Translation, Replication, Transcription

PROTEIN SYNTHESIS

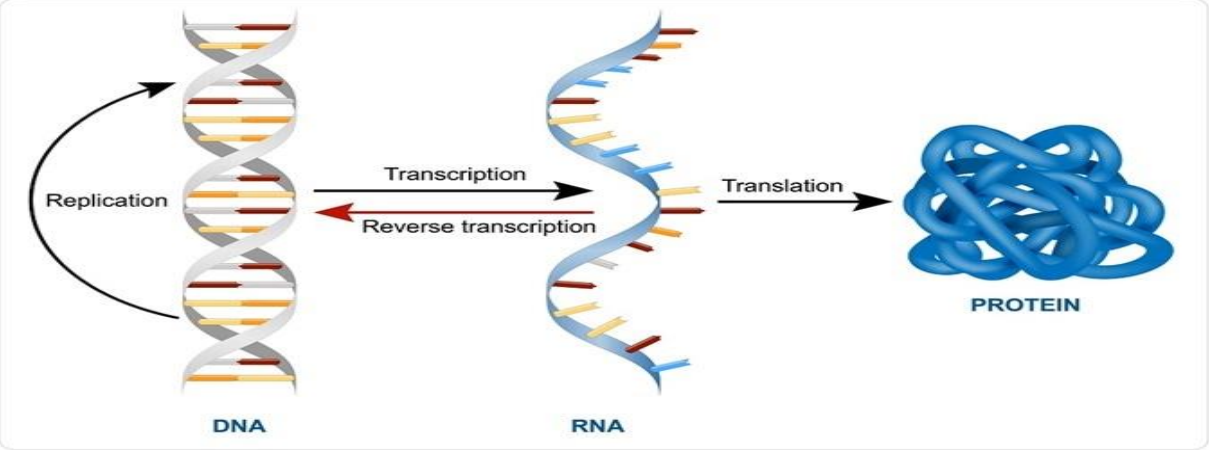

Fig.1. Stages of protein synthesis

Synthesis of protein (translation) proceeds in 4 stages:

1) Transcription

2) Amino acid activation and recognition

3) Translation: A) initiation, B) elongation, C) Termination.

4) Post-translational processing.

Cystrone is a structural DNA gene, which stores information about amino acid sequence in protein.

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.

rRNA is the ribosome framework.

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.

Brought tRNA amino acid is placed in a hole of A-center. Near the A-center is located peptidyl center, or P-center. 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 - 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.

When the codon in the A site is a termination codon, a releasing factor binds to

the site, stopping translation and releasing the ribosomal complex and mRNA.Termination is encoded by codons UAA, UGA, UAG.

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 expended on its formation.
Post-translational modification, or processing includes following steps:

1) Deformylase cleaves formyl from formylmethionine. 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.

Phosphate binds to serine and threonine.
Gamma-carboxyl group binds with glutamic or aspartic acids.

Lysine and proline are hydroxylated.

Carbohydrates are associated with serine, threonine, asparagine.

3) Disulfide bridge is formed between cystein amino acids.

4) a prosthetic group is associated with its apoenzyme.

5) partial proteolysis from procollagen forms collagen, from proinsulin - insulin, proparat-hormone - parat-hormone.
Regulation of synthesis:

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.

DNA SYNTHESIS

1. Reproduction of DNA of its analogue is replication. It proceeds on the basis of complementarity.
2. Transcription is the synthesis of RNA on DNA. When DNA is synthesized on RNA, it is called “reverse transcription”.
3. Translation is the transfer of information from NA to the structure of a polypeptide chain of protein.

Genetic information is transmitted by the mechanism of the matrix.

The transcriptional template can be DNA and RNA.

According to Watson and Crick theory, replication follows a semi-conservative path.

DNA polymerase synthesizes DNA. One of polymyrases is primase, i.e. it synthesizes an oligoribonucleotide primer.

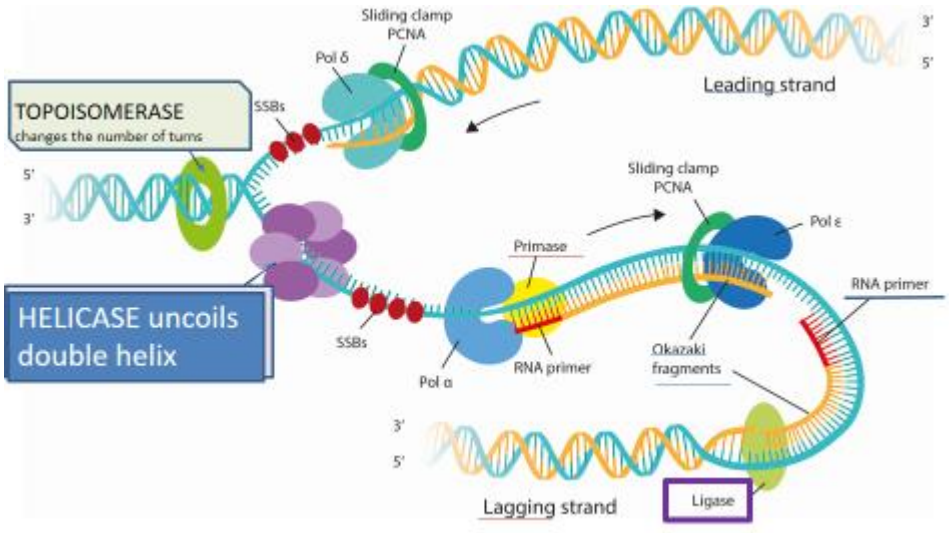

Fig.2. Scheme of replication

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 of replication DNA, 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, ...).

DNA replication proceeds in 3 steps: 1) initiation; 2) elongation; 3) termination.

Firstly, the primer-oligo-RN is synthesized.

The lead chain is synthesized in the direction of DNA unwinding, i.e. 5' 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:

Replication stops after the formation of a complete complementary to the DNA template copy.

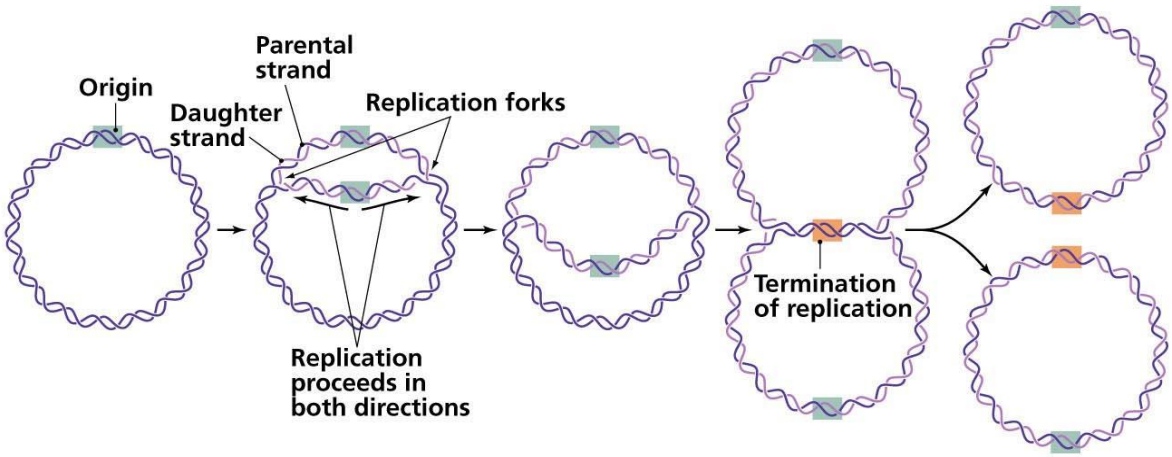

Fig. 3. Separation of daughter strand from parental after termination.

TRANSCRIPTION. RNA SYNTHESIS

Transcribed DNA is located in the fragment between the nucleosomes, or on the linear nucleosome. Globular nucleosome DNA is always inactive.

RNA synthesis is produced by RNA polymerase, or transcriptase. RNA polymerase I synthesizes rRNA, RNA polymerase II - mRNA, RNA polymerase III- tRNA. First are formed pre-rRNA, pre-mRNA and pre-tRNA.
A transcripton is a transcribed region of DNA.

At the beginning of the transcript, 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. TRANSCRIPTON (operon) the is transcribed region of DNA (between promoter and terminator).

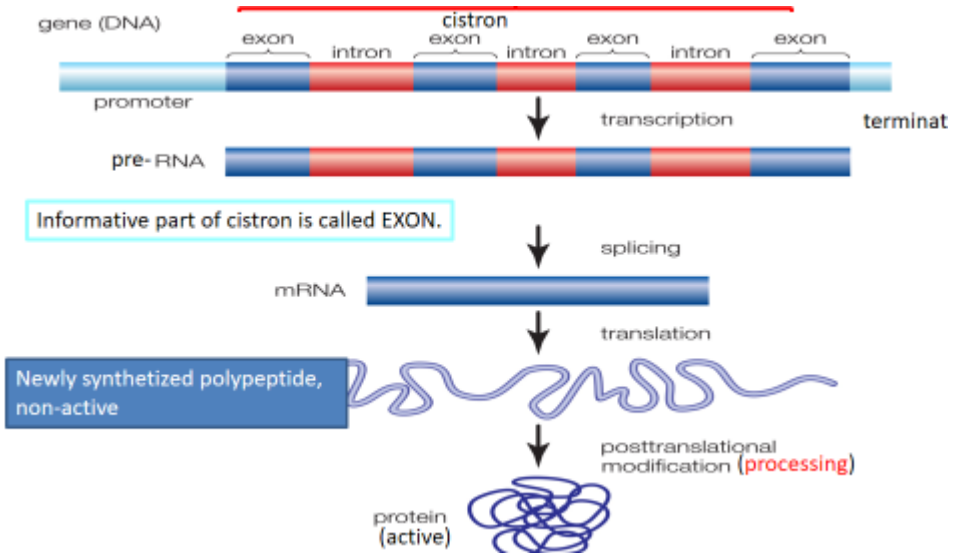

Fig. 4. Transmission of information about the protein structure from transcripton

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.
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.

RNA synthesis also occurs 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. As always, 3'- end remains with free OH-, while 5' end has triphosphate.

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

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.

Reverse transcriptase ("revertase") synthesizes DNA on RNA templet. It is

also called “RNA-dependent DNA polymerase”. Revertase occurs in oncoviruses, tumor cells.

There is also **RNA replicase**, that synthesizes RNA on an RNA template.

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.

XERODERMA PIGMENTOSUM- disease based on insufficiency of DNA reparative enzymes. These patients do not have ***DNA-nucleases*** to break off the error nitrogenous bases. 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 this disease. Its severe form is called De Sanctis-Kakkone.

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).