



Expression of eukaryotic genes. The structure of eukaryotic genes. Organization of genomes, as well as genetic elements that regulate gene expression in pro- and eukaryotes. The role of RNA (rRNA, mRNA, tRNA, U-family RNA, 7S-RNA, etc.) in gene expression.

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Subject: Genetic engineering

(Lecture 6)

Lecture Goal:

To examine the organization of genomes and the genetic elements that regulate gene expression in both prokaryotes and eukaryotes, along with the role of various types of RNA in gene expression.

Tasks:

1. Compare the genome organization in prokaryotes and eukaryotes, emphasizing the differences in regulatory genetic elements.
2. Discuss the role of various types of RNA (rRNA, mRNA, tRNA, U-family RNA, 7S-RNA) in the process of gene expression.
3. Analyze how these RNA molecules contribute to transcription, translation, and post-transcriptional regulation in gene expression.

Keywords: *Genome organization, gene regulation, prokaryotes, eukaryotes, rRNA, mRNA, tRNA, U-family RNA, 7S-RNA, transcription, translation, post-transcriptional regulation*

Summary

1. Genome organization

- ❑ Prokaryotic genomes are usually single circular chromosomes; genes often organized in **operons**.
- ❑ Eukaryotic genomes are larger, linear, contain introns and exons, regulatory sequences, and chromatin structure.

2. Gene regulation

- ❑ In prokaryotes: regulation is mainly at the transcriptional level (operons, repressors, activators, inducers).
- ❑ In eukaryotes: regulation is complex, occurring at **transcriptional**, **post-transcriptional**, **translational**, and **post-translational** levels.

3. RNA types

- ❑ **rRNA**: forms the core of ribosomes and catalyzes protein synthesis.
- ❑ **mRNA**: carries the genetic code from DNA to ribosomes.
- ❑ **tRNA**: delivers amino acids to ribosomes during translation.
- ❑ **U-family RNA (snRNA)**: involved in pre-mRNA splicing in eukaryotes.
- ❑ **7S-RNA**: part of the signal recognition particle for targeting proteins to membranes.

4. Transcription and translation

- ❑ Prokaryotes: transcription and translation are coupled; RNA polymerase synthesizes mRNA directly from DNA.
- ❑ Eukaryotes: transcription occurs in the nucleus; mRNA undergoes capping, splicing, and polyadenylation before translation in the cytoplasm.

5. Post-transcriptional regulation

- ❑ Includes **RNA splicing**, **RNA editing**, **RNA stability**, and **microRNA-mediated regulation**.
- ❑ Ensures precise control of gene expression in eukaryotic cells.

Key questions

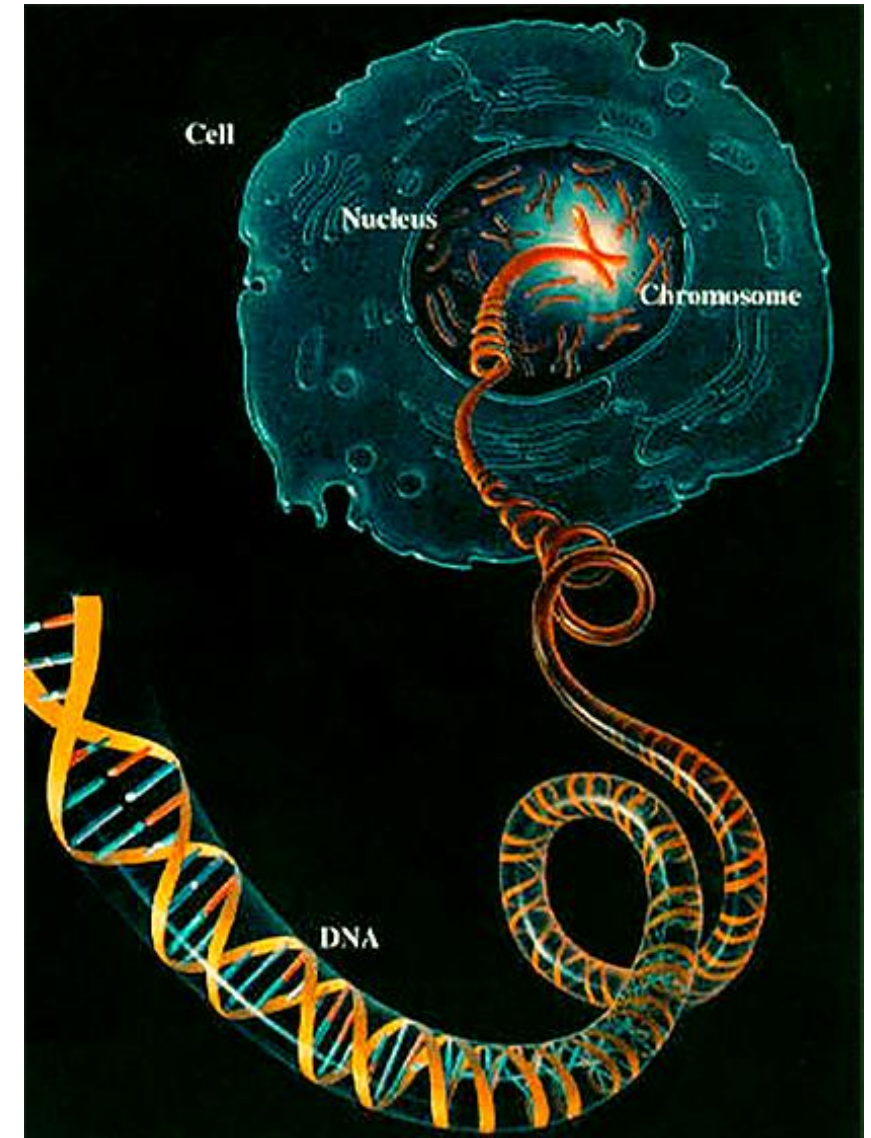
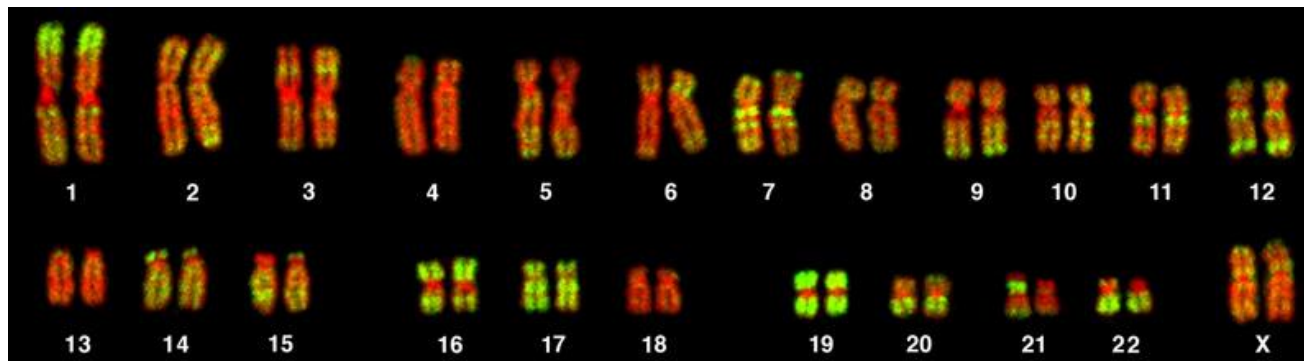
1. How do prokaryotic and eukaryotic genome organizations differ?
2. What are the main regulatory mechanisms of gene expression in prokaryotes? In eukaryotes?
3. What are the functions of rRNA, mRNA, tRNA, U-family RNA, and 7S-RNA?
4. How does transcription differ between prokaryotes and eukaryotes?
5. What is **post-transcriptional regulation**, and why is it important in eukaryotes?
6. How do operons facilitate gene regulation in prokaryotes?
7. Give examples of regulatory RNA molecules in eukaryotes and their functions.

What is genome?

✓ **A GENOME** IS ALL THE GENETIC INFORMATION OF AN ORGANISM

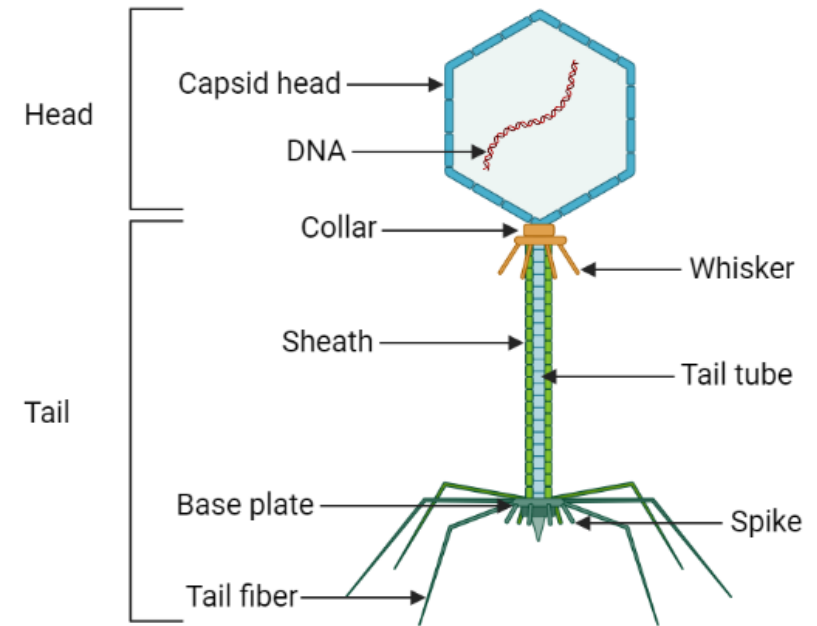
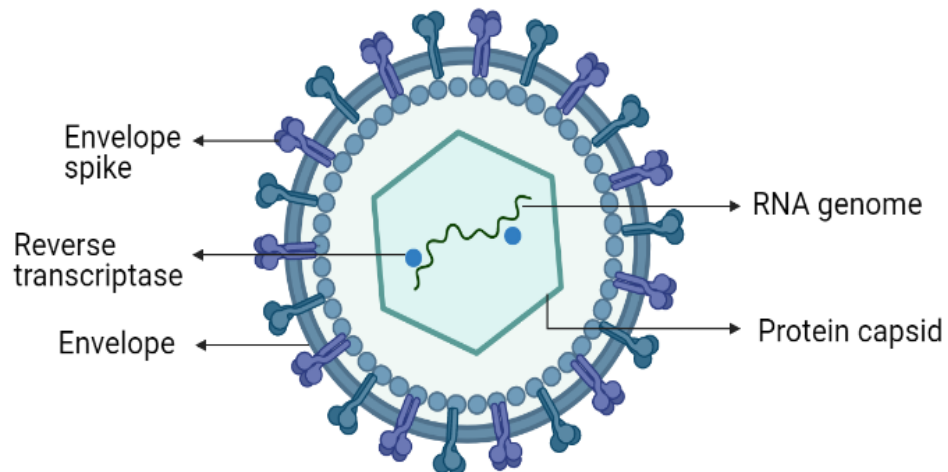
- It provides all of the information the organism requires to **function**.
- In living organisms, the genome is stored in long molecules of DNA called **chromosomes**.
- In eukaryotes, each cell's genome is contained within a membrane-bound structure called the **nucleus**.
- Prokaryotes, which contain no inner membranes, store their genome in a region of the cytoplasm called the **nucleoid**.
- The study and analysis of genomes is called **genomics**.

Note: There are 23 pairs of chromosomes in the human genome. Between 1990 and 2003, all twenty-three pairs were fully sequenced through an international research undertaking known as the Human Genome Project.



Organization of genomes

- ✓ The term "genome" was proposed by G. Winkler in 1920 to describe the totality of genes contained in the haploid set of chromosomes of organisms of the same biological species.
- ✓ The genome includes the entire set of DNA molecules of the cell (in the case of a number of viruses, they speak of genomic RNA).



- **Features:**

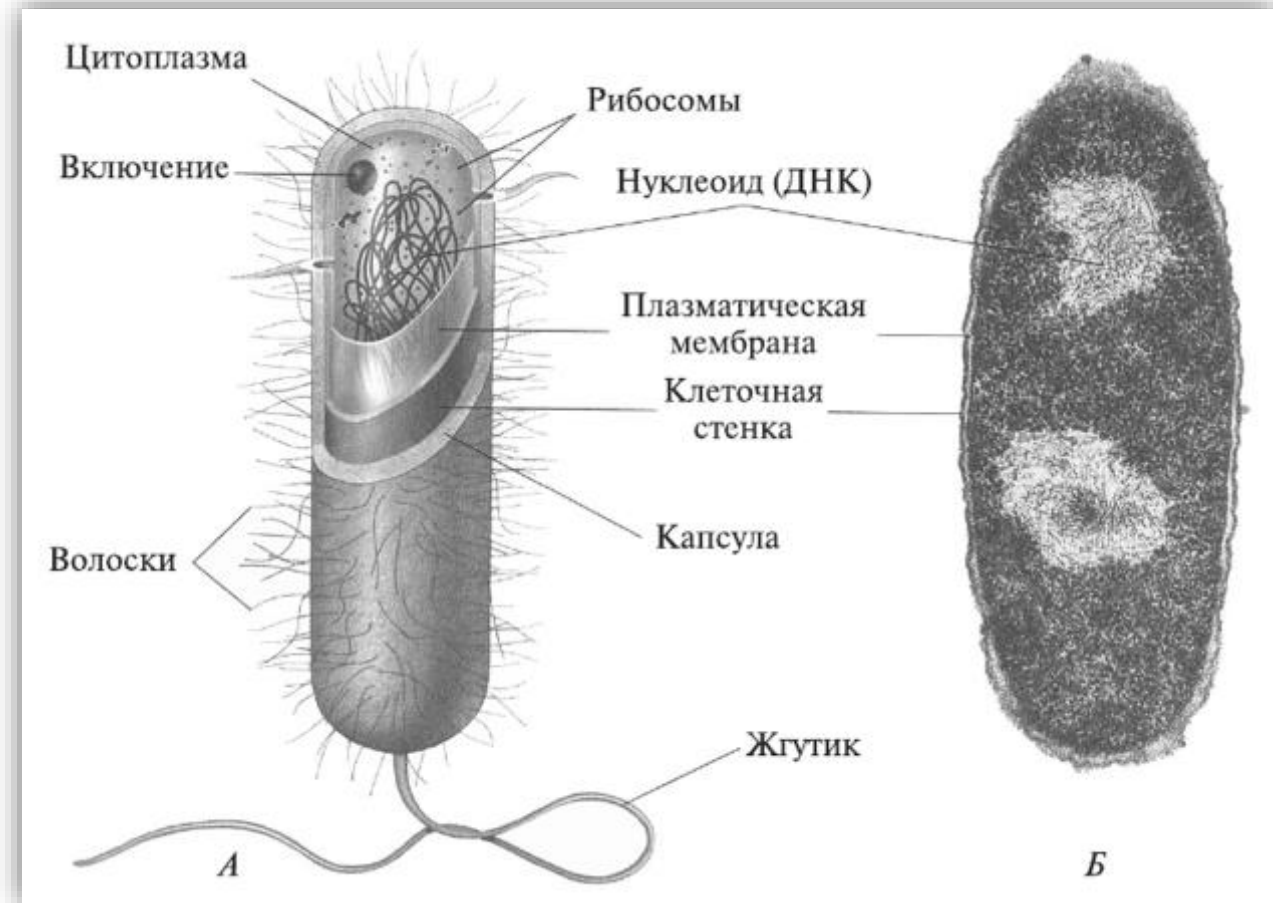
1. *Smaller size*
2. *Diverse genome types* (DNA or RNA; Single-stranded (ss) or double-stranded (ds); Positive-sense (+) or negative-sense (–) RNA)
3. *different physical forms* (Linear or circular; Segmented or non-segmented)
4. *Compactness and gene density* (Overlapping genes; Polyprotein strategy; Alternative splicing)
5. *High mutation rates* in RNA viruses
6. *Lower mutation rates* in DNA viruses
7. *Recombination and reassortment*

Prokaryotic genome

- **Features:**

1. *Small genome size (0.5–10 Mb).*
2. *Circular DNA is most common, often with plasmids.*
3. *Haploid, with a single copy of each gene.*
4. *High gene density, with few non-coding sequences.*
5. *Operon structure for coordinated gene expression.*
6. *Horizontal gene transfer is a key factor in evolution.*
7. *Rapid replication with a single origin of replication.*
8. *No nucleus; the DNA is found in the nucleoid.*
9. *Mobile genetic elements such as transposons are common.*

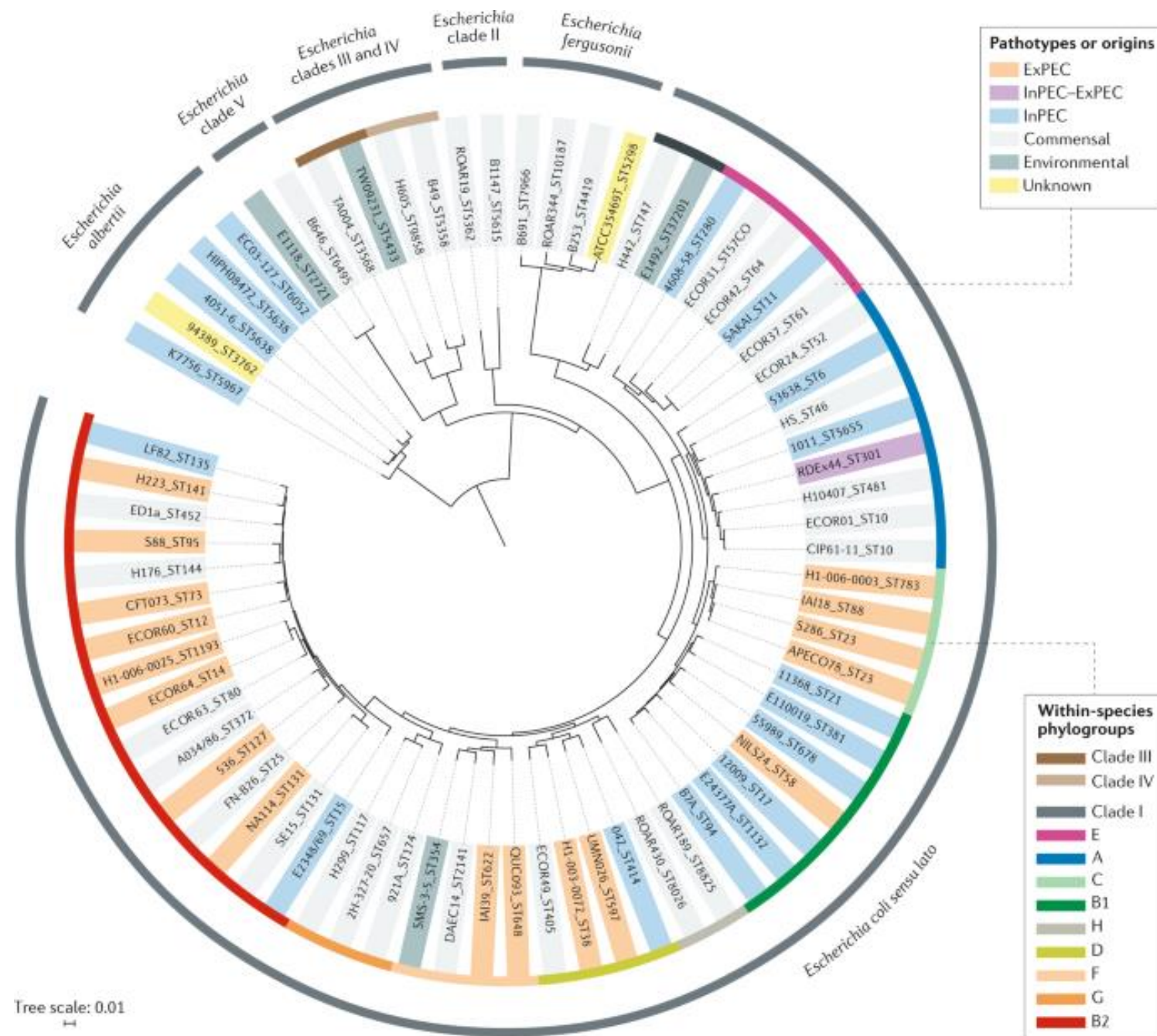
In 1956, F. Jacob and E. Volman proposed a ring model for the organization of bacterial chromosomes.





The genome size is 4.6 million bp.

Most of the **prokaryotic genome** (usually 80-90%) is made up of sequences encoding proteins and RNA



Organisms		Source	Genome size (Mb)	Total genes ¹
Phage/viruses, organelle				
1	mitochondrion	Human mitochondrion	16.6 x10 ³	40
2	SV40	Simian virus 40	5.2 x 10 ³	8
3	TMV	Tobacco Mosaic Virus (RNA)	6.4 x10 ³	4
4	HIV	AIDS virus (RNA)	9.3 x10 ³	10
5	adenovirus 2	Human adenovirus	35.9 x10 ³	11
6	λ phage	lysogenic phage	48.5 x10 ³	50
7	T4 phage	DNA virus E. coli	169 x10 ³	300
Bacteria				
8	Mycoplasma genitalium		580 x10 ³	470
9	Rickettsia prowazeki		1.11x10 ⁶	834
10	Streptococcus pyogenes		1.85 x10 ⁶	1752
11	Bacillus subtilis		4.21 x10 ⁶	4100
12	Escherichia coli		4.64 x10 ⁶	4288
13	Mycobacterium tuberculosis		4.41 x10 ⁶	3924
14	Helicobacter pylori		1.668 x10 ⁶	1590 ¹
			1.644 x10 ⁶	1495 ¹
Eucaryotes				
15	Saccharomyces cerevisiae	budding yeast	12 x10 ⁶	5885
16	Caenorhabditis elegans	worm, Nematoda	97 x10 ⁶	19100
17	Arabidopsis thaliana	plant, Angiospermae	120 x 10 ⁶	25500
18	Drosophila melanogaster	fruit fly, Diptera	165 x10 ⁶	13000
19	Mus musculus	mouse	2.5 x10 ⁹	30000
20	Homo sapiens	humans	3.2 x10 ⁹	31000

¹ Two strains isolated from two geographically separated regions of USA (32).

Eukaryotic genome



- The **eukaryotic genome** is much more complex than that of prokaryotes.
- The **genetic apparatus** of a eukaryotic cell is isolated in the form of a cell nucleus, inside which the main carriers of heredity, the chromosomes, are located.
- The **number of chromosomes** is species-specific and ranges from two (horse roundworm) to a thousand (lower plants).

Organism	Chromosomes	Estimated genome size	Estimated gene number	Estimated gene density gene/ base pairs
Homo Sapiens (human)	46	290×10^6	30 000	1 / 100 000
Mus musculus (mouse)	40	250×10^6	30 000	1 / 100 000
Drosophila melanogaster (fruit fly)	8	180×10^6	13 600	1 / 9000
Arabidopsis thaliana (plant)	5	125×10^6	25 500	1 / 4000
Caenorhabditis elegans (roundworm)	6	97×10^6	19 100	1 / 5000
Saccharomyces cerevisiae (yeast)	16	12×10^6	6 300	1 / 2000
Escherichia coli (bacteria)	1	4.7×10^6	3 200	1 / 1400
Haemophilus influenzae (bacteria)	1	1.8×10^6	1 700	1 / 1000

2.5.1 Human-mouse genome sequence comparisons

The C-paradox is the lack of correlation between the physical size of the genome and the complexity of organisms.

Reasons for redundancy:

1. Large size of genes (due to the presence of introns).
2. Presence of repeated sequences. Both genes and non-coding regions are repeated.
3. The presence of a large number of non-coding sequences

Disadvantages of "excessive" DNA: - increase in DNA synthesis time; - it is more difficult to organize DNA duplication; - high energy intensity - for 1 nucleotide to be included in the DNA chain, you need to spend ~ 60 ATP molecules.
Indeterminate consequence: - due to the dependence of the size of the nucleus on the amount of DNA, an increase in the size of the cell occurs.

Pluses of "excessive" DNA: - it becomes possible to create a complex regulatory apparatus that allows you to raise the body to a higher evolutionary level.

Organization of genomes:

❖ **Genes:**

- Genes are segments of DNA that carry the information for synthesizing RNA and proteins. Proteins are key components responsible for carrying out cellular functions.
- Genes contain coding sequences (**exons**) and non-coding sequences (**introns**), which are removed during RNA processing.

❖ **Regulatory Elements:**

- ❖ **Promoters** and **enhancers** are DNA regions that regulate gene activity. Promoters determine where and when a gene will be active, while enhancers boost the transcription of genes.

❖ **Non-coding Sequences:**

These regions of DNA do not code for proteins but may play important regulatory and structural roles. For example, they can include sequences that regulate gene expression or regions responsible for maintaining chromosome structure.

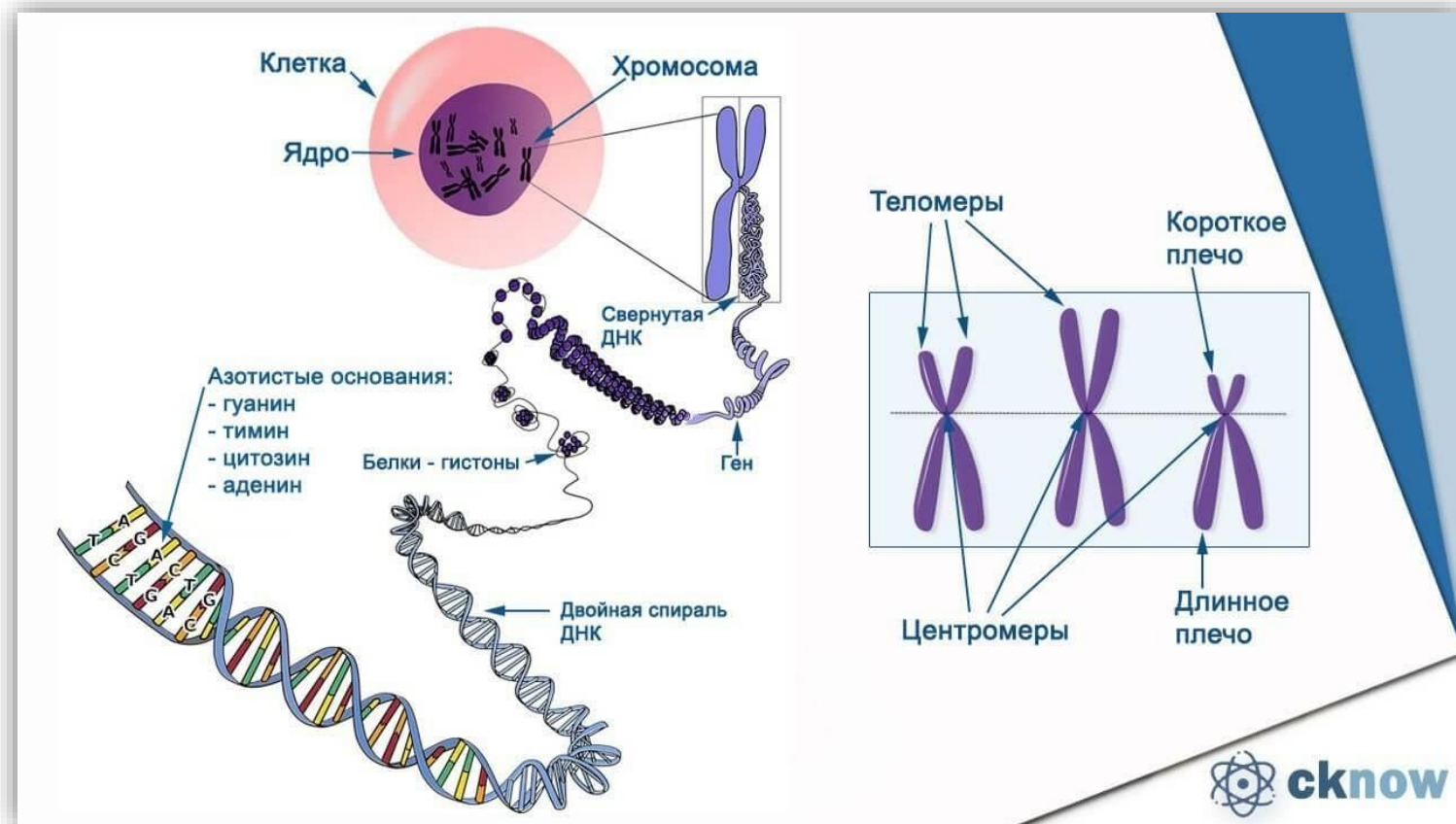
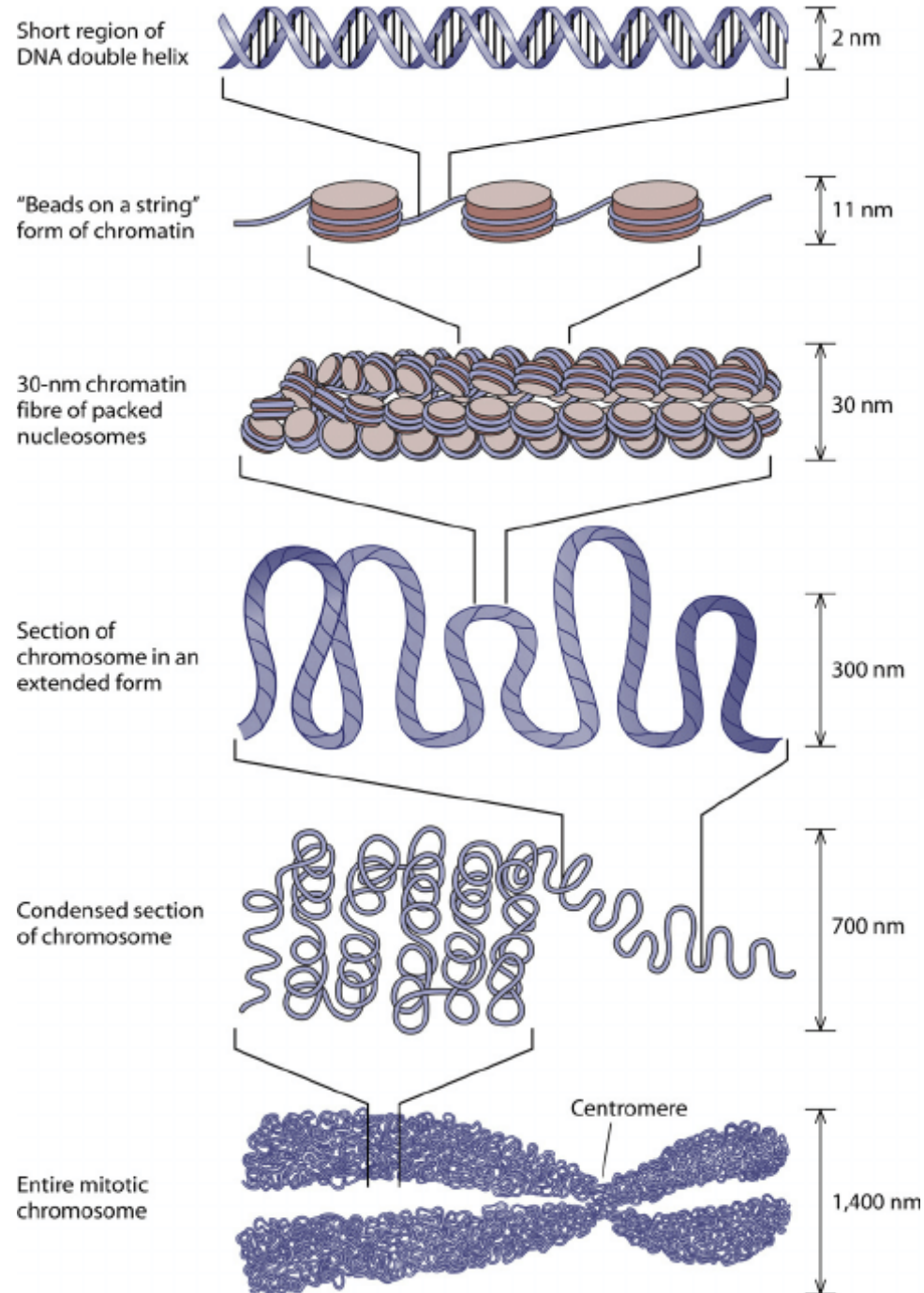
❖ **Chromosomes:**

DNA is packaged in the cell nucleus as chromosomes, which allow it to take up less space and facilitate the distribution of genetic material during cell division. In eukaryotes (such as humans), each chromosome is made up of a linear DNA molecule associated with proteins like histones.

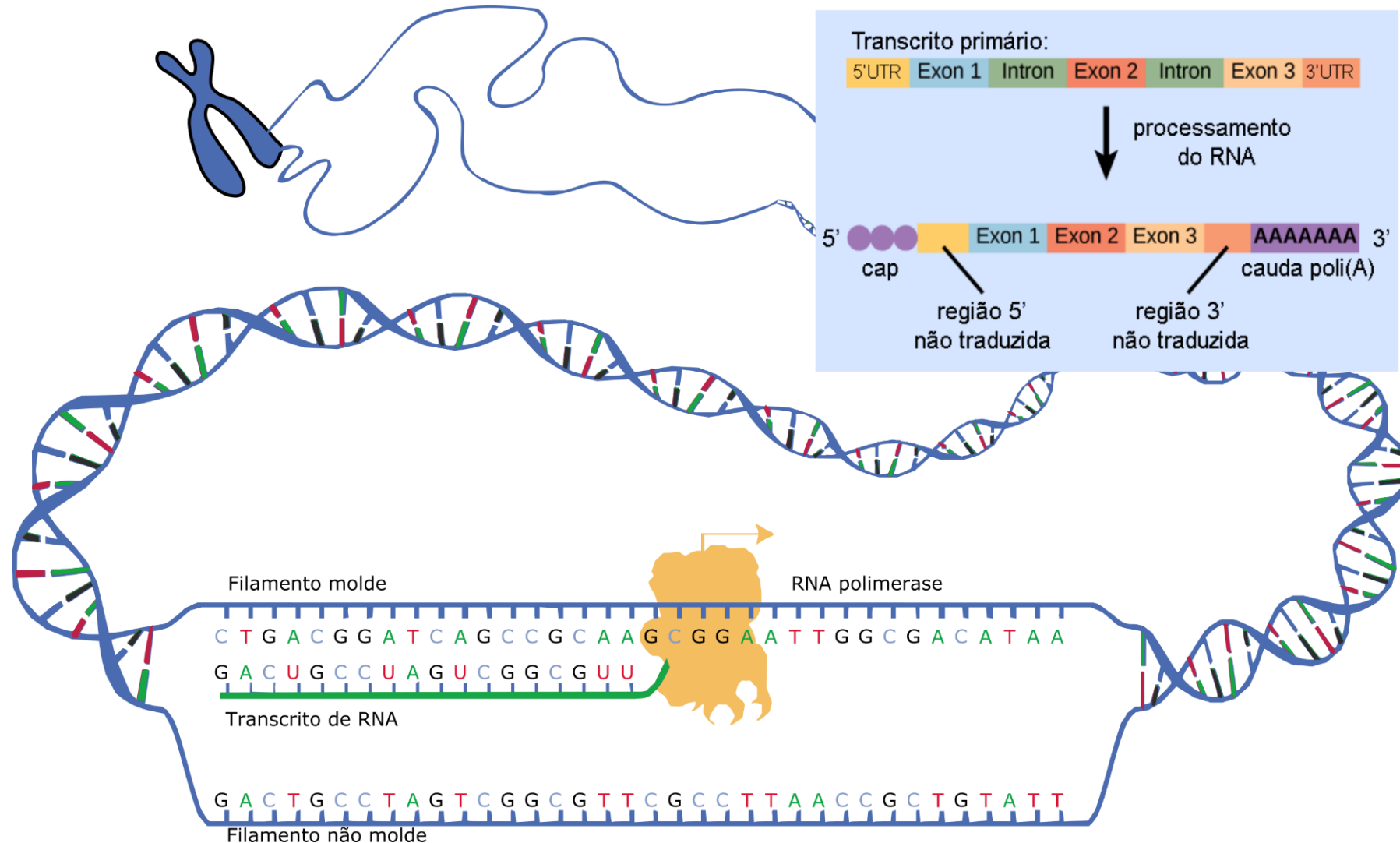
❖ **Genomes in Different Organisms:**

In prokaryotes (bacteria and archaea), genomes typically take the form of a circular DNA molecule found in the cytoplasm, while in eukaryotes, genomes are usually linear and stored in the nucleus.

The size of genomes can vary significantly, from a few thousand to billions of base pairs, depending on the organism.

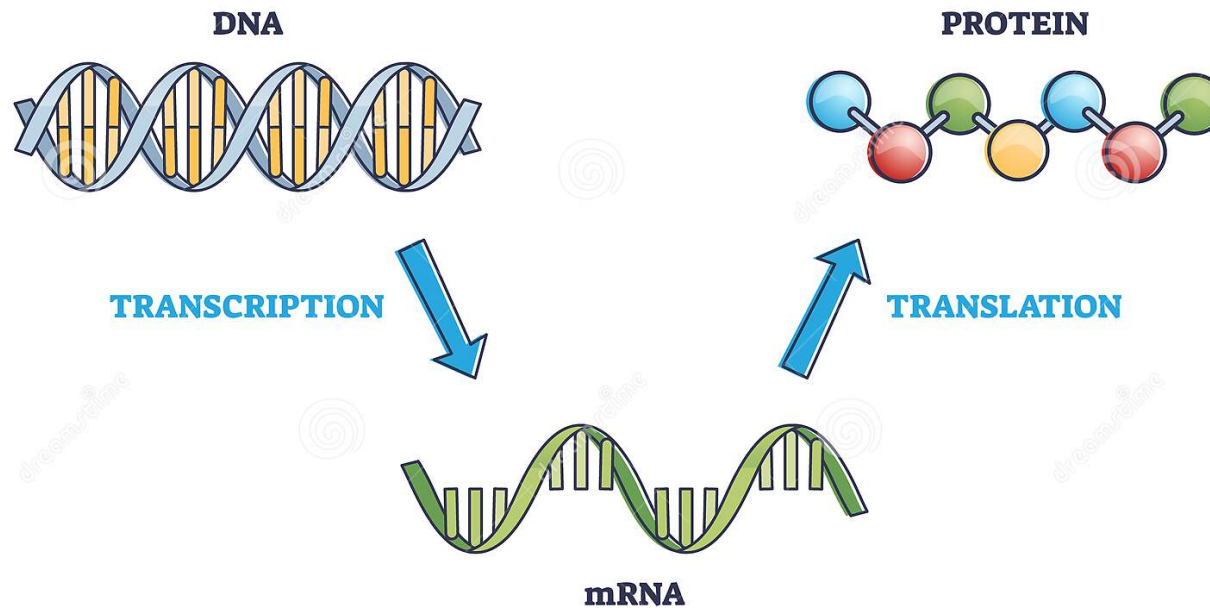


Individual genetic elements with a strictly **specific nucleotide sequence** encoding certain products are **genes**. Some of them code for **proteins**, others only for **RNA** molecules.



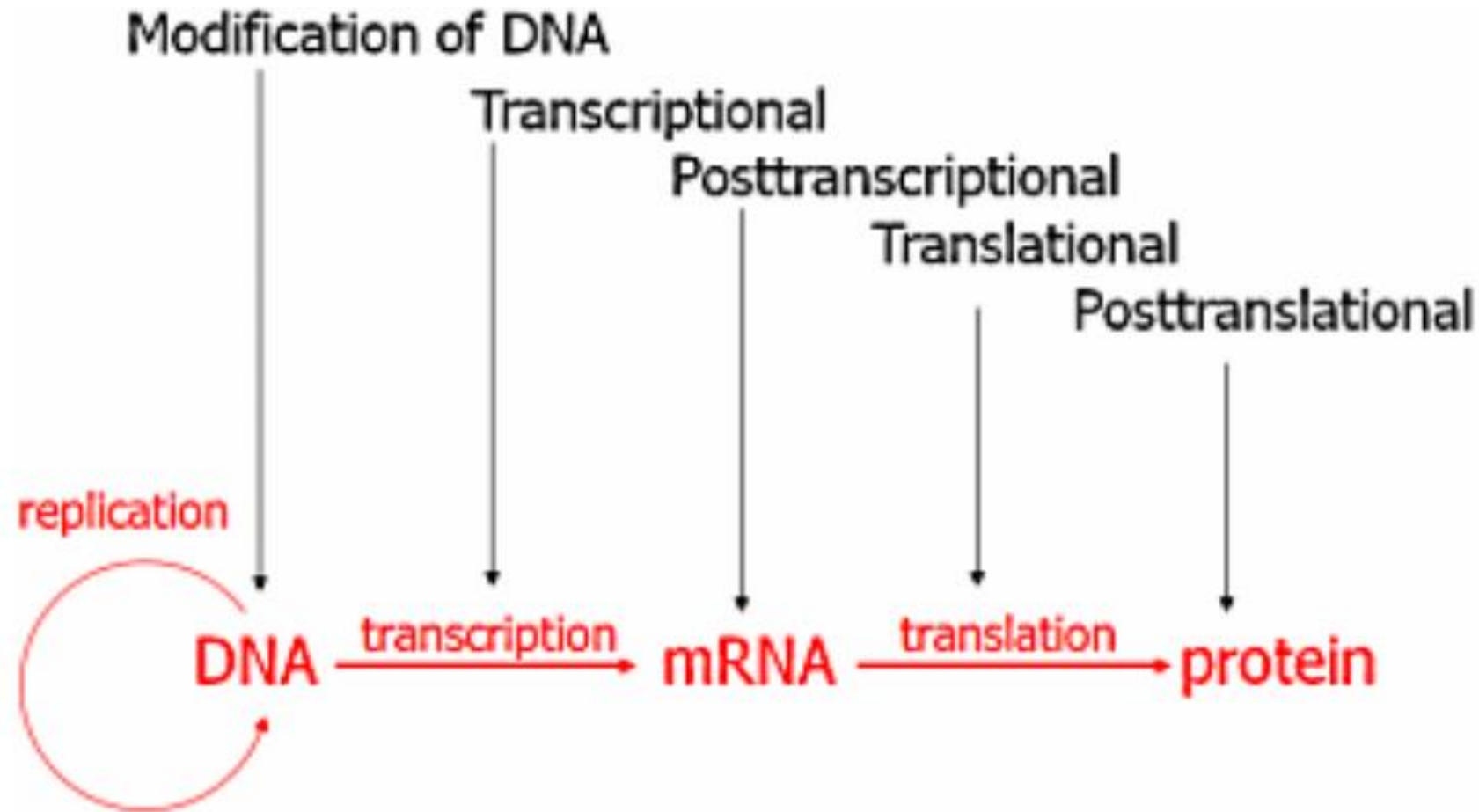
Genetic information moves from DNA to RNA to protein

GENE EXPRESSION

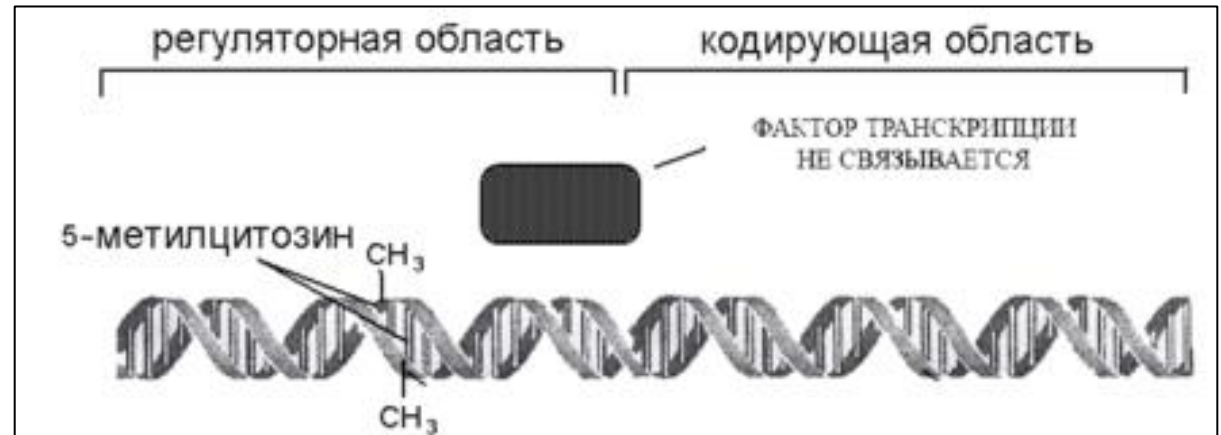
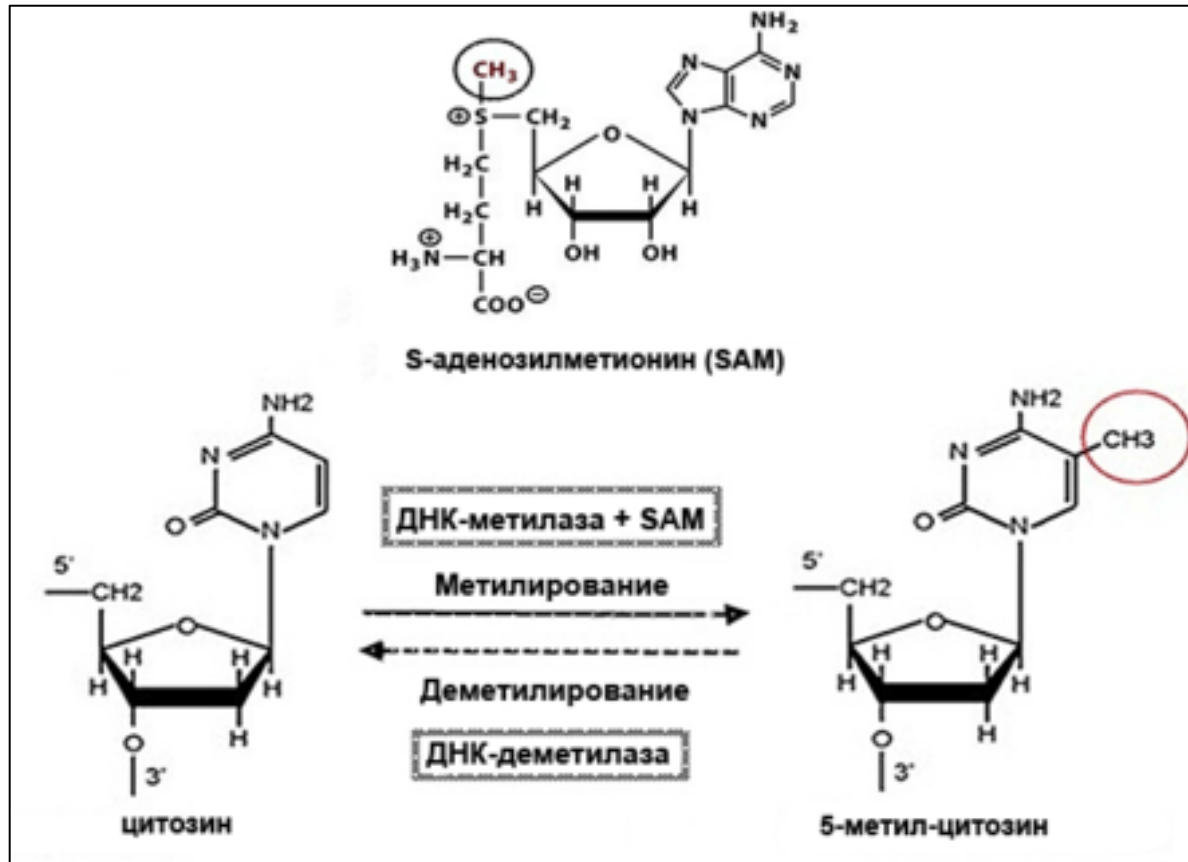


Gene expression is the process by which DNA directs the synthesis of functional products such as proteins. Cells can regulate gene expression at various stages. This allows organisms to generate different types of cells and allows cells to adapt to internal and external factors.

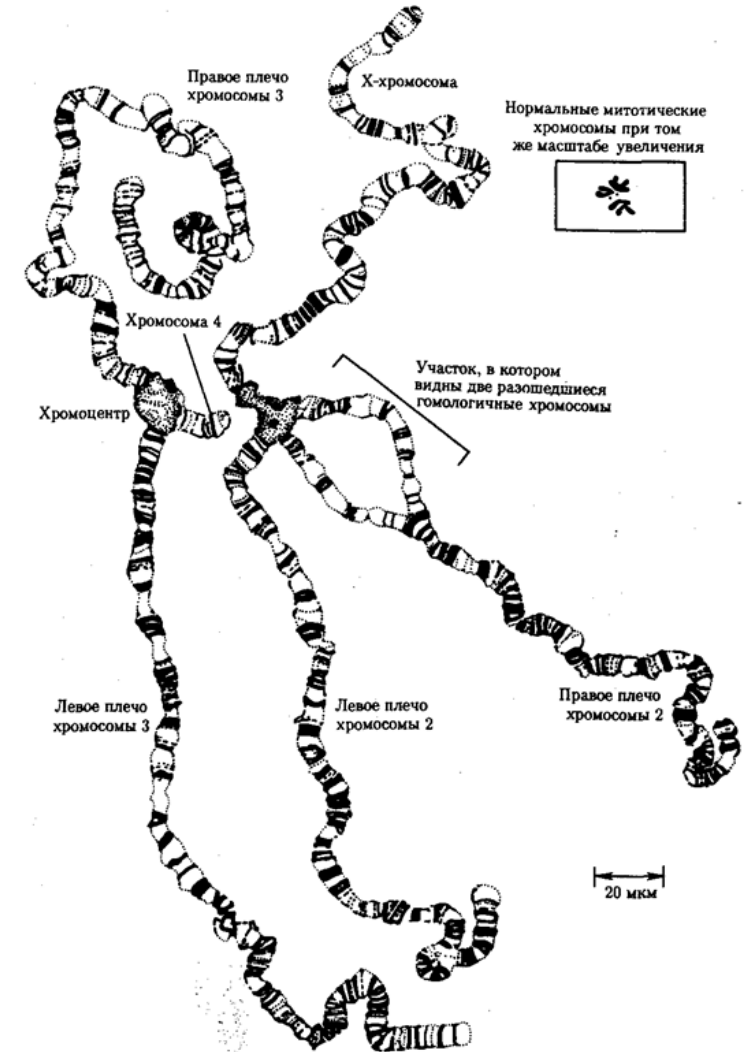
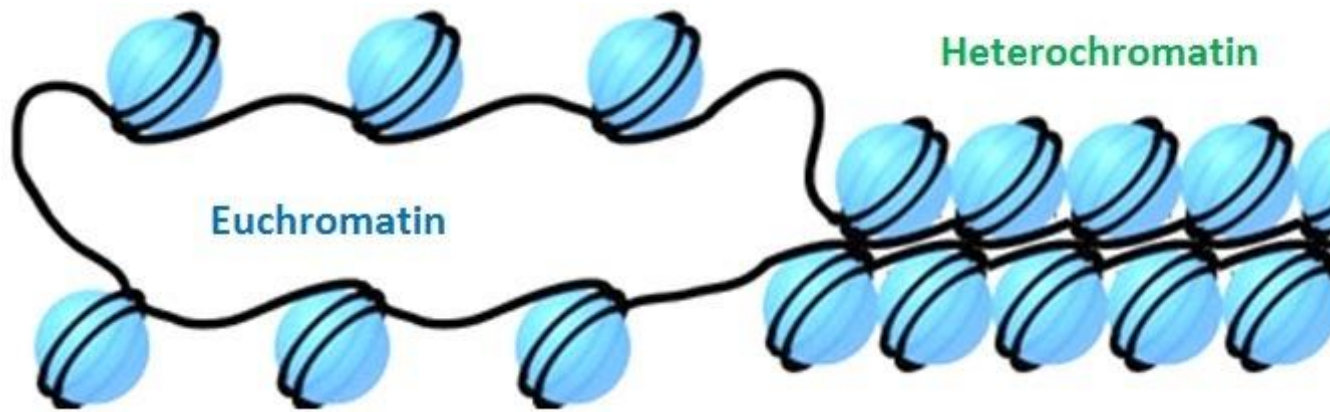
Types of regulation of eukaryotic gene expression



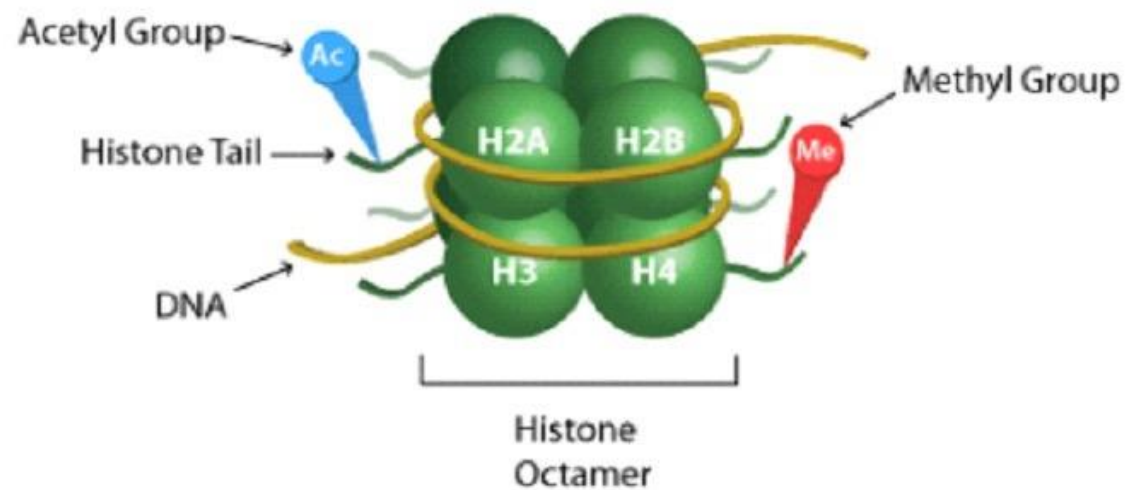
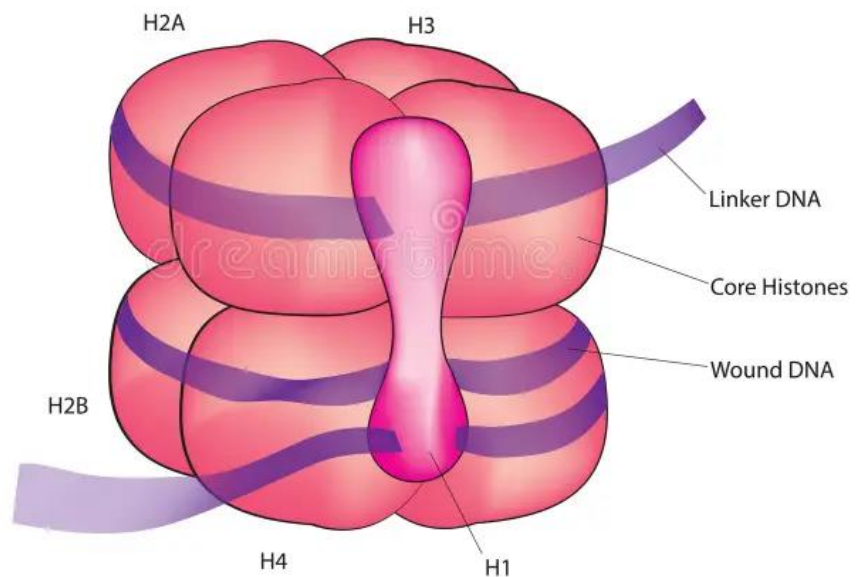
- **DNA methylation and demethylation.**
- DNA methylation occurs in the regulatory regions of the gene. Cytosine is methylated in the CG sequence, after which the gene is inactivated.
- Demethylation restores gene activity. The process is regulated by the enzyme methyltransferase.



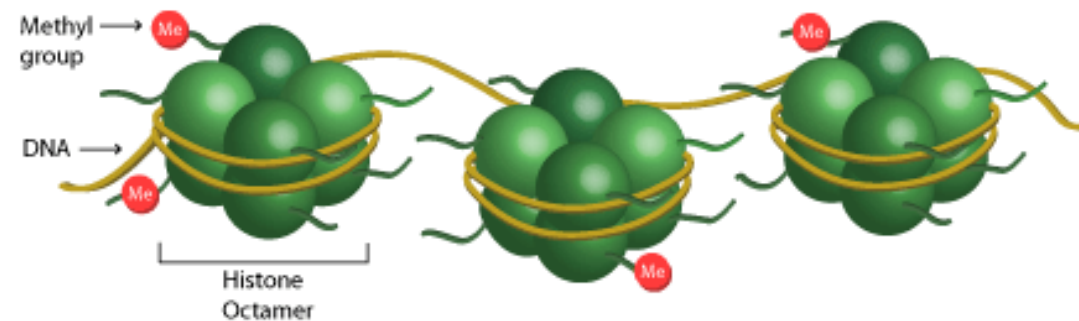
- **Condensation and decondensation of chromatin.**
- This is the most versatile method for regulating transcription. When certain genes need to be expressed, the chromatin decondenses at that location.



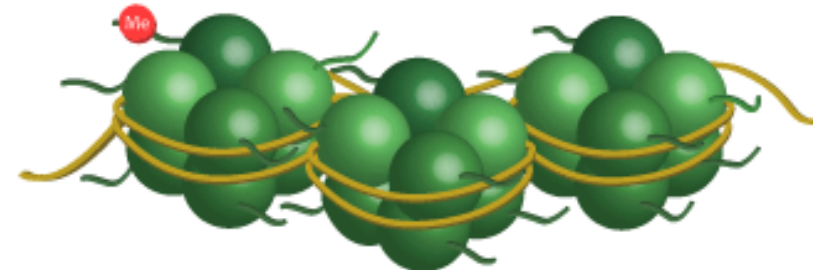
NUCLEOSOME



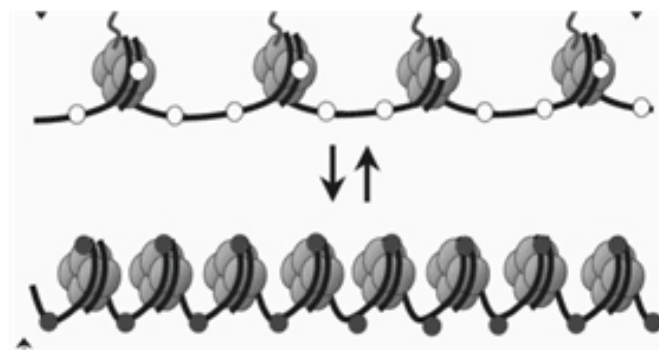
Euchromatin



Heterochromatin



нуклеосома

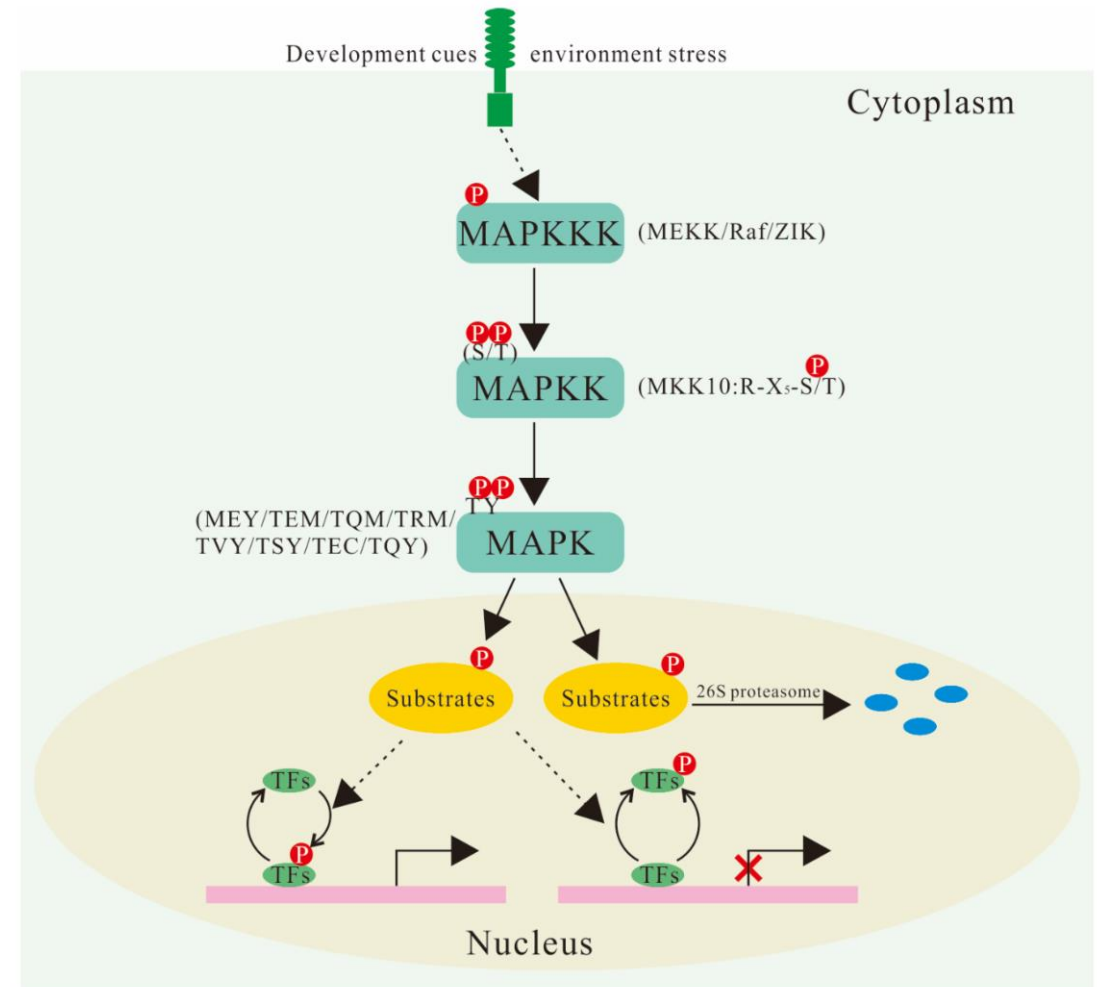


ДНК деметилирована, гистоны ацетилированы, транскрипция возможна

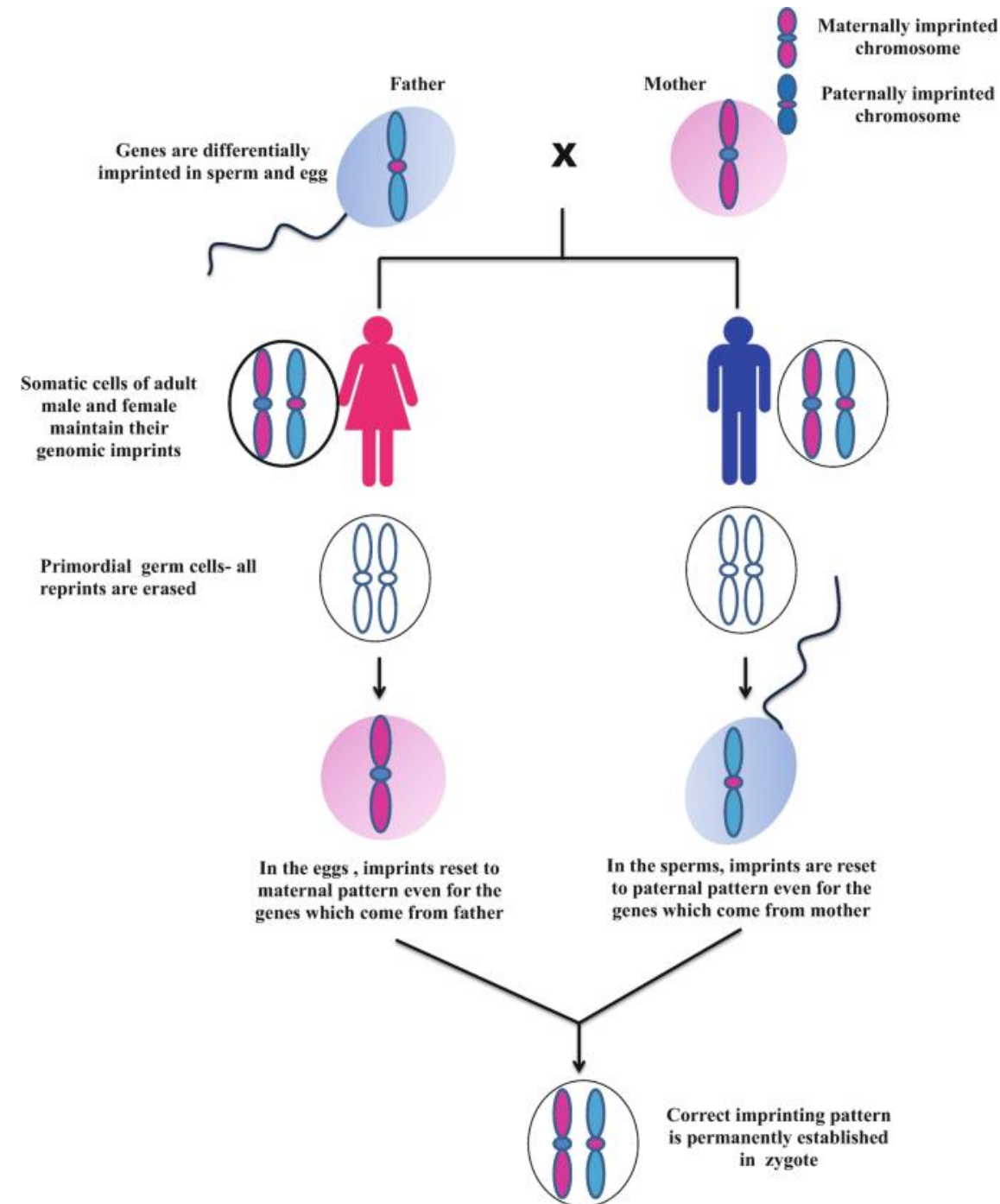
ДНК метилирована, гистоны деацетилированы, транскрипция невозможна (черные точки – метилцитозин)

- **Hormonal regulation.**

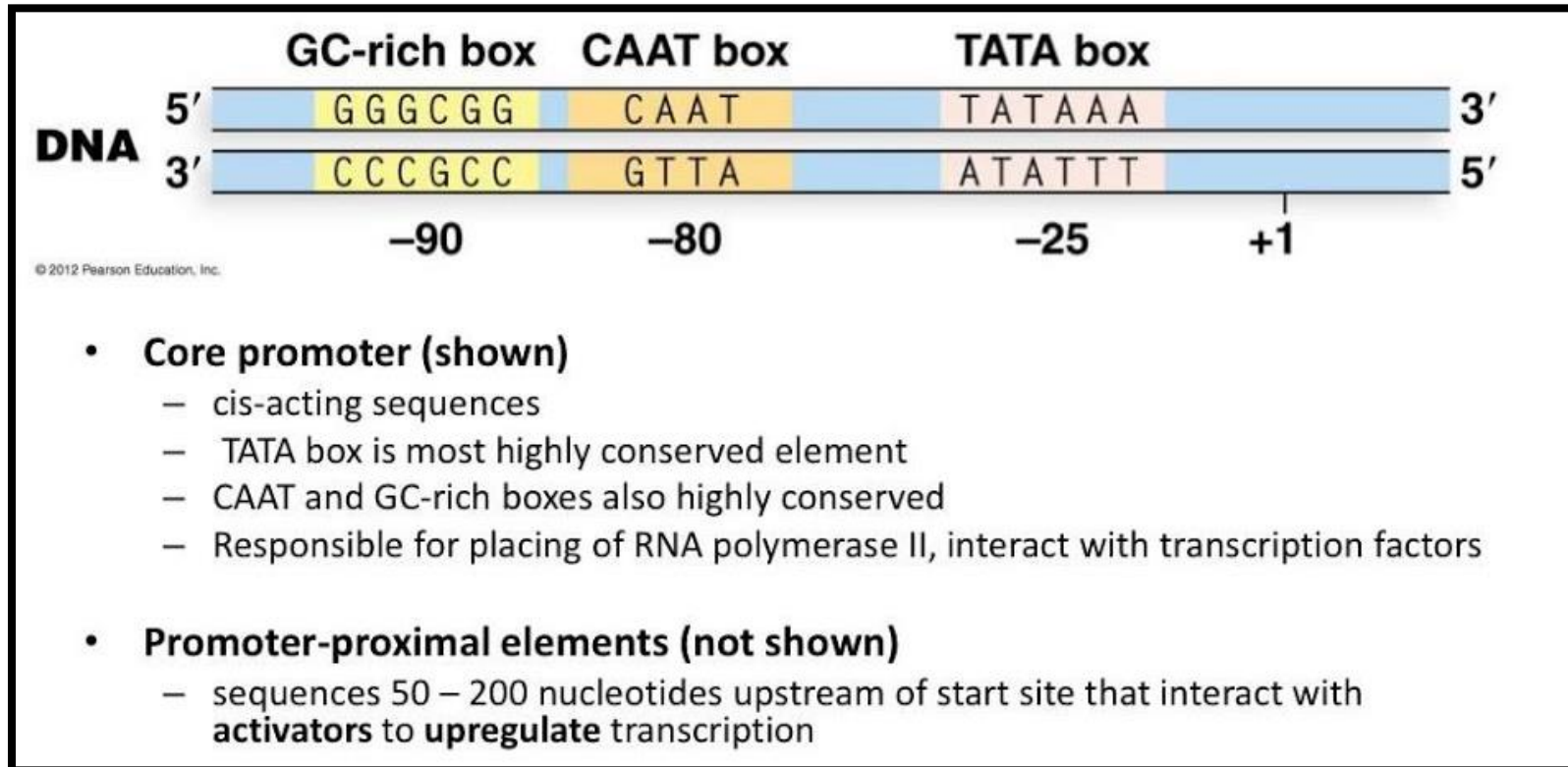
- With hormonal regulation, genes are activated in response to an external chemical signal (the entry of a certain hormone into the cell). This hormone starts those genes that have specific nucleotide sequences in the regulatory regions.



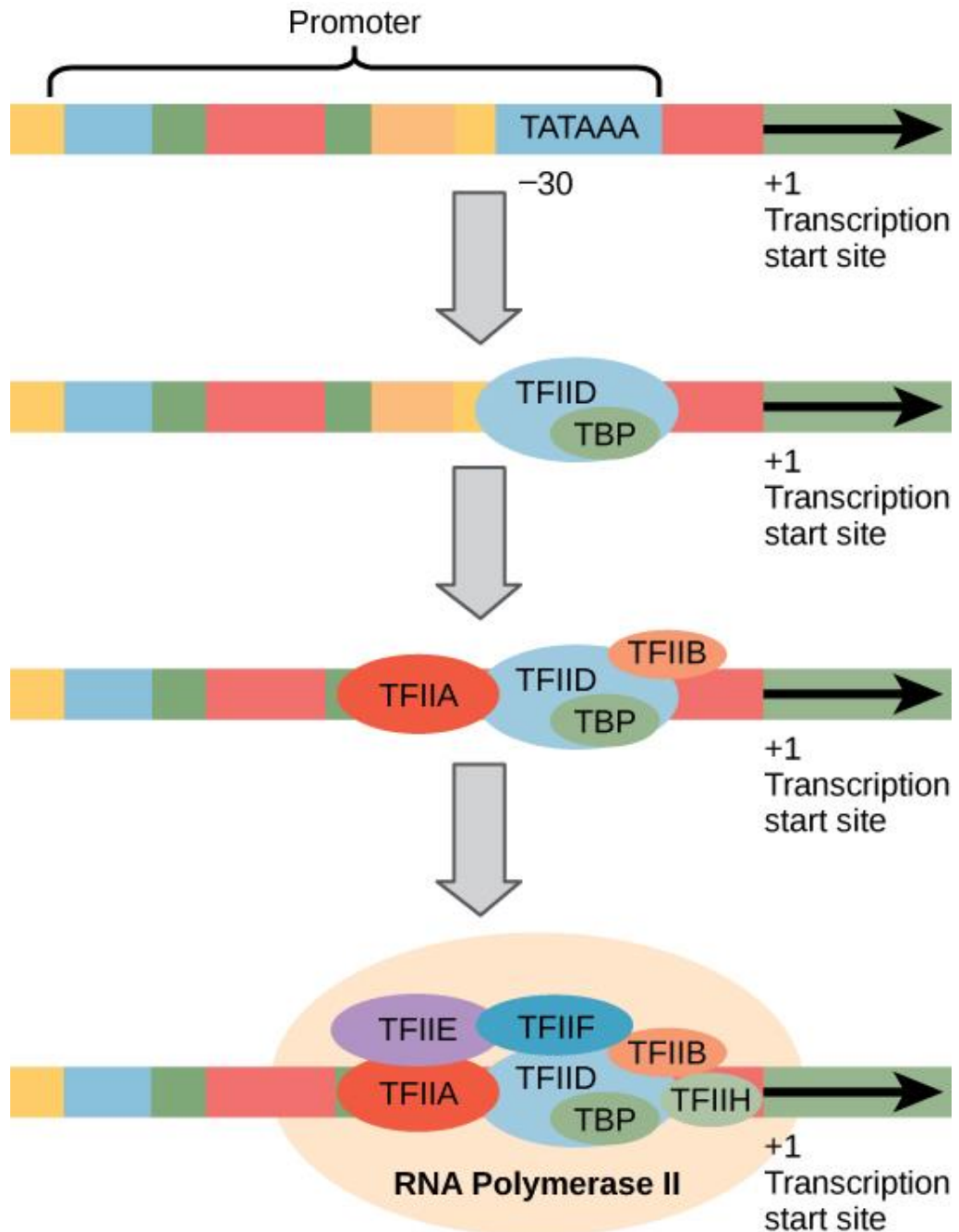
- **Genomic imprinting.**
- This is a poorly understood way of regulating gene expression in eukaryotes. It is possible only in diploid organisms and is expressed in the fact that the activity of genes depends on which parent they were obtained from. Turning off genes is carried out by DNA methylation.



Despite the individuality of the set of regulatory elements in the structural genes of eukaryotes, each of them has a promoter region (TATA box, or Hogness box) of eight nucleotides, including the TATA sequence; CCAAT sequence (CAT box); a section of repeated GC dinucleotides (GC-box). These elements are located at a distance of 25, 75 and 90 bp. from the site of initiation, respectively.

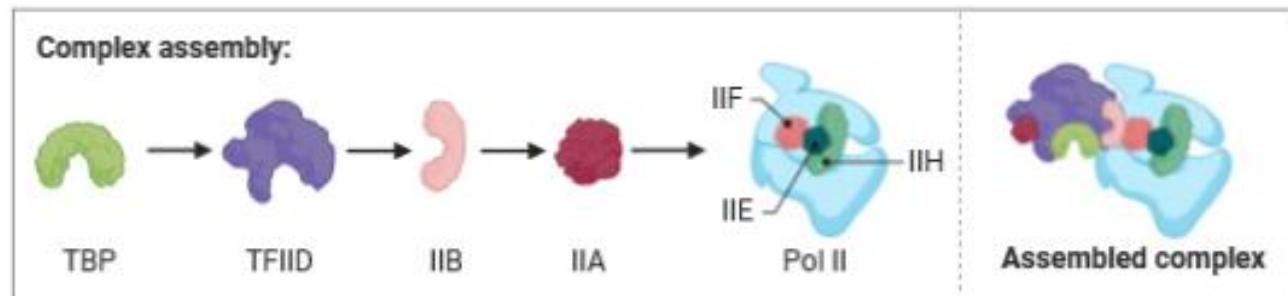
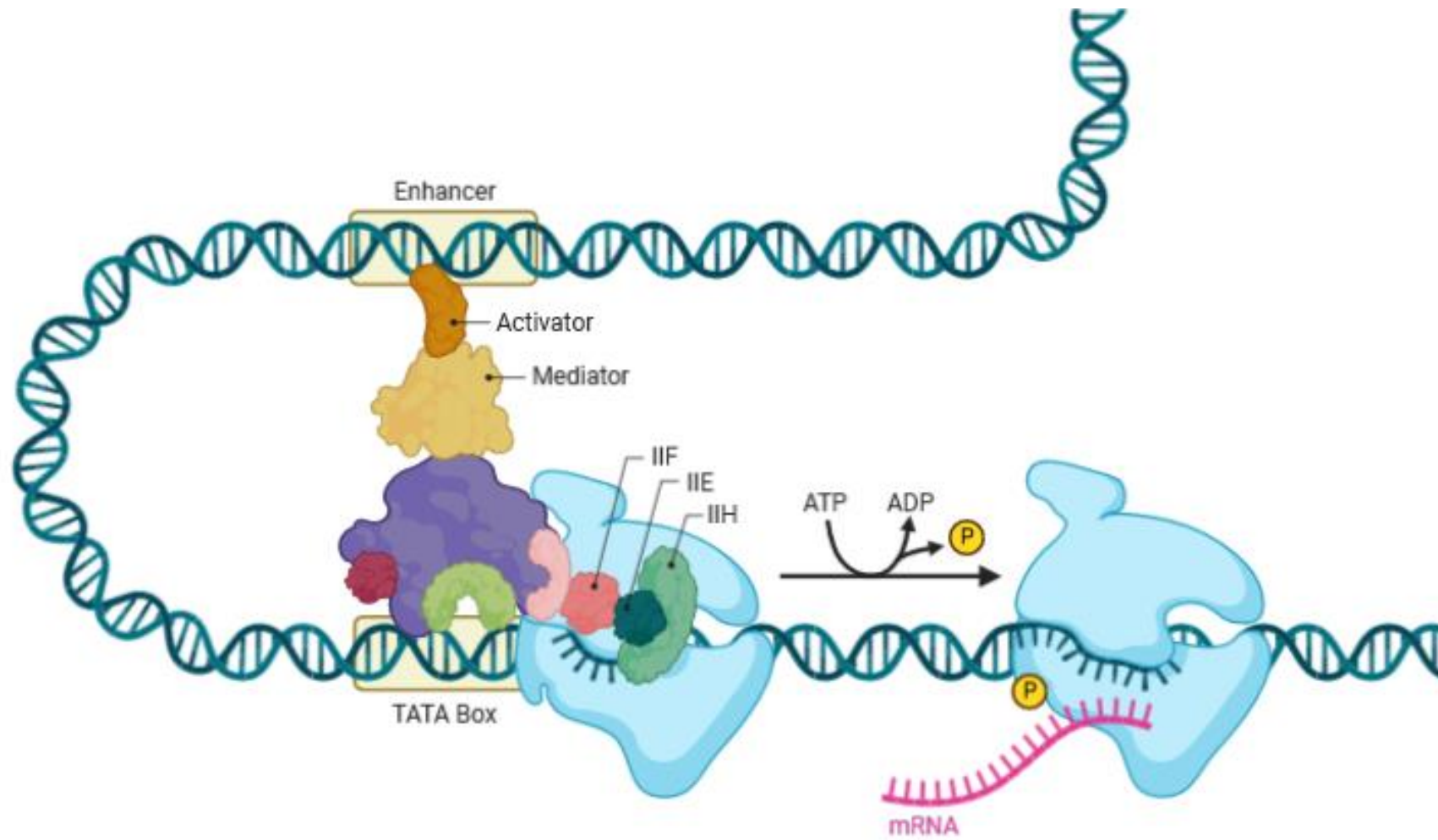


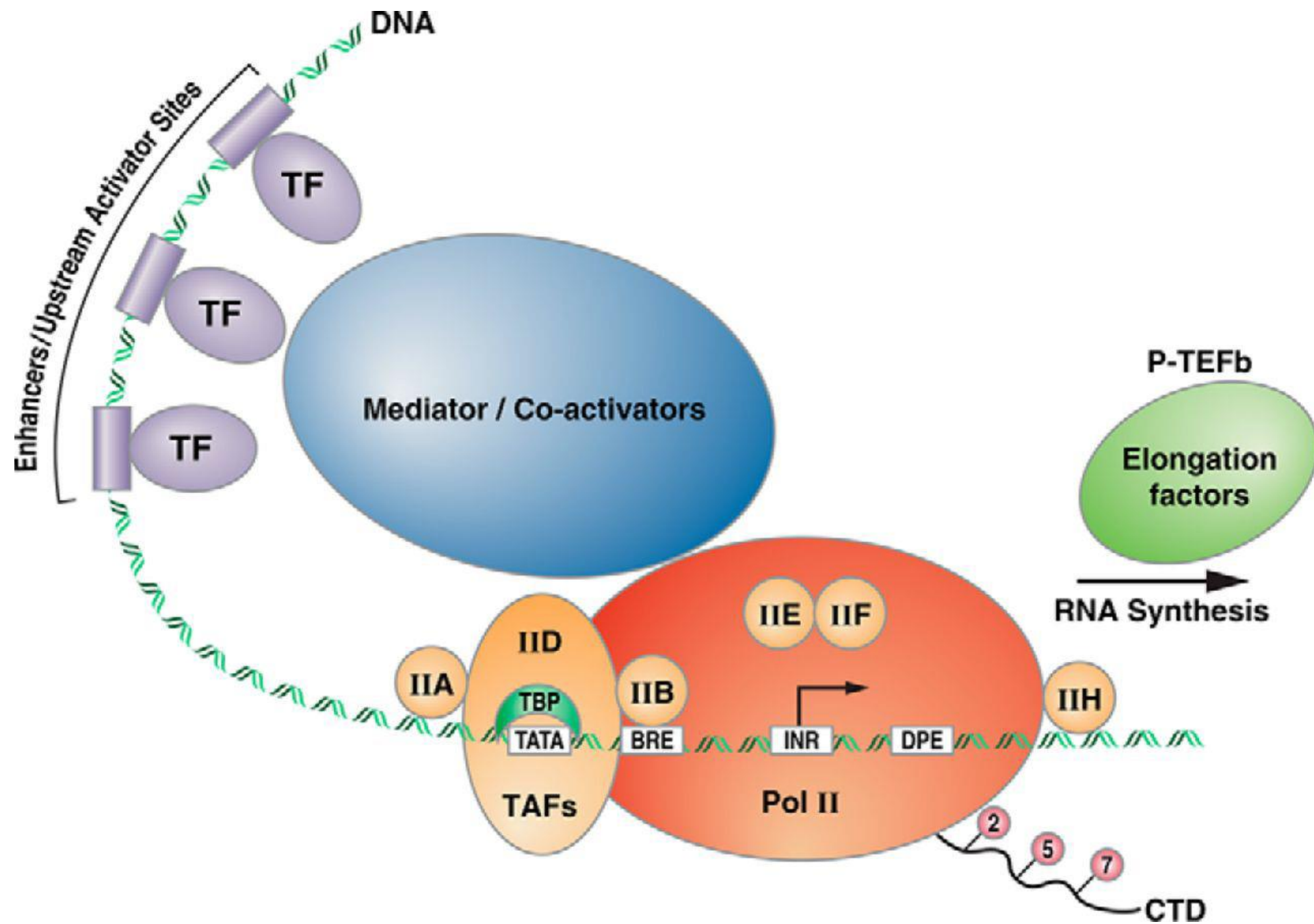
Unlike prokaryotes, eukaryotes mostly lack operons, i.e., each eukaryotic structural gene has its own set of regulatory elements. A significant role in the regulation of transcription in eukaryotes, in addition to being mediated by the interaction between DNA and proteins, is also played by protein-protein interactions.



Transcription initiation of a structural gene in eukaryotes.

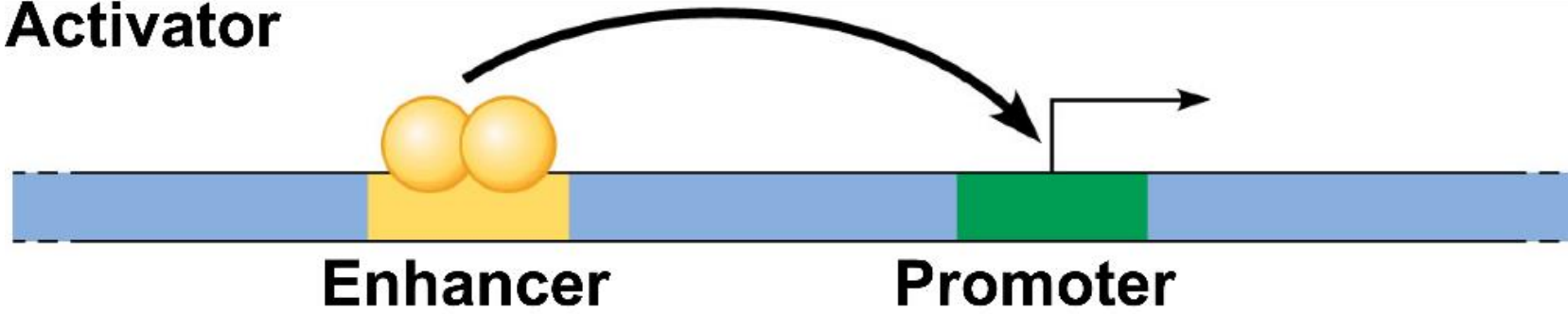
First, the transcription factor TFIID binds to the TATA box, then other transcription factors and RNA polymerase II are attached, and finally, auxiliary factors that trigger transcription. Arrow direction of transcription.



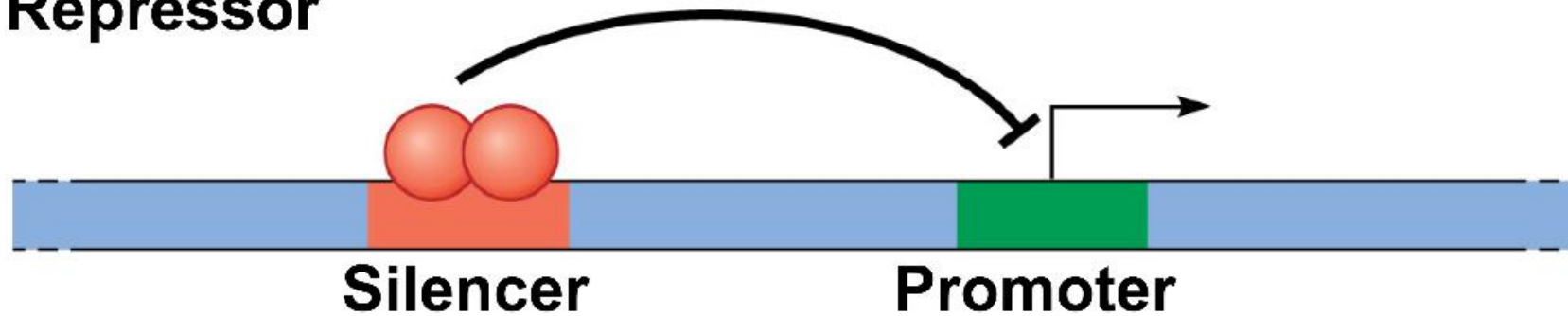


Enhancer and Silencer DNA elements.

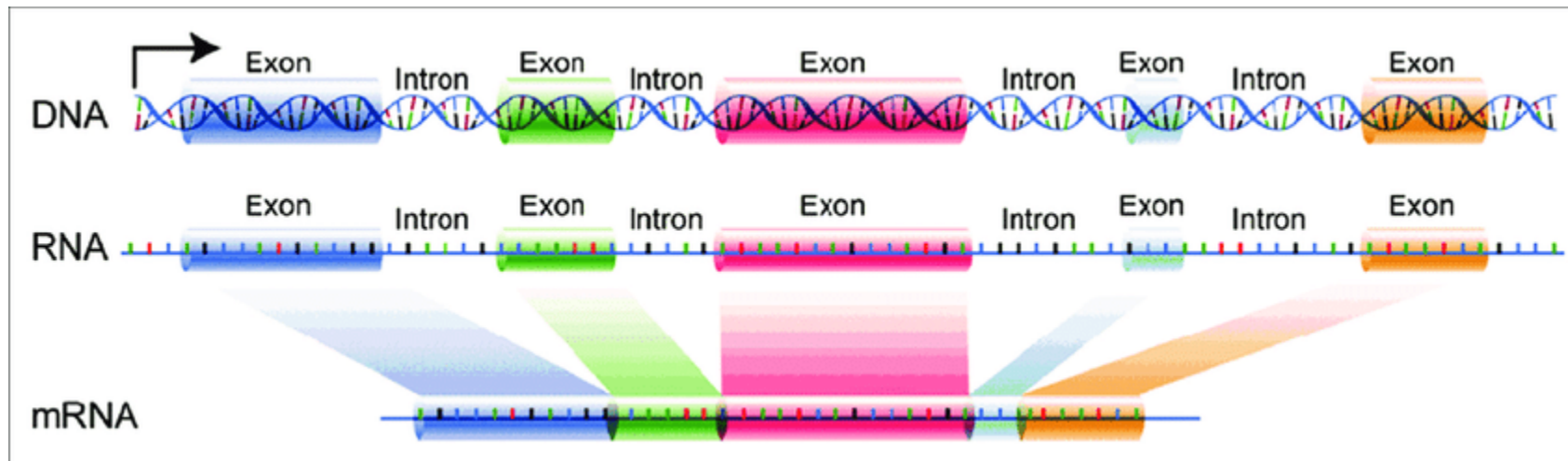
Activator



Repressor

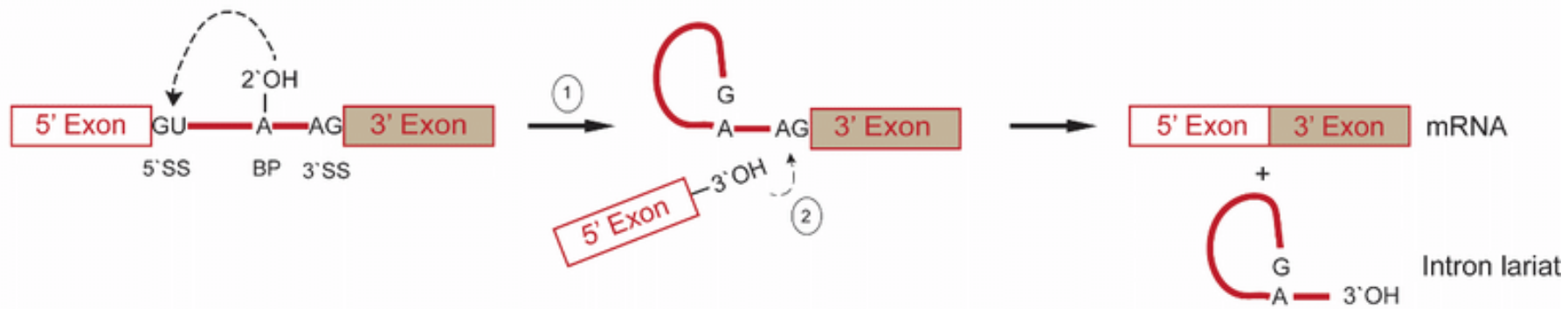
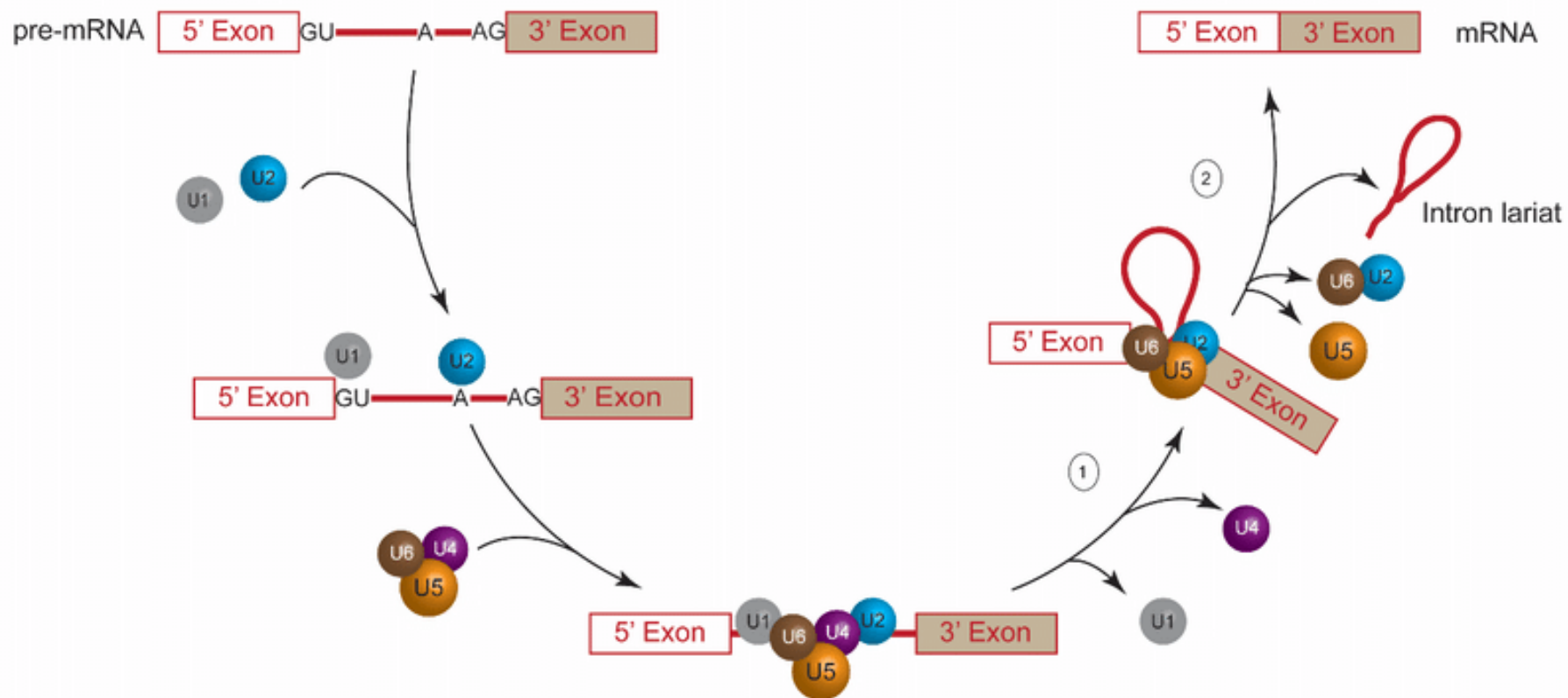


- In eukaryotes, most structural genes consist of several discrete coding regions (exons) separated by non-coding regions (introns). Upon completion of transcription of a eukaryotic structural gene, introns are excised from the primary transcription product with the help of enzymes, and exons are sutured together end to end (splicing) to form functional mRNA
- Typically, exons are 150 to 200 nucleotides long and introns are 40 to 10,000 nucleotides long.

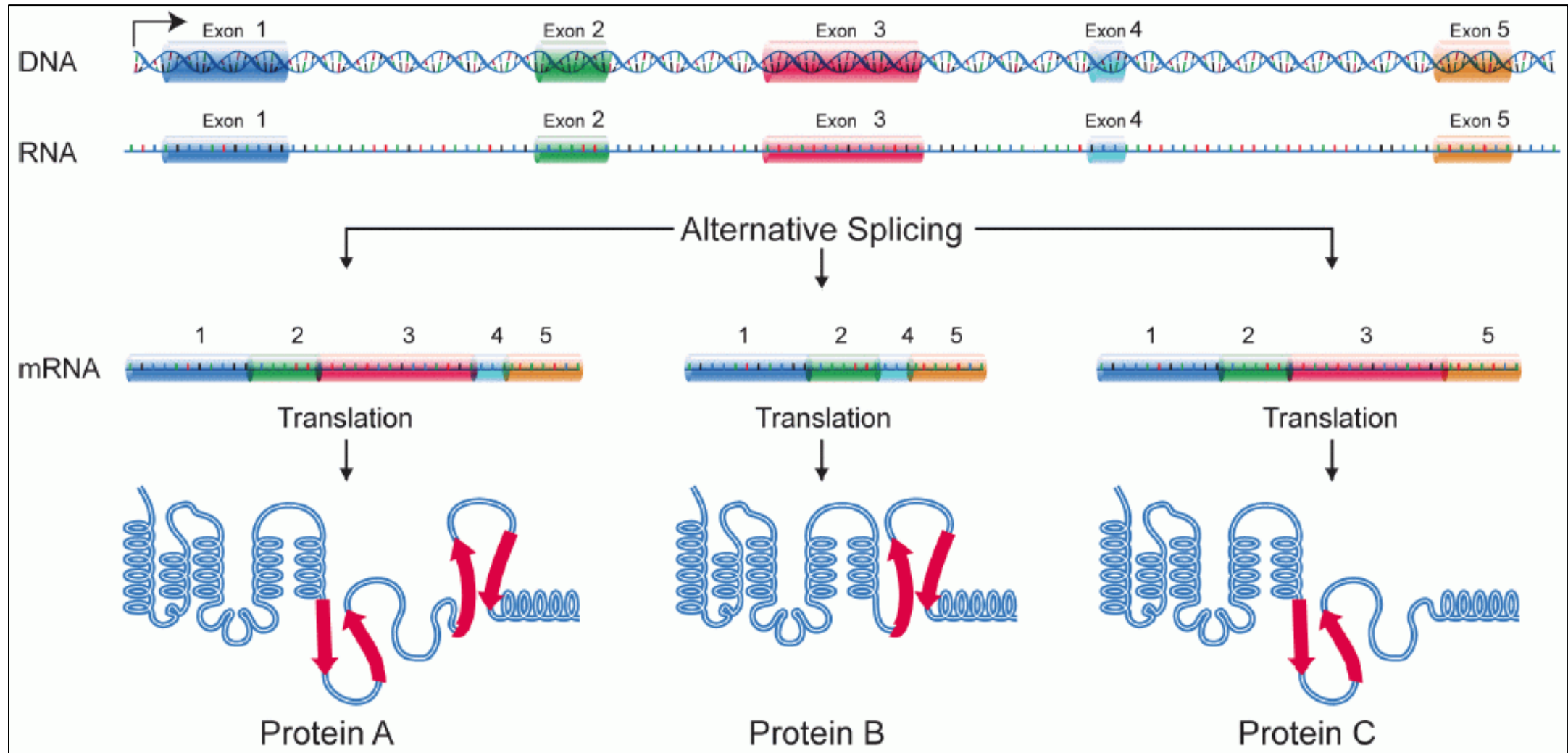


Regulation of mRNA stability.

In eukaryotes, there is also regulation at the level of translation, when ready-made mRNAs are not “allowed” to ribosomes or are destroyed. Other mRNAs can be further stabilized for repeated use.

A**B**

Due to alternative splicing, different products of the same structural gene can be formed in different tissues.

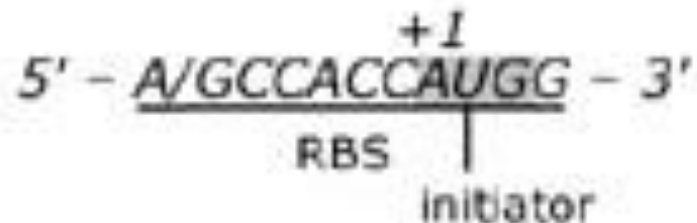


- The Kozak consensus sequence is a nucleotide sequence in eukaryotic mRNA (as well as the corresponding sequence in their genes) that surrounds the start codon and is important for translation initiation.
- Described by American biochemist and molecular biologist Marilyn Kozak in 1986.

Prokaryotic (Shine-Dalgarno sequence)



Eukaryotic (Kozak sequence)



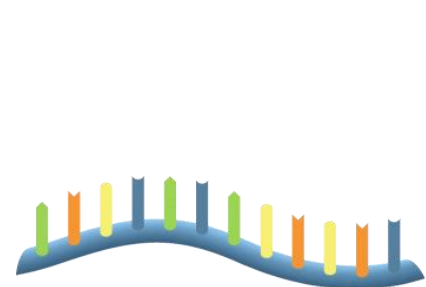
Main types of RNA: mRNA, rRNA and tRNA

All of them play an important role in the process of deciphering genetic information.

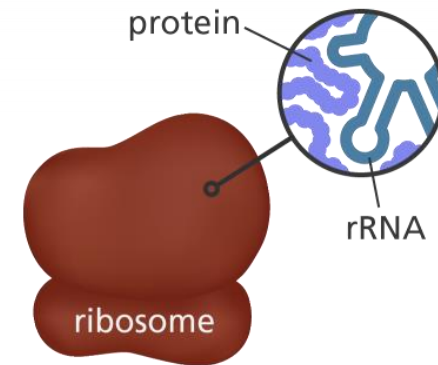
The synthesis of RNA on a DNA template is called transcription.

In most prokaryotes, transcription of all RNA is carried out using the same RNA polymerase.

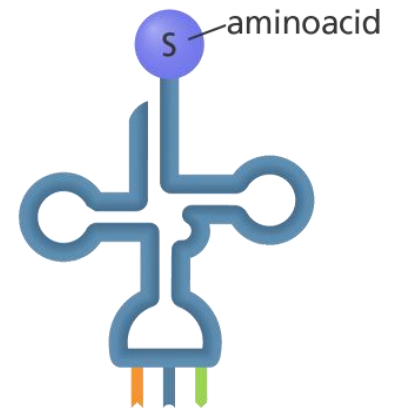
In eukaryotes, mRNA, rRNA, and tRNA are transcribed by different RNA polymerases.



messenger RNA
(mRNA)



ribosomal RNA
(rRNA)

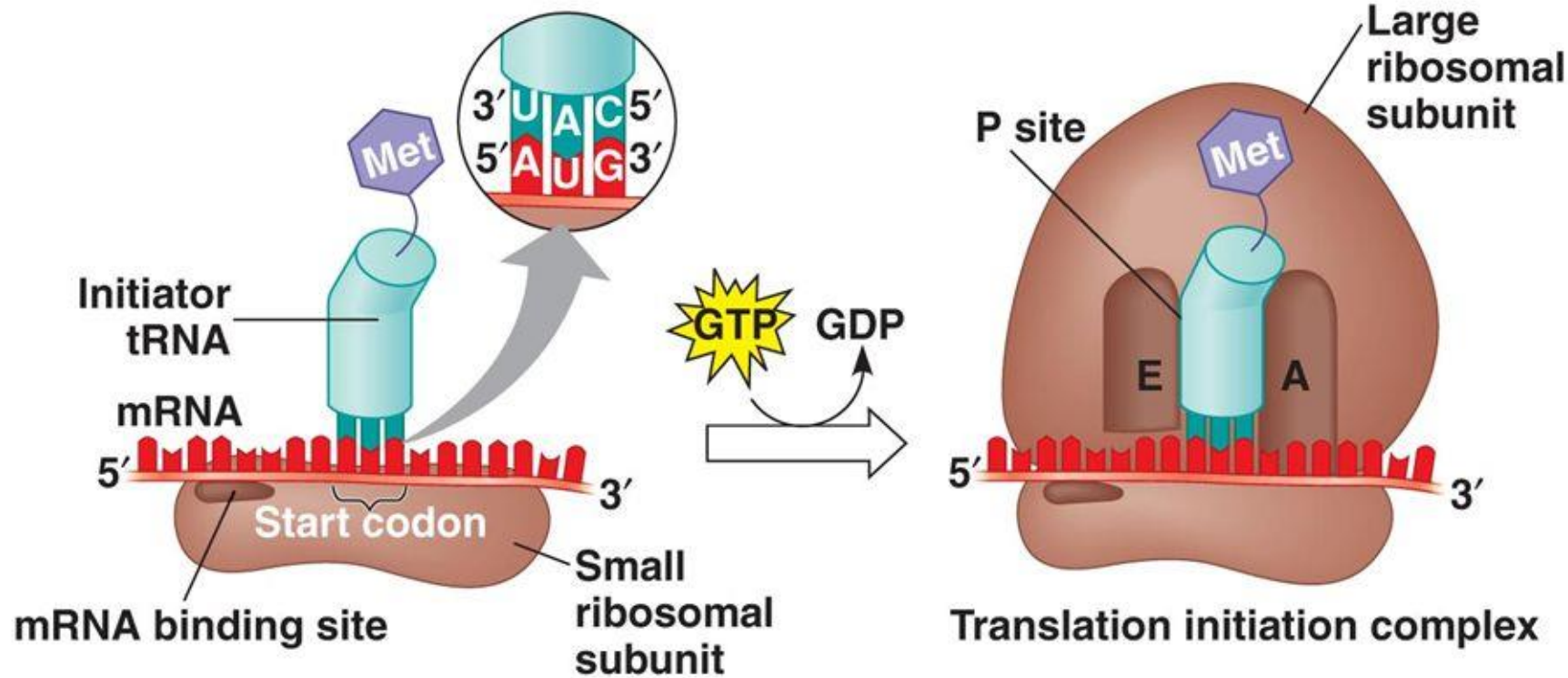


transfer RNA
(tRNA)

In an actively functioning cell, approximately 3-5% of total RNA is mRNA, 90% is rRNA, and 4% is tRNA.

- As a result of transcription of discontinuous genes, the primary product is formed - pro-mRNA, which is a complete copy of the gene and contains sections corresponding to both exons and introns.
- The transcription process involves three different types of RNA polymerases that read different genes.
- RNAP-I reads genes encoding the structure of various forms of rRNA (5.8S, 18S, 28S).
- RNAP-II transcribes genes encoding the structure of proteins and some snRNAs.
- Finally, RNAP-III reads the 5S rRNA, transfer RNA, and snRNA genes.

Translation – Initiation

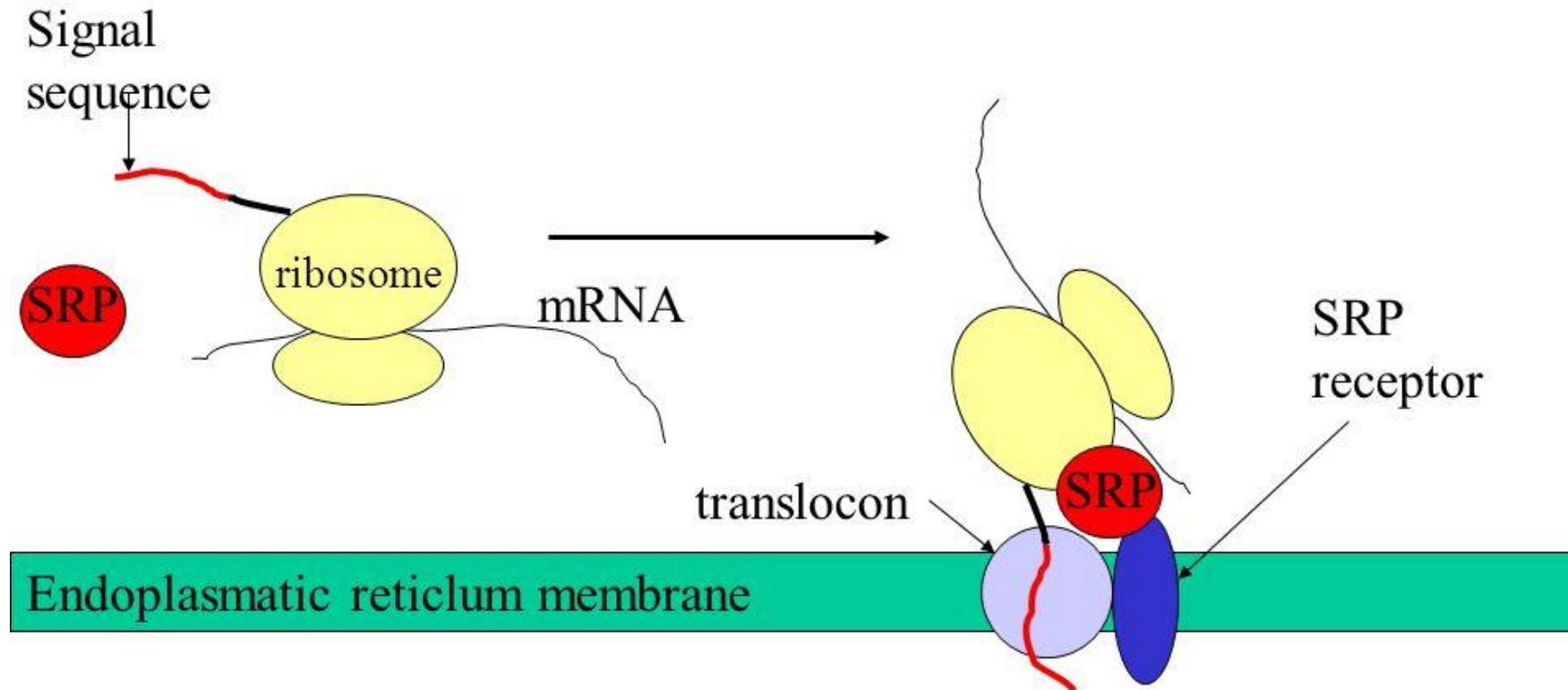


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Steps of initiation:

- Small ribosomal subunit binds to mRNA
- An initiator tRNA with the anticodon UAC base pairs with the start codon AUG
- The large ribosomal subunit (rRNA) completes the initiation complex, the energy molecule GTP provides energy for assembly.

4.5S RNA and 7S RNA – a part of signal recognition particle (SRP)



- SRP recognizes signalling amino acid sequence in the N-terminus of growing polypeptide chain
- Upon signal recognition, ribosome is attached to endoplasmatic reticulum so that the protein, made by ribosome, enters the secretory pathway

References

- 1) Shchelkunov, S. N. Genetic Engineering: A Reference Manual. 3rd Edition. Novosibirsk: Siberian University of Information, 2008.
- 2) Zhimulev, I. F. General and Molecular Genetics: A Textbook. Novosibirsk: Siberian University of Information, 2007.
- 3) Sambrook, J., & Russell, D. W. Molecular Cloning: A Laboratory Manual. 3rd Edition. Cold Spring Harbor Laboratory Press, New York, 2001.
- 4) Madigan, M. T., Martinko, J. M., Bender, K., Buckley, D., & Stahl, D. Brock Biology of Microorganisms. 15th Edition. Pearson, 2017.
- 5) Alberts, B., Johnson, A., Lewis, J., Morgan, D., Raff, M., Roberts, K., & Walter, P. Molecular Biology of the Cell. 7th Edition. Garland Science, 2015.