NUCLEOPROTEINS BIOCHEMISTRY

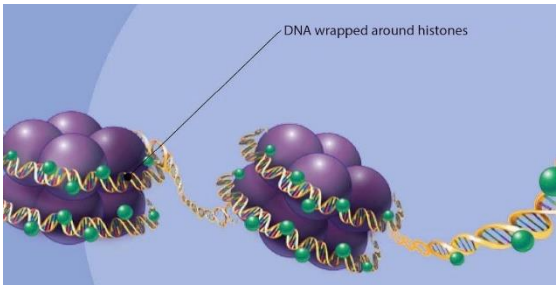
Structural properties of nucleic acids.

Nucleoproteins: their types, composition and functions

Nucleic acids (NA) store and transmit genetic information. Two different types of nucleic acids are distinguished: deoxyribonucleic acid (DNA) and ribonucleic acid (RNA). DNA, that stores genetic information, is present not only in the chromosomes of eukaryotes, but also in mitochondria and chloroplasts of plants. Prokaryotic cells lack a nucleus; they have a single chromosome, or non-chromosomal DNA in the form of a plasmid.

Nucleoprotein is a nucleic acid associated with proteins. In eukaryotic cells, DNA is associated with proteins forming nucleoprotein. The protein part of nucleoproteins is represented by alkaline proteins, such as protamines and histones. Histones and protamines, that are connected with DNA are simple proteins of alkaline nature present in cell nucleus. According to modern classification, due to a molecular weight of less than 5000 (4000), they are considered not true proteins, but peptides. Mono-, di- and tri-protamines are distinquished.

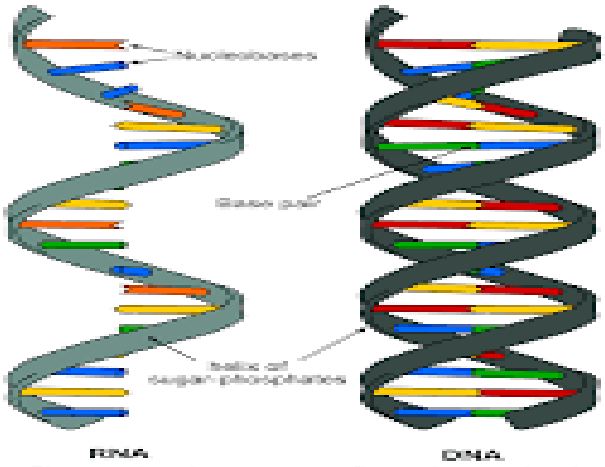
Examples of protamines from fish are: salmine from salmon, scumbrin from mackerel, clupeine from herring, stelline from starry sturgeon, scylliorhinine from dogfish, thinnine from tunafish etc.



The protein part of nucleoproteins in human is histone. To determine the composition of the nucleoprotein protein part composition, laboratories can separate histones from the nucleic acid with a solution of chloroform.

Histones contain a lot of arginine and lysine, so their isoelectric point (pİ) equals 9-12. Tryptophan does not occur in histones.

All NPs are sort into 2 main groups:



1) deoxyribonucleoproteins

2) ribonucleoproteins.

With complete hydrolysis of nucleic acid, phosphoric acid, monosaccharide pentose

Fig.1. Single-stranded RNA & double stranded DNA

and nitrogen base is formed. Nitrogen bases in nucleic acids are represented in the form of purines and pyrimidines. Purines are adenine and guanine, and pyrimidines are cytosine, uracil and thymine. In DNA is thymine instead of uracil, so uracil is absent in DNA.

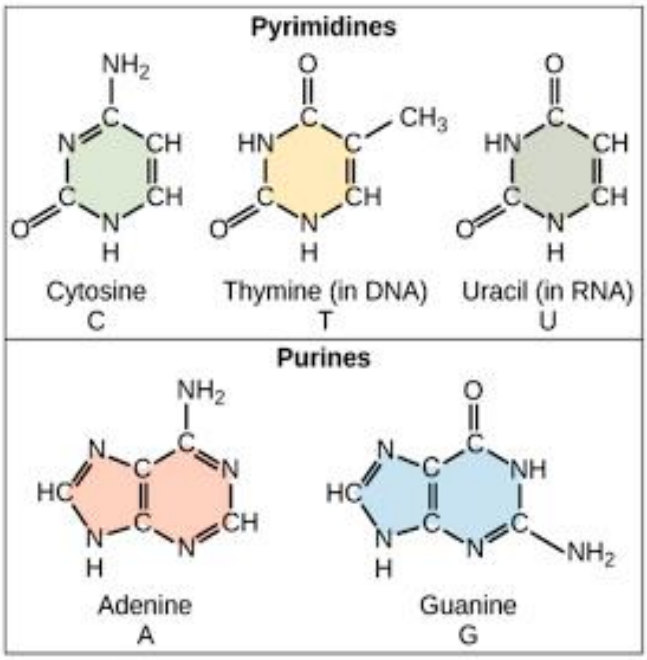


Fig.2. Purine and pyrimidine *nitrogenous bases* of DNA and RNA

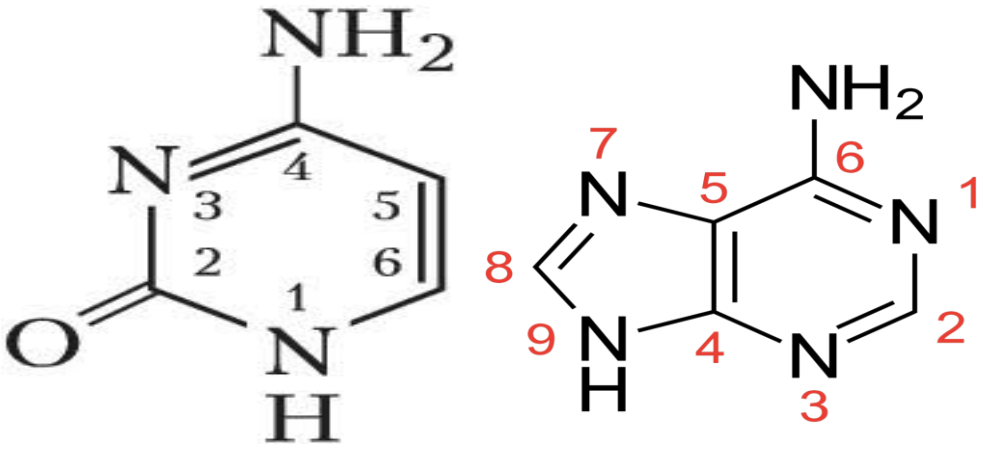


Fig.3. Purine and pyrimidine *nitrogenous bases* ring numbering

Pentose in RNA is ribose, but DNA pentose is deoxyribose.

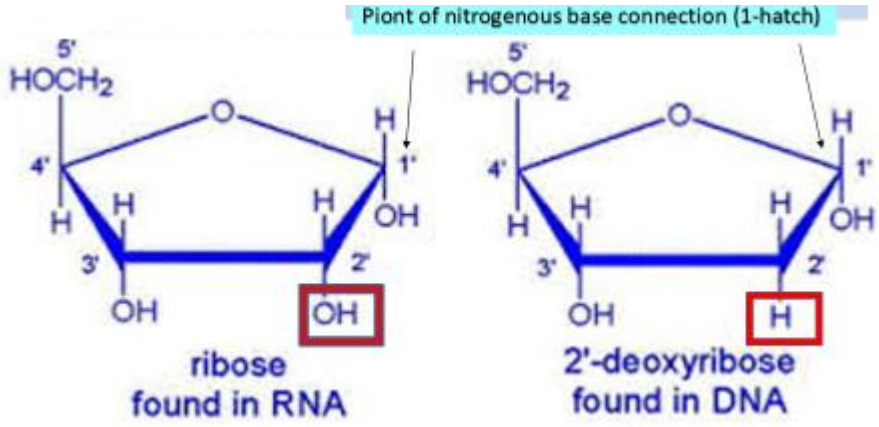


Fig.4. Ribose and deoxyribose composition. In order not to mix atoms of nitrogenous base with carbohydrate ring atoms, the latter are indicated by the sign ` (hatch)

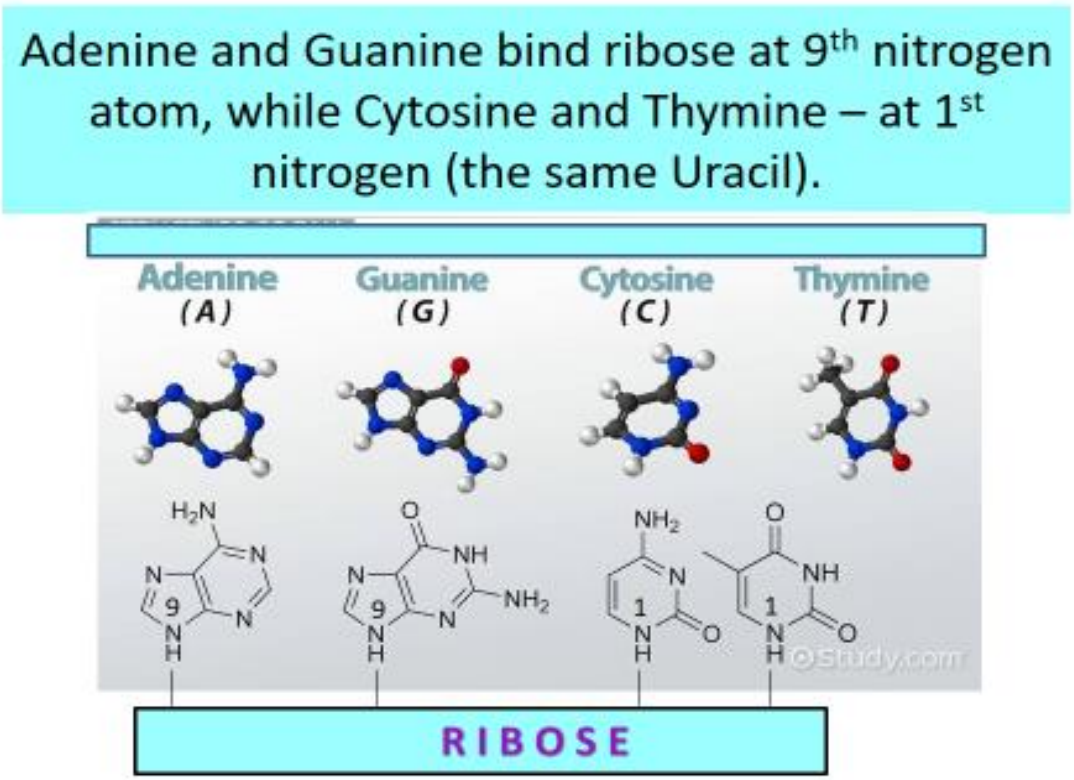


Fig.5. Point of nitrogenous base c onnection with ribose

Some NA have in their content minor purines: methyl adenine, methyl guanine and dimethyl guanine. Minor forms ***of pyrimidines*** are:

methyl uracil,

thio-uracil,

dihydro-uracil.

Minor nucleosides are:

-pseudouridine,

-hydroxymethyl-cytosine.

The minor bases mostly present in transfer RNA (t-RNA).

Purines and pyrimidines that are not part of nucleic acids

Xanthine and hypoxanthine are also purines, but they <u>are not costituents</u> of nucleic acids. Uric acid also is a purine that is not present in nucleic acids, but

a waste product washed out with urine. Food purines enter the body mainly with tea, coffee and chocolate. Theobromine is a plant purine, alkaloid that is similar in structure to caffeine mainly found in the cocoa bean. Theobromine stimulates the activity of the heart, but it also has a diuretic effect, thereby lowering blood pressure. Tea also contains theobromine, and along with it theophylline also closely related to caffeine. Theophylline relaxes the smooth muscles of the airways. This leads to easier breathing. Theophylline stimulates the frequency and force of heart contractions. Purines are associated with an increased risk of gout. With gout, it is better to stop eating chocolate as it increases the amount of purines in the body and thereby exacerbates the symptoms of the disease. Caffeine is abundant in tea leaves, and is significantly higher in green tea than in black tea leaves. Nevertheless, green tea can reduce uric acid in the body, which is important in gout when the level of uric acid is elevated in the blood. Normal urine contains xanthine along with uric acid, but no guanine.

Orotic acid is pyrimidine, that does not present in nucleic acids, but is used as an anabolic drug.

Nitrogenous base associated with pentose (ribose or deoxyribose) forms nucleoside. Nucleosides are formed at incomplete hydrolysis of nucleic acids. The following nucleosides are distinquished:

-(deoxy) adenosine, (deoxy) guanosine, (deoxy) cytidine, (deoxy) thymidine, uridine.

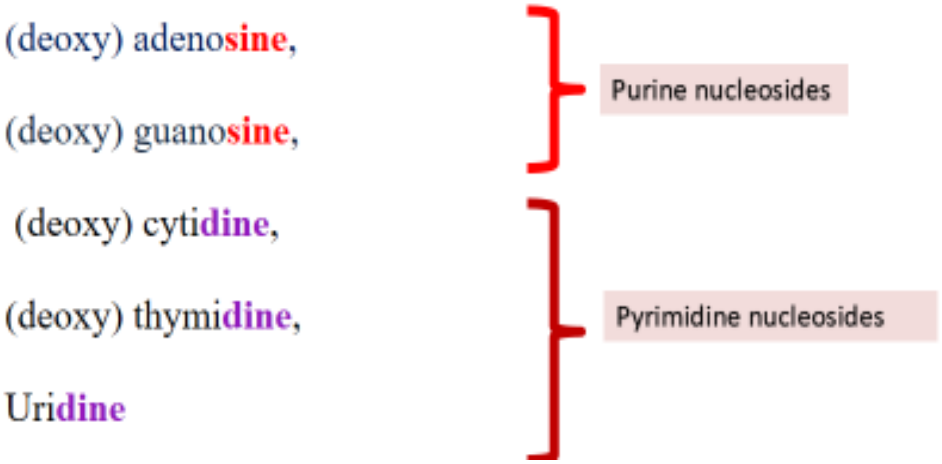


Fig.6. Purine and pyrimidine nucleosides

Nucleotides are nucleosides associated with phosphoric acid. The following nucleotides are distinguished in DNA and RNA:

(d) -adenylic acid - (d) AMP, (d) ADP, (d) ATP.

(d) -guanilic acid - (d) GMP, (d) GDF, (d) GTP.

(d) -cytidyl acid - (d) CMP, (d) CDP, (d) CTP.

(d) thymidyl acid - (d) TMF, (d) TDF, (d) TTF

Uridylic acid: UMF, UDF, UTP.

Structure of nucleotide mono-, di-, triphosphates is shown in the picture below.

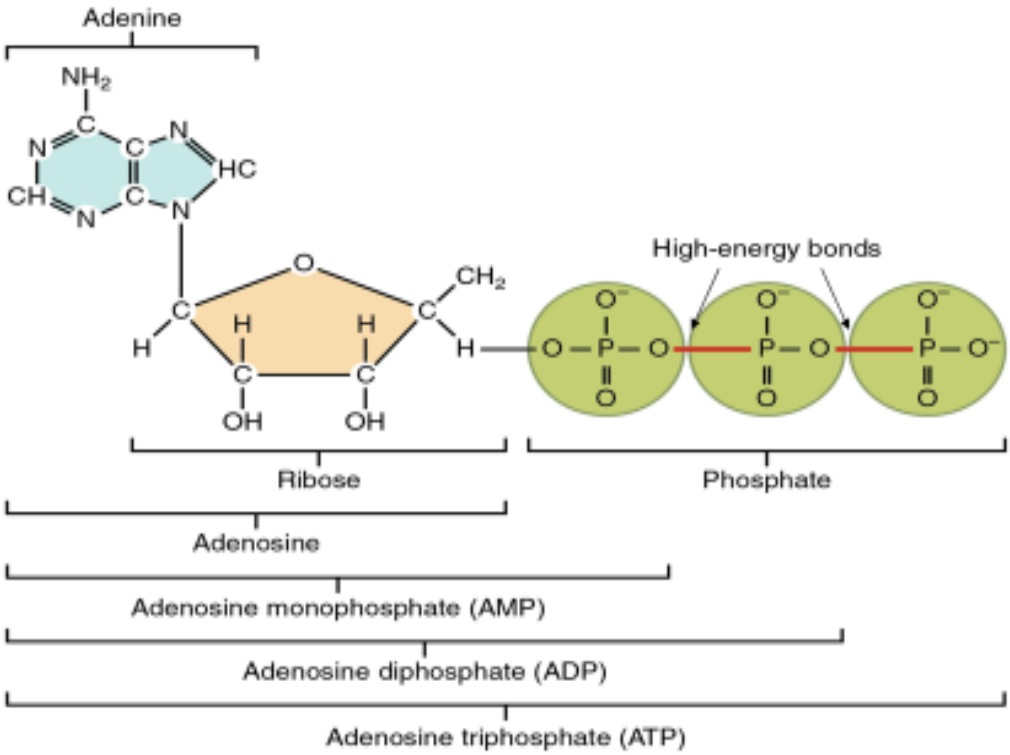


Fig.7. In nucleotide triphosphates, the second and third phosphate groups are attached by relatively unstable high energy bonds.

The cells also have cyclic nucleotides, such as cAMP, cGMP. These are cyclic 3’5’monophosphates: cyclic Adenosine Mono-Phosphate and cyclic Guanosine Mono-Phosphate. They are secondary messengers, that transmit a hormonal signal to the cell for regulation its metabolism.

Primary, secondary and tertiary structures of DNA. Chargaff’s rule.

Eukaryotic DNA (Deoxyribo Nucleic Acid) is found in the chromosome of the nucleus. DNA is also found in mitochondria, plant plastids. DNA is a polymer, namely polynucleotide composed of linked mononucleotides. İts mononucleotides are represented by deoxyribonucleoside monophosphates. Mononucleotides are linked in polynucleotides via covalent 3'5'-phosphodiester bond. Phosphodiester bond binds the 3-hydroxyl group of the pentose of one nucleotide to the 5-hydroxyl group of the pentose of the adjacent nucleotide through the phosphoryl group. Bases located along the chain are always written in sequence from the 5’-end of the chain to the 3’- end.

For example, the DNA base sequence shown in picture below (Fig. 8) is 5-AGCT-3, and reads "adenine, guanine, cytosine, thymine".

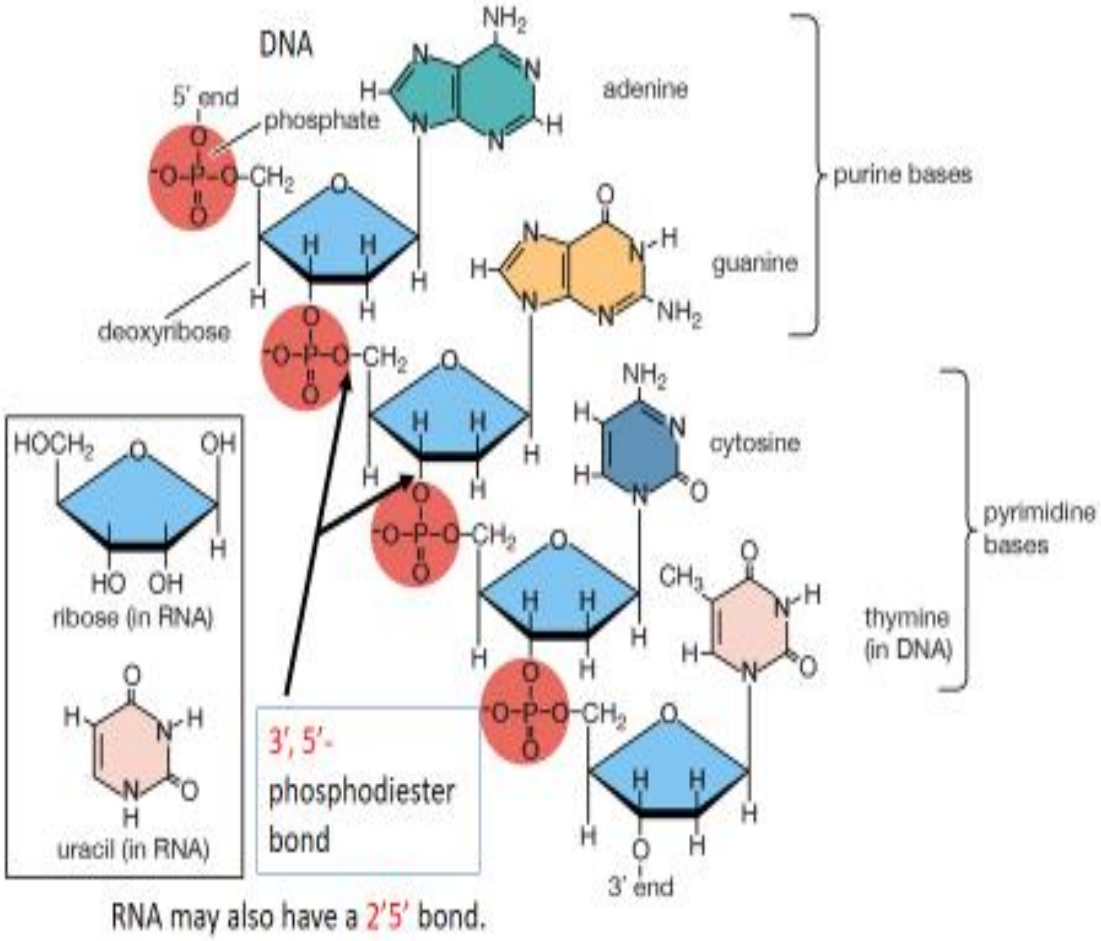


Fig.8. Primary structure of DNA: sequence of mononucleotides combined into polynucleotide via 3', 5'-phosphodiester bond.

The native polynucleotide has 3 structural levels: primary, secondary and tertiary. The primary structure of each NA is the sequence of mononucleotides in the polynucleotide chain. In the laboratories, the primary structure of DNA is determined by splitting DNA with *restrictase* enzymes into small fragments. Each restrictase is specific for a certain nucleotide sequence. DNA is a double stranded, while RNA has a single strand.

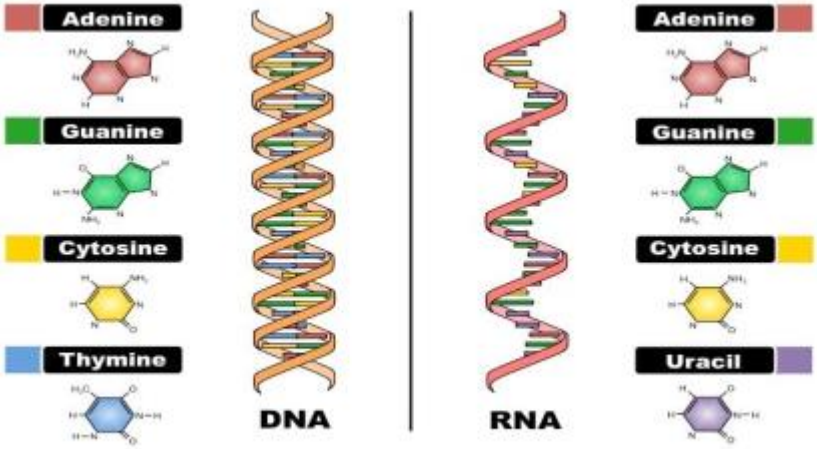


Fig.9. Double stranded DNA and single stranded RNA

The sequence of mononucleotides in the single DNA strand means primary structure of DNA. With the exception of a few viruses containing single-stranded DNA, DNA usually exists as a double-stranded molecule. The secondary structure of the NA is the spatial configuration of the NA. İn DNA secondary structure, two strands wind around each other with formation of double helix. The bases of the DNA strand are paired according to the adjacent strand. İn this double stranded helix, the two chains of DNA are coiled around a common axis termed the helical axis. The chains are arranged in an antiparallel manner, that is, the 5-end of one strand is paired with the 3-end of an adjacent strand.

There are three main secondary structural DNA forms: form B, form A and form Z. Form B is a right-handed helix with 10 base pairs per turn in spiral. The chromosomal DNA consists mainly B-DNA. Form A is also a right helix, but there are 11 base pairs in each turn; it is obtained with moderate dehydration of B form. Z-form of DNA is a left-handed helix that contains 12 base pairs per turn.

The hydrogen bonds, as well as hydrophobic interactions between folded bases stabilize the double helix structure. İn DNA, Adenine (A) is always paired with thymine (T) and cytosine (C) is always paired with guanine (G). The binding of A only to T and G only to C is a manifestation of complementarity. This specific base pairing in DNA results in formation of Chargaff's rule. According to Chargaff's rule,

in any sample of double-stranded DNA, the amount of A equals T, the quantity G is equal to the quantity C, and the total purines are equal to the total number of pyrimidines. Schematically, Gargaff's law is written as following:

1. Sum of purines equals to pyrimidines: A + G = C + T

2. A = T, G = C

3. A + C = G + T. But for RNA A + C= G + U.

G+C/ A+T is the coefficient of DNA specificity. In human, the DNA is of the adenine-thymine type, since its coefficient is 0.66.

There are 2 hydrogen bonds between adenine and thymine in NA, while between guanine and cytosine - 3.

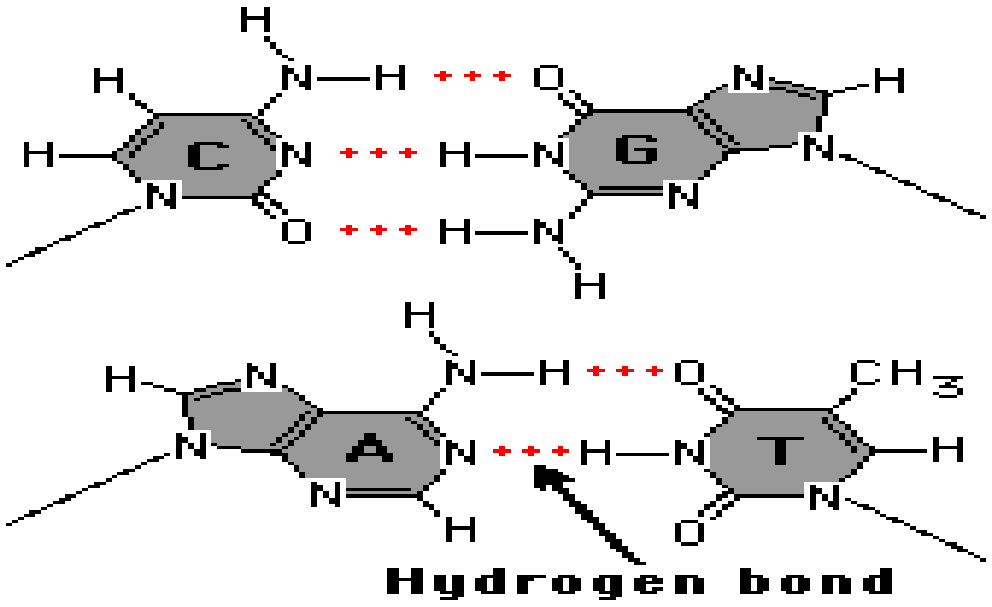


Fig.10. Hydrogen bonds formed between purine & pyrimidine bases in DNA

Eukaryotes have closed circular double-stranded DNA molecules. The chromosome contains one long linear molecule DNA. Additional supercoiling of DNA, its binding to proteins leads to the formation of the tertiary structure of DNA. DNA is associated with a complex mixture of histone and non-histone proteins to form chromatin. In the tertiary structure of DNA, its 2 helices bind to histone proteins H_1, H_{2a}, H_{2b}, H_3, H_4.

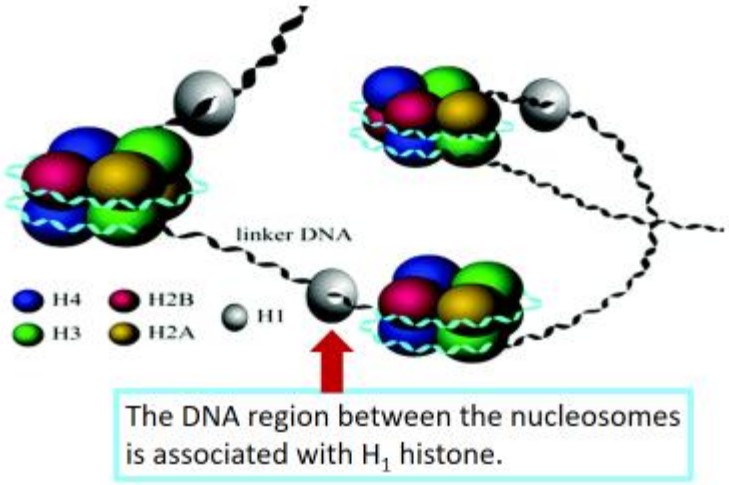


Fig.11. Tertiary structure of DNA

These low-weight proteins are positively charged at physiological pH as a result of high amount of lysine and arginine in them. Due to their positive charge, they form ionic bonds with negatively charged DNA. Histones and Mg^{2+} ions, neutralize the negatively charged phosphate groups of DNA. Histones help condensation of chromosome and DNA turning. Two of each H2A, H2B, H3, and H4 form an octameric core of nucleosome, around which appropriate segment DNA is wound. In the nucleosome, the DNA helix is wrapped 1.75 times around 8 histone molecules. The DNA region between the nucleosomes is associated with H_1 histone. Reversible phosphorylation, acetylation, methylation changes the strength of histone binding to DNA, thereby affecting gene expression. This histone modification is an example of "epigenetics", which means changes in gene expression without changing the nucleotide sequence. Neighboring nucleosomes join together via linear DNA about 50 base pairs long. Linear DNA is associated with H1.

The nucleosomes then pack more tightly, forming polynucleosomes. This structure takes the form of coil. Additional levels of organization lead to the final chromosome structure as follows. Nucleosomes fold up to form a 30-nanometer chromatin fiber. The chromatin fiber forms loops averaging 300 nanometers in length. During prophase of mitosis, chromatin fibers become coiled into

chromosomes. Ergo, chromatin is the material, of which the chromosomes of eukaryotes are composed (the base of the chromosome is chromatin). In chromatin present DNA, histones, non-histone proteins, which regulate chromatin's activity, and some RNA. In non-histone proteins, dicarboxylic amino acids predominate, so they are acidic and help to regulate chromatin's activity.

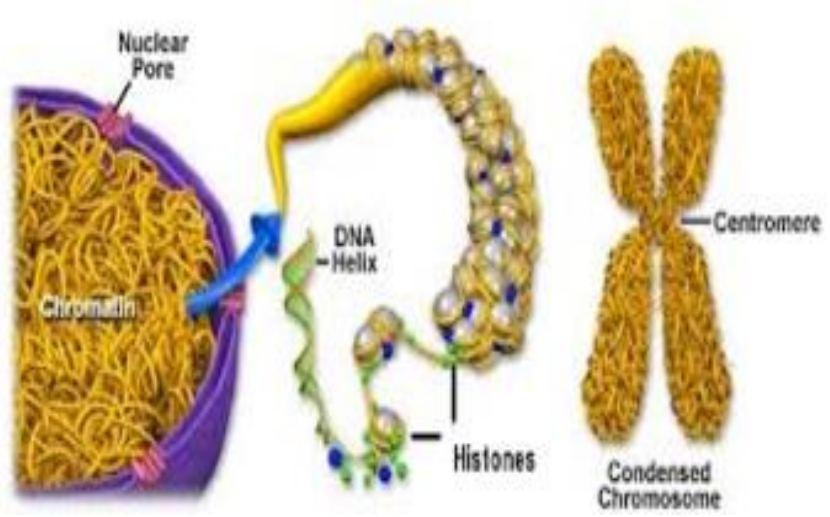


Fig.12. Package of DNA into nucleosomes, then – polynucleosomes, then – to chromatin, and finally – condensation of chromatin into chromosome during prophase

In eukaryotes, all chromosomes are located in the nucleus. A prokaryotic organism usually contains a single circular double-stranded DNA molecule. In addition, most bacterial species also contain small, round, extra-chromosomal DNA molecules called plasmids. Plasmid DNA carries the genetic information and undergoes replication, which may be synchronized with division of chromosomes, or sometimes is not synchronized. The genes of plasmids can make bacteria resistant to antibiotics. Plasmids can also transfer genetic information from one bacterium to another. Plasmids are used in laboratories as vectors in recombinant DNA technology.

Eukaryotic cells face phenomenon termed shortening of telomeres. This means shortening of their linear DNA molecules ends after cell division. İt is due to fact that

DNA replication cannot completed fully in 5'end of the lagging strand. As a result, in most normal somatic cells, telomeres shorten with each division of somatic cells.

Once the telomeres shorten to a certain length, the cell can no longer divide, and is considered senescent. In germ cells and stem cells, as well as in cancer cells, telomeres are not shortened. This is due to the presence of telomerase ribonucleoprotein in them. Telomerase helps to restore telomere length in these cells, and these cells do not age.

RNA – types and structural features.

RNA (Ribo Nucleic Acid) is a single stranded nucleic acid used for synthesis of protein. Three main types of RNA involved in the process of protein synthesis are: rRNA (ribosomal RNA), tRNA (transfer RNA) and mRNA (messenger RNA). They are significantly smaller than DNA. RNA differs from DNA in that:

1) its pentose is ribose instead of deoxyribose in DNA.

2) there is uracil in RNA instead of thymine, so there is no thymine in RNA.

3) the rules of Chargaff are not valid in it, only rule A + C = G + U applies to RNA.

4) Since there is no complementarity, then thymine bases are not equal to adenine, and guanine is not equal to cytosine: T (G ≠ C).

5) The secondary structure of RNA is formed due to the binding of single strand nitrogenous bases in some **sites** according to the principle of complementarity.

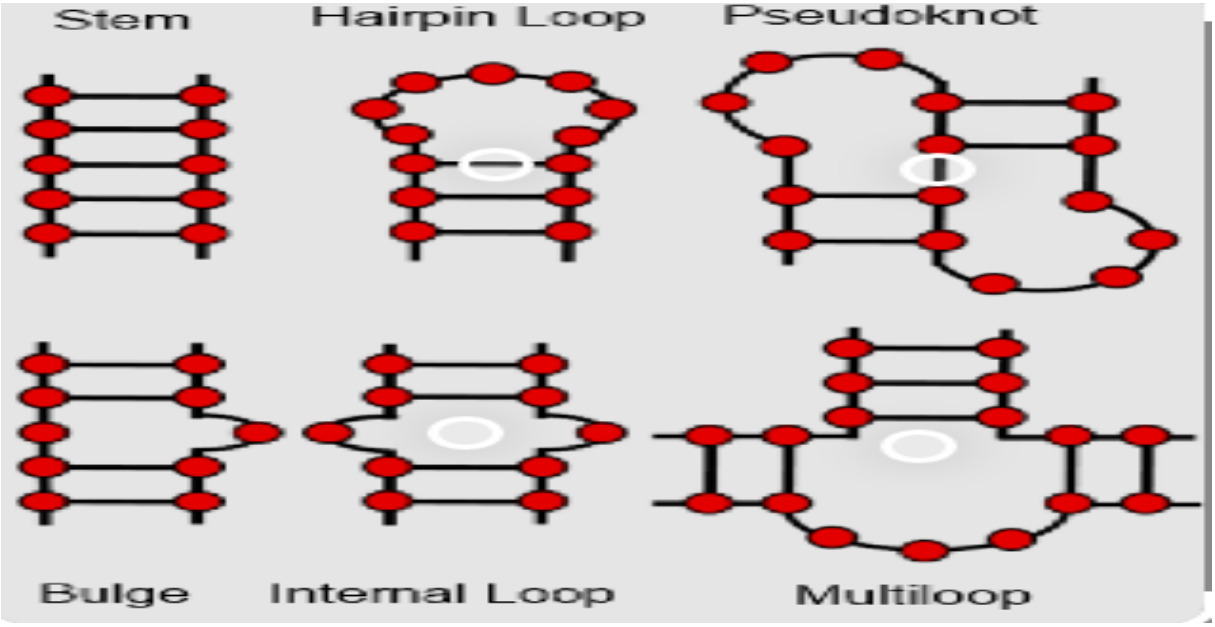


Fig.13. Sticking of chains during the formation of a secondary structure

In addition, for linkage between mononucleotides, besides the 3'5' bond RNA may also have a 2'5' bond. The three main types of RNA also differ from each other in size, functions and special structural modifications.

Transfer RNA (tRNA)

tRNA is located in cytoplasm and is called soluble RNA. tRNAs are the smallest of the three main types of RNA molecules (4S). S is the Svedberg unit, indicating the rate of sedimentation. Together, tRNAs make up about 15% of all RNA in a cell. tRNA contains minor nucleotides more than any other RNA type. For example, tRNA molecules contain a high percentage of unusual, dihydrouracil bases, and have extensive intra-chain base pairing (Fig. 13), which leads to the formation of their characteristic secondary and tertiary structure. tRNA transports amino acids to the ribosome for protein synthesis. Each tRNA carries its specific amino acids covalently attached to its 3rd end. Entering ribosome, tRNA recognizes the genetic mRNA coding sequence. The brought amino acid is linked to growing peptide chain. Each t-RNA can bind only one amino acid. So there are at least 20 tRNAs in the cell.

The secondary structure of tRNA resembles a “clover leaf”. tRNA has 4 loops, the most important of them is the anticodon (Fig.14).

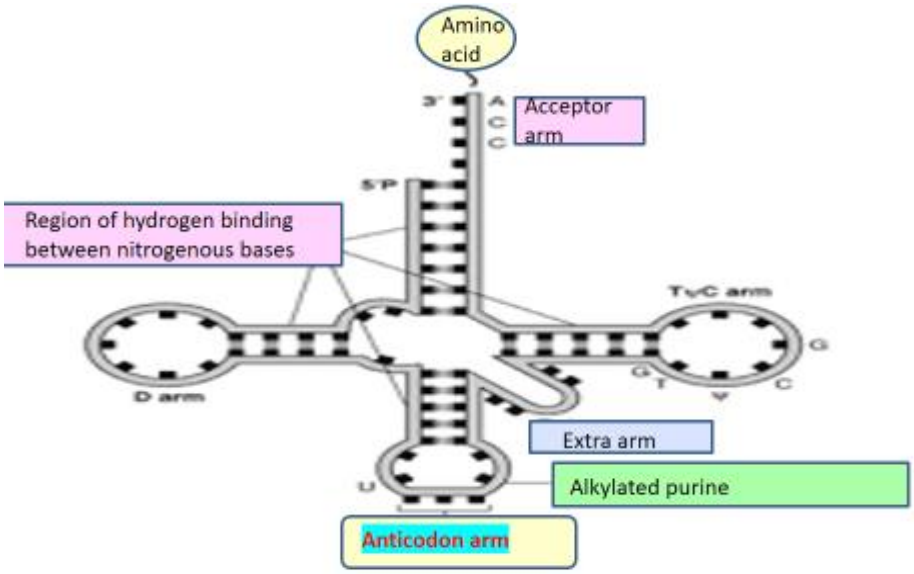


Fig. 14. Secondary structure of tRNA: “clover leaf”. Acceptor and anticodone loops.

At the 3’-end of tRNA is always Adenine, at the 5’-end – Guanine . 3 ’-terminus of tRNA ends with a C-C-A triplet. An amino acid is attached here to the OH group of adenine. Since CCA 3’-end accepts amino acid, this CCA 3’end is called the “acceptor zone”. Anticodon is a sequence of three nucleotide bases. This three nucleotide containing triplet in tRNA determines the type of tRNA, to be correct, the amino acid carried by this tRNA. Anticodon binds to a specific codon on an mRNA. The anticodon triplet corresponds to the mRNA codon on the principle of complementarity.

The tertiary structure of tRNA resembles an elbow bend. This structure is formed by van der Waals forces (Fig.14).

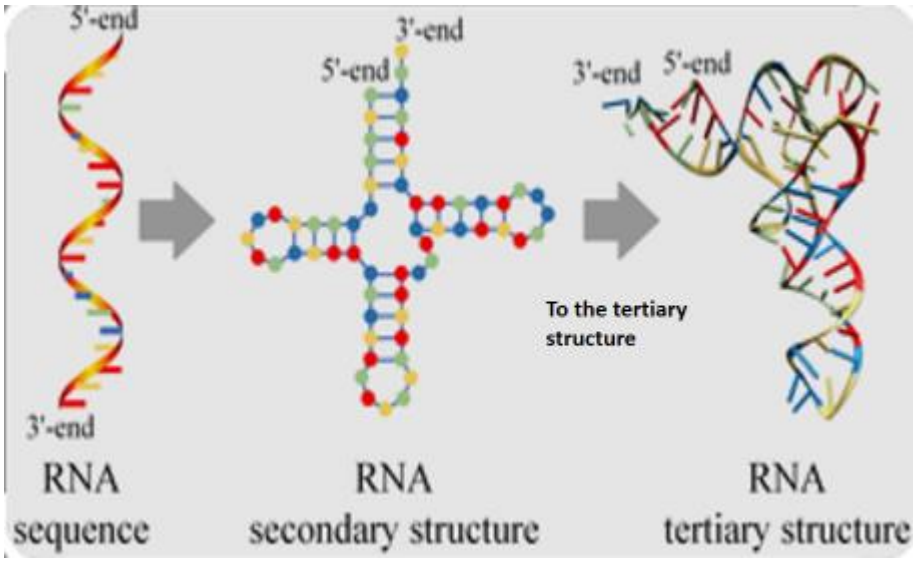


Fig.15. From primary to 3D tertiary structure of t-RNA

Ribosomal RNA (rRNA)

rRNA is the most abundant type of RNAs. Together, rRNA make up more than 80% of all cellular RNA. rRNA accumulates in the ribosome, has the largest molecular weight. In ribosome, rRNAs are in association with proteins and serve as ribosome framework. In prokaryotic cells, there are three types of rRNA of different sizes: 5S, 16S 23S. In eukaryotes, there are four types of rRNA: 5S, 5.8S, 18S, 28S. Some RNAs act as catalysts, such as rRNAs in proteins synthesis. These RNA with catalytic activity are termed *ribozymes.*

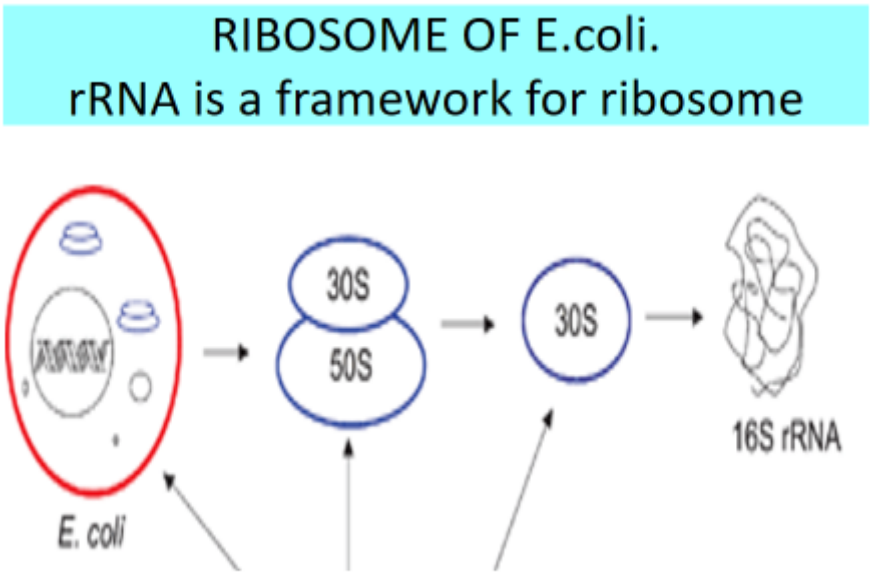


Fig. 16. Ribosome of E.coli

Messenger RNA (mRNA)

mRNA is synthesized on the basis of DNA genes (cistrons) and carries information about the sequence of amino acids that should be included in the newly synthesized peptide chain. If mRNA contains information from more than one gene, it is said to be polycistronic. Polycistronic mRNA is characteristic for prokaryotes. If mRNA carries information from only one gene, it is considered monocistronic and is characteristic for eukaryotes.

Messenger RNA (mRNA) makes up minimum amount of cell RNAs, only 5% of total cell RNAs. İt is the most heterogeneous type of RNA in size and base sequence. mRNA carries the genetic information for protein synthesis. The mRNA consists of the codon triplets (tri-nucleotides). Each triplet encodes certain amino acid. The amino acid sequence of synthesized on ribosome protein is determined by the codon-anticodon relationship. Codon is provided by mRNA, while anticodon– by tRNA carrying an amino acid.

The genetic code on mRNA consists of 61 amino-acid coding codons and three termination codons, which stop the process of translation. Since the genetic code of RNA has multiple codes to the same amino acid, thus it is considered redundant. For example, glycine is encoded by GGU, GGC, GGA, and GGG codons.

For protein synthesis in eukaryotes, mRNA is transferred from the nucleus to the cytosol. In addition to protein coding region, mRNA contains untranslated regions in its 5- and 3-ends. mRNA of eukaryotes has some features. The special structural characteristics includes a long sequence of adenine nucleotides termed poly-A-tail at the 3rd end of the RNA chain. Additionally, mRNA of eucariots comprises a "cap". at its 5'-end. This cap consists 7-methylguanosine attached to rest part of mRNA by an unusual (5→5) triphosphate bond. These features help prevent mRNA cleavage by enzymes.

Synthesis in nuclear matrix

For procreation and development of the organism, DNA is copied and passed on to daughter cells through DNA replication. Reproduction by DNA (RNA) its own analogue is termed replication. The template for replication (and transcription) can be DNA or RNA. According to Watson and Crick model, replication follows semi-conservative path. It proceeds according to the complementarity of nitrogenous bases. So, replication means formation of new DNA (RNA) copy, while transcription is the synthesis of RNA on a DNA template. Both these processes occur in nuclear matrix. As can be seen, the genetic information is transmitted by the mechanism of the template, and the transcriptional template can be DNA and RNA.

DNA synthesis

Reproduction of DNA of its analogue is replication. It proceeds on the basis of complementarity. New DNA is synthesized by DNA polymerase. One of polymyrases is primase, i.e. it synthesizes an oligoribonucleotide primer.

First, helicase uncoils double chain of DNA at the expense of ATP.

DNA topoisomerase changes the number of turns in the DNA helix. So it weakens or strengthens the structure of DNA. At initiation stage of DNA replication, topoisomerase breaks 3'5'phosphodiester bonds in DNA. When replication ends, the same enzyme stitches the break point.
DNA is synthesized from deoxy nucleoside triphosphates (dATP, dGTP, etc.).
DNA replication proceeds in 3 steps: 1) initiation; 2) elongation; 3) termination.
Firstly, the primer-oligo-ribonucleotide is synthesized.

The lead chain is synthesized in the direction of DNA unwinding, i.e. from 5' to 3 '. So, the lead chain is synthesized faster. The second chain is synthesized more slowly - it is a lagging chain, because it is synthesized in the opposite direction in the form of Okazaki fragments. About 200 mononucleotides present in each Okazaki fragment.

For each Okazaki fragment a primer, a short oligo-ribonukeotide, is also synthesized. Here the synthesis also occurs in the direction of 5'3 '.
Termination stage: replication stops after the formation of a complete complementary to the DNA template copy.

Mutations mean any change in the DNA. We can refine that definition: a mutation is a change in the DNA base sequence, that results in a change of amino acids in the polypeptide, coded by that gene.

Spontaneous DNA damage are:

1) replication errors

2) **depurination**

3) **deamination,** when adenine turns into hypoxanthine, guanine - into xanthine, cytosine - into uracil.

Damages, which are results of physical and chemical factors, are:

1. Alkylation of nitrogenous bases. At this process the formation of **methyl guanine**, methyl adenine occur.

2. Dimerization of pyrimidines under the UV radiation. 2 neighboring pyrimidine form a dimer called **thymine dimers**.

Reparative enzymes change the "mistaken" nucleotides to the "healthy" ones.Their insufficiency leads to severe diseases, for example xeroderma pigmentosum. Violation of reparative system breaks renovation of damaged DNA. Reparative, antimutagenic enzyme system includes:

- DNA **exonuclease**, which cleaves the damaged area.
- Uracil-DNA-**glucosidase**, which **cleaves uracile** from deoxyribose.
- β- **DNA polymerase**

  * **DNA ligase**.

Severe sunburn after only a few minutes in the sun, freckling in sun exposed areas, dry skin, changes in skin pigmentation are only few symptomes of xeroderma pigmentosum disease. Its severe form is called De Sanctis-Kakkone. These patients do not have ***DNA-nuclease*** to break off the erroneous base.

Addition, deletion, or addition of nucleotides can alter the polypeptide. Point mutations are the result of the substitution of a single base**.** Addition or deletion of one or more bases leads to shifted reading frame of the gene, and is called **frame-shift mutations**.

Alleles are alternate sequences of DNA bases (genes), and thus at the molecular level

the products of alleles differ (often by only a single amino acid, which can have a ripple effect on an organism by changing).

Transcription as the stage of protein biosynthesis.

The process of RNA synthesis on the DNA template (or DNA on RNA template) is termed transcription. Transcription is the first step of genetic information expression. Transcribed DNA is located in the fragment between the nucleosomes, or on the linear nucleosome. Globular nucleosome DNA is always inactive.

A transcribed region of DNA is termed transcripton. At the beginning of the transcripton, there is a DNA region, called promoter, that binds RNA polymerase. At the end of the transcript there is a terminator site. It stores information about the end of transcription. The informative section of transcription is called cystrone. Additionally, an informative site of cystron is called exon, while an uninformative one is called intron. The uninformative site, an intron, does not store genetic information.

The acceptor, or regulatory zone, is a following the promoter zone site. It is sensitive to regulatory factors. In prokaryotes, this zone is called the **operator** zone and binds the **repressor,** which stops the transcription of the protein. But **enhancer zone** is an **inductor zone** and enhances transcription rate.

RNAs are synthesized on DNA cistrons according to the principle of complementarity. RNA synthesis is produced by RNA polymerase, or transcriptase. RNAs are produced by RNA-polymerases, or trancriptases. First, immature pre-r RNA, pre-m RNA and pre-t RNA are formed on DNA template. RNA-polymerase I synthesizes rRNA, RNA-polymerase II synthesizes mRNA, while RNA-polymerase III synthesizes tRNA. When DNA is synthesized on RNA, it is termed reverse transcription. **Reverse**

transcriptase (”revertase”) synthesizes DNA on RNA templet. It is also called “RNA-dependent DNA polymerase”. Revertase occurs in oncoviruses, tumor cells.

Reverse transcriptase synthesize double-stranded DNA on the template of the RNA of retroviruses. This enzyme first makes RNA:DNA hybrid by synthesis of complementary to RNA strand of DNA. There is also **RNA replicase**, that synthesizes RNA on an RNA template.

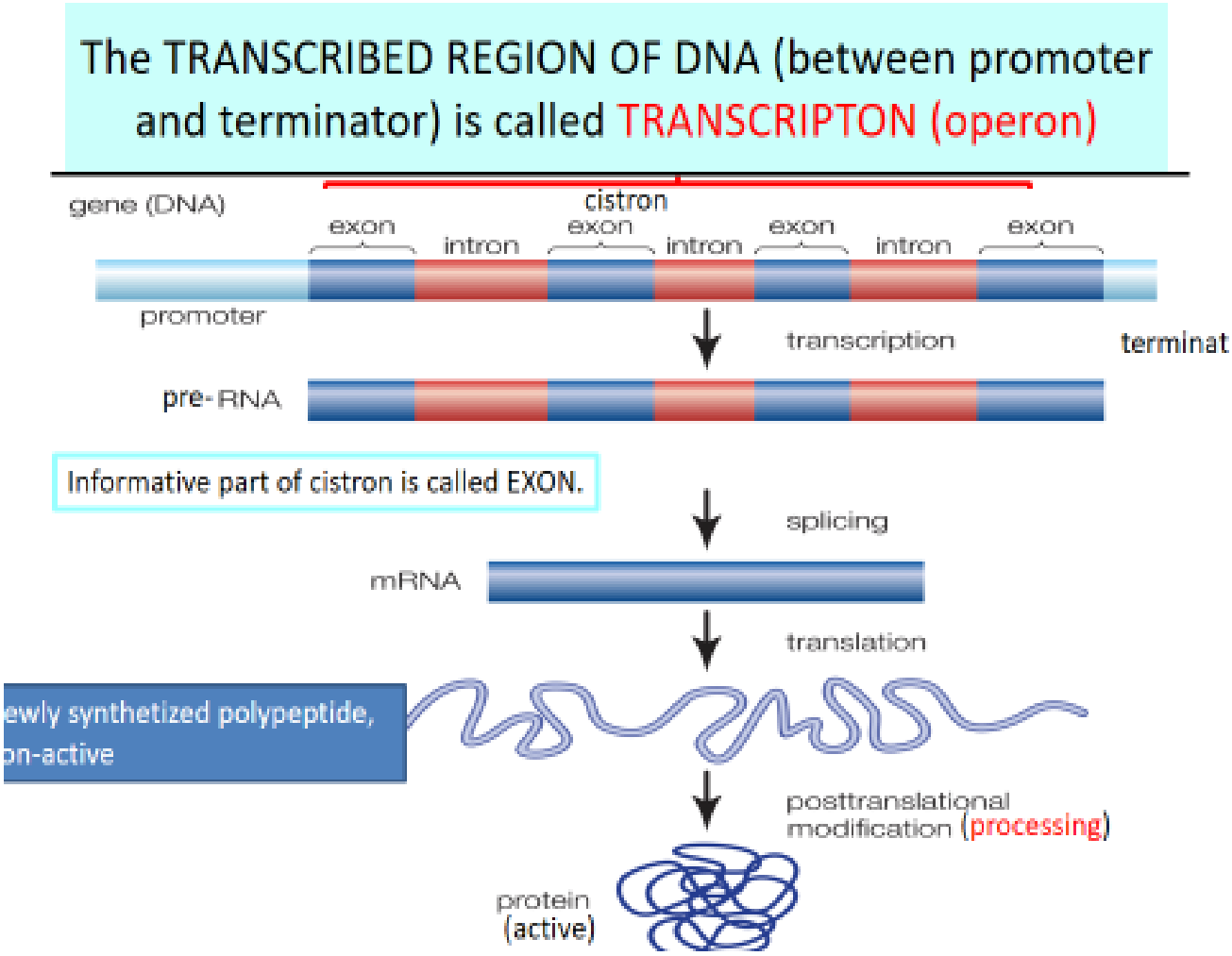


Fig.17. Trancribed mRNA is further used as a template for protein synthesis

Transcription proceeds in 3 steps: 1) initiation, 2) elongation, 3) termination.

As all polymerases, RNA polymerase moves in the direction of 5'3 ', i.e. in the direction of unwinding. In the synthesis of NA, as the primary material are used nucleotides in the form of 5'-triphosphate.

The synthesis of all RNA begins with purine, i.e. adenine or guanine.

As always in newly synthesized RNA, 3'- end remains with free OH-, while 5' end has triphosphate. Depending on the gene, the final product of gene expression may be protein (only 2% of DNA encodes proteins) or RNA. Initially, RNA transcripts are exact copies of one of the two DNA strands, but later they undergo various modifications. These modifications include removal of non-informative segments - introns. This will convert the inactive primary transcript into functionally active RNA.

Post-transcriptional processing of RNA includes 4 changes in pre-RNA:

1) Cleavage of non-informative **introns** from pre-RNA.

2) **splicing of exons** by RNA **ligases**.

3) polyadenylation of the 3'OH end.

4) Keeping of 5'end – this step increases the resistance of RNA to nucleases. At keeping, a 5'-5'phosphodiester bond is formed with the methyl GTP.
Mature RNA is transferred from the nucleus to the cytoplasm. The protein, called informer, helps to transfer mRNA from nucleus to the cytoplasm.

Translation (protein synthesis)

For protein synthesis, first mRNA should be synthesized. Information of the protein structure is saved in DNA nucleotides. Cistron is a structural DNA gene, that stores information about amino acid sequence in protein. Information from DNA gene (cistoron) is transferred to transcribed RNA. Transcribed RNA is used in translation (protein synthesis). During translation, the nucleotide sequence of messenger RNA molecules is transferred to the protein structure. Ergo, protein synthesis proceeds in 4 stages:

1) Transcription

2) Amino acid activation and recognition

3) Translation:

A) initiation, B) elongation, C) Termination.

4) Post-translational processing.

Since translation is the transfer of the code contained in RNA to the translated protein, protein synthesis occurs in accordance with the sequence of trinucleotides in RNA. Codons are universal, i.e. they act in all living organisms.
The initiation codon is the codon of the first amino acid. In eukaryotes, this is the N-terminal methionine, in prokaryotes - formyl-methionine.

Amino acid is activated in 2 stages:

1st stage:

$$\text{amino acid} + \text{ATP} \xrightarrow{Mg^{2+}} \text{aminoacyl adenylate}$$

2nd stage:

the formation of aminoacyl-tRNA, i.e. binding of amino acid to 3 ’ OH end of tRNA.

The initiation of protein synthesis begins with the assembly of components

for translation. These components include: two subunits of the ribosome, mRNA, aminoacyl-tRNA, GTP that provides energy for the process, and

initiation factors. İn prokaryotes, three initiation factors are known: IF-1, IF-2 and IF-3, whereas eukaryotes have many initiation factors designated eIF. Letter “e” is added to indicate eukaryotic origin of initiation factor.

The mRNA program is always read from the 5'end to the 3'end.
Synthesis begins with a **GUG and AUG** triplet. Therefore, GUG and AUG are called “initiating codons”.

Aminoacyl center, called A-center, is the binding site of an aminoacyl-tRNA with a ribosome.

Amino acid brought by tRNA is placed in a hole of A-center. Near the A-center is located peptidyl center, or P-center. First amino acid, formyl methionine enters directly the peptidyl center after the formylation.
Elongation proceeds in 3 stages from the N-end to the C-end:

1) Recognition of the codon, which leads to the connection of aminoacyl tRNA with mRNA.

2) Transpeptidation – formation of peptide bond.

3) Translocation, when mRNA moves 1 triplet. At translocation tRNA leaves P-zone, mRNA moves on 1 codon and peptidyl-tRNA moves from the A-zone to the P-zone.
Termination occurs when one of these three termination codons moves into the A

site. Termination codons are UAA, UGA, UAG. After the termination codon enters the A site, a releasing factor binds to the site, stopping translation and releasing the ribosomal complex and mRNA.

Each peptide bond is formed at the expense of 4 high energy bonds (2ATP, 2GTP). Despite the fact that the peptide bond contains only 21 kJ of energy, 140 kJ of energy is invested on its formation.

Post translational processing of polypeptide chain

Post-translational modification of protein, or processing includes following steps:

1) Deformylase cleaves formyl from formylmethionine, the first amino acid included into peptide chain during synthesis on ribosome. In eukaryotes, peptidase removes methionine from newly synthesized peptide. The N-terminal amino acid is acetylated - this is a modification of the N-end.

2) Modification of radicals: addition of phosphate, carboxyl, hydroxyl, acetyl, glycosyl groups, $I_{2.}$ to proteins if required.

In phosphoproteins, phosphate binds to serine and threonine.

Gamma-carboxyl group binds with glutamic or aspartic acids in synthesis of bone proteins..

Lysine and proline are hydroxylated in collagen.

Carbohydrates are associated with serine, threonine, asparagine in membrane proteins.

3) Disulfide bridge is formed between cystein amino acids, for example in insulin.

4) a prosthetic group is associated with its apoenzyme to form holoenzyme.

5) During partial proteolysis from procollagen forms collagen, from proinsulin - insulin, proparat-hormone - parat-hormone.

Regulation of protein biosynthesis, the effect of activators and inhibitors on biosynthesis.

Proteins, that are continuously synthesized in a cell, are called constitutive; for them, an inductor is not needed. And adaptive, or inductive proteins are synthesized depending on the conditions of life, if the inductor presents in the cell. An inductor is a substance, that enhances protein synthesis.

Sample of anabolic drugs are analogues of male hormones. Hypoxanthine - riboside and potassium ororate are non-hormonal anabolics. Antibiotics are inhibitors of protein synthesis.

Regulation of protein synthesis

Some chemicals are used for regulation protein synthesis. Protein synthesis inducers are used to restore between a weakened body or for regeneration, treatment of injuries. This group of drugs belongs to anabolics (activators of protein synthesis). Steroid and non-steroidal anabolics are distinguished. Anabolic steroids include methandrostenolone, phenobolin, retabolil, methylandrostenediol, silabolin. These drugs are powerful stimulators of protein synthesis, but their negative side effect is a steroid effect on metabolism and a shift in hormonal levels towards masculinization. Abuse of steroids is hepatotoxic, promotes bile stasis and hepatomas. The good news is that the disturbances are reversible upon discontinuation of anabolic steroids. Suppression of the hypothalamic-adrenal axis leads to changes in the adrenal glands, which are also reversible. Rare kidney side effects lead to acute kidney failure and even Wilms' tumor. Insulin and growth hormone also have anabolic effects, but insulin causes hypoglycemia, and growth hormone after puberty causes acromegaly. Potassium orotate and inosine are non-steroidal and non-hormonal anabolics. Orotic

acid and inosine serve as precursors for the synthesis of purines. Branched-chain amino acids, namely valine, leucine and isoleucine, especially leucine are known to specifically stimulate muscle protein synthesis and to decrease catabolism in this tissue.

İnhibitors of protein synthesis disrupt the generation of new proteins. By this way they stop or slow growth and proliferation of cells. They affect transcription, translation, recognition processes. For example, amanitin is a poison of a fungus that stops transcription. Antitumor medications, such as vinblastine, vincristine, 5-fluorouracil and some antibiotics (rifampicin, actinomycin D, olivomycin) also inhibit transcription. Trimtylmethane, puromycin inhibit translation.

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