unique to circular DNA. The axis of the double helix can itself be twisted or supercoiled into a superhelix (Fig. 9). Supercoiling is biologically important for two reasons. First, a supercoiled DNA molecule has a more compact shape than does its relaxed counterpart. Second, supercoiling may hinder or favor the capacity of the double helix to unwind and thereby affect the interactions between DNA and other molecules.
In general, adenine base containing DNA tracts are rigid and straight. **Bent** conformation of DNA occurs when A-tracts are replaced by other bases or a collapse of the helix into the minor groove of A-tract. Bending in DNA structure has also been reported due to photochemical damage or mispairing of bases. Certain antitumor drugs (e.g. cisplatin) produce bent structure in DNA. Such changed structure can take up proteins that damage the DNA.

**Figure 8.** Circular DNA.

**Figure 9.** Supercoiling in a Prokaryotic Cell.

**Triple-stranded DNA** formation may occur due to additional hydrogen bonds between the bases. Thus, a thymine can selectively form two **Hoogsteen hydrogen bonds** to the adenine of A-T pair to form **T-A-T**. Likewise, a protonated cytosine can also form two hydrogen bonds with guanine of C-C pairs that results in **C-G-C**.
Triple-helical structure is less stable than double helix. This is due to the fact that the three negatively charged backbone strands in triple helix results in an increased electrostatic repulsion.

Polynucleotides with very high contents of guanine can form a novel tetrameric structure called G-quartets. These structures are planar and are connected by Hoogsteen hydrogen bonds. Antiparallel four-stranded DNA structures, referred to as G-tetraplexes have also been reported. The ends of eukaryotic chromosomes namely telomeres are rich in guanine, and therefore form G-tetraplexes. In recent years, telomeres have become the targets for anticancer chemotherapies. G-tetraplexes have been implicated in the recombination of immunoglobulin genes, and in dimerization of double-stranded genomic RNA of the human immunodeficiency virus (HIV).

RNA. RNA molecules are synthesized in a process referred to as transcription. During transcription, new RNA molecules are produced by a mechanism similar to DNA synthesis, that is, through complementary base pair formation. The sequence of bases in RNA is therefore specified by the base sequence in one of the two strands in DNA. For example, the DNA sequence 5′-CCGATTACG-3′ is transcribed into the RNA sequence 3′-GGCUAAUGC-5′. Complementary DNA and RNA sequences are antiparallel. RNA molecules differ from DNA in the following ways:

1. The sugar moiety of RNA is ribose instead of deoxyribose in DNA.

2. The nitrogenous bases in RNA differ somewhat from those observed in DNA. Instead of thymine, RNA molecules use uracil. In addition, the bases in some RNA molecules are modified by a variety of enzymes (e.g., methylases, thiolases, and deaminases).

3. In contrast to the double helix of DNA, RNA exists as a single strand. For this reason, RNA can coil back on itself and form unique and often quite complex three-dimensional structures (Fig. 10). The shape of these structures is determined by complementary base pairing by specific RNA sequences, as well as by base stacking. In addition, the 2′-OH of ribose can form hydrogen bonds with nearby molecular groups. Because RNA is single stranded, Chargaff's rules do not apply. An RNA molecule’s contents of A and U, as well as C and G, are usually not equal.
The most prominent types of RNA are transfer RNA, ribosomal RNA, and messenger RNA.

**Transfer RNA.** Transfer RNA (tRNA) molecules transport amino acids to ribosomes for assembly into proteins. Comprising about 15% of cellular RNA the average length of a tRNA molecule is 75 nucleotides. Because each tRNA molecule becomes bound to a specific amino acid, cells possess at least one type of tRNA for each of the 20 amino acids commonly found in protein. The three-dimensional structure of tRNA molecules, which resembles a warped cloverleaf (Fig. 11), results primarily from extensive intrachain base pairing. tRNA molecules contain a variety of modified bases. Examples include pseudouridine, 4-thiouridine, 1-methylguanosine, and dihydouridine:

![Modified Bases](image)

The structure of tRNA allows it to perform two critical functions involving the most important structural components: the 3′-terminus and the anticodon loop.
Figure 11. Transfer RNA. (a) Three-dimensional structure of a tRNA molecule. (b) A schematic view of a tRNA molecule.

The 3′-terminus forms a covalent bond to a specific amino acid. The anticodon loop contains a three-base-pair sequence that is complementary to the DNA triplet code for the specific amino acid. The conformational relationship between the 3′-terminus and the anticodon loop allows the tRNA to align its attached amino acid properly during protein synthesis. tRNAs also possess three other prominent structural features, referred to as the D loop, the TψC loop (ψ is an abbreviation for the modified base pseudouridine), and the variable loop. The function of these structures is unknown, but they are presumably related to the alignment of tRNA within the ribosome and/or the binding of a tRNA to the enzyme that catalyzes the attachment of the appropriate amino acid. The D loop is so named because it contains dihydrouridine. Similarly, the TψC loop contains the base sequence thymine,
pseudouridine, and cytosine. tRNAs can be classified on the basis of the length of their variable loop. The majority (approximately 80%) of tRNAs have variable loops with four to five nucleotides, whereas the others have variable loops with as many as 20 nucleotides.

**Ribosomal RNA.** Ribosomal RNA (rRNA) is the most abundant form of RNA in living cells. In most cells, rRNA constitutes approximately 80% of the total RNA. The secondary structure of rRNA is extraordinarily complex. Although there are species differences in the primary nucleotide sequences of rRNA, the overall three-dimensional structure of this class of molecules is conserved. As its name suggests, rRNA is a component of ribosomes.

Ribosomes are cytoplasmic structures that synthesize proteins. Because they are composed of both protein and rRNA, the ribosomes are sometimes described as ribonucleoprotein bodies. The ribosomes of prokaryotes and eukaryotes are similar in shape and function, although they differ in size and their chemical composition. Several different kinds of rRNA and protein are found in each type of ribosomal subunit. The large ribosomal subunit of E. coli, for example, contains 5 S and 23 S rRNAs and 34 polypeptides. The small ribosomal subunit of E. coli contains a 16 S rRNA and 21 polypeptides. A typical large eukaryotic ribosomal subunit contains three rRNAs (5 S, 5.8 S, and 28 S) and 49 polypeptides; the small subunit contains an 18 S rRNA and approximately 30 polypeptides. The functions of the rRNA and polypeptides in ribosomes are poorly understood and are being investigated.

**Messenger RNA.** As its name suggests, messenger RNA (mRNA) is the carrier of genetic information from DNA for the synthesis of protein. mRNA molecules, which typically constitute approximately 5% of cellular RNA, vary considerably in size. For example, mRNA from E. coli varies from 500 to 6000 nucleotides.

Prokaryotic mRNA and eukaryotic mRNA differ in several respects. First, many prokaryotic mRNAs are polycistronic, that is, they contain coding information for several polypeptide chains. In contrast, eukaryotic mRNA typically codes for a single polypeptide and is therefore referred to as monocistronic. A cistron is a DNA
sequence that contains the coding information for a polypeptide and several signals that are required for ribosome function. Second, prokaryotic and eukaryotic mRNAs are processed differently. In contrast to prokaryotic mRNAs, which are translated into protein by ribosomes during or immediately after they are synthesized, eukaryotic mRNAs are modified extensively. These modifications include capping (linkage of 7-methylguanosine to the 5′-terminal residue), splicing (removal of introns), and the attachment of an adenylate polymer referred to as a poly A tail.

**Heterogeneous RNA and small nuclear RNA.** Heterogeneous RNA and small nuclear RNA play complementary roles in eukaryotic cells. Heterogeneous nuclear RNA (hnRNA) molecules are the primary transcripts of DNA and are the precursors of mRNA. HnRNA is processed by splicing and modifications to form mRNA. Splicing is the enzymatic removal of the introns from the primary transcripts. A class of small nuclear RNA (snRNA) molecules (containing between 90 and 300 nucleotides), which are complexed with several proteins to form small nuclear ribonucleoprotein particles (snRNP or snurps), are involved in splicing activities and other forms of RNA processing.

**Chromatin Organization in Nucleus**

The fact that DNA in eukaryotic chromosomes is not bare. Instead, eukaryotic DNA is tightly bound to a group of small basic proteins called histones. Histones constitute half the mass of a eukaryotic chromosome. The entire complex of a cell’s DNA and associated protein is called chromatin. Five major histones are present in chromatin: four histones, called $H2A$, $H2B$, $H3$, and $H4$, associate with one another; the other histone is called $H1$. Histones have strikingly basic properties because a quarter of the residues in each histone are either arginine or lysine.

Chromatin is made up of repeating units, each containing 200 bp of DNA and two copies each of $H2A$, $H2B$, $H3$, and $H4$, called the histone octamer. These repeating units are known as nucleosomes. Strong support for this model comes from the results of a variety of experiments, including observations of appropriately prepared samples of chromatin viewed by electron microscopy. Chromatin viewed
with the electron microscope has the appearance of beads on a string; each bead has a
diameter of approximately 100 Å. Partial digestion of chromatin with DNase yields
the isolated beads. These particles consist of fragments of DNA about 200 bp in
length bound to the eight histones. More-extensive digestion yields a shorter DNA
fragment of 145 bp bound to the histone octamer. The smaller complex formed by the
histone octamer and the 145-bp DNA fragment is the nucleosome core particle. The
DNA connecting core particles in undigested chromatin is called linker DNA. Histone
H1 binds, in part, to the linker DNA (Fig. 12).

Figure 12. Chromatin organization.

The overall structure of the nucleosome was revealed through electron
microscopic and x-ray crystallographic studies pioneered by Aaron Klug and his
colleagues. More recently, the three-dimensional structure of a reconstituted
nucleosome core was determined to higher resolution by x-ray diffraction methods.
As was shown by Evangelos Moudrianakis, the four types of histone that make up the
protein core are homologous and similar in structure. The eight histones in the core
are arranged into a \((\text{H3})_2(\text{H4})_2\) tetramer and a pair of \(\text{H2A-H2B}\) dimers. The tetramer
and dimers come together to form a left-handed superhelical ramp around which the
DNA wraps. In addition, each histone has an amino-terminal tail that extends out
from the core structure. These tails are flexible and contain a number of lysine and
arginine residues. As we shall see, covalent modifications of these tails play an
essential role in modulating the affinity of the histones for DNA and other properties.
The DNA forms a left-handed superhelix as it wraps around the outside of the histone octamer. The protein core forms contacts with the inner surface of the DNA superhelix at many points, particularly along the phosphodiester backbone and the minor groove. Nucleosomes will form on almost all DNA sites, although some sequences are preferred because the dinucleotide steps are properly spaced to favor bending around the histone core. A histone with a different structure from that of the others, called histone H1, seals off the nucleosome at the location at which the linker DNA enters and leaves. The amino acid sequences of histones, including their aminoterminal tails, are remarkably conserved from yeast through human beings.

The winding of DNA around the nucleosome core contributes to the packing of DNA by decreasing its linear extent. An extended 200-bp stretch of DNA would have a length of about 680 Å. Wrapping this DNA around the histone octamer reduces the length to approximately 100 Å along the long dimension of the nucleosome. Thus the DNA is compacted by a factor of 7. However, human chromosomes in metaphase, which are highly condensed, are compacted by a factor of $10^4$. Clearly, the nucleosome is just the first step in DNA compaction. What is the next step? The nucleosomes themselves are arranged in a helical array approximately 360 Å across, forming a series of stacked layers approximately 110 Å apart (Fig. 13). The folding of these fibers of nucleosomes into loops further compacts DNA.

**Figure 13.** Steps of chromatin organization.
The wrapping of DNA around the histone core as a left-handed helix also stores negative supercoils; if the DNA in a nucleosome is straightened out, the DNA will be underwound. This underwinding is exactly what is needed to separate the two DNA strands during replication and transcription.

**Metabolism of Purine and Pyrimidine Nucleotides.**

**Disorders of Nucleotide Metabolism.**

The metabolism of nucleic acids is composed from anabolic pathways (DNA synthesis - replication; RNA synthesis – transcription) and their catabolic pathways (degradation) up to terminal products for humans (uric acid, urea, carbon dioxide and water). All these pathways are associated with the metabolism of nucleotides: their synthetic ways and degradation, too.

The breakdown of nucleoprotein containing DNA or RNA may be in the beginning both in gastrointestinal tract (GIT) and in tissues. Complete way destruction of nucleoprotein in GIT is represented in figure 14. Enzymes which are in cleavage of phosphor diester bonds of polynucleotide chains are named respectively the type of nucleic acid: **DNA-nuclease or RNA-nuclease.** The removal of phosphate from nucleotidie molecules is catalyzed by **special phosphatases** (may be **5'-nucleotidase** in name), and nucleosides are formed. Destruction of nucleosides is found mainly in human tissues. But about 3% of their total content is derived into terminal products of destruction in the large intestine.

All the nucleosides are absorbed in GIT and transported across blood to all the tissues to be destructed there mainly. Only d-thymidine may be involved in the synthesis of corresponded nucleotide – this is exception.

Therefore more then 95% of dietary nucleosides are destructed in human tissues. Their structures are shown in figure 15.
DIGESTION OF DEOXYRIBONUCLEOPROTEIN (DNP) IN GIT

**Figure 14.** Digestion steps of DNP in GIT.

**Figure 15.** Main nucleosides discussed for their metabolism in humans.

**Degradation of Purine Nucleosides in Cells of Tissues**

Adenosine is involved in hydrolytic deamination to form inosine (Fig. 16, step 1), then we have to consider its dephosphorylation due to special phosphorylase. Hypoxanthine is formed as a product (Fig. 16, step 2).

Xanthine oxidase catalyzed two reactions: conversion of hypoxanthine to xanthine (Fig. 16, step 3), and then there is formation of uric acid (Fig. 3, step 4). The latter enzyme is flavoprotein, keeps Mo$^{2+}$ and four Fe$^{2+}$-centres. Allopurinol is inhibitor of Xanthine oxidase. Guanosine is converted to guanine due to special
phosphorylase (Fig. 16, step 5), and then we can consider the deamination of guanine (Fig. 16, step 6) to form xanthine.

**Figure 16.** Degradation of purine nucleosides up to uric acid.

Uric acid may be in two forms: enol- and keto-form. Sodium ions form with enol-form of uric acid salt that is known as sodium urate (Fig. 17).

**Figure 17.** Enol- and keto- forms of uric acid; the formation of monosodium urate.
Terminal product of purine catabolism in non-primate mammals is allantoin, formed from uric acid.

**Degradation of Pyrimidine Nucleosides**

Uridine is formed due to deamination of cytidine, and then there are two catabolic pathways for two nucleosides in human tissues (Fig. 18): uridine and thymidine destructions.

**Figure 18.** Degradation of pyrimidine nucleosides.

Step by step they are destructed to give the end-products: urea (using the way for ammonia utilization), carbon dioxide, beta-alanine and β-aminoisobutyrate. β-alanine is involved in transamination with pyruvate to give formyl acetate that is cleaved into acetyl-CoA. Other way for its utilization may be: it is involved in the
synthesis of Anserine and Carnosine. The latter substances are used in muscles for to increase the myosin-ATPase activity.

β-Aminoisobutyrate is involved in direct deamination to form hydroxybutyrate, acetyl-CoA is formed after its oxidation. Due to the formation of acetyl-CoA in both cases we can consider β-alanine and β-aminoisobutyrate as energy sources, that is because acetyl-CoA is involved in Krebs cycle in any type of cell. Excretion of β-aminoisobutyrate increases in leukemia and severe X-ray radiation exposure due to increased destruction of nucleic acids.

**Synthesis de novo of Purine Nucleotides**

This type of synthesis is very important, first of all, for strong vegetarians (in all types of tissue) and for tissues, where we have to consider the high rate of regeneration processes (epithelial tissues, skin, bone marrow, liver).

The first three reactions are very important for regulation of this process (Fig. 19).

1) \[ \text{PRPP} + \text{ATP} \xrightarrow{\text{PRPP Synthetase}} \text{PRPP} \]

2) \[ \text{PRPP} + \text{Glutamine} \xrightarrow{\text{Phosphoribosyl aminotransferase}} \text{Phosphoribosylamine} \]

3) \[ \text{PRPP} + \text{Glycine} \xrightarrow{\text{Glycine amide-ribosyl-5-P synthetase}} \text{Glycinamide-ribosyl-5-P} \]

**Figure 19.** The initial reactions of purine nucleotide synthesis.
Then there are eight reactions that give the metabolite – Inosine monophosphate (IMP, Fig. 20). Nitrogen atom N\(^{(9)}\) is from Glutamine (reaction 2). Carbon atoms C\(^{(4)}\) and C\(^{(5)}\) and nitrogen N\(^{(7)}\) are from Glycine (reaction 3). Carbon C\(^{(8)}\) is from methenyl-Tetra Hydro Folic Acid (THFA) (formyl fragment is formed, reaction 4).

![Diagram of metabolite structure](image)

**Figure 20.** A structure of key intermediate metabolite for synthesis of purine nucleotides - Inosine Mono Phosphate (IMP): THFA- Tetra Hydro Folic Acid; PRPP - phosphoribosyl pyrophosphate.

N\(^{(3)}\) is incorporated from Glutamine (reaction 5). Then there is formation of imidazole fragment, the bond C\(^{(8)}\)–N\(^{(9)}\) is formed due to dehydration (reaction 6). After that aminimidazol-ribosyl-5-Phosphate is carboxylated in position C\(^{(5)}\) (reaction 7), C\(^{(6)}\) is incorporated in the structure from carbon dioxide. Reaction 8 is due to synthetase that catalyzed the attachment of the fragment from Aspartate. The product Amino imidazole succinyl carboxamide ribosyl-5-Phosphate is formed and N\(^{(1)}\) is incorporated.

Reaction 9 is the liberation of succinyl group as fumarate. Carbon atom C\(^{(2)}\) is added (reaction 10) from formyl-THFA. Reaction N11 is a ring closure by enzyme – IMP cyclohydrolase and IMP is formed.

Four ATP are used for IMP synthesis (reactions 1, 3, 5, 6). The formation of IMP is discussed as first phase of synthetic pathway.
The second phase of purine nucleotide synthesis is the formation of AMP or GMP from IMP (Fig. 21). Two reactions (1, 3, Fig. 21) are used for AMP synthesis from IMP, and GTP is used as energy source for reaction (1). Aspartate is used in the step (1, Fig. 21) to add nitrogen in a form of amino group instead of keto-group in position 6 of inosine fragment in IMP.

\[
\text{IMP} \xrightarrow{(1)} \text{AMP} \\
\text{Adenosyl succinate} \xrightarrow{(3)} \text{Fumarate} \\
\text{GTP} \xrightarrow{(2)} \text{NADH}+(\text{2})
\]

\[
\text{Mg}^{2+} \quad \text{NAD} \\
\text{GDP} \xrightarrow{(2)} \text{Xanthosine monophosphate} \\
\text{H}_3\text{PO}_4 \xrightarrow{(4)} \text{Glu} \\
\text{ATP} \quad \text{Gln}
\]

1 -- Adenosyl succinate synthetase  
2 -- IMP-dehydrogenase  
3 -- Adenosyl succinate lyase  
4 -- GMP-synthetase

**Figure 21.** The second phase of purine nucleotide synthesis.

Two reactions are used for GMP synthesis from IMP, and ATP is used as energy source in the step (4, Fig. 21). Glutamine is a donor of amine group for position 2 of guanine fragment in GMP.

It is in need to remember:

1. **Energy requirement for complete pathway per 1 mol of AMP or GMP is 5 ATP.**
2. **Special vitamins intake is for complete pathway:** B₉, B₁₂, B₅ (or PP); B₁₂ is required for formation of folic acid derivatives.

It should be noted that THFA derivatives formation requires the presence of special enzyme named dihydrofolate reductase, whose activity may be inhibited by competitive inhibitor - a preparation Methotrexate. The synthesis of mononucleotides is inhibited under the this condition, too.
Two reactions (Fig. 8, steps 1, 3) are used for AMP synthesis from IMP, and GTP is used as energy source for reaction (1). Aspartate is used in the step (1) to add nitrogen in a form of amino group instead of keto group in position 6 of inosine fragment in IMP.

GMP and AMP are considered as precursors for ATP and GTP, the latter compounds may be produced due to substrate phosphorylation due to special kinases. For example:

\[
\begin{array}{c}
\text{GMP} \xrightarrow{\text{ATP, ADP, kinase}} \text{GDP} \\
\xrightarrow{\text{ATP, ADP, kinase}} \text{GTP}
\end{array}
\]

But the most important way for ATP formation in aerobic cells is oxidative phosphorylation placed in the inner membrane of mitochondria.

**Salvage Reactions**

Some metabolites from purine nucleotide degradation may be involved in the synthesis of IMP, AMP, GMP. Those reactions are named as salvage reactions (Fig. 22). They are found in the liver, brain, polymorphonuclear leukocytes, lymphocytes:

**Figure 22.** Salvage reactions duration in humans.
Hypoxanthine guanine phosphoribosyl pyrophosphate transferase (HGPRT) catalyzed two reactions using hypoxanthine and guanine to form respectively IMP and GMP. Sometimes this enzyme may be disturbed in synthesis to cause pathologies development in humans. It will be discussed later.

**Pyrimidine Nucleotide de novo Synthesis**

Uridine monophosphate is synthesized in five reactions from carbamoyl phosphate and aspartic acid (Fig. 23). Carbamoyl phosphate may be synthesized in our tissues in two ways:
1) from ammonia due to carbamoyl phosphate synthetase I (placed in the liver, only);
2) from glutamine as donor of amine group for carbamoyl phosphate due to carbamoyl phosphate synthetase II, found in all the tissues, except nervous tissue.

So, this synthesis we can consider as the way for ammonia utilization, too, but in the liver, only.

![Diagram of UMP synthesis](image)

**Figure 23.** The synthesis of UMP; R – ribose-5-phosphate fragment.

The UMP is considered as the precursor for UTP (reaction 1) and CTP (reaction 2 below):
The synthesis of dTMP requires three steps (Fig. 24):

**Step 1.** The function of special multienzyme system that is used for transformation of riboderivative (UDP) to deoxyriboderivative (dUDP). This multienzyme system includes two enzymes:
- Ribonucleoside diphosphate reductase containing Thioredoxin as a non-protein part. During the first reaction the reduced form of Thioredoxin becomes the oxidized one;
- Thioredoxin reductase (NADPH – the non-protein part of enzyme) is used for transformation of oxidized form of Thioredoxin again to the reduced one.

**Step 2.** The function of dUDP phosphatase to form dUMP as a product.

**Step 3.** The function of Deoxythymidilate synthetase to form dTMP using special derivative of THFA.

Then again we can consider the transformation of dTMP to dTTP due to the action of special kinases.

**Figure 24.** The synthesis of dTMP from UDP.

**The Regulation of Nucleotide Synthesis**

- AMP, ADP GMP, GDP, TDP are considered as allosteric inhibitors for PRPP-synthetase when they are accumulated in cytoplasm (Fig. 25).
- High concentration of GMP is allosteric inhibitor for Phosphoribosyl aminotransferase (Fig. 25) in the purine nucleotide synthesis.
- The regulation of second stage of synthesis (Fig. 21), respectively: GTP accumulation causes the stimulation of AMP synthesis (adenosyl succinate lyase), ATP accumulation causes the stimulation of GMP synthesis (GMP-synthetase)
- The accumulation of PRPP is a positive factor in stimulation of Carbamoyl phosphate synthesis and then the synthesis of UMP. But the accumulation of UDP in a cell is considered as a factor for inhibition of discussed reaction.
- The accumulation of CTP is the factor for inhibition of Carbamoyl aspartate formation during the synthesis of UMP (Fig. 25).

**Figure 25.** The regulation of purine nucleotide synthesis
Clinical Disorders of Nucleotide Metabolism

Net excretion of total uric acid in healthy humans averages 400-600 mg/day (24 hours). Many pharmacologic and naturally occurring compounds influence renal absorption and secretion of sodium urate. High doses of aspirin competitively inhibit both urate excretion and reabsorption.

Urates (monosodium urate salts) are present in human fluids. Urates are far more soluble in water than uric acid. The lower the temperature of the medium the lower the solubility of urates in water.

Normal value of urates content in the blood plasma is not more than 0.42 mmol/l (for men) and 0.3 mmol/l (for women). The values, which are higher, provide the state named hyperuricemia.

In hyperuricemia, serum urate levels exceed the solubility limit; this causes the crystallization of sodium urate in soft tissues and joints to form deposits named tophi. This event causes an inflammatory reaction, later acute gouty arthritis, which can progress to chronic gouty arthritis. Inflammation and erosion of the joints occur when leukocytes engage the deposited crystals and consequently rupture, releasing lysosomal enzymes. Sodium urate crystals in the urinary tract impair renal function, too.

Hyperuricemia is the obligatory component of gout appearance, but only in 15% of patients from all having this state.

Factors, which can cause the gout in patients with hyperuricemia:
1. Overcooling of human organism. The solubility of sodium urate is lower under low temperature. The rate of urate accumulation in joints is higher in this case.
2. The sharp change of diet in patient with hyperuricemia. If you are patient with hyperuricemia and have the diet with animal food products you cannot become strong vegetarian before consultation with doctor.

Hyperuricemia may be secondary to other disease such as cancer, psoriasis, chronic renal deficiency.

Treatment of gout. Allopurinol is the drug that blocks the action of Xanthine oxidase for production of uric acid. This drug is oxidized by xanthine oxidase to
oxypurinol. Oxypurinol binds tightly to xanthine oxidase, inhibiting its ability to oxidase xanthine or hypoxanthine. It is an example of suicide inhibition.

The reaction of allopurinol action with PRPP used in HGPRT reaction results in decrease in PRPP levels and thus a decrease in de novo purine synthesis.

Colchicine is an anti-inflammatory drug that is used to treat gout. It inhibits leukocyte movement by affecting microtubules thus it blocks the development of inflammation.

Inherited Disorders of Purine Metabolism

PRPP synthetase may be with abnormal features:
1. Superactive (increased Vmax) → purine overproduction → gout;
2. Resistance to feedback inhibition → purine overproduction → gout;
3. Low Km for ribose-5-P→ purine overproduction → gout.

Hypoxanthine guanine phosphoribosylpyrophosphate transferase (HGPRT)
1. Partial deficiency → purine overproduction → gout
2. Complete deficiency (Lesch-Nyhan syndrome) → purine overproduction, the main clinical symptoms: self-mutilation, mental retardation, and death in yearly childhood.

Lesch-Nyhan syndrome: Several forms of HGPRT deficiency have been identified:
1) in one form patients have normal levels of this enzyme, but the enzyme is inactive;
2) the patients have an enzyme that is apparently unstable; its activity is higher in young red cells than in old.

The symptoms: hyperuricemia, gout, urinary tract stones, and neurological symptoms of mental retardation, self-mutilation, and then death in young age.

The basis of neurological symptoms is unknown. However, brain cells normally have much higher levels of purine salvage enzymes than other cells and may normally use salvage pathways to a greater extent.
Treatment by allopurinol reduces the uric acid formation but does not alleviate the neurological symptoms.

**Xanthine oxidase** complete deficiency: → xanthine renal lithiasis, hypouricemia associated with **xanthinuria**.

**von Gierke’s disease** *(glucose-6-phosphatase deficiency)*. Purine overproduction and hyperuricemia in von Gierke’s disease (glucose-6-phosphatase deficiency) occurs secondarily to enhanced generation of the PRPP precursor-ribose-5-phosphate. In addition, associated lactic acidosis elevates the renal threshold for urates, elevating total body urates.

**Biosynthesis of Nucleic Acids**

The metabolism of nucleic acids is composed from anabolic pathways (DNA synthesis - replication; RNA synthesis – transcription) and their catabolic pathways (degradation) up to terminal products for humans (uric acid, urea, carbon dioxide and water). All these pathways are associated with the metabolism of nucleotides: their synthetic ways and degradation, too.

**Replication**

Replication is the synthesis of two complementary DNA strands from deoxyribomononucleoside triphosphates on parental DNA template due to the function of special multienzyme system named **Replisome**.

The double-helical model of DNA suggested that the strands can separate and act as templates for the formation of a new, complementary strands.

**Conservative replication** would occur if, after replication and cell division, the parental DNA strands remained together in one of the daughter cells and the newly synthesized DNA strands went to the other daughter cell.

**Semi conservative replication** would occur if, after replication and cell division, each daughter cell received one parental DNA strand and one newly synthesized complementary strand for which the parental strand was the template (Fig. 26).
Prokaryotic Replication. Replication of prokaryotes is much better understood than is replication in eukaryotes. The basic requirements and components of replication are the same for prokaryotes as for eukaryotes. Therefore, an understanding of how prokaryotes replicate provides much insight into the understanding of how eukaryotes replicate.

Consideration of the influence of chromosome structure on DNA replication in bacteria and eukaryotes must also take into account the different organization of DNA in the cell. The bacterial chromosome is associated with the cell membrane but otherwise is exposed to the entire intracellular environment. A similar initiation
complex may exist at the membrane-bound bacterial replicator, since Dna A protein from E. coli is a lipid-binding protein and is associated with the membrane (Sekimizu and Kornberg 1988; Sekimizu et al. 1988; Fig. 27). Thus, in both cell types, initiation may actually occur on a solid-state support, albeit that the supports and environment may be quite different.

**Basic requirements for DNA synthesis:**

1. **Substrates** - deoxynucleoside triphosphates: d-ATP, d-GTP, d-CTP, d-TTP. Cleavage of the high energy phosphate bonds (two) provides the energy for the phospho diester bond formation in a new strand.

2. **Template** - DNA replication can’t occur without a template. A template is required to direct the addition of the appropriate complementary nucleotide to the newly synthesized DNA strand. In semi conservative replication, each strand of parental DNA serves as a template. Then, each template strand and its complementary strand serve as the DNA in daughter cells.

**Initiation phase of replication in E.coli.** DNA replication in Escherichia coli initiates at ori C, the unique origin of replication, and proceeds bidirectionally. This creates two replication forks (Fig. 27), that invade the duplex DNA on either side of the origin.

![Figure 27](image)

**Figure 27.** Circular DNA molecule found in E.coli; its replication is from ori C, bidirectional, it is finished in 40 minutes due to ten termination sequences (Ter) placed opposite ori C.
The forks move around the circular chromosome at a rate of about 1,000 nucleotides per second and so meet about 40 min after initiation in a region opposite ori C (Fig. 27). In this region are located a series of sites, called termination or Ter sites, that block replication forks moving in one direction but not the other (Fig. 27). This event creates a "replication fork trap" that allows forks to enter but not to leave the terminus region (Fig. 28, a,b).

(b)  
**Figure 28.** (a) - direction of replication\(^\wedge\) synthesis of leading and lagging strands; (b) - steps of replication in E.coli.

*Enzymes for replication*

**The Replisome.** It is believed that all replication enzymes and factors are part of a large macromolecular complex named a Replisome. It has been suggested that the replisome may be attached to the membrane, and that instead of the replisome moving along DNA during replication, DNA is passed through the stationary Replisome. The Replisome is a multiprotein complex made up of the dna A protein,
DnaB helicase, the DnaG primase, and the Pol III holoenzyme. Each replicated strand commences with a short RNA primer synthesized by DnaG primase recruited from solution by interaction with DnaB. Single-stranded DNA is protected by SSB proteins.

**Dna A protein (E.coli)** is required for proper initiation of replication at the origin C. When Dna A-ATP binds to ori C it twists the DNA and promotes the separation of DNA-strands in the AT-rich region to produce a single-stranded bubble or “open complex” (Fig.3). The next step is the recruitment of the (DnaBC) complex to DnaA to obtain the pre-replicative Complex, which is stimulator of primosome complex. Four or five Dna A-ATP molecules interact with the (DnaBC) complex via the N-terminal of the replicative DnaB helicase and their common binding to oriC (Seitz et al., 2000).

**Dna B is a monohexameric helicase.** Its function is the unwinding of double-stranded DNA employing the hydrolysis of ATP, this activity is maintained as the elongation phase proceeds. Helicase activity provides single-strand templates for replication. dna B protein is the principal helicase of E.coli replication. It is a component of a primosome. In the normal process of replication, DnaB is at the front of the replisome. It is a ring-shaped homohexameric enzyme that translocates in the 5‘-to-3’ direction on the lagging-strand template to unwind double-stranded DNA in front of the DNA polymerase III holoenzyme, the multisubunit replicase that simultaneously synthesizes both strands.

**Primosome**. DNA synthesis can’t start without a primer which prepares the template strand for the addition of nucleotides. Because, new nucleotides are added to the 3’ end of a primer, new synthesis is said occur in a 5′ to 3′ direction.

Primosome is a complex of proteins, a hexamer of dna B protein, dna C protein and several other proteins n, n’, n”, i. Primosome complex may be named as Primase. The primosome complex primes DNA synthesis at the origin. Using ATP hydrolysis, the primosome moves with the replication fork, making RNA primers for Okazaki fragment synthesis. It also makes the primer that initiates leading strand synthesis at the origin. Primers are not shorter than 12 and up to 29 ribonucleotides.
One strand (the leading strand) is replicated continuously, while the other (lagging) strand is synthesized discontinuously in a series of Okazaki fragments (Fig. 28,b). The replicative RNA-priming enzyme, DnaG primase is recruited by DnaB for the priming of each new fragment on the discontinuous strand. DnaB is physically associated with the replicase through the special subunit of the holoenzyme.

SSBP. The single-stranded sections that result from helicase action are coated with single-stranded DNA-binding proteins (SSBP). Their functions are:
- to enhance the activity of helicase and to bind to single-stranded template DNA until it can serve as a template.
- to protect single-stranded DNA from degradation by nucleases and may block formation of intra-strand duplexes of hairpins that can slow replication.
SSBP is displaced from single-stranded DNA when DNA undergoes replication.

Topoisomerase. Forks for replication represent unwound parental template DNA strands to which newly synthesized complementary DNA are paired. Positive super coils would build up in advance of a moving replication fork if it was not for the action of topoisomerase. It introduces “nicks” in one strand of the unwinding double helix allowing the unwinding process to proceed; alters the supercoiling of DNA. Topoisomerase is named as gyrase in some microbial organisms.

Initiation phase of replication requires the presence of all enzymes described before to produce first primer. The elongation phase starts from the moment of first round of DNA-polymerase action to make complementary linkage of first deoxyribonucleoside monophosphate to the chain of primer.

Elongation phase; Leading strand synthesis. It is the continuous synthesis of the daughter strand in a 5’ to 3’ direction. DNA-polymerase III catalyzes leading strand synthesis, continuously (in prokaryotes) (Fig. 29).

Elongation phase; Lagging strand synthesis. This strand is made discontinuously (Fig.4). The resulting short fragments are named Okazaki fragments, and they are synthesized by DNA-Polymerase III, too. Synthesis of each new Okazaki fragment takes place until it reaches the RNA primer of the preceding Okazaki
fragment. This effectively leaves a nick between the newly synthesized Okazaki fragment and the RNA primer.

Figure 29. Replisome complex function in elongation phase of replication (E.coli).

**DNA polymerase I** uses its nick-translation properties to hydrolyse the RNA primer (5’ to 3’ exonuclease activity) and replace it with DNA fragment. These fragments are later joined by **DNA ligase** to make a continuous piece of DNA.

This lagging strand synthesis also occurs in a 5’ to 3’direction.

**DNA ligase** catalyses the formation of phospho diester bond between the adjoining fragments by the following reaction:

\[
\text{DNA-3'}-\text{OH} + \text{HO} \rightarrow \text{O} - \text{5'}-\text{DNA} \xrightarrow{\text{Ligase}} \text{DNA-3'} - \text{O} + \text{O} - \text{5'}-\text{DNA}
\]

**DNA polymerase II** is a minor polymerase in E.coli. It may be involved in some DNA repair processes, but E.coli mutants lacking this enzyme show no replication or growth deficiencies. Polymerase II has proofreading activity (3’ to 5’ exonuclease activity) but lacks excision-repair activity.
**The Termination of replication.** Termination sequences in the parental DNA strands direct the termination of replication are placed in circular parental DNA molecule quite oppositely the ori C region (Fig. 2). A specific protein, Ter-binding protein (TUS), binds to these sequences and prevents the Helicase (or dna B protein) from further unwinding of DNA. This facilitates the termination of replication. In the *E. coli* chromosome, the *Ter* sequences were placed so as to form a "replication fork trap" that would allow a replisome to enter the region between the two Ter sites but not to leave. *Ter* sequences were also found in a variety of other plasmids as well as in other bacteria (30), and the number of *Ter* sites identified in the *E. coli* chromosome also increased, first to 4, then to 5, and finally to 10, after the publication of the entire genome sequence and an in-depth study of nucleotide substitutions by Coskun-Ari and Hill (1998).

**All the ways to control replication in E.Coli may be across:**

- Dna A gene regulation
- Dna A activity regulation
- Ori C blocking
- DNA methylation

**Eukaryotic replication.** In contrast, eukaryotic DNA replication occurs in a distinct compartment in the separation of proteins that may influence the initiation of DNA replication. Within the nucleus, initiation of eukaryotic DNA replication occurs at pre-replicative complexes that are established prior to the beginning of S-phase, and these presumably contain the initiator and other replication proteins (Adachi and Laemmli 1994; Diffley et al. 1994). The mechanism is similar to that of prokaryotic replication. It is semi conservative and proceeds bidirectionally from many origins. Replicons are basic units of replication. A replicon encompasses of all the DNA replicated from the growing replication forks originating from a single origin. There are estimated to be about 100000 replicons per cell in mammal. The large number of replicons is needed to replicate the large mammal genomes in a reasonable period of time. It takes about 8 hours to replicate the human genome. The duration of
replication in eukaryotic cell may only once and during the S-phase of cell life. Factors for stimulation: Cyclins E, A (special proteins for regulation) and cyclin-dependent protein kinases involved in the initiation phase of replication.

The eukaryotic replication rate is about ten times slower than prokaryotic replication rate. Eukaryotes contain at least three different nuclear DNA polymerases: \( \alpha \), \( \beta \) and \( \delta \); and one mitochondrial DNA polymerase - \( \gamma \).

**DNA polymerase \( \alpha \)** is probably analogous to polymerase I but it plays no role in DNA repair.

**DNA polymerase \( \beta \)** acts in DNA repair synthesis.

**DNA polymerase \( \delta \)** is probably analogous to polymerase III and responsible for leading strand synthesis.

**DNA polymerase \( \gamma \)** replicates mitochondrial DNA.

**Telomerase**

Whereas the genomes of essentially all prokaryotes are circular, the chromosomes of human beings and other eukaryotes are linear. The free ends of linear DNA molecules introduce several complications that must be resolved by special enzymes. In particular, it is difficult to fully replicate DNA ends, because polymerases act only in the 5′ → 3′ direction. The lagging strand would have an incomplete 5′ end after the removal of the RNA primer. Each round of replication would further shorten the chromosome. The first clue to how this problem is resolved came from sequence analyses of the ends of chromosomes, which are called telomeres (from the Greek telos, “an end”). Telomeric DNA contains hundreds of tandem repeats of a hexanucleotide sequence. One of the strands is G rich at the 3′ end, and it is slightly longer than the other strand. In human beings, the repeating G-rich sequence is AGGGTT.

How are the repeated sequences generated? An enzyme, termed telomerase, that executes this function has been purified and characterized. When a primer ending in GGTT is added to the human enzyme in the presence of deoxynucleoside triphosphates, the sequences GGTTAGGGTT and GGTTAGGGTTAGGGTT, as well as longer products, are generated. Elizabeth Blackburn and Carol Greider
discovered that the enzyme contains an RNA molecule that serves as the template enzyme carries the information necessary to generate the telomere sequences. The exact number of repeated sequences is not crucial from its amino acid sequence, this component is clearly related to reverse transcriptases, enzymes first discovered in retroviruses that copy RNA into DNA. Thus, telomerase is a specialized reverse transcriptase that carries its own template. Telomeres may play important roles in cancer-cell biology and in cell aging.

Cells which have an unlimited capacity such as male germ cells and the majority of human cancers have high levels of telomerase activity, the level and frequency of telomerase activity in more than 85% of all cancers highlights the critical role telomerase plays in tumor progression. Telomerase activation is the most common general marker for cancer cell to date making it in attractive target for new cancer diagnostics and therapeutics.

Reverse transcription is observed also for some viruses or some foreign microorganisms which can use their RNA to produce their DNA due to this process in human cells. For example: RNA that contains AIDS virus penetrated into a leukocyte and by means of reverse transcriptase forced a cell to synthesize a viral DNA.

Something about Regulation of Replication in Eukaryotes

The opening of the DNA helix by locked CMG-complex (Fig. 30), and stabilization of separated DNA strands after the binding of replication protein A (RPA, it is similar to SSB-proteins in action) facilitates the recruitment of DNA replication enzymes to begin DNA synthesis.

CMG-complex is composed from:
- MCM2-7 – multichain protein complex - twisted dimmer; it is in conformation as two rings. The central channel, formed by these two staggered rings, has four constriction points that would restrict the movement of duplex DNA with tight grips and a kink at the interface of the two rings that would deform the bound DNA
• GINS – multichain protein complex linked with other key proteins at the fork to maintain an active replisome progression complex (RPC).
• Cdc45 - is a protein that in humans is encoded by the CDC45L gene; required to the initiation of DNA replication.

![Diagram of GINS and Cdc45](image)

**Figure 30.** CMG-complex composition and function at initiation of replication in eukaryotic cell.

Regulation of CMG-complex formation is due to Cdt1, S phase-kinases ad regulatory peptide Geminin.

Cdt1 is a protein encoded by the gene Cdt1, and it is a key licensing factor which, along with the protein Cdc6, functions to license DNA by forming the pre-replication complex.

The interplay of S phase-kinases with Cdt1 and other components on the pre-replicative complex prevents the reformation of this complex, thus ‘licensing’ occurs only once per cell cycle at any given origin.
1) Cdt1 is subject to proteolysis as the cell cycle progresses through S-phase and G2-phase.

2) Cdt1 is inhibited by Geminin which specifically binds to Cdt1 during S, G2, and early mitosis. Geminin both inhibits Cdt1 activity during S phase in order to prevent re-replication of DNA and prevents it from ubiquitination and subsequent proteolysis.

**Drugs that effect replication**

1. **Antimetabolites which reduce or inhibit the production of the substrate for replication:**
   - 5-**Fluorouracil** (analog of thymine according position of fluoride atom)
   - **Methotrexate** (analog of folic acid) that inhibits dihydrofolate reductase, regeneration of tetrahydrofolate is blocked, d-TMP synthesis is damaged
   - 6-**Mercaptopurine, 8-azoguanine and thioguanine.**

2. **Substrate analogs: Azidothymidine**

3. **Antiviral drugs used to treat human immunodeficiency virus (HIV) infections**
   - **Cytosine arabinoside (cytoribine):** it is a potent myelonic antileukemia drug. Upon incorporation into DNA, it is believed to alter the structure of DNA and make it more prone to breakage.

4. **Intercalators** are drugs, usually with aromatic ring, that insert between adjacent, stacked base pairs. Intercalation causes a physical block as well as disruption or change in the DNA conformation that inhibits the action of replication enzymes.

   - **Anthracycline glycosides** – antibiotics produced by a strain of Streptomycetes.
   - **Actinomycin D** (anticancer activity), it is beneficial in treating Wilm’s tumor in children when used in combination with surgery, radiotherapy and other chemotherapeutic drugs.

   - **Xeroderma pigmentosum** occurs when the human person has deficiency of special enzyme - exonuclease (may be endonuclease). Endonuclease of healthy person recognizes defect in DNA, unwinds partially DNA helix, and exonuclease...
cuts the DNA above and below the defective region. This gap is then filled in by a special polymerase (δ/ε in humans) and relegated (Fig. 31).

<table>
<thead>
<tr>
<th>Disease</th>
<th>Symptoms</th>
<th>Genetic defect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Xeroderma pigmentosum</td>
<td>Frecklelike spots on skin, sensitivity to sunlight, predisposition to skin cancer</td>
<td>Defects in nucleotide-excision repair</td>
</tr>
<tr>
<td>Cockayne syndrome</td>
<td>Dwarfism, sensitivity to sunlight, premature aging, deafness, mental retardation</td>
<td>Defects in nucleotide-excision repair</td>
</tr>
<tr>
<td>Trichothiodystrophy</td>
<td>Brittle hair, skin abnormalities, short stature, immature sexual development, characteristic facial features</td>
<td>Defects in nucleotide-excision repair</td>
</tr>
<tr>
<td>Hereditary nonpolyposis colon cancer</td>
<td>Predisposition to colon cancer</td>
<td>Defects in mismatch repair</td>
</tr>
<tr>
<td>Fanconi anemia</td>
<td>Increased skin pigmentation, abnormalities of skeleton, heart, and kidneys, predisposition to leukemia</td>
<td>Possibly defects in the repair of interstrand cross-links</td>
</tr>
<tr>
<td>Ataxia telangiectasia</td>
<td>Defective muscle coordination, dilation of blood vessels in skin and eyes, immune deficiencies, sensitivity to ionizing radiation, predisposition to cancer</td>
<td>Defects in DNA damage detection and response</td>
</tr>
<tr>
<td>Li-Fraumeni syndrome</td>
<td>Predisposition to cancer in many different tissues</td>
<td>Defects in DNA damage response</td>
</tr>
</tbody>
</table>

**Figure 31.** Genetic diseases associated with defects in DNA repair system.

**Drugs that damage DNA**

a) *Alkylating agents* (strong electrophils) become linked to many cellular nucleophils, in particular to the seventh nitrogen in the guanine residue of DNA. After replication mutation can be, or cross-linking of double helix.

b) *Platinum-coordination complexes (cis-platin).* They lead to the formation of cross-links between adjacent guanines in DNA. They are drugs for testicular and ovarian cancers.

c) *Bleomycins* bind to DNA and interact with oxygen and Fe²⁺ to cause DNA breakage.

It should be noted that some exogenous factors like XR-radiation or ultraviolet radiation can cause the damage of DNA molecule structure, the latter factor causes formation of thymidine dimmers in DNA to provoke abnormalities in replication.

Transcription

Multiple steps are required to produce functional cellular RNAs. Although some of these steps are common to the production of all RNAs others depend on the class of RNA being produced. Three functionally distinct classes of RNA are produced in prokaryotes, and four are produced in eukaryotes.

m-RNA of prokaryotes

It is in need to know that:
1) most prokaryotic m-RNAs are polycistronic. That is they carry the information for the production of multiple polypeptides;
2) not all portions of prokaryotic m-RNA code for polypeptides:
   a) the 5’-ends of m-RNA contain sequences that are never translated into protein (leader sequences or 5’-untranslated regions);
   b) the 3’-ends contain sequences that are never translated into protein (trailer seq. or 3’-untranslated regions);
   c) if the m-RNA is polycistronic, the sequences between that code for proteins (cistrons) are called the intercistronic regions or spacers.

m-RNA accounts for only 5% of the total cellular RNA in prokaryotes. Their life-time is short (just a few minutes).

mRNAs of eukaryotic cell are monocistronic. They are formed from large precursors that are named heterogenous nuclear RNA (hnRNA). Like prokaryotic m-RNA, eukaryotic m-RNA contains leader and trailer sequences.

1) Leader seq. has 7-methylguanylate attached 5’to 5’ triphosphate linkage –a cap.
2) Trailer sequence is a polyadenylate tail (200-300 adenylate residues at the 3’end) (Fig. 32)
3) m-RNA accounts for only 3% of the total cellular RNA in eukaryotes;
4) they exhibit half-lives on the order of hours to days.

**m-RNA of eukaryotes**

![Diagram](image)

**Figure 32.** Schematic representation of typical prokaryotic and eukaryotic messenger RNAs (mRNAs). The coding regions are indicated with open arrows.

A polycistronic prokaryotic mRNA with three coding regions is shown (Fig. 32 A). The coding regions are separated by noncoding spacer sequences. Flanking the proximal and distal coding sequences are noncoding 5' and 3' untranslated regions (UTRs). The 5' end of the mRNA is a purine nucleoside triphosphate.

A monocistronic eukaryotic mRNA is shown (Fig. 32 B). The single coding region is flanked by a 5' and 3' UTR. The 5' end has a 7-methylguanylate cap and the 3' end has a polyadenylate (poly A) tail.

**r-RNA of prokaryotes**

Three kinds: 23S r-RNA; 16s r-RNA; 5S r-RNA. They arise from the processing of a large 30S precursor r-RNA. r-RNAs account for 80% of the total cellular RNA in prokaryotes.

**r-RNA of eukaryotes**

They are typically bigger than prokaryotic.

Four kinds: 28S r-RNA, 18S r-RNA, 5,8S r-RNA, 5S r-RNA. They arise from 45 precursor r-RNA; the 5S r-RNA is a transcription product of separate gene.

**t-RNA of prokaryotes**

1. Average size of t-RNAs is about 80 nucleotides.
2. All t-RNAs arise from processing of large precursor.
3. They are heavily modified post-transcriptionally.
4. They account for 15% of the total cellular RNA in prokaryotes.

**t-RNA of eukaryotes**

They are very similar in positions 1-4 to prokaryotic t-RNA.

Besides t-RNAs of eukaryotes have numerous other small RNAs that serve a variety of functions. These RNAs are divided in two groups according to their location: cytoplasmic and nuclear. The latter are associated with proteins in small nuclear ribonucleoprotein or snurps. Snurps function in splicing reactions needed to process hnRNA to m-RNA.

**Transcription in E. coli (Prokaryotic Cell)**

The process of *RNA synthesis from nucleoside triphosphates* (ATP, GTP, UTP, CTP) *directed by a DNA template due to function of RNA-polymerase* is termed *Transcription and it proceeds in three phases:*

**Initiation**

*Transcriptional initiation does not require a primer.*

Promoter sequences (Fig. 33) are responsible for directing RNA polymerase to initiate transcription at a particular point of one DNA strand, only. In the Figure 34 (below) you can see the sequence features of typical prokaryotic promoter. Conserved promoter sequence elements (*enclosed boxes*), are shown relative to the start point of transcription. The start point precedes the coding region so that the transcripts have a 5' untranslated region (**UTR**).

**Figure 33.** Prokaryotic promoter sequences.
For most prokaryotic genes, they are conserved sequences that are necessary to promote accurate initiation of transcription.

*The promoters have three sequence elements:*

1) **Initiation site (start point).** The start point is usually purine nucleotide residue.

2) **Pribnow box.** There is a sequence called so which exists 9 to 18 base pairs upstream of the start point. A typical Pribnow box is either identical to or very similar to the sequence TATAAT. It has also been called -10 sequence because it is usually found 10 base pairs upstream of the start point.

3) **The –35 sequence** is a component of typical prokaryotic promoters. This sequence is very similar to the sequence TTGACA. It is named -35 sequence because it is typically found 35 base pairs upstream of the start point.

Initiation factors are needed to initiate transcription:

*The prokaryotic σ-factor placed in holo-enzyme of RNA-polymerase is required for accurate initiation of transcription.* σ-factor enables the RNA-polymerase holoenzyme (Fig. 34) to recognize and bind tightly to the promoter sequences.

*Process of initiation:*

a) upon binding, the σ-factor facilitates the opening or melting of the DNA double helix; Sigma (σ) factor mediates initiation of prokaryotic transcription. Sigma factor enables the RNA polymerase holoenzyme to recognize and bind
tightly to the promoter where it facilitates the initiation of transcription. After initiation, the sigma factor dissociates within the time it takes for new chain growth to proceed 10 nucleotides.

b) then holoenzyme catalyzes the formation of a phospho diester bond between the first two monomers. The first nucleotide is usually purine nucleoside triphosphate (ATP or GTP); A holoenzyme is a core enzyme with an additional subunit σ, it is required for proper initiation of transcription. A core enzyme consists of two α₂-subunit and two β̂-subunit, it is required for the elongation steps of RNA synthesis.

c) the released σ-factor can combine with free core-enzyme to form another holoenzyme that can initiate transcription.

Elongation

This phase proceeds after the formation of the first phosphodiester bond. By the time 10 nucleotides have been added, the σ-factor dissociates. The core enzyme then continues the elongation of the transcription A single strand of DNA acts as a template to direct the formation of complementary RNA during transcription. Substrates are four ribonucleoside triphosphates : ATP, GTP, UTP, CTP.

Except for the first nucleoside triphosphate, subsequent nucleotides are added to the 3'-hydroxyl of the preceding nucleotide. Therefore, RNA chain growth proceeds in the 5` to 3` direction.

Termination

There are two basic classes of termination events in prokaryotes:

1) Factor-independent termination.

   Particular sequences of DNA strand can:
   a) cause the core enzyme to terminate transcription;
   b) share several common features.

   All these sequences can code an inverted repeat, which – when transcribed – can form a stable stem-loop structure (Fig. 35). While transcription pauses at the guanine-cytosine (GC)-rich sequences, a stable stem – loop structure forms in the RNA. This causes displacement – and subsequent termination – while the uracil
(U)-rich sequence, which is only weakly base-paired to the template, is being synthesized. The formation of a stable stem-loop induces the displacement of the transcript when RNA polymerase synthesizes the U-rich segment. Displacement occurs easily because only weak adenine-uracil bonds hold the transcript to the template.

![Diagram of RNA transcription](image)

**Figure 35.** A formation of a stable stem-loop in termination phase of transcription.

2) **Factor-dependent termination**

Particular sequences act as termination sequences in the presence of factor rho (ρ). Rho-dependent termination sequences do not appear to share common structural features as do the factor-independent termination sequences. Rho binds as a hexamer to the forming transcript at these unique sequences. Rho is an ATPase. The exact mechanism that Rho uses to terminate transcription is unknown but it requires the cleavage of ATP by Rho.

**Transcription and Processing of mRNA in Eukaryotes**

The process of transcription in eukaryotes is similar to that in prokaryotes, although there are some differences:

- **Eukaryote genes are not grouped in operons as are prokaryote genes.**
- Each eukaryote gene is transcribed separately, with separate transcriptional controls on each gene.

- Whereas prokaryotes have one type of RNA polymerase for all types of RNA, eukaryotes have a separate RNA polymerase for each type of RNA. One enzyme RNA-polymerase B (II) for mRNA-coding genes such as structural proteins. One enzyme for large rRNAs. A third enzyme for smaller rRNAs and tRNAs.

- Prokaryote translation begins even before transcription has finished, while eukaryotes have the two processes separated in time and location (remember the nuclear envelope). In prokaryotes, m-RNA is not post-transcriptionally processed. It may be only for precursor of r-RNA and t-RNA. Enzymes ribonucleases P, D, III are used.

_After eukaryotes transcribe an RNA, the RNA-transcript is extensively modified before export to the cytoplasm_ Eukaryotic m-RNA is formed from extensive processing of a large precursor named hn-RNA:

- A cap of 7-methylguanine (a series of an unusual base) is added to the 5' end of the mRNA; this cap is essential for binding the mRNA to the ribosome. Cap formation is a multistep process that begins during transcription or immediately after. Caps serve two functions:
  1. m-RNAs with caps are translated more efficiently;
  2. caps help stabilize m-RNAs by protecting them from digestion by ribonucleases that degradate RNA from 5'-end

- A string of adenines (as many as 200 nucleotides known as poly-A) is added to the 3' end of the mRNA after transcription. Polyadenylation is made (Fig. 36).

- The function of a poly-A tail is not known, but it can be used to capture mRNAs for study. The signal that identifies the site of polyadenylation lies within the hn-RNA. The sequence AAUAAA (cleavage polyadenylation signal) directs a cleavage of the RNA being transcribed to a point 11 to 30 nucleotides downstream. Polyadenylation occurs after capping before splicing; it helps to stabilize m-RNA. (Poly A polymerase adds several hundred adenylate residues to the free 3’ end of the RNA formed from the cleavage reaction.)
Figure 36. Polyadenylation of hn-RNA during processing.

- **Introns are cut out of the message and the exons are spliced together before the mRNA leaves the nucleus.** There are several examples of identical messages being processed by different methods, often turning introns into exons and vice-versa. Protein molecules are attached to mRNAs that are exported, forming ribonucleoprotein particles (mRNPs) which may help in transport through the nuclear pores and also in attaching to ribosomes. The process by which non-coding sequences are removed to form a functional m-RNA is named *splicing*. Splicing occurs through a multistep process that is catalyzed by a large ribonucleoprotein complex called *splicesome*. Splicosomes are made of five snurps that contain five snRNA ($u_1, u_2, u_4, u_5, u_6$). The *snRNAs* are responsible for recognition of conserved sequences in introns and the bringing together of RNA sequences into perfect alignment for splicing. The first step is a cleavage at the 5' intron/exon junction. The 5'-phosphate of the conserved guanylate of the 5' intron/exon junction is then covalently linked to the 2'-hydroxide of the adenylate located in the branch site. After formation of this intermediate, lariat-like structure, a second cleavage at the 3' intron/exon junction occurs. The two exons are then ligated together and the lariat-like structure is lost, to eventually be degraded.
Transport of m-RNA from nucleus to the cytoplasm is coupled to splicing and does not occur until the splicing is complete. Regulation of gene expression is often at the level of splicing.

The Regulation of Transcription in E. coli. LAC-operon Theory

Experimental investigation of LAC-operon in E.coli proved some notions in the regulation of transcription.

Some terms for understanding of this subchapter:

Cistron (structural gene) – the sequence of DNA strand that codes the structure of one polypeptide chain of protein.

Inducible gene – transcription of this gene can be in the presence of inducer, only.

Inducer – special regulator-substance. It has affinity to special protein-repressor to block its linkage to gene-operator sequence.

Constitutive expression of genes – there is independent transcription of genes

Operon – a site of DNA strand that contains promoter sequence, gene-operator and one or more cistrons.


Protein-repressor – protein that can bind to gene-operator to stop RNA-polymerase action.

Gene-operator - sequence of DNA strand placed between promoter sequence and structural genes, and it has affinity to protein-repressor.

LAC-operon of E.coli contains three structural genes that keep information about enzymes: Gene X – β-Galactosidase; Gene Y – Galactoside permiase; Gene Z – Galactoside acetylase (Fig. 37).

All three genes are transcribed in a single m-RNA. Lactose is inducer of this transcription.
In the presence of inducer (lactose) protein-repressor cannot attach the gene-operator, and holoenzyme of RNA-polymerase can move along the DNA single strand to form primary transcript. Inducer blocks the conformation of protein-repressor (active form) to allow the transcription of LAC-operon (Fig. 38).

Positive regulators of transcription are: CRP-protein – catabolite gene reactive protein; cAMP (Fig. 39).
Figure 39. CRP-cAMP complex is enhancer of RNA-polymerase linkage to promoter sequence.

These two factors in a complex are required for activation of Sigma ($\sigma$) factor in the structure of holo-enzyme of RNA-polymerase to attach to promoter sequence when the inducer is present. Their content depends on the content of sources for carbon atom in the cell such as glucose or glycerol. The higher the content of glucose in bacteria cell the lower the content of cAMP. Glucose and glycerol are considered as suppressor for transcription on LAC-operon.

**Transcription Regulation in Eukaryotes**

An eukaryotic cell contains in DNA molecules about 20,000–25,000 genes.

- Some of these are expressed in all cells all the time. These so-called housekeeping genes are responsible for the routine metabolic functions (e.g. respiration) common to all cells.
- Some are expressed as a cell enters a particular pathway of differentiation.
- Some are expressed all the time in only those cells that have differentiated in a particular way. For example, a plasma cell expresses continuously the genes for the antibody it synthesizes.
- Some are expressed only as conditions around and in the cell change. For example, the arrival of a hormone may turn on (or off) certain genes in that cell.

Protein-coding genes have:
• **exons** whose sequence encodes the polypeptide;
• **introns** that will be removed from the mRNA before it is translated;
• **a transcription start site**
  • a promoter is represented by two types:
    o **the basal or core promoter** located within about 40 bp of the start site
    o an "upstream" promoter, which may extend over as many as 200 bp farther upstream
• **enhancers**
• **silencers**

Adjacent genes (RNA-coding as well as protein-coding) are often separated by an **insulator** which helps them avoid cross-talk between each other's promoters and enhancers (and/or silencers).

**Transcription start site.** This is where a molecule of RNA polymerase II (pol II, also known as RNAP II) binds. Pol II is a complex of 12 different proteins (shown in the figure 16 in yellow with small colored circles superimposed on it).

The start site is where transcription of the gene into RNA begins is the basal promoter (Fig. 39).

![Figure 39. The composition of basal promoter in DNA of eukaryotic cell.](image)

The basal promoter contains a sequence of 7 bases (TATAAAAA) called the TATA box. It is bound by a large complex of some 50 different proteins, including:
1) Transcription Factor IID (TFIID) which is a complex of TATA-binding protein (TBP), which recognizes and binds to the TATA box (Fig. 39);
2) 14 other protein factors which bind to TBP — and each other — but not to the DNA.

3) Transcription Factor IIB (TFIIB) which binds both the DNA and pol II.

   The basal or core promoter is found in all protein-coding genes. This is in sharp contrast to the upstream promoter whose structure and associated binding factors differ from gene to gene.

   Although the figure 16 is drawn as a straight line, the binding of transcription factors to each other probably draws the DNA of the promoter into a loop.

   Many different genes and many different types of cells share the same transcription factors — not only those that bind at the basal promoter but even some of those that bind upstream. What turns on a particular gene in a particular cell is probably the unique combination of promoter sites and the transcription factors that are chosen.

   Transcription factors represent only a small fraction of the proteins in a cell. Hormones exert many of their effects by forming transcription factors. The complexes of hormones with their receptor represent one class of transcription factor. Hormone "response elements", to which the complex binds, are promoter sites.

   **Enhancers.** Some transcription factors ("Enhancer-binding protein") bind to regions of DNA that are thousands of base pairs away from the gene they control. Binding increases the rate of transcription of the gene. Enhancers can be located upstream, downstream, or even within the gene they control. How does the binding of a protein to an enhancer regulate the transcription of a gene thousands of base pairs away?

   - One possibility is that enhancer-binding proteins – in addition to their DNA-binding site, have sites that bind to transcription factors ("TF") assembled at the promoter of the gene.

       This would draw the DNA into a loop (as shown in the Figure 40).
Enhancers can turn on promoters of genes located thousands of base pairs away. Like promoter-proximal elements, many enhancers are cell-type-specific. For example, the genes encoding antibodies (immunoglobulins) contain an enhancer within the second intron that can stimulate transcription from all promoters tested, but only in B lymphocytes, the type of cells that normally express antibodies. Analyses of the effects of deletions and linker scanning mutations in cellular enhancers have shown that they generally are composed of multiple elements that contribute to the overall activity.

What is to prevent an enhancer from inappropriately binding to and activating the promoter of some other gene in the same region of the chromosome? One answer: an insulator.

Insulators are stretches of DNA (as few as 42 base pairs may do the trick) which located between the enhancer(s) and promoter or silencer(s) and promoter of adjacent genes or clusters of adjacent genes, their function is to prevent a gene from being influenced by the activation (or repression) of its neighbors.

Silencers. Silencers are control regions of DNA that, like enhancers, may be located thousands of base pairs away from the gene they control. However, when transcription factors bind to them, expression of the gene they control is repressed.
Features of transcription control by different factors

Transcription activators and repressors are generally modular proteins containing a single DNA-binding domain and one or a few activation domains (for activators) or repression domains (for repressors). The different domains frequently are linked through flexible polypeptide regions.

Among the most common structural motifs found in the DNA-binding domains of eukaryotic transcription factors are the C2H2 zinc finger, homeodomain, basic helix-loop-helix (bHLH), and basic zipper (leucine zipper). All these and many other DNA-binding proteins contain one or more helices that interact with major grooves in their cognate site in DNA.

The transcription-control regions of most genes contain binding sites for multiple transcription factors. Transcription of such genes varies depending on the particular repertoire of transcription factors that are expressed and activated in a particular cell at a particular time.

Combinatorial complexity in transcription control results from alternative combinations of monomers that form heterodimeric transcription factors and from cooperative binding of transcription factors to composite control sites.

Activation and repression domains in transcription factors exhibit a variety of amino acid sequences and three-dimensional structures. In general, these functional domains interact with co-activators or co-repressors, which are critical to the ability of transcription factors to modulate gene expression.

Cooperative binding of multiple activators to nearby sites in an enhancer forms a multiprotein complex called an enhancesome. Assembly of enhancesomes often requires small proteins that bind to the DNA minor groove and bend the DNA sharply, allowing bound proteins on either side of the bend to interact more readily. The function of some hormones is associated with enhancer-genes function.

Steroid Hormone Receptors and their Response Elements

Steroid hormone receptors are proteins that have a binding site for a particular steroid molecule. Their response elements are DNA sequences that are bound by the complex of the steroid bound to its receptor. The response element is part of the
promoter of a gene. Binding by the receptor activates or represses, as the case may be, the gene controlled by that promoter.

Glucocorticoid receptor, like all steroid hormone receptors, is a zinc-finger transcription factor; The DNA sequence of the glucocorticoid response element is

\[
\begin{align*}
5' & \quad \text{AGAACAnnnTGTTCT} \quad 3' \\
3' & \quad \text{TCTTGTnnnACAAGA} \quad 5'
\end{align*}
\]

where \( n \) represents any nucleotide.

For a steroid hormone to turn gene transcription on, its receptor must:

- bind to the hormone
- bind to a second copy of itself to form a homodimer
- be in the nucleus, moving from the cytosol if necessary
- bind to its response element
- activate other transcription factors to start transcription.

Each of these functions depends upon a particular region of the protein (e.g., the zinc fingers for binding DNA). Mutations in any one region may upset the function of that region without necessarily interfering with other functions of the receptor.
EXERCISES FOR INDEPENDENT WORK

1. Name organic compounds which may be used for the purine nucleotide structure creation:
   A. Erythrose-5-phosphate
   B. Ribose-5phosphate
   C. Guanine
   D. Substances placed in positions B, C
   E. Thymine

2. Name organic compounds formed after cleavage of N-glycosidic bond in deoxythymidine-5′-monophosphate:
   A. Thymine and ribose
   B. Thymine, deoxyribose and phosphoric acid
   C. Thymine and deoxyribose
   D. Thymine and deoxyribose-5′-phosphate
   E. Thymidine and phosphoric acid

3. Name the processes which are in need for participator DNA molecule:
   A. Translation
   B. Transcription
   C. Processes placed in positions B, D
   D. Replication
   E. Post-translational modification

4. cAMP is special nucleotide produced by adenylate cyclase to be secondary messenger for hormone action in the intracellular space of target cell. Name the substrate for adenylate cyclase:
   A. Adenosine-5′-triphosphate
   B. 3′,5′ Adenosine triphosphate
   C. 3′,5′- Adenosine monophosphate
5. Find out the nucleotides which are found in function as allosteric regulators for enzymes with trivial name “kinases”:
A. UTP, CTP and ATP, only
B. cAMP, cGMP, ATP, ADP, AMP
C. cAMP, cGMP and AMP, only
D. NADH, NADPH and ATP, only
E. ADP, AMP, only

6. t-RNA molecule is in need to transfer amino-acid residue to the site of protein synthesis in a cell. The linkage of amino acyl to t-RNA is due to special triplet in t-RNA structure. Name it:
A. 5′-CCA triplet
B. 3′-GGA triplet
C. 3′-CCA triplet
D. 5′-AUG triplet
E. 5′-UUC triplet

7. Name organic compounds which may be used for the pyrimidine nucleotide structure creation:
A. Ribulose-5-phosphate
B. Deoxyribose-5-phosphate
C. Adenine
D. Thymine
E. Substances placed in positions B, D

8. Name organic compounds formed after cleavage of N-glycosidic bond in guanosine-5′-monophosphate:
A. Guanosine and ribose
B. Guanine and ribose-5’-phosphate
C. Guanine and deoxyribose-5’-phosphate
D. Guanosine and phosphoric acid
E. Guanine, ribose and phosphoric acid

9. Name the processes which are in need for the use of messenger RNA molecule:
   A. Translation and transcription
   B. Translation, only
   C. Transcription, only
   D. Transcription and processing
   E. Replication, only

10. 3’-Polyadenyllate trailer is found as a fragment in the structure of:
    A. DNA
    B. m-RNA
    C. t-RNA
    D. r-RNA
    E. Primary transcript

11. The phosphorylation of organic compound is in need the donor of phosphate group. Name the most probable donor of this group in any type of a cell:
    A. ADP
    B. TTP
    C. GTP
    D. ATP
    E. AMP
12. All the types of ribonucleic acids are involved in protein synthesis but only one among them is not in a free form (there is the linkage of it with proteins) during the whole duration of the process. Name it:
A. t-RNA
B. m-RNA
C. r-RNA
D. snurp-RNA
E. There is no any right answer

13. Choose the proteins, which are included into the deoxyribonucleoprotein (DNP) structure of eukaryotic cells:
A. Albumins
B. Globulins
C. Histones
D. Collagen
E. Glutelines

14. Point out bonds that stabilize the DNA double helix and are formed between the complementary nitrogenous bases:
A. Phosphodiester
B. Hydrophobic
C. Hydrogen
D. Peptide
E. Disulfide

15. Deoxyribose is different from ribose by:
A. The presence of hydroxyl-group at the second carbon atom
B. The absence of hydroxyl-group at the second carbon atom
C. The quantity of carbon atoms
D. The presence of amino group
E. The presence of hydroxyl-group at the third carbon atom

16. Point the blood plasma index whose value is determined in patients with gout:
A. Urea
B. Uric acid
C. α-Alanine
D. β-Alanine
E. β-Aminoisobutyric acid

17. Choose the compartment of a cell that contains the biggest part of deoxynucleoproteins:
A. Cytoplasm
B. Nucleus
C. Mitochondria
D. Cellular membrane
E. Lysosome

18. Hereditary gout appears when the activity of certain enzyme of purine nucleotide de novo synthesis rises. Point out it:
A. Adenylosuccinate lyase
B. PRPP aminotransferase
C. Hypoxanthine guanine phosphoribosyl transferase
D. Adenylic acid deaminase
E. 5’-nucleotidase

19. One of the secondary reasons of hyperuricemia development is:
A. An abnormally high fat consumption
B. An abnormally high carbohydrate intake
C. A folic acid hypovitaminosis
D. An abnormally high meat provisions intake
E. The utilization of protein-free products in a diet

20. Two carbon atoms and the nitrogen one of the imidazole fragment in purine base skeleton are got from only one amino acid in de novo synthesis. Name it:
   A. Glutamic acid
   B. Aspartic acid
   C. Glycine
   D. Folic acid
   E. Asparagine

21. The first stage of purine nucleotide de novo synthesis is dependent on energy. Point out the ATP moles quantity needed for synthesis of 1 mole of Inosine monophosphate (IMP):
   A. 1
   B. 2
   C. 3
   D. 4
   E. 5

22. Name the tissue type where the nucleotide de novo synthesis is the most active:
   A. Nervous
   B. Myocardium
   C. Skeletal muscle
   D. Smooth muscle
   E. Blood tissue

23. A conversion of ribonucleotides into deoxyribo nucleotides is an important reaction of de novo synthesis. Point out one enzyme of this conversion:
   A. AMP dehydrogenase
   B. Thioredoxine reductase
C. Thymidylate synthetase
D. CTP-synthetase
E. β-oxy-β-methylglutaryl-CoA-reductase

24. Allopurinol is a drug used in gout treatment. Point out the enzyme of purine nucleotide metabolism that is inhibited by it:
A. 5'-nucleotidase
B. Phosphoribosyl pyrophosphate synthetase
C. Phosphoribosyl aminotransferase
D. Xanthine oxidase
E. Adenosine deaminase

25. Purine nucleotide catabolic pathways finish in human organism in forming of:
A. β-Alanine
B. β-aminoisobutyric acid
C. Uric acid
D. Oxaloacetate
E. Pyruvate

26. Concentration of uric acid in the blood serum was determined at five patients. Point the index value that is estimated as hyperuricemia in the patient:
A. 0,14 mmol/l
B. 0,44 mmol/l
C. 0,54 mmol/l
D. 0,34 mmol/l
E. 0,24 mmol/l

27. A d-TMP synthesis from UMP consists of some successive transformations. Point out the correct order of these transformations:
A. UMP->UDP->UTP->d-UTP->d-TTP->d-TMP
B. UMP->UDP->d-UDP->d-UMP->d-TMP
C. UMP->d-UMP->d-TMP
D. UMP->UTP->CTP->d-CTP->d-TTP->d-TMP
E. UMP->UDP->CDP->d-CDP->d-TDP->d-TMP

28. Choose the enzyme of the first stage of purine nucleotide de novo synthesis inhibited during the accumulation of ATP:
A. PRPP aminotransferase
B. Adenylsuccinate lyase
C. Hypoxanthine guanine phosphoribosyl transferase
D. Adenylic acid deaminase
E. 5’-nucleotidase

29. Point out the inhibitor for xanthine oxidase:
A. AMP
B. Allopurinol
C. Hypoxanthine
D. GTP
E. β-Alanine

30. Choose the intermediate metabolite of purine nucleoside degradation used in de novo synthesis:
A. PRPP
B. Hypoxanthine
C. Adenylic acid
D. Phosphoribosylamine
E. Imidasole

31. Point out the amino acid used both in AMP and UMP de novo synthesis:
A. Glutamic acid
B. Aspartic acid
C. Serine
D. Inosinic acid
E. Alanine

32. Point out the vitamin that is actively used in the purine and pyrimidine nucleotide de novo syntheses:
A. Pangamic acid
B. Ascorbic acid
C. Pantothenic acid
D. Folic acid
E. Linolinic acid

33. The reason of Lesch-Nyhan syndrome is a genetic defect of enzyme:
A. Phosphoribosyl pyrophosphate synthetase
B. Phosphoribosyl aminotransferase
C. Hypoxanthine guanine phosphoribosyl transferase
D. Guanylate synthetase
E. Adenine phosphoribosyl transferase

34. The pyrimidine nucleotide synthesis may be considered in the liver as the way of toxic ammonium utilization because:
A. Synthesis starts with the aspartic acid
B. The initial substrate (carbomoyl-phosphate) may be formed from ammonia
C. Phosphoribosyl pyrophosphate contains the NH$_3$ molecule fragment
D. All the UMP nitrogen atoms are got from the NH$_3$ molecules
E. The synthesis of all the pyrimidine nucleotides starts with the reaction with NH$_3$

35. The pyrimidine nucleotide UMP degradation in human organism finishes by the formation of:
A. β-Alanine
B. β-Aminoisobutyric acid
C. Aspartic acid
D. Inosinic acid
E. Uric acid

36. DNA- and RNA-polymerases are the enzymes of two different processes. Please, find the likeness in their action:
A. They form an equal quantity of polynucleotide chains
B. They don't take part in the correction of process mistakes
C. They take part in the formation of the same type of bonds
D. They use the same substrate
E. The inhibitors of their action are absent

37. DNA biosynthesis in prokaryotes may be on RNA - template. Please, name the main enzyme of this process:
A. DNA - polymerase
B. RNA - polymerase
C. Oligoribonuclease
D. Primase
E. Reverse transcriptase

38. The formation of template single strands for the replication process in DNA of prokaryotic cells is due to the enzyme. Please, point out it:
A. Topoisomerase
B. Transcriptase
C. DNA ligase
D. Helicase
E. Exonuclease
39. Three DNA polymerases (I, II, III) take part in the replication process in prokaryotic cells. Please, point out the type of enzyme, which can remove the RNA-primer from the Okazaki fragment:
A. DNA polymerase I
B. DNA polymerase II
C. DNA polymerase III
D. All types of DNA polymerase
E. This function is for DNA ligase

40. 8-Azoguanine is used for the treatment of many oncology diseases with the aim to suppress cells proliferation of tumor tissue. Please, point out the possible mechanism of this drug action:
A. It inhibits the DNA - polymerase in the replication
B. It inhibits the RNA - polymerase in the transcription of genes
C. It is a protein - repressor
D. It is an inducer of regulator-gene’s transcription
E. It is an antimetabolite, used in the structure of DNA of the tumor cells

41. There is fragment in the structure of DNA strand, which RNA - polymerase attaches to in the phase of transcription initiation. Please, find the name of this fragment:
A. Primary transcript
B. Promotor
C. Operator gene
D. Regulator gene
E. Structural gene

42. DNA polymerase III makes Okazaki fragments on the lagging strand of replication fork. Please, name the enzyme that removes primers from them:
A. DNA polymerase I
B. RNA polymerase
C. Primase
D. DNA ligase
E. Exonuclease

43. A Splicing is the important stage in the primary transcript processing to mRNA in eukaryotes. Please, find the right definition of the Splicing:
A. The connection of exons and removal of introns
B. The removal of exons and connection of introns
C. The methylation of nucleoside residues
D. The polyadenylic acid attaching from the 3’-end of the chain
E. The guanylate nucleotide attaching from the 5’-end

44. According to the LAC operon hypothesis the induction of transcription is possible without protein - repressor, only. Please, point out the term of DNA site that saves the information about the primary structure of this protein:
A. Promotor
B. Operator gene
C. Regulator gene
D. Operon
E. Structural gene

45. The RNA polymerase attaching to the promoter of DNA strand in E.coli is possible if two important compounds are present, only. Please, find out them:
A. cAMP and ATP
B. Protein - repressor and gene
C. CAP - protein and cAMP
D. Glucose and ATP
E. Helicase and topoisomerase
46. Choose the type of bonds formed during the processes of replication and transcription
A. Peptide
B. 1,4-glycoside
C. N-glycoside
D. 3',5'-Phosphodiester
E. Disulfide

47. The complimentary nature of pairs A - T; G - C in double helical structure of DNA is promoted by:
A. The similarity of each couple structure
B. The formation of hydrogen bonds between them
C. The equal concentration of A=T; G=C
D. The capability to form glycoside bonds between them
E. The capability to form covalent bonds between them

48. Name, please, the term used for modification type of primary transcript at 3'-end during the formation of mRNA in eukaryotic cell:
A. Splicing
B. Polyadenylation
C. Capping
D. Methylation
E. Deamination

49. Name, please, the fragment of RNA-polymerase structure (E. coli) that is represented in holo-enzyme and is absent in core-enzyme:
A. Promotor
B. ρ-Protein
C. σ-Factor
D. Operon
E. Exon

50. Three DNA polymerases (I, II, III) take part in the replication process in prokaryotic cell. Please, point out the type of enzyme producing the leading strand for the daughter DNA:
A. DNA polymerase I
B. DNA polymerase II
C. DNA polymerase III
D. All the types of DNA polymerase
E. This function is for DNA ligase, only

51. Three DNA polymerases (I, II, III) take part in the replication process in prokaryotic cell. Please, point out the type of enzyme producing the Okazaki fragments:
A. DNA polymerase I
B. DNA polymerase II
C. DNA polymerase III
D. All the types of DNA polymerase
E. This function is for DNA ligase, only

52. Choose the term of the uninformative site of the initial transcript:
A. Cistron
B. Intron
C. Exon
D. Operon
E. Structural gene

53. Point out the enzyme participating in the stabilization of the replication fork at E. coli:
A. Topoisomerase
B. Endonuclease  
C. DNA-polymerase I  
D. DNA-ligase  
E. Restrictase  

54. Point out the site of polynucleotide chain of DNA in eukaryotic cell, which may be linked to transcription factors to stimulate this process:  
A. Reverse repeat sequence  
B. Primer  
C. Enhancer  
D. Operon  
E. Gene-operator  

55. Point out the enzyme that is capable to connect DNA fragments after primers restriction in E. coli:  
A. Topoisomerase  
B. Endonuclease  
C. DNA-polymerase I  
D. DNA-ligase  
E. Restrictase
LITERATURE

Basic:


Additional:
