Biological chemistry

A manual for independent work at home and in class preparation for licensing examination "KROK 1" on semantic modules 6, 7 of module 2

for students of International Faculty
(the second year of study)

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This manual is recommended for II year students of International Faculty of specialty "General medicine" studying biological chemistry, as additional material to prepare for practical training semantic modules 6, 7 of module 2 and licensing exam "KROK 1: General medical training".

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INTRODUCTION

The handbook "Biological chemistry. A manual for independent work at home and in class preparation for licensing examination "KROK 1" on semantic modules 6 "Metabolism of chromoproteins and nucleaproteins. Basis of molecular biology and genetics", and 7 "Molecular mechanisms of hormones action" of module 2 "Molecular Biology. Biochemistry of cell-to-cell interactions. Of tissues and physiological functions" for students of International Faculty (the second year of study) speciality «General Medicine» contains a summary of the theory, which facilitates finding the right answer test tasks.

Tests of this manual are similar in content and form to the test tasks, provided Testing Center of Ministry of Health of Ukraine. Each test task has only one either correct or more correct answer that must be chosen among the available ones by a student. As a self-study students are invited to give rationale for the choice of the correct answer, identify key words for case described in a test task.

The authors hope that this special form of student work with test tasks, with detailed explanation described in these tasks mostly clinical situations allow foreign English-speaking students to prepare properly and pass licensing exam "KROK 1: General medical training".

NUCLEOPROTEINS.
NUCLEOTIDES AND NUCLEIC ACID FUNCTIONS. MEMBRANES OF CELLS: THEIR STRUCTURE, COMPOSITION AND FUNCTIONS (IVANCHENKO D.H., ROMANENKO M.I.)

INFORMATIONAL MATERIAL

Nucleoproteins

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 Nucleic Acids

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*.
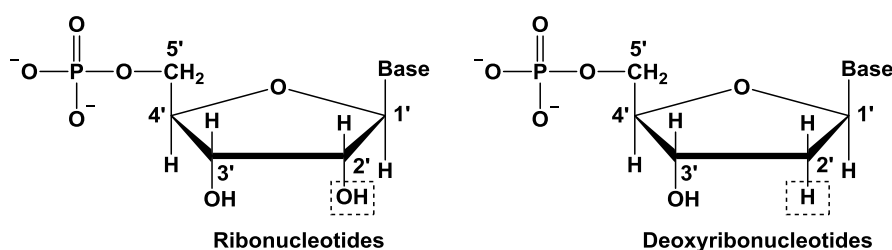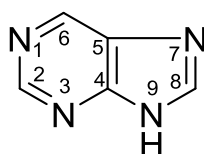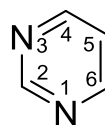

Figure 1. Chemical Structures of Ribonucleotides and Deoxyribonucleotides.

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

Purine   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.

Base Formula	Base (X = H)	Nucleoside (X = ribose*)	Nucleotide (X = ribose phosphate*)
	Adenine Ade A	Adenosine Ado A	Adenylic acid Adenosine monophosphate AMP
	Guanine Gua G	Guanosine Guo G	Guanylic acid Guanosine monophosphate GMP
	Cytosine Cyt C	Cytidine Cyd C	Cytidylic acid Cytidine monophosphate CMP
	Uracil Ura U	Uridine Urd U	Uridylic acid Uridine monophosphate UMP
	Thymine Thy T	Deoxythymidine dThd dT	Deoxythymidylic acid Deoxythymidine monophosphate dTMP

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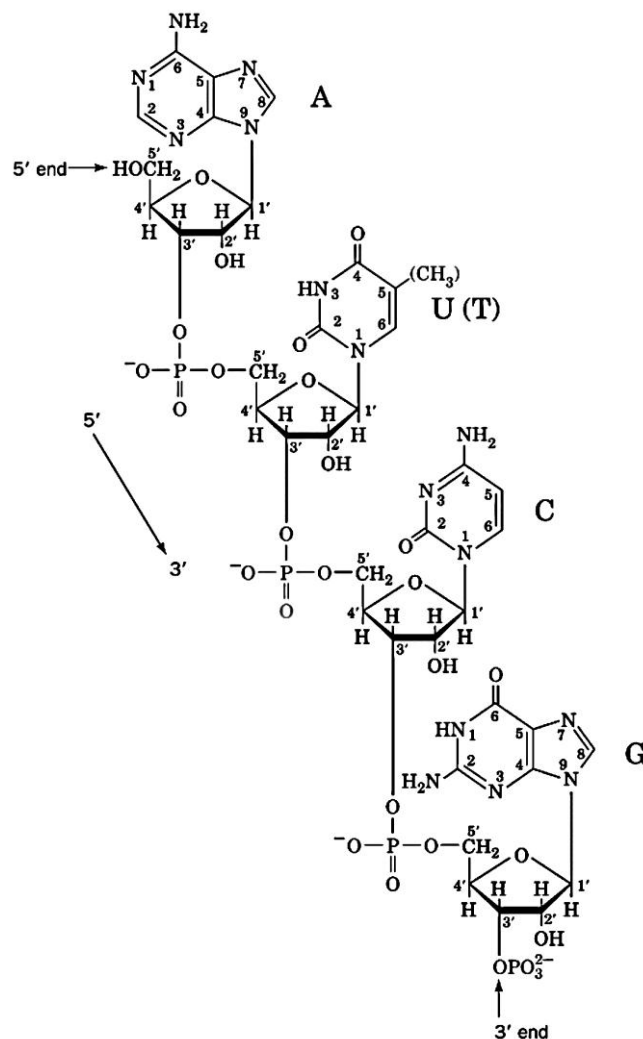
Figure 2. The Tetranucleotide Adenyl-3′,5′-uridyl-3′,5′-cytidyl-3′,5′-guanylyl-3′-phosphate.

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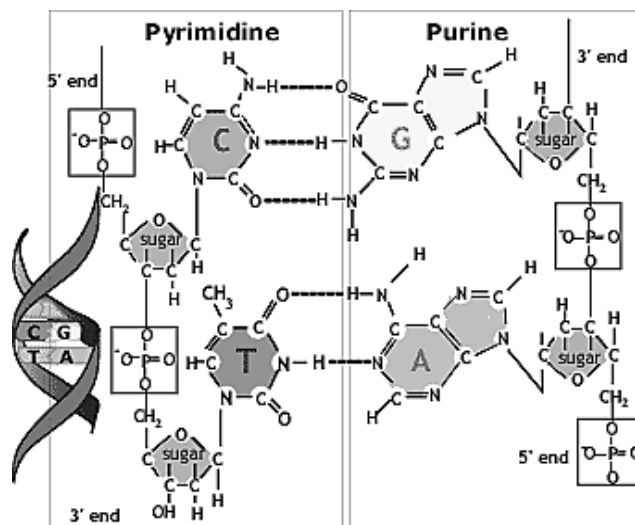
DNA's base composition varies widely among different organisms. It ranges from ~25% to 75% G+C in different species of bacteria. It is, however, more or less constant among related species; for example, in mammals G+C ranges from 39% to 46%.

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.

N^6-Methyl-dA   5-Methyl-dC
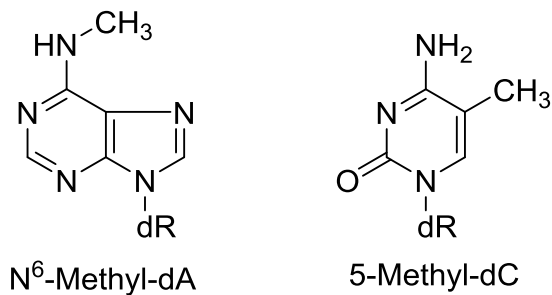
The altered bases are generated by the sequence-specific enzymatic modification of normal DNA. The modified DNAs obey Chargaff's rules if the derivatized bases are taken as equivalent to their parent bases. Likewise, many bases in RNAs and, in particular, those in **transfer RNAs** (**tRNAs**) are derivatized.

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.
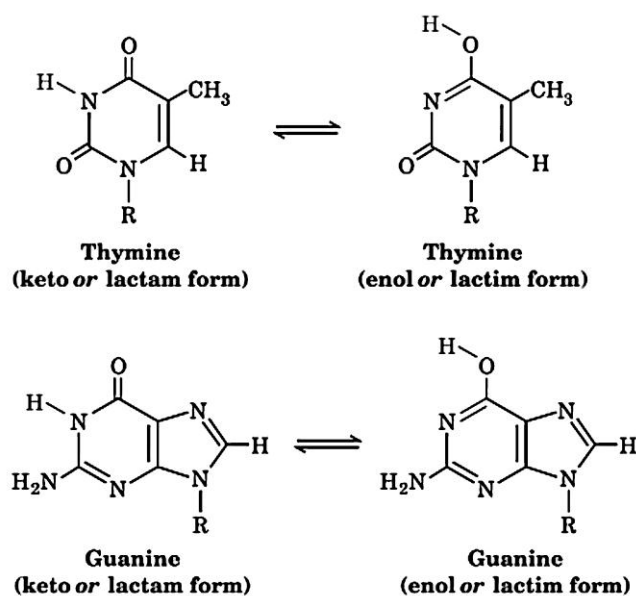
Figure 4. Some Possible Tautomeric Conversions for Bases.

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.
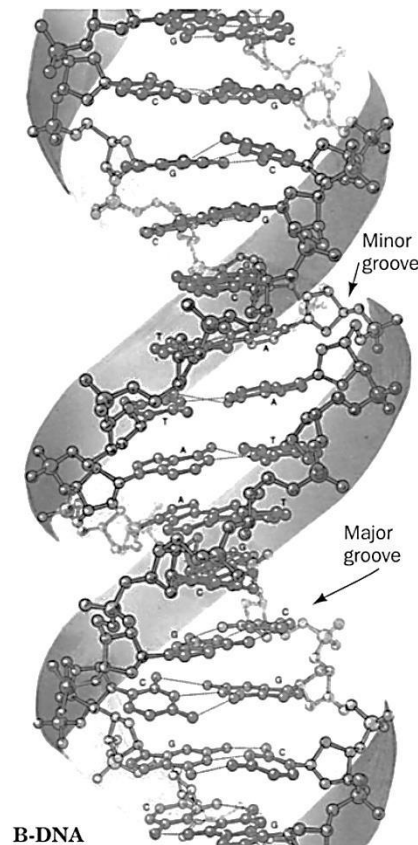
Figure 5. Three-dimensional Structure of B-DNA.

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.

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.
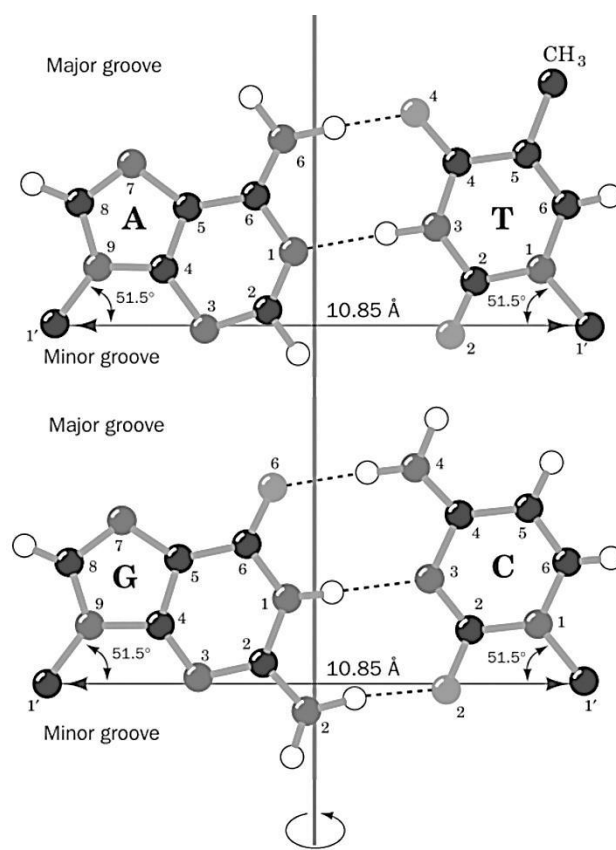
Figure 6. Watson-Crick Base Pairs.

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(GGCCGGCC) and d(GGTATACC), 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 2. Structural Features of Ideal A-, B-, and Z-DNA.

|	**A-DNA**	**B-DNA**	**Z-DNA**
Helical sense	Right-handed	Right-handed	Left-handed
Diameter	~26 Å	~20 Å	~18 Å
Base pairs per helical turn	11.6	10	12 (6 dimers)
Helical twist per base pair	31°	36°	9° for pyrimidine-purine steps; 51° for purine-pyrimidine steps
Helix pitch (rise per turn)	34 Å	34 Å	44 Å
Helix rise per base pair	2.9 Å	3.4 Å	7.4 Å per dimer
Base tilt normal to the helix axis	20°	6°	7°
Major groove	Narrow and deep	Wide and deep	Flat
Minor groove	Wide and shallow	Narrow and deep	Narrow and deep
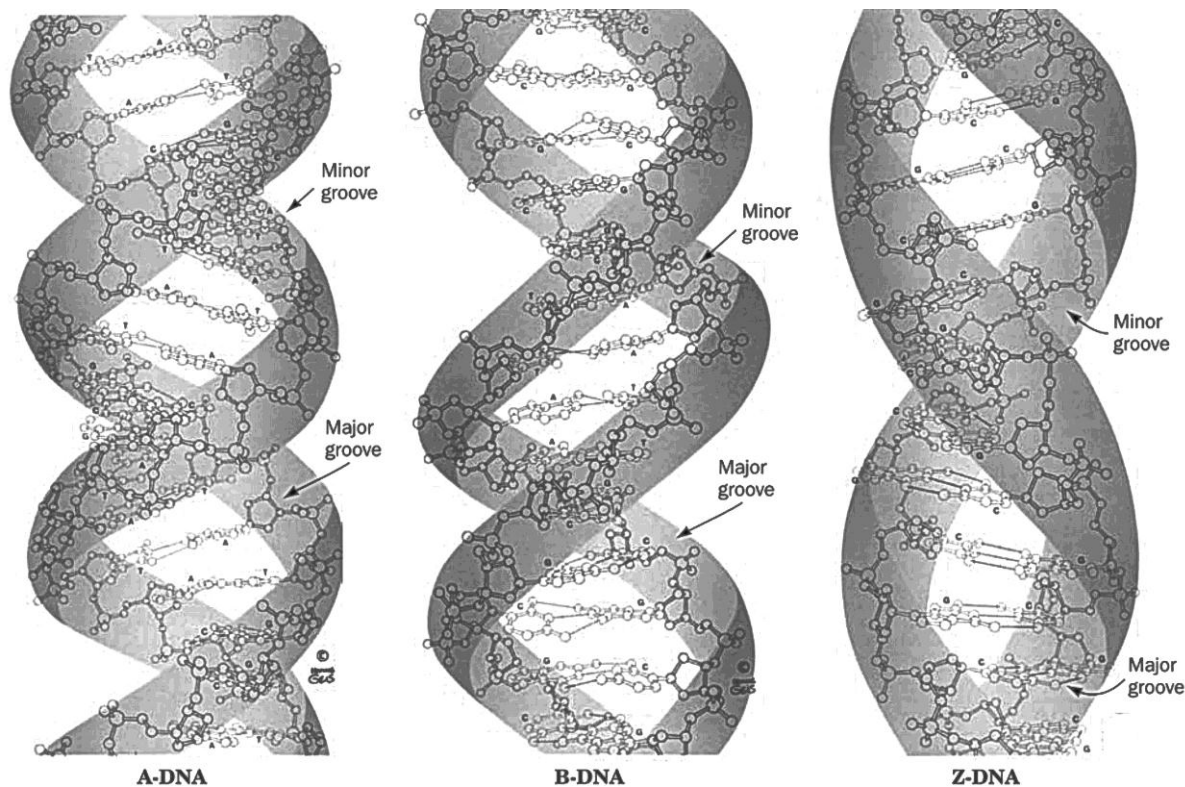
Figure 7. Structures of A-, B-, and Z-DNAs.

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.

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 demon-strating 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.
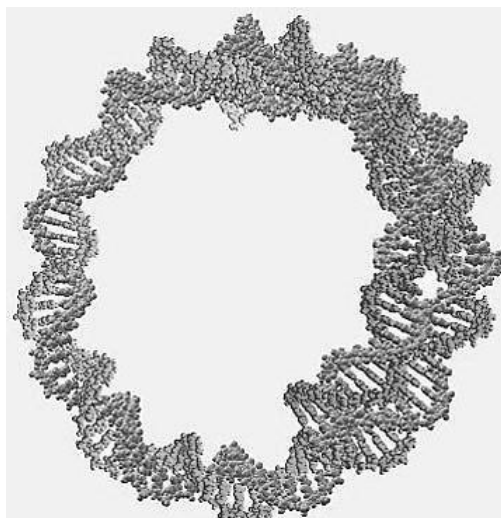

Figure 8. Circular DNA.

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.

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.
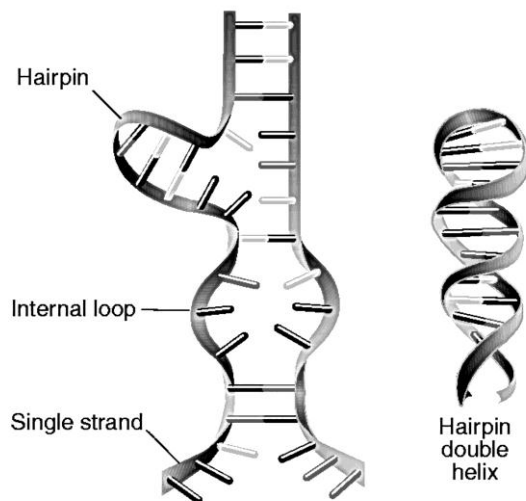
Figure 10. Secondary Structure of RNA.

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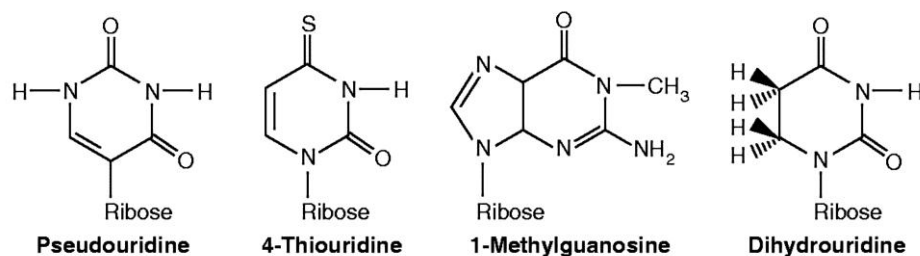
The structure of tRNA allows it to perform two critical functions involving the most important structural components: the 3′-terminus and the anticodon loop.
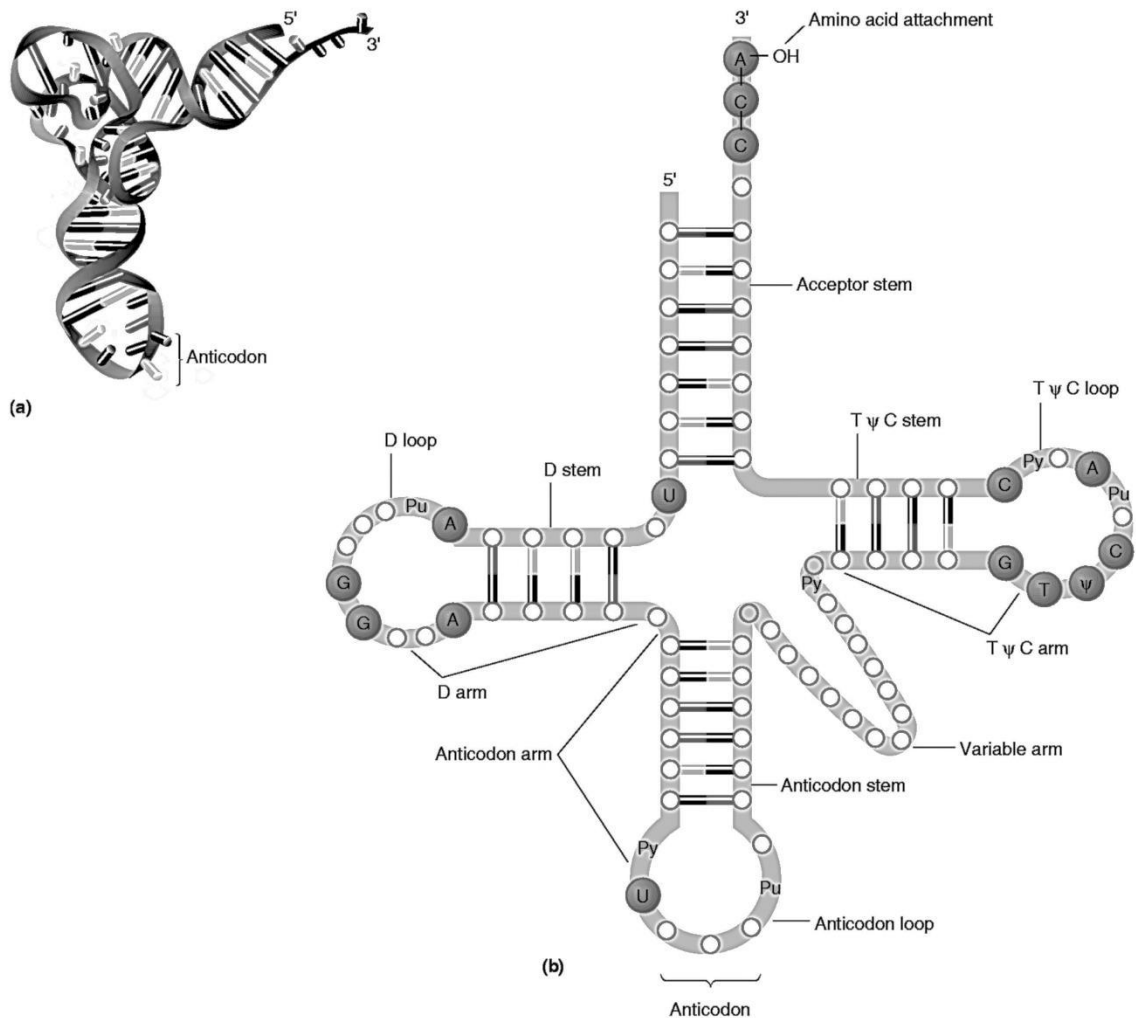
Figure 11. Transfer RNA. (a) Three-dimensional structure of a tRNA molecule. (b) A schematic view of a tRNA molecule.

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 Hl binds, in part, to the linker DNA (Fig. 12).
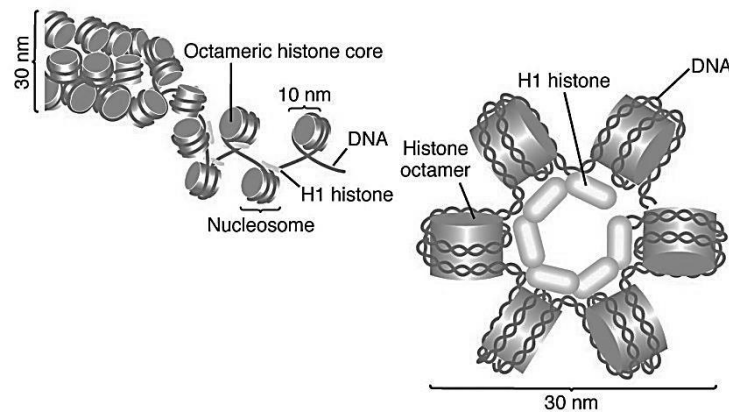
Figure 12. Chromatin organization.

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 $(H3)_2(H4)_2$ tetramer and a pair of 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. N ucleosomes 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.

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.
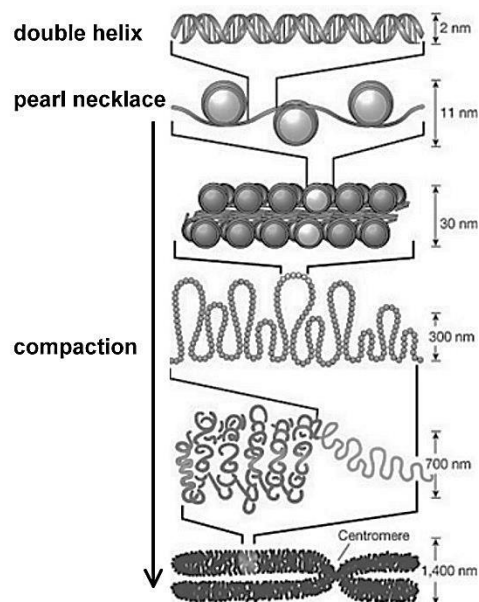
Figure 13. Steps of chromatin organization.

Biological Membranes

Most of the properties attributed to living organisms (e.g., movement, growth, reproduction, and metabolism) depend, either directly or indirectly, on membranes. A *lipid bilayer model* originally proposed for membrane structure in 1935 by Davson and Danielle has been modified. *Fluid mosaic model*, proposed by Singer and Nicolson, is a more recent and acceptable model for membrane structure. All biological membranes have the same general structure. The biological membranes usually have a thickness of 5-8 nm. A membrane is essentially composed of a lipid bilayer. The hydrophobic (nonpolar) regions of the lipids face each other at the core of the bilayer while the hydrophilic (polar) regions face outward. Globular proteins are irregularly embedded in the lipid bilayer. The proteins, most of which float within the lipid bilayer, largely determine a membrane's biological functions.

Membrane Functions

Enclosure and insulation of cells and organelles. The enclosure provided by the plasma membrane protects cells from their environment both mechanically and chemically. The plasma membrane is essential for maintaining differences in

the concentration of many substances between the intracellular and extracellular compartments.

Membrane Transport. Membrane transport mechanisms are vital to living organisms. Ions and molecules constantly move across cell plasma membranes and across the membranes of organelles. This flux must be carefully regulated to meet each cell's metabolic needs. For example, a cell's plasma membrane regulates the entrance of nutrient molecules and the exit of waste products. Additionally, it regulates intracellular ion concentrations. Because lipid bilayers are generally impenetrable to ions and polar substances, specific transport components must be inserted into cellular membranes.

Biological transport mechanisms are classified according to whether they require energy. Major types of biological transport are illustrated in Figure 14. In *passive transport*, there is no direct input of energy. In contrast, *active transport* requires energy to transport molecules against a concentration gradient.
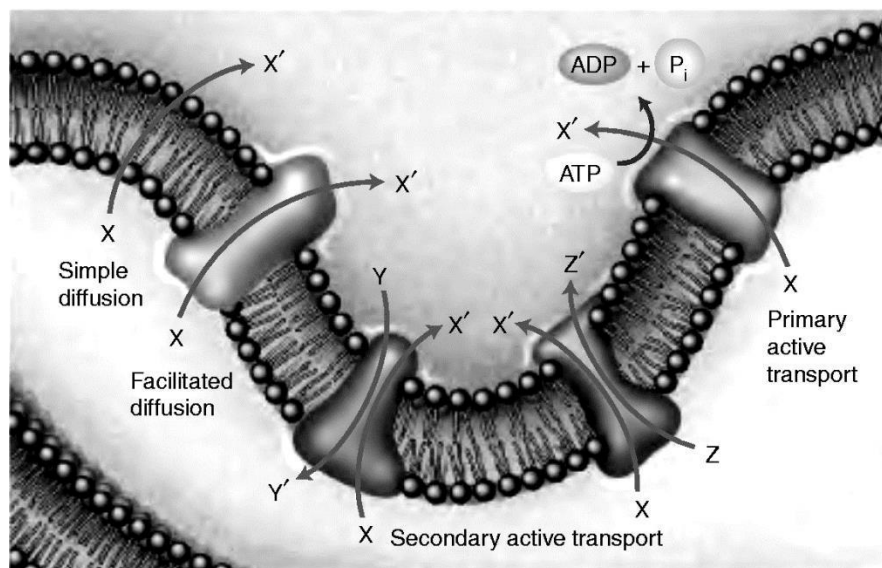
Figure 14. Transport Across Membranes.

In *simple diffusion*, each solute, propelled by random molecular motion, moves down its concentration gradient (i.e., from an area of high concentration to an area of low concentration). In this spontaneous process, there is a net movement of solute until an equilibrium is reached. A system reaching equilibrium becomes more disordered, that is, entropy increases. Because there is no input of energy,

transport occurs with a negative change in free energy. In general, the higher the concentration gradient, the faster the rate of solute diffusion. The diffusion of gases such as O_2 and CO_2 across membranes is proportional to their concentration gradients. The diffusion of organic molecules also depends on molecular weight and lipid solubility.

In *facilitated diffusion*, the second type of passive transport, the transport of certain large or charged molecules occurs through special channels or carriers. Channels are tunnel-like transmembrane proteins. Each type is designed for the transport of a specific solute. Many channels are chemically or voltage-regulated. Chemically regulated channels open or close in response to a specific chemical signal. For example, a chemically gated Na^+ channel in the nicotinic acetylcholine receptor complex (found in muscle cell plasma membranes) opens when acetylcholine binds. Na^+ rushes into the cell and the membrane potential falls. Because membrane potential is an electrical gradient across the membrane, a decrease in membrane potential is membrane depolarization. Local depolarization caused by acetylcholine leads to the opening of nearby Na^+ channels. Repolarization, the reestablishment of the membrane potential, begins with the diffusion of K^+ ions out of the cell through voltage-gated K^+ channels. The diffusion of K^+ ions out of the cell makes the inside less positive, that is, more negative.

Another form of facilitated diffusion involves membrane proteins called *carriers* (sometimes referred to as *passive transporters*). In carrier-mediated transport, a specific solute binds to the carrier on one side of a membrane and causes a conformational change in the carrier. The solute is then translocated across the membrane and released. The red blood cell *glucose transporter* is the best characterized example of passive transporters. It allows D-glucose to diffuse across the red blood cell membrane for use in glycolysis and the pentose phosphate pathway. Facilitated diffusion increases the rate at which certain solutes move down their concentration gradients. This process cannot cause a net increase in solute concentration on one side of the membrane.

The two forms of active transport are primary and secondary. In *primary active transport*, energy is provided by ATP. Transmembrane ATP-hydrolyzing enzymes use the energy derived from ATP to drive the transport of ions or molecules. The Na^+-K^+ pump (also referred to as the Na^+-K^+ ATPase) is a prominent example of a primary transporter. In *secondary active transport*, concentration gradients generated by primary active transport are harnessed to move substances across membranes. For example, the Na^+ gradient created by the Na^+-K^+ ATPase pump is used in kidney tubule cells and intestinal cells to transport D-glucose.

Membrane Receptors. Membrane receptors play a vital role in the metabolism of all living organisms. They provide mechanisms by which cells monitor and respond to changes in their environment. In multicellular organisms the binding of chemical signals, such as the hormones and neurotransmitters of animals, to membrane receptors is a vital link in intracellular communication. Other receptors are engaged in cell-cell recognition or adhesion. For example, lymphocytes perform a critical role in the immune system function of identifying and then destroying foreign or virus-infected cells when they transiently bind to cell surfaces throughout the body. Similarly, the capacity of cells to recognize and adhere to other appropriate cells in a tissue is of crucial importance in many organismal processes, such as embryonic and fetal development.

Enzymatic catalysis of reactions. Important enzymes are located in membranes at the interface between the lipid and aqueous phases. This is where reactions with apolar substrates occur. Examples include lipid biosynthesis and the metabolism of apolar xenobiotics. The most important reactions in energy conversion – i. e., oxidative phosphorylation.

Anchoring of the cytoskeleton to maintain the shape of cells and organelles and to provide the basis for movement processes.

Membrane Structure

Because each type of living cell has its own functions, it follows that the structure of its membranes is also unique. Not surprisingly, the proportion of lipid and protein varies considerably among cell types and among organelles within each cell (Table 3). The types of lipid and protein found in each membrane also vary.

Table 3. Chemical Composition of Some Cell Membranes

Membrane	Protein, %	Lipid, %	Carbohydrate, %
Human erythrocyte plasma membrane	49	43	8
Mouse liver cell plasma membrane	46	54	2-4
Amoeba plasma membrane	54	42	4
Mitochondrial inner membrane	76	24	1-2
Spinach chloroplast lamellar membrane	70	30	6
Halobacterium purple membrane	75	25	0

Membrane Lipids. The fundamental building blocks of all cell membranes are phospholipids, which are amphipathic molecules, consisting of two hydrophobic fatty acid chains linked to a phosphate-containing hydrophilic head group (Fig. 15). Because their fatty acid tails are poorly soluble in water, phospholipids spontaneously form bilayers in aqueous solutions, with the hydrophobic tails buried in the interior of the membrane and the polar head groups exposed on both sides, in contact with water (Fig. 16). Such phospholipid bilayers form a stable barrier between two aqueous compartments and represent the basic structure of all biological membranes.

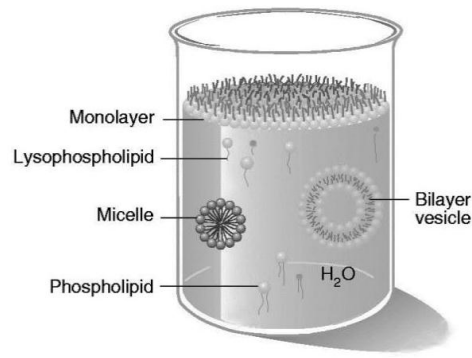**Figure 15.** Structure of phospholipids.

Figure 16. Phospholipid Molecules in Aqueous Solution.

Membrane lipids are largely responsible for several other important features of biological membranes:

1. *Membrane fluidity.* The term fluidity describes the resistance of membrane components to movement. Rapid lateral movement (Fig. 17) of lipid molecules is apparently responsible for the proper functioning of many membrane proteins. The movement of lipid molecules from one side of a lipid bilayer to the other is relatively rare. A membrane's fluidity is largely determined by the percentage of unsaturated fatty acids in its phospholipid molecules. A high concentration of unsaturated chains results in a more fluid membrane. Because of its hydrocarbon ring structure, cholesterol plays a distinct role in determining membrane fluidity. Cholesterol molecules insert into the bilayer with their polar hydroxyl groups close to the hydrophilic head groups of the phospholipids (Fig. 18). The rigid hydrocarbon rings of cholesterol therefore interact with the regions of the fatty acid chains that are adjacent to the phospholipid head groups. This interaction decreases the mobility of the outer portions of the fatty acid chains, making this part of the membrane more rigid. On the other hand, insertion of cholesterol interferes with interactions between fatty acid chains, thereby maintaining membrane fluidity at lower temperatures.
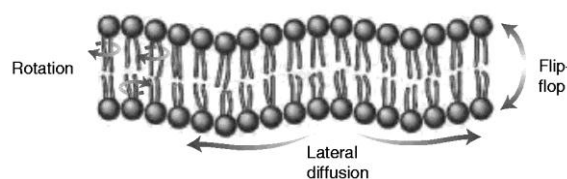
Figure 17. Diffusion in Biological Membranes.
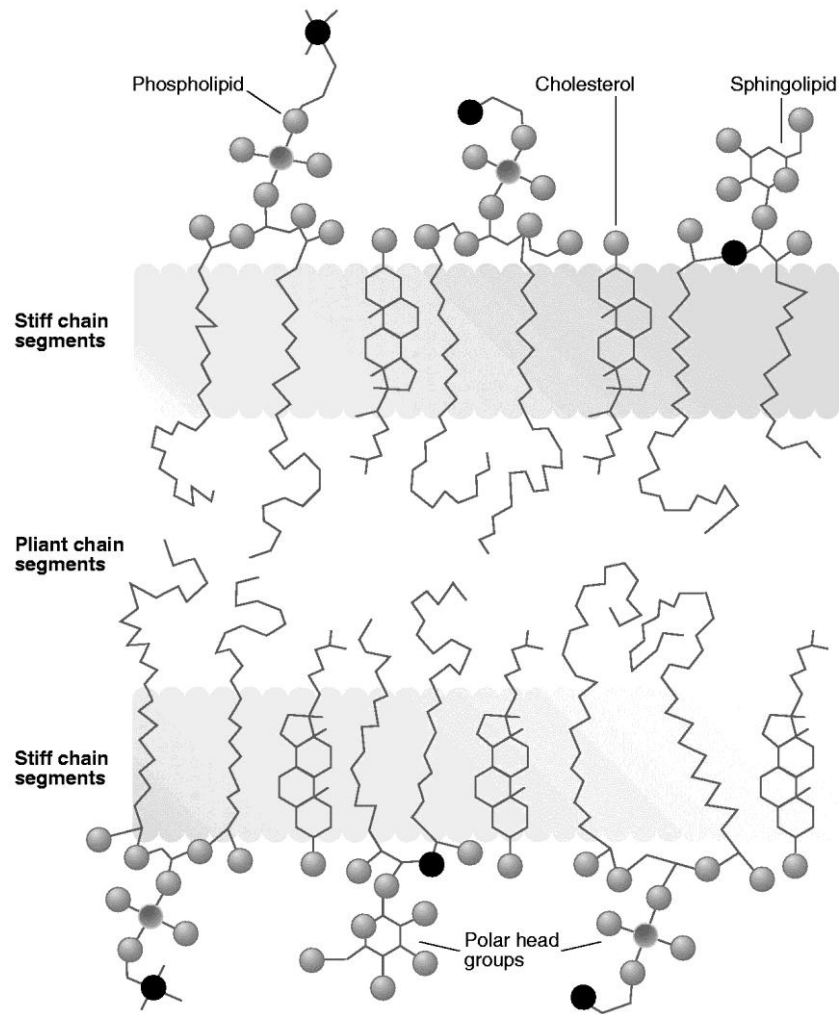
Figure 18. Lipid Bilayer.

2. ***Selective permeability.*** Because of their hydrophobic nature, the hydrocarbon chains in lipid bilayers provide a virtually impenetrable barrier to the transport of ionic and polar substances. Specific membrane proteins regulate the movement of such substances into and out of cells. To cross a lipid bilayer, a polar substance must shed some or all of its hydration sphere and bind to a carrier protein for membrane translocation or pass through an aqueous protein channel. Both methods shield the hydrophilic molecule from the hydrophobic core of the membrane. Most transmembrane water movement accompanies ion transport. Nonpolar substances simply diffuse through the lipid bilayer down their concentration gradients. Each membrane exhibits its own transport capability or selectivity based on its protein component.

3. ***Self-sealing capability.*** When lipid bilayers are disrupted, they immediately and spontaneously reseal (Fig. 19) because a break in a lipid bilayer exposes the hydrophobic hydrocarbon chains to water. Because breaches in cell membranes can be lethal, this resealing property is critical. In living cells, certain protein components of membrane and the cytoskeleton, as well as calcium ions, also assist in membrane resealing.

Figure 19. Membrane Self-Sealing.

4. ***Asymmetry.*** Biological membranes are asymmetric; that is, the lipid composition of each half of a bilayer is different. For exam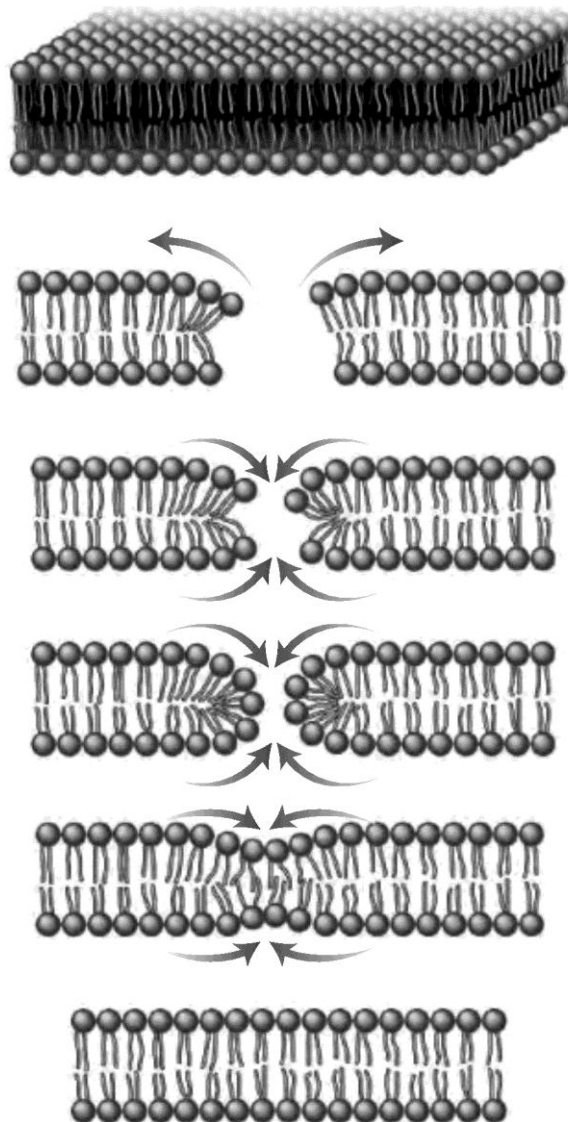ple, the human red blood cell membrane possesses substantially more phosphatidylcholine and sphingomyelin on its outside surface. Most of the membrane's phosphatidylserine

and phosphatidylethanolamine are on the inner side. Membrane asymmetry is not unexpected, because each side of a membrane is exposed to a different environment. Asymmetry originates during membrane synthesis, because phospholipid biosynthesis occurs on only one side of a membrane. The protein components of membranes also exhibit considerable asymmetry with distinctly different functional domains within membrane and on the cytoplasmic and extracellular faces of membrane.

Membrane Proteins. Most of the functions associated with biological membranes require protein molecules. Membrane proteins are often classified by the function they perform. Most of these molecules are structural components, enzymes, hormone receptors, or transport proteins.

Membrane proteins are also classified according to their structural relationship to membrane. Proteins that are embedded in and/or extend through a membrane are referred to as *integral proteins* (Fig. 20). Such molecules can be extracted only by disrupting the membrane with organic solvents or detergents. Many integral membrane proteins (called *transmembrane proteins*) span the lipid bilayer, with portions exposed on both sides of the membrane. The membrane-spanning portions of these proteins are usually α-helical regions of 20 to 25 nonpolar amino acids. The hydrophobic side chains of these amino acids interact with the fatty acid chains of membrane lipids, and the formation of an α-helix neutralizes the polar character of the peptide bonds. Like the phospholipids, transmembrane proteins are amphipathic molecules, with their hydrophilic portions exposed to the aqueous environment on both sides of the membrane. Some transmembrane proteins span the membrane only once; others have multiple membrane-spanning regions. Most transmembrane proteins of eukaryotic plasma membranes have been modified by the addition of carbohydrates, which are exposed on the surface of the cell and may participate in cell-cell interactions.
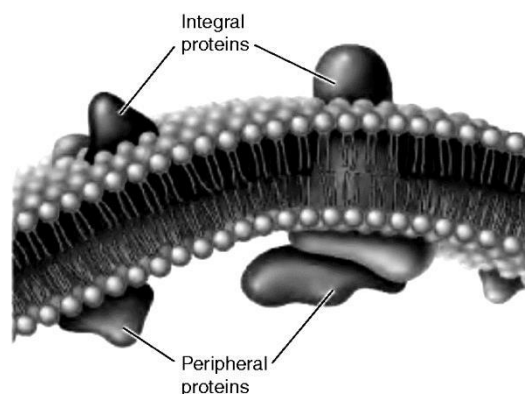
Figure 20. Integral and Peripheral Membrane Proteins.

Peripheral proteins are bound to membrane primarily through interactions with integral membrane proteins. Some peripheral proteins interact directly with the lipid bilayer. Typically, peripheral proteins can be released from membrane by relatively gentle methods (e.g., concentrated salt solutions or pH changes alter noncovalent interactions between amino acid side chains).

Proteins can also be anchored in membranes by lipids that are covalently attached to the polypeptide chain. Distinct lipid modifications anchor proteins to the cytosolic and extracellular faces of the plasma membrane. Proteins can be anchored to the cytosolic face of the membrane either by the addition of a 14-carbon fatty acid (myristic acid) to their amino terminus or by the addition of either a 16-carbon fatty acid (palmitic acid) or 15- or 20-carbon prenyl groups to the side chains of cysteine residues. Alternatively, proteins are anchored to the extracellular face of the plasma membrane by the addition of glycolipids to their carboxy terminus.

EXERCISES FOR INDEPENDENT WORK. In the table with test tasks emphasize keywords, choose the correct answer and justify it:

№	Test:	Explanation:
1.	Nowadays about 50 minor bases have found in the t-RNA structure besides the main four nitrogenous bases. Choose the minor nitrogenous base: A. Dihydrouracil	

№	Test:	Explanation:
	B. Cysteine	
	C. Uracil	
	D. Adenine	
	E. Cytosine	
2.	Point out constituents of nucleoside:	
	A. Pentose, H_3PO_4	
	B. Nitrogenous base, H_3PO_4	
	C. Pentose, $H_4P_2O_7$	
	D. Nitrogenous base, pentose	
	E. Nitrogenous base, $H_4P_2O_7$	
3.	Choose the proteins that are included into the deoxyribonucleoprotein (DNP) structure of eukaryotic cells: A. Albumins B. Globulins C. Histones D. Collagen E. Glutelines	
4.	Point out the type of bonds that stabilize the DNA double helix and are formed between the complementary pairs of bases: A. Phosphodiester B. Hydrophobic C. Hydrogen D. Peptide E. Disulfide	
5.	Nucleic acids have acid properties due to the presence of residue in their structure: A. Adenosine B. Guanine C. Deoxyribose D. Ribose E. Phosphoric acid	
6.	Name the main important class of lipids used for formation of any type of membrane: A. Triacylglycerols B. Cholesterol C. Steroids D. High fatty acids E. Phospholipids	

№	Test:	Explanation:
7.	Find out the position for lipid whose high content in the membrane can decrease the fluidity of this membrane: A. Triacylglycerol B. Cholesterol C. Oleic acid D. Arachidonic acid E. Phosphatidyl ethanol amine	
8.	Nitrogenous bases are linked by hydrogen bonds at the formation of DNA double helix. Point out the quantity of the hydrogen bonds between adenine (A) and thymine (T): A. Three B. One C. Four D. Two E. Five	
9.	Cytosine is involved in oxidative deamination (the removal of amino group) to form the other nitrogenous base residue in the DNA molecule. Point out this nitrogenous base: A. Adenine B. Uracil C. Guanine D. Inosine E. Thymine	
10.	The secondary tRNA structure (the model of "clover leaf") has several sites which are responsible for a certain biologic function. Name the site, including the special order of three nucleotides that are complementary to the mRNA triplet: A. Codon B. Supplementary loop C. Pseudouracil loop D. Anticodon sequence E. Acceptor part	
11.	The length of a complete turn in the DNA double helix (form B) is 3.4 nm. Point out how many nucleotide residues can be	

№	Test:	Explanation:
	packed in this turn: A. 8 B. 10 C. 15 D. 20 E. 6	
12.	Name the component of the phospholipid structure whose big quantity in the membrane correlates with its high fluidity: A. Saturated high fatty acid B. Glycerol C. Unsaturated high fatty acid D. Phosphoric acid E. Nitrogenous derivative	
13.	Point out the name of the scientist who gave some conclusions (or rules) about quantitative correlations between nitrogenous bases in the DNA chains: A. Belozersky A.N. B. Ochoa S. C. Nirenberg M. D. Chargaff E. E. Jakob L.	
14.	Point out the type of the bond that stabilizes the primary structure of nucleic acid: A. Hydrogen B. Disulfide C. Peptide D. Phosphodiester E. Van der Waal's forces	
15.	In a human genome project scientists notice, that one strand of the DNA molecule contains 20 thymine (T), 25 cytosine (C), 30 guanine (G) and 22 adenine (A) residues. How many of each of the bases is found in complete double-stranded molecule? A. T=44, C=60, G=50, A=40 B. T=22, C=30, G=25, A=20	

№	Test:	Explanation:
	C. T=40, C=50, G=60, A=44	
	D. T=42, C=55, G=55, A=42	
	E. T=42, C=50, G=60, A=42	
16.	Membrane fluidity is increased by increased content of: A. Stearic acid B. Palmitic acid C. Cholesterol D. Linoleic acid E. Lauric acid	
17.	Chargaff rule states that A. A+G=T+C B. A/T=G/C C. A=U=T=G=C D. A+T=G+C E. None of the above	
18.	At the physiological pH the DNA molecules are: A. Positively charged B. Negatively charged C. Neutral D. Amphipathic E. Uncharged	
19.	Which one of the following is the complementary sequence of 5'-TTAAGCTAC-3'? A. 5'GTAGCTTAA3' B. 5'AATTCGCATG3' C. 5'CATGCGAATT3' D. 5'TTAAGCGTAC3' E. 5'CCGGATCGT3'	
20.	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	

THE METABOLISM OF PURINE AND PYRIMIDINE NUCLEOTIDES. THE DISORDERS OF NUCLEOTIDE METABOLISM
(KRISANOVA N. V.)

INFORMATIONAL MATERIAL

INTRODUCTION

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 fig. 21. 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 nucleotide 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.

DIGESTION OF DEOXYRIBONUCLEOPROTEIN (DNP) IN GIT

STOMACH → Denaturation in two parts : DNA and Proteins; the unwinding of double helix of DNA; proteins degradation beginning

DUODENUM SMALL INTESTINE → Continuation of protein degradation; DNA degradation up to nucleosides; Absorption of nucleosides

LARGE INTESTINE → Partial nucleosides transformation (~3%) up to uric acid, ammonia and carbon dioxide under the influence of microflora enzymes
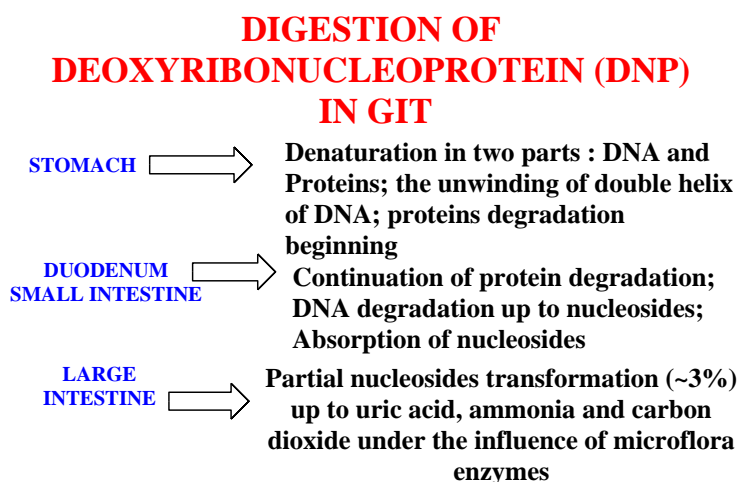
Figure 21. Digestion steps of DNP in GIT.

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 fig.22.
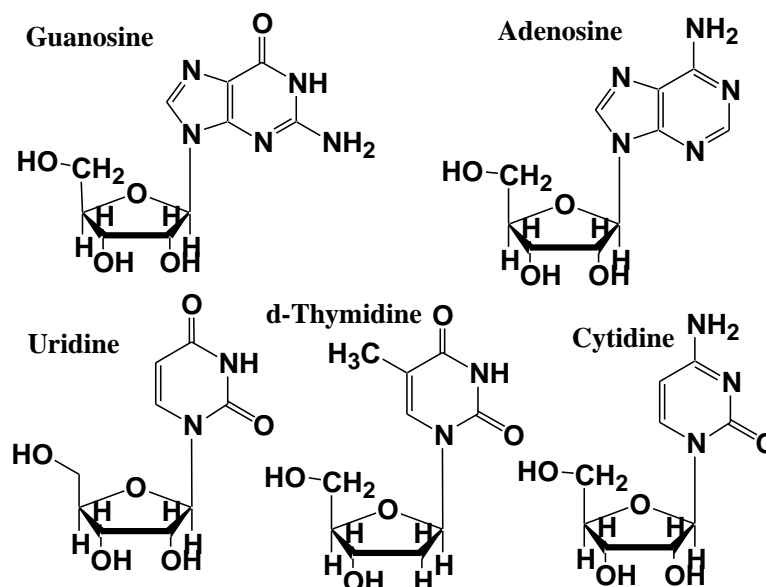
Figure 22. Main nucleosides discussed for their metabolism in humans.

DEGRADATION OF PURINE NUCLEOSIDES IN CELLS OF TISSUES

Adenosine is involved in hydrolytic deamination to form inosine (fig.3, step 1), then we have to consider its dephosphorylation due to special phosphorylase. Hypoxanthine is formed as a product (fig.23, step 2).

Xanthine oxidase catalyzed two reactions: conversion of hypoxanthine to xanthine (fig.23, step 3), and then there is formation of uric acid (fig.23, 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.3, step 5), and then we can consider the deamination of guanine (fig.3, step 6) to form xanthine.

Figure 23. Degradation of purine nucleosides up to uric acid.

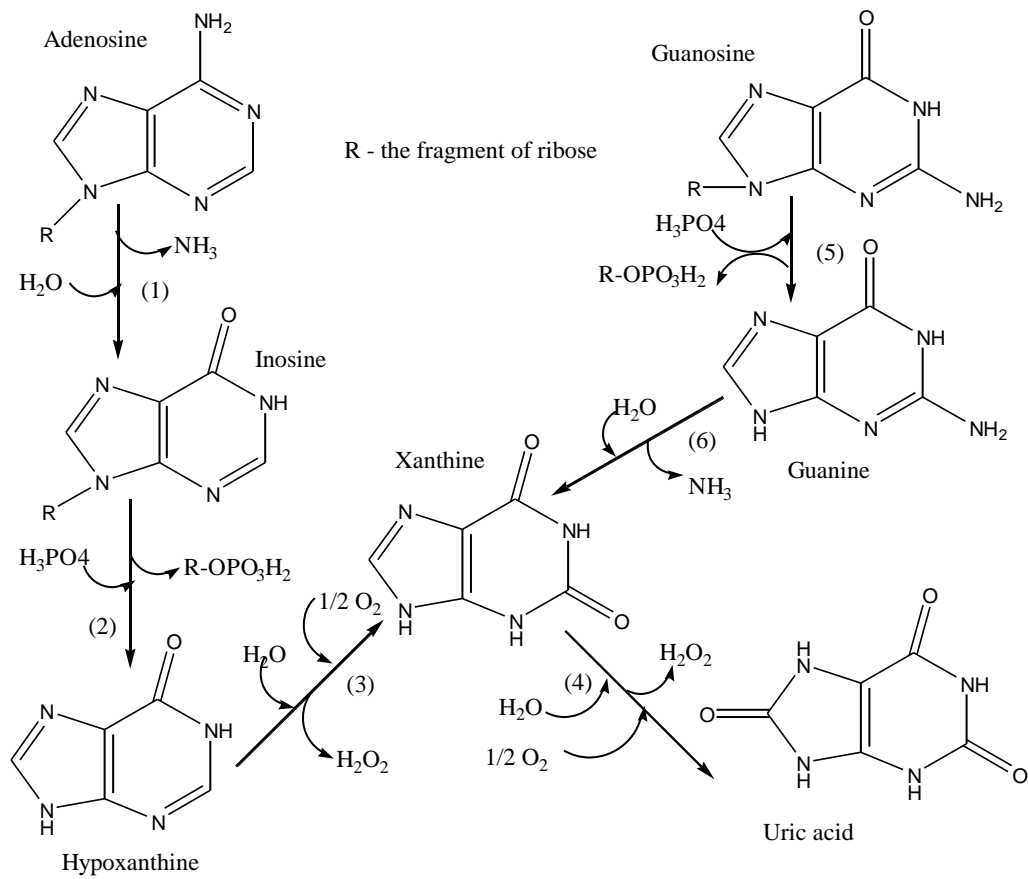Uric acid may be in two forms: enol- and keto-form. Sodium ions form with enol-form of uric acid salt that is known as sodium urate (fig. 24).

Conversion for uric acid and formation of sodium urate
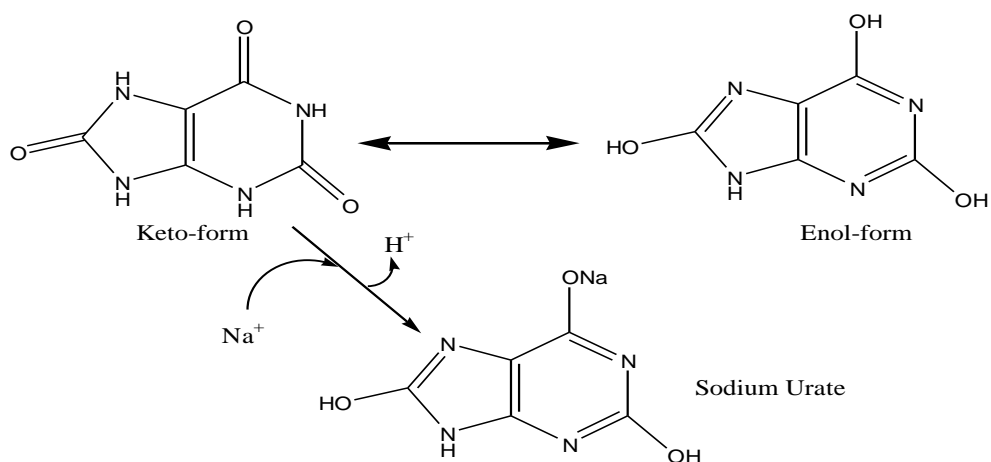
Figure 24. Enol- and keto- forms of uric acid; the formation ofmonosodium urate.

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.25): uridine and thymidine destructions.
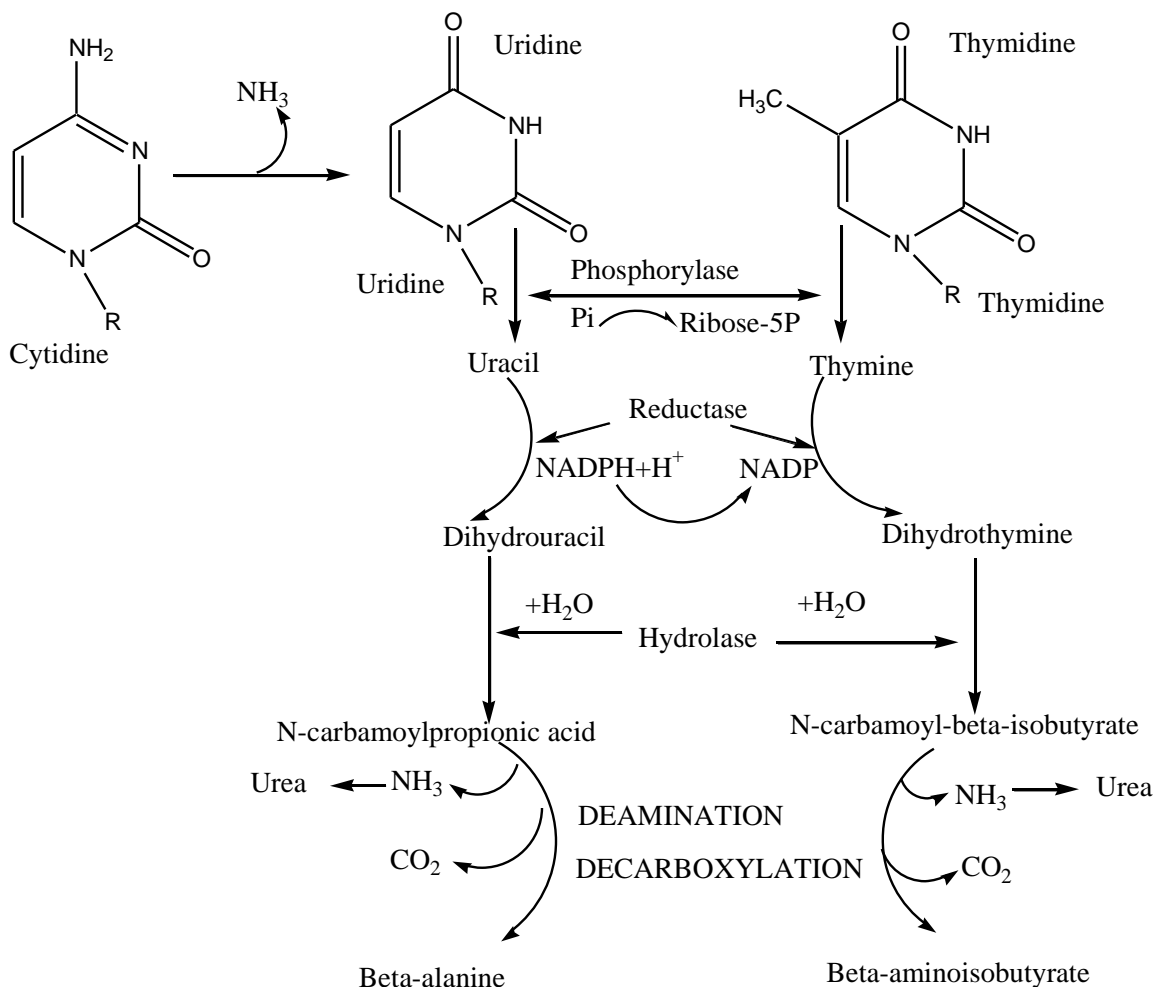
Figure 25. Degradation of pyrimidine nucleosides

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).

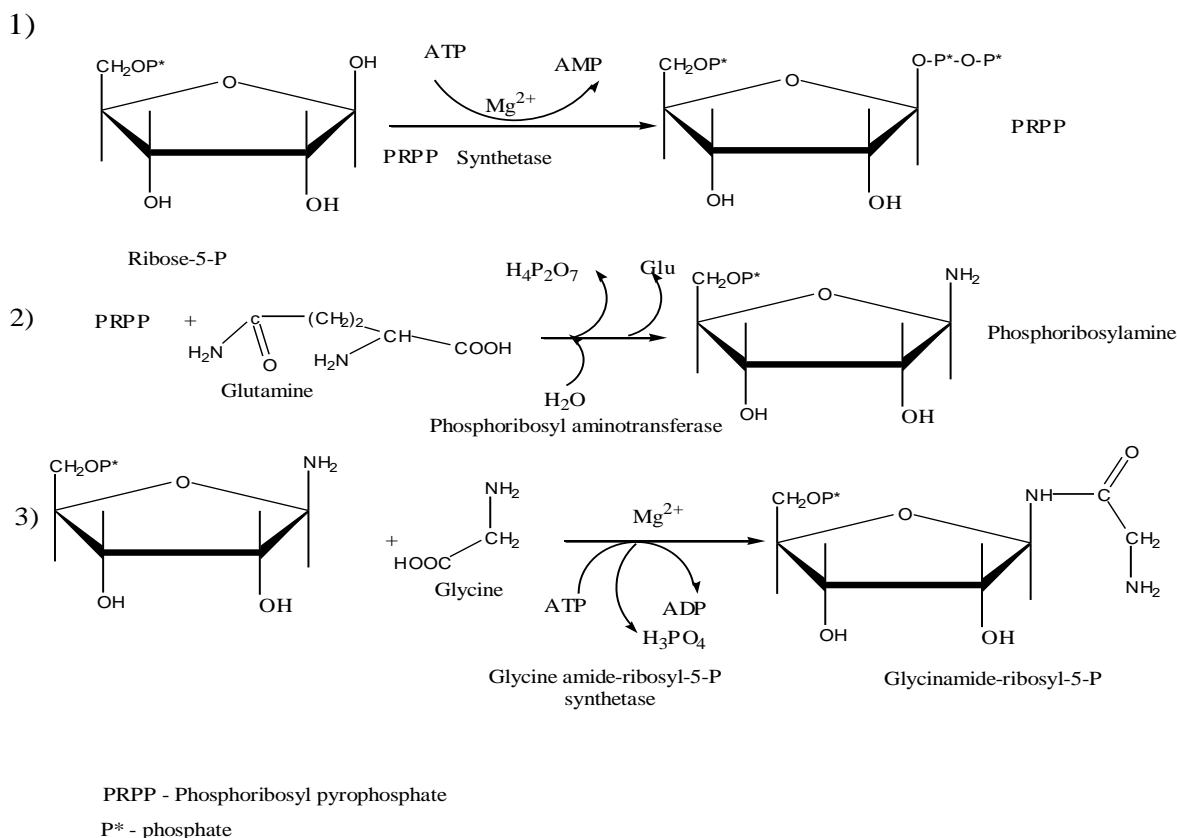The first three reactions are very important for regulation of this process (figure 26).

PRPP - Phosphoribosyl pyrophosphate

P* - phosphate

Figure 26. The initial reactions of purine nucleotide synthesis

43

Then there are eight reactions that give the metabolite – Inosine monophosphate (IMP, fig.27). 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).

$N^{(3)}$ 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 aminoimidazol-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
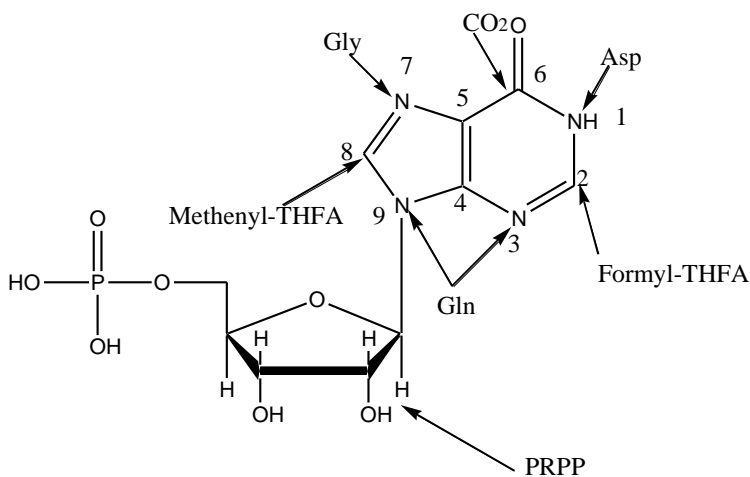
Figure 27. A structure of key intermediate metabolite for synthesis of purine nucleotides - Inosine Mono Phosphate (IMP): THFA- Tetra Hydro Folic Acid; PRPP - phosphoribosyl pyrophosphate

The second phase of purine nucleotide synthesis is the formation of AMP or GMP from IMP (fig. 28). Two reactions (1, 3, fig.28) 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.8) to add nitrogen in a form of amino group instead of keto-group in position 6 of inosine fragment in IMP.

Two reactions are used for GMP synthesis from IMP, and ATP is used as energy source in the step (4, fig.28). Glutamine is a donor of amine group for position 2 of guanine fragment in GMP.
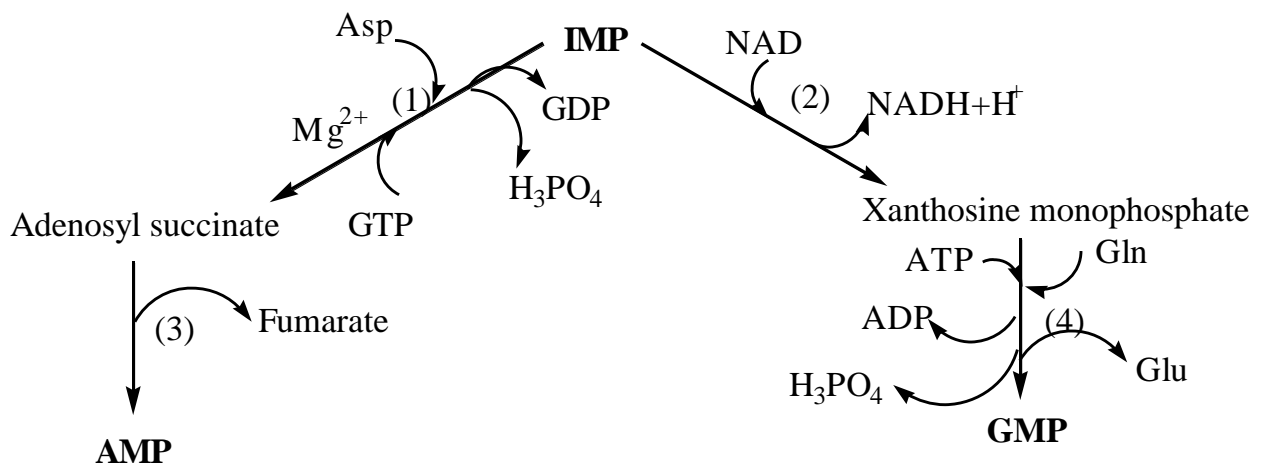
1 -- Adenosyl succinate synthetase   3 -- Adenosyl succinate lyase

2 -- IMP-dehydrogenase   4 -- GMP-synthetase

Figure 28. The second phase of purine nucleotide synthesis.

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:* B_9, B_{12}, B_5 (or PP); B_{12} 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:

$$GMP \xrightarrow[\text{kinase}]{ATP \quad ADP} GDP \xrightarrow[\text{kinase}]{ATP \quad ADP} GTP$$

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

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.29). They are found in the liver, brain, polymorphonuclear leukocytes, lymphocytes:

$$Hypoxanthine \xrightarrow{PRPP \quad H_4P_2O_7} IMP$$

Hypoxanthine guanine phosphoribo-
syl pyrophosphate transferase ∗

$$Guanine \xrightarrow{PRPP \quad H_4P_2O_7} GMP$$
∗

$$Adenine \xrightarrow{PRPP \quad H_4P_2O_7} AMP$$

Adenine phosphoribosyl pyro-
phosphate transferase
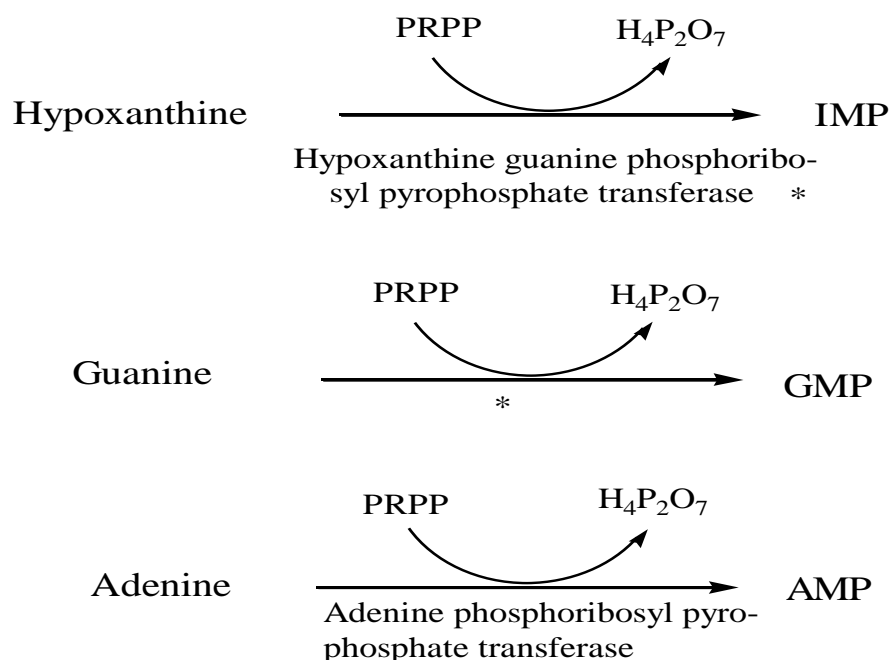
Figure 29. Salvage reactions duration in humans.

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. 30). 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.
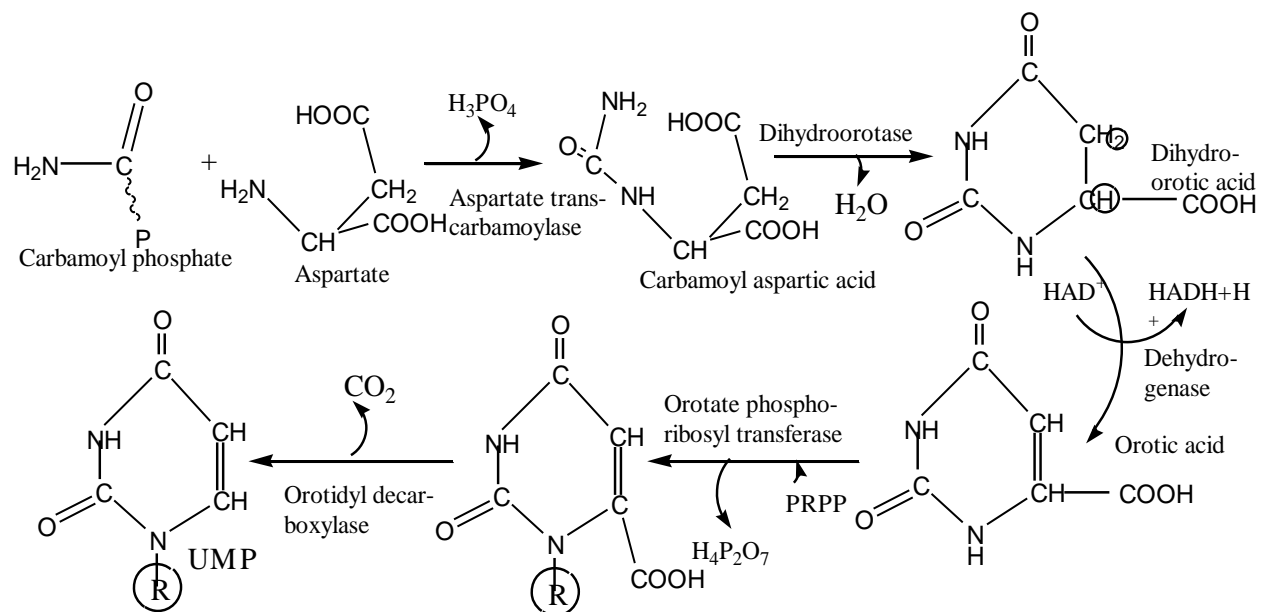
Figure 30. The synthesis of UMP; R – ribose-5-phosphate fragment.

The UMP is considered as the precursor for UTP (reaction 1) and CTP (reaction 2 below):

$$UMP \xrightarrow[ATP \quad ADP]{kinase} UDP \xrightarrow[ATP \quad ADP]{kinase} UTP \quad (1)$$

$$UTP + Gln + ATP \xrightarrow[Synthetase]{} CTP + ADP + H_3PO_4 + Glu \quad (2)$$
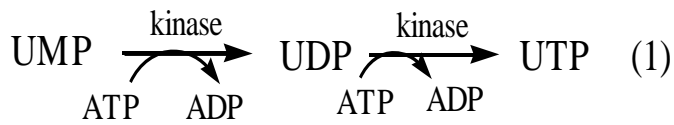
The synthesis of dTMP requires three steps (fig. 31):

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.
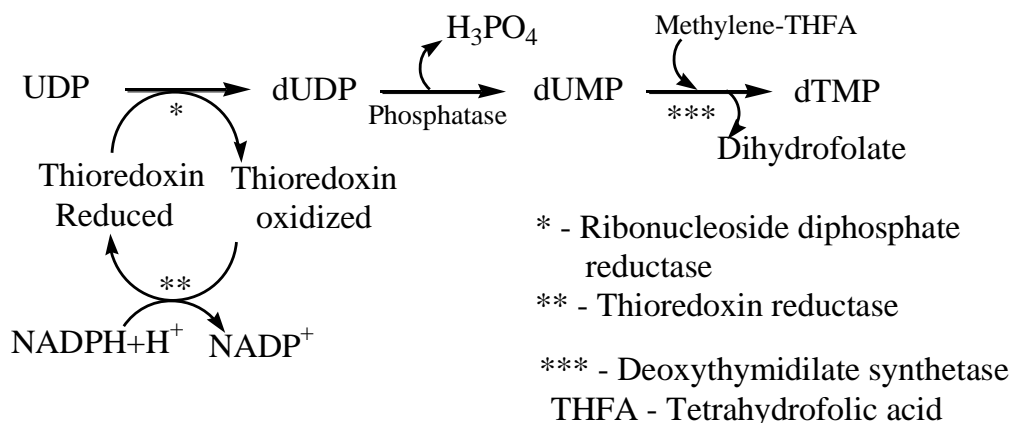
Figure 31. The synthesis of dTMP from UDP.

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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. 32).

• High concentration of GMP is allosteric inhibitor for Phosphoribosyl aminotransferase (fig. 32) in the purine nucleotide synthesis.

• The regulation of second stage of synthesis (fig.28), 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.32).
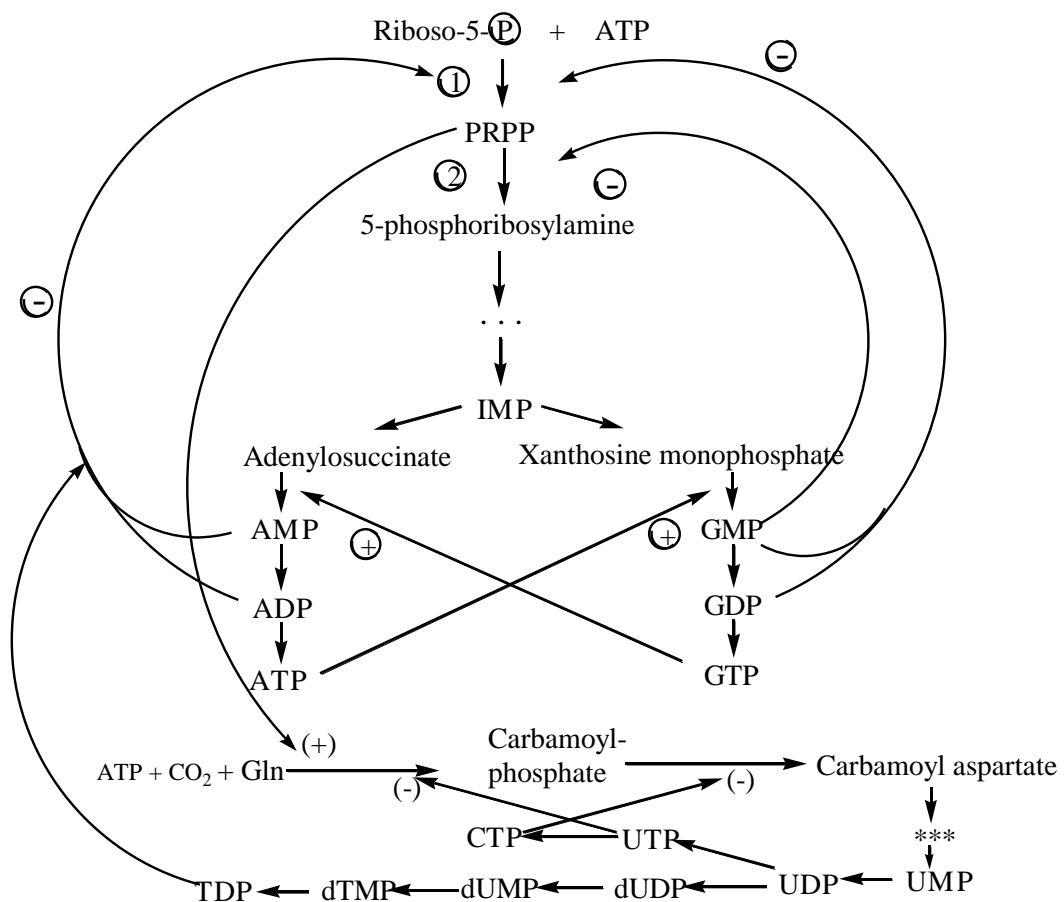
Figure 32. The regulation of purine nucleotide synthesis

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 then 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 inflamation.

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 en 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.

EXERCISES FOR INDEPENDENT WORK. In the table with test tasks emphasize keywords, choose the correct answer and justify it:

№	Test tasks:	Explanations:
1.	A doctor administered Allopurinol to a 26-year-old young man with the symptoms of gout. What pharmacological action of Allopurinol ensures therapeutical effect? A. By inhibiting uric acid formation B. By general analgetic effect C. By increasing uric acid excretion D. By general anti-inflammatory effect E. By inhibiting leucocyte migration into the joint	
2.	Methotrexate (structural analogue of the folic acid which is competitive inhibitor of the dihydrofolate	

№	Test tasks:	Explanations:
	reductase) is prescribed for treatment of the malignant tumour. On which level does methotrexate hinder synthesis of the nucleic acids? A. Reparation B. Mononucleotide synthesis C. Replication D. Processing E. Transcription	
3.	A 42-year-old man suffering from gout has increased level of urinary acid in blood. Allopurinol was prescribed to decrease the level of urinary acid. Competitive inhibitor of what enzyme is allopurinol? A. Adenosine deaminase B. Hypoxanthine phosphoribosil transferase C. Xanthine oxidase D. Guanine deaminase E. Adenine phosphoribosiltransfe-rase	
4.	46-year-old patient complains of pain in joints that becomes stronger the day before the weather changes. Blood examination revealed an increased concentration of uric acid. This substance is accumulated in the blood o the patient due to intensive degradation of the following substance: A. Cytidine monophosphate B. Adenosine monophosphate C. Uridine triphosphate D. Uridine monophosphate E. Thymidine monophosphate	
5.	Children with Lesch-Nyhan syndrome have a severe form of hyperuricemia accompanied by the	

№	Test tasks:	Explanations:
	formation of tophi, urate calculi in the urinary tracts, as well as serious neuro-psychiatric disorders. The cause of this disease is the reduced activity of the following enzyme: A. Hypoxanthine guanine phosphoriboxyl transferase B. Xanthine oxidase C. Dihydrofolate reductase D. Thymidylate synthase E. Carbamoyl phosphate synthetase	
6.	A 46-year-old female patient consulted a doctor about pain in small joints of the upper and lower limbs. The joints are enlarged and shaped like thickened nodes. Serum test revealed an increase in urate concentration. This might be caused by a disorder in metabolism of: A. Pyrimidines B. Carbohydrates C. Lipids D. Purines E. Amino acids	
7.	A patient with hereditary hyperammonemia due to a disorder of Ornithine cycle has developed secondary orotaciduria. The increased synthesis of orotic acid is caused by an increase in the following metabolite of Ornithine cycle: A. Carbamoyl phosphate B. Citrulline C. Argininosuccinate D. Urea E. Ornithine	

№	Test tasks:	Explanations:
8.	A 65-year-old man suffering from gout complains of pain in his kidneys. Ultrasonic examination revealed kidney stones. A certain substance in increased concentration can cause kidney stones formation. Name this substance: A. Uric acid B. Urea C. Cholesterol D. Cystine E. Bilirubin	
9.	What enzyme deficiency will develop in a young male X-linked recessive disorder with hyperuricemia and mild retardation? A. Branch chain amino acids metabolites deficiency B. Homogentisate oxidase defective enzymes C. Hypoxanthine phosphoribosyltransferase deficiency D. Phenylalanine hydroxylase deficiency E. Hypoxanthine phosphoribosyl oxidase deficiency	
10.	Terminal product of purine catabolism in non-primate mammals is: A. Uric acid B. Ammonia C. Urea D. Allantoin E. CO_2 and H_2O	
11.	Due to complete hydrolysis of DNA, we will get all of the following except:	

№	Test tasks:	Explanations:
	A. Adenosine B. Purine bases C. Pyrimidine bases D. Phosphoric acid E. Deoxyribose	
12.	Increased serum uric acid levels occur in: A. Von Gierke`s disease B. Leukemia C. Disturbances of PRPP synthase regulation D. Lesh-Nyhan syndrome E. All the above	
13.	Beta-alanine is a degradation product of: A. Uridylate B. Albumin C. Adenosine D. Thymidylate E. Guanylate	
14.	A gout is developed in patients when the activity of certain enzyme of purine nucleotide de novo synthesis is higher (genetic defect of enzyme) then normal. Point out it: A. Adenylsuccinate lyase B. PRPP amino transferase C. Adenylate cyclase D. Adenylic acid deaminase E. 5′-nucleotidase	
15.	Von Gierke`s disease (Glycogen storage disease I) is a observed with overproduction of purine nucleotides in patient. Name pathological state which may be found for patient in this case: A. Hypoglycemia and hyperuricemia B. Hyperglycemia and hyperuricemia	

№	Test tasks:	Explanations:
	C. Avitaminosis of folic acid D. Renal Azotemia E. Hyperglycemia, only	
16.	Uric acid salts have special behavior under the change of temperature of the medium around: the lower the temperature value: A. The higher their solubility in water B. The lower their solubility in water C. The higher their stability D. The lower their stability E. The higher the rate of their destruction	
17.	High intake of animal food products by healthy person increases the content of this compound in the urine of this person. Name it: A. Glucose B. Pyruvate C. Sodium ions D. Calcium ions E. Uric acid	
18.	Name the most important vitamin needed for nucleotide synthesis de novo: A. Folic acid B. Thiamine C. Ascorbic acid D. Retinol E. Cholecalciferol	
19.	Xanthine oxidase complete deficiency in patients will cause this state: A. Xanthine renal lithiasis B. Hypouricemia C. Xanthinuria D. Everything placed above E. The right answer is absent	

№	Test tasks:	Explanations:
20.	The gout may be developed due to PRPP synthetase abnormal features. Find out them: A. PRPP synthetase may be superactive B. PRPP synthetase has resistance to feedback inhibition C. PRPP synthetase has very low Km for ribose-5-P D. The right answer is absent E. All the features proposed above	

BIOSYNTHESIS OF NUCLEIC ACIDS
(KRISANOVA N. V.)

INFORMATIONAL MATERIAL

INTRODUCTION

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.

A 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.
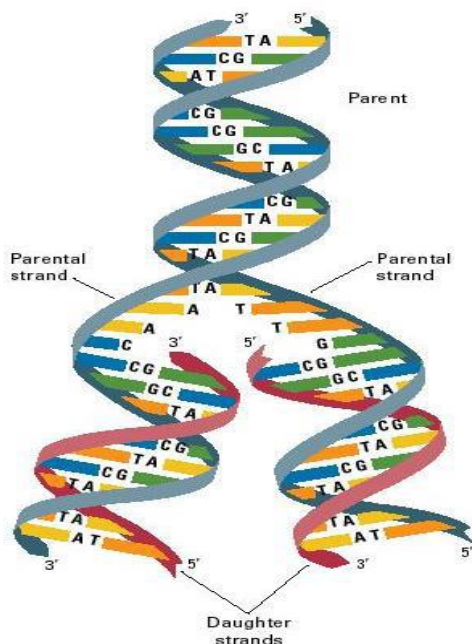

Figure 33. A picture for formation of two double helical structures of DNA in semi conservative replication.

59

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.

1. 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. 1988a,b). 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: <u>d-ATP, d-GTP, d-CTP, d-TTP.</u> 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.
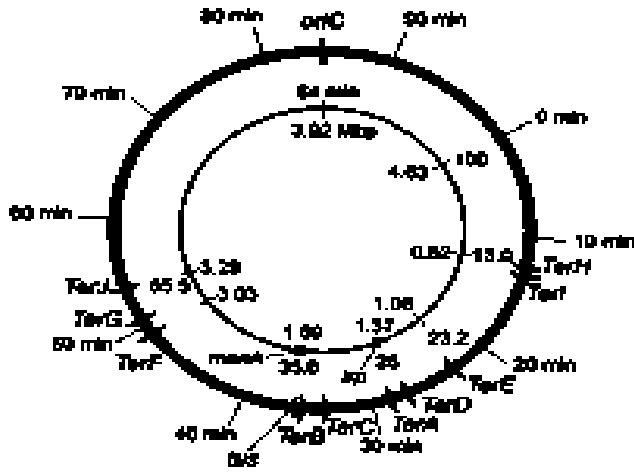
Figure 34. Circular DNA molecule found in E.coli; its replication is from ori C, bidirectional, it is finished in 40 minutes due to ten termination sequences (Ter) placed opposite ori C.

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. 2), that invade the duplex DNA on either side of the origin.
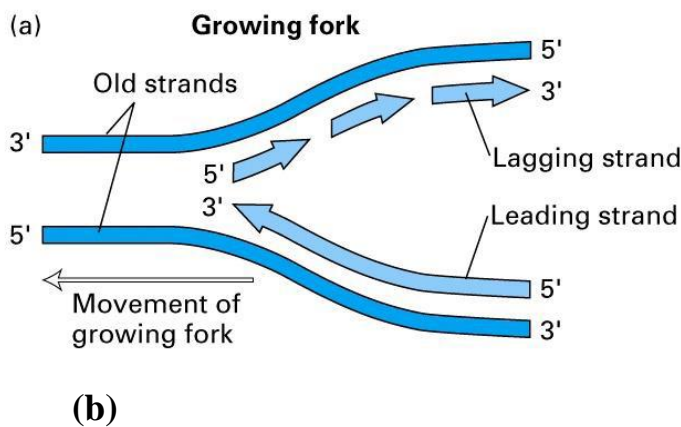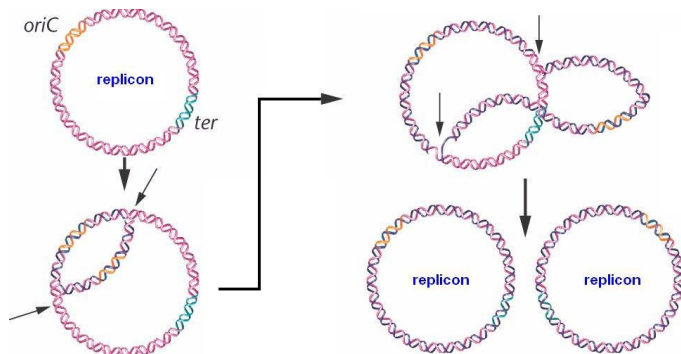
Figure.35 . (a) - steps of replication in E.coli; (b) - direction of replication^ synthesis of leading and lagging strands

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.34). 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. 2). This event creates a "replication fork trap" that allows forks to enter but not to leave the terminus region (fig.35, a,b).

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.35). 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 <u>hexamer of dna B protein, dna C protein and several other proteins n, n′, n″, i.</u> 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.35,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 save 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 <u>topoisomerase</u>. 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. 36)
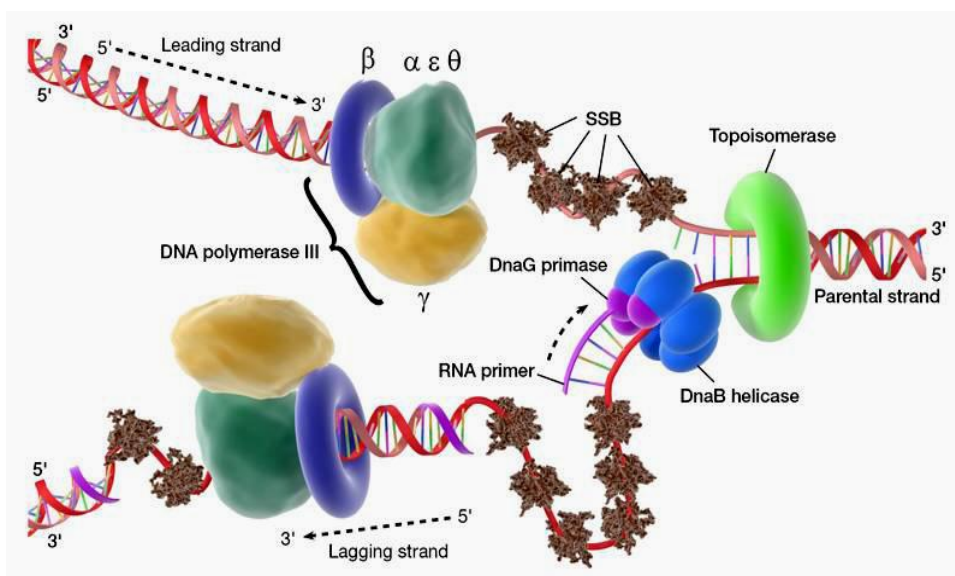
Figure 36. Replisome complex function in elongation phase of replication (E.coli).

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.

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:

$$\text{——DNA-3`-OH} + \text{HO——P——O-5`-DNA} \xrightarrow[\text{+ATP}]{\text{Ligase}} \text{DNA-3`-O ——P——O-5`-DNA}$$

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.34). A specific protein, Ter-binding p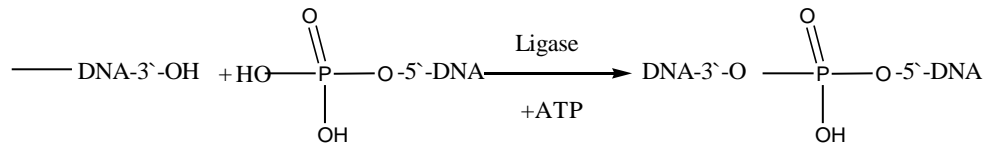rotein (TUS), binds to these sequences and prevents the Helicase (or dna B protein) from further unwinding of DNA. This facilitates the termination of replication. In the *E. coli* chromosome, the *Ter* sequences were placed so as to form a "replication fork trap" that would allow a replisome to enter 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

2. 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: α, β and δ; and one mitochondrial DNA polymerase - γ .

DNA polymerase α is probably analogous to polymerase I but it plays no role in DNA repair.

DNA polymerase β acts in DNA repair synthesis.

DNA polymerase δ is probably analogous to polymerase III and responsible for leading strand synthesis.

DNA polymerase γ 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 $5' \rightarrow 3'$ 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.5), 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.

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.
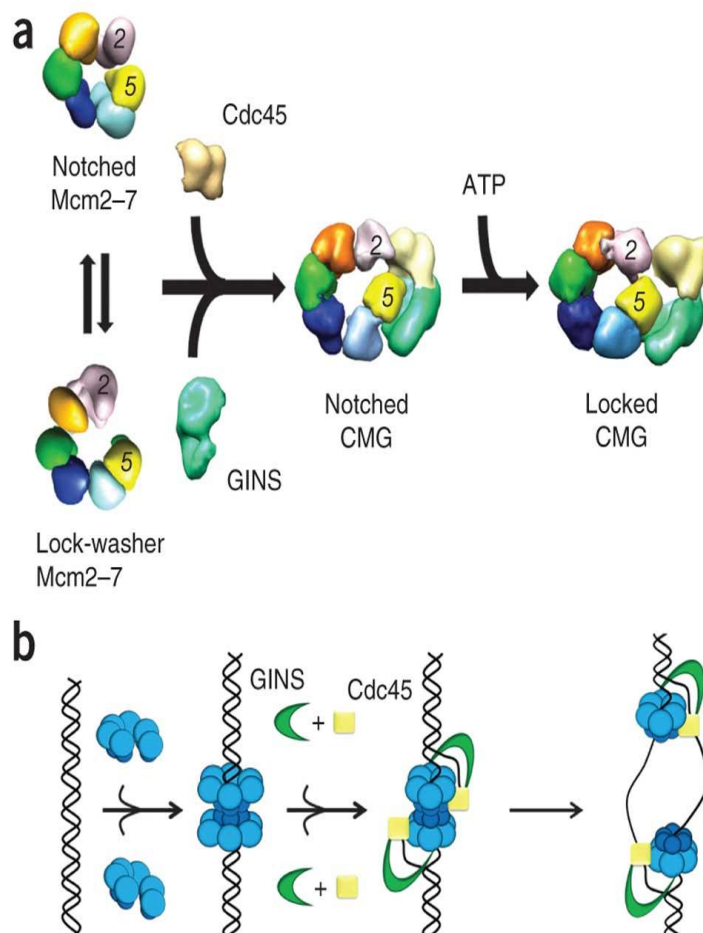

Figure 37. CMG-complex composition and function at initiation of replication in eukaryotic cell.

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): i*t 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.

3. 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 chemiotherapeutic drugs.

Genetic diseases associated with defects in DNA repair systems

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

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.

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 Fe^{2+} to cause DNA breakage.

d) **Inhibitors of replicative enzymes**: DNA polymerase inhibitors or topoisomerase inhibitors (**Nalidixic acid and Fluoroquinolones**). Use: treatment of urinary tract infections.

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.38)

3) m-RNA accounts for only 3% of the total cellular RNA in eukaryotes;

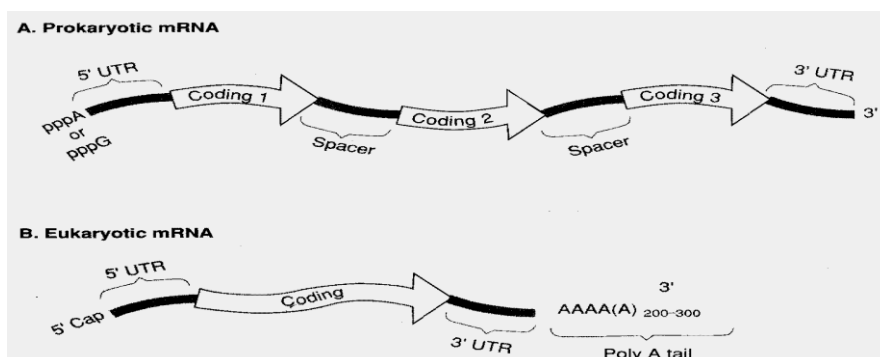4) they exhibit half-lives on the order of hours to days.

m-RNA of eukaryotes

Figure 38. 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(A,fig.38). 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 (B, fig.38). 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.

1. 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.39) are responsible for directing RNA polymerase to initiate transcription at a particular point of one DNA strand, only. In the figure 40 (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).*

For most prokaryotic genes, they are conserved sequences that are necessary to promote accurate initiation of transcription.

A. Prokaryotic promoter
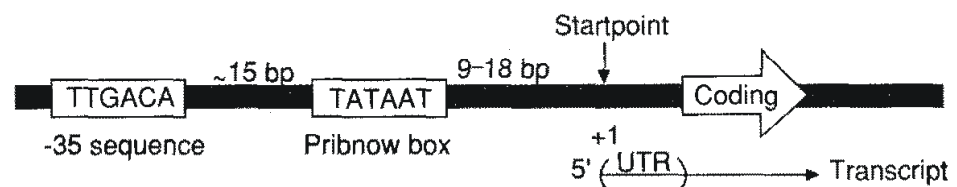
Figure 39. Prokaryotic promoter sequences

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. 40) 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.
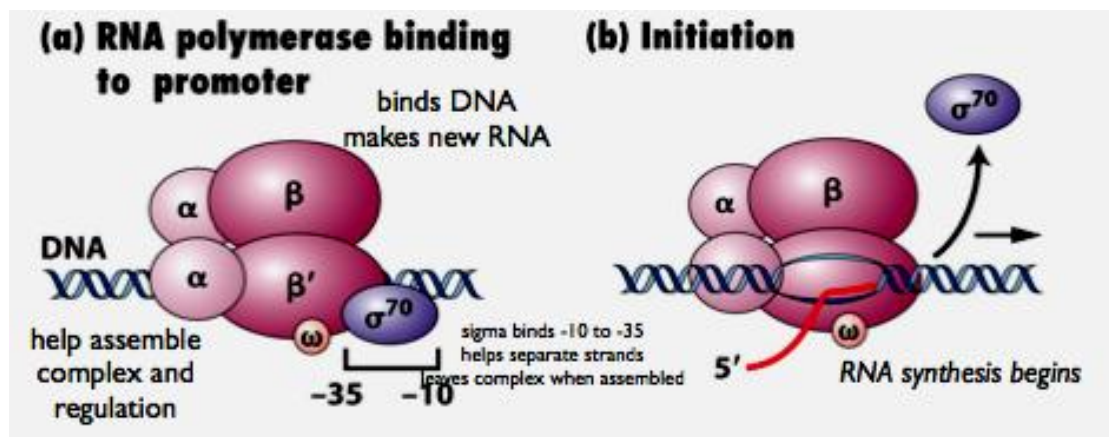
Figure 40. RNA polymerase of E.coli in initiation phase of transcription.

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 α_2-subunit and two β`-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 phospho diester 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. 41). 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.
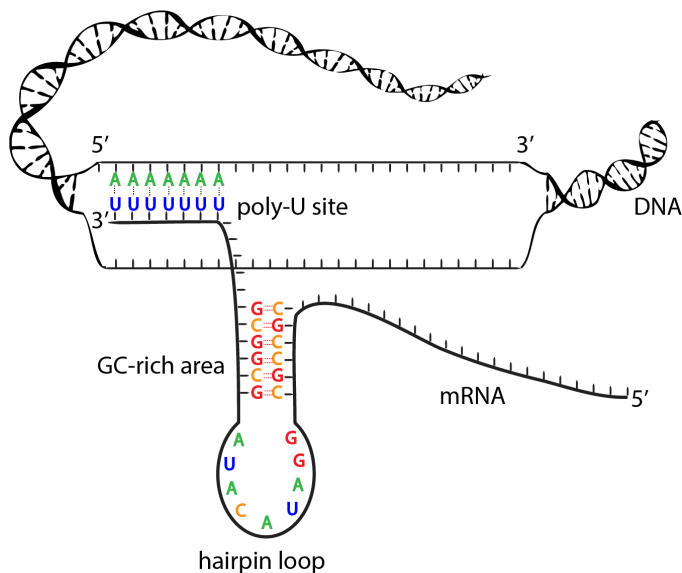
Figure 41. A formation of a stable stem-loop in termination phase of transcription.

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.

2. 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.42)

- 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.
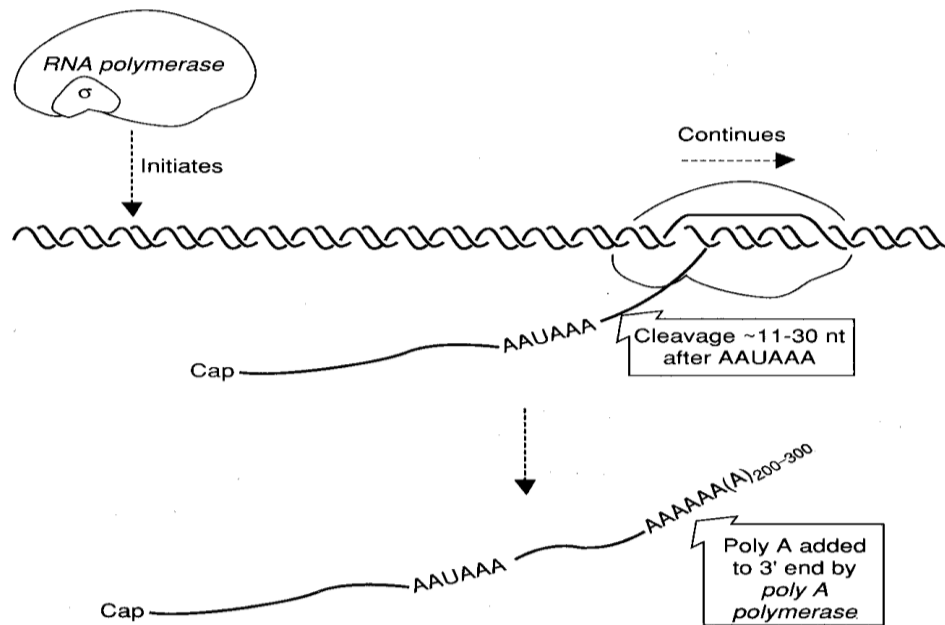
Figure 42. Polyadenylation of hn-RNA during processing.

- ***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***. Splicesomes 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.

3. 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 cystrons.*

Gene-regulator – gene, coding the structure of protein-repressor.

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.43).

All three genes are transcribed in a single m-RNA. *Lactose is inducer* of this transcription.
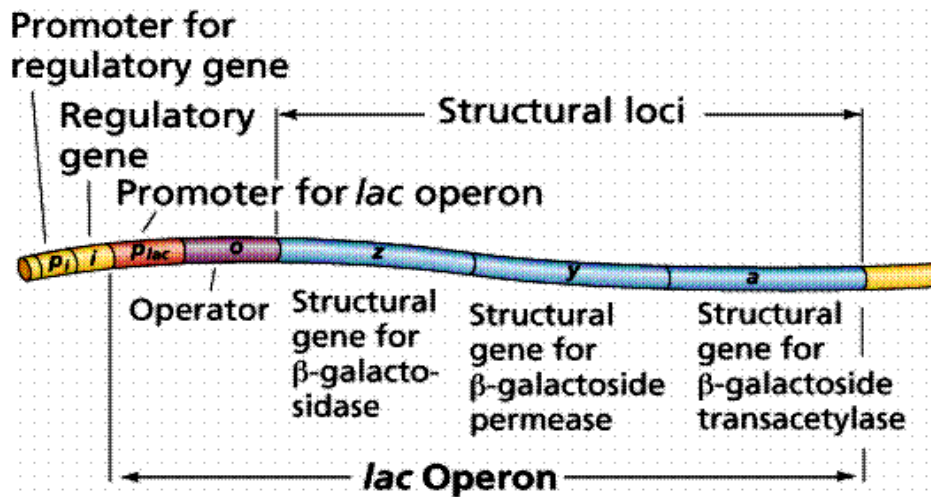
Figure 43. The composition of LAC-operon in E.coli.

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.44)
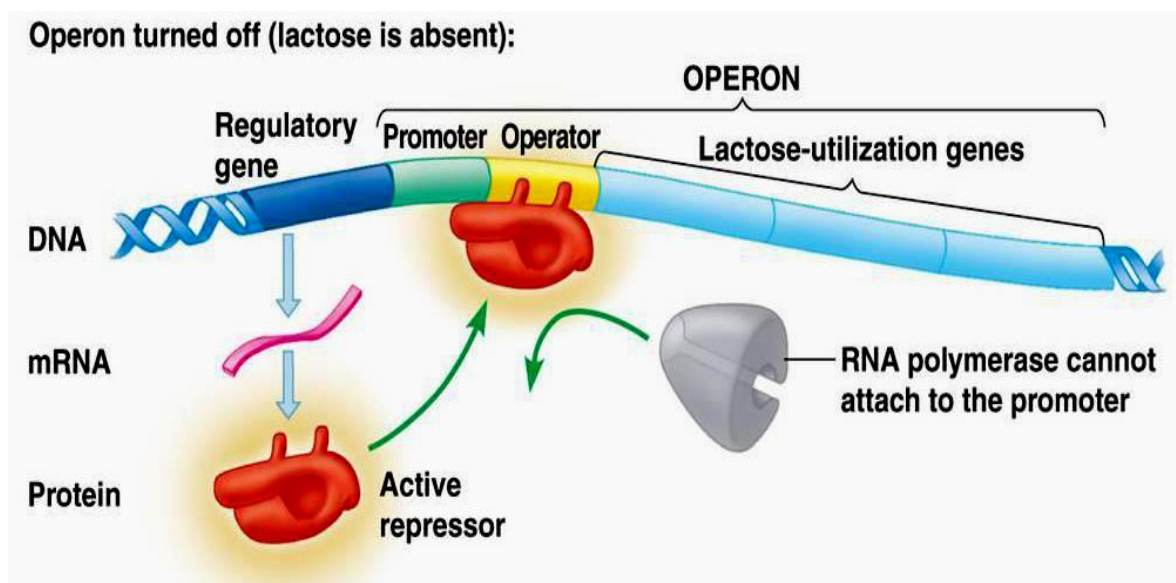
Figure 44. The function of protein-repressor in control of transcription of LAC-operon.

Positive regulators of transcription are: CRP-protein –catabolite gene reactive protein; cAMP (fig.45).
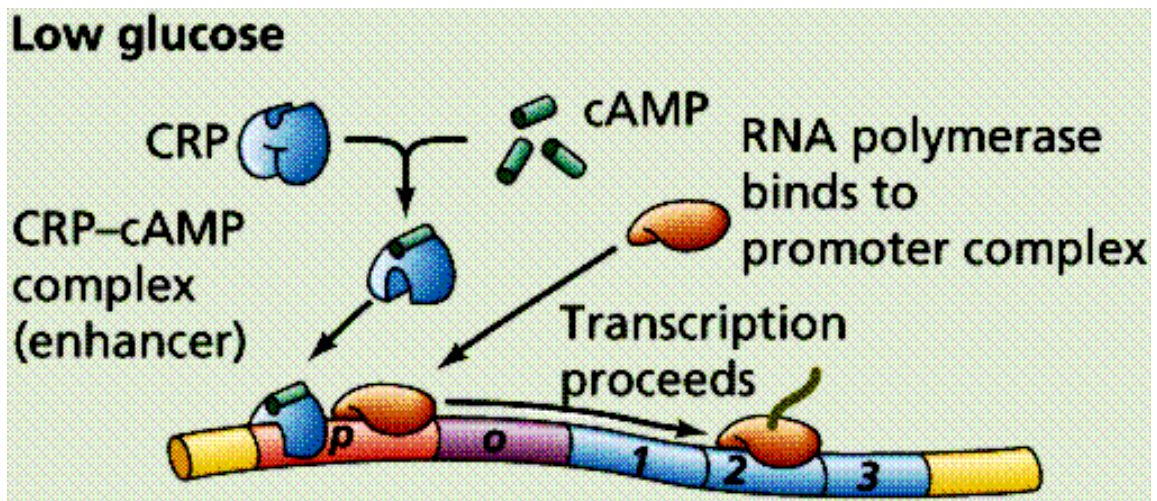
Figure 45. CRP-cAMP complex is enhancer of RNA-polymerase linkage to promoter sequence.

These two factors in a complex are required for activation of Sigma (σ) 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.

4. 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;

82

- *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 47 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.46).
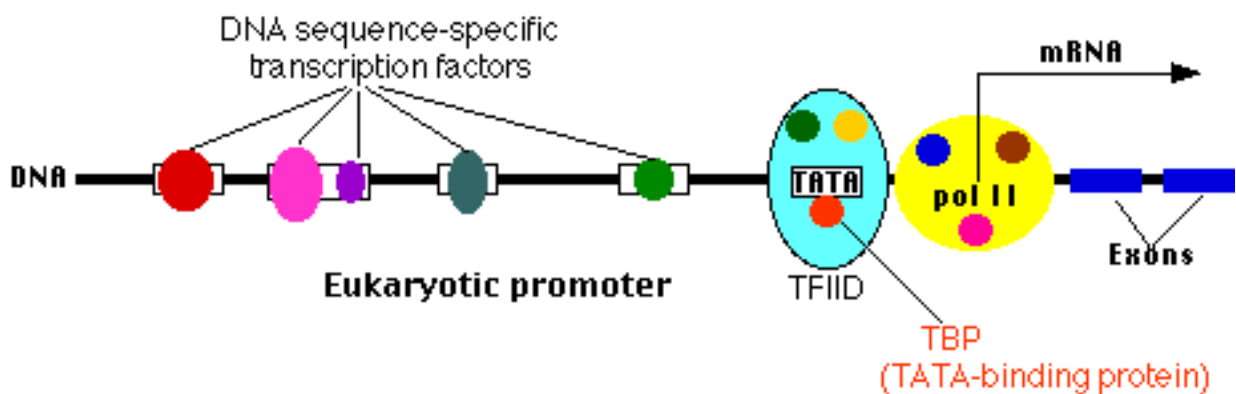
Figure 46. The composition of basal promoter in DNA of eukaryotic cell.

The basal promoter contains a sequence of 7 bases (TATAAAA) 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.46);

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 47).
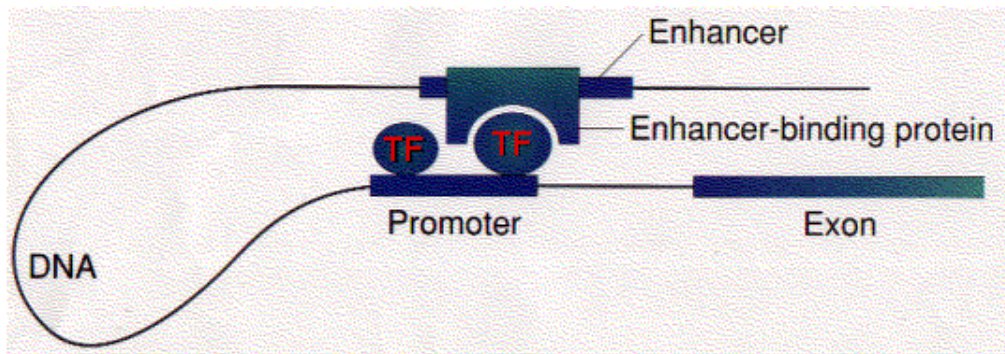
Figure 47. The change of DNA strand conformation when transcription factor (TF) binds to enhancer sequence.

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

5'   AGAACAnnnTGTTCT   3'

3'   TCTTGTnnnACAAGA   5'
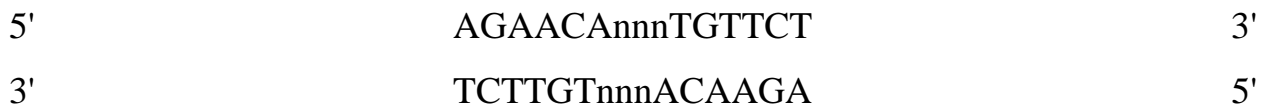
where *n* represents any nucleotide.

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. In the table with test tasks emphasize keywords, choose the correct answer and justify it:

№	Test tasks:	Explanations:
1.	RNA that contains AIDS virus penetrated into a leukocyte and by means of reverse transcriptase forced a cell to synthesize a viral DNA. This process is based upon: A. Reverse transcription B. Convariant replication C. Operon depression D. Operon repression E. Reverse translation	
2.	RNA-polymerase B (II) is blocked	

№	Test tasks:	Explanations:
	due to amanitin poisoning (poison of death-cup). What process is disturbed?: A. Primer synthesis B. Synthesis of t-RNA C. Reverse transcription D. Synthesis of m-RNA E. Maturation of m-RNA	
3.	An experiment proved that UV-radiated cells of patients with xeroderma pigmentosum restore the native DNA structure slower than cells of healthy individuals as a result of reparation enzyme defection. What enzyme helps this process? A. DNA polymerase III B. DNA gyrase C. RNA ligase D. Endonuclease E. Primase	
4.	According to the model of double DNA helix that was suggested by Watson and Greek, it was established that one of chains would not be lost during replication and the second chain would be synthesized complementary to the first one. What way of replication is it? A. Analogous B. Dispersed C. Semiconservative D. Identical E. Conservative	
5.	It was found out that some compounds for instance fungi toxins and some antibiotics can inhibit activity of RNA-polymerase II. What process will be disturbed in	

№	Test tasks:	Explanations:
	eukaryotic cell in a case of inhibition of this enzyme? A. Transcription B. Reparation C. Replication D. Processing E. Translation	
6.	You are studying functioning of a bacteria operon. The operator gene has been released from the repressor. Immediately after this the following process will start in the cell: A. Translation B. Replication C. Repression D. Processing E. Transcription	
7.	An oncological patient was administered methotrexate. With the lapse of time the target cells of the tumor lost sensitivity to the preparation. We can observe changes in the gene expression of the following enzyme: A. Dihydrofolate reductase B. Folate decarboxylase C. Folate oxidase D. Desaminase E. Thiminase	
8.	Pterin derivatives (aminopterin and methotrexate) are the inhibitors of dihydrofolate reductase so that they inhibit the regeneration of tetrahydrofolic acid from dihydrofolate. These drugs inhibit	

№	Test tasks:	Explanations:

	the intermolecular transfer of monocarbon groups, thus suppressing the synthesis of following polymer:
	    A. Glycosaminoglycans
	    B. Homopolysaccharides
	    C. DNA
	    D. Protein
	    E. Gangliosides	

9.	In cancer patients who have been continuously receiving methotrexate, the target cells of tumor with a time became insensitive to this drug. In this case, gene amplification of the following enzyme is observed:
	    A. Dihydrofolate reductase
	    B. Thioredoxin reductase
	    C. Deaminase
	    D. AlAT
	    E. Thiaminase	

10.	During cell division, DNA replication occurs by a signal from the cytoplasm, and a certain portion of the DNA helix unwinds and splits into two individual strains. What enzyme facilitates this process?
	    A. Restrictase
	    B. DNA polymerase
	    C. RNA polymerase
	    D. Ligase
	    E. Helicase	

11.	Formation of Okazaki fragments occurs in: A. Transcription B. Reverse Transcription C. Translation D. Replication E. Reparation	

№	Test tasks:	Explanations:
12.	Okazaki fragment is: A. DNA fragment B. RNA fragment C. DNA fragment with RNA head D. RNA fragment with DNA head E. None	
13.	In mammals, DNA synthesis occurs in which part of the cell cycle: A. S phase B. G1 phase C. M phase D. G2 phase E. G0 phase	
14.	Transcription is inhibited by: A. Amanitin B. Streptomycin C. Sulfonylamide D. Chloramphenicol E. Puromycin	
15.	In E. coli structural gene of LAC operon is stimulated in: A. Presence of glucose only B. Presence of galactose only C. Presence of lactose only D. Presence of glucose and absence of lactose E. Presence of lactose and absence of glucose	
16.	Which of the following is an example of a reverse transcriptase? A. Gyrase B. Helicase C. Telomerase	

№	Test tasks:	Explanations:
	D. RNA Polymerase E. DNA Polymerase	
17.	Xeroderma pigmentosum is produced as a result of a defect in: A. DNA polymerase III B. DNA polymerase II C. DNA polymerase I D. DNA exonuclease E. DNA ligase	
18.	The sigma subunit of prokaryotic RNA polymerase: A. Binds the antibiotic rifampicin B. Is inhibited by α-amanitin C. Specifically recognizes the promoter site D. Is part of the core enzyme E. Specifically recognizes the operator site	
19.	Excessive ultraviolet radiation is harmful to life. The damage caused to the biological systems by ultraviolet radiation is by: A. Inhibition of DNA synthesis B. Formation of thymidine dimmers C. Ionization D. DNA fragmentation E. Deamination of DNA	
20.	Steroid-receptor complex binds to a specific region on DNA through: A. Zinc finger motif B. Leucine zipper motif C. Helix turn helix D. Histidine E. Histone	

BIOSYNTHESIS OF PROTEINS AND ITS REGULATION
(IVANCHENKO D.H., RUDKO N.P.)

INFORMATIONAL MATERIAL

Proteins are the most dynamic and varied class of biomolecules. In addition to providing structural components, proteins are largely responsible for promoting many of the most dynamic aspects of living processes.

Protein synthesis is an extraordinarily complex process in which genetic information encoded in the nucleic acids is translated into the 20 amino acid "alphabet" of polypeptides. In addition to translation (the mechanism by which a nucleotide base sequence directs the polymerization of amino acids), protein synthesis can also be considered to include the processes of posttranslational modification and targeting. Posttranslational modification consists of chemical alterations cells use to prepare polypeptides for their functional roles. Several modifications assist in targeting, which directs newly synthesized molecules to a specific intracellular or extracellular location.

In all, at least 100 different molecules are involved in protein synthesis. Among the most important of these are the components of the ribosome, a supramolecular structure composed of RNA and protein that rapidly and precisely decodes genetic messages. Speed is required because organisms must respond expeditiously to ever-changing environmental conditions. In prokaryotes such as *E. coli*, for example, a polypeptide of 100 residues is synthesized in about 6 s. Precision in mRNA translation is critical because the accurate folding, and therefore the proper functioning, of each polypeptide is determined by the molecule's primary sequence.

The genetic code

It became apparent during early investigations of protein synthesis that translation is fundamentally different from the transcription process that precedes it. During transcription the language of DNA sequences is converted to the closely related dialect of RNA sequences. During protein synthesis, however, a nucleic

acid base sequence is converted to a clearly different language (i.e., an amino acid sequence), hence the term *translation*. Because mRNA and amino acid molecules have little natural affinity for each other, researchers predicted that a series of adaptor molecules must mediate the translation process. This role was eventually assigned to tRNA molecules.

Before adaptor molecules could be identified, however, a more important problem had to be solved: deciphering the genetic code. *The genetic code* can be described as a coding dictionary that specifies a meaning for base sequence. Once the importance of the genetic code was recognized, investigators speculated about its dimensions. Because only four different bases (G, C, A, and U) occur in mRNA and 20 amino acids must be specified, it appeared that a combination of bases coded for each amino acid. A sequence of two bases would specify only a total of 16 amino acids (i.e., $4^2 = 16$). However, a three-base sequence provides more than sufficient base combinations for translation (i.e., $4^3 = 64$).

The first major breakthrough in assigning mRNA triplet base sequences (later referred to as *codons*) came in 1961, when Marshall Nirenberg and Heinrich Matthaei performed a series of experiments using an artificial test system containing an extract of *E. coli* fortified with nucleotides, amino acids, ATP, and GTP. They showed that poly U (a synthetic polynucleotide whose base components consist only of uracil) directed the synthesis of polyphenylalanine. Assuming that codons consist of a three-base sequence, Nirenberg and Matthaei surmised that UUU codes for the amino acid phenylalanine. Subsequently, they repeated their experiment using poly A and poly C. Because polylysine and polyproline products resulted from these tests, the codons AAA and CCC were assigned to lysine and proline, respectively.

Most of the remaining codon assignments were determined by using synthetic polynucleotides with repeating sequences. Such molecules were constructed by enzymatically amplifying short chemically synthesized sequences. The resulting polypeptides, which contained repeating peptide segments, were then analyzed. The information obtained from this technique, devised by Har Gobind

Khorana, was later supplemented with a strategy used by Nirenberg. This latter technique measured the capacity of specific trinucleotides to promote tRNA binding to ribosomes.

The codon assignments for the 64 possible trinucleotide sequences are presented in Table 4. Of these, 61 code for amino acids. The remaining three codons (UAA, UAG, and UGA) are *stop* (polypeptide chain terminating) signals. AUG, the codon for methionine, also serves as a *start* signal (sometimes referred to as the *initiating codon*). The genetic code is now believed to possess the following properties: *Universal*, *Specific*, *Degenerate*, *Nonoverlapping and without punctuation*.

Table 4. The Genetic Code.

First position (5′ end)	Second Position				Third position (3′ end)
	U	C	A	G	
U	UUU Phe, UUC, UUA Leu, UUG	UCU, UCC, UCA Ser, UCG	UAU Tyr, UAC, UAA STOP, UAG	UGU Cys, UGC, UGA STOP, UGG Trp	U C A G
C	CUU, CUC Leu, CUA, CUG	CCU, CCC Pro, CCA, CCG	CAU His, CAC, CAA Gln, CAG	CGU, CGC Arg, CGA, CGG	U C A G
A	AUU, AUC Ile, AUA, AUG Met	ACU, ACC Thr, ACA, ACG	AAU Asn, AAC, AAA Lys, AAG	AGU Ser, AGC, AGA Arg, AGG	U C A G
G	GUU, GUC Val, GUA, GUG	GCU, GCC Ala, GCA, GCG	GAU Asp, GAC, GAA Glu, GAG	GGU, GGC Gly, GGA, GGG	U C A G

Universal. With a few minor exceptions the genetic code is universal. In other words, examinations of the translation process in the species that have been investigated have revealed that the coding signals for amino acids are always the same.

Mitochondria contain DNA, as double-stranded DNA circles, and the mitochondrial genome codes for about 10-20 proteins. Surprisingly, in mitochondrial mRNAs, some codons have different meanings from their

counterparts in mRNA in the cytosol. A few examples are given below (N denotes any of the four nucleotides A, G, C or U):

mitochondria AUA = Met not Ile

mitochondria UGA = Trp not Stop

some animal mitochondria AGA and AGG = Stop not Arg

plant mitochondria CGG = Trp not Arg

yeast mitochondria CUN = Thr not Leu

Specific. Each codon is a signal for a specific amino acid. The majority of codons that code for the same amino acid possess similar sequences. For example, in each of the four serine codons (UCU, UCC, UCA, and UCG) the first and second bases are identical. Consequently, a point mutation in the third base of a serine codon would not be deleterious.

Degenerate. Any coding system in which several signals have the same meaning is said to be degenerate. The genetic code is partially degenerate because most amino acids are coded for by several codons. For example, leucine is coded for by six different codons (UUA, UUG, CUU, CUC, CUA, and CUG). In fact, methionine (AUG) and tryptophan (UGG) are the only amino acids that are coded for by a single codon.

Nonoverlapping and without punctuation. The mRNA coding sequence is "read" by a ribosome starting from the initiating codon (AUG) as a continuous sequence taken three bases at a time until a stop codon is reached. A set of contiguous triplet codons in an mRNA is called **a reading frame**. The term *open reading frame* describes a series of triplet base sequences in mRNA that do not contain a stop codon.

Ribosomes

The efficiency of translation is greatly increased by the binding of the mRNA and the individual aminoacyl-tRNAs to the most abundant RNA-protein complex in the cell, the ribosome, which directs elongation of a polypeptide at a rate of three to five amino acids added per second. Small proteins of 100-200

amino acids are therefore made in a minute or less. On the other hand, it takes 2-3 hours to make the largest known protein, titin, which is found in muscle and contains about 30,000 amino acid residues. The cellular machine that accomplishes this task must be precise and persistent.

With the aid of the electron microscope, ribosomes were first discovered as small, discrete, RNA-rich particles in cells that secrete large amounts of protein. However, their role in protein synthesis was not recognized until reasonably pure ribosome preparations were obtained. In vitro radiolabeling experiments with such preparations showed that radioactive amino acids first were incorporated into growing polypeptide chains that were associated with ribosomes before appearing in finished chains.

A ribosome is composed of three (in bacteria) or four (in eukaryotes) different rRNA molecules and as many as 83 proteins, organized into a large subunit and a small subunit (Fig. 48). The ribosomal subunits and the rRNA molecules are commonly designated in Svedberg units (S), a measure of the sedimentation rate of suspended particles centrifuged under standard conditions. The small ribosomal subunit contains a single rRNA molecule, referred to as small rRNA. The large subunit contains a molecule of large rRNA and one molecule of 5S rRNA, plus an additional molecule of 5.8S rRNA in vertebrates. The lengths of the rRNA molecules, the quantity of proteins in each subunit, and consequently the sizes of the subunits differ in bacterial and eukaryotic cells. The assembled ribosome is 70S in bacteria and 80S in vertebrates. But more interesting than these differences are the great structural and functional similarities between ribosomes from all species. This consistency is another reflection of the common evolutionary origin of the most basic constituents of living cells.

The sequences of the small and large rRNAs from several thousand organisms are now known. Although the primary nucleotide sequences of these rRNAs vary considerably, the same parts of each type of rRNA theoretically can form basepaired stem-loops, which would generate a similar three-dimensional structure for each rRNA in all organisms. The actual three-dimensional structures

of bacterial rRNAs from Thermus thermopolis recently have been determined by x-ray crystallography of the 70S ribosome. The multiple, much smaller ribosomal proteins for the most part are associated with the surface of the rRNAs. Although the number of protein molecules in ribosomes greatly exceeds the number of RNA molecules, RNA constitutes about 60 percent of the mass of a ribosome.
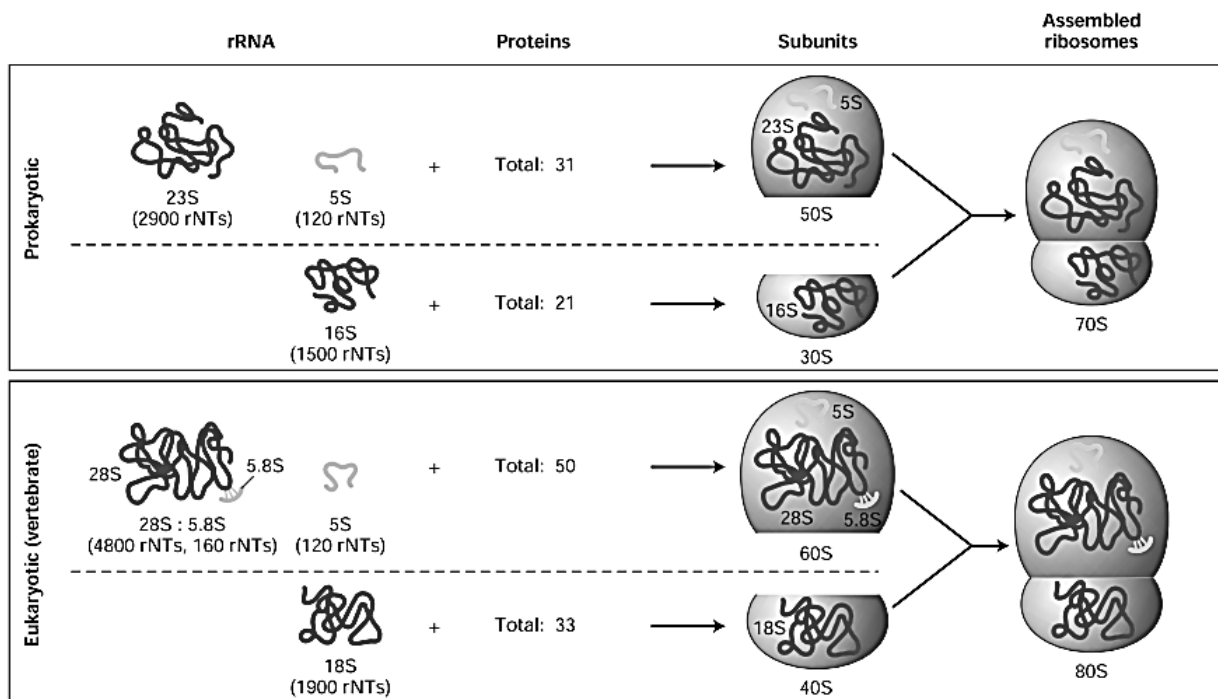
Figure 48. The general structure of ribosomes in prokaryotes and eukaryotes.

During translation, a ribosome moves along an mRNA chain, interacting with various protein factors and tRNAs and very likely undergoing large conformational changes. Despite the complexity of the ribosome, great progress has been made in determining the overall structure of bacterial ribosomes and in identifying various reactive sites. X-ray crystallographic studies on the T. thermophilus 70S ribosome, for instance, not only have revealed the dimensions and overall shape of the ribosomal subunits but also have localized the positions of tRNAs bound to the ribosome during elongation of a growing protein chain.

Amino Acid Activation

The activation of the amino acid and the formation of the aminoacyl-tRNA take place in two separate steps, both of which are catalyzed by the aminoacyl-

tRNA synthetase (Fig. 49). First, the amino acid forms a covalent bond to an adenine nucleotide, producing an aminoacyl-AMP. The free energy of hydrolysis of ATP provides energy for bond formation. The aminoacyl moiety is then transferred to tRNA, forming an aminoacyl-tRNA.
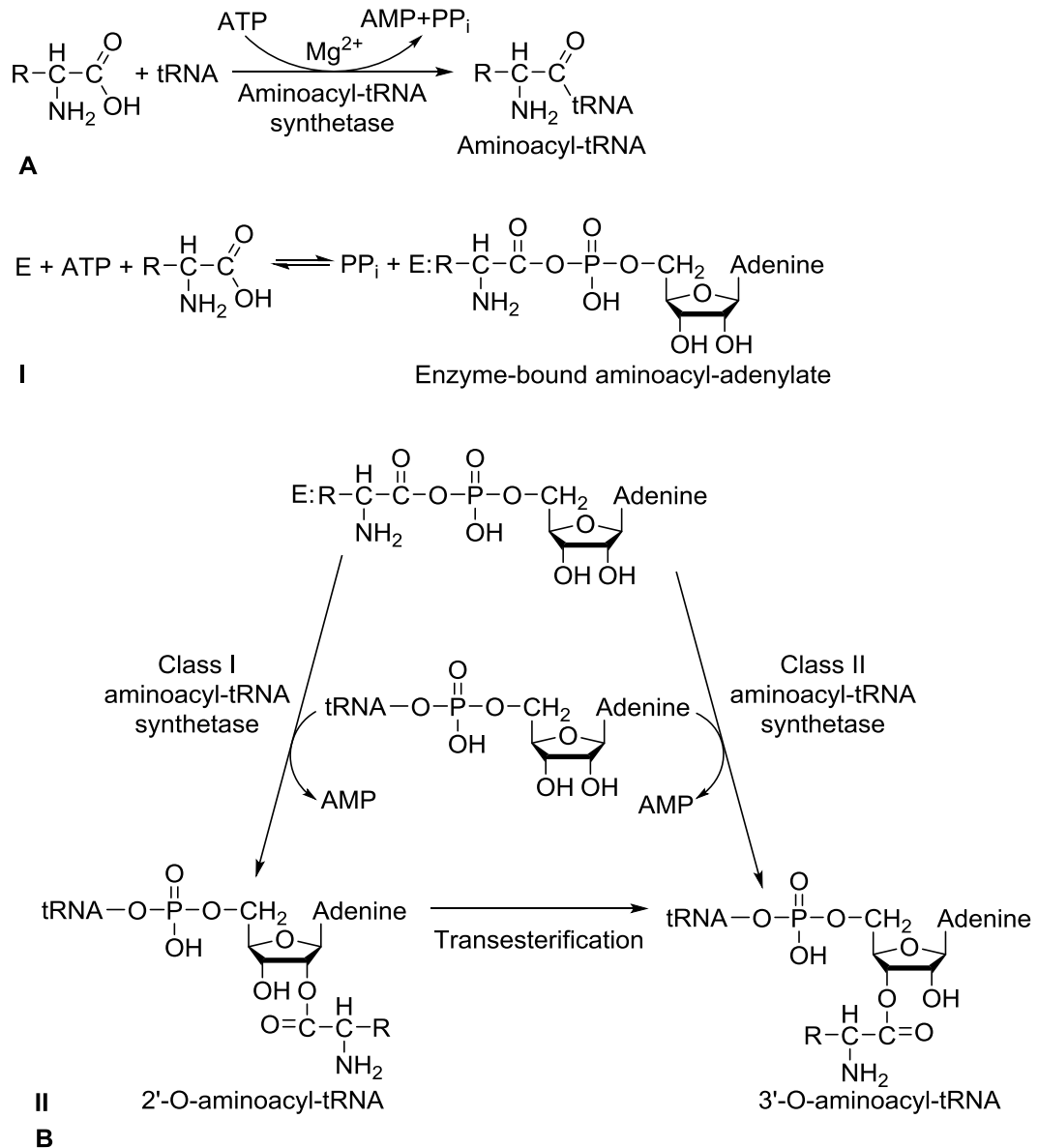
Figure 49. The aminoacyl-tRNA synthetase reaction. (A) The overall reaction. Everpresent pyrophosphatases in cells quickly hydrolyze the PPi produced in the aminoacyl-tRNA synthetase reaction, rendering aminoacyl-tRNA synthesis thermodynamically favorable and essentially irreversible. (B) The overall reaction commonly proceeds in two steps: (I) formation of an aminoacyl-adenylate and (II) transfer of the activated amino acid moiety of the mixed anhydride to either the 2'-OH (class I aminoacyl-tRNA synthetases) or 3'-OH (class II aminoacyl-tRNA

synthetases) of the ribose on the terminal adenylic acid at the 3'-OH terminus common to all tRNAs. Those aminoacyl-tRNAs formed as 2'-OH esters undergo a transesterification that moves the aminoacyl group to the 3'-OH of tRNA. Only the 3'-esters are substrates for protein synthesis.

Aminoacyl-AMP is a mixed anhydride of a carboxylic acid and a phosphoric acid. Because anhydrides are reactive compounds, the free-energy change for the hydrolysis of aminoacyl-AMP favors the second step of the overall reaction. Another point that favors the process is the energy released when pyrophosphate (PPi) is hydrolyzed to orthophosphate (Pi) to replenish the phosphate pool in the cell.

In the second part of the reaction, an ester linkage is formed between the amino acid and either the 3'-hydroxyl or the 2'-hydroxyl of the ribose at the 3' end of the tRNA. There are two classes of aminoacyl-tRNA synthetases. Class I loads the amino acid onto the 2' hydroxyl. Class II uses the 3' hydroxyl. These two classes of enzyme appear to be unrelated and indicate a convergent evolution. Several tRNAs can exist for each amino acid, but a given tRNA does not bond to more than one amino acid. The synthetase enzyme requires Mg^{2+} and is highly specific both for the amino acid and for the tRNA. A separate synthetase exists for each amino acid, and this synthetase functions for all the different tRNA molecules for that amino acid. The specificity of the enzyme contributes to the accuracy of the translation process.

Protein synthesis

An overview of protein synthesis is illustrated in Figure 50. Despite its complexity and the variations among species, the translation of a genetic message into the primary sequence of a polypeptide can be divided into three phases: initiation, elongation, and termination.
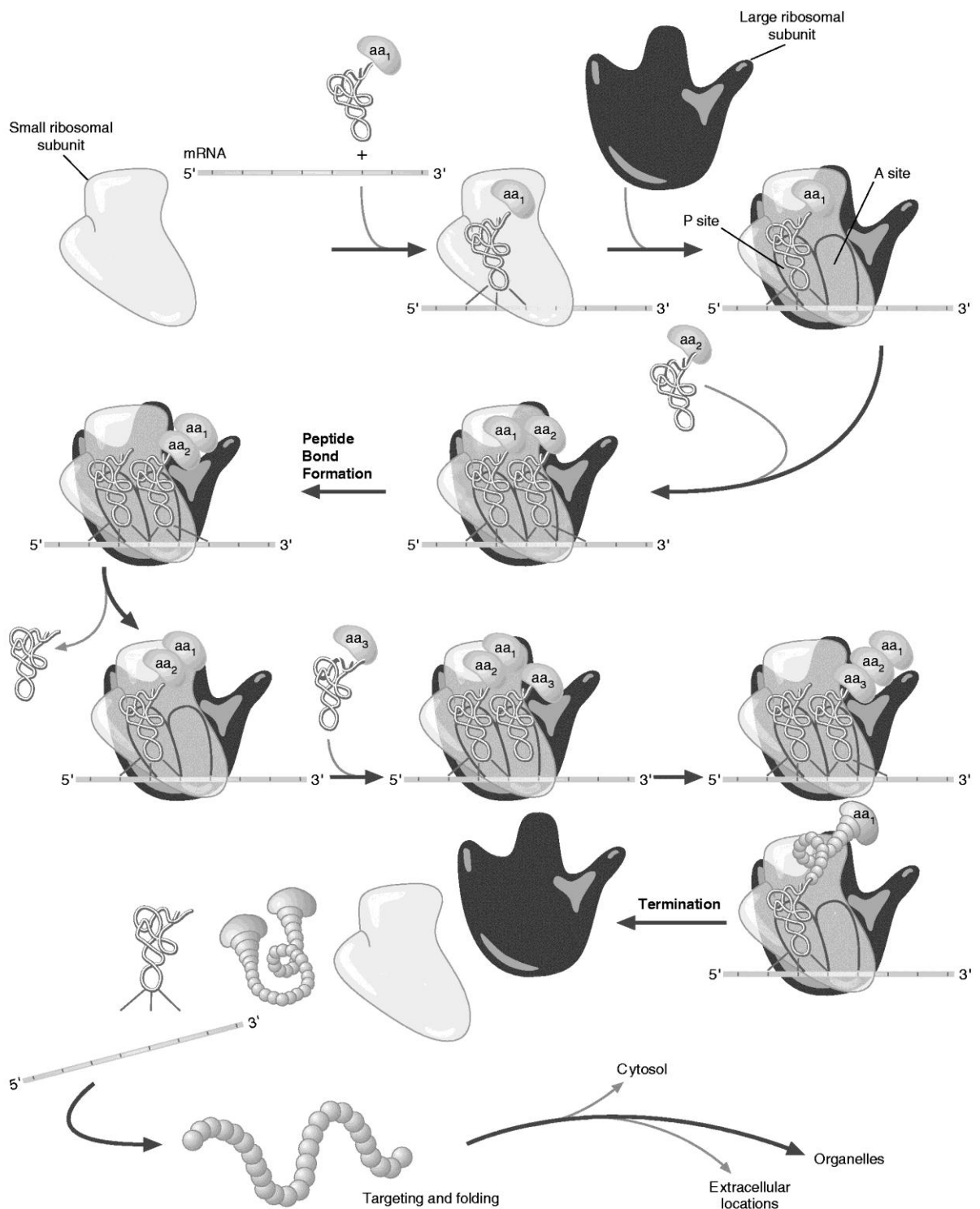
Figure 50. General conception of protein synthesis.

1. Initiation. Translation begins with initiation, when the small ribosomal subunit binds an mRNA. The anticodon of a specific tRNA, referred to as an initiator tRNA, then base pairs with the initiation codon AUG. Initiation ends as the large ribosomal subunit combines with the small subunit. There are two sites

on the complete ribosome for codon-anticodon interactions: the P (peptidyl) site (now occupied by the initiator tRNA) and the A (aminoacyl) site. In both prokaryotes and eukaryotes, mRNAs are read simultaneously by numerous ribosomes. An mRNA with several ribosomes bound to it is referred to as a polysome. In actively growing prokaryotes, for example, the ribosomes attached to an mRNA molecule may be separated from each other by as few as 80 nucleotides.

2. Elongation. During the elongation phase the polypeptide is actually synthesized according to the specifications of the genetic message. The message is read in the 5'→3' direction, polypeptide synthesis proceeds from the N-terminal to the C-terminal. Elongation begins as a second aminoacyl-tRNA becomes bound to the ribosome in the A site because of codon-anticodon base pairing. Peptide bond formation is then catalyzed by peptidyl transferase. During this reaction (referred to as transpeptidation) the α-amino group of the A site amino acid (acting as a nucleophile) attacks the carbonyl group of the P site amino acid (Fig. 51). Because of peptide bond formation, both amino acids are now attached to the A site tRNA. The now uncharged P site tRNA is released from the ribosome. There is some evidence that a discharged tRNA lingers briefly in another site within the ribosome referred to as the E, or exit, site. The next step in elongation involves translocation, whereby the ribosome is moved along the mRNA. As the mRNA moves, the next codon enters the A site, and the tRNA bearing the growing peptide chain moves into the P site. This series of steps, referred to as the elongation cycle, is repeated until a stop codon enters the A site.

3. Termination. During termination the polypeptide chain is released from the ribosome. Translation terminates because a stop codon cannot bind an aminoacyl-tRNA. Instead, a protein releasing factor binds to the A site. Subsequently, peptidyl transferase (acting as an esterase) hydrolyzes the bond connecting the now-completed polypeptide chain and the tRNA in the P site. Translation ends as the ribosome releases the mRNA and dissociates into the large and small subunits.
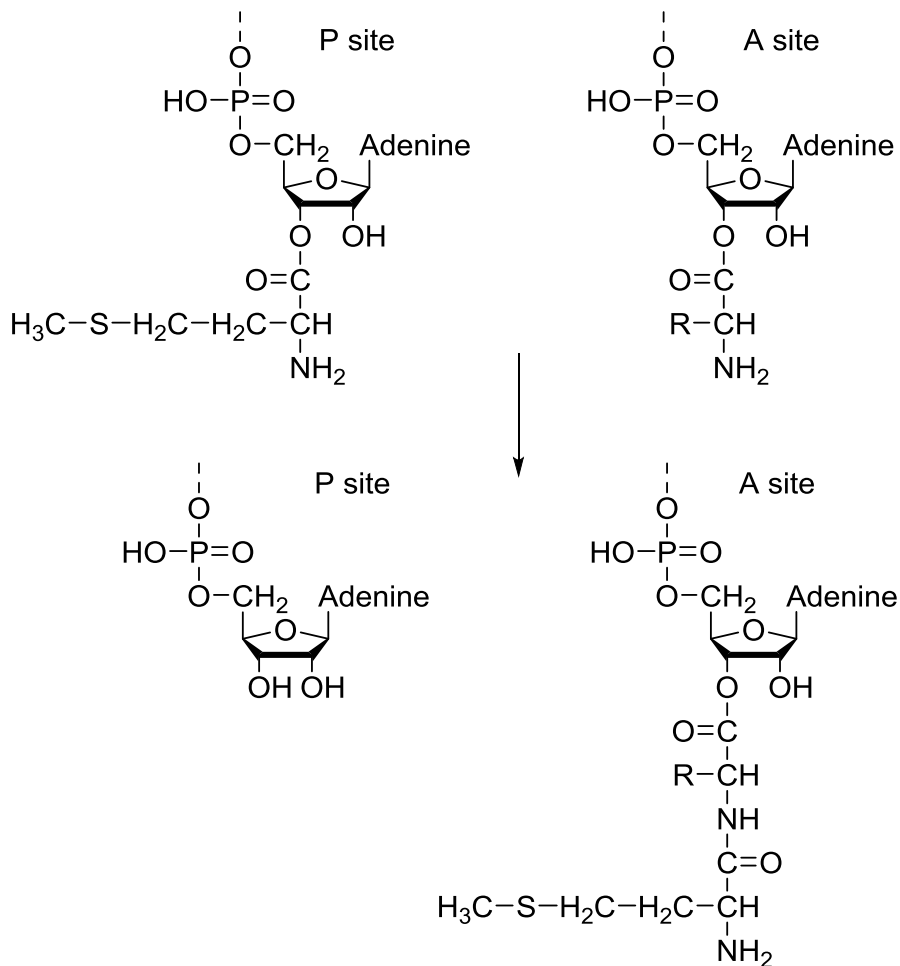
Figure 51. Peptide Bond Fonnation.

In addition to the ribosomal subunits, mRNA, and aminoacyl-tRNAs, translation requires an energy source (GTP) and a wide variety of protein factors. These factors perform several roles. Some have catalytic functions; others stabilize specific structures that form during translation. Translation factors are classified according to the phase of the translation process that they affect, that is, initiation, elongation, or termination. The major differences between prokaryotic and eukaryotic translation appear to be due largely to the identity and functioning of these protein factors.

Regardless of the species, immediately after translation, some polypeptides fold into their final form without further modifications. Frequently, however, newly synthesized polypeptides are modified. These alterations, referred to as posttranslational modifications, can be considered to be the fourth phase of translation. They include removal of portions of the polypeptide by proteases,

modification of the side chains of certain amino acid residues, and insertion of cofactors. Often, individual polypeptides then combine to form multisubunit proteins. Posttranslational modifications appear to serve two general purposes: (1) to prepare a polypeptide for its specific function and (2) to direct a polypeptide to a specific location, a process referred to as targeting. Targeting is an especially important process in eukaryotes because proteins must be directed to many destinations. In addition to cytoplasm and the plasma membrane (the principal destinations in prokaryotes), eukaryotic proteins may be sent to a variety of organelles (e.g., mitochondria, chloroplasts, lysosomes, or peroxisomes).

Although there are many similarities between prokaryotic and eukaryotic protein synthesis, there are also notable differences. In fact, these differences are the basis for the therapeutic and research uses of several antibiotics (Table 5). Consequently, the details of prokaryotic and eukaryotic processes are discussed separately.

Table 5. Selected Antibiotic Inhibitors of Protein Synthesis.

Antibiotic	Action
Chloramphenicol	Inhibits prokaryotic peptidyltransferase
Cycloheximide	Inhibits eukaryotic peptidyltransferase
Erythromycin	Inhibits prokaryotic peptide chain elongation
Streptomycin	Binding to 30S subunit causes mRNA misreading
Tetracycline	Binding to 30S subunit interferes with aminoacyl-tRNA binding
Aurintricarboxylic acid	Inhibits the attachment of mRNA to the ribosome (initiation of protein synthesis) in prokaryotes
Amicetin	Inhibits prokaryotic and eukaryotic peptidyltransferase (binds to 23S rRNA)
Actinomycin D	Binding to DNA at the transcription initiation complex prevents elongation of RNA chain by RNA polymerase

Rifamycin	Binding to bacterial RNA polymerase inhibits synthesis of RNA
Puromycin	Inhibits prokaryotic and eukaryotic peptide chain elongation. Binding to A site causes peptide chain release
Anisomycin	Inhibits eukaryotic peptidyltransferase (the 80S ribosome system)
Lincomycin	Binding reversibly to the P site on the subunit 50S of the bacterial ribosome prevents peptidyltransferase from adding the growing peptide attached to tRNA to the next amino acid

Prokaryotic Protein Synthesis

INITIATION. As described, translation begins with forming an initiation complex (Fig. 52). In prokaryotes this process requires three initiation factors (IFs). IF-3 and IF-1 have previously bound to the 30S subunit. IF-3 prevents it from binding prematurely to the 50S subunit. IF-1 binds to the A site of the 30S subunit, thereby blocking it during initiation. As an mRNA binds to the 30S subunit, it is guided into a precise location (so that the initiation codon AUG is correctly positioned) by a purine-rich sequence referred to as **the Shine-Dalgarno sequence**.

The Shine-Dalgamo sequence (named for its discoverers, John Shine and Lynn Dalgamo) occurs a short distance upstream from AUG. It binds to a complementary sequence contained in the 16S rRNA component of the 30S subunit. Base pairing between the Shine-Dalgamo sequence and the 30S subunit provides a mechanism for distinguishing a start codon from an internal methionine codon. Each gene on a polycistronic mRNA possesses its own Shine-Dalgamo sequence and an initiation codon. The translation of each gene appears to occur independently; that is, translation of the first gene in a polycistronic message may or may not be followed by the translation of subsequent genes.
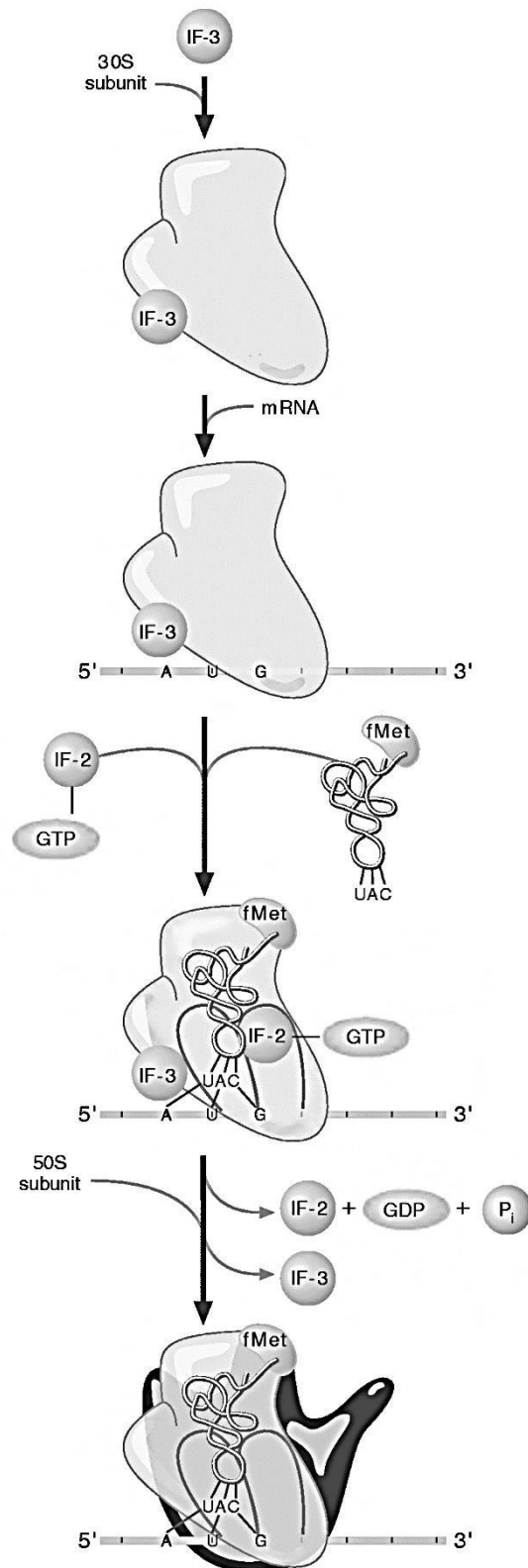
Figure 52. Formation of the Prokaryotic Initiation Complex.

In the next step in initiation, IF-2 (a GTP-binding protein with a bound GTP) binds to the 30S subunit, where it promotes the binding of the initiating tRNA to the initiation codon of the mRNA. The initiating tRNA in prokaryotes is N-

formylmethionine-tRNA (fmet-tRNA^{fmet}). After a special initiator tRNA is charged with methionine, the amino acid residue is formylated in an N^{10}-THF-requiring reaction. The enzyme that catalyzes this reaction binds met-tRNA^{fmet} but not met-tRNA^{met}.

The initiation phase ends as the GTP molecule bound to IF-2 is hydrolyzed to GDP and Pi' GTP hydrolysis presumably causes a conformational change that binds the 50S subunit to the 30S subunit. Simultaneously, IF-2 and IF-3 are released.

ELONGATION. Elongation consists of three steps: (1) positioning an aminoacyl-tRNA in the A site, (2) peptide bond formation, and (3) translocation. As noted, these steps are referred to collectively as an elongation cycle.

The prokaryotic elongation process begins when an aminoacyl-tRNA, specified by the next codon, binds to the A site. Before it can be positioned in the A site, the aminoacyl-tRNA must first bind EF-Tu-GTP. The elongation factor EF-Tu is a GTP-binding protein involved in positioning aminoacyl-tRNA molecules in the A site. After the aminoacyl-tRNA is positioned, the GTP bound to EF-Tu is hydrolyzed to GDP and Pi' GTP hydrolysis releases EF-Tu from the ribosome. Then a second elongation factor, referred to as EF-Ts, promotes EF-Tu regeneration by displacing its GDP moiety. EF-Ts is then itself displaced by an incoming GTP molecule (Fig. 53).

After EF-Tu delivers an aminoacyl-tRNA to the A site, the formation of a peptide bond is catalyzed by peptidyl transferase. Recall that the peptidyl transferase activity is now known to reside in the 23S rRNA component of the 50S subunit. The energy required to drive this reaction is provided by the high-energy ester bond linking the P site amino acid to its tRNA. During the first elongation cycle, this amino acid is formylmethionine. As described, the now uncharged tRNA occupying the P site leaves the ribosome.

For translation to continue, the mRNA must move, or translocate, so that a new codon-anticodon interaction can occur. Translocation requires binding another GTP-binding protein, referred to as EF-G. GTP hydrolysis provides the energy

required for the ribosomal conformation change that is apparently involved in moving the peptidyl-tRNA (the tRNA bearing the growing peptide chain) from the A site to the P site. The unoccupied A site then binds an appropriate aminoacyl-tRNA to the new A site codon. After EF-G is released, the ribosome is ready for the next elongation cycle. Elongation continues until a stop codon enters the A site.
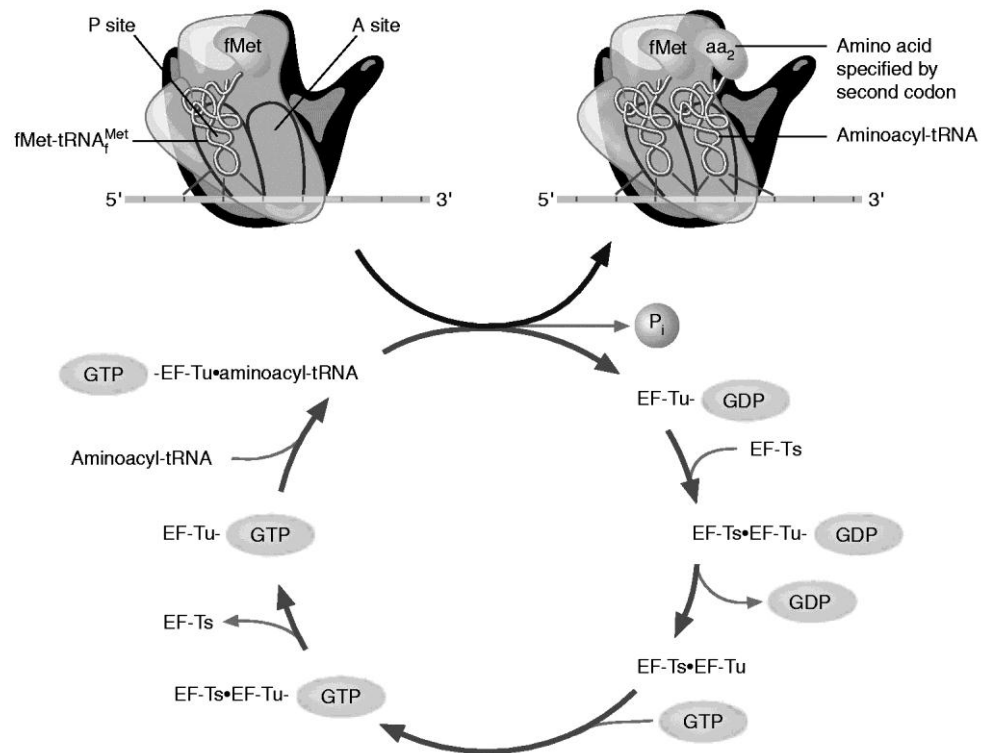
Figure 53. The EF-Tu-EF-Ts Cycle in *E. coli*.

TERMINATION. The termination phase begins when a termination codon (UAA, UAG, or UGA) enters the A site. Three releasing factors (RF- 1, RF-2, and RF-3) are involved in termination. The codons UAA and UAG are recognized by RF-1, whereas UAA and UGA are recognized by RF-2. The role of RF-3 is unclear. It may promote RF-1 and RF-2 binding. This recognition process, which involves GTP hydrolysis, alters ribosome function. The peptidyl transferase, which is transiently transformed into an esterase, hydrolyzes the bond linking the completed polypeptide chain and the P site tRNA. Following the polypeptide's release from the ribosome, the mRNA and tRNA also dissociate. The termination phase ends when the ribosome dissociates into its constituent subunits.

Eukaryotic Protein Synthesis

INITIATION. Most of the major differences between the prokaryotic and eukaryotic versions of protein synthesis occur during the initiation phase. Among the reasons for the additional complexity of eukaryotic initiation are the following:

1. mRNA secondary structure. Recall that eukaryotic mRNA is processed by the addition of a methylguanosine cap and a poly A tail and by the removal of introns. In addition, eukaryotic mRNA does not associate with a ribosome until it leaves the nucleus and, as a result, is free to interact with a number of cellular proteins. An mRNA that is complexed with these proteins is sometimes referred to as a ribonucleoprotein particle.

2. mRNA scanning. In contrast to prokaryotic mRNA, eukaryotic molecules lack Shine-Dalgamo sequences, which allow for the identification of the initiating AUG sequence. Instead, eukaryotic ribosomes "scan" each mRNA. This scanning is a complex (and poorly understood) process in which ribosomes bind to the capped 5' end of the molecule and migrate in a 5'→3' direction searching for a translation start site.

Eukaryotes use a more complex spectrum of initiation factors than prokaryotes. There are at least nine eukaryotic initiating factors (elFs), several of which possess numerous subunits. The functional roles of most of these factors are still under investigation.

Eukaryotic initiation (Fig. 54) begins when the small 40S ribosomal subunit binds to a complex composed of *elF-2* (a GTP-binding protein), GTP, and an initiating species of methionyl-tRNA^{met} (met-tRNA$_i$). elF-2-GTP, which mediates the binding of the initiating tRNA to the 40S subunit, is regenerated from inactive elF-2-GDP by *elF-2B*, a guanine nucleotide-releasing protein. After GDP is released from elF-2, GTP binding occurs. The small (40S) subunit is prevented from binding to the large (60S) subunit during this phase of initiation because it is associated with *elF-3*, a multisubunit protein. Subunit assembly is also prevented by the association of elF-6 with the 60S subunit. The complex consisting of the small subunit, elF-2-GTP, elF-3, and methionyl-tRNA^{met} is referred to as a ***40S***

preinitiation complex. Subsequently, mRNA binds to the 40S preinitiation complex to form a *40S initiation complex*. This is an ATP-requiring process that involves several additional initiation factors (e.g., elF-4A, elF-4B, elF-1, elF-4F). *elF-4F* binds to the cap structure at the 5' end of the mRNA, whereas the binding of *elF-4A* (an ATPase) and *elF-4B* (a helicase) is believed to reduce the secondary structure of the bound mRNA molecule. Identifying eukaryotic initiation factors has been confusing. For example, some factors have been revealed to be subunits of larger factors. *elF-4E*, also referred to as cap-binding protein or *CBP I*, is one of several subunits of elF-4F. *elF-4F* is often referred to as *CBP II*.
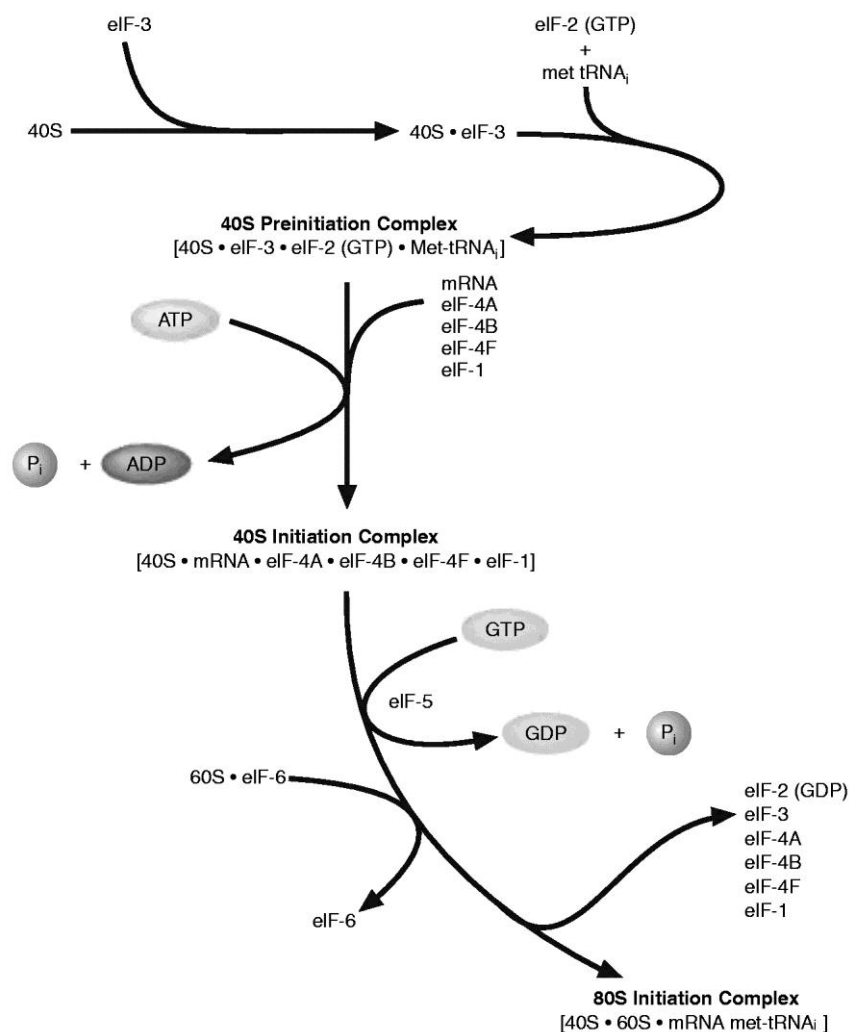
Figure 54. Formation of the Eukaryotic Initiation Complex.

Once the 40S initiation complex is formed, it scans the mRNA for a suitable initiation codon, which is usually an AUG near the 5' end. The 40S complex then binds the 60S subunit (now dissociated from elF-6) to form an *80S initiation*

complex. The formation of the 80S complex involves the hydrolysis of the GTP associated with elF-2, a process that requires elF-5. The initiation phase ends as the initiation factors elF-2, elF-3, elF-4A, elF-4B, elF-4F, and elF-1 are released from the initiation complex.

ELONGATION. Figure 55 illustrates the eukaryotic elongation cycle as it is currently understood. Several elongation factors (eEFs) are required during this phase of translation. *eEF-lα*: is a 50 kDa polypeptide that mediates the binding of aminoacyl-tRNAs to the A site. After a complex is formed between eEF-1α, GTP, and the entering aminoacyl-tRNA, codon-anticodon interactions are initiated. If correct pairing occurs, eEF-1α hydrolyzes its bound GTP and subsequently exits the ribosome, leaving its aminoacyl-tRNA behind. If correct pairing does not occur, the complex leaves the A site, thereby preventing incorrect amino acid residues from being incorporated. This process has been referred to as **kinetic proofreading**. In various fungi (e.g., yeast), another elongation factor, referred to as *eEF-3*, is also required in combination with eEF-1α for A site aminoacyl-tRNA binding.
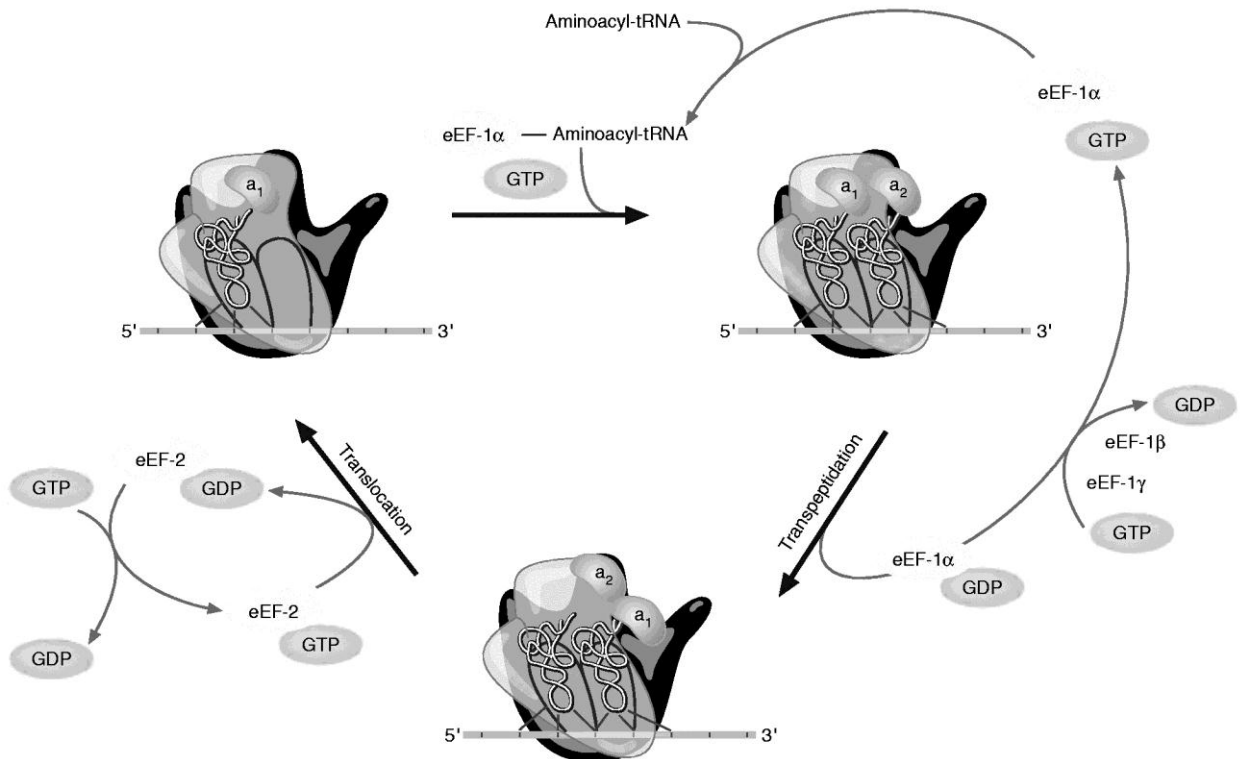
Figure 55. The Elongation Cycle in Eukaryotic Translation.

During the next elongation step (i.e., peptide bond formation) the peptidyl transferase activity of the large ribosomal subunit catalyzes the nucleophilic attack of the A site α-amino group on the carboxyl carbon of the P site amino acid residue. Apparently eEF-1α dissociates from the ribosome immediately before transpeptidation. eEF-lβ and eEF-1γ mediate the regeneration of eEF-1α by promoting an exchange of GDP for GTP. Recall that a similar process involving EF-Tu and EF-Ts occurs in bacteria such as *E. coli*.

Translocation in eukaryotes requires a 100 kDa polypeptide referred to as *eEF-2*, which is also a GTP-binding protein. eEF-2-GTP binds to the ribosome at some as yet undetermined site during translocation. GTP is then hydrolyzed to GDP, and eEF-2-GDP is released. As noted, GTP hydrolysis provides the energy needed to physically move the ribosome along the mRNA. At the end of translocation a new codon is exposed in the A site.

TERMINATION. In eukaryotic cells two releasing factors, *eRF-1* and *eRF-3* (a GTP-binding protein), mediate the termination process. When GTP binds to eRF-3, its GTPase activity is activated. eRF-1 and eRF-3-GTP form a complex that bind in the A site when UAG, UGA, or UAA enter. Then GTP hydrolysis promotes the dissociation of the releasing factors from the ribosome. This step is soon followed by the release of mRNA and the separation of the functional ribosome into its subunits. As described, the release of the newly synthesized polypeptide is catalyzed by peptidyl transferase.

Gene Expression in Prokaryotes

The highly regulated metabolism of prokaryotes such as *E. coli* allows these organisms to respond rapidly to a changing environment to promote growth and survival. The timely synthesis of enzymes and other gene products only when needed prevents wasting energy and nutritional resources. At the genetic level, the control of inducible genes is often effected by groups of linked structural and regulatory genes called ***operons***. Investigations of operons, especially the lac

operon, have provided substantial insight into how gene expression can be altered by environmental conditions.

The lac operon (Fig. 56) consists of a control element and structural genes that code for the enzymes of lactose metabolism. The control element contains the promoter site, which overlaps the operator site. In prokaryotes the operator is a DNA sequence involved in the regulation of adjacent genes that binds to a repressor protein. The promoter site also contains the CAP site. The structural genes Z, Y, and A specify the primary structure of β-galactosidase, lactose permease, and thiogalactoside transacetylase, respectively. β-Galactosidase catalyzes the hydrolysis of lactose, which yields the monosaccharides galactose and glucose, whereas lactose permease promotes lactose transport into the cell. Because lactose metabolism proceeds normally without thiogalactoside transacetylase, its role is unclear. A repressor gene i, directly adjacent to the lac operon, codes for the lac repressor protein, a tetramer that binds to the operator site with high affinity. There are about 10 copies of lac repressor protein per cell. The binding of the lac repressor to the operator prevents the functional binding of RNA polymerase to the promoter (Fig. 57).
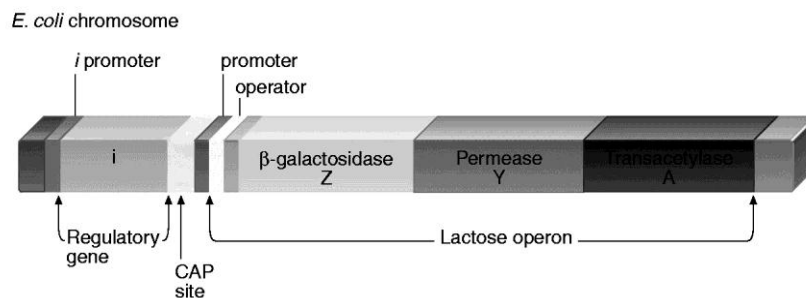
Figure 56. The bac Operon in *E. coli*.
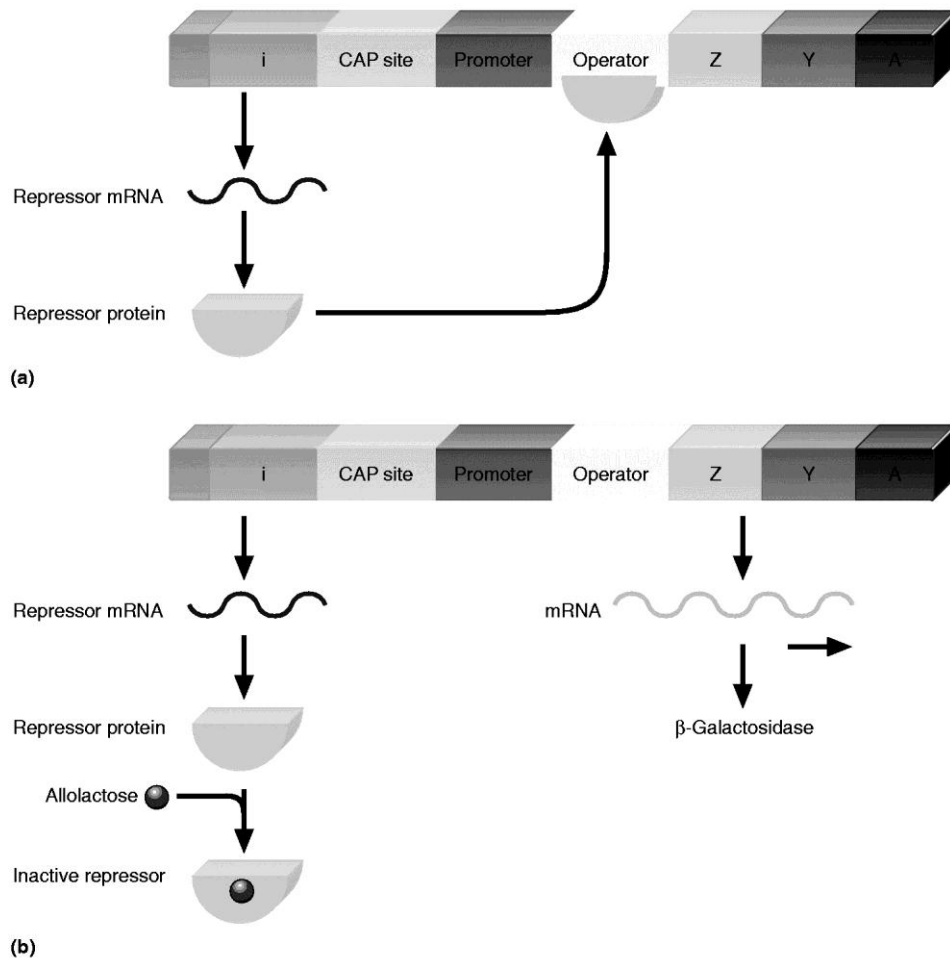
Figure 57. Function of the Lac Operon.

(a) The repressor gene i encodes a repressor that binds to the operator when lactose (the inducer) is not present. (b) When lactose is present, its isomer allolactose binds to repressor protein, thereby inactivating it.

Without its inducer (allolactose, a β-l,6-isomer of lactose) the lac operon remains repressed because the lac repressor binds to the operator. When lactose becomes available, a few molecules are converted to allolactose by β-galactosidase. Allolactose then binds to the repressor, changing its conformation and promoting dissociation from the operator. Once the inactive repressor diffuses away from the operator, the transcription of the structural genes begins. The lac operon remains active until the lactose supply is consumed. Then the repressor reverts to its active form and rebinds to the operator.

Glucose is the preferred carbon and energy source for *E. coli*. If both glucose and lactose are available, the glucose is metabolized first. Synthesis of the lac operon enzymes is induced only after the glucose has been consumed. This makes sense because glucose is more commonly available and has a central role in cellular metabolism. The delay in activating the lac operon is mediated by a catabolite gene activator protein (CAP). CAP is an allosteric homodimer that binds to the chromosome at a site directly upstream of the lac promoter when glucose is absent. CAP is an indicator of glucose concentration because it binds to cAMP. The cell's cAMP concentration increases when the cell is in energy deficit, that is, when the primary carbon source (glucose) is absent. The binding of cAMP to CAP, which occurs only when glucose is absent and cAMP levels are high, causes a conformational change that allows the protein to bind to the lac promoter. CAP binding promotes transcription by increasing the affinity of RNA polymerase for the lac promoter. In other words, CAP exerts a positive or activating control on lactose metabolism.

EXERCISES FOR INDEPENDENT WORK. In the table with test tasks emphasize keywords, choose the correct answer and justify it:

№	Test:	Explanation:
1.	At the stage of translation in the rough endoplasmic reticulum, the ribosome moves along the mRNA. Amino acids are joined together by peptide bonds in a specific sequence, and thus polypeptide synthesis takes place. The sequence of amino acids in a polypeptide corresponds to the sequence of: A. rRNA nucleotides B. rRNA anticodons C. mRNA codons D. tRNA nucleotides E. tRNA anticodons	
2.	Infection diseases are treated with antibiotics (streptomycin, erythromycin, chloramphenicol). They inhibit the following stage of protein synthesis: A. Translation B. Replication C. Processing D. Transcription E. Splicing	
3.	A patient has decreased concentration of magnesium ions that are required for ribosomes connection to granular endoplasmic reticulum. This condition is known to disrupt the process of protein biosynthesis. Disruption occurs at the following state: A. Translation B. Processing C. Replication D. Amino acid activation E. Transcription	
4.	Several parts are distinguished in the secondary tRNA structure. Each part has a single function. Name the part that provides tRNA recognition by amino acyl-	

№	Test:	Explanation:
	tRNA-synthetase: A. Anticodon loop B. Pseudouracil loop C. Extra loop D. Acceptor loop E. Dihydrouracil loop	
5.	Each amino acid is coded for by the triplet of nucleotide residues in the structure of DNA. Point out the total quantity of triplets that keeps a genetic code: A. 20 B. 16 C. 64 D. 32 E. 40	
6.	Initiation complex is formed at the last step of initiation stage of protein biosynthesis. Point out the site of this complex, where formyl methionyl-tRNA is attached to at this moment: A. Peptide site B. Amino acyl site C. 50S subunit of the ribosome D. Protein factor IF-3 E. Protein factor IF-1	
7.	Protein biosynthesis depends on energy. Point out the type of high-energy bonds containing substances that are used in this process: A. GDP B. ADP C. UTP D. GTP E. CTP	
8.	Point out the enzyme that takes part in amino acid activation at the first stage of protein biosynthesis: A. Peptidyltransferase B. Translocase C. Amino acyl-tRNA-synthetase D. Amino peptidase E. Transaminase	

№	Test:	Explanation:
9.	The fragment of DNA that keeps the information about the synthesis of functionally united proteins is named as operon. Point out the gene, which is able to attach a protein-repressor in the operon: A. Promotor B. Operator gene C. Regulator gene D. Structural gene E. Terminator	
10.	Several antibiotics are applied in medical practice. They can act as the inhibitors for protein synthesis at the different stages of this process. Point out the inhibitor of the initiation stage of translation: A. Aurintricarboxylic acid B. Neomycin C. Chloramphenicol D. Tetracycline E. Puromycin	
11.	Protein biosynthesis consists of several stages. Point out the stage of peptide bond formation: A. Initiation B. Elongation C. Post-translation modification D. Termination E. Activation of amino acids	
12.	Point out the derivative of N- terminal amino acid used at initiation stage in the protein synthesis (E. coli): A. Isoleucine B. Formyl threonine C. Methionine D. Formyl methionine E. Leucine	
13.	Point out the enzyme that takes part in the peptide bond formation at the elongation stage: A. Translocase B. Peptidase C. Peptidyltransferase	

№	Test:	Explanation:
	D. Aminoacyl-tRNA-synthetase E. Hydrolase	
14.	Choose the component that doesn't take part in the initiation stage of protein synthesis: A. mRNA B. ATP C. Amino acyl-tRNA D. 30S subunit of the ribosome E. 50S subunit of the ribosome	
15.	Several antibiotics are applied in medical practice. They can act as the inhibitors for protein synthesis at the different stages of this process. Point out the inhibitor of the termination stage of translation: A. Aurintricarboxylic acid B. Neomycin C. Cycloheximide D. Tetracycline E. Erythromycin	
16.	The degeneration of Genetic code is explained as such: A. There are two or more triplets for one amino acid B. Genetic Code is composed of various triplets C. Each amino acid is coded for one triplet, only D. "Punctuation marks" are absent in the Genetic code E. Genetic code is a single for all biologic systems	
17.	Point out the function for amino acyl-tRNA-synthetase in protein synthesis: A. It forms the peptide bond B. It promotes the ribosome moving along the mRNA C. It binds tRNA with amino acid residue D. It takes part in the ribosome structure E. It binds tRNA with the ribosome	

№	Test:	Explanation:
18.	Point out the maintenance of information keeping in the triplet A-U-G of mRNA: A. Protein synthesis beginning from initial amino acid B. Protein synthesis beginning and protein synthesis termination C. Doesn't posses any information D. Protein synthesis termination E. The operation of post-translational modification of proteins	
19.	Peptidyl-tRNA transport from amino acyl site to peptide site takes place during the elongation stage of protein synthesis. Name the enzyme that promotes this operation: A. Aminoacyl-tRNA-synthetase B. Translocase C. Peptidyltranferase D. Transaminase E. Amino peptidase	
20.	In E. coli structural gene of LAC operon is stimulated in: A. Presence of glucose only B. Presence of galactose only C. Presence of lactose only D. Presence of glucose and absence of lactose E. Presence of lactose and absence of glucose	

CHROMOPROTEINS.
HEMOGLOBIN METABOLISM AND ITS DISORDERS.
PORPHYRINS METABOLISM
(IVANCHENKO D.H.)

INFORMATIONAL MATERIAL

The transition from anaerobic to aerobic life was a major step in evolution because it uncovered a rich reservoir of energy. Fifteen times as much energy is extracted from glucose in the presence of oxygen than in its absence. For single-celled and other small organisms, oxygen can be absorbed into actively metabolizing cells directly from the air or surrounding water. Vertebrates evolved two principal mechanisms for supplying their cells with an adequate supply of oxygen. The first is a circulatory system that actively delivers oxygen to cells throughout the body. The second is the use of the oxygen-transport and oxygen-storage proteins, hemoglobin and myoglobin. Hemoglobin, which is contained in red blood cells, is a fascinating protein, efficiently carrying oxygen from the lungs to the tissues while also contributing to the transport of carbon dioxide and hydrogen ions back to the lungs. Myoglobin, located in muscle, provides a reserve supply of oxygen available in time of need.

Indeed, the early history of protein chemistry is essentially that of hemoglobin. The observation of crystalline hemoglobin was first reported by Friedrich Hünefeld in 1840, and by 1909 Edward Reichert and Amos Brown had published a photographic atlas of hemoglobin crystals from several hundred species. Hemoglobin was one of the first proteins to have its molecular mass accurately determined, the first protein to be characterized by ultracentrifugation, the first to be associated with a specific physiological function (that of oxygen transport), and, in sickle-cell anemia, the first in which a point mutation was demonstrated to cause a single amino acid change. Theories formulated to account for the cooperative binding of oxygen to hemoglobin have also been successful in explaining the control of enzyme activity. The first protein X-ray structures to be

elucidated were those of hemoglobin and myoglobin. This central role in the development of protein chemistry together with its enzymelike O_2-binding properties have caused hemoglobin to be dubbed an "honorary enzyme".

Hemoglobin Structure and the Gas Exchange

Hemoglobin is a roughly spherical molecule of dimensions 64 x 55 x 50 Å found in red blood cells, where its primary function is to transport oxygen from the lungs to every tissue in the body. Recall that HbA is composed of two α-chains and two β-chains (Fig. 58). The HbA molecule is commonly designated $\alpha_2\beta_2$. There is another type of adult hemoglobin. Approximately 2% of human hemoglobin is HbA_2, which contains δ(delta)-chains instead of β-chains.
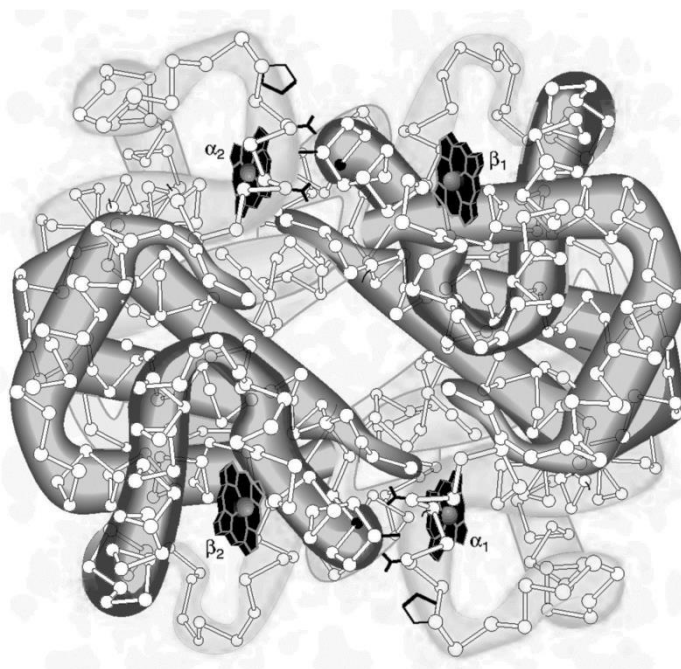
Figure 58. Hemoglobin structure.

Although the three-dimensional configurations of myoglobin and the α- and β-chains of hemoglobin are very similar, their amino acid sequences have many differences. Comparison of these molecules from dozens of species has revealed nine invariant amino acid residues. Several invariant residues directly affect the oxygen-binding site, whereas others stabilize the α-helical peptide segments. The

remaining residues may vary considerably. However, most substitutions are conservative. For example, each polypeptide's interior remains nonpolar.

The four chains of hemoglobin are arranged in two identical dimers, designated as $\alpha_1\beta_1$ and $\alpha_2\beta_2$. Each globin polypeptide has a heme-binding unit similar to that described for myoglobin.

Hemoglobin can exist in an oxygen-free form called *deoxymyoglobin* or in a form with an oxygen molecule bound called *oxymyoglobin*. The ability of myoglobin, and hemoglobin as well, to bind oxygen depends on the presence of a bound prosthetic group called *heme*. The heme group gives muscle and blood their distinctive red color. It consists of an organic component and a central iron atom. The organic component, called *protoporphyrin*, is made up of four pyrrole rings linked by methine bridges to form a tetrapyrrole ring. Four methyl groups, two vinyl groups, and two propionate side chains are attached (Fig. 59).
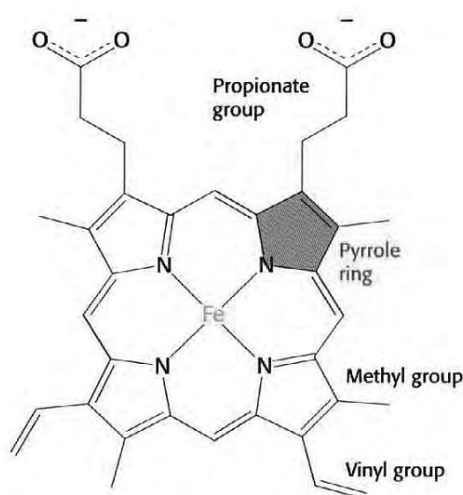
Figure 59. Structure of Heme (Fe-protoporphyrin IX).

The iron atom lies in the center of the protoporphyrin, bonded to the four pyrrole nitrogen atoms. Under normal conditions, the iron is in the ferrous (Fe^{2+}) oxidation state. The iron ion can form two additional bonds one on each side of the heme plane. These binding sites are called the fifth and sixth coordination sites. Tn myoglobin, the fifth coordination site is occupied by the imidazole ring of a histidine residue from the protein. This histidine is referred to as the *proximal histidine*. In deoxymyoglobin, the sixth coordination site remains unoccupied; this

position is available for binding oxygen. The iron ion lies approximately 0,4 Å outside the porphyrin plane because an iron ion, in this form, is slightly too large to fit into the well-defined hole within the porphyrin ring (Fig. 60, left). The binding of the oxygen molecule at the sixth coordination site of the iron ion substantially rearranges the electrons within the iron so that the ion becomes effectively smaller, allowing it to move into the plane of the porphyrin (Fig. 60, right).
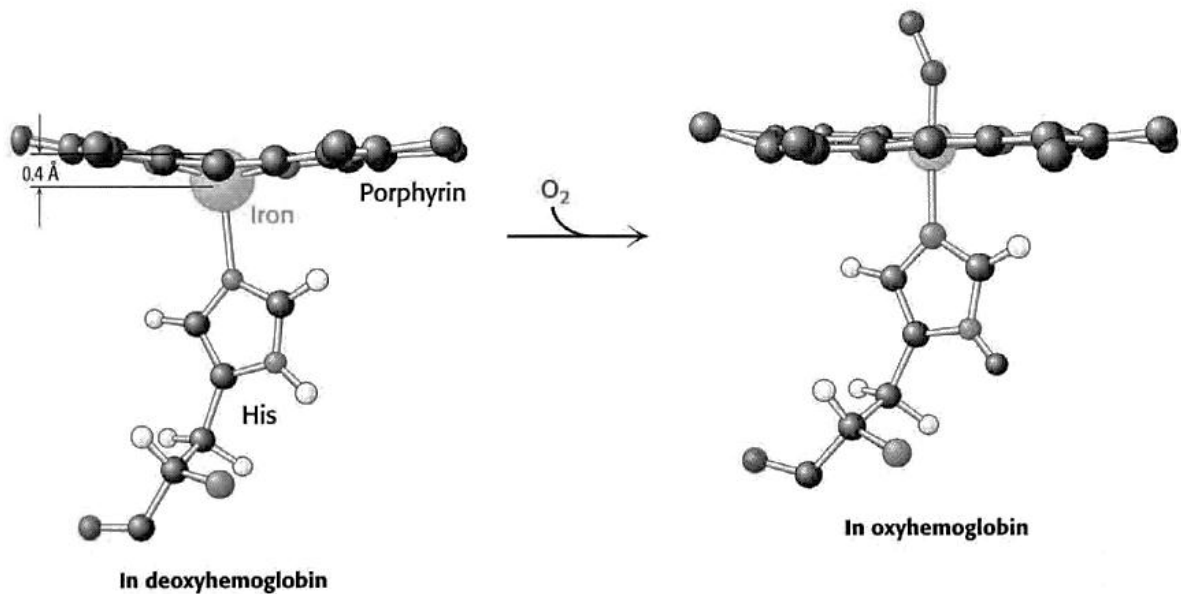
Figure 60. Oxygen Binding Changes the Position of the Iron Ion.

Hemoglobin Derivatives

There are two groups of hemoglobin derivatives: *normal* and *pathological*.

Normal derivatives of hemoglobin are *oxyhemoglobin* (HbO_2) and *carbhemoglobin* ($HbCO_2$, deoxyhemoglobin).

Pathological derivatives of hemoglobin are *methemoglobin* (MtHb) and *carboxyhemoglobin* (HbCO).

Methemoglobin. In hemoglobin Fe^{2+} does not change its valency during binding or release of oxygen. But it can be oxidized by oxidation agents (H_2O_2, free radicals, drugs) to Fe^{3+}, giving rise to hemming. The resulting compound is called methemoglobin and it can't function as oxygen carrier. The oxidation of hemoglobin is corrected by the enzyme *methemoglobin reductase* present in erythrocytes.

Carboxyhemoglobin is forming during carbon monooxide (CO) poisoning. CO is very toxic gas because it binds with a higher affinity (much tighter) to heme does oxygen. Since CO has a higher affinity than oxygen, oxygen cannot displace it. In this way, CO acts as much like a potent competitive inhibitor. There can be plenty of oxygen available, but the hemoglobin bond to CO will not carry it; therefore the oxygen is not available for tissues. CO effectively binds irreversibly to the heme in hemoglobin molecule.

Hemoglobin Types in Healthy Adults

Hemoglobin in healthy adults is in three isoforms:

HbA$_1$: four polypeptide chains $\alpha_2\beta_2$ (content about 96%);

HbA$_2$: four polypeptide chain $\alpha_2\delta_2$ (minor adult hemoglobin, content about 3%)

HbF: four polypeptide chain $\alpha_2\gamma_2$ (fetal hemoglobin, content about 0,2-1%). HbF content in newborns is about 60-80%.

Abnormal Hemoglobins

Mutant hemoglobins provided the original opportunity to study structure–function relationships in proteins because Hb is a readily isolated protein of known structure that has a large number of well-characterized naturally occurring variants. The examination of individuals with physiological disabilities, together with the routine electrophoretic screening of human blood samples, has led to the discovery of over 1000 variant hemoglobins, >90% of which result from single amino acid substitutions in a globin polypeptide chain. It should be noted that ~300,000 individuals with serious hemoglobin disorders are born every year and that ~5% of the world's population are carriers of an inherited variant hemoglobin.

Molecular Pathology of Hemoglobin. The physiological effect of an amino acid substitution on Hb can, in most cases, be understood in terms of its molecular location:

1. Changes in surface residues. Changes of surface residues are usually innocuous because most of these residues have no specific functional role

(although sickle-cell Hb (HbS) is a glaring exception to this generalization). For example, **HbE** (Glu B8(26)β → Lys), the most common human Hb mutant after HbS (possessed by up to 10% of the population in parts of Southeast Asia), has no clinical manifestations in either heterozygotes or homozygotes. About half of the known Hb mutations are of this type and have been discovered only accidentally or through surveys of large populations.

2. Changes in internally located residues. Changing an internal residue often destabilizes the Hb molecule. The degradation products of these hemoglobins, particularly those of heme, form granular precipitates (known as **Heinz bodies**) that hydrophobically adhere to the erythrocyte cell membrane. The membrane's permeability is thereby increased, causing premature cell lysis. Carriers of unstable hemoglobins therefore suffer from hemolytic anemia of varying degrees of severity.

The structure of Hb is so delicately balanced that small structural changes may render it nonfunctional. This can occur through the weakening of the heme-globin association or as a consequence of other conformational changes. For instance, the heme group is easily dislodged from its closely fitting hydrophobic binding pocket. This occurs in **Hb Hammersmith** (Hb variants are often named after the locality of their discovery), in which Phe CD1(42)β, an invariant residue that wedges the heme into its pocket (Fig. 61), is replaced by Ser. The resulting gap permits water to enter the heme pocket, which causes the hydrophobic heme to drop out easily (Phe CD1 and the proximal His F8 are the only invariant residues among all known hemoglobins). Similarly, in **Hb Bristol**, the substitution of Asp for Val E11(67)β, which partially occludes the O_2 pocket, places a polar group in contact with the heme. This weakens the binding of the heme to the protein, probably by facilitating the access of water to the subunit's otherwise hydrophobic interior.

3. Changes stabilizing methemoglobin. Changes at the O_2-binding site that stabilize the heme in the Fe(III) oxidation state eliminate the binding of O_2 to the defective subunits. Such methemoglobins are designated **HbM** and individuals carrying them are said to have **methemoglobinemia**. These individuals usually

have bluish skin, a condition known as **cyanosis**, which results from the presence of deoxyHb in their arterial blood.
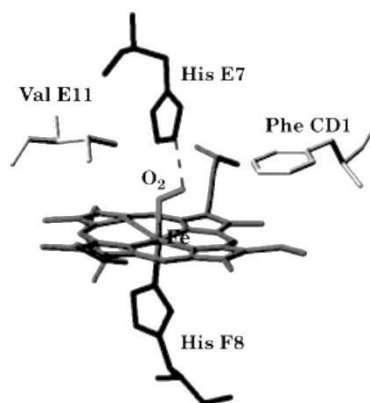
Figure 61. The Heme Complex in HbO_2.

4. Changes at the α_1-β_2 contact. Changes at the α_1-β_2 contact often interfere with hemoglobin's quaternary structural changes. Most such hemoglobins have an increased O_2 affinity so that they release less than normal amounts of O_2 in the tissues. Individuals with such defects compensate for it by increasing their hematocrit (concentration of erythrocytes in their blood). This condition, which is named **polycythemia**, often gives them a ruddy complexion. Some amino acid substitutions at the α_1-β_2 interface instead result in a reduced O_2 affinity. Individuals carrying such hemoglobins are cyanotic.

Molecular Basis of Sickle-Cell Anemia. Most harmful Hb variants occur in only a few individuals, in many of whom the mutation apparently originated. However, ~10% of American blacks and as many as 25% of African blacks are heterozygotes for **sickle-cell hemoglobin** (HbS). HbS arises from the substitution in the β chain of a hydrophobic Val residue for the hydrophilic surface residue Glu in position 6. The prevalence of HbS results from the protection it affords heterozygotes against malaria, a disease carried by a parasite, *Plasmodium falciparum*, that lives within red blood cells at one stage in its life cycle.. However, homozygotes for HbS are severely afflicted by hemolytic anemia together with painful, debilitating, and sometimes fatal blood flow blockages caused by the irregularly shaped and inflexible erythrocytes characteristic of the disease (Fig. 62).

Figure 62. Structure of Normal and Sickled Red Blood Cell.

Examination of the contents of these red cells reveals that the hemoglobin molecules have formed large fibrous aggregates (Fig. 63). These fibers extend across the red blood cells, distorting them so that they clog small capillaries and impair blood flow. The results may be painful swelling of the extremities and a higher risk of stroke or bacterial infection (due to poor circulation). The sickled red cells also do not remain in circulation as long as normal cells do, leading to anemia.

Examination of the structure of hemoglobin S reveals that the new Val residue lies on the surface of the T-state molecule. This new hydrophobic patch interacts with another hydrophobic patch formed by Phe 85 and Val 88 of the 13 chain of a neighboring molecule to initiate the aggregation process. More-detailed analysis reveals that a single hemoglobin S fiber is formed from 14 chains of multiple interlinked hemoglobin molecules. Why do these aggregates not form when hemoglobin S is oxygenated? Oxygenated hemoglobin S is in the R state, and residues Phe 85 and Val 88 on the 13 chain are largely buried inside the hemoglobin assembly. Without a partner with which to interact, the surface Val residue in position 6 is benign.
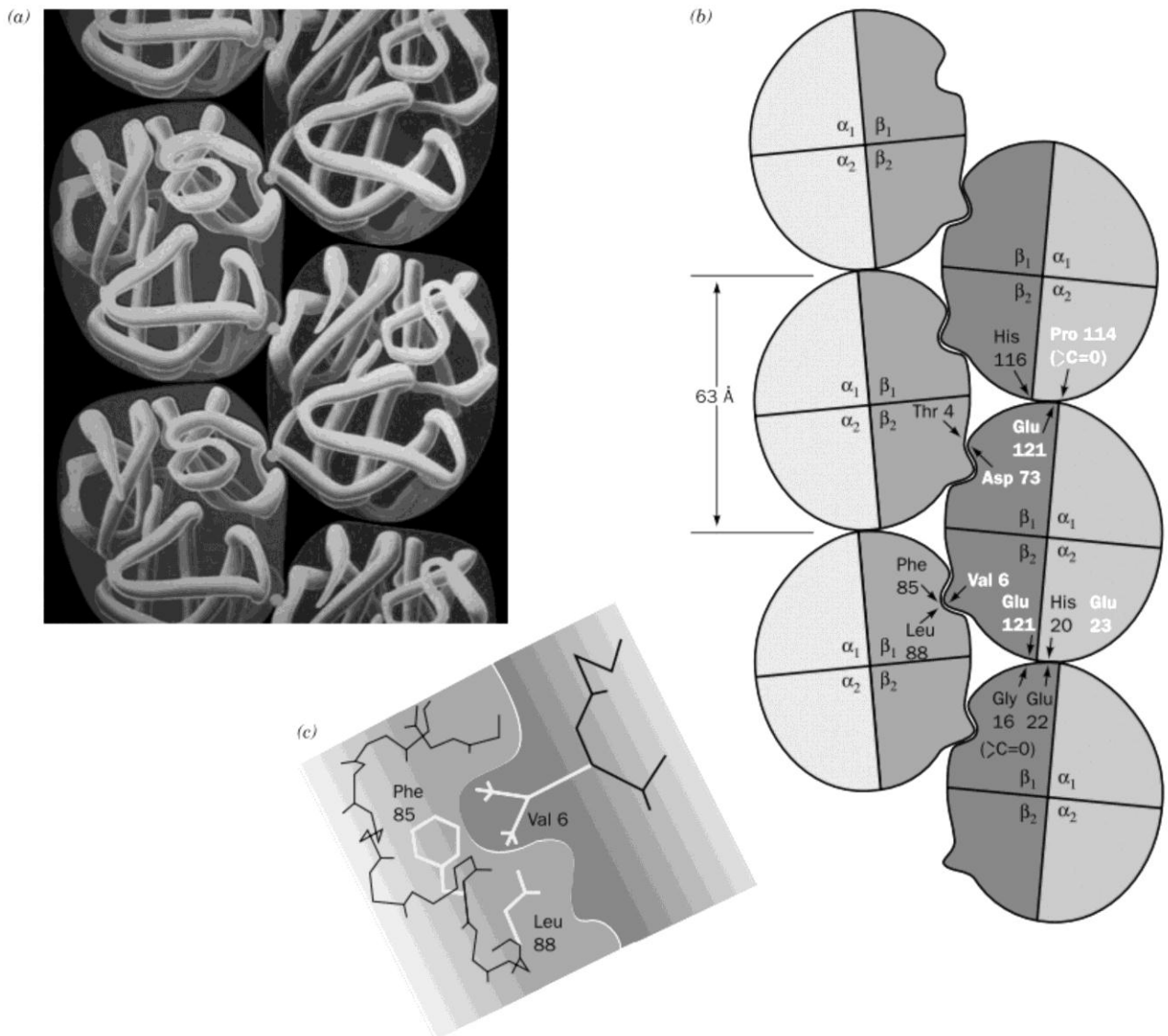
Figure 63. Structure of the DeoxyHbS Fiber.

(a) The arrangement of the deoxyHbS molecules in the fiber.

(b) A schematic diagram indicating the intermolecular contacts in the crystal structure of deoxyHbS.

(c) The mutant Val $6\beta_2$ fits neatly into a hydrophobic pocket formed mainly by Phe 85 and Leu 88 of an adjacent β_1 subunit.

Thalassemia. Sickle-cell anemia is caused by the substitution of a single specific amino acid in one hemoglobin chain . *Thalassemia*, the other prevalent inherited disorder of hemoglobin, is caused by the loss or substantial reduction of a single hemoglobin chain. The result is low levels of functional hemoglobin and a decreased production of red blood cells, which may lead to anemia, fatigue, pale

skin, and spleen and liver malfunction. Thalassemia is a set of related diseases. In *α-thalassemia*, the a chain of hemoglobin is not produced in sufficient quantity. Consequently, hemoglobin tetramers form that contain only the β chain. These tetramers, referred to as **hemoglobin H** (HbH), bind oxygen but with high affinity and no cooperativity. Thus, oxygen release in the tissues is poor. In *β-thalassemia*, the β chain of hemoglobin is not produced in sufficient quantity. In the absence of β chains, the α chains form insoluble aggregates that precipitate inside immature red blood cells. The loss of red blood cells results in anemia. The most severe form of β-thalassemia is called *thalassemia major* or *Cooley anemia*.

Both α- and β-thalassemia are associated with many different genetic variations and display a wide range of clinical severity. The most severe forms of α-thalassemia are usually fatal shortly before or just after birth. However, these forms are relatively rare. An examination of the repertoire of hemoglobin genes in the human genome provides one explanation. Normally, human beings have not two but four alleles for the α chain, arranged such that a pair of genes are located adjacent to each other on one end of each chromosome 16. Thus, the complete loss of α-chain expression requires the disruption of four alleles. β-Thalassemia is more common because we normally have only two alleles for the β chain, one on each copy of chromosome 11.

Heme Biosynthesis

Elucidation of the heme biosynthesis pathway involved some interesting detective work. David Shemin and David Rittenberg, who were among the first to use isotopic tracers in the elucidation of metabolic pathways, demonstrated, in 1945, that all of heme's C and N atoms can be derived from acetate and glycine. Only glycine, out of a variety of ^{15}N-labeled metabolites they tested (including ammonia, glutamate, leucine, and proline), yielded ^{15}N-labeled heme in the hemoglobin of experimental subjects to whom these metabolites were administered. Similar experiments, using acetate labeled with ^{14}C in its methyl or carboxyl groups, or [^{14}C$_\alpha$]glycine, demonstrated that 24 of heme's 34 carbon atoms are

derived from acetate's methyl carbon, 2 from acetate's carboxyl carbon, and 8 from glycine's C_α atom (Fig. 64). None of the heme atoms is derived from glycine's carboxyl carbon atom.

Figure 64 indicates that heme C atoms derived from acetate methyl groups occur in groups of three linked atoms. Evidently, acetate is first converted to some other metabolite that has this labeling pattern. Shemin and Rittenberg postulated that this metabolite is succinyl-CoA based on the following reasoning:

1. Acetate is metabolized via the citric acid cycle.

2. Labeling studies indicate that atom C3 of the citric acid cycle intermediate succinyl-CoA is derived from acetate's methyl C atom, whereas atom C4 comes from acetate's carboxyl C atom.

3. After many turns of the citric acid cycle, C1 and C2 of succinyl-CoA likewise become fully derived from acetate's methyl C atom.
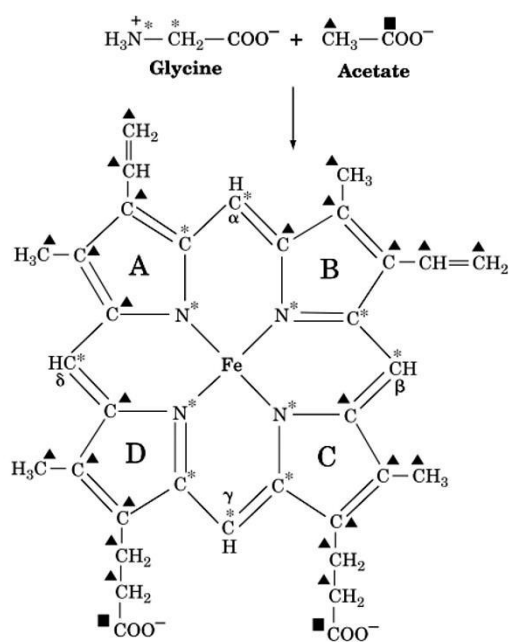
Figure 64. Heme's C and N Atoms are Derived from those of Glycine and Acetate.

In the mitochondria of yeast and animals as well as in some bacteria, the first phase of heme biosynthesis is a condensation of succinyl-CoA with glycine followed by decarboxylation to form δ-aminolevulinic acid (ALA) as catalyzed by the PLP-dependent enzyme δ-aminolevulinate synthase (ALA synthase or ALAS;

Fig. 65). The carboxyl group lost in the decarboxylation (Fig. 65, Reaction 5) originates in glycine, which is why heme contains no label from this group.

The pyrrole ring is formed in the next phase of the pathway through linkage of two molecules of ALA to yield **porphobilinogen** (**PBG**). The reaction is catalyzed by *porphobilinogen synthase* [*PBGS*; alternatively, *δ-aminolevulinic acid dehydratase* (*ALAD*)], which in yeast and mammals, is Zn^{2+}-dependent and involves Schiff base formation of one of the substrate molecules with an enzyme amine group. One possible mechanism of this condensation-elimination reaction involves formation of a second Schiff base between the ALA-enzyme Schiff base and the second ALA molecule (Fig. 66). At this point, if we continue tracing the acetate and glycine labels through the PBG synthase reaction (Fig. 66), we can begin to see how heme's labeling pattern arises.

Inhibition of PBG synthase by Pb^{2+} (a competitor of its active site Zn^{2+} ion) is one of the major manifestations of lead poisoning, which is among the most common acquired environmental diseases. Indeed, it has been suggested that the accumulation, in the blood, of ALA, which resembles the neurotransmitter γ-aminobutyric acid, is responsible for the psychosis that often accompanies lead poisoning.
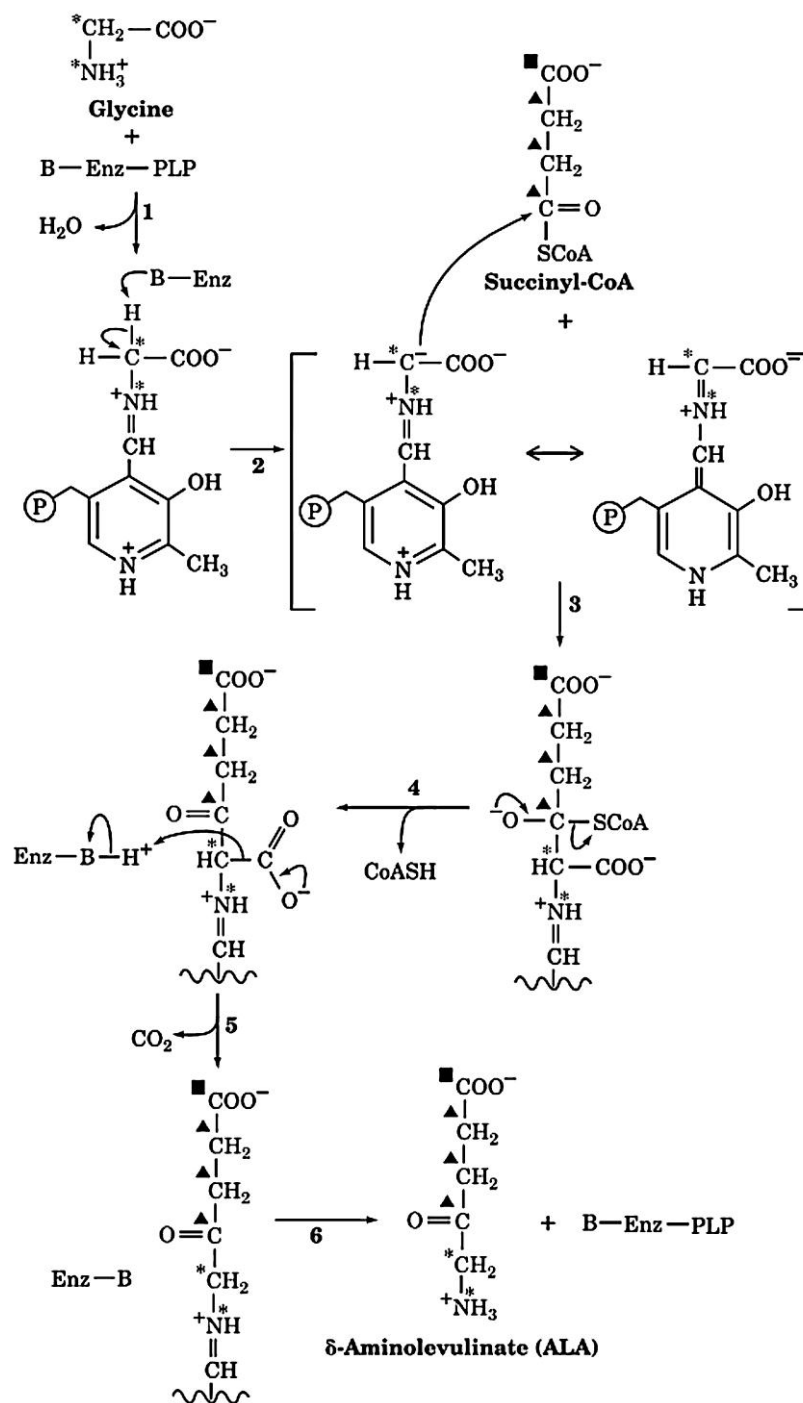
Figure 65. The Mechanism of Action of the PLP-dependent Enzyme δ-Aminolevulinate Synthase (ALAS).

The reaction steps are (1) transimination, (2) PLP-stabilized carbanion formation, (3) C-C bond formation, (4) CoA elimination, (5) decarboxylation facilitated by the PLP–Schiff base, and (6) transimination yielding ALA and regenerating the PLP-enzyme.
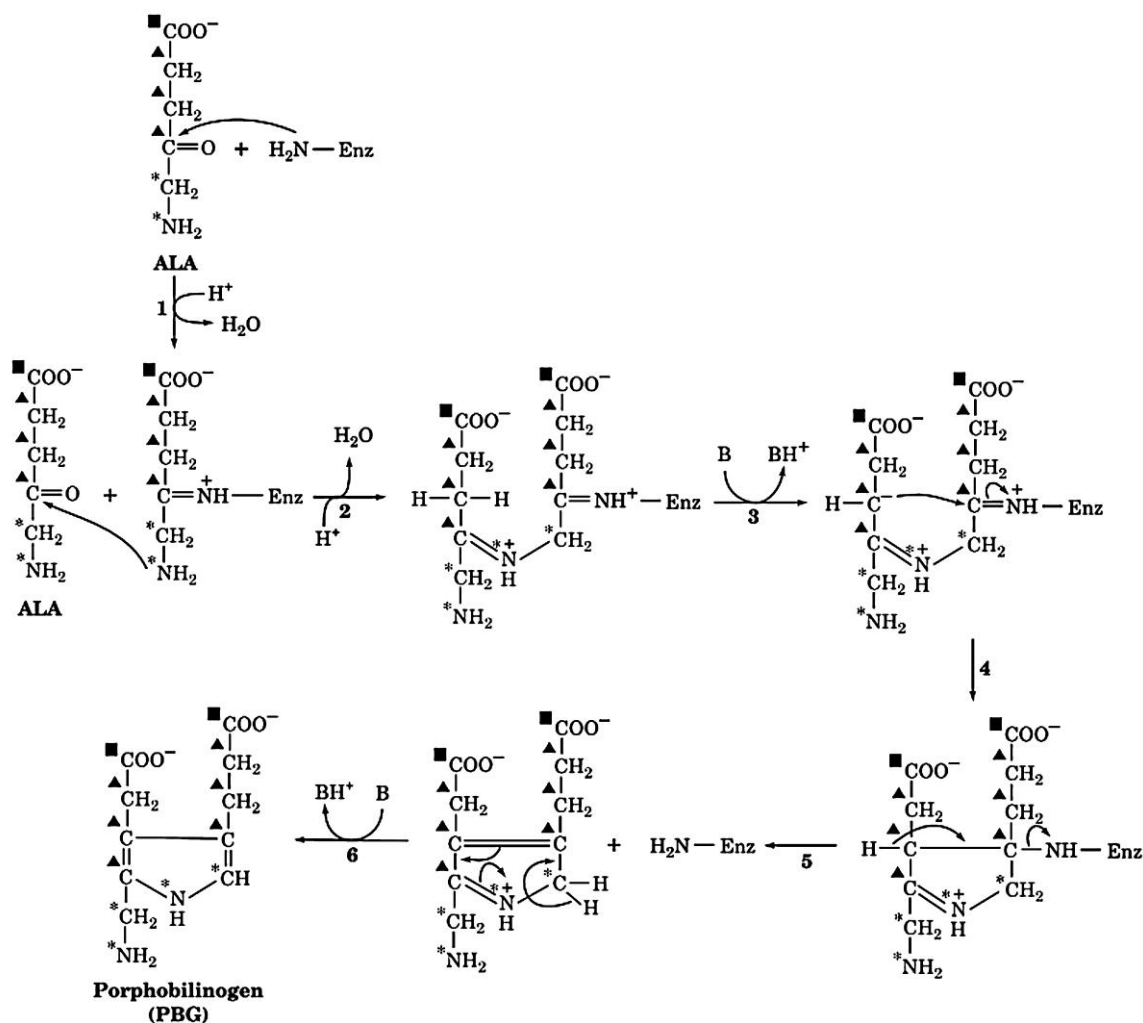
Figure 66. A Possible Mechanism for Porphobilinogen Synthase.

The reaction involves (1) Schiff base formation, (2) second Schiff base formation, (3) formation of a carbanion α to a Schiff base, (4) cyclization by an aldol-type condensation, (5) elimination of the enzyme -NH$_2$ group, and (6) tautomerization.

The next phase of heme biosynthesis is the condensation of four PBG molecules to form **uroporphyrinogen III**, the porphyrin nucleus, in a series of reactions catalyzed by *porphobilinogen deaminase* (alternatively, *hydroxymethylbilane synthase* or *uroporphyrinogen synthase*) and *uroporphyrinogen III synthase*. The reaction (Fig. 67) begins with the enzyme's displacement of the amino group in PBG to form a covalent adduct. A second, third, and fourth PBG then sequentially add through the displacement of the primary amino group on one PBG by a carbon atom on the pyrrole ring of the

succeeding PBG to yield a linear tetrapyrrole that is hydrolyzed and released from the enzyme as *hydroxymethylbilane* (also called *preuroporphyrinogen*).
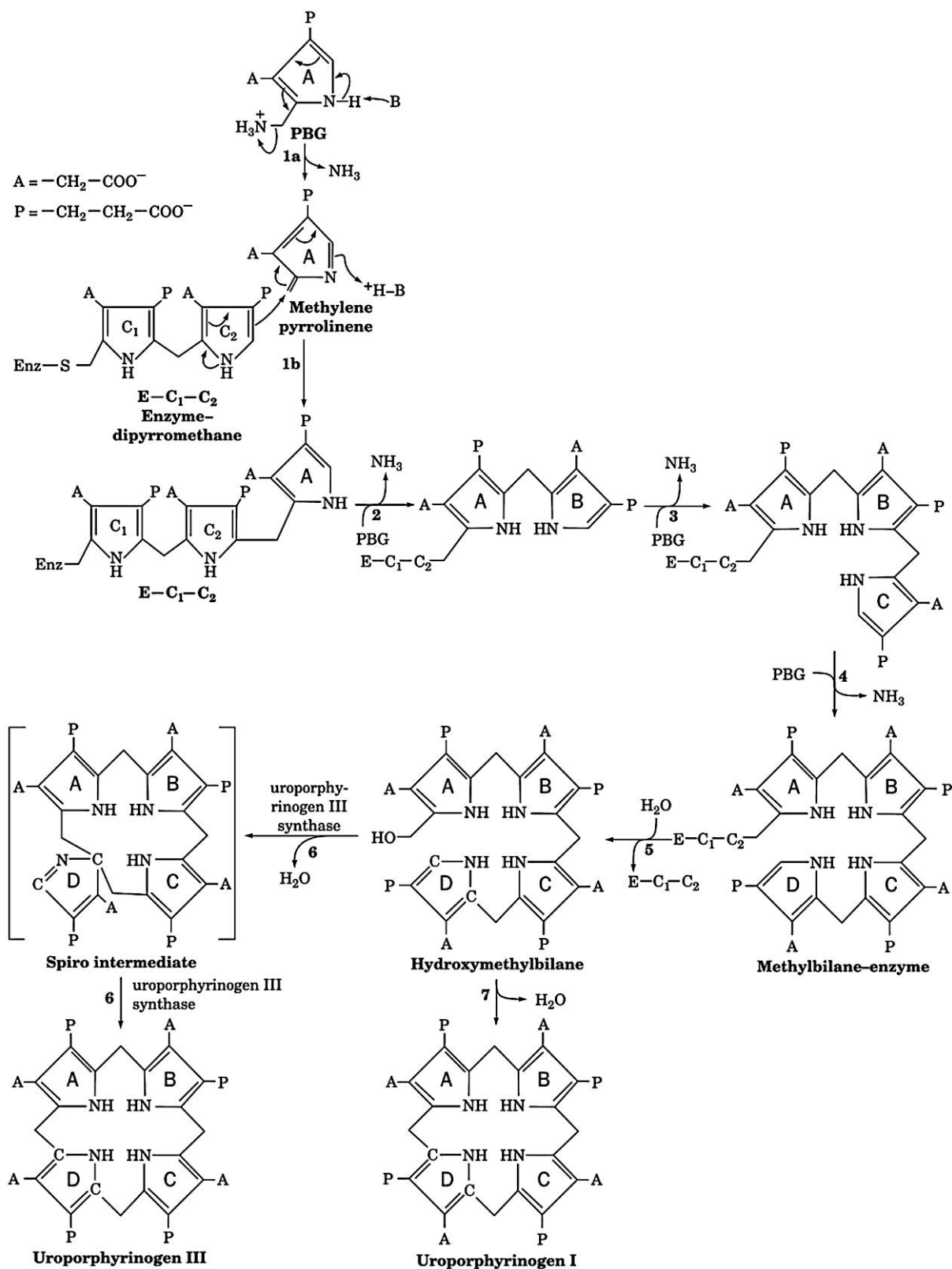
Figure 67. The Synthesis of Uroporphyrinogen III from PBG as Catalyzed by Porphobilinogen Deaminase and Uroporphyrinogen III Synthase.

Cyclization of the hydroxymethylbilane product requires *uroporphyrinogen III synthase* (Fig. 67). In the absence of this enzyme, hydroxymethylbilane is released from the synthase and rapidly cyclizes nonenzymatically to the symmetric **uroporphyrinogen I**. Heme, however, is an asymmetric molecule; the methyl substituent of pyrrole ring D has an inverted placement compared to those of rings A, B, and C. This ring reversal to yield uroporphyrinogen III has been shown by Battersby to proceed through attachment of the methylenes from rings A and C to the same carbon of ring D so as to form a spiro compound (a bicyclic compound with a carbon atom common to both rings).

Heme biosynthesis takes place partly in the mitochondrion and partly in the cytosol (Fig. 68). ALA is mitochondrially synthesized and is transported to the cytosol for conversion to PBG and then to uroporphyrinogen III. **Protoporphyrin IX**, to which Fe is added to form heme, is produced from uroporphyrinogen III in a series of reactions catalyzed by (1) *uroporphyrinogen decarboxylase*, which decarboxylates all four acetate side chains (A) to form methyl groups (M); (2) *coproporphyrinogen oxidase*, which oxidatively decarboxylates two of the propionate side chains (P) to vinyl groups (V); and (3) *protoporphyrinogen oxidase*, which oxidizes the methylene groups linking the pyrrole rings to methenyl groups. Altogether, six carboxyl groups originally from carboxyl-labeled acetate are lost as CO_2 .The only remaining C atoms from carboxyl-labeled acetate are the carboxyl groups of heme's two propionate side chains (P). During the coproporphyrinogen oxidase reaction, the macrocycle is transported back into the mitochondrion for the pathway's final reactions.

Protoporphyrin IX is converted to heme by the insertion of Fe(II) into the tetrapyrrole nucleus by *ferrochelatase*, a protein that is associated with the inner mitochondrial membrane on the matrix side.
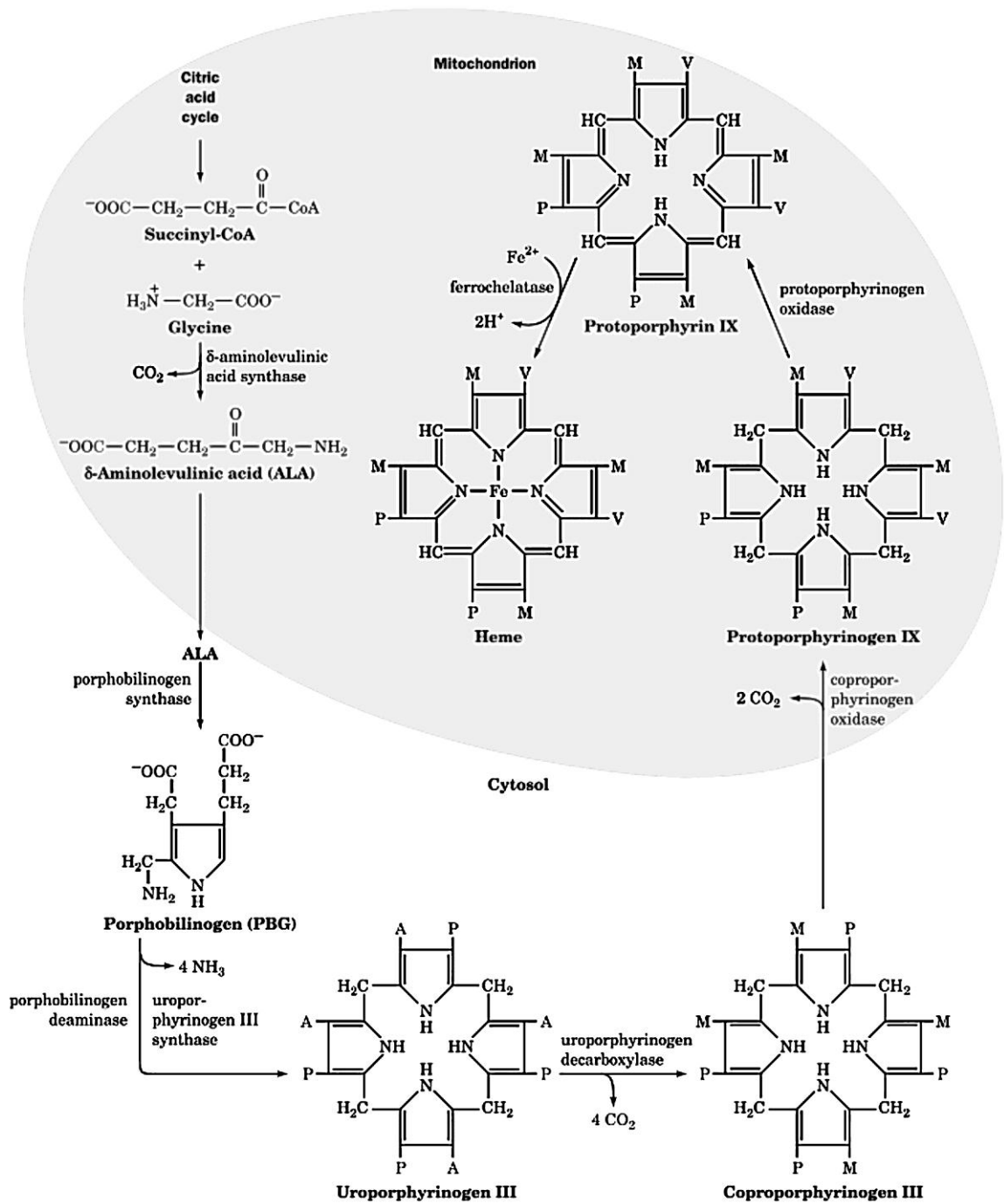
Figure 68. The Overall Pathway of Heme Biosynthesis.

Hemoglobin Polypeptide Chains Synthesis and HbA$_1$ Formation

All polypeptide chains (α_1, α_2, β_1, β_2) are synthesized as any others in EPR of cells (bone marrow, nucleated erythrocytes, first of all). After this synthesis we have to consider their post-translation modification in cytoplasm of the cell to form the molecule of HbA$_1$. The order of steps to form the molecule was investigated by

non-complete denaturation of HbA_1 by urea solution and renaturation after the removal of urea:

Step 1: the formation of four single subunits (α_1, α_2, β_1, β_2). Each subunit accommodates in its interior one heme.

Step 2: the formation of dimmers $\alpha_1\beta_1$ and $\alpha_2\beta_2$, disulfide bonds are formed between subunits.

Step 3: two dimmers are united together by disulfide bonds to form the molecule HbA_1.

Porphyrias

Seven sets of genetic defects in heme biosynthesis, in liver or erythroid cells, are recognized. All involve the accumulation of porphyrin and/or its precursors and are therefore known as **porphyrias** (Greek: *porphyra*, purple). Two such defects are known to affect erythroid cells: *uroporphyrinogen III synthase* deficiency (**congenital erythropoietic porphyria**) and *ferrochelatase* deficiency (**erythropoietic protoporphyria**). The former results in accumulation of uroporphyrinogen I and its decarboxylation product **coproporphyrinogen I**. Excretion of these compounds colors the urine red, their deposition in the teeth turns them a fluorescent reddish brown, and their accumulation in the skin renders it extremely photosensitive such that it ulcerates and forms disfiguring scars. Increased hair growth is also observed in afflicted individuals such that fine hair may cover much of the face and extremities. These symptoms have prompted speculation that the werewolf legend has a biochemical basis.

The most common porphyria that primarily affects liver is *porphobilinogen deaminase* deficiency (**acute intermittent porphyria**). This disease is marked by intermittent attacks of abdominal pain and neurological dysfunction, often brought about by infection, fasting, certain drugs, alcohol, steroids, and other chemicals, all of which induce the expression of ALAS-1. Excessive amounts of ALA and PBG are excreted in the urine during and after such attacks. The urine may become red resulting from the excretion of excess porphyrins synthesized from PBG in

nonhepatic cells although the skin does not become unusually photosensitive. King George III, who ruled England during the American Revolution, and who has been widely portrayed as being mad, in fact had attacks characteristic of acute intermittent porphyria, was reported to have urine the color of port wine, and had several descendants who were diagnosed as having this disease. American history might have been quite different had George III not inherited this metabolic defect.

Hemoglobin Catabolism

The time life of erythrocytes is about 120 days. After that there is their destruction – hemolysis.

Some factors can cause hemolysis:

1) X-ray radiation;

2) Toxins and poisons.

The destruction of red cells usually occurs in the spleen or in the liver.

In the event that red cell destruction occurs at a site other then spleen or liver (in hemolytic anemia), two carrier proteins are available to bind hemoglobin, in which the iron is in ferric state (methemoglobin) or exists as a free heme, in order to prevent the loss iron via the kidney that could otherwise occur:

a) Haptoglobin binds methemoglobin dimmers;

b) Hemopexin binds free heme.

Under physiologic conditions in the human adult $1\text{-}2 \cdot 10^8$ erythrocytes are destroyed per hour. Thus, in a day, a 70 kg human turns over approximately 6 g of hemoglobin. When hemoglobin is destroyed in the body, the protein part is degraded to its constituent amino acids which are reused, and the iron of heme enters the iron pool, also for reuse. The iron-free porphyrin portion of heme is also degraded, mainly in the reticulo endothelial cells of the liver, spleen, and bone marrow.

Heme catabolism (Fig. 69) begins with oxidative cleavage, by *heme oxygenase*, of the porphyrin between rings A and B to form **biliverdin**, a green linear tetrapyrrole. Biliverdin's central methenyl bridge (between rings C and D) is

then reduced to form the red-orange **bilirubin**. The changing colors of a healing bruise are a visible manifestation of heme degradation. Hemoglobin oxygenase system is located in close proximity to the microsomal electron transport system.
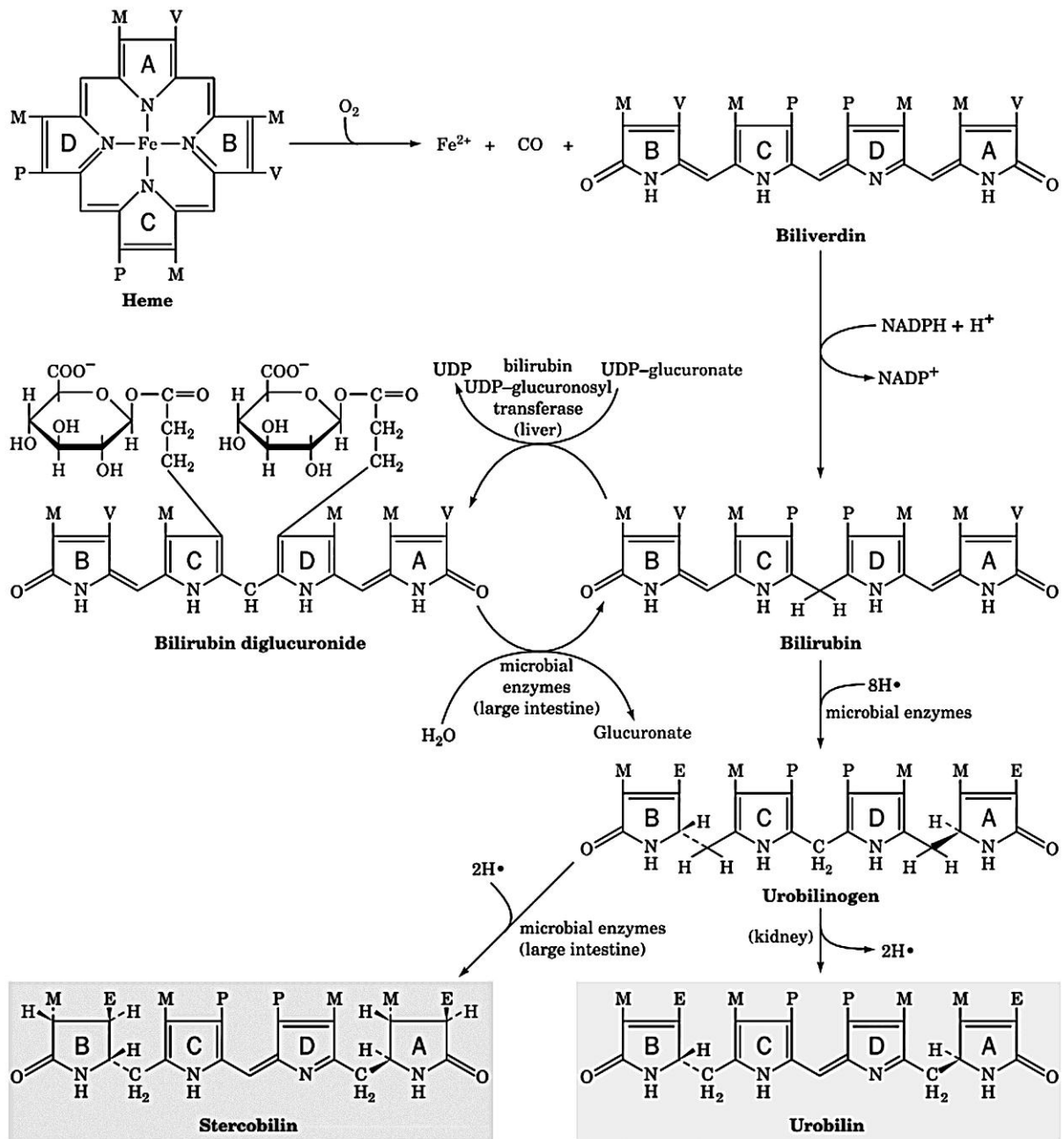
Figure 69. The Heme Degradation Pathway.

The highly lipophilic bilirubin is insoluble in aqueous solutions. Like other lipophilic metabolites, such as free fatty acids, it is transported in the blood in complex with serum albumin. This complex called **unconjugated bilirubin** or **indirect bilirubin** (the measurement of **direct bilirubin** (see below) depends on

its reaction with *diazosulfanilic acid – Erlich's reagent –* to create *azobilirubin*. However, unconjugated bilirubin can be converted into a soluble form by addition of *caffeine reagent* to the blood serum and then reacts with diazosulfanilic acid). In the liver, its aqueous solubility is increased by esterification of its two propionate side groups with glucuronic acid, yielding **bilirubin diglucuronide** (**conjugated bilirubin** or **direct bilirubin**), which is secreted into the bile. Bacterial enzymes in the large intestine hydrolyze the glucuronic acid groups and, in a multistep process, convert bilirubin to several products, most notably **urobilinogen**. Some urobilinogen is reabsorbed and transported via the bloodstream to the kidney, where it is converted to the yellow **urobilin** and excreted, thus giving urine its characteristic color. Most of the urobilinogen, however, is microbially converted to the deeply red-brown **stercobilin**, the major pigment of feces.

Disorders of Hemoglobin Breakdown. Jaundices

When bilirubin in the blood plasm exceeds more then 17,1 μmol/Lit, **hyperbilirubinemia** exists.

When bilirubin reaches a certain concentration: about 35 μmol/Lit, it diffuses into the tissues which then become yellow. The condition is called **jaundice** (French: *jaune*, yellow) or **icterus**.

Depending on the type of bilirubin present in plasma (unconjugated or conjugated) hyperbilirubinemia may be classified as:

1) **retention hyperbilirubinemia**, due to overproduction of bilirubin (unconjugated bilirubin levels are very high);

2) **regurgitation hyperbilirubinemia**, due to reflux of bilirubin diglucuronides (conjugated bilirubin) into the blood stream because of **biliary obstruction**.

Only unconjugated bilirubin can cross the blood-brain barrier into CNS; thus, encephalopathy due to hyperbilirubinemia (kern icterus) can occur only in connection with unconjugated bilirubin, as found in retention hyperbilirubinemia.

Only conjugated bilirubin can appear in urine. Accordingly, **choluric jaundice** (choluria – presence of biliary derivatives in the urine) occurs only in regurgitation hyperbilirubinemia, and acholuric jaundice occurs only in the presence of an excess of unconjugated bilirubin.

Hemolytic Anemia. Extensive hemolysis causes the high levels of unconjugated bilirubin in the blood plasm (~68,4 μmol/Lit); the conjugated bilirubin may be slight higher. Urobilinogen is in high concentration in the urine in this case.

Reasons of hemolytic anemia:

1. Genetic defect of some enzymes of heme synthesis.
2. Megaloblastic anemia (pernicious anemia) appears when absorption of vitamin B_{12} is prevented by lack of intrinsic factor.
3. Folic acid deficiency causes megaloblastic anemia.
4. Hemolytic anemia may by causes by exogenous factors:
   - Prolonged treatment by antibiotics;
   - Poisoning by some products of chemical industry;
   - X-ray radiation.
5. Some special disorders of brain marrow: ostheomyelosclerosis, ostheopetrosis.
6. Hemolytic anemia can be caused by deficiency of two enzymes in erythrocytes:
   - Glucose-6-phosphate Dehydrogenase,
   - Pyruvate kinase.
7. Methemoglobinemia: intake of excess oxidants (various chemicals and drugs).

Neonatal Physiologic Jaundice. High levels of unconjugated bilirubin are indicated in infants, because: UDP-glucuronyl transferase activity is reduced, or UDP-glucose dehydrogenase activity is lower then normal.

Crigler-Najjar syndrome. Type I: Congenital Non-hemolytic Jaundice. A rare autosomal recessive disorder of humans is due to a primary metabolic defect

in the conjugation of bilirubin (Fig. 70). Inherited absence of bilirubin UDP-glucuronyl transferase activity is considered in hepatic tissues. The disease is usual total within the first 15 months of life, but a few teenagers have been reported who did not develop difficulties until puberty. These children have been treated with phototherapy with some reduction in plasma bilirubin levels. Phenobarbital has no effect on the formation of bilirubin glucuronides in patients with type I.

Serum bilirubin usually exceeds 20 mg/dL (in 20 times higher the normal value).

Type II: There is a milder defect in the bilirubin conjugating system and has a more benign course. The serum bilirubin concentrations usually do not exceed 20 mg/dL. Patients are treated with large doses of phenobarbital.

Gilbert's disease. It is a heterogenous group of disorders, many of which are now recognized to be due to a compensated hemolysis associated with unconjugated hyperbilirubinemia. There is a defect in the hepatic clearance of bilirubin, possibly due to a defect in the uptake of bilirubin by the liver parenchymal cells. UDP-glucuronyl transferase activity also was found to be reduced.

Toxic hyperbilirubinemia. It can be as a result from toxin induced liver dysfunction such as that caused by chloroform, arsphenamines, carbon tetrachloride, acetaminophen, hepatitis virus, cirrhosis, and Amanita mushroom poisoning. There is hepatic parenchyma damage, which impairs conjugation, there is frequently a component of obstruction of the biliary tree within the liver that results in the presence of some conjugated hyperbilirubinemia.

Obstruction of the biliary tree. Because of the obstruction, bilirubin diglucuronides cannot be excreted in the intestine. In thus regurgitates into the hepatic ulins and lymphatics, and conjugated bilirubin appears in the blood and urine (choluric jaundice).

The term **cholestatic jaundice** is used to include all cases of extrahepatic obstructive jaundice. It can be also due to micro-obstruction of intrahepatic biliary ductules by swollen, damaged hepatocytes (may occur in infections hepatitis).

In the complete obstruction of bile duct no urobilinogen is found in the urine, but conjugated bilirubin in urine suggests obstruction.

Dubin-Johnson syndrome. Conjugated hyperbilirubinemia in childhood and in adults, caused by a defect in the hepatic secretion of conjugated bilirubin into the bile.

Heme

Hemoglobin oxigenase

Verdoglobin

→ Fe^{3+}

→ Globin

Biliverdin (green colour)

Biliverdin reductase

Bilirubin IXa (yellow colour)

Albumins of blood plasm

Indirect/unconjugated bilirubin (bilirubin complex with Albumins)

→ Albumins

✕ ← *Gilbert disease*

Bilirubin

2 UDP-glucuronate

UDP-glucuronyl transferase   ✕   *Neonatal jaundice*
"Toxic" jaundice
Crigler-Najjar syndrome
Gilbert disease

Direct/conjugatsd bilirubin (mono-, diglucoronide of bilirubin)

✕ defect in the hepatic secretion ← *Dublin-Johnson syndrome*
Rotor's syndrome

β-Glucuronidase

→ Glucuronate

Bilirubin

Spleen / **Liver** / **Duodenum**

Figure 70. The Heme Degradation Pathway and Disorders of Heme Breakdown.

Rotor's syndrome is a rare condition characterized by chronic conjugated hyperbilirubinemia and normal liver histology. Its precise cause has not been identified, but it also may be due to a defect in transport by hepatocytes of organic ions, including bilirubin.

EXERCISES FOR INDEPENDENT WORK. In the table with test tasks emphasize keywords, choose the correct answer and justify it:

№	Test:	Explanation:
1.	A patient who suffers from congenital erythropoietic porphyria has skin photosensitivity. The accumulation of what compound in the skin cells can cause it? A. Uroporphyrinogen I B. Uroporphyrinogen II C. Protoporphyrin D. Coproporphyrinogen III E. Heme	
2.	A denaturation of proteins can be found in some substances. Specify the substance that is used for the incomplete denaturation of hemoglobin: A. Sulfuric acid B. Nitric acid C. Toluene D. Sodium hydroxide E. Urea	
3.	A patient is ill with diabetes mellitus that is accompanied with hyperglycemia of over 7,2 millimole/L on an empty stomach. The level of what blood plasma protein can estimate the hyperglycemia rate retrospectively {4-8 weeks before examination}? A. C-reactive protein B. Ceruloplasmin C. Fibrinogen D. Albumin E. Glycosylated haemoglobin	
4.	Examination of initial molecular structure	

№	Test:	Explanation:
	revealed substitution of the glutamic acid by valine. What inherited pathology is it typical for? A. Minkowsky-Shauffard disease B. Hemoglobinosis C. Thalassemia D. Favism E. Sickle-cell anemia	
5.	A 48 y.o. patient was admitted to the hospital with complaints about weakness, irritability, sleep disturbance. Objectively: skin and scleras are yellow. In blood: conjugated bilirubin, cholamia. Feces are acholic. Urine is of dark colour (bilirubin). What jaundice is it? A. Hemolytic B. Gilbert's syndrome C. Mechanic (obstractive) D. Crigler-Najjar syndrome E. Parenchymatous	
6.	A full-term newborn child has yellowish skin and mucous membranes. This might be probably caused by temporary deficiency of following enzyme: A. UDP glucoronyltransferase B. Uridine transferase C. Biliverdin reductase D. Heme oxygenase E. Heme synthetase	
7.	Blood analysis of a patient with jaundice reveals hyperbilirubinemia, increased concentration of bile acids in the blood plasma. There is no stercobilinogen in urine. What type of jaundice is it? A. Hemolytic jaundice B. Hepatocellular jaundice C. Cythemolytic jaundice D. Parenchymatous jaundice E. Obstructive jaundice	
8.	A patient presents with icteritiousness of skin, sclera and mucous membranes.	

№	Test:	Explanation:
	Blood plasma total bilirubin content is increased, stercobilin is increased in feces, urobilin is increased in the urine of this patient. What type of jaundice is this one: A. Obturational B. Gilbert`s disease C. Hemolytic D. Cholestatic E. Parenchymatous	
9.	Jaundice treatment involves administration of barbiturates inducing the synthesis of UDP-glucuronyl transferase. Effects of barbiturates cause the production of : A. Protoporphyrin B. Indirect (unconjugated) bilirubin C. Heme D. Biliverdin E. Direct (conjugated) bilirubin	
10.	A 48-year-old patient was admitted to the hospital with complaints about weakness, irritability, sleep disturbance. Objectively: skin and scleras are of yellow colour. In blood: increased concentration of total bilirubin with prevailing direct bilirubin. The feces are acholic. The urine is dark (contains bile pigments). What type of jaundice is it? A. Gilbert's syndrome B. Mechanic C. Crigler-Najjar syndrome D. Haemolytic E. Parenchmatous	
11.	A patient presents with icteritiousness of skin, scleras and mucous membranes. Blood plasma total bilirubin is increased, stercobilin is increased in feces, urobilin is increased in urine. What type of jaundice is it? A. Gilbert's disease B. Cholestatic C. Haemolytic	

№	Test:	Explanation:
	D. Parenchymatous	
	E. Obturational	
12.	A mother consulted a doctor about her 5-year-old child who develops erythemas, vesicular rash and skin itch under the influence of sun. Laboratory studies revealed decreased iron concentration in the blood serum, increased uroporphyrinogen I excretion with the urine. What is the most likely inherited pathology in this child? A. Intermittent porphyria B. Coproporphyria C. Hepatic porphyria D. Methemoglobinemia E. Erythropoietic porphyria	
13.	Hemoglobin catabolism results in release of iron which is transported to the bone marrow by a certain transfer protein and used again for the synthesis of hemoglobin. Specify this transfer protein: A. Albumin B. Ceruloplasmin C. Haptoglobin D. Transferrin (siderophilin) E. Transcobalamin	
14.	Enzymatic jaundices are accompanied by abnormal activity of UDP-glucuronyltransferase. What compound is accumulated in blood serum in case of these pathologies? A. Unconjugated bilirubin B. Dehydrobilirubin C. Conjugated bilirubin D. Choleglobin E. Hydrobilirubin	
15.	A patient with jaundice has high bilirubin that is mainly indirect (unconjugated), high concentration of stercobilin in the stool and urine. The level of direct (conjugated) bilirubin in the blood plasma	

№	Test:	Explanation:
	is normal. What kind of jaundice can you think about? A. Neonatal jaundice B. Gilbert's disease C. Parenchymal (hepatic) D. Mechanical E. Hemolytic	
16.	Patients with erythropoietic porphyria (Gunther's disease) have teeth that fluoresce with bright red color when subjected to ultraviolet radiation; their skin is light-sensitive, urine is red-colored. What enzyme can cause this disease, when it is deficient? A. Ferrochelatase B. Uroporphyrinogen decarbozylase C. Uroporphyrinogen I synthase D. Delta-aminolevulinate synthase E. Uroporphyrinogen III cosynthase	
17.	Along with normal hemoglobin types there can be pathological ones in the organism of an adult. Name one of them; A. HbS B. HbA_1 C. HbA_2 D. HbO_2 E. HbF	
18.	A 16-year-old adolescent is diagnosed with hereditary UDP–glucuronyl-transferase deficiency. Laboratory tests revealed hyperbilirubinemia caused mostly by increase blood content of the following substance: A. Unconjugated bilirubin B. Conjugated bilirubin C. Biliverdine D. Stercobilinogen E. Urobilinogen	

№	Test:	Explanation:
19.	One of the hemoglobin forms is dominated after the child's birth. This form retains in adults, but in smaller concentration. Point out it: A. HbA$_1$ B. HbA$_2$ C. HbF D. HbS E. HbC	
20.	Point out the derivative of hemoglobin that is produced after the CO poisoning: A. Oxyhemoglobin B. Carbhemoglobin C. Methemoglobin D. Carboxyhemoglobin E. HbS	

A CLASSIFICATION AND PROPERTIES OF HORMONES. THE MECHANISMS OF HORMONES ACTION (PROTEIN-PEPTIDES AND BIOGENIC AMINES)
(LEVICH S.V.)

INFORMATIONAL MATERIAL

Hormone – *a substance which, produced in any one part of an organism, is transferred to another part and there influences a specific physiological process.*

The tissues or organs where they are produced are called as **effectors** and those where they exert their influence as **targets.**

Based on their site on action, the hormones are of two types: local and general. The *local hormones,* obviously, have specific local effects, whence their nomenclature. These may be exemplified by acetylcholine, secretin, cholecystokinin etc. The *general hormones*, on the other hand, are secreted by specific endocrine glands and are transported in the blood to cause physiologic actions at points remote from their place of origin. A few of the general hormones affect almost all cells of the body, *e.g.*, growth hormones (GH) and thyroid hormones ; whereas other general hormones, however, affect specific tissues far more than other tissues, *e.g.*, adrenocorticotropin (a hormone secreted from adenohyprophysis and stimulating the adrenal cortex) and ovarian hormones (affecting the uterine endometrium).

The hormones conduct a wide variety of functions ranging from growth, vegetative and sexual development, cellular oxidation to thermal production and the metabolism of carbohydrates, proteins and fats. The various functions performed by hormones may, in general, be discussed under following heads: *1) regulatory or homeostatic function; 2) permissive function; 3) integrative function; 4) morphogenetic function.*

Chemically, a hormone may be any kind of organic molecule. Most known hormones are either **steroids** or **peptides** with usually high molecular weights. A

third group of hormones, which is less common, consists of **amino acid derivatives** (or phenolic derivatives) with relatively low molecular weights. Example of hormone - amino acid derivative is *neurohormone melatonin.* Thus, three categories of hormones may be recognized: steroids, peptides and amino acid derivatives (figure 71).

Vertebrate Hormones

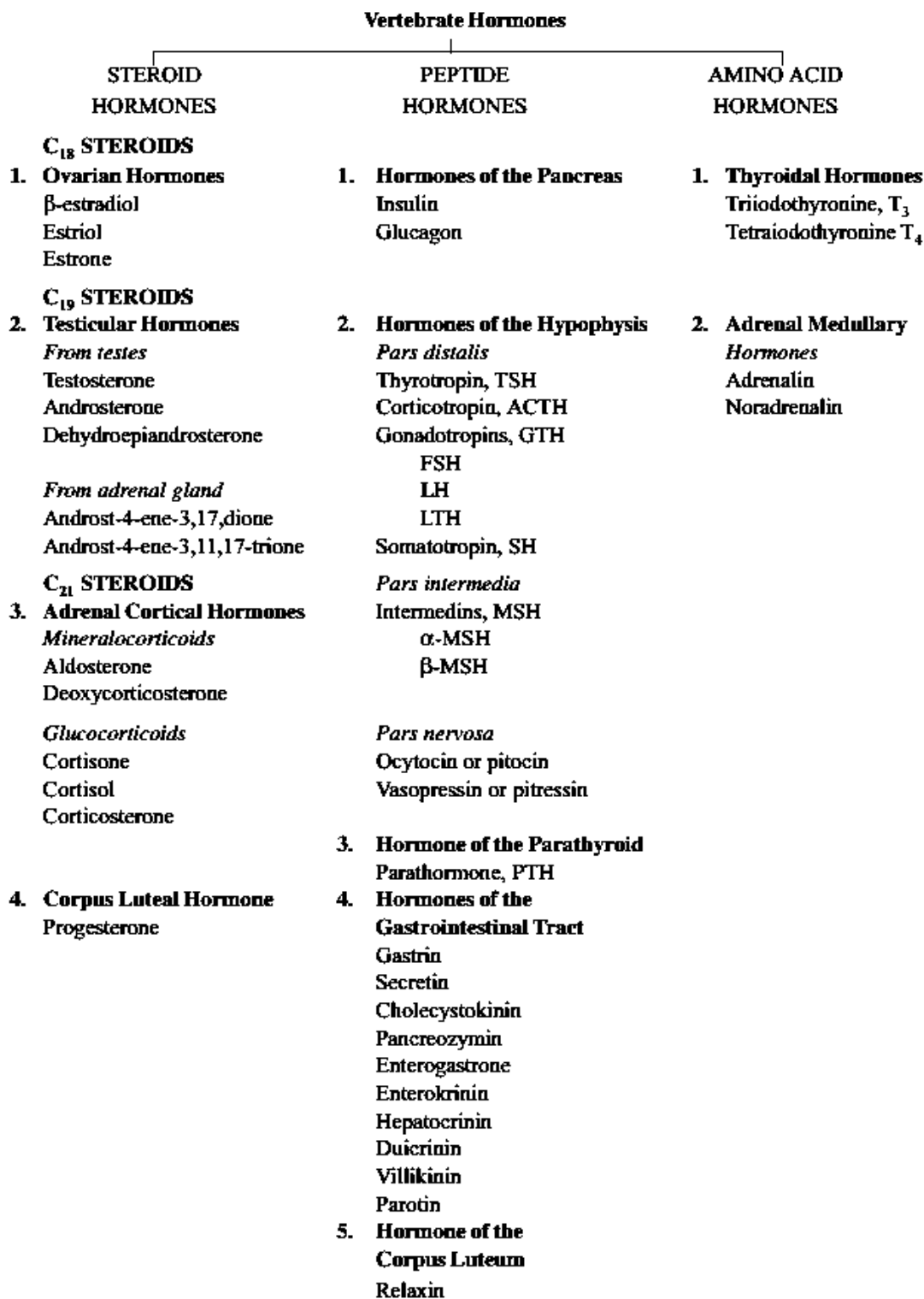| STEROID HORMONES	PEPTIDE HORMONES	AMINO ACID HORMONES
C_{18} STEROIDS		
1. **Ovarian Hormones**	1. **Hormones of the Pancreas**	1. **Thyroidal Hormones**
β-estradiol	Insulin	Triiodothyronine, T_3
Estriol	Glucagon	Tetraiodothyronine T_4
Estrone		
C_{19} STEROIDS		
2. **Testicular Hormones**	2. **Hormones of the Hypophysis**	2. **Adrenal Medullary**
From testes	*Pars distalis*	*Hormones*
Testosterone	Thyrotropin, TSH	Adrenalin
Androsterone	Corticotropin, ACTH	Noradrenalin
Dehydroepiandrosterone	Gonadotropins, GTH	
	FSH	
From adrenal gland	LH	
Androst-4-ene-3,17,dione	LTH	
Androst-4-ene-3,11,17-trione	Somatotropin, SH	
C_{21} STEROIDS	*Pars intermedia*	
3. **Adrenal Cortical Hormones**	Intermedins, MSH	
Mineralocorticoids	α-MSH	
Aldosterone	β-MSH	
Deoxycorticosterone		
Glucocorticoids	*Pars nervosa*	
Cortisone	Ocytocin or pitocin	
Cortisol	Vasopressin or pitressin	
Corticosterone		
	3. **Hormone of the Parathyroid**	
	Parathormone, PTH	
4. **Corpus Luteal Hormone**	4. **Hormones of the**	
Progesterone	**Gastrointestinal Tract**	
	Gastrin	
	Secretin	
	Cholecystokinin	
	Pancreozymin	
	Enterogastrone	
	Enterokrinin	
	Hepatocrinin	
	Duicrinin	
	Villikinin	
	Parotin	
	5. **Hormone of the**	
	Corpus Luteum	
	Relaxin	

Figure 71. Classification of hormones by chemical nature

MECHANISMS OF HORMONE ACTION

The function of different hormones is to *control* the activity of levels of target tissues. To achieve this, the hormones may alter either the permeability of the cells or they may activate some other specific cellular mechanism. Although the exact site of action of any hormone is not established, five *general sites* have been proposed.

A. Hormonal Action at Cyclic Nucleotides Level. Many hormones exert their effect on cells by first causing the formation of a substance, cyclic 3′, 5′-adenosine monophosphate in the cell.

Once formed, the cyclic AMP causes the hormonal effects inside the cell. Thus, *cyclic AMP acts as an intracellular hormonal mediator*. It is also frequently referred to as the *second messenger* for hormone mediation; the *first messenger* being the original hormone itself.

The effects of cyclic AMP on the action of a hormone was first described by Earl W. Sutherland and T.W. Rall in 1960. They found that the effect of epinephrine on hepatic glycogenolysis (breakdown of glycogen) is a result of the conversion of inactive phosphorylase b into an active form by cyclic AMP. Epinephrine was found to activate the enzyme, adenyl cyclase which, in turn, converts ATP to cAMP. Besides epinephrine, other hormones like glucagon, parathormone, ACTH, TSH, ICSH, LH, α-MSH and vasopressin are now known to have a stimulatory effect on cAMP levels. Several hormones, on the contrary, decrease cAMP levels and thus produce an opposite effect. These include insulin, melatonin and the prostaglandins. From the many names of hormones given above, it appears that hormone action not mediated by cAMP may be an exception rather than the rule.

Figure 73 depicts, in a schematic way, the effect of cAMP on hormone action. The cell contains receptor for hormones in the plasma membrane. The stimulating hormone acts at the plasma membrance of the target cell and combines with a specific receptor for that particular type of hormone. The specificity of the

receptor determines which hormone will affect the target cell. The combination of the hormone with its receptor leads to the activation of the enzyme, adenyl cyclase, which is also bound to the plasma membrane. The portion of the adenyl cyclase that is exposed to the cytoplasm causes immediate conversion of cytoplasmic ATP into cAMP. The reaction representing cAMP synthesis may, thus, be written as:

$$ATP \rightarrow cAMP + PP_i + H^+$$

The reaction is slightly endergonic and has a $\Delta G^{\circ\prime}$ value of about 1.6 kcal/mol.

The cAMP then acts inside the cell to initiate a number of cellular functions before it itself is destroyed. The various functions initiated include:

(*a*) activating the enzymes

(*b*) altering the cell permeability

(*c*) synthesizing the intracellular proteins

(*d*) contracting or relaxing the muscles

(*e*) releasing other hormones (*third messengers*).
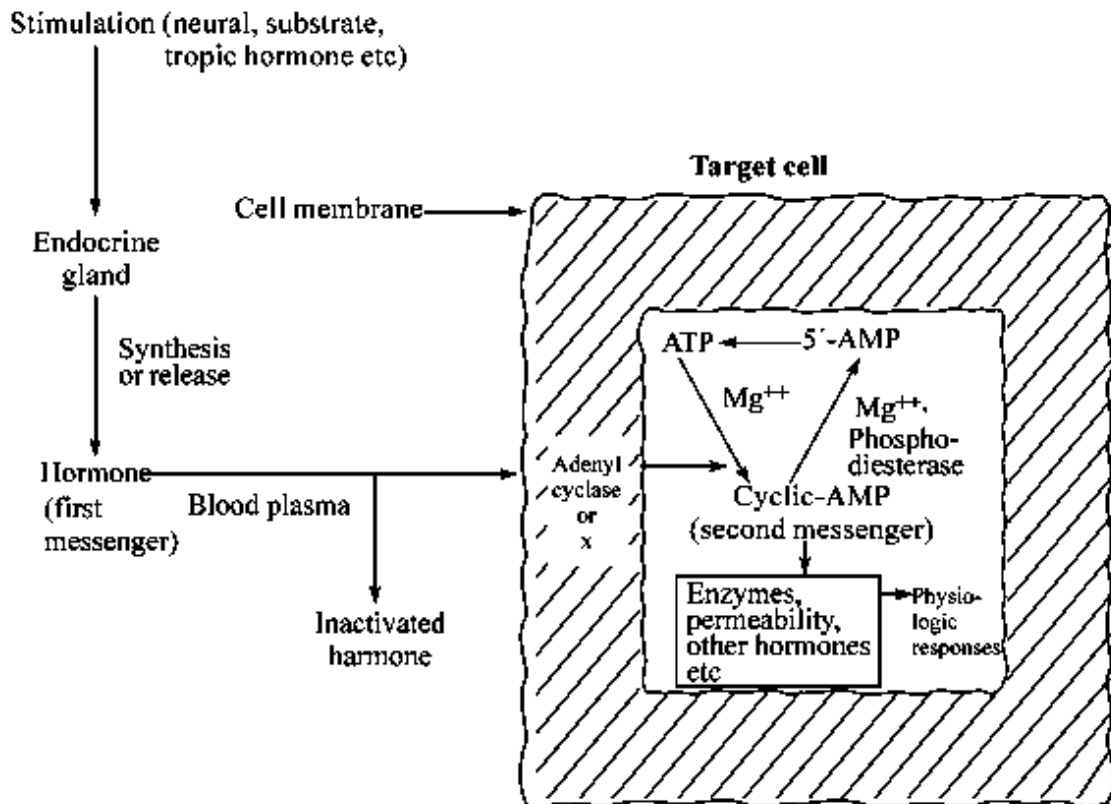
Figure 72. Role of cAMP

It should, however, be emphasized that *what cAMP does in a particular effector cell is determined by the cell itself, rather than by cAMP.*

Cyclic AMP is, however, destroyed (or inactivated) by a specific enzyme called *phosphodiesterase*, which hydrolyzes it to AMP. Like adenyl cyclase, the phosphodiesterase is present in practically all tissues.

$$\text{Cyclic AMP} + H_2O \xrightarrow{Mg^{2+}} AMP + H^+$$
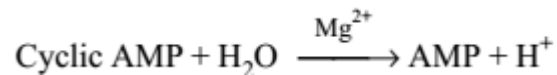
Cyclic AMP is a very stable compound unless hydrolyzed by a specific phosphodiesterase. An important feature of the second messenger model is that *the hormone need not enter the cell and its impact is made at the cell membrane*. The biological effects of the hormone are mediated inside the cell by cAMP rather than by the hormone itself. *cAMP and the Protein Kinases* — Cyclic AMP elicits many of its effects by activating protein kinases. Protein kinases are ubiquitous in nature and are activated by cAMP at extremely low concentrations of 10–8 M. These kinases molecule the activities of different proteins in different cells by phosphorylating them. The enzyme *protein kinase* (figure 74) consists of two subunits: a catalytic subunit and a regulatory subunit which can bind cAMP. In the absence of cAMAP, the catalytic and regulatory subunits form a complex that is enzymatically inactive.

Figure 73. Influence of cAMP on Protein kinase

Other Intracellular Hormonal Mediators — It has been postulated that, besides cAMP, other types of intracellular hormonal mediators also exist.

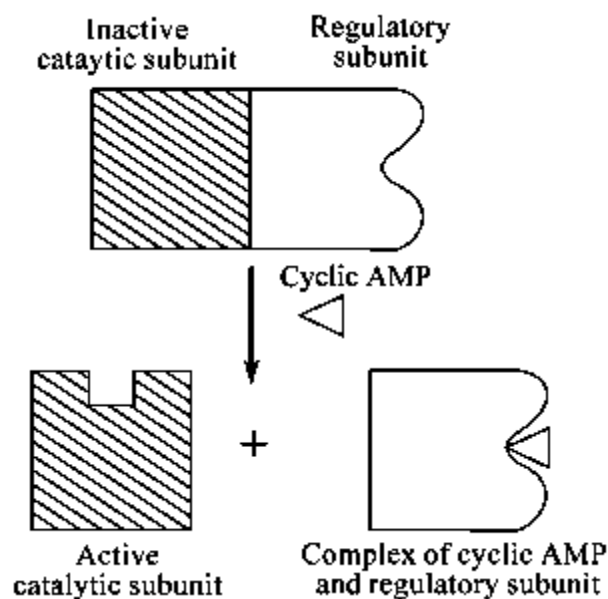1. One almost-certain mediator is **cyclic guanosine monophosphate** (= cyclic GMP). Cyclic GMP is a nucleoside similar to cAMP and is found in most tissues. It can probably catalyze some intracellular functions in a manner similar to that of cAMP.

2. Another type of intracellular hormonal mediator is a group of compounds referred to as **prostaglandins**. These substances frequently cause intracellular inhibition, in contrast to the activation usually caused by cAMP.

Also as secondary messengers are used diacylglycerol, inositol-3,4,5-triphosphate and ions of Ca^{2+}. Tissue inositol triphosphate is generated as a result of the phosphatidylinositol diphosphate hydrolysis. It`s effect as secondary messenger in cells is directed at calcium ion liberation from cellular deport.

B. Induction of Enzyme Synthesis at the Nuclear Level. A second major mechanism by which the hormones, *esp*., the steroidal and thyroidal ones, act is to cause synthesis of proteins in the target cell. These proteins are presumably the enzymes which, in turn, activate other functions of the cells. The mechanism behind the **steroidal hormones** is depicted in figure 74.

The sequence of events is as follows :

1. The steroidal hormone enters the cytoplasm of the target cell where it binds with a specific, high- affinity receptor protein.

2. The receptor protein- hormone complex, so formed, then diffuses into (or is transported into) the nucleus, where it reacts with the nuclear chromatin.

3. Somewhere along this route, the receptor protein is structurally altered to form a smaller protein with low molecular weight. Or else the steroid hormone is transferred to a second smaller protein.

4. The combination of the small protein and hormone is now the active factor that stimulates the specific genes to form messenger RNA (mRNA) in the nucleus.

5. The mRNA diffuses into the cytoplasm where it accelerates the translation process at the ribosomes to synthesize new proteins. It is, however, noteworthy that a direct chemical reaction of the hormone with DNA or RNA polynucleotide is not likely. Instead, the hormone must first combine with a specific receptor protein and it is this combination that acts on DNA chromatin. It is possible that the chromatin proteins may influence hormonal activity by modifying the ability of the receptor complex to bind with DNA.
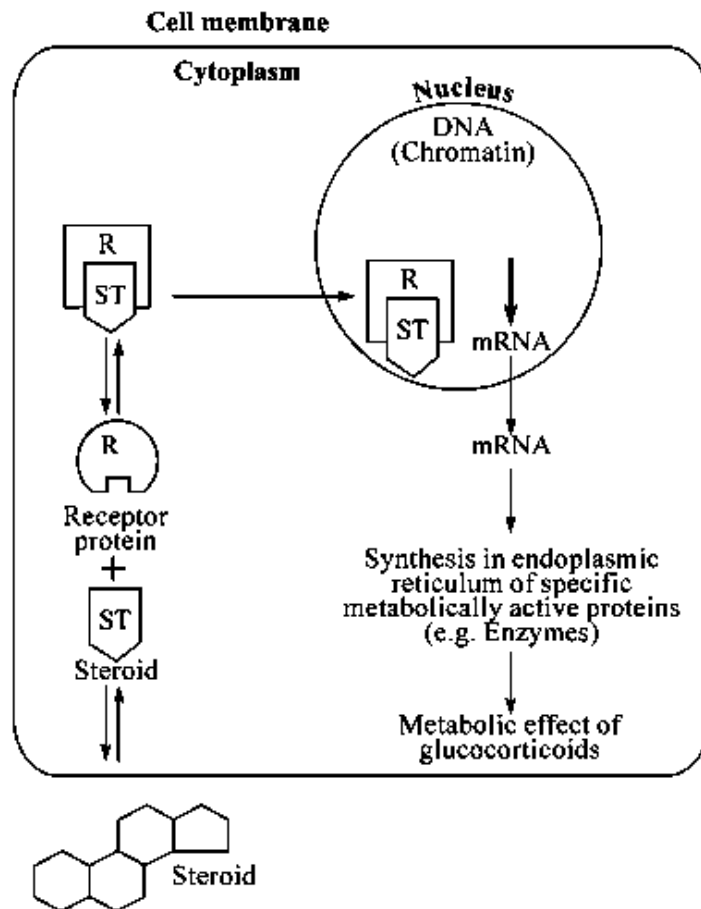
Figure 74. Mechanism of action of steroidal hormones

To cite an example, the aldosterone, one of the mineralocorticoids secreted by adrenal cortex, enters the cytoplasm of the renal tubular cells. These tubular cells contain its specific receptor protein and hence above sequence of events follows. After about 45 minutes, the proteins begin to appear in the renal tubular cells that promote sodium reabsorption from the tubules and potassium secretion into the tubules. This characteristic delay, of about 45 minutes, in the final action

of this steroid hormone is in marked contrast to the almost instantaneous action of some of the peptide hormones.

The **thyroidal hormones** act similarly to enhance RNA and enzyme synthesis but may do so by directly binding with the specific receptor proteins present in the nuclear chromatin. The receptors present in the cytoplasm are less effective in this regard.

C. Stimulation of Enzyme Synthesis at Ribosomal Level. In the case of some hormones, the activity is at the level of translation of information carried by the mRNA on the ribosomes to the production of enzyme protein. For example, the ribosomes taken from animals, which have been given growth hormone, have a capacity for protein synthesis in the presence of normal mRNA.

D. Direct Activation at the Enzyme Level. It has been experimentally observed that treatment of the intact animal (or of isolated tissue) with some hormones results in a change in enzyme behavior which is not related to *de novo* synthesis. The cell membrane is usually required for such activity. Henceforth, it is possible that activation of a membrane receptor might be an initial step in hormone action.

E. Hormone Action at the Membrane Level. Many hormones appear to transport a variety of substances, including carbohydrates, amino acids and nucleotides, across cell membranes. These hormones, in fact, bind to cell membranes and cause rapid metabolic changes in the tissues.

Catecholamines (epinephrine and norepinephrine) and many protein hormones stimulate different membrane enzyme systems by direct binding to specific receptors on cell membrane rather than in the cytoplasm.

A schematic representation of the two principal mechanisms of action involving water soluble hormones and steroid hormones is presented in figure 75.
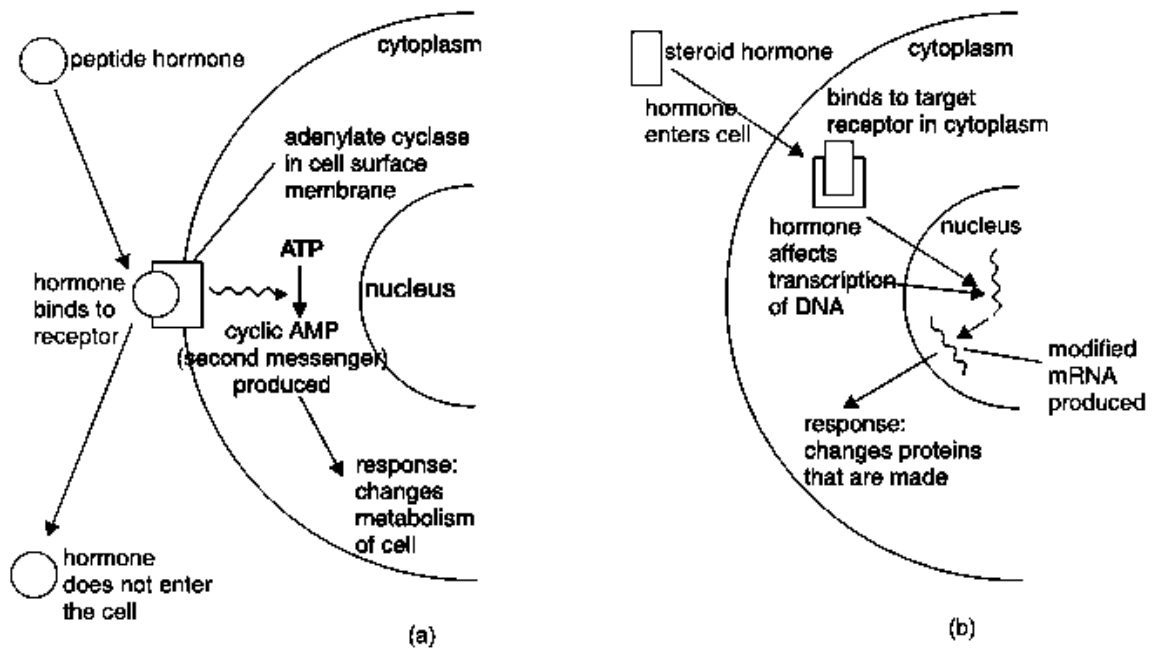
Figure 75. Mechanism of hormone action

Hormones of the Pancreas

Insulin

Structure. Insulin (*insula*L = island) was first isolated in 1922 from the pancreas of dogs by Banting and Best, both of the University of Toronto, Canada. They also demonstrated the curative effect of pancreatic extract in dogs ailing with diabetes mellitus. Abel and his associates (1926) obtained insulin in crystalline form (Fig. 31–27) and also demonstrated its protein nature. *Insulin is, in fact, the first hormone to be recognized as a protein.*

The two polypeptide chains are held together by cross linkages of two disulfide bonds. The acidic chain A contains 21 residues and the peptide chain B having 30 residues. It has a molecular weight of 5,733 and is isoelectric at pH 5.4. Human insulin, however, has a molecular weight of 5,808. Insulin is destroyed by alkali but is relatively stable in acid solutions. Reduction of the disulfide bond results in a loss of biologic activity. Zinc is always found with this hormone but is not a part of the insulin molecule.

Biosynthesis. β-cells of the pancreas synthesize insulin by the ribosomes of the endoplasmic reticulum. Previously, it was suggested that the two chains of

insulin are synthesized independently and later these combine by disulfide bonds. But now Donald F. Steiner *et al* (1967) have shown that it is formed from its precursor, *proinsulin*. Proinsulin has been isolated and purified from pancreatic extracts. It is a linear protein with 84 amino acid residues and has a molecular weight of about 9,100.

The transformation of proinsulin to insulin takes place in the granules and not in the endoplasmic reticulum where synthesis of proinsulin takes place. The conversion, which is brought about by lysosomal proteolytic enzymes, consists in cleavage of a 33 amino acid-connecting peptide chain from the proinsulin molecule leaving behind insulin.

$$\text{Proinsulin} \xrightarrow{\text{Proteolytic cleavage}} \text{Insulin} + \text{Peptide}$$
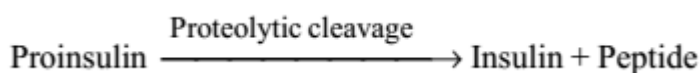
Functions. Insulin has a profound influence on carbohydrate metabolism. It facilitates entry of glucose and other sugars into the cells, by increasing penetration of cell membranes and augmenting phosphorylation of glucose. This results in lowering the sugar content of the blood – a fact leading to its common name, **hypoglycemic factor**, which may be abbreviated as **hG-factor.** Insulin administration promotes protein synthesis (proteogenesis) by assisting incorporation of amino acids into proteins. This effect is not dependent on glucose utilization. At the same time, it also acts as an *antiproteolytic agent*, *i.e.*, discourages excessive breakdown of tissue protein. This action is similar to that of GH and testosterone. Synthesis of lipids (lipogenesis) is also stimulated by administration of insulin. Insulin also influences the inorganic metabolism *esp.*, that of phosphate and potassium. Insulin administration lowers the blood phosphate level and facilitates absorption of inorganic phosphate by the cells. This phosphate appears within the cells as ATP. A similar mechanism operates in potassium uptake.

It may be said, in general, that insulin promotes anabolic processes (synthesis of glycogen, fatty acids and proteins) and inhibits catabolic ones (breakdown of glycogen and fat).

Insulin deficiency. The deficiency of insulin caused either by inadequate insulin production or by accelerated insulin destruction, leads to *diabetes mellitus* in man.

This disease is characterized by:

1. an increase in blood sugar or glucose (hyperglycemia) from a normal value of 80 mg/100 ml of plasma to abnormal value ranging between 150-200 mg/100 ml of plasma.

2. the appearance of sugar in the urine (glycosuria); with the result, the victim's urine tastes sweet.

3. an increase in concentration of ketone bodies in the blood (ketonemia) and in the urine (ketonuria).

4. the excretion of large quantities of urine (polyuria), frequently at night (nocturia), leading to dehydration.

5. the excessive drinking of water (polydipsia) on account of an unrelenting thirst.

6. the excessive eating (polyphagia) due to feeling constant hunger. This is because the tissues cannot utilize glucose normally, even though they need fuel.

7. the lack of energy (asthenia) which is apparently caused mainly by loss of body protein.

Diabetes mellitus is of 2 types: type I (insulin-dependent) and type II (noninsulin-dependent). The use of Roman numerals probably dignifies the importance of this classification.

Type I (Insulin-dependent diabetes mellitus, IDDM): IDDM occurs because the insulin producing β-cells are destroyed and there is not enough insulin produced. In the past, there was treatment that had some measure of success. This was relative starvation. People with IDDM had very short careers. The former synonyms for this disease are brittle diabetes or juvenile diabetes. Type II Noninsulin-dependent diabetes mellitus, NIDDM) : NIDDM is caused by a relative insulin insufficiency due to insulin resistance– the inability of the insulin to tell the cells to use glucose– plus insufficient insulin to overcome this resistance. Patients

with NIDDM often lived for many years, if they heroically reduced their weight to live with the small amount of insulin that might be available. In a sense, this was organised starvation. NIDDM is also known by the former names, stable diabetes or adult-onset diabetes. In case of type II diabetes, it may be said that heredity may load the cannon, but stress or obesity pulls the trigger.

Glucagon

Structure. Glucagon was first isolated in crystalline form by Behrens and others. This peptide hormone has a molecular weight of 3,485 and is isolectric at pH 8. It has 29 amino acid residues (of 15 different types) arranged in a linear row. Histidine is the N-terminal amino acid and threonine, the C-terminal amino acid. Unlike insulin, it contains no cystine, proline or isoleucine, but possesses Parenteral is any route outside the gastrointestinal tract, including subcutaneous, intramuscular, intraperitoneal and intravenous injections or infusions. In practice, parenteral feeding is done intravenously. The small amount of sulfur present is, thus, in the form of methionine rather than cystine.

Functions. Like insulin, glucagon also influences carbohydrate metabolism but in an opposing way. Glucagon (as also the other hormone, epinephrine) activates the enzyme adenyl cyclase which converts ATP to cyclic AMP. The latter compound activates phosphorylase *b* kinase which, in its turn, activates phosphorylase *b* to yield phosphorylase *a*. This releases glucose-lphosphate from glycogen of liver. Glucose-l-phosphate then yields free glucose in blood, whereby increasing blood sugar contents. It is because of this reason that the hormone is also termed as **hyperglycemic factor** or **HG-factor**.

In contrast to epinephrine, glucagon does not cause an increase in blood pressure. Therefore, glucagon and not epinephrine has found clinical applications and is administered in patients with acute hypoglycemia.

Acting in the liver, it stimulates glycogenolysis (glycogen breakdown) and gluconeogenesis (production of glucose from noncarbohydrate sources such as proteins and fats). The former function is similar to that of ACTH and epinephrine.

Glucagon also affects lipid metabolism by accelerating ketogenesis and inhibiting synthesis of fatty acids. Glucagon has a catabolic action on proteins. Its administration in the body results in excretion of enough nitrogen and phosphorus, in decrease of liver tissues and in loss of body weight.

Adrenal Medullary Hormones

As already stated, the adrenal medulla forms the central core of adrenal gland and originates from the neural canal. It is composed of densely packed polyhedral cells containing chromaffin granules. It is highly vascular and receives 6-7 ml of blood per gram of tissue per minute. The chromaffin granules store large quantities of adrenal medullary hormones.

Structure. Adrenal medulla, whose secretion is under nervous control, produces two hormones (figure 76): (*a*) epinephrine or adrenalin ($C_9H_{13}O_3N$) and (*b*) norepinephrine or noradrenalin ($C_8H_{11}O_3N$).

Epinephrine
(Adrenalin)

Norepinephrine
(Noradrenalin or arterenol)
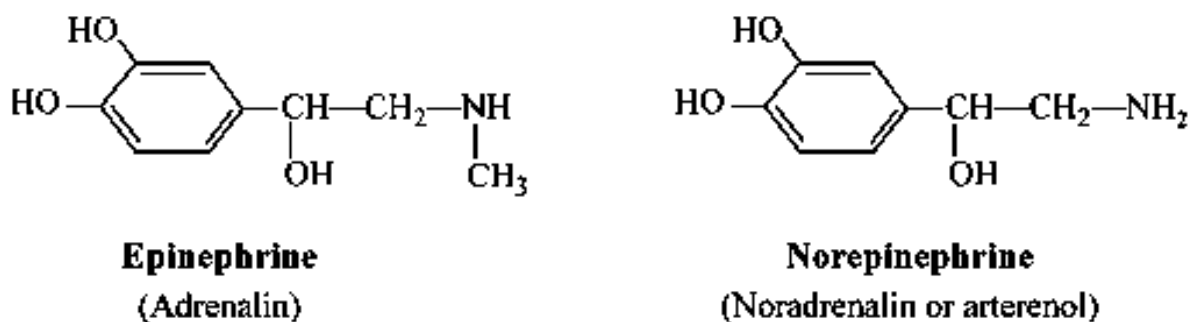
Figure 76. Structures of Epinephrine and Norepinephrine

Functions. In general, the adrenal medullary hormones reinforce the functions performed by the sympathetic nervous system. Although both these hormones exert similar effects in regulating carbohydrate metabolism and blood pressure, yet epinephrine is more closely related to carbohydrate metabolism and norepinephrine to blood pressure. *Epinephrine* conducts a wide variety of functions, which are as follows:

1. It promotes glycogenolysis in muscles and liver, resulting in an increase of blood glucose level and an increased lactic acid formation in muscles. These changes are then followed by an increase in oxygen consumption.

2. It causes an increase in blood pressure because of arteriolar vasoconstriction of the skin and splanchnic vessels.

3. It brings about an increase in the heart rate and in the cardiac output.

4. It causes dilation of vessels (= vasodilation) of skeletal muscles, corona and the viscera. This results in an increase of blood flow in these areas.

5. It relaxes the muscles of gastrointestinal tract and bronchials of the lungs but causes contraction of the pyloric and ileocecal sphincter muscles.

6. It also serves in cases of emergency. Under emotional stress, fear or anger, it is secreted in the blood stream and the blood is shifted from the viscera to the brain and the muscles so that the individual becomes ready for fight. It is for this reason that the adrenals are frequently referred to as the '*emergency glands*' or the '*glands of flight*, fright and fight' and the two adrenal medullary hormones as **'emergency hormones'**.

Norepinephrine, on the other hand, does not relax bronchiolar muscles and has little effect on cardiac output. It augments both systolic and diastolic blood pressure.

Adrenal demedullation. Despite the varied and definite physiologic effects of its characteristic hormones, the adrenal medulla does not appear to be essential to life. Hence, removal of only the medullary portion of the adrenal gland leads to no specific physiologic disorder. This is because the autonomous nervous system may take over in its absence. Consequently, the exact importance of the adrenal medulla is really undetermined. However, certain tumours of the medullary cells result in **pheochromocytoma**, characterized by hypertension, hyperglycemia, increasing of basal metabolic rate, tachycardia and ultimately leading to death due to coronary insufficiency and pulmonary edema. Treatment is to remove the tumour surgically.

EXERCISES FOR INDEPENDENT WORK. In the table with test tasks emphasize keywords, choose the correct answer and justify it:

№	Test:	Explanation:
1.	The formation of a secondary mediator is obligatory in membrane-intracellular mechanism of hormone action. Point out the substance that is unable to be a secondary mediator: A. Diacylglycerol B. cAMP C. Inositol-3,4,5-triphosphate D. Ca^{2+} E. Glycerol	
2.	There is only one hormone among neurohormones which refers to the derivatives of amino acids according to classification. Point out it: A. Melatonin B. Vasopressin C. Oxytocin D. Thyroliberin E. Somatotropin	
3.	Tissue inositol triphosphate is generated as a result of the phosphatidylinositol diphosphate hydrolysis and act as secondary agent (mediator) in the mechanism of hormone action. It`s effect in cells is directed at: A. Phosphodiesterase inhibition B. Protein kinase A inhibition C. Protein kinase A activation D. Calcium ion liberation from cellular deport E. Adenylate cyclase activation	

№	Test:	Explanation:

4.	A 41-year-old male patient has a history of recurrent attacks of heartbeats (paroxysms), profuse sweating, headaches, Examination reveled hypertension, hyperglycemia, increased basal metabolic rate, and tachycardia. These clinical presentations are typical for the following adrenal pathology:
	    A. Hyperfunction of the adrenal cortex
	    B. Hyperfunction of the medulla
	    C. Hyperfunction of the adrenal cortex
	    D. Primaty aldosteronism
	    E. Hypofunction of the medulla	

5.	Prior to glucose utilization in cells it is transported inside cells from extracellular space through plasmatic membrane. This process is stimulated by the following hormone:
	    A. Insulin
	    B. Adrenalin
	    C. Thyroxin
	    D. Aldosterone
	    E. Glucagon	

6.	A patient suffering from pheochromocytoma complains of thirst, dry mouth, hunger. Blood test for sugar revealed hyperglycemia. What type of hyperglycemia is it?
	  A. Hypercorticoid
	  B. Adrenal	

№	Test:	Explanation:
	C. Alimentary D. Hypoinsulinemic E. Somatotropic	
7.	A middle-aged man went to a foreign country because he had been offered a job there. However he had been unemployed for quite a long time. What endocrine glands were exhausted most of all in this man? A. Substernal gland B. Parathyroid glands C. Seminal glands D. Thyroid gland E. Adrenal glands	

STEROID AND THYROID HORMONES: THE MECHAMISM OF ACTION AND THE INFLUENCE ON METABOLIC PROCESSE. THE REGULATION OF CALCIUM LEVELS BY HORMONES IN THE BLOOD (ALEKSANDROVA K. V., LEVICH S.V.)

INFORMATIONAL MATERIAL

Steroid hormones

These include the sex hormones and the hormones from adrenal cortex. These are synthesized in mammals by the ovary (or testis), adrenal cortex, corpus luteum and the placenta. Three types of sex hormones are recognized:

(*a*) the estrogens (female or ovarian or follicular hormones)

(*b*) the androgens (male or testicular hormones)

(*c*) the gestogens (corpus luteal hormones).

Based on the number of carbon atoms present in the molecule, the steroid hormones may be named as C18, C19 or C21 steroids.

C18 STEROIDS

Chemically, the estrogens are derivatives of a C18 hydrocarbon, estrange. The three compounds of this group (figure 77) with hormonal activity are:

1. β-estradiol (= dihydrotheelin), $C_{18}H_{24}O_2$

2. Estriol (= theelol), $C_{18}H_{24}O_3$

3. Estrone (= theelin), $C_{18}H_{22}O_2$
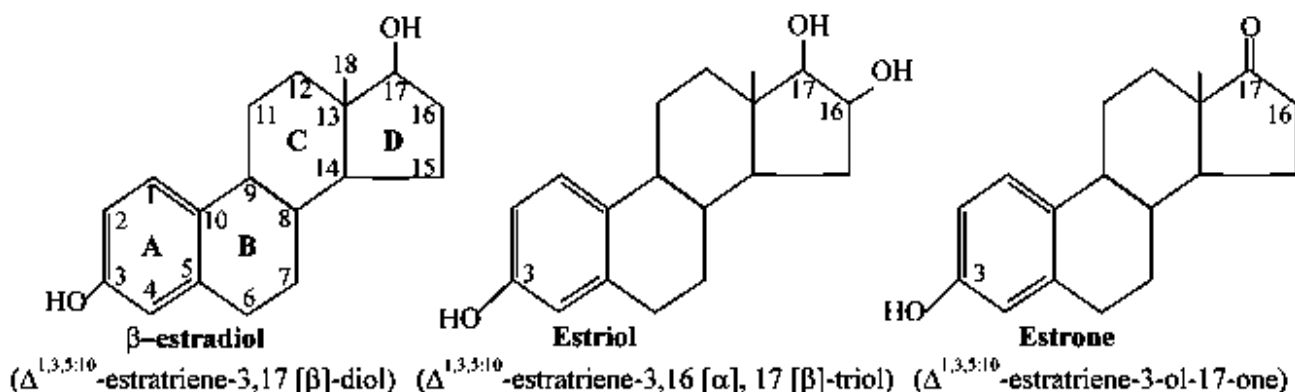
Figure 77. Structures of β-estradiol, estriol and estrone

Biosynthesis. In nonepregnant females, estrogen is mainly synthesized in the ovary. The estrogen (as well as the androgen) are, in part, transported by binding to a specific plasma protein called sex steriod binding protein SBT. The amount of this protein increases in pregnancy or estrogen therapy which results in reduced androgenic action. Curiously enough, testosterone, a male hormone, is the precursor of estrogens.

Functions. "In women, the follicular hormones (estrogens) prepare the uterine mucosa for the later action of the progestational hormones (produced by the corpus luteum). The changes in the uterine include proliferative growth of the lining of the endometrium, deepening of uterine glands, and increased vascularity; changes in the epithelium of the fallopian tubes and of the vagina also occur. All of these changes begin immediately after menstrual bleeding has ceased."

Estrogen preserves the elasticity of the skin and possibly improves the memory in women at postmenopausal stages. It also protects women from osteoporosis by slowing the rate at which calcium is leached from their bones. Estrogen supplement also preserves the flexibility of blood vessels, thus helping to prevent cardiac diseases. Nowadays, estrogen in combination with the hormone progestin is considered an important tool for helping women remain healthy. The combination is known as **hormone replacement therapy (HRT)**, which has indeed become the closest in medicine to a woman's elixir of youth. HRT, when used by menopausal women, relieves hot splashes, dry sweats and vaginal dryness. However, the long-term use of HRT or estrogen therapy hightens the risk of ovarian cancer.

The estrogens are also effective in the development of *secondary sex characters in females*.

C 19 STEROIDS

These hormones are secreted mainly by the testes, the male reproductive organs and are called as androgens (*andros*G = male). Chemically, these are derivatives of a C19 hydrocarbon, *androstane*.

There are many hormones secreted from testes with androgenic activity. The three important ones (figure 78) are:

1. Testosterone, $C_{19}H_{28}O_2$

2. Androsterone, $C_{19}H_{30}O_2$

3. Dehydroepiandrosterone, $C_{19}H_{25}O_2$
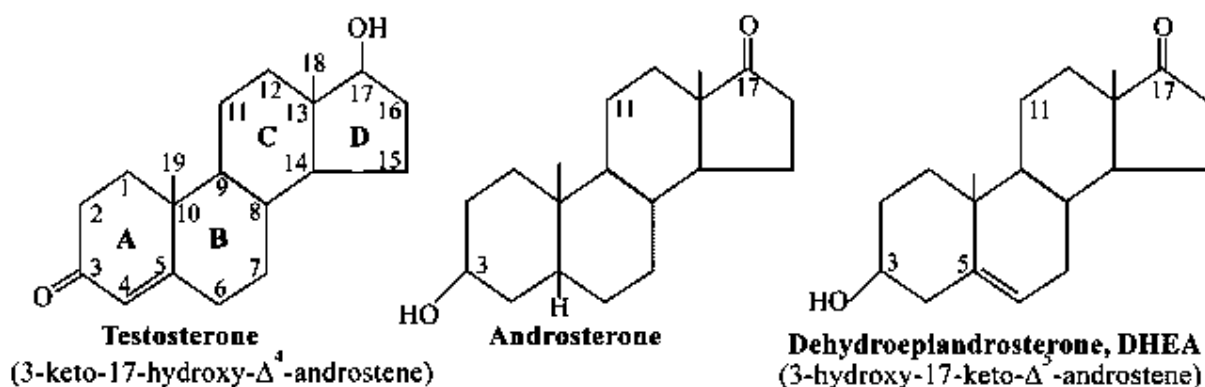
Figure 78. Structure of testosterone, androsterone, dehydroepiandrosterone

A few testicular hormones are also produced by the adrenal gland.

Metabolism. Most of the metabolic transformations of androgens takes place in the liver. Two major reactions occurring in the liver are:

(*a*) Conversion of testosterone to androst-4-ene-3,17-dione.

(*b*) Interconversion of 3-hydroxy and 3-keto derivatives.

Functions. Testosterone has often been considered to be a 'youth horomone', because of its effects on the musculature (by the stimulation special *nuclear receptors*), and it is occasionally used for treatment of persons who have poorly developed muscles. Because of the ability of testosterone to increase the size and strength of bones, it is often used in old age to treat osteoporosis. Like estrogens,

the androgens are also responsible for the development of secondary sex characters in males. These are listed in Table 6.

Table 6. Secondary sex characters in males

Characters	Changes
1. External genitalia	Penis increases in length and width; Scrotum becomes pigmented and rugose.
2. Internal genitalia	Seminal vesicles enlarge and secrete– they also begin to form fructose ; Prostate and bulbourethral glands also enlarge and secrete.
3. Voice	Larynx enlarges and the vocal cords increase in length and thickness; Voice becomes deeper.
4. Hair growth	General body hair increases; Hair line on scalp recedes anterolaterally (Fig. 31–10); Hair appear in axillae (axillary hair) and around anus; Pubic hair have a characteristic male pattern, i.e., triangle with apex up; Hair on face grow as beard.
5. Mental	More aggressive; Active attitude; Interest in opposite sex more pronounced.
6. Body conformation	Shoulders broaden and hips remain unaltered, i.e., narrow; Thighs that diverge and arms that converge, i.e., a narrow carrying angle; No distribution of body fat in the chests and buttocks; Muscles enlarge, leading to a muscular body contour.
7. Skin	Sebaceous gland secretion thickens and increases (predisposing to acne).
8. Weight gain	Tend to gain weight in the abdominal region.

The androgens constitute one factor in the production of baldness. Age and inheritence are other factors involved in causing this condition. But baldness does not ensue without androgenic stimulation. In cases where the testes fail to descend in a normal manner (cryptorchidism), the testosterone is of considerable value. But the long usage and higher dosage of testosterone (*e.g.*, 25 mg per day for 4−6 weeks) in individuals often leads to atrophy of the sperm. The effect can, however be reversed by discontinuing the treatment for a similar period. Testosterone also controls the libido, and also the development of muscle mass and bone density

C 21 STEROIDS

Adrenal cortex secretes some 40-50 closely related C21 steroids, collectively called as corticosteroids.

From physiological viewpoint, the corticosteroids may be grouped under two categories:

A. *Mineralocorticoids.* — concerned primarily with the transport of electrolytes and the distribution of water in tissues, *e.g.*, aldosterone and deoxycorticosterone.

B. *Glucocorticoids.*—concerned primarily with the metabolism of carbohydrates, proteins and fats, *e.g.*, cortisone (= compound E), cortisol (= hydrocortisone) and corticosterone.

Functions. A *mineralocorticoid*, aldosterone is chiefly concerned with water-salt balance of the body. It stimulates the reabsorption of Na+ ion from the kidney tubules and as such regulates NaCl contents of the blood. This also causes excretion of K in the urine. Aldosterone is also more potent in maintaining the life of adrenalectomized animals. Angiotensin is involved in regulating aldosterone and is the core regulation. Angiotensin II acts synergistically with potassium, and the potassium feedback is virtually inoperative when no angiotensin II is present. A small portion of the regulation resulting from angiotensin II must take place indirectly from decreased blood flow through the liver due to constriction of capillaries. When the blood flow decreases so does the destruction of aldosterone by liver enzymes. Blockage of angiotensin II production could lead to the hypo excretion of aldosterone and could be a reasone of hyperkalemia, bradycardia and arrhythmia.

Glucocorticoids, on the contrary, govern many other processes. They perform the following physiological functions:

1. Influence the carbohydrate metabolism firstly by increasing release of glucose from the liver and secondly by promoting the transformation of amino acids to carbohydrates.

2. Inhibit protein synthesis in muscle tissues.

3. Control eosinophil cells of the blood.

4. Regulate lipogenesis.

5. Reduce the osteoid matrix of bone, thus favouring osteoporosis (weak bones) and heavy loss of calcium from the body.

6. Decrease immune responses associated with infection and anaphylaxis (immunosuppressive effects).

7. Cause increased secretion of hydrochloric acid and pespinogen by the stomach and that of trypsinogen by the pancreas (exocrine secretory effects).

8. Cause retention of sodium(and water) and loss of potassium to some extent. In this respect, it resembles aldosterone in action.

Hypoadrenocorticism. A decrease in the amount of corticosteroids in the body (hypoadrenocorticism) leads to the decreased metabolic rate, excessive pigmentation, loss of appetite (anorexia), muscular weakness, deficiency of blood (anemia), eosinophilia and decreased blood sugar (hypoglycemia) with fasting.

Hyperadrenocorticism. The excessive supply of adrenal cortical steroids (hyperadrenocorticism) results from cortical cell tumours which may arise in or outside the adrenal gland. Oversecretion of cortisol in man leads to a rare disease, **Cushing's syndrome**, after its discoverer, Harvey Cushing. The most common cause of the symptoms of Cushing's syndrome is the prolonged administration of glucocorticoids for medical treatment. The syndrome is characterized by profound disturbance of carbohydrate, protein, fat and calcium metabolism. There occurs mobilization of fat from the lower part of the body, with the concomitant extra deposition of fat in the thoracic region. The obesity becomes visible on the neck (buffalo hump) and on the face (moon face). Weakness and muscle wastings with marked osteosis become evident. Hypertension, pigmentation of the hair and excessive growth of hair are other symptoms. In men, there is impotence, and in women, amenorrhea and masculinization. Thus, Cushing's syndrome resembles somewhat adrenogenital syndrome. Hypersecretion of aldosterone leads to a marked Na+ and water retention, resulting in edema and hypertension causing heart failure. The adrenal cortex also produces androgenic steroids known as *adrenosterones*. Their hypersecretion has effects varying according to the age and sex of the patient. In adult female, it leads to **adrenal virilism.** In it menstruation stops, breasts atrophy, hair on breast and face develop and the voice deepens. In all, the adult woman becomes masculine. In adult males, there occurs excessive hair

growth, enlargement of the sex organ and increased sexual desire. However, in children excessive supply of adrenosterones results in precocious development of sex organs and the secondary sexual characters.

Adrenal decortication. Removal of adrenal cortex (*adrenalectomy*) leads to a fatal human disease known as **Addison's disease**, named after its discoverer Thomas Addison.

Symptoms now attributed to this disease are low blood pressure, lowered basal metabolic rate (BMR), subnormal temperature and a disturbed water and electrolyte balance. This includes loss of sodium and chloride ions and a loss of body water. The person develops hyperkalemia and acidosis because of failure of potassium and hydrogen ions to be secreted in exchange for sodium reabsorption.

The patient becomes hypoglycemic. The kidneys are also affected, resulting in urea retention. Skin pigmentation occurs in areas of greatest normal pigmentation. Frequently, the face and neck and backs of the hands are so deeply bronzed as to cause the afflicted individual to look like a mulatto.

<div align="center">Thyroidal Hormones Secretory gland.</div>

Thyroid contains large amounts of elemental iodine which is bound to a protein named iodothyroglobulin or simply thyroglobulin. It is a glycoprotein with a molecular weight of about 650,000 and iodine content from 0.5 to 1.0%. This protein represents the storage form of the hormone in the gland.

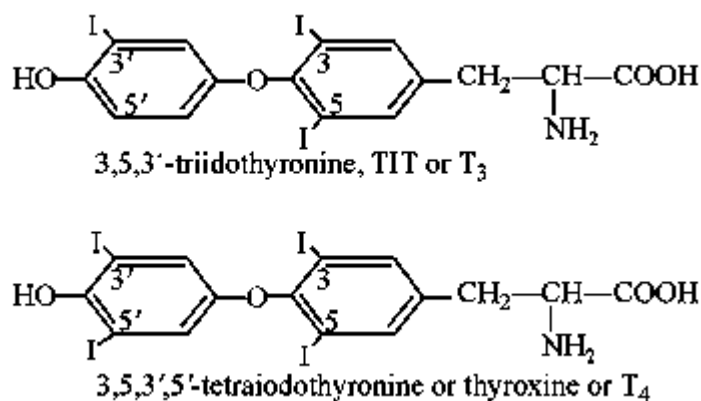The structure of triiodothyronine and tetraiodothyronine is given in figure 80.

$3,5,3'$-triidothyronine, TIT or T_3

$3,5,3',5'$-tetraiodothyronine or thyroxine or T_4

Figure 79. The structure of triiodothyronine and tetraiodothyronine

Functions. Thyroid hormones have widespread effects on the ossification of cartilage, the growth of teeth, the contours of the face and the proportions of the body. They also carry out following functions:

1. They bring about deamination reactions in the liver.

2. They also carry on deiodination in the extrahepatic tissues.

3. These influence oxidative phosphorylation by altering the permeability of the mitochondrial membrane and plays a role of an adaptive thermoregulatory hormone.

4. Their presence accelerates metamorphosis in amphibians. This is so a sensitive test that tadpole has been widely used for the assay of the potency of these hormones.

5. These may increase the level of cytochrome c in the tissues. It is, thus, apparent that these hormones affect the general metabolism, regardless of the nature of its specific activity. It is for this reason that the thyroid gland has rightly been called as the '*pace setter*' of the endocrine system.

Hypothyroidism. Underactivity of the thyroid may result from two causes: a degeneration of thyroid cells or a lack of sufficient iodide in the diet. The disease that results from thyroid cell degeneration is cretinism in children and myxedema in adults.

Myxedema is characterized by an abnormally low basal metabolic rate (BMR). In it, the adults become mentally lethargic and possess thick puffy skin (edema) and dry hair. The patient shows bagginess under the eyes and swelling of the face. The hair thin on the eyebrows and scalp. As there is deposition of semi-fluid material under the skin, the name myxedema (*myxa*G = mucus; *oidema*G = swelling) is given to this condition. Myxedema also responds well to administration of thyroid-active compounds.

Lack of sufficient iodide in the diet results in thyroid gland enlargement, known as **simple goiter.** It is also associated with a low BMR. This type of goiter is also known as **endemic goiter**, since it is prevalent in areas where the soil and drinking water lack iodide. Simple goiter was once fairly common in some

mountainous parts of Switzerland and the United States, where soil and water are deficient in iodine compounds.

Resection of the thyroid gland lead to reducing of the thyroxin production, that caused appetite loss, dyspepsia, increased neuromuscular excitement. Differences between such state and mexidema: unchanged body weight and normal body temperature.

Hyperthyroidism or **Thyrotoxicosis.** Abnormally high activity of this gland may occur due to either oversecretion of the gland or an increase in size of the gland. Swelling of the gland results in an **exophthalmic goiter**, characterized by protrusion of the eye balls. In it, the BMR increases considerably above the normal figures ; 80% above normal is not unusual. Consequently, appetite is increased in hyperthyroid individuals. In spite of this, they lose weight and often feel hot because of the increased heat production. Heat production increased because thyroxine in high concentration could <u>uncouple</u> of <u>oxidation</u> and <u>phosphorylation</u> in the mitochondria. The clinical syndrome is generally termed **Graves' disease**. **Basedow's disease** and **thyrotoxic exophthalmos** are other names of this disease. Hyperthyroidism can be cured by surgical removal of the thyroid (thyroidectomy), treatment with x-rays, injection of radioactive iodide (131I) or by treating with antithyroid drugs or with agents like thiocyanate or perchlorate which compete with iodide for the uptake mechanism. Propylthiouracil is being particularly used against the Graves' disease.

Thyrocalcitonin or Calcitonin

Thyrocalcitonin acts by causing a transfer of calcium from blood into bone either by increasing calcification of the bones or by diminishing decalcification or by both processes. In other words, itrapidly inhibits calcium withdrawal from bones. This characteristic property, however, promises it to be a therapeutic agent for the treatment of certain types of bone diseases. This action of thyrocalcitonin is counterbalanced by the hypercalcemic hormone, the parathormone which is secreted by the parathyroids.

<center>**Hormone of the Parathyroid Secretory gland.**</center>

Parathyroids secrete a hormone called parathyroid hormone (parathormone, PTH) or **Collip's hormone.**

Functions. The principal sites of parathyroid action are bones, kidney and gastrointestinal tract. Following physiological functions are attributed to this hormone:

1. *Bone resorption* – It exerts a direct influence on the metabolism of bone, leading to an increased release of bone Ca^{2+} into the blood. The exact mechanism behind this phenomenon is not truly known. It has, however, been suggested that the hormone stimulates the production of citric acid in the bone tissues and an increased concentration of citrate ions leads to the removal of phosphate from calcium phosphate, the bone material. The bone is, thus, made soluble.

2. *Renal reabsorption of calcium* – In the kidney, parathormone affects renal tubular reabsorption of calcium and reabsorption or secretion of phosphate. It *decreases* the elimination of *calcium* and *increases* elimination of *phosphorus* in the urine. It is interesting to note that the secretion of this hormone is controlled by Ca^{2+} ion concentration of the blood itself. As the Ca^{2+} ion concentration increases, PTH secretion decreases tending to preserve the original condition. This affords an excellent example of feedback mechanism of metabolic control.

3. *Increase in osteoelastic activity* – Parathormone increases osteoelastic activity with augmented growth of the connective tissue.

4. *Calcium homeostasis* – An optimum Ca^{2+} concentration is necessary for various functions of the body, *viz.*, normal transmission of impulses, the contraction of the muscles, the formation of the bones, the coagulation of the blood etc.

Hypoparathyroidism. Undersecretion of PTH causes a decrease in Ca contents of the blood from the normal 10 mg per 100 ml to 7 mg per 100 ml (hypocalcemia) which leads to excessive contraction of the muscles (convulsions). In fact, convulsions occur when the calcium is further decreased to 4 mg per 100

ml of plasma. As the calcium decreases in the blood, there is a decrease in the urine.

However, during this period, the phosphorus in plasma increases from a normal 5 mg per 100 ml to 9 mg per 100 ml and even higher (hyperphosphotemia). These changes develop into a fatal disease called **muscular twitchings** or **tetany**. It is characterized by locking up of the jaw, rapid breathing, increased heart beat, rise in temperature and ultimately death due to asphyxia. The signs of tetany in man include **Chvostek's sign**, a quick contraction of the ipsilateral facial muscles elicited by tapping over the facial nerve at the angle of the jaw; and **Trousseau's sign**, a spasm of the muscles of the upper extremity that causes flexion of the wrist and thumb with extension of the fingers. Tetany can be relieved either by the administration of a soluble calcium salt or of PTH.

Hyperparathyroidism. An increase in PTH production is usually due to a tumour of the gland (parathyroid adenoma). Oversecretion of PTH in man results in a cystic bone disease variously called as **osteitis fibrosa cystica** or **von Recklinghausen's disease** or **neurofibromatosis.** It is an autosomal dominant condition. The disease is characterized by increased calcium contents of the blood (hypercalcemia) usually up to 20 mg calcium per 100 ml palsma, decreased phosphate concentration and increased renal excretion of calcium. Overproduction of parathormone causes calcium and phosphorus to move out of the bones and teeth, making them soft and fragile. Such patients, therefore, suffer fractures of the bones very frequently. Cysts in the bones are another characteristic of this disease.

Calcitriol

Calcitriol, also called 1,25-dihydroxycholecalciferol or 1,25-dihydroxyvitamin D_3, is the hormonally active metabolite of vitamin D with three hydroxyl groups (abbreviated 1,25-$(OH)_2D_3$ or simply 1,25$(OH)_2D$), It was first identified by Michael F. Holick in work published in 1971. Calcitriol increases the level of calcium (Ca^{2+}) in the blood by increasing the uptake of calcium from the gut into the blood, and possibly increasing the release of calcium into the blood from bone.

Calcitriol increases blood calcium levels ($[Ca^{2+}]$) by promoting absorption of dietary calcium from the gastrointestinal tract and increasing renal tubular reabsorption of calcium, thus reducing the loss of calcium in the urine. Calcitriol also stimulates release of calcium from bone by its action on the specific type of bone cells referred to as osteoblasts, causing them to release RANKL, which in turn activates osteoclasts.

Calcitriol acts in concert with parathyroid hormone (PTH) in all three of these roles.

Many of the effects of calcitriol are mediated by its interaction with the calcitriol receptor, also called the vitamin D receptor or VDR.

The observation that calcitriol stimulates the release of calcium from bone seems contradictory, given that sufficient levels of serum calcitriol generally prevent overall loss of calcium from bone. It is believed that the increased levels of serum calcium resulting from calcitriol-stimulated intestinal uptake causes bone to take up more calcium than it loses by hormonal stimulation of osteoclasts. Only when there are conditions, such as dietary calcium deficiency or defects in intestinal transport, which result in a reduction of serum calcium does an overall loss of calcium from bone occur.

A diet deficient in vitamin D in conjunction with inadequate sun exposure causes osteomalacia (or rickets when it occurs in children), which is a softening of the bones. In the developed world, this is a rare disease. However, vitamin D deficiency has become a worldwide issue in the elderly and remains common in children and adults. Low blood calcidiol (25-hydroxy-vitamin D) can result from avoiding the sun or liver or kidneys deseases. Deficiency results in impaired bone mineralization and bone damage which leads to bone-softening diseases, including rickets.

EXERCISES FOR INDEPENDENT WORK. In the table with test tasks emphasize keywords, choose the correct answer and justify it:

№	Test:	Explanation:
1.	A patient is followed up in an endocrinological dispensary on account of hyperthyroidism. Weight loss, tachycardia, finger tremor are accompanied with hypoxia similar symptoms – headache, fatigue, eye flicker. Find out the result for the influence of high level of thyroid hormones on tissue respiration causing the development of hypoxia similar symptoms: A. Specific binding of active centers of respiratory enzymes B. Intensification of respiratory enzymes synthesis C. Competitive inhibition of respiratory enzymes D. Inhibition of respiratory enzymes synthesis E. Uncoupling of oxidation and phosphorylation	
2.	A 2 y.o child has convulsions as a result of reduced concentration of calcium ions in the blood plasma. It is caused by the reduced function of: A. Adrenal cortex B. Pineal gland C. Parathyroid glands D. Hypophysis E. Thymus	
3.	A 4 year old child with hereditary renal lesion has signs of rickets, vitamin D concentration in blood is	

№	Test:	Explanation:
	normal. What is the most probable cause of rickets development? A. Impaired synthesis of calcitriol B. Inreased excretion of calcium C. Hypofunction of parathyroid glands D. Hyperfunction of parathyroid glands E. Lack of calcium in food	
4.	Some diseases reveal symptoms of aldosteronism with hypertension and edema due to sodium retention in the organism. What organ of the internal secretion is affected on aldosteronism? A. Hypophysis B. Testicle C. Ovaries D. Pancreas E. Adrenal glands	
5.	Testosterone and its analogs increase the mass of skeletal muscles that allows to use them for treatment of dystrophy. Due to interaction of the hormone with what cell substrate is this action caused? A. Membrane receptors B. Chromatin C. Ribosomes D. Proteins-activators of transcription E. Nuclear receptors	

№	Test:	Explanation:
6.	Periodic renal colics attacks are observed in a woman with primary hyperparathyroidizm. Ultrasonic examination revealed small stones in the kidneys. What is the most plausible reason of the stones's formation? A. Hyperphosphatemia B. Hypercalcemia C. Hypercholesterinemia D. Hyperuricemia E. Hyperkalemia	
7.	Parents of a 10 y.o. boy consulted a doctor about extension of hair-covering, growth of beard and moustache, low voice. Intensified secretion of which hormone must be assumed? A. Of oestrogen B. Of progesterone C. Of somatotropin D. Of testosterone E. Of cortisol	
8.	Thyrotoxicosis leads to increased production of thyroidal hormones T3 and T4, weight loss, tachycardia, psychic excitement and so on. How do thyroidal hormones influence energy metabolism in the mitochondrion of cells? A. Stop respiratory chain B. Activate oxidative phosphorylation C. Stop substrate phosphorylation D. Activate substrate phosphorylation E. Disconnect oxidation and	

№	Test:	Explanation:
	oxidative phosphorylation	
9.	Kidneys of a man under examination show increased resorbtion of calcium ions and decreased resorbtion of phosphate ions. What hormone causes this phenomenon? A. Vasopressin B. Hormonal form D_3 C. Thyrocalcitonin D. Aldosterone E. Parathormone	
10.	To prevent the transplant rejection after organ transplantation it is required to administer hormonotherapy for the purpose of immunosuppression. What hormones are used for this purpose? A. Thyroid B. Sexual hormones C. Glucocorticoids D. Catecholamines E. Mineralocorticoids	
11.	A 46-year-old patient suffering from the diffuse toxic goiter underwent resection of the thyroid gland. After the surgery the patient presents with appetite loss, dyspepsia, increased neuromuscular excitement. The body weight remained unchanged. Body temperature is normal. Which of the following has caused such a condition in this patient? A. Reduced production of parathormone B. Increased production of thyroliberin	

№	Test:	Explanation:
	C. Reduced production of thyroxin	
	D. Increased production of calcitonin	
	E. Increased production of thyroxin	
12.	A 19-year old male was found to have an elevated level of potassium in the secondary urine. These changes might have been caused by the increase in the following hormone level: A. Oxytocin B. Glucagon C. Aldosterone D. Adrenalin E. Testosterone	
13.	A patient with the signs of osteoporosis and urobithiasis has been admitted to the endocrinology department. Blood test revealed hypercalcemia and hypophosphatemia. These changes are associated with abnormal synthesis of the following hormone: A. Calcitonin B. Cortisol C. Calcitriol D. Aldosterone E. Parathyroid hormone	
14.	A patient who had been continuously taking drugs blocking the production of angiotensin II developed bradycardia and arrhythmia. A likely cause of these disorders is:	

№	Test:	Explanation:
	A. Hypercalcemia B. Hypernatremia C. Hypocalcemia D. Hypokalemia E. Hyperkalemia	
15.	Examination of a patient revealed hyperkalaemia and hyponatraemia. Low secretion of which hormone may cause such changes? A. Aldosterone B. Parathyroid hormone C. Atrial natriuretic peptide D. Cortisol E. Vasopressin	
16.	Inhabitants of territories with cold climate have high content of an adaptive thermoregulatory hormone. What hormone is it? A. Glucagon B. Insulin C. Cortisol D. Thyroxin E. Somatotropin	
17.	A 44-year-old woman complains of common weakness, heart pain, increase of body weight. Objectively: moon-like face, hirsutism, AP- 165/100 mm Hg, height -164 cm, weight -103 kg; fat is mostly accumulated in the region of neck, upper shoulder girdle, stomach. What is the main	

№	Test:	Explanation:
	pathogenic mechanism of obesity?	
	A. Decreased production of glucagon	
	B. Decreased production of thyroid hormones	
	C. Increased production of mineral corticoids	
	D. Increased production of insulin	
	E. Increased production of glucocorticoids	
18.	A 19-year-old female suffers from tachycardia in rest condition, weight loss, excessive sweating, exophtalmos and irritability. What hormone would you expect to find elevated in her serum? A. ACTH B. Mineralocorticoids C. Cortisol D. Insulin E. Thyroxine	
19.	A person has reduced diuresis, hypernatremia, hypokalemia. Hypersecretion of what hormone can cause such changes? A. Adrenalin B. Aldosterone C. Vasopressin D. Auricular sodiumuretic factor E. Parathormone	
20.	The patient with complaints of permanent thirst applied to the doctor. Hyperglycemia, polyuria and	

№	Test:	Explanation:

	increased concentration of 17-ketosteroids in the urine were revealed. What disease is the most likely in patient?
	
	    A. Glycogen storage disease type I
	    B. Steroidal subtype of diabetes mellitus
	    C. Addison`s disease
	    D. Myxedema
	    E. Insulin- dependent diabetes mellitus	

21.	People adapted to high external temperatures have such pecularity: profuse sweating isn't accompanied by loss of large volumes of sodium chloride. This is caused by the effect of the following hormone upon the respiratory glands: A. Cortisol B. Natriuretic C. Aldosterone D. Vasopressin E. Thyroxin	

THE ROLE OF HORMONES IN THE REGULATION OF METABOLIC PROCESSES
(LEVICH S.V.)

INFORMATIONAL MATERIAL

Hormones of the Hypophysis or Pituitary Gland

*Secretory gland***.** Hypophysis (meaning undergrowth) is so named because of its location below the brain as an undergrowth. Its synonym pituitary gland is, however, misleading as the gland is not concerned with the secretion of mucus or phlegm (*pituita*L = phlegm), as was thought previously.

It consists of 3 lobes:

(*a*) an anterior richly vascular largest lobe, pars distalis or adenohypophysis.

(*b*) an intermediate relatively avascular smallest lobe, pars intermedia.

(*c*) a posterior neural lobe, pars nervosa or neurohypophysis.

The anterior lobe produces hormones which govern the production of hormones secreted by other glands. These hormones are called as **tropins** (*tropos*G = turning) or **trophic** (*trophikos*G = nursing) **hormones**. Evidences available indicate that the rate of secretion of a trophic hormone is inversely proportional to the concentration, in the blood, of the hormone with which it is related. For example, a high blood level of thyroid hormone tends to inhibit the secretion of TSH from the adenohypophysis and a low level causes an increased production of it.

Neurohormones – The secretion of thyrotropin (as of almost all other pituitary hormones) is controlled by the hormones (or factors) released from hypothalamus, a region of the brain immediately proximal to the pituitary. These hormones are called as **hypothalamo-releasing hormones** or **hypothalamic factors.** These have been classified as *neurohormones*, *i.e*., those produced by the nerve cells. These are unlike *neurohumors* (*e.g*., acetylcholine, serotonin,

norepinephrine etc) which are released at nerve endings and activate the adjacent nerve bodies. The neurohormones, on the contrary, are released into the blood and activate cells a little far from their point of release. These are introduced into the capillary of the hypothalamo-hypophyseal portal system at the floor of the hypothalamus called median eminence. In addition, the release of anterior pituitary hormone may be inhibited by *a release inhibiting* factor which passes down the same hypothalamo-hypophyseal portal veins.

The various hypothalamic factors controlling the release of pituitary hormones have been listed in Table 7.

Table 7. Hypothalamic factors controlling the release of pituitary hormones

S.N.	Pituitary Hormones	Hypothalamic Releasing Factors*
1.	Thyrotropin, TSH	Thyrotropin-releasing factor, TRF
2.	Corticotropin, ACTH	Corticotropin-releasing factor, CRF
3.	Follicle-stimulating hormone, FSH	Follicle-stimulating hormone-releasing factor, FSH-RF
4.	Luteinizing hormone, LH	Luteinizing hormone-releasing factor, LH-RF
5	Prolactin, PL	Prolactin-releasing factor, PRF
6.	Growth hormone, GH	Growth hormone-releasing factor, GH-RF
7.	Melanocyte-stimulating hormone, MSH	Melanocyte-stimulating hormone-releasing factor, MRF

Pro-opiomelanocortin (**POMC**). is a precursor polypeptide with 241 amino acid residues. POMC is synthesized in the pituitary from the 285-amino-acid-long polypeptide precursor **pre-pro-opiomelanocortin** (pre-POMC), by the removal of a 44-amino-acid-long signal peptide sequence during translation. POMC is cleaved to give rise to multiple peptide hormones. Each of these peptides is packaged in large dense-core vesicles that are released from the cells by exocytosis in response to appropriate stimulation:

- α-MSH produced by neurons in the arcuate nucleus has important roles in the regulation of appetite and sexual behavior, while α-MSH secreted from the intermediate lobe of the pituitary regulates the production of melanin.

- ACTH is a peptide hormone that regulates the secretion of glucocorticoids from the adrenal cortex.

- β-Endorphin and [Met]enkephalin are endogenous opioid peptides with widespread actions in the brain.

The large molecule of POMC is the source of several important biologically active substances. POMC can be cleaved enzymatically into the following peptides: *N*-Terminal Peptide of Proopiomelanocortin (NPP, or pro-γ-MSH), γ-Melanotropin (γ-MSH), Corticotropin (Adrenocorticotropic Hormone, or ACTH), α-Melanotropin (α-Melanocyte-Stimulating Hormone, or α-MSH), Corticotropin-like Intermediate Peptide (CLIP), β-Lipotropin (β-LPH), Lipotropin Gamma (γ-LPH), β-Melanotropin (β-MSH), β-Endorphin, [Met]Enkephalin.

Some 30 tropins are secreted from the anterior lobe. The four important tropins are described below.

1. Thyrotropin or thyroid-stimulating hormone, TSH. It is a glycoprotein with molecular weight about 30,000. In general, it stimulates the activity of thyroid gland and enhances the rate of certain reactions such as:

(*a*) removal of iodide from blood by thyroid

(*b*) conversion of iodide to thyroid hormones

(*c*) release of hormonal iodine from thyroid.

The release of thyrotropin is controlled by another hormone from hypothalamus called thyrotropinreleasing factor, TRF.

2. Corticotropin or adrenocorticotrophic hormone, ACTH.

ATCH, in general, has a stimulatory effect on the hormone-producing capacity of the adrenal cortex. ATCH administration leads to accelerated gluconeogenesis with accompanied retardation of protein synthesis in all tissues except liver. It also possesses an intrinsic melanocyte-stimulating activity, causing darkening of the skin in a manner similar to that of another hormone, MSH. Over secretion of ACTH results in Cushing's disease, already described earlier. Certain peptides found in hypothalamus and also in neurohypophysis have ACTH-releasing activity. These have been termed as corticotropin-releasing factor, CRF.

3. Gonadotropins or gonadotrophic hormones, GTH. These hormones control the development and functioning of the gonads which remain dormant until the age of 12-14 years in the human beings.

Damage to certain areas of the hypothalamus greatly decreases the secretion of gonadotrophic hormones by the anterior pituitary. If this occurs prior to puberty, it causes typical eunuchism. The damage often causes simultaneous overeating because of its effect on the feeding centre of the hypothalamus.

Consequently, the person develops severe obesity along with the eunuchism. This condition is called **adiposogenital syndrome** or **Frohlich's syndrome** or **hypothalamic eunuchism**. Three gonadotropins are known. Oral contraceptives inhibited the secretion of GTH.

A. Follitropin or follicle-stimulating hormone, FSH. In human females, it induces the growth of graafian follicles resulting in an increased weight of the ovary. In males, however, FSH promotes spermatogenesis by stimulating the development of seminiferous tubules, thus leading to the formation of a large number of spermatocytes. The release of this hormone is controlled by another hormone from hypothalamus called folliclestimulating hormone-releasing factor, FSH-RF. Oral contraceptives inhibited the secretion of FSH.

B. Luteinizing hormone, LH or interstitial cell-stimulating hormone, ICSH. In females, LH is concerned with the ripening and rupturing of ovarian follicles, which later transform into corpus lutea. It also induces the development of interstitial cells of both the ovaries and the testes – a fact responsible for its nomenclature.

The secretion of LH is controlled by luteinizing hormone-releasing factor, LH-RF, a secretion from hypothalamus. The long-acting analogue of LH-RF has been found useful in the treatment of precocious puberty in females. Puberty is initiated by pulsed nocturnal secretions of gonadotropins that result from the release of the hormone LH-RF by the hypothalamus gland in the brain. The administration of LH-RF analogue "initially stimulated but subsequently inhibited" the release of LH and FSH, the two sex hormones that initiate puberty.

C. Luteotropin or luteotrophic hormone, LTH. Because of its broad spectrum of effects on vertebrates in general, *luteotropin is the most versatile of all the adenohypophyseal hormones*. However, in association with estrogen, luteotropin promotes the growth of the mammary glands (mammogenesis) and also induces secretion of milk (lactation) at the time of child birth (parturition). Henceforth, this hormone is variously called as *prolactin*, PL or *lactogenic hormone or mammotrophic hormone*, MH. It also stimulates glucose uptake and lipogenesis. Along with androgens, it causes the development of secondary male sex characters. In rat, at least, prolactin also has gonadotrophic activities in that it maintains functional corpora lutea in hypophysecto-mized animals. It also acts as an anabolic agent mimicking the effects of growth hormone.

In fact, prolactin is credited with performing some more than 80 functions and it is for the same reason that it has been jocularly termed as a "*jack-of-all-trades.*"

4. Somatotropin or somatotrophic hormone, STH or growth hormone, GH.

Unlike other adenohypophyseal hormones, the various effects of somatotropin are not due to its influence on other endocrine glands. It acts rather directly upon various tissues to produce diverse effects. *It is, therefore, not a true tropic hormone*. The various metabolic activities particularly attributed to this hormone are listed below.

(*a*) It affects the rate of skeletal growth and gain in body weight. In adult animals with closed epiphyses, SH stimulates chondrogenesis followed by ossification.

(*b*) It causes abnormal increase in blood sugar by producing degenerative changes in islets of Langerhans (*diabetogenic effect*).

(*c*) It stimulates the growth of the islets of Langerhans (*pancreatotropic effect*).

(*d*) It controls the production of fat in the body and its deposition in the liver (*ketogenic effect*).

(*e*) It prevents the fall of muscle glycogen in fasting and hypophysectomized animals.

(*f*) It also stimulates milk secretion in cows and also the growth of mammary glands in hypophysectomized rats (*galactopoietic effect*).

(*g*) STH is also known to cause adrenal enlargement (*corticotropic effect*).

Pituitary diabetes − A general increase in the secretion of all the adenohypophyseal hormones causes elevated blood glucose concentration. This condition is clinically designated as pituitary diabetes. It, however, differs from diabetes mellitus, which results from insulin deficiency, in the following respects:

I. In pituitary diabetes the rate of glucose utilization by the cells is only moderately depressed whereas in diabetes mellitus almost no utilization of glucose takes place.

II. Many of the side effects that result from reduced carbohydrate metabolism in diabetes mellitus are, however, lacking in pituitary diabetes.

Pars Nervosa or Neurohypophysis. The neurohypophysis develops from an outgrowth of the hypothalamus. This explains the presence of glial type cells in the gland. The posterior pituitary secretes 2 hormones: ocytocin and vasopressin.

1. Ocytocin (*ocy*G = quick ; tokosG = birth), **<u>oxytocin</u>** or **pitocin.** Ocytocin stimulates the contraction of smooth muscles, *esp*., those of uterus, thus facilitating childbirth. Commercial form of ocytocin is frequently used to induce 'labour'. In general, it also causes contraction of other smooth muscles like those of intestine, urinary bladder and the ducts of mammary glands resulting in milk ejection. It is, therefore, also called as *milk-let-down-ejection factor*. Ocytocin levels are increased by suckling which is necessary for the continued formation of milk by the breasts. Optimum milk secretion lasts for about 8 to 10 months after which it gradually falls and ultimately ceases. Oxytocin is used as drug for labuor stimulation.

2. Vasopressin or **pitressin.**

Vasopressin regulates many functions:

I. Circulatory or pressor action. Vasopressin causes a rise in blood pressure by contraction of vessels. The term 'insipidus' (= tasteless) dates from the time when the only method of testing urine was to taste it after diluting, and in this condition the urine is tasteless of peripheral blood vessels. It is, thus, a vasopressor substance and for this reason it is termed 'vasopressin'. It has been used in surgical shock as an adjuvant in elevating blood pressure. It may also be used at the time of delivery to overcome uterine inertia. The effect is similar to that caused by adrenalin. However, the rise in blood pressure caused by vasopressin lasts much longer than that caused by adrenalin which wears off in a few minutes.

II. Antidiuretic action. It brings about a reduction in the urine volume by causing renal tubules to withhold more water. Consequently, the urine passed is rich in sodium chloride, phosphate and total nitrogen. It was earlier thought that this antidiuretic effect was due to a different hormone called *antidiuretic hormone*, *ADH*. But it is now established that the two hormones (vasopressin and ADH) are one and the same. Liquid loss stimulating secretion of vasopressin, that lead to diuresis suppression. Vasopressin, therefore, finds use against persons suffering from **diabetes insipidus**, a disease characterized by excretion of large quantities of urine (polyuria) and a marked thirst (polydipsia). The urine specific gravity remains almost constant between 1.002 and 1.006. The urine output becomes 4 to 6 litres a day but can be sometimes as high as 12 to 15 litres a day, depending mainly on the amount of water taken by the patient. Furthermore, the rapid loss of fluid in the urine creates a constant thirst which keeps the water flushing throughout the body. The patient, thus, has a tendency to become dehydrated. But this tendency is quite well offset by the increased thirst. The disease may be controlled by administration of posterior pituitary extracts subcutaneously or even by nasal instillation.

Hypopituitarism. Insufficient secretion of pituitary hormones (or hypopituitarism) may occur as a result of pituitary tumours or atrophy of the gonad. Hypoactivity of this gland may lead to the following disorders:

1. **Dwarfism.** It refers to the arrested growth of the individuals. It is of 2 types:

(*a*) Lorain type – short statured individuals with a head large in comparison to the rest of the body; usually intelligent but unattractive.

(*b*) Fröhlich type – obesity and arrested sexual development; men-tally below normal and usually lethargic.

2. **Panhypopituitarism.** It is caused because of the destruction of the gland, thus leading to cessation of all hypophyseal functions.

3. **Pituitary myxedema.** It is caused due to the lack of TSH and produces symptoms similar to those described for primary hypothyroidism.

Hyperpituitarism. It refers to the overproduction of hypophyseal hormones. Hypersecretion of this gland leads to **gigantism** during childhood or adolescence, *i.e*., before closure of the epiphyses. The disease is characterized by overgrowth of the bones, especially at joints, leading to a tall individual with 2 to 2.5 M height. The limbs usually become disproportionately large. In human adults with closed epiphyses, excessive secretion of pituitary hormones causes **acromegaly** (*acron*G = extremity). The chief symptoms of this disease are: 1) consistent overgrowth of the bones of face, hands and feet (*acral* parts; hence the term acromegaly) so that the patient often complains of having have to change globes and shoes frequently as they no longer fit; 2) protrusion of the lower jaw (prognathism). Overgrowth of the malar, frontal, and basal bones combines with prognathism to produce the coarse facial features called *acromegalic facies;* 3) bowing of the spine (kyphosis); 4) overgrowth of the body hair; 5) thickening of the soft tissues of nose, lips and forehead; 6) enlargement of the visceral organs such as lungs, heart, liver and spleen; 7) increased sexual activity in the beginning which is ultimately followed by atrophy of the gonads.

Hypophysectomy. The effects of hypophysectomy (removal of hypophysis) appear to be almost entirely due to the loss of adenohypophysis. Removal of only the neurohypophysis exerts no striking dysfunctions. Hypophysectomy, in general, leads to: 1) gonadal atrophy in either sex; 2) atrophy of the thyroid; 3) atrophy of

the adrenal cortex; 4) loss of body tissue with some reversion to younger characters, *i.e.*, appearance of juvenile hair.

TISSUE HORMONES

Cholecystokinin (CCK), formerly called pancreozymin, a digestive hormone released with secretin when food from the stomach reaches the first part of the small intestine (duodenum). Cholecystokinin and pancreozymin were once considered two separate hormones because two distinct actions had been described: the release of enzymes from the pancreas, an action ascribed to pancreozymin; and the contraction of the gallbladder, which forces bile into duodenum, an action ascribed to cholecystokinin. However, today these two actions are recognized as belonging to one enzyme, now known solely as cholecystokinin.

Cholecystokinin is secreted by cells of the upper small intestine. Its secretion is stimulated by the introduction of hydrochloric acid, amino acids, or fatty acids into the stomach or duodenum. Cholecystokinin stimulates the gallbladder to contract and release stored bile into the intestine. It also stimulates the secretion of pancreatic juice and may induce satiety. There are several hypotheses regarding cholecystokinin's ability to induce satiety. One hypothesis is that meal-induced secretion of cholecystokinin activates the satiety centre of the hypothalamus in the brain so that the person feels full and stops eating. A second hypothesis is that, because cholecystokinin inhibits emptying of the stomach, the sensation of satiety may be the result of distension of the stomach.

Atrial natriuretic peptide (**ANP**), also called **atrial natriuretic factor** (**ANF**), **atrial natriuretic hormone** (**ANH**), **cardionatrine**, **cardiodilatin** (**CDD**), or **atriopeptin**, is a powerful vasodilator, and a protein (polypeptide) hormone secreted by heart muscle cells. It is involved in the homeostatic control of body water, sodium, potassium and fat (adipose tissue). It is released by muscle cells in the upper chambers (atria) of the heart (atrial myocytes) in response to high blood volume. ANP acts to reduce the water, sodium and adipose loads on the circulatory system, thereby reducing blood pressure.[3] ANP has exactly the opposite function

of the aldosterone secreted by the zona glomerulosa in regard to its effect on sodium in the kidney – that is, aldosterone stimulates sodium retention and ANP generates sodium loss.

ANP binds to a specific set of receptors – ANP receptors. Receptor-agonist binding causes a reduction in blood volume and, therefore, a reduction in cardiac output and systemic blood pressure. Lipolysis is increased and renal sodium reabsorption is decreased. The overall effect of ANP on the body is to counter increases in blood pressure and volume caused by the renin-angiotensin system.

Renal: Dilates the afferent glomerular arteriole, constricts the efferent glomerular arteriole, and relaxes the mesangial cells. This increases pressure in the glomerular capillaries, thus increasing the glomerular filtration rate (GFR), resulting in greater filter load of sodium and water; Increases blood flow through the vasa recta, which will wash the solutes (NaCl and urea) out of the medullary interstitium. The lower osmolarity of the medullary interstitium leads to less reabsorption of tubular fluid and increased excretion; Decreases sodium reabsorption in the distal convoluted tubule (interaction with NCC) and cortical collecting duct of the nephron via guanosine 3',5'-cyclic monophosphate (cGMP) dependent phosphorylation of ENaC; Inhibits renin secretion, thereby inhibiting the renin–angiotensin–aldosterone system.

Adrenal: Reduces aldosterone secretion by the zona glomerulosa of the adrenal cortex.

Vascular: Relaxes vascular smooth muscle in arterioles and venules.

Eicosanoid Hormones

A. Structure and Metabolic Roles. Eicosanoid hormones (or simply eicosanoids) are fatty acid derivatives with a variety of extremely potent hormonelike actions on various tissues of vertebrates. Eicosanoids, in general, are known to be involved in reproductive function; in the inflammation, fever and pain ; in the formation of blood clots and the regulation of blood pressure ; in gastric acid secretion ; and in a variety of other human processes.

Eicosanoids are all derived form 20-carbon polyunsaturated fatty acid, **arachidonic acid** (20:4; 5,8,11,14), from which they take their general name (*eikosi*G = twenty). Arachidonic acid could be synthesized in the body from linoleic and linolenic acids. Deficiency of which could cause deficiency of eicosanoids. There are 3 classes of eicosanoids (or the *signal molecules*, as they are also called) : prostaglandins, prostacyclins and thromboxanes, and leukotrienes (figure 80).
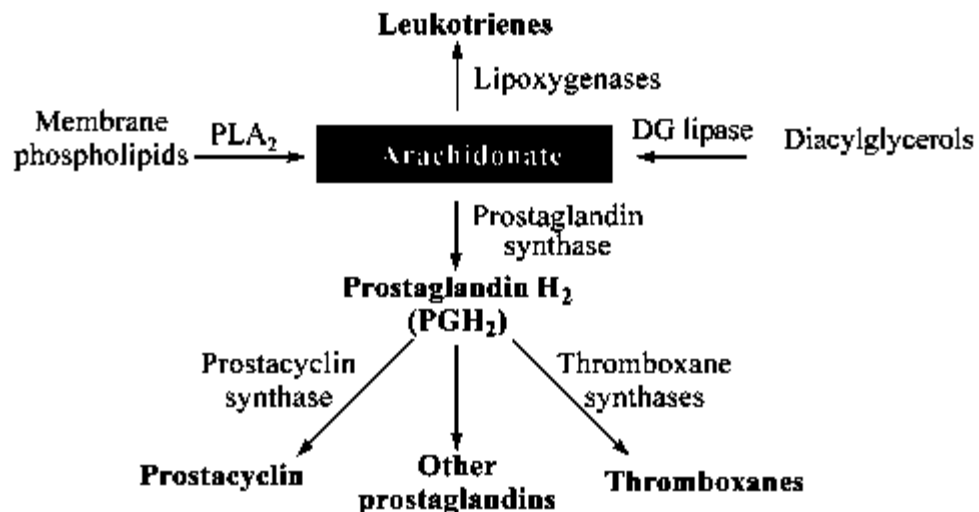
Figure 80. Biosynthesis of eicosanoids

Prostaglandins.

Chemically, the prostaglandins resemble prostanoic acid.

In fact, two groups of prostaglandins were originally recognized: **PGE** which is ether-soluble (hence the nomenclature, E from ether) and has a keto group at C9, and **PGF** which is phosphate-buffer-soluble (hence the nomenclature, F from *fosfat* in Swedish) and has a hydroxyl group at C9. These are the 6 primary prostaglandins and are abbreviated as PGE 1, PGE2, PGE3, PGF1 α, PGF2α and PGF3α. The prostaglandins perform a wide variety of biologic activities:

1. As mentioned earlier, they bring about contraction or relaxation of the smooth muscles of the uterus, *esp*., at the time of ovulation. This may be due to a chelation of calcium ions. As little as 1 ng/ml can cause contraction of the smooth muscles. They, thus, resemble ocytocin in this regard. They are modulators of hormone action.

2. They lower down blood pressure.

3. They inhibit lipolysis in adipose tissue, possibly by inhibiting the conversion of ATP to cyclic AMP and inhibition of platelet aggregation. The prostaglandins, thus, have the opposite effect of epinephrine, norepinephrine, glucagon and corticotropin on the release of fatty acids from adipose tissue.

4. They behave both as pressor and depressor agents under different conditions and thus affect the cardiovascular system.

5. They appear to control the secretion of gastric hydrochloric acid.

6. They also have some beneficial effect in the control of the acid-induced gastric ulcers.

7. Prostaglandins are best known for their effects on reproductive system. There seems to be a strong link between male fertility and seminal prostaglandin content. Human semen is rich in prostaglandins, which when deposited in vagina through coitus, facilitate conception. Thus, low prostaglandin content in the human semen is related to infertility.

8. They are effective labour inducers in pregnant women also.

9. Recent work indicates that the prostaglandins are also involved in the inflammatory reaction and pain. Anti-inflammatory drugs such as aspirin, in part, act by inhibiting the synthesis of prostaglandins. However, paracetamol (an analgesic drug like aspirin) is not anti-inflammatory as it does not inhibit the synthesis of prostaglandins. The widespread distribution of prostaglandins and their capacity to carry out varied metabolic effects have, however, led some to question the propriety of their being called hormones.

Thromboxanes and Prostacyclins

Thromboxanes (TXAs) and prostacyclins (PGIs) are structurally-related compounds that arise from a nascent prostaglandin. In compounds of both these categories, carbons 8 and 12 are joined and an oxygen atom is added to form the six-membered ring (*cf* prostaglandins, where a five- membered ring is formed).

The striking similarity and diversity in the physiological roles of thromboxanes and prostacyclins displays a critical balance required for the normal

functioning in the body. TXA2 and PGI2 are medically important examples of how such a balance operates *in vivo*. TXA2 is a highly effective vasoconstrictor (blood vessel constrictor) and platelet aggregator ; conversely, PGI2 is a potent vasodilator and inhibitor of platelet aggregation. Platelets are the blood cells that first appear and aggregate at the site of injury to produce a temporary plug that serves as a base on which the strong fibrin clot ultimately forms. However, for maintenance of normal blood flow, TXA2-induced aggregation of platelets would quickly prove fatal. Thus, a vital opposing role of PGI2 is to prevent platelets from aggregating on blood vessel walls, a site of PGI2 production.

Unlike other eicosanoids, PGI2 is not metabolized during passage through the lungs. Thus, TXA2 and PGI 2 are continuously engaged in a 'tug of war' with respect to platelet aggregation.

Leukotrienes.

Leukotrienes (LTs), first found in leukocytes, are cysteinyl-containing derivatives of arachidonic acid.

Leukotriene B4 (LTB4) has two hydroxyl groups at C-5 and C-12 and three conjugated double bonds at C-6, C-8 and C-10. An additional double bond is present between C-14 and C-15. **Leukotriene C4** (LTC4) contains the tripeptide glutathione (γ-Glu-Gly-Cys) covalently bonded to a derivative of arachidonic acid. **Leukotriene D4** (LTD4) possesses the dipeptide, Gly-Cys (Glu residue is eliminated) and **leukotriene E4** (LTE4), the amino acid Cys (Gly residue eliminated).

Neutrophils make one class of leukotrienes to alter mobility and act as chemotactic agents. *Mast cells* make another class, formerly known as **slow-reacting substances**, which is responsible for bronchial constriction and other anaphylactic allergic reactions. Leukocytes are powerful biological signals; for example, they induce contraction of the muscle lining the airways to the lung. They also cause a slow and persistent contraction in the smooth muscle of blood vessels. Overproduction of leukotrienes causes asthmatic attacks and also stimulates mucus secretion, and increases *body temperature*.

B. Biosynthesis Various eicosanoids are produced in different cell types by different synthetic pathways, and have different target cells and biological activities. While prostaglandins are made everywhere in the body, synthesis of thromboxanes and leukotrienes occurs in restricted locations. Both compounds are synthesized in platelets, neutrophils and the lung. Some amount of thromboxane production also takes place in the brain.

Arachidonic acid is generated from phospholipids by the action of *phospholipase A* 2 (PLA2), or from diacylglycerol by the action of a *lipase*. The biosynthesis of most eicosanoids starts at arachidonate, which has 4 double bonds at C5, C8, C11 and C14. In the first key reaction, arachidonate gets converted to prostaglandin H2 (PGH2) by the enzyme *prostaglandin synthase*, which is made up of two components, cyclooxygenase and hydroperoxidase. This is a two-step reaction. In *first step*, cyclooxygenase component of prostaglandin synthase catalyzes the addition of one mole of oxygen to C-9 of arachidonate and of a second mole to C-15.

The bond formation between C-8 and C-12 accompanying this oxygenation produces the 5-membered endoperoxide ring structure, characteristic of eicosanoids. The compound so formed is called as **prostaglandin G2** (PGG2). The 4 oxygen atoms introduced into PGG2 come from 2 moles of oxygen. In the second step, the hydroperoxidase component of prostaglandin synthase, then, catalyzes a two-electron reduction of the 15-hydroperoxy group of PGG2 to a 15-hydroxyl group, producing **prostaglandin H2** (PGH2). The highly unstable PGH2 is rapidly transformed into other prostaglandins, prostacyclins and thromboxanes. In fact, the biochemical fate of the PGH2 synthesized is determined by tissue-specific enzymes. For example, in a tissue producing prostaglandin E2, the enzyme endoperoxide isomerase is present and converts PGH2 into **prostaglandin E2** (PGE2). The wondrous drug aspirin has been used for centuries to decrease inflammation, pain and fever. Its mode of action was an enigma until John Vane, in 1975, discovered that *aspirin inhibits the synthesis of prostaglandins by inactivating prostaglandin synthase*. Specifically, aspirin (acetylsalicylate)

irreversibly inhibits the cyclooxygenase activity of this enzyme by acetylating a specific serine hyydroxyl group. Aspirin is a potent antiinflammatory agent because it blocks the first step in the synthesis of prostaglandins. This drug is also widely used to prevent excessive blood clotting, which can lead to heart attacks and strokes. Aspirin is antithrombotic also because it blocks the formation of thromboxane A 2 (TXA2), a potent aggregator of blood platelets. Inhibition of the cyclooxygenase blocks the formation of prostaglandin H2, PGH2. PGH2, which is produced in the platelets, is also the precursor of thromboxane A2 (TXA2); the reaction being catalyzed by the enzyme *thromboxane synthase*. **Prostacyclin I2 (**PGI2) is synthesized from PGH2 and the reaction is mediated by *prostacyclin synthase*. Thus, we see that the tissues are differently endowed with enzymes that transform endoperoxides into sepcific types of eicosanoids.

Leukotrienes are made from arachidonate by another pathway, beginning with the addition of oxygen to C-5 of arachidonate ; the reaction being catalyzed by *lipoxygenase*. This reaction is not affected by antiinflammatory drugs.

EXERCISES FOR INDEPENDENT WORK. In the table with test tasks emphasize keywords, choose the correct answer and justify it:

№	Test:	Explanation:
1.	The intake of oral contraceptives containing sex hormones inhibits secretion of the hypophysial hormones. Secretion of which of the indicated hormones is inhibited while taking oral contraceptives? A. Thyrotropic B. Somatotropic C. Oxytocin D. Follicle-stimulating E. Vasopressin	
2.	A man after 1,5 liter blood loss has suddenly reduced diuresis. The increased secretion of what hormone caused this diuresis alteration? A. Vasopressin B. Corticotropin C. Cortisol D. Parathormone E. Natriuretic	
3.	Secretion of what gastrointestinal hormones will be primarily decreased as a result of duodenum removal? A. Neurotensin B. Gastrin and histamine C. Cholecystokinin and secretin D. Gastrin E. Histamine	
4.	A patient who suffers from pneumonia has high body temperature. What biologically active substances play the leading	

№	Test:	Explanation:
	part in origin of this phenomenon? A. Leukotrienes B. Histamine C. Serotonin D. Bradykinin E. Interleukin	
5.	Utilization of arachidonic acid via cyclooxygenase pathway results in formation of some bioactive substances. Name them: A. Biogenic amines B. T_3 and T_4 C. Somatomedins D. Insulin-like growth factors E. Prostaglandins	
6.	A 0.9% solution of sodium chloride was intravenously injected to an animal. This caused decreased reabsorption of sodium ions in renal tubules. It is the result of the following changes in endocrine system: A. Increase of Aldosterone secretion B. Decrease of Aldosterone secretion C. Increase of Vasopressin secretion D. Vasopressin synthesis is disturbed E. Increase of atrial natriuretic factor synthesis	
7.	A patient has osmotic pressure of blood plasma at the rate of 350 mOsmol/l (norm is 300 mOsmol/l). This will cause hypersecretion of the following hormone: A. Aldosterone B. Adrenocorticotropin	

№	Test:	Explanation:
	C. Natriuretic D. Cortisol. E. Vasopressin	
8.	Examination of a patient revealed overgrowth of facial bones and soft tissues, tongue enlargement, wide interdental spaces in the enlarged dental arch. What changes of the hormonal secretion are the most likely? A. Hyposecretion of thyroxin B. Hypersecretion of the somatotropic hormone C. Hypersecretion of insulin D. Hyposecretion of insulin E. Hyposecretion of the somatotropic hormone	
9.	What pituitary gland hormone secretion will be inhibited in female after taking the oral contraceptives containing sex hormones? A. Growth hormone B. Gonadotropin C. Thyroid stimulating hormone D. Vasopressin E. Oxytocin	
10.	Atria of an experimental animal were superdistended with blood which resulted in decreased reabsorption of Na+ and water in renal tubules. This can be explained by the influence of the following factor on kidneys: A. Natriuretic peptide B. Vasopressin C. Renin D. Aldosterone E. Angiotensin	
11	As a result of home injury, a patient	

№	Test:	Explanation:
.	suffered a significant blood loss, which led to a fall in blood pressure. Rapid blood pressure recovery after the blood loss is provided by the following hormones: A. Cortisol B. Aldosterone C. Adrenalin, vasopressin D. Sex hormones E. Oxytocin	
12.	A patient has been administrated an anti-inflammatory drug that blocks the action of cyclooxygenase. Specify this anti-inflammatory agent: A. Analgene B. Thiamin C. Aspirin D. Allopurinol E. Creatine	
13.	A 60-year old patient with a long history of stenocardia takes coronarodilator agents. He has also been administrated acetylsalicylic acid to reduce platelet aggregation. What is the mechanism of anti-platelet action of acetylsalicylic acid? A. It has membrane stabilizing effect B. It enhances the synthesis of prostacyclin C. It reduces the activity of cyclooxygenase D. It reduces the activity of phosphodiesterase E. It enhances the activity of platelet adenylate cyclase	

№	Test:	Explanation:
14.	Products of some proteins hydrolysis and modification are the biologically active substances called hormones. Lipotropin, corticotrophin, melanotropin and endorphins are synthesized in the hypophysis of the following protein: A. Neuroglobulin B. Proopiomelanocortin (POMC) C. Neuroalbumin D. Neurostromin E. Thyroglobulin	
15.	A 26-year old woman at 40 weeks pregnant has been delivered to the maternity ward. Objectively: the uterine cervix is opened, but the contractions are absent. The doctor has administrated her a hormonal drug to stimulate the labor. Name this drug: A. Testosterone B. Oxytocin C. Hydrocortisone D. ACTH E. Estrone	
16.	Deficiency of linoleic and linolenic acids in the body leads to the skin damage, hear loss, delayed wound healing, thrombocytopenia, low resistance to infections. These changes are most likely to be caused by impaired synthesis of the following substance: A. Corticosteroids B. Interferons C. Interleukins D. Eicosanoids E. Catecholamines	
17.	A 32-year-old female patient consulted a doctor about the absence	

№	Test:	Explanation:
	of lactation after parturition. Such disorder might be explained by the deficit of the following hormone: A. Glucagon B. Prolactin C. Vasopressin D. Somatotropin E. Thyrocalcitonin	

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ANSWERS TO TESTS TASKS:

Nucleoproteins. Nucleotides and nucleic acid functions. membranes of cells: their structure, composition and functions

1	2	3	4	5	6	7	8	9	10
A	D	C	C	E	E	A	D	B	D
11	12	13	14	15	16	17	18	19	20
B	C	D	D	D	D	A	B	A	B

The metabolism of purine and pyrimidine nucleotides. The disorders of nucleotide metabolism

1	2	3	4	5	6	7	8	9	10
A	B	C	B	A	D	A	A	C	D
11	12	13	14	15	16	17	18	19	20
A	E	A	B	A	B	E	A	D	E

Biosynthesis of nucleic acids

1	2	3	4	5	6	7	8	9	10
A	D	D	C	A	E	A	C	A	E
11	12	13	14	15	16	17	18	19	20
D	C	A	A	C	C	D	C	B	A

Biosynthesis of proteins and its regulation

1	2	3	4	5	6	7	8	9	10
C	A	A	D	C	A	D	C	A	B
11	12	13	14	15	16	17	18	19	20
B	D	C	E	B	A	C	A	B	E

Chromoproteins. Hemoglobin metabolism and its disorders. Porphyrins metabolism

1	2	3	4	5	6	7	8	9	10
A	C	A	E	C	A	E	C	E	B
11	12	13	14	15	16	17	18	19	20
C	A	D	A	E	C	A	A	C	D

A Classification and properties of hormones. the mechanisms of hormones action (protein-peptides and biogenic amines)

1	2	3	4	5	6	7
E	A	D	B	A	B	E

Steroid and thyroid hormones: the mechamism of action and the influence on metabolic processe. The regulation of calcium levels by hormones in the blood

1	2	3	4	5	6	7	8	9	10
E	C	A	E	E	B	D	E	E	C
11	12	13	14	15	16	17	18	19	20
C	C	E	E	A	D	E	E	D	B
21									
E									

The role of hormones in the regulation of metabolic processes

1	2	3	4	5	6	7	8	9	10
D	A	D	E	E	E	E	B	B	A
11	12	13	14	15	16	17			
C	C	C	B	B	D	B			

CONTENT