
# STRUCTURE AND EXPRESSION OF GENES

Azerbaijan Medical University

Medical biology and genetics department

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# Lecture plan:

1. Structure of DNA
2. *A*-, *B*- and *Z*- forms of DNA
3. Replication of DNA
4. Reparation of DNA
5. Molecular-biological determination of gene
6. Basic properties of gene
7. Classification of genes
8. Gene structure of prokaryotes
9. Gene expression in prokaryotes
10. Gene structure of eukaryotes
11. Gene expression in eukaryotes
12. Alternative splicing
13. Post-translational modification of protein
14. DNA methylation (epigenetics).
15. Imprinted genes

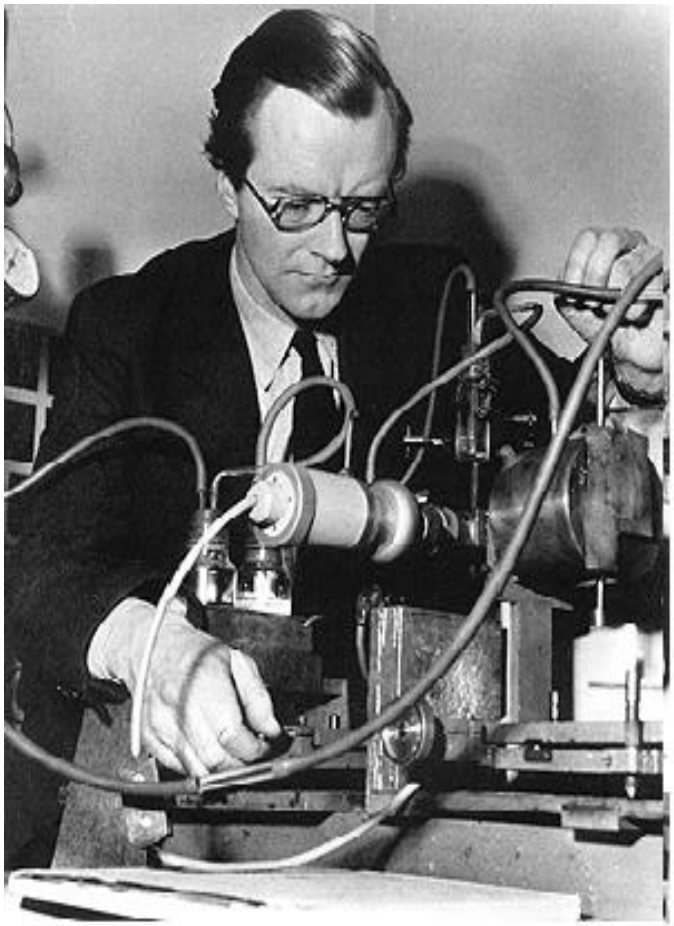
# Discovery of DNA

- The first X-ray picture of DNA was made by Rosalind Franklin **(1920-1958)** in 1952 using a technique called X-ray crystallography, which revealed the helical shape of the DNA molecule. And her senior partner, Wilkins, showed some of Franklin's findings to Watson in January 1953 without her knowledge.

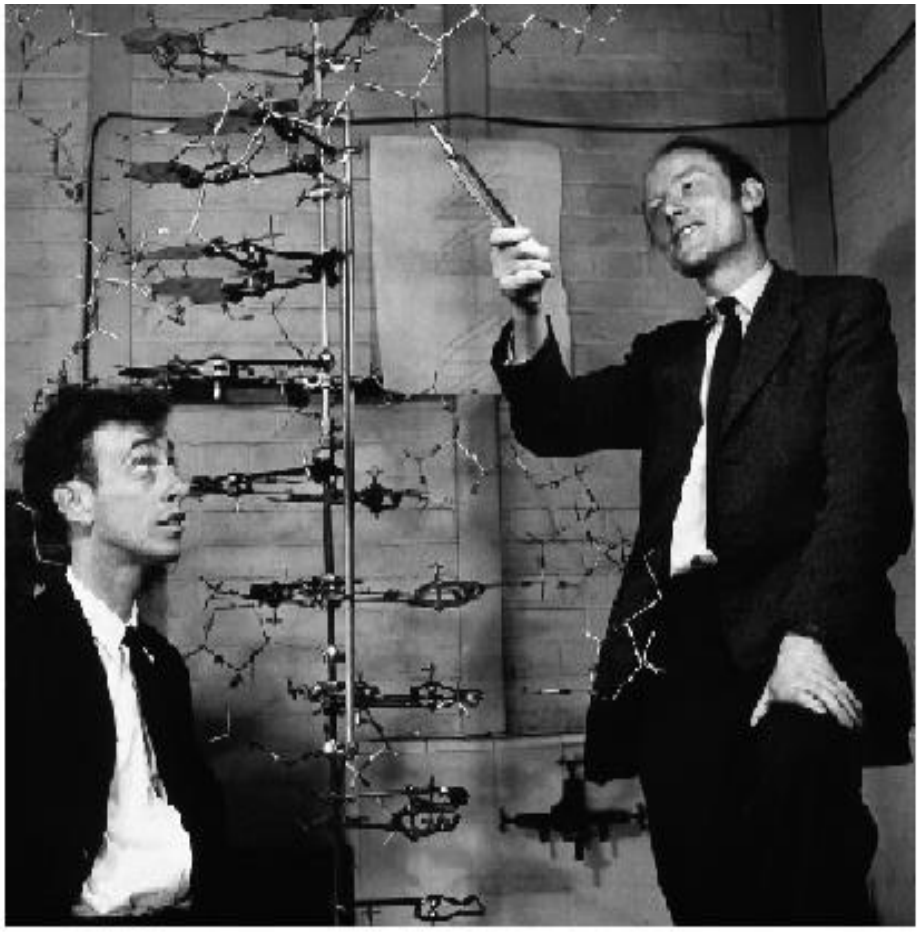
# Discovery of DNA

- Shortly after, Watson and Crick made a crucial advance when they proposed that the DNA molecule was made up of two chains of nucleotides paired in such a way to form a double helix. This structure, announced in their famous paper in the April 1953 issue of Nature, explained how the DNA molecule could replicate itself during cell division, enabling organisms to reproduce themselves with amazing accuracy except for occasional mutations. For their work, Watson, Crick, and Wilkins received the Nobel Prize in 1962. Unfortunately despite her contribution to the discovery of DNA's helical structure, Rosalind Franklin was not named a prize winner.

# Discovery of DNA

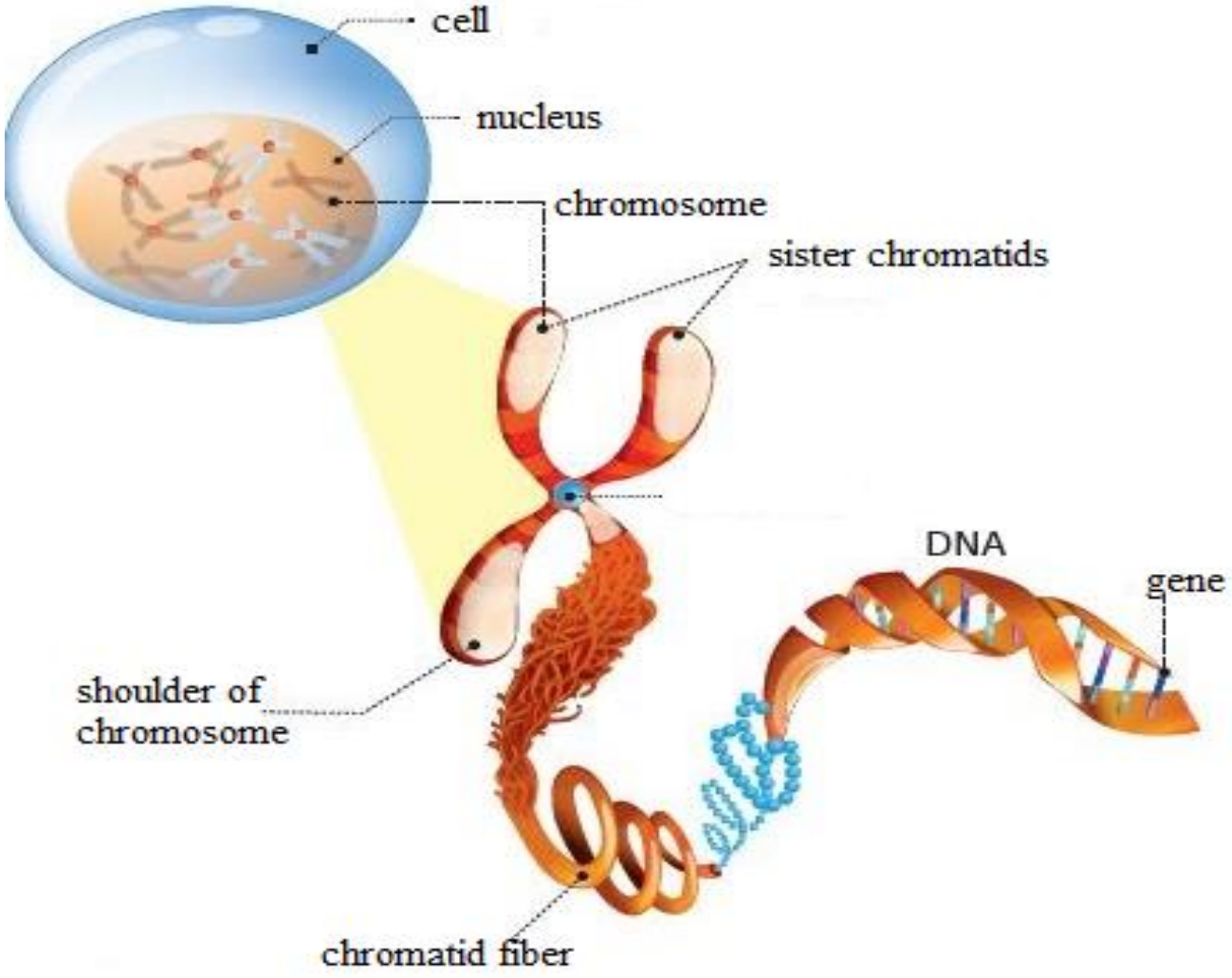


Maurice Wilkins



James Watson and Francis Crick

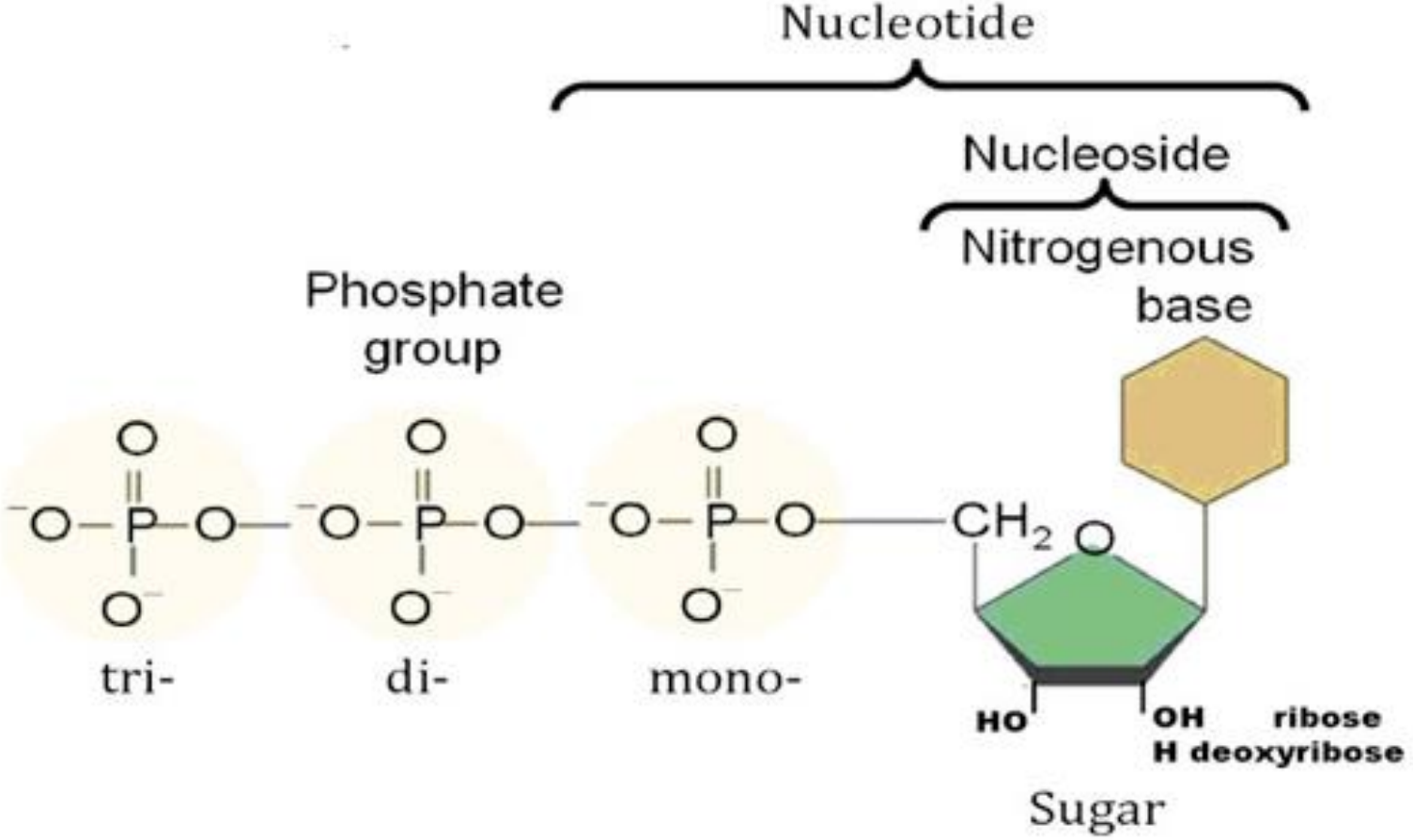
# Chromosome and DNA



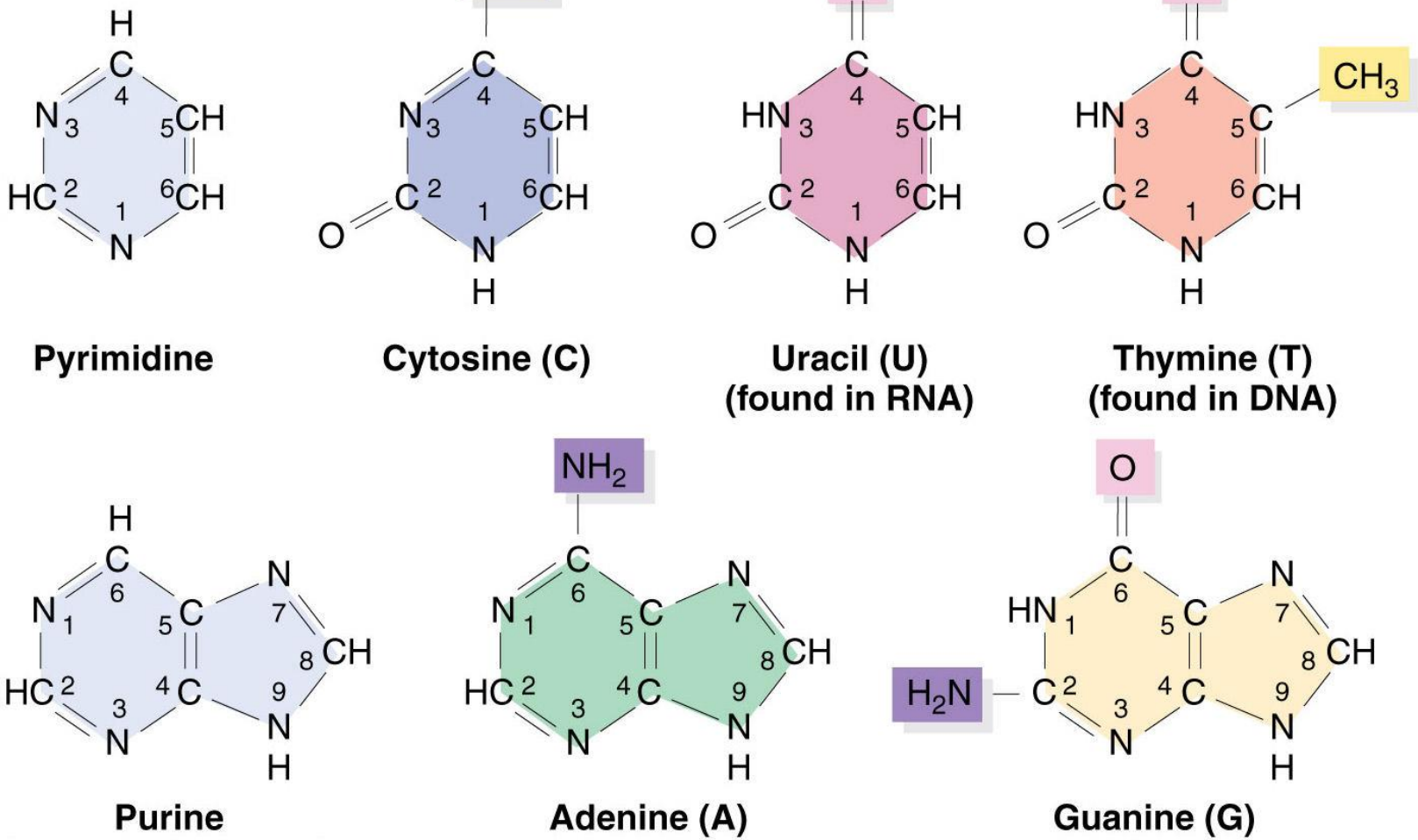
# Nucleotide Structure

- A **nucleotide** is an organic molecule that is the building block of DNA and RNA.
- A nucleotide is made up nitrogenous base, 5-carbon sugar and one phosphate group.
- A **nucleoside** is a nitrogenous base and a 5-carbon sugar.
- Adenine forms a base pair with thymine with two hydrogen bonds, while guanine pairs with cytosine with three hydrogen bonds.
- Each strand of DNA has a "backbone" of phosphate-sugar-phosphate-sugar-phosphate…. The backbone has a 5' end (with a free phosphate) and a 3' end (with a free OH group). The sugar – phosphate backbone is hold together by phosphodiester bonds.
- Nitrogenous base is attached to the 1' carbon of the ribose by a glycosidic bond.

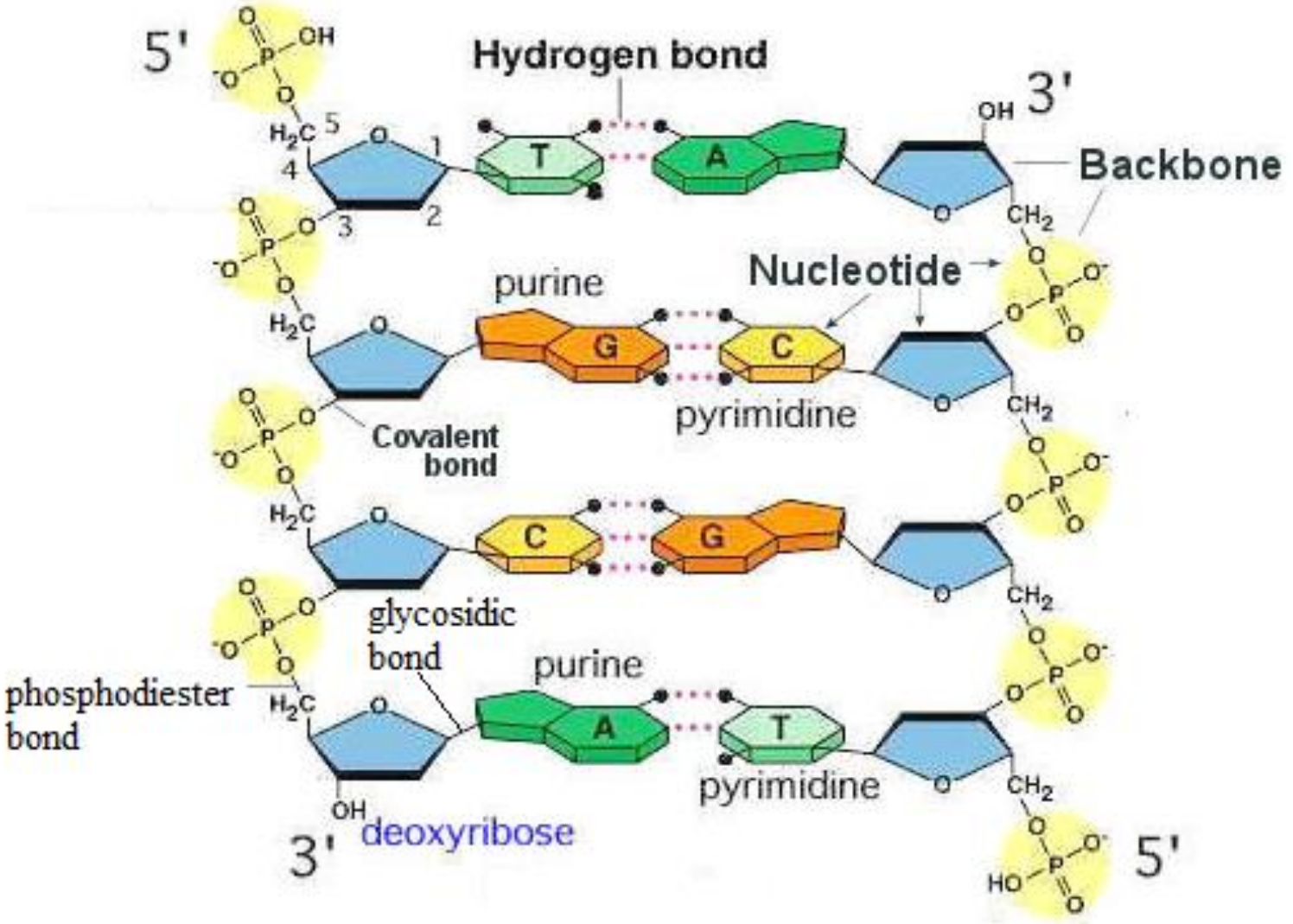
# Nucleotide and nucleoside structure



# Nitrogenous bases

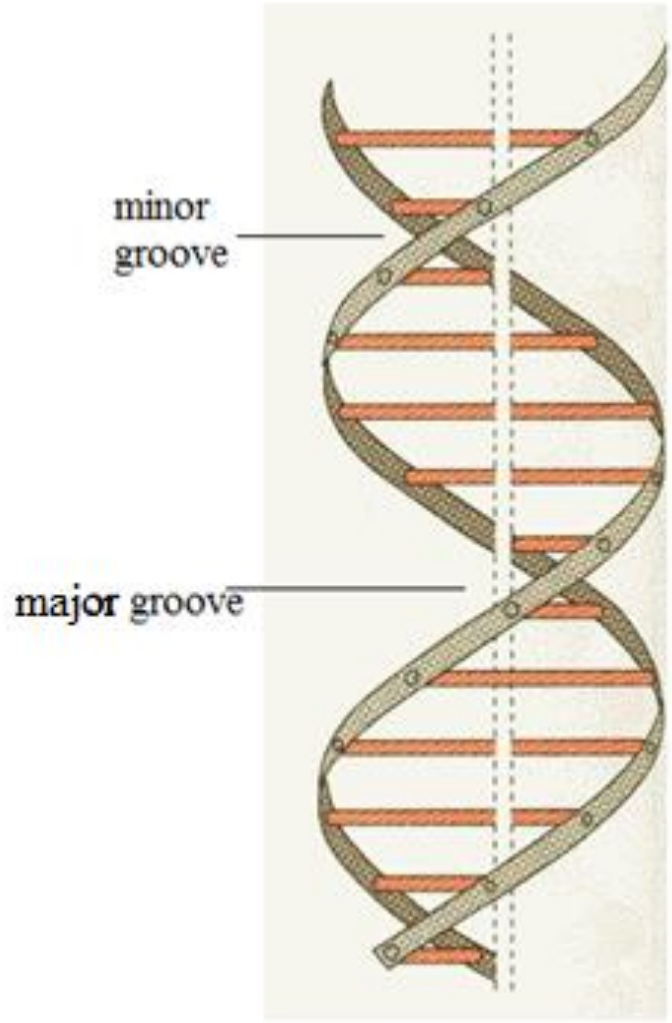


# Chemical bonds in DNA



# B-form of DNA

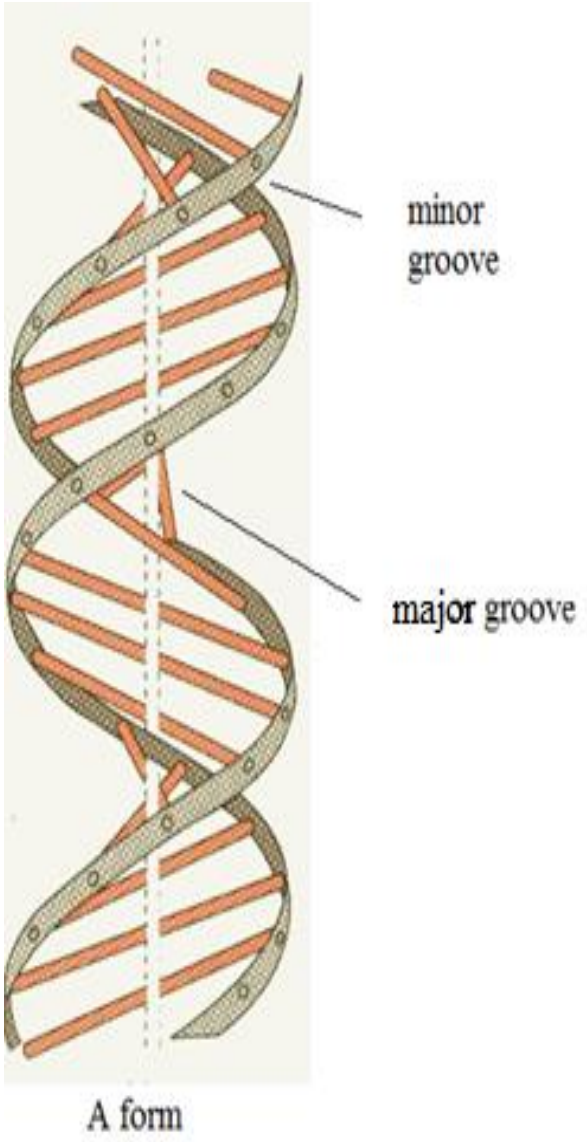
- The B-form of DNA predominates in the cell. The double helix B-DNA is right-handed with about 10–10.5 base pairs per turn.
- The double helix structure of B-DNA contains a major groove and minor groove: the major groove is wider than the minor groove. In B-form, the base-pairs are almost centered over the helical axis and perpendicular to the helix-axis.



B form

# A-form of DNA

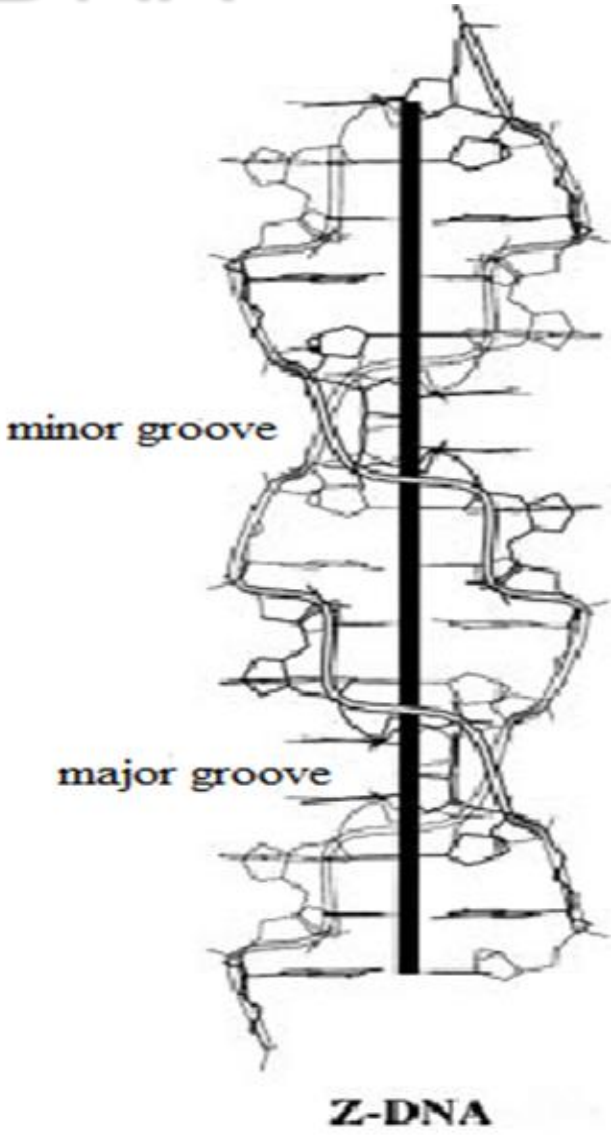
- A-DNA is a right-handed double helix with about 11 base pairs per turn, fairly similar to the more common B-DNA form, but with a shorter, more compact helical structure whose base pairs are not perpendicular to the helix-axis as in B-DNA.
- The major groove of A-DNA is deep and narrow, while the minor groove is wide and shallow.
- In A-form the base-pairs are displaced away from the central axis and closer to the major groove.
- Dehydration of DNA drives it into the A form, and this apparently protects DNA under conditions such as the extreme desiccation of bacteria. Protein binding can also strip solvent off of DNA and convert it to the A form, as revealed by the structure of a rod-shaped virus.
- Also, the A-form of DNA is more resistant to UV radiation, and therefore fungal spores contain just such form of DNA.



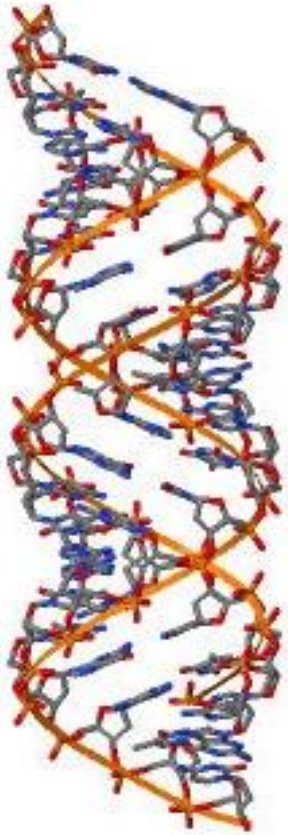
A form

# Z-form of DNA

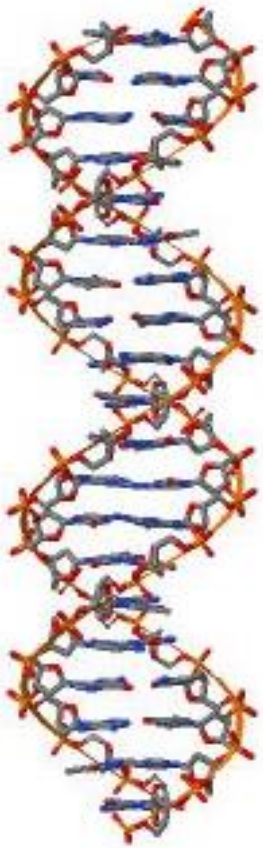
- Z-DNA is one of the possible double helical structures of DNA with about 12 base pairs per turn.
- It is a left-handed double helical structure in which the helix winds to the left in a zigzag pattern, instead of to the right, like the more common B-DNA form.
- A small amount of the DNA in a cell exists in the Z form. It has been tantalizing to propose that this different structure is involved in some way in regulation of some cellular function, such as transcription or regulation, but conclusive evidence for or against this proposal is not available yet.



# Determine form of DNA



1



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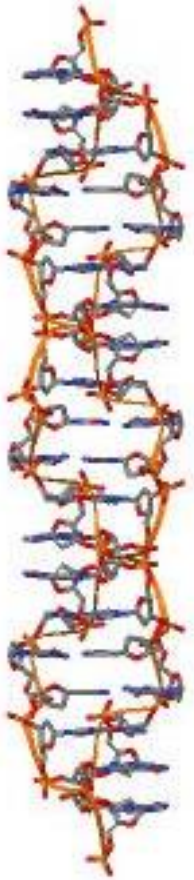
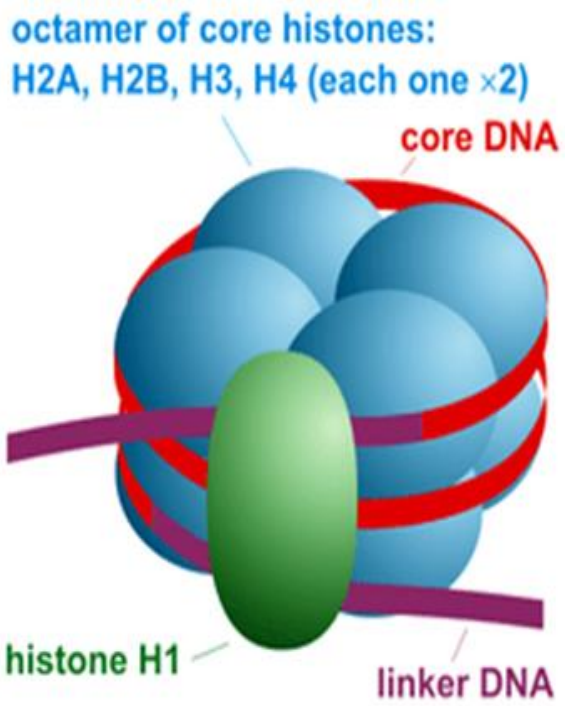


3

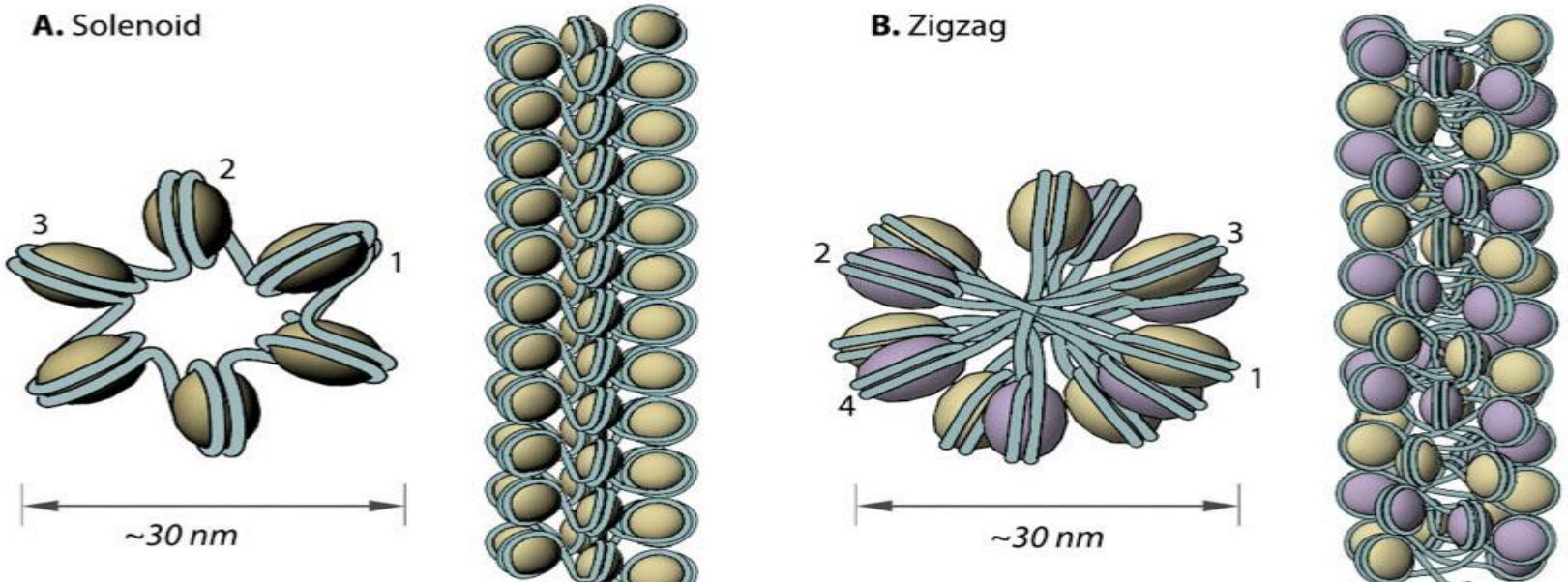
# DNA packaging

- Nucleosomes are the repeated primary units of chromatin and are composed of two copies of each of the four core histone proteins - octamers (H2A and H2B, H3, and H4), around which 147 base pairs of DNA are wrapped, and that after a series of folding, form a chromosome.
- H1 is not a part of histone core. It facilitates the binding of DNA to histones.

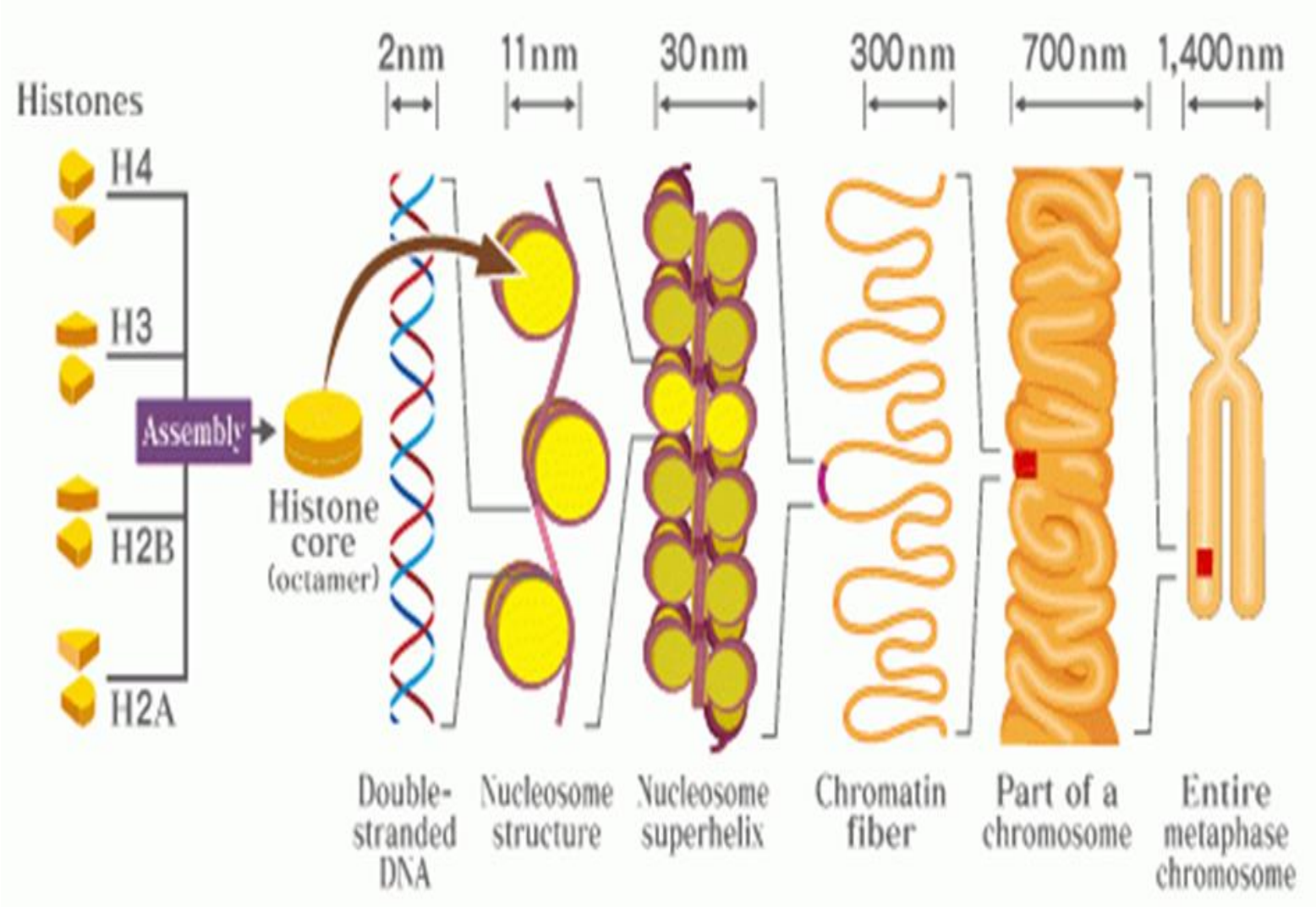


# DNA packaging

- 30 nm chromatin fibers are considered to exist in the form of so called solenoid or zigzag. The main feature of solenoid model is that nucleosomes follow each other along the same helical path, and interactions between the histone cores occur sequentially (1, 2, 3 and so on). Therefore, solenoid is also referred to as "one start model".
- In zigzag, on the other hand, linker DNA connects two opposing nucleosomes, creating a structure where the alternate histone cores become interacting partners (i.e., 1 and 3, 2 and 4 and so on). Therefore, zigzag is considered as a "two start model", which is indicated in the figure (B) by two different colors of histone cores: yellow interacting nucleosome partners (1, 3, etc.) as opposed to the violet nucleosome row (2, 4, etc.).



# DNA packaging



# DNA replication

- DNA replication occurs in the S phase of interphase during the cell cycle.

  Step 1: Replication fork formation

  Step 2: Primer Binding

  Step 3: Elongation

  Step 4: Termination

# Step 1

- Before DNA can be replicated, the double stranded molecule must be “unzipped” into two single strands. This process is initiated at particular points in the DNA, known as "origins" or ori-locus. This is performed by an enzyme known as DNA **helicase**. As helicase unwinds DNA at the replication fork, the DNA ahead is forced to rotate.
- T**opoisomerases** (including DNA gyrase) unwinds and rewinds DNA strands to prevent the DNA from becoming tangled or supercoiled.
- In order to unwind DNA, interactions between base pairs must be broken. DNA helicase disrupts the hydrogen bonding between base pairs to separate the strands into a Y shape known as the replication fork. The replication fork is bi-directional; one strand is oriented in the 3' to 5' direction (leading strand) while the other is oriented 5' to 3' (lagging strand).
- Bare single-stranded DNA tends to fold back on itself forming secondary structures; these structures can interfere with the movement of DNA polymerase. To prevent this, **single-strand binding proteins** bind to the DNA until a second strand is synthesized, preventing secondary structure formation.

# Step 2

- The leading strand is the simplest to replicate. Once the DNA strands have been separated, a short piece of RNA called a **primer** binds to the 3' end of the strand. The primer always binds as the starting point for replication. Primers are generated by the enzyme **DNA primase**.
- The lagging strand begins replication by binding with multiple primers. Each primer is only several bases apart. DNA polymerase then adds pieces of DNA, called **Okazaki fragments**, to the strand between primers. This process of replication is discontinuous as the newly created fragments are disjointed.

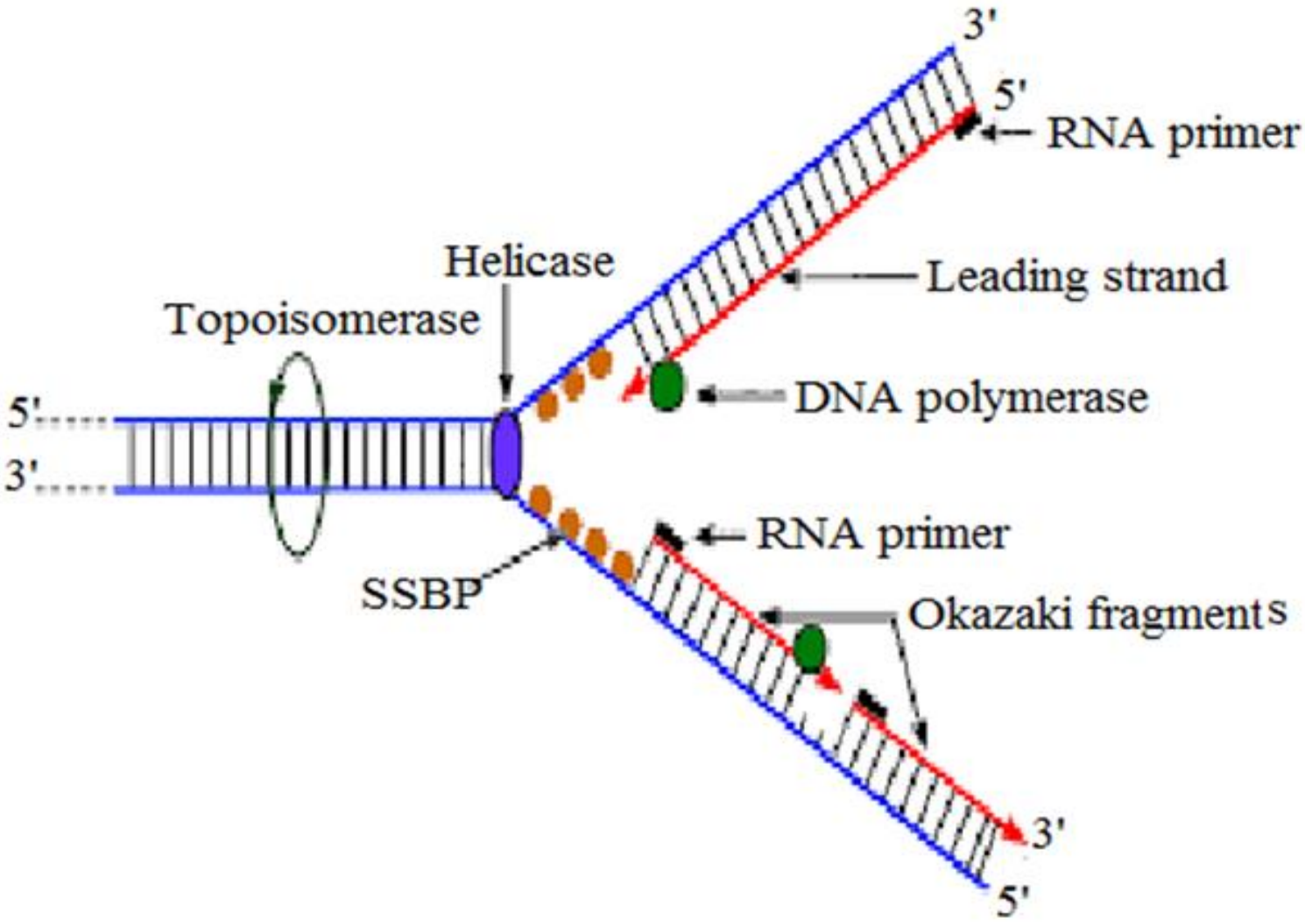
# Step 3

- Enzymes known as **DNA polymerases** (there exist many different types of DNA polymerase, each of which perform different functions in different types of cells) are responsible creating the new strand by a process called elongation. There are five different known types of DNA polymerases in bacteria and human cells. In bacteria such as E. coli, polymerase III is the main replication enzyme, while polymerase I, II, IV and V are responsible for error checking and repair.
- DNA polymerase III binds to the strand at the site of the primer and begins adding new base pairs complementary to the strand during replication. In eukaryotic cells, polymerases alpha, delta, and epsilon are the primary polymerases involved in DNA replication. Because replication proceeds in the 5' to 3' direction on the leading strand, the newly formed strand is continuous.

# Step 4

- Once both the continuous and discontinuous strands are formed, an enzyme called **DNA polymerase I** removes all RNA primers from the original strands. These primers are then replaced with appropriate bases.
- Another enzyme called **DNA ligase** joins Okazaki fragments together forming a single unified strand.
- The ends of the linear DNA present a problem as DNA polymerase can only add nucleotides in the 5′ to 3′ direction. The ends of the parent strands consist of repeated DNA sequences called telomeres. Telomeres act as protective caps at the end of chromosomes to prevent nearby chromosomes from fusing. A special type of DNA polymerase enzyme called **telomerase** catalyses the synthesis of telomere sequences at the ends of the DNA. Once completed, the parent strand and its complementary DNA strand coils into the familiar double helix shape. In the end, replication produces two DNA molecules, each with one strand from the parent molecule and one new strand.

# DNA replication

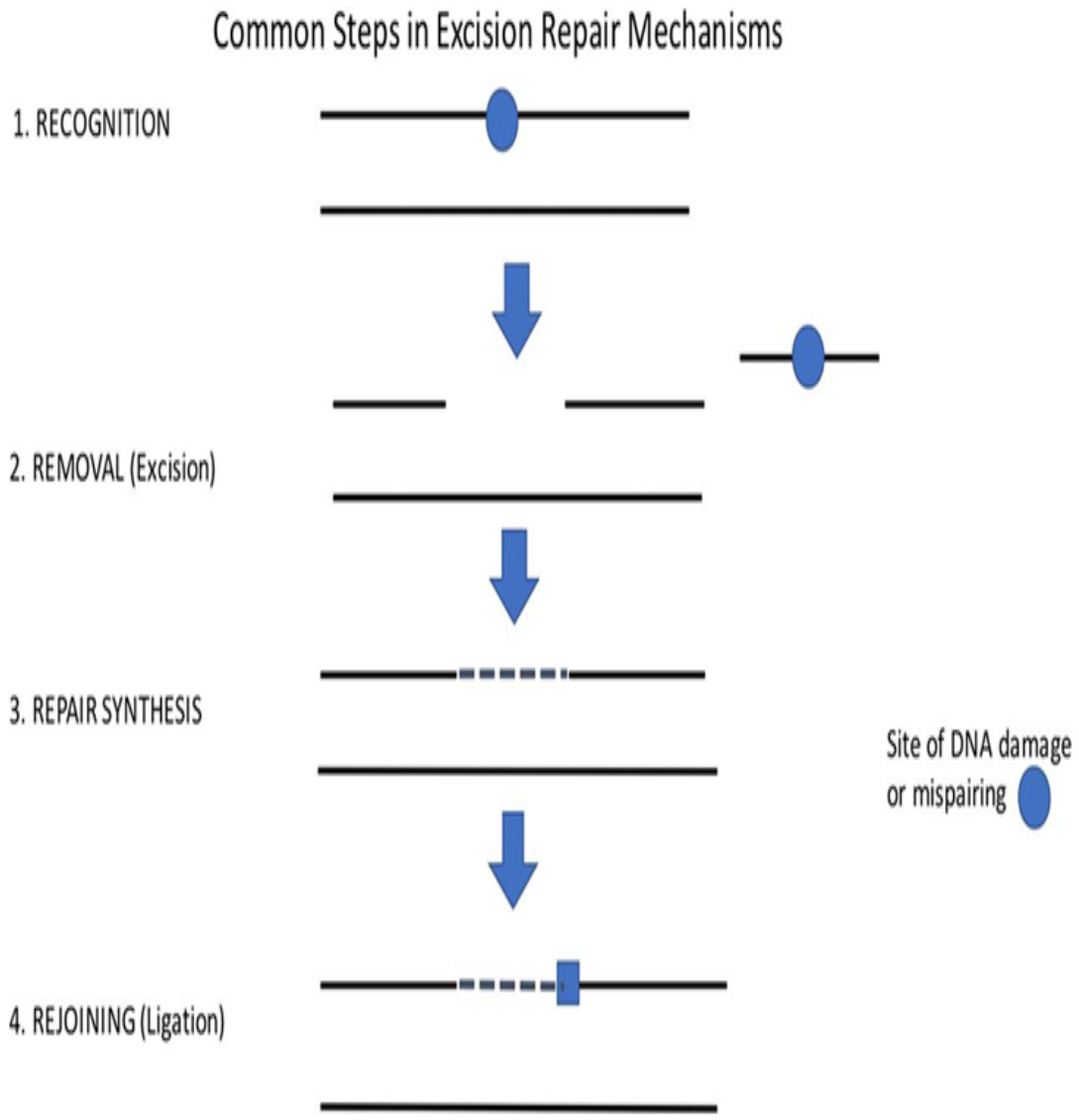


# DNA repair

- Excision repair - when only one of the two strands of a double helix has a defect, the other strand can be used as a template to guide the correction of the damaged strand. In order to repair damage to one of the two paired molecules of DNA, there exist a number of excision repair mechanisms that remove the damaged nucleotide and replace it with an undamaged nucleotide complementary to that found in the undamaged DNA strand.

# DNA repair: Excision repair

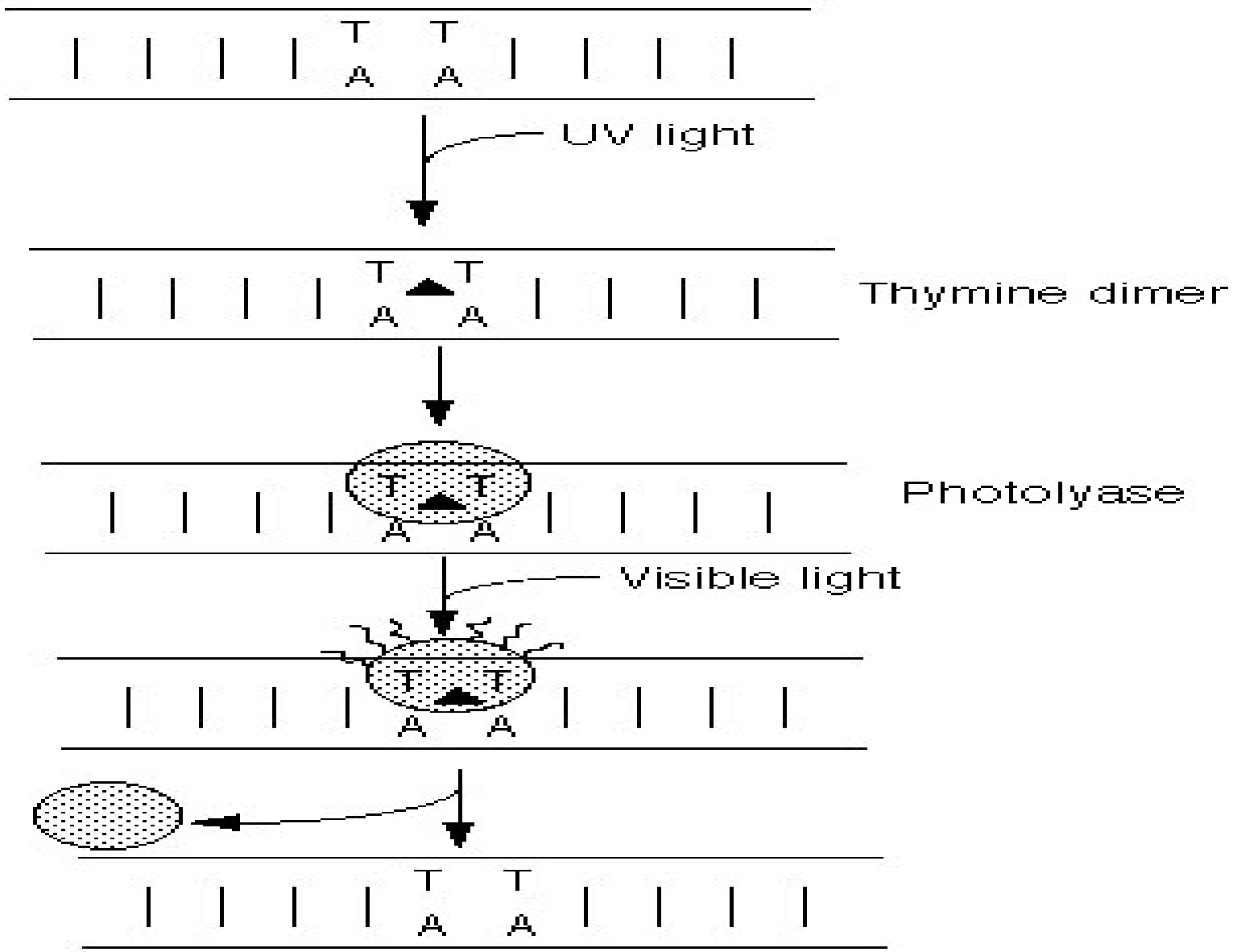
- Base excision repair (BER)
- Nucleotide excision repair (NER)
- Mismatch repair (MMR)

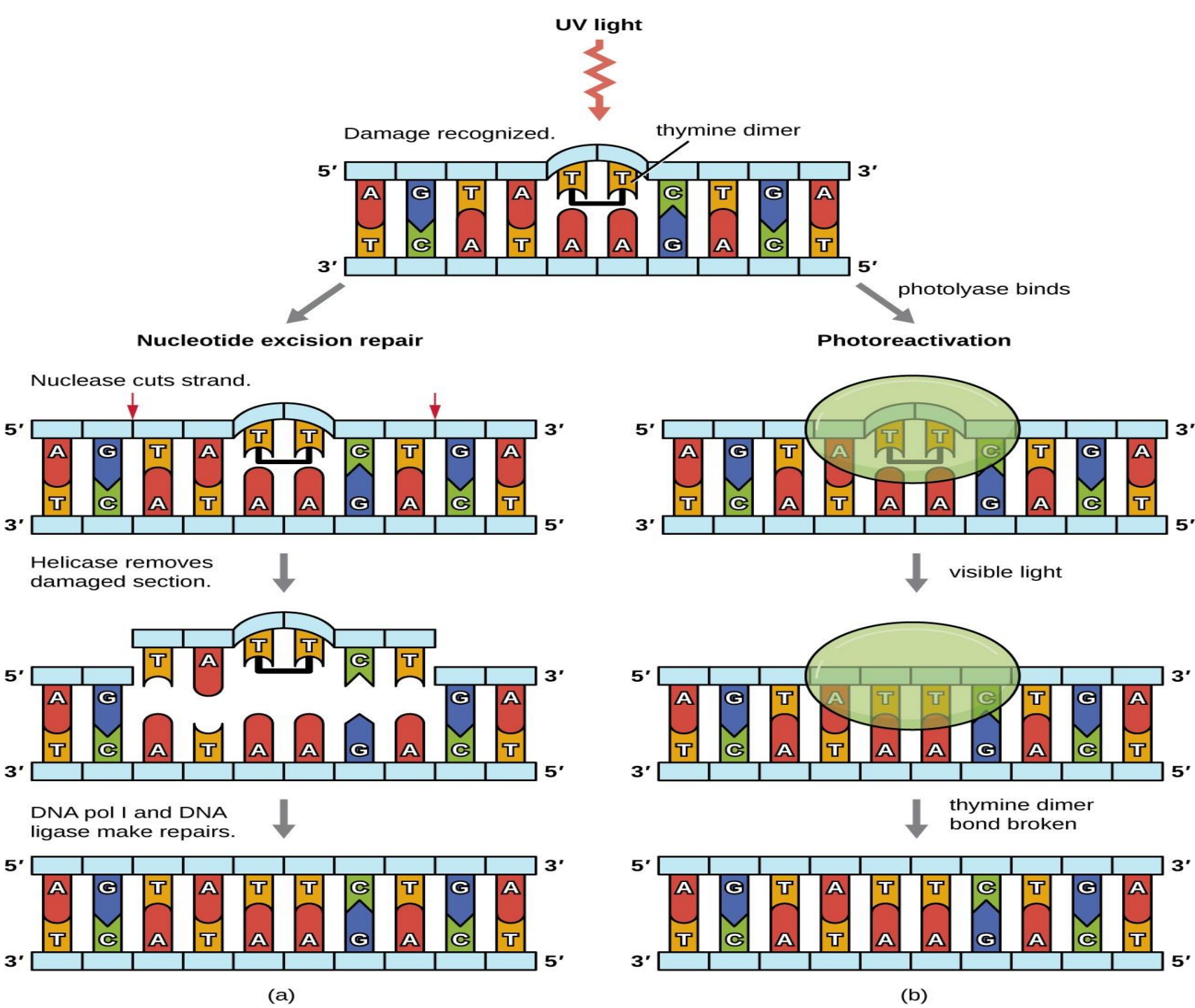


# DNA repair: Photoreactivation

- Photolyases are DNA repair enzymes that repair damage caused by exposure to ultraviolet light. These enzymes require visible light (from the violet/blue end of the spectrum) both for their own activation and for the actual DNA repair. The DNA repair mechanism involving photolyases is called photoreactivation. They mainly convert pyrimidine dimers into a normal pair of pyrimidine bases.

# Photoreactivation Repair




UV light
Damage recognized.
thymine dimer
5′
3′
3′
5′
photolyase binds
Nucleotide excision repair
Photoreactivation
Nuclease cuts strand.
Helicase removes damaged section.
visible light
DNA pol I and DNA ligase make repairs.
thymine dimer bond broken
(a)
(b)

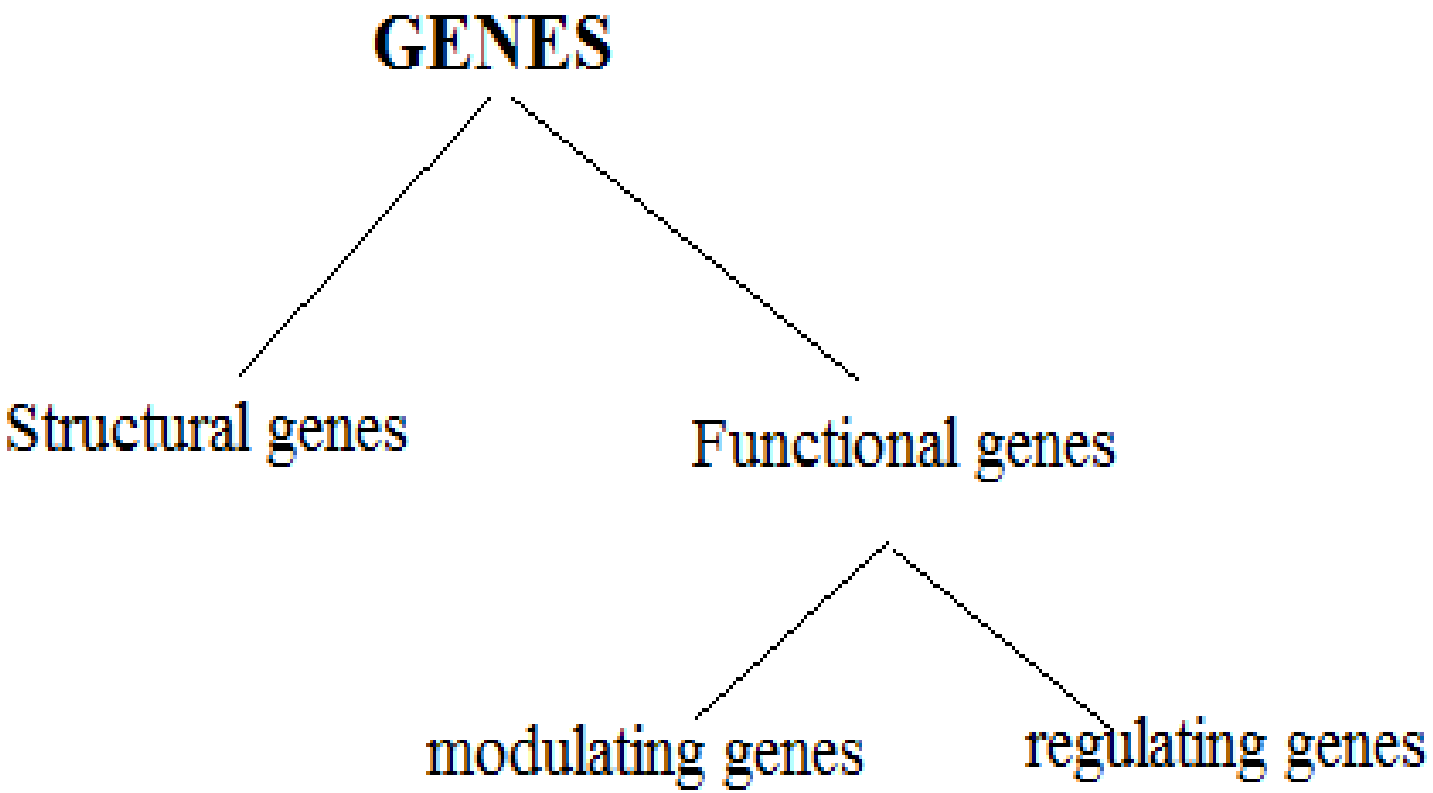
# Molecular definition of gene

- In molecular terms, a gene commonly is defined as the entire nucleic acid sequence that is necessary for the synthesis of a functional polypeptide.
- A gene also includes all the DNA sequences required for synthesis of a particular RNA transcript. In some prokaryotic genes, DNA sequences controlling the initiation of transcription by RNA polymerase can lie thousands of base pairs from the coding region. In eukaryotic genes, transcription-control regions known as enhancers can lie 50 kb or more from the coding region.
- Other critical noncoding regions in eukaryotic genes are the sequences that specify 3′ cleavage and polyadenylation and splicing of primary RNA transcripts. Mutations in these RNA processing signals prevent expression of a functional mRNA and thus of the encoded polypeptide.
- Although most genes are transcribed into mRNAs, which encode proteins, clearly some DNA sequences are transcribed into RNAs that do not encode proteins (e.g., tRNAs and rRNAs).

# Gene and its properties

- The gene as a unit of functioning of the hereditary material has a number of properties.
- 1. **Specificity** is a unique nucleotide sequence for each structural gene, i.e. each gene encodes its trait;
- 2. **Integrity** - as a functional unit (programming of protein synthesis) the gene is indivisible;
- 3. **Discreteness** - there are subunits in the gene: a muton is a subunit responsible for mutation, a recon is responsible for recombination. Their minimum value is a pair of nucleotides;
- 4. **Stability** - a gene, as a discrete unit of heredity is distinguished by stability (constancy) - in the absence of mutation, it is transmitted in a number of generations in an unchanged form. The frequency of spontaneous mutation of one gene is approximately 1 • 10 -5 per generation.
- 5. **Lability** - the stability of genes is not absolute, they can change, mutate;
- 6. **Pleiotropy** - the multiple effect of a single gene (one gene is responsible for several signs);
- 7. **Expressiveness** - the degree of expression of a gene in a trait or the degree of phenotypic manifestation of a gene.
- For example, the alleles of the AB0 blood group in a person have constant expressiveness (always occurring 100%), and the alleles that determine the color of the eyes are variable expressiveness. Recessive mutation, which reduces the number of eye facets in Drosophila, in different individuals in different ways reduces the number of facets up to their complete absence.
- 8. **Penetrance** - the frequency of phenotypic manifestation of a trait in the presence of the corresponding gene (the ratio (in %) of the number of individuals with a given trait to the number of individuals with a given gene);
- For example, the penetrance of congenital hip dislocation in humans is 25%, i.e. only 1/4 of recessive homozygotes are affected. Medico-genetic significance of penetrance: a healthy person, in which one of the parents suffers from a disease with incomplete penetrance, may have a non-developing mutant gene and pass it on to children.

# Gene classification



# Gene classification

- All genes by function are divided into structural and functional:

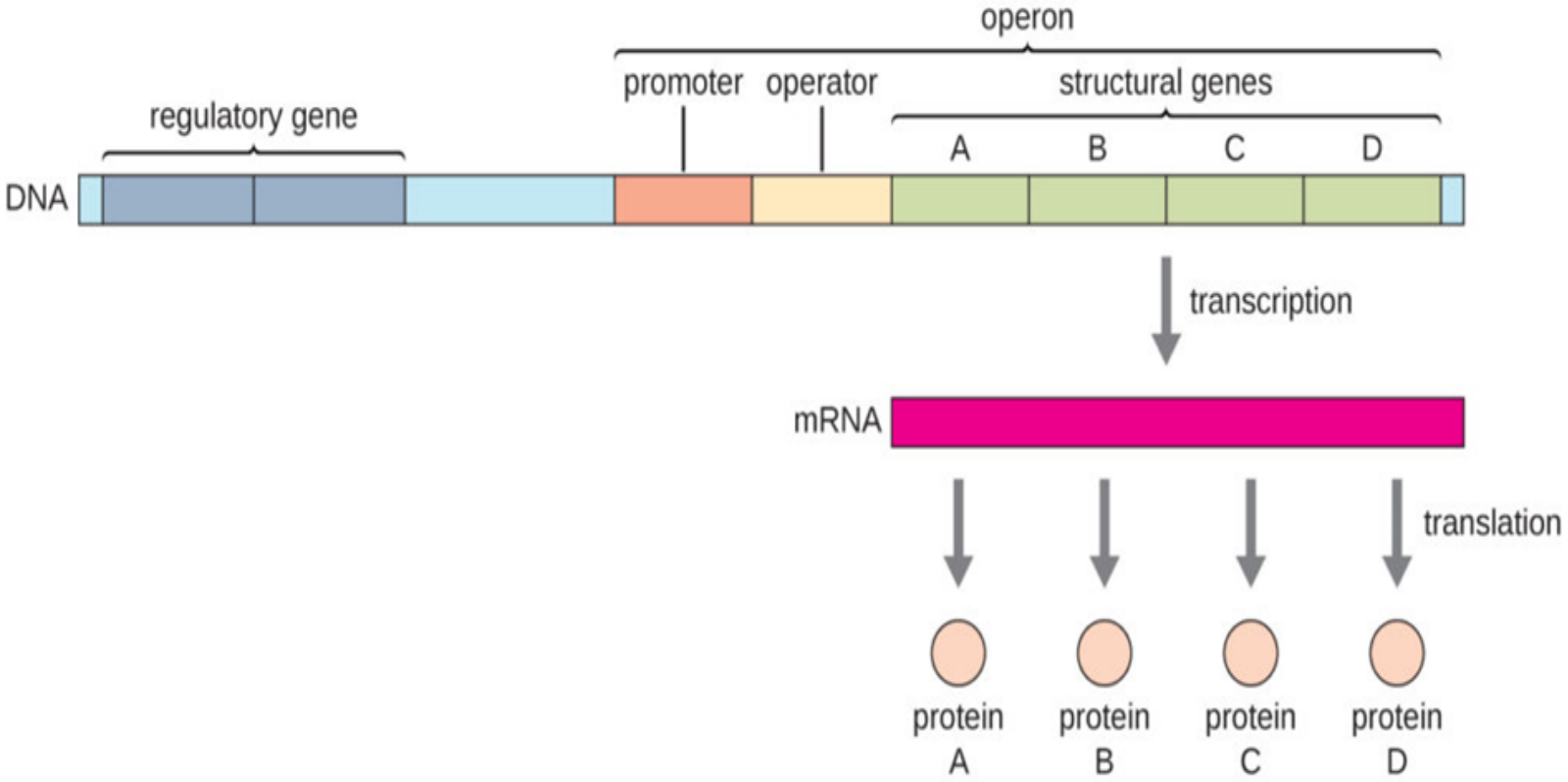
**- Structural genes** carry information about enzyme proteins and histones, about the sequence of nucleotides in various types of RNA.

- Among the **functional genes** emit:

- - *modulating genes* that enhance or weaken the action of structural genes (suppressors (inhibitors), activators, modifiers);
- - *genes regulating* the work of structural genes (regulators and operators).

# Gene structure of prokaryotes

- Genes of prokaryotic organisms are often organized into operons.
- Operon is a structure consisting of several structural genes. It allows prokaryotes to synthesize products of several genes at once.
- Structural genes in the operon are located one after the other and for all - one common promoter, one common terminator and one common operator, which regulates its work.

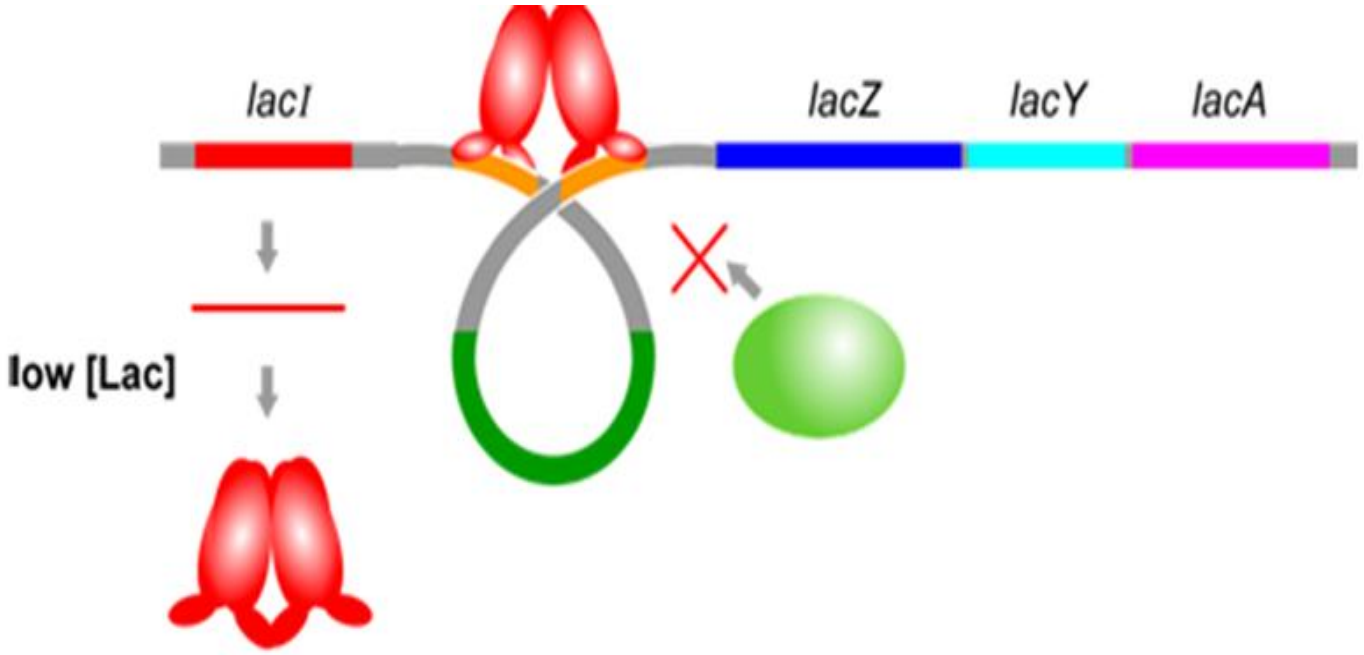


# E.coli lactose operon

- Regulation of gene expression of lactose metabolism in E. coli was first described in 1961 by scientists F. Jacob and J. Mono (received the Nobel Prize in 1965 together with A. Lvov ).
- The lac operon consists of three structural genes, and a promoter, a terminator, regulator, and an operator. The three structural genes are: lacZ, lacY, and lacA.

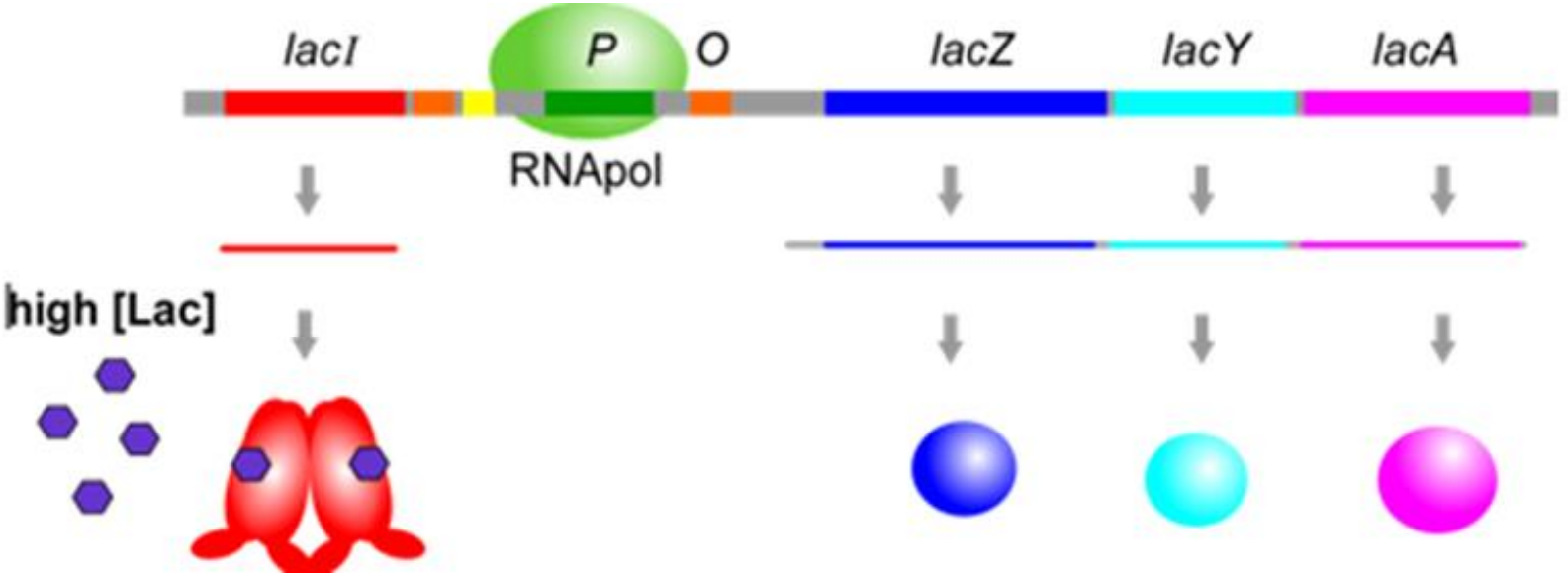
# Negative control of gene expression

- In the absence or low concentration of lactose in the cell, the repressor protein, named lacI, which found near the lac operon, but it is not a part of the operon and is expressed separately (lacI is continually transcribed, so its protein product – the lac repressor – is always present), connects to the operator region and RNA polymerase cannot bind to the promoter and start transcription.



# Positive control of gene expression

- When lactose is present, the lac repressor (lacI) loses its DNA-binding ability. This clears the way for RNA polymerase to bind to the promoter and transcribe the lac operon.



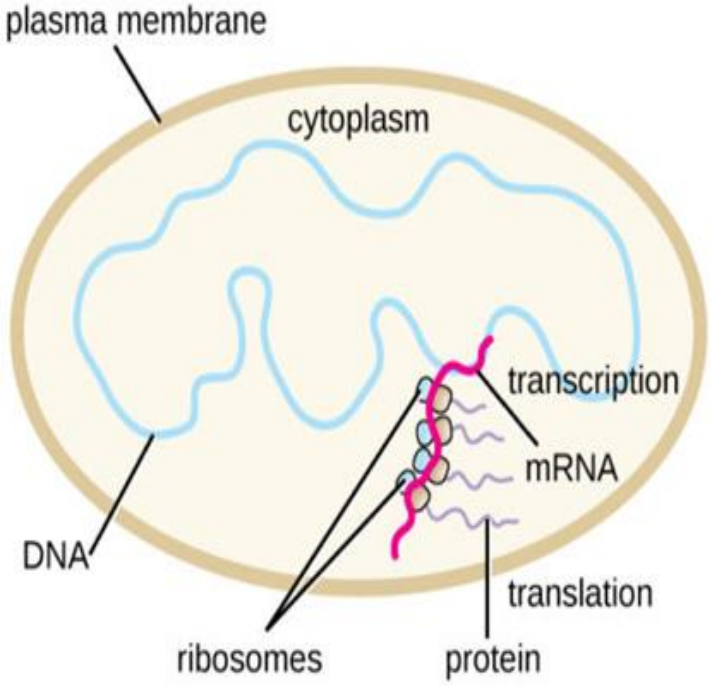
# Gene expression

- Gene expression is the process by which information from a gene is used in the synthesis of a functional gene product – RNA or protein.
- There are several steps in the gene expression: transcription, RNA splicing, translation, and post-translational modification of a protein.

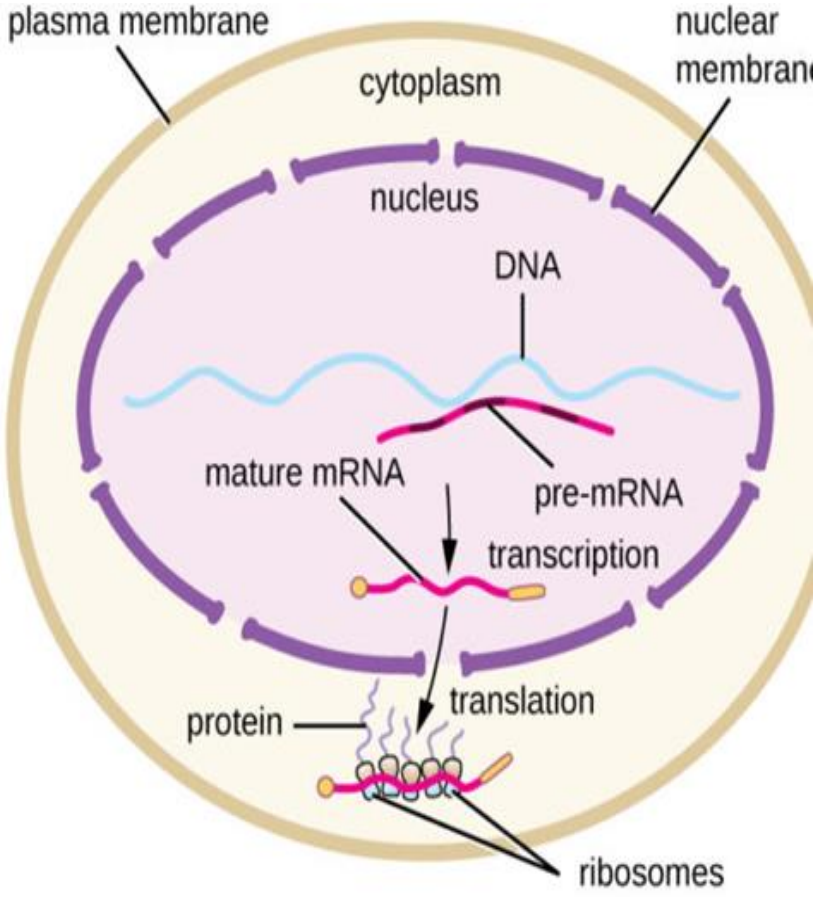
# Gene expression in prokaryotes and eukaryotes

- **In prokaryotes**, the processes of transcription and translation occur simultaneously in the cytoplasm, allowing for a rapid cellular response to an environmental cue.
- **In eukaryotes,** transcription is localized to the nucleus and translation is localized to the cytoplasm, separating these processes and necessitating RNA processing for stability.

# Gene expression in prokaryotes and eukaryotes



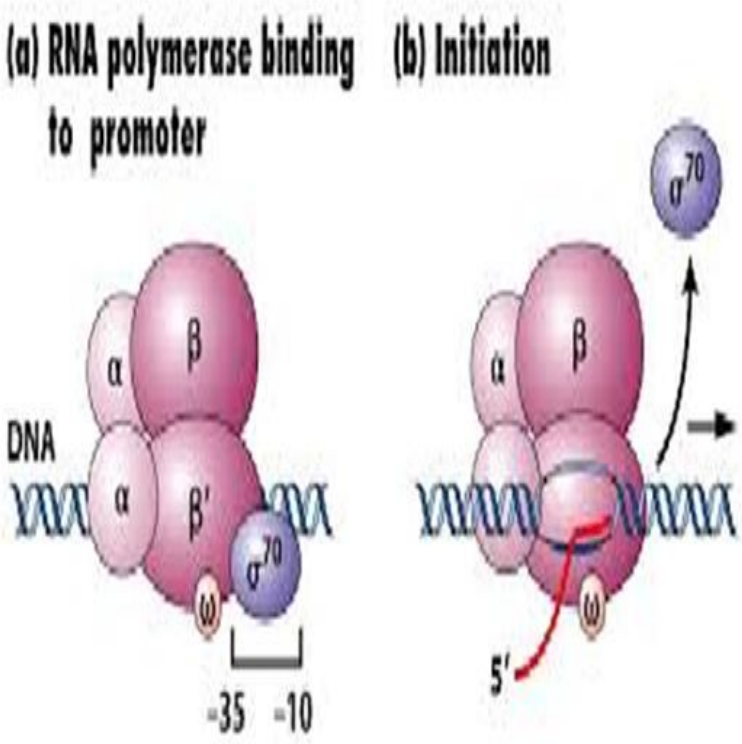

prokaryote



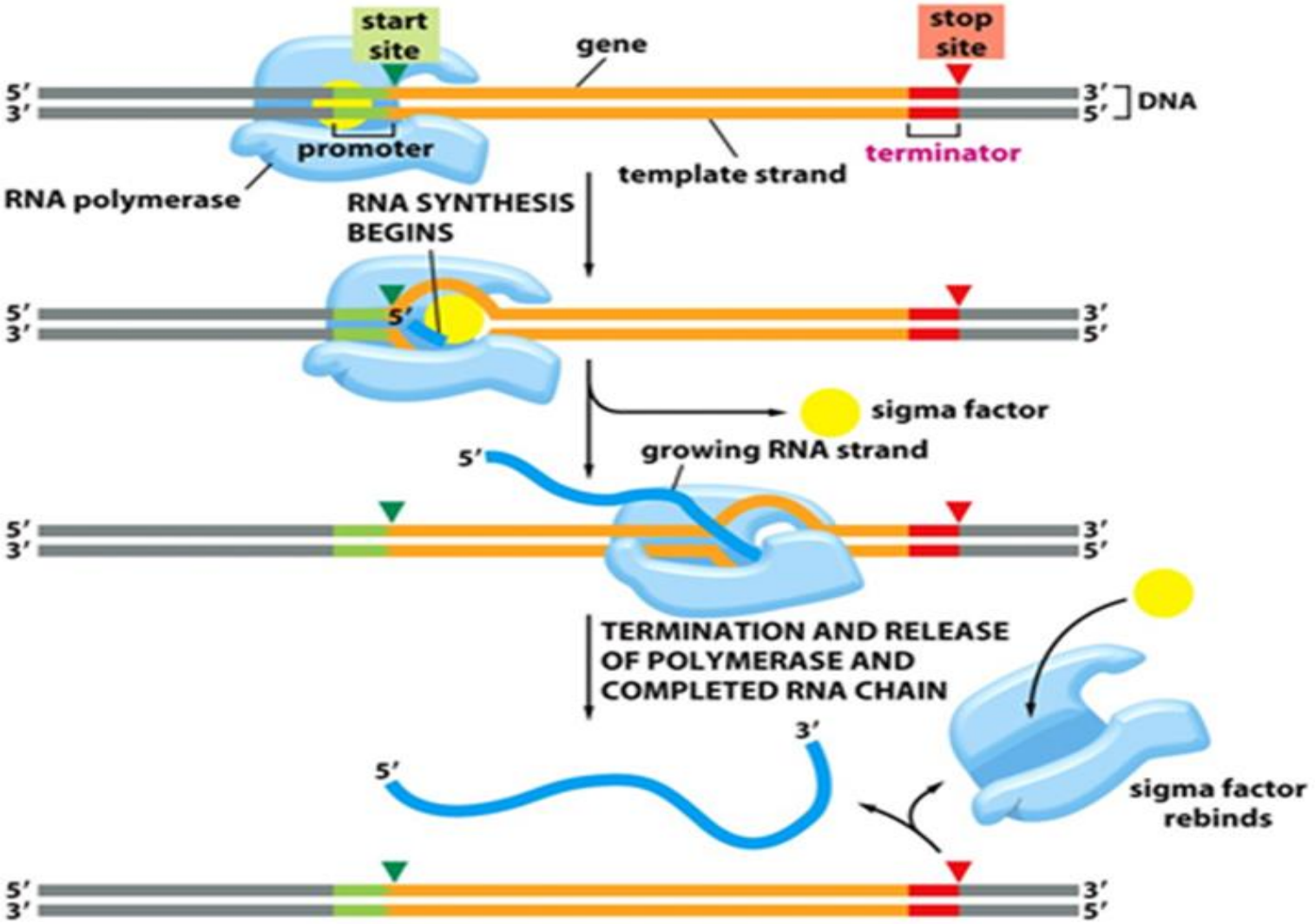

eukaryote

# Gene expression in prokaryotes

- The principal enzyme responsible for RNA synthesis is **RNA polymerase.**
- The E. coli complete RNA polymerase is known as the holoenzyme.
- The holoenzyme consists of the following two components: (1) the core enzyme consisting of two α, one β, and one β′ subunits and (2) the sigma factor.



# Transcription in prokaryotes



# Translation in prokaryotes

1. **Initiation**

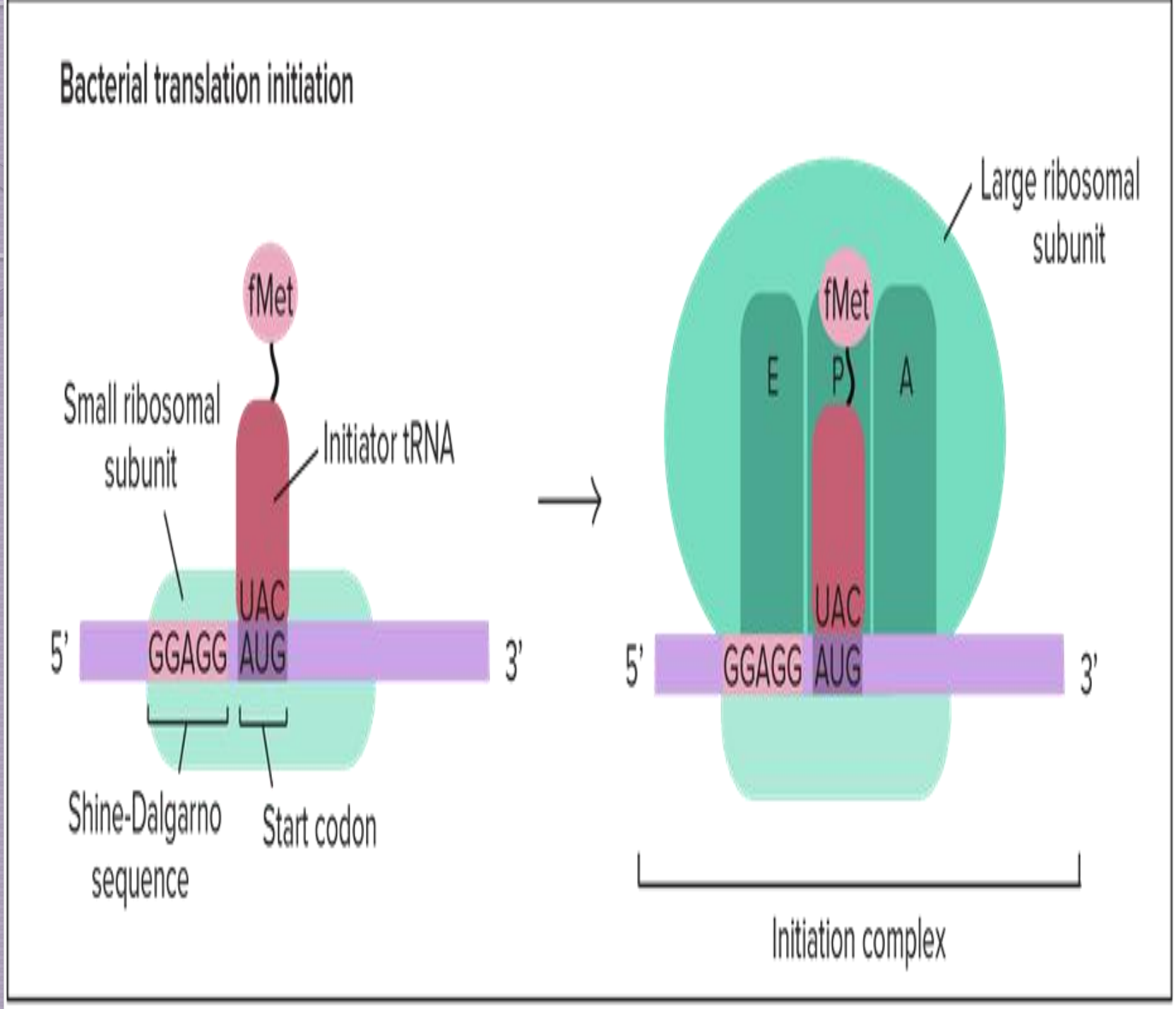
The initiation of protein synthesis begins with the formation of an initiation complex. The small ribosomal subunit attaches directly to certain sequences in the mRNA. These Shine-Dalgarno sequences come just before start codons and "point them out" to the ribosome.

2. **Elongation**

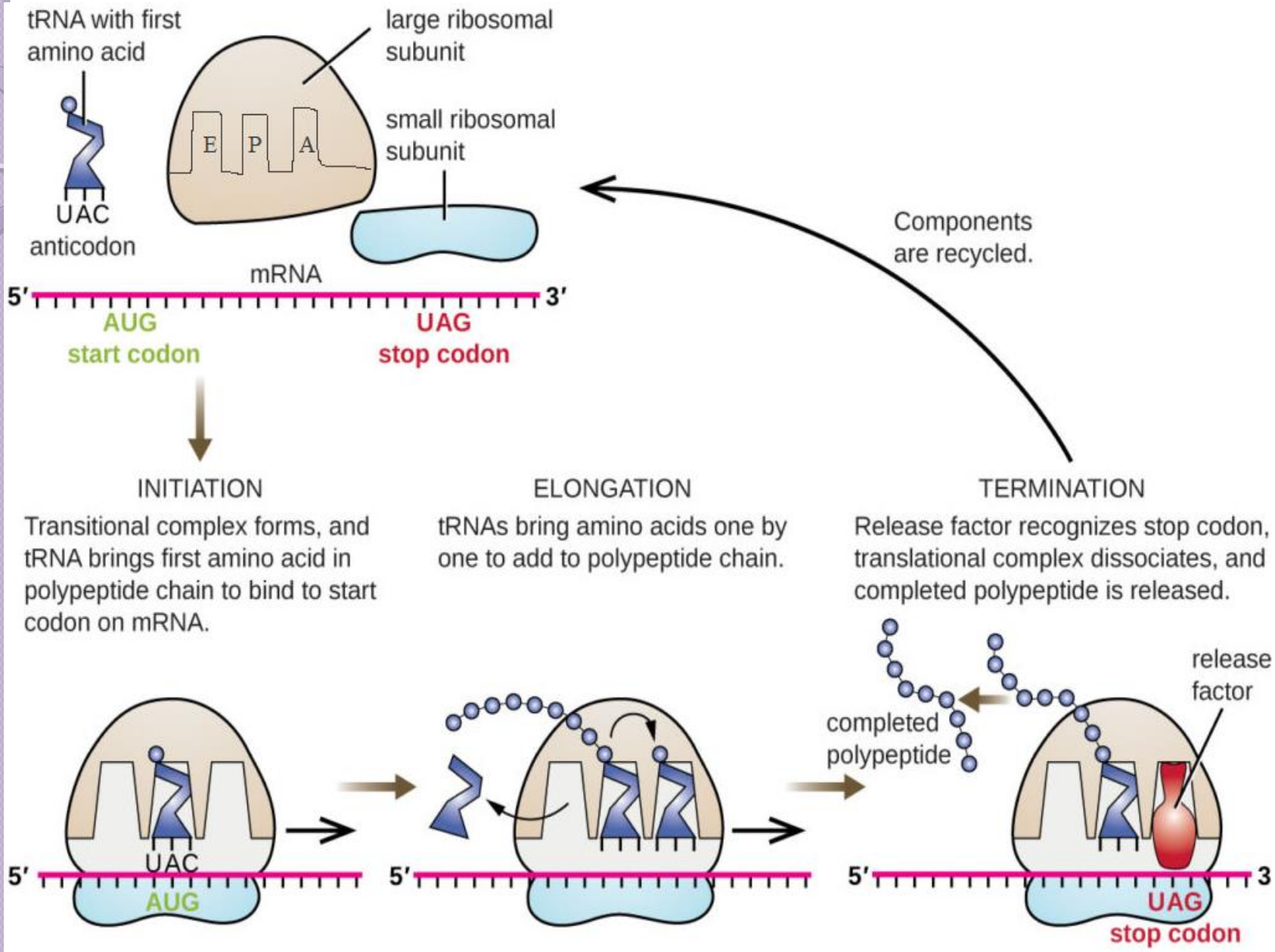
In prokaryotes and eukaryotes, the basics of elongation of translation are the same. In E. coli, the binding of the 50S ribosomal subunit to produce the intact ribosome forms three functionally important ribosomal sites: The A (aminoacyl) site binds incoming charged aminoacyl tRNAs. The P (peptidyl) site binds charged tRNAs carrying amino acids that have formed peptide bonds with the growing polypeptide chain but have not yet dissociated from their corresponding tRNA. The E (exit) site releases dissociated tRNAs so that they can be recharged with free amino acids.

3. **Termination**

The termination of translation occurs when a nonsense codon (UAA, UAG, or UGA) is encountered for which there is no complementary tRNA.


Bacterial translation initiation
fMet
Small ribosomal subunit
Initiator tRNA
UAC
5'
GGAGG
AUG
3'
Shine-Dalgarno sequence
Start codon
Large ribosomal subunit
fMet
E
P
A
UAC
5'
GGAGG
AUG
3'
Initiation complex

# Translation in prokaryotes



# Gene structure of eukaryotes

- An important role in the regulation of the genome is played non-coding sequences — enhancers, silencers, and insulators. The activity of these sequences determines the level of gene transcription, and hence the level of the protein synthesized from them.

# Gene structure of eukaryotes

- **Enhancer** is a short (50–1500 bp) region of DNA that can be bound by proteins (activators) to increase the rate of transcription of genes. Enhancers of higher eukaryotes are able to activate genes at large distances (up to tens of thousands of base pairs).
- **Silencer** is a DNA sequence with which repressor proteins (transcription factors) bind. Binding of repressor proteins to silencers leads to a decrease or complete suppression of RNA synthesis by the enzyme DNA-dependent RNA polymerase.
- **Insulators** are DNA sequences, special regulatory elements that have the ability to block signals from the environment. This function of insulators includes two activities. First, they block the interaction between the enhancer and the promoter, if it is between them. In this case, the insulator performs only the separation function and does not affect the activity of the enhancer and promoter. Secondly, the insulator performs a barrier function for propagating condensed chromatin. It is shown that there are insulators, both performing one of the two functions, and both.

# Transcription in eukaryotes

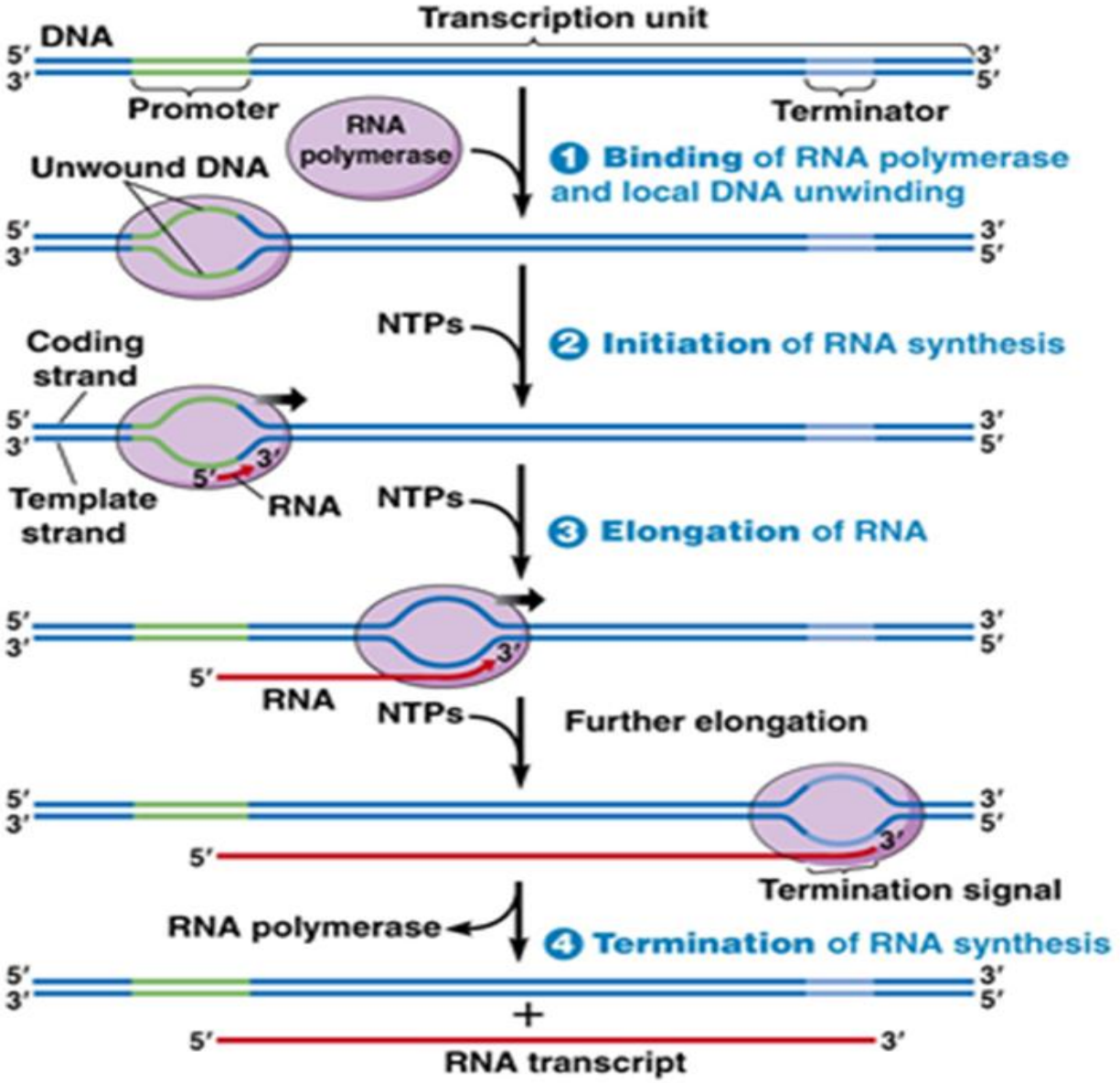
- During **initiation**, RNA polymerase recognizes a specific site on the DNA, upstream from the gene that will be transcribed, called a promoter site and then unwinds the DNA locally. The start site which has the consensus TATA(A/T)A(A/T) and is called the TATA box (located at about position -25).
- First, an RNA polymerase along with general transcription factors binds to the promoter region of the gene to form a closed pre-initiation complex.
- Second, transformation into an open complex. The DNA helix at a distance of about 13 nucleotide pairs from the start point of transcription is melted, that is, the DNA chains are separated from each other. The region of separated DNA helices is called a transcription bubble.

# Transcription in eukaryotes

- The transition from initiation to **elongation** is accompanied by breaking of bonds between the enzyme and promoter. The elongation phase ends after the release of the growing transcript and the dissociation of the enzyme from the matrix (termination).

# Transcription in eukaryotes

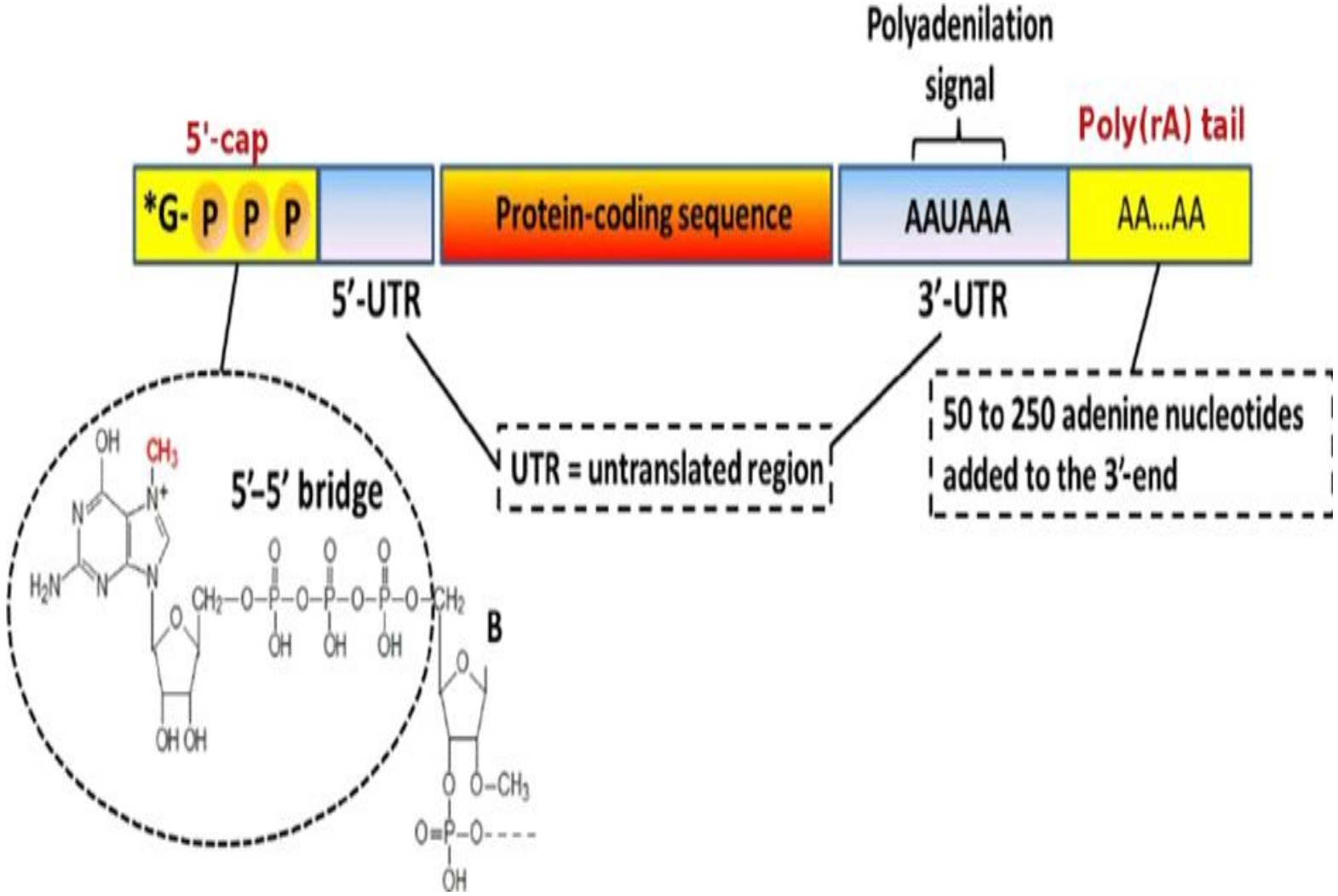
- The **termination** of transcription in eukaryotes is less studied. It ends by cutting RNA, after which to its 3 ′ end the enzyme adds several adenines (... AAAA), the number of which determines the stability of the transcript.


Transcription unit
DNA
5′
3′
3′
5′
Promoter
Terminator
RNA polymerase
Unwound DNA
1 Binding of RNA polymerase and local DNA unwinding
NTPs
2 Initiation of RNA synthesis
Coding strand
Template strand
RNA
3 Elongation of RNA
Further elongation
Termination signal
RNA polymerase
4 Termination of RNA synthesis
+
RNA transcript

# RNA processing

- 1. 5' capping, which is set of enzymatic reactions that add 7-methylguanosine (m7G) to the 5' end of pre-mRNA and thus protect the RNA from degradation by exonucleases.
- 2. 3' cleavage and polyadenylation. They occur if polyadenylation signal sequence (5'-AAUAAA-3') is present in pre-mRNA, which is usually between protein-coding sequence and terminator. The pre-mRNA is first cleaved and then a series of ~200 adenines (A) are added to form poly(A) tail, which protects the RNA from degradation.

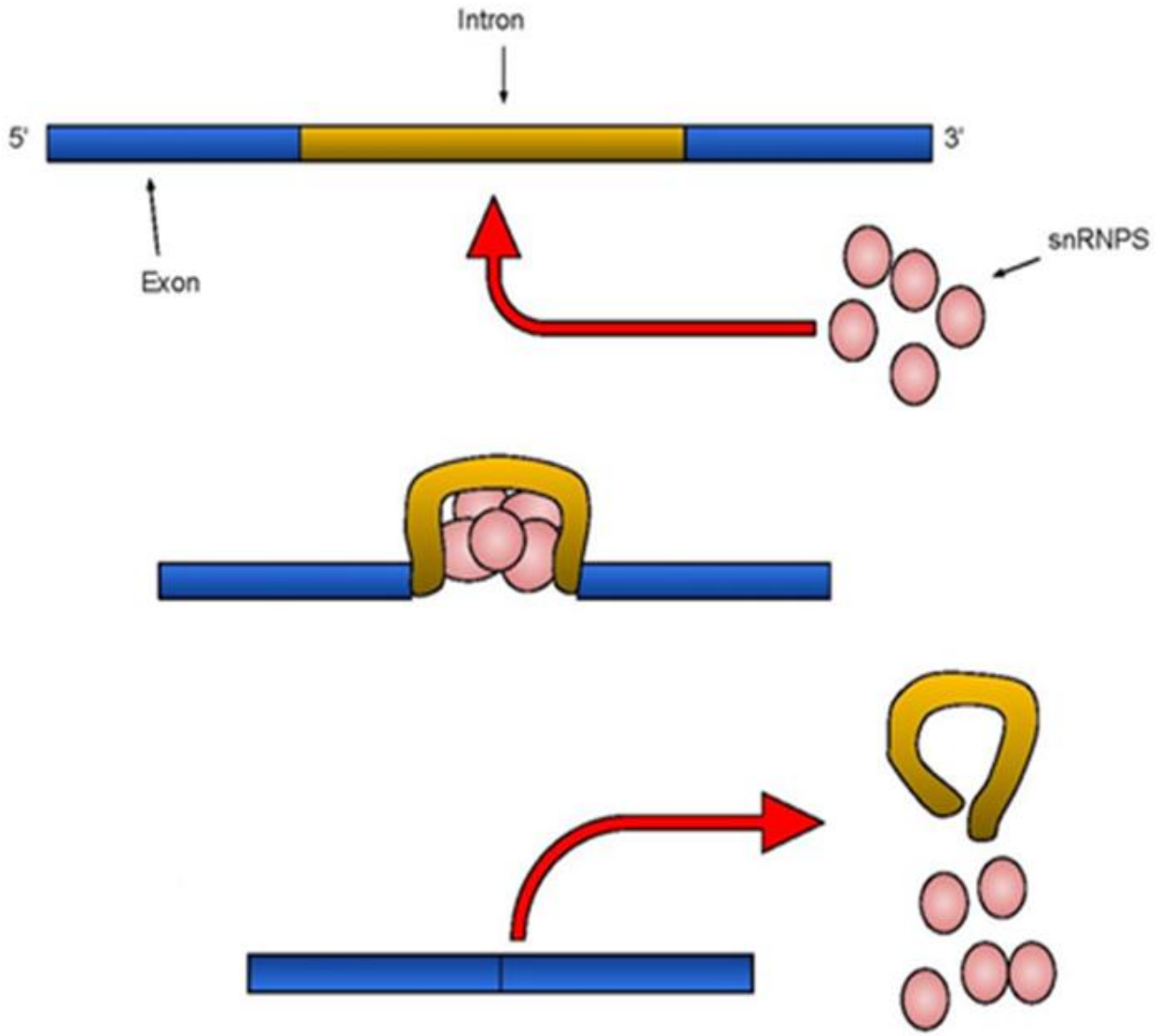
# 5' capping and polyadenylation



# RNA processing

- 3. Splicing. The majority of eukaryotic pre-mRNAs consist of alternating segments called exons and introns. During the process of splicing, an RNA-protein complex (snRNPs) known as spliceosome remove an intron, and then splice neighbouring exons together. In certain cases, some introns or exons can be either removed or retained in mature mRNA. This so-called alternative splicing creates series of different transcripts originating from a single gene. Because these transcripts can be potentially translated into different proteins, splicing extends the complexity of eukaryotic gene expression.

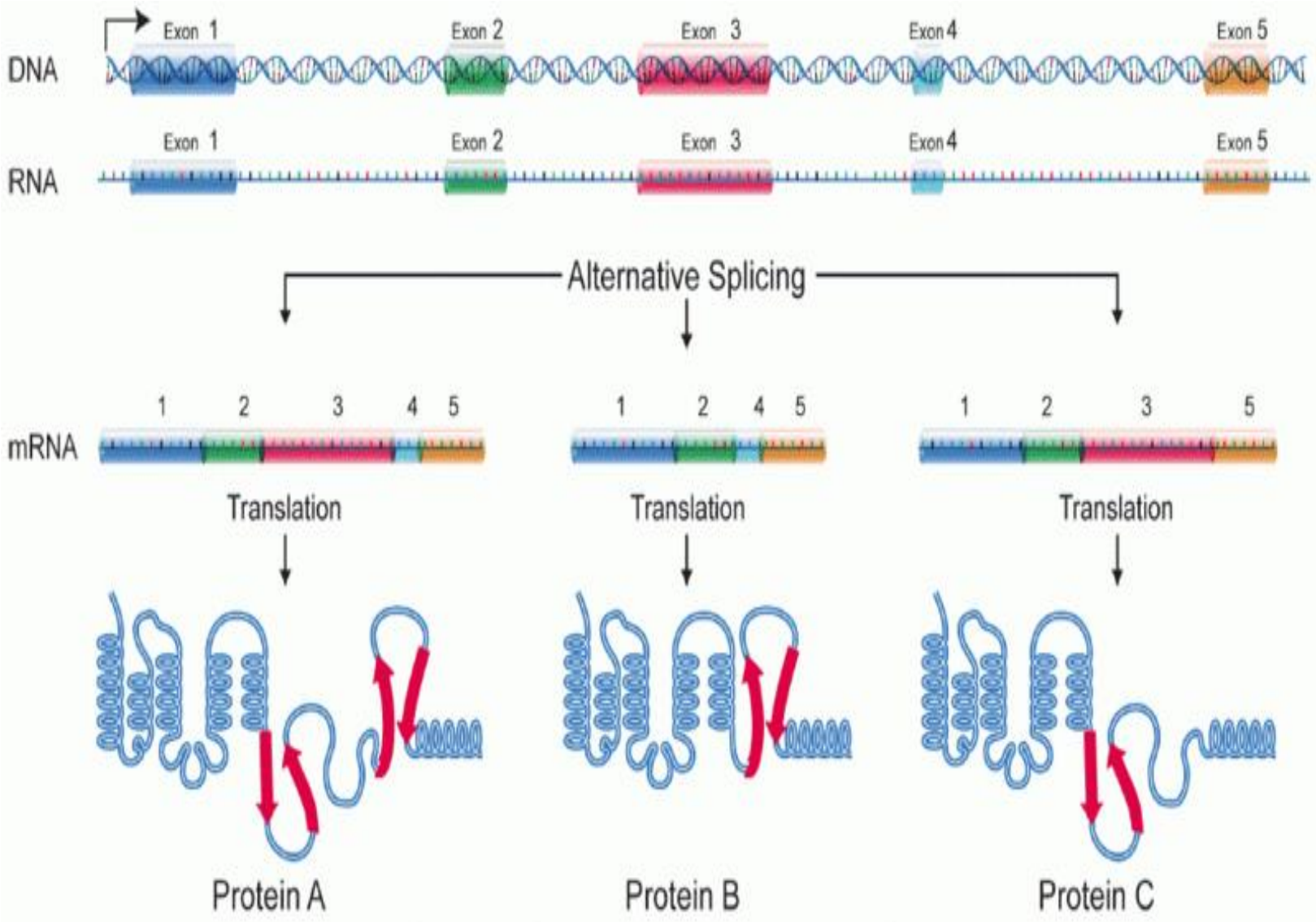
# Splicing



# Alternative splicing

- **Alternative splicing** is a variant of splicing of messenger RNA (mRNA), in which several mature mRNA is formed during gene expression based on the same primary transcript (pre-mRNA). Structural and functional differences of the resulting transcripts can be caused both by selective inclusion of exons of the primary transcript into the mature mRNA, and by preserving parts of introns in it. The most common type of alternative splicing involves skipping exon: individual exons of the transcript under certain conditions can be either included in the mature mRNA or skipped.
- The proteins produced by the translation of such mRNA result in different amino acid sequences; thus, with alternative splicing, a single transcript provides for the synthesis of several proteins.

# Alternative splicing

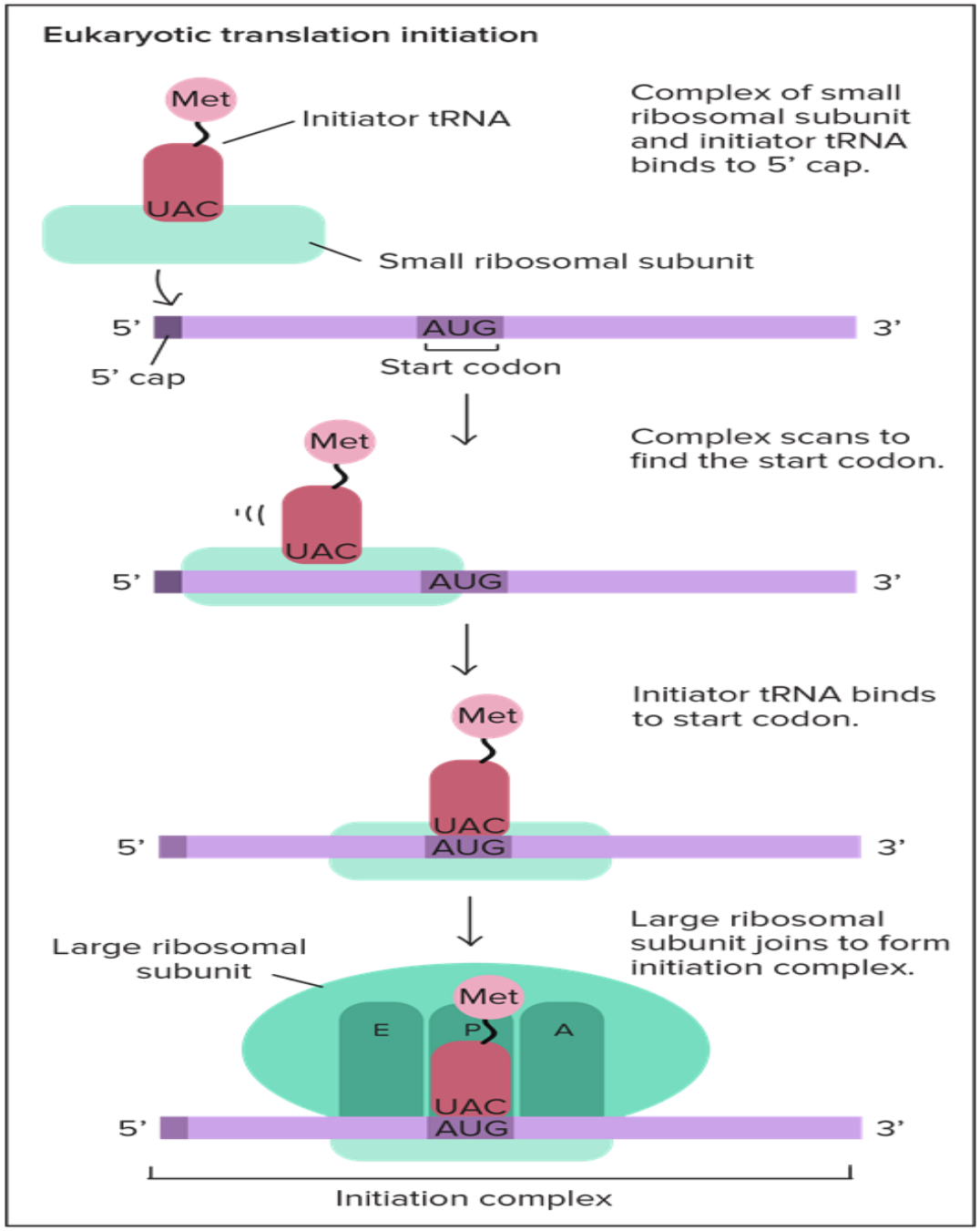


# Translation in eukaryotes

- Eukaryotic translation is the biological process by which messenger RNA is translated into proteins in eukaryotes.
- It consists of four phases: initiation, elongation and termination.

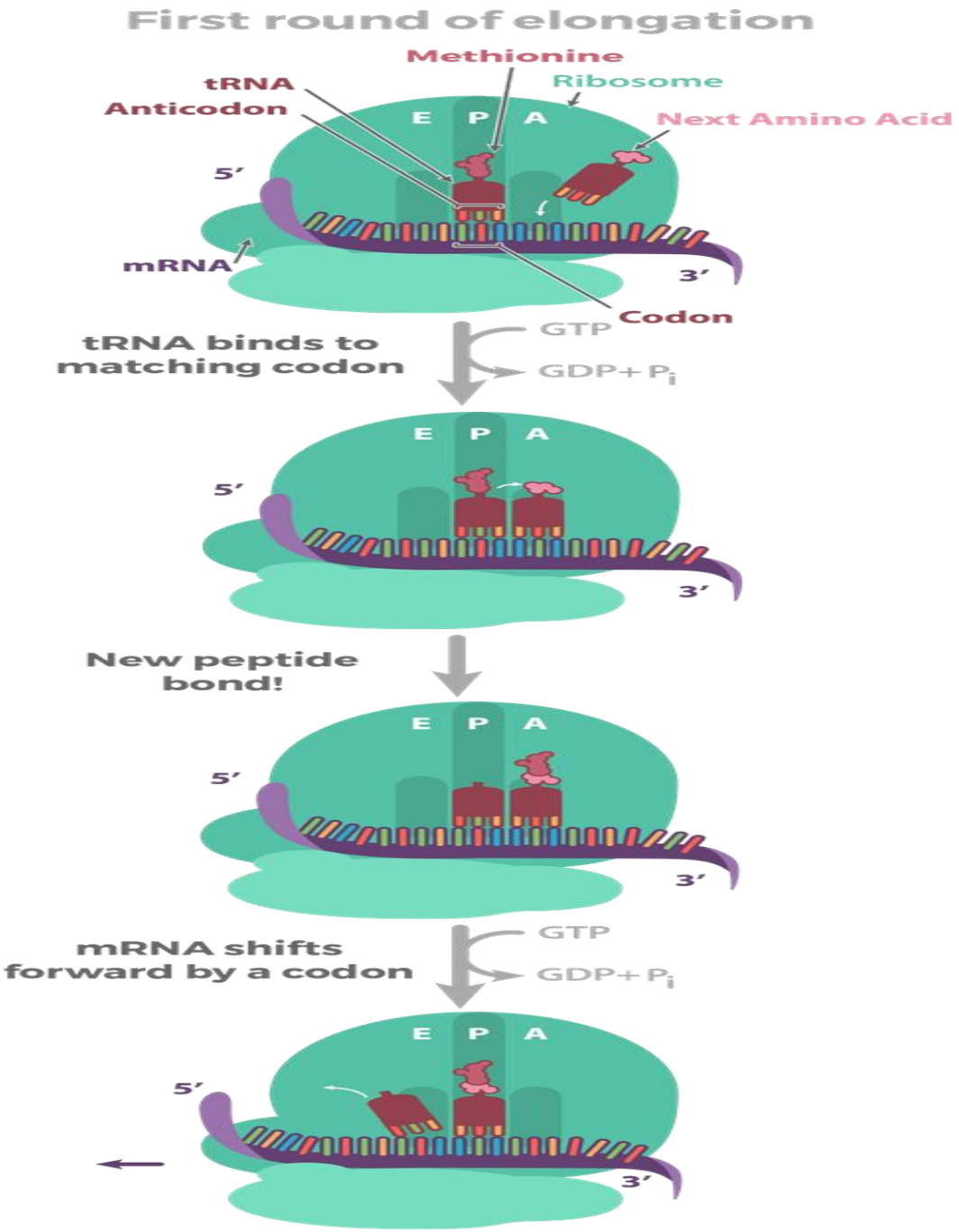
# Translation in eukaryotes

- **Initiation** depends on certain key proteins, called initiation factors. Their job is to help the ribosome subunits, tRNA, and mRNA find each other in an orderly and predictable way.
- Initiation goes like this: first, the tRNA carrying methionine attaches to the small ribosomal subunit. Together, they bind to the 5' end of the mRNA by recognizing the 5' GTP cap (added during processing in the nucleus). Then, they "walk" along the mRNA in the 3' direction, stopping when they reach the start codon (often, but not always, the first AUG).


Eukaryotic translation initiation
Met
Initiator tRNA
UAC
Complex of small ribosomal subunit and initiator tRNA binds to 5' cap.
Small ribosomal subunit
5'
AUG
3'
5' cap
Start codon
Met
UAC
Complex scans to find the start codon.
5'
AUG
3'
Met
UAC
Initiator tRNA binds to start codon.
5'
AUG
3'
Large ribosomal subunit
Large ribosomal subunit joins to form initiation complex.
Met
E
P
A
UAC
5'
AUG
3'
Initiation complex

# Translation in eukaryotes

- **Elongation**
- First, methionine-carrying tRNA starts out in the middle slot of the ribosome, called the P site. Next to it, a fresh codon is exposed in another slot, called the A site. The A site will be the "landing site" for the next tRNA, one whose anticodon is a perfect (complementary) match for the exposed codon.
- Once the matching tRNA has landed in the A site, it's time for the action: that is, the formation of the peptide bond that connects one amino acid to another. This step transfers the methionine from the first tRNA onto the amino acid of the second tRNA in the A site. Once the peptide bond is formed, the mRNA is pulled onward through the ribosome by exactly one codon. This shift allows the first, empty tRNA to drift out via the E ("exit") site. It also exposes a new codon in the A site, so the whole cycle can repeat.


First round of elongation
Methionine
Ribosome
tRNA
Anticodon
Next Amino Acid
E P A
5′
mRNA
3′
Codon
tRNA binds to matching codon
GTP
GDP+ $P_i$
E P A
5′
3′
New peptide bond!
E P A
5′
3′
mRNA shifts forward by a codon
GTP
GDP+ $P_i$
E P A
5′
3′

# Translation in eukaryotes

- Translation ends in a process called **termination**. Polypeptides must eventually come to an end. Termination happens when a stop codon in the mRNA (UAA, UAG, or UGA) enters the A site.
- Stop codons are recognized by proteins called release factors. Release factors mess with the enzyme that normally forms peptide bonds: they make it add a water molecule to the last amino acid of the chain. This reaction separates the chain from the tRNA, and the newly made protein is released.

# Post-translational modification of protein

- After completion of translation, most proteins undergo further chemical modifications, which are called post-translational modifications. More than two hundred variants of post-translational protein modifications are known.
- Post-translational modifications can regulate the duration of the existence of proteins in the cell, their enzymatic activity and interactions with other proteins. In some cases, post-translational modifications are an obligatory stage of protein maturation, otherwise it is functionally inactive. For example, in the maturation of insulin and some other hormones, limited proteolysis of the polypeptide chain is necessary, and in the maturation of plasma membrane proteins, glycosylation is required.

# Post-translational modifications

- Post-translational modifications are divided into:

1. **Modifications of the main chain**:

-cleavage of the N-terminal methionine residue;

-limited proteolysis — removal of a protein fragment that can occur from the ends (cleavage of signal sequences) or, in some cases, in the middle of the molecule (insulin maturation);

-addition of various chemical groups to free amino and carboxyl groups (N-acylation, etc.);

2. **Modification of amino acid side chains**:

-joining or cleavage of small chemical groups (glycosylation, phosphorylation , etc.);

-addition of lipids and hydrocarbons;

-change of standard amino acid residues to non-standard (formation of citrulline);

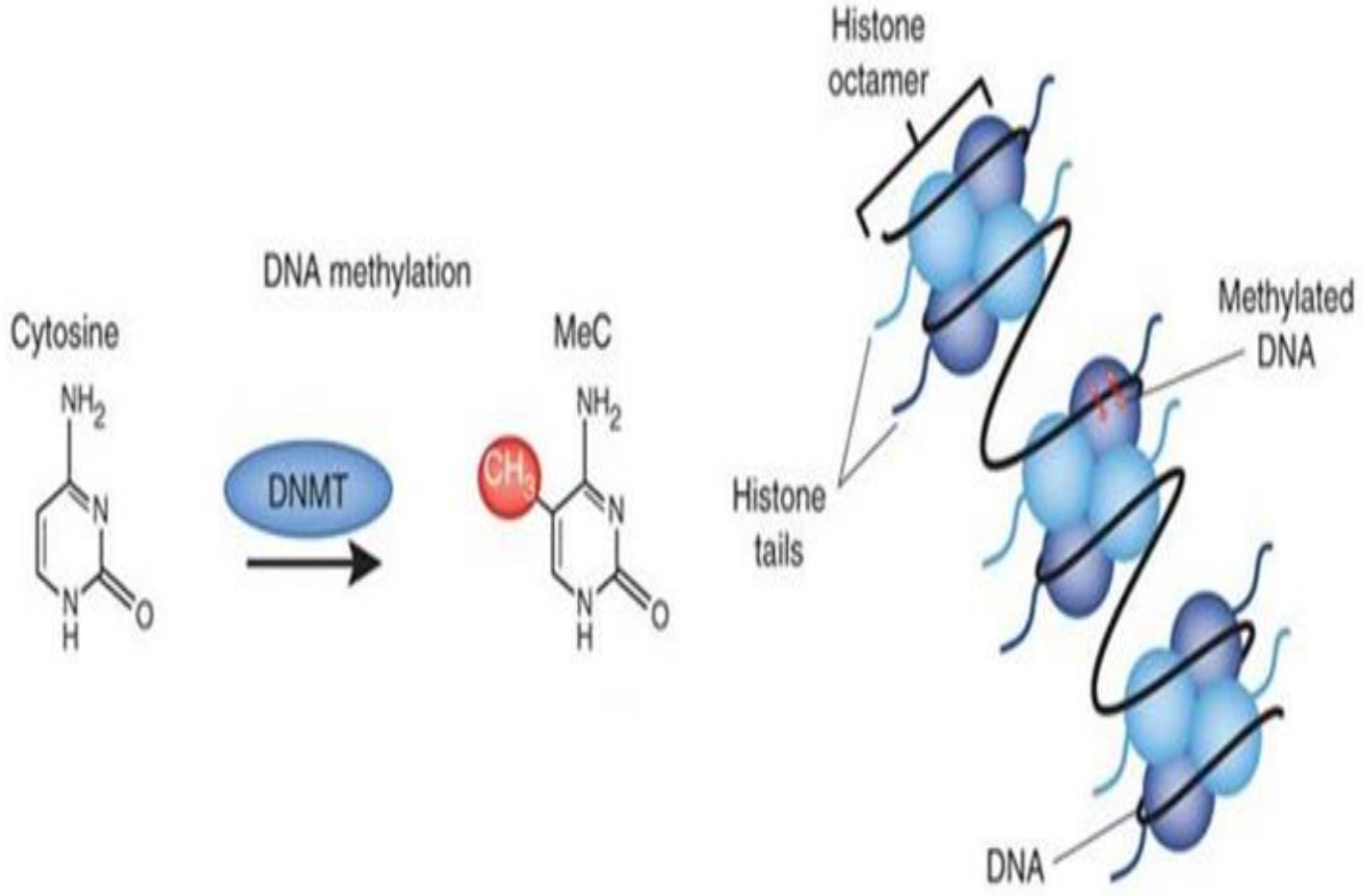
-the formation of disulphide bridges between cysteine residues ;

3. **The addition of small proteins**.

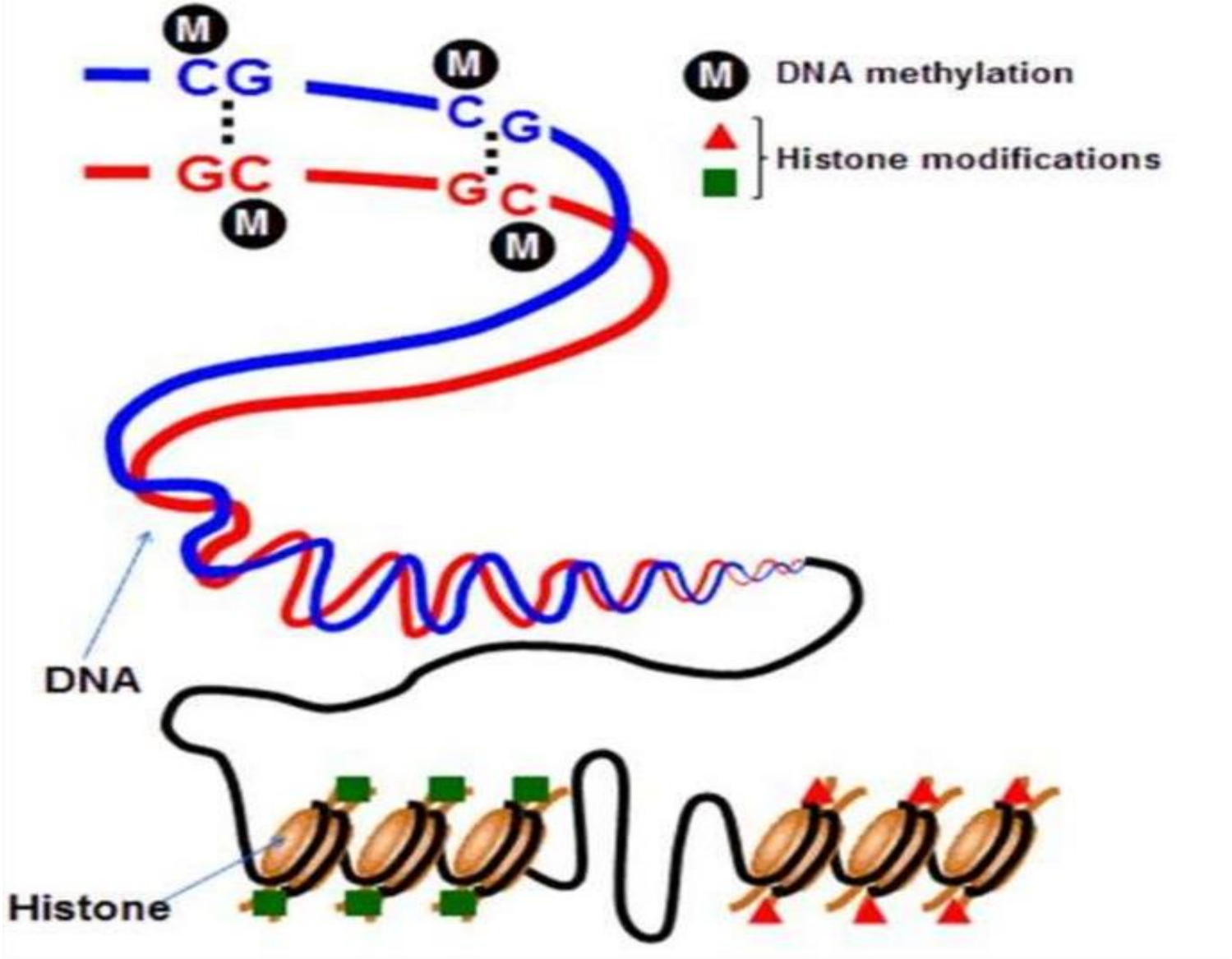
# DNA methylation

- **Methylation** is a method of regulating gene activity by attaching methyl group (-CH3) to the cytosine bases of DNA. Methylation inhibits gene activity: the synthesis of RNA (respectively, and protein) in such matrix becomes impossible. This is a kind of "plug" that the organism uses by inactivating certain genes whose work does not currently need or may be dangerous.

# DNA methylation



# DNA methylation



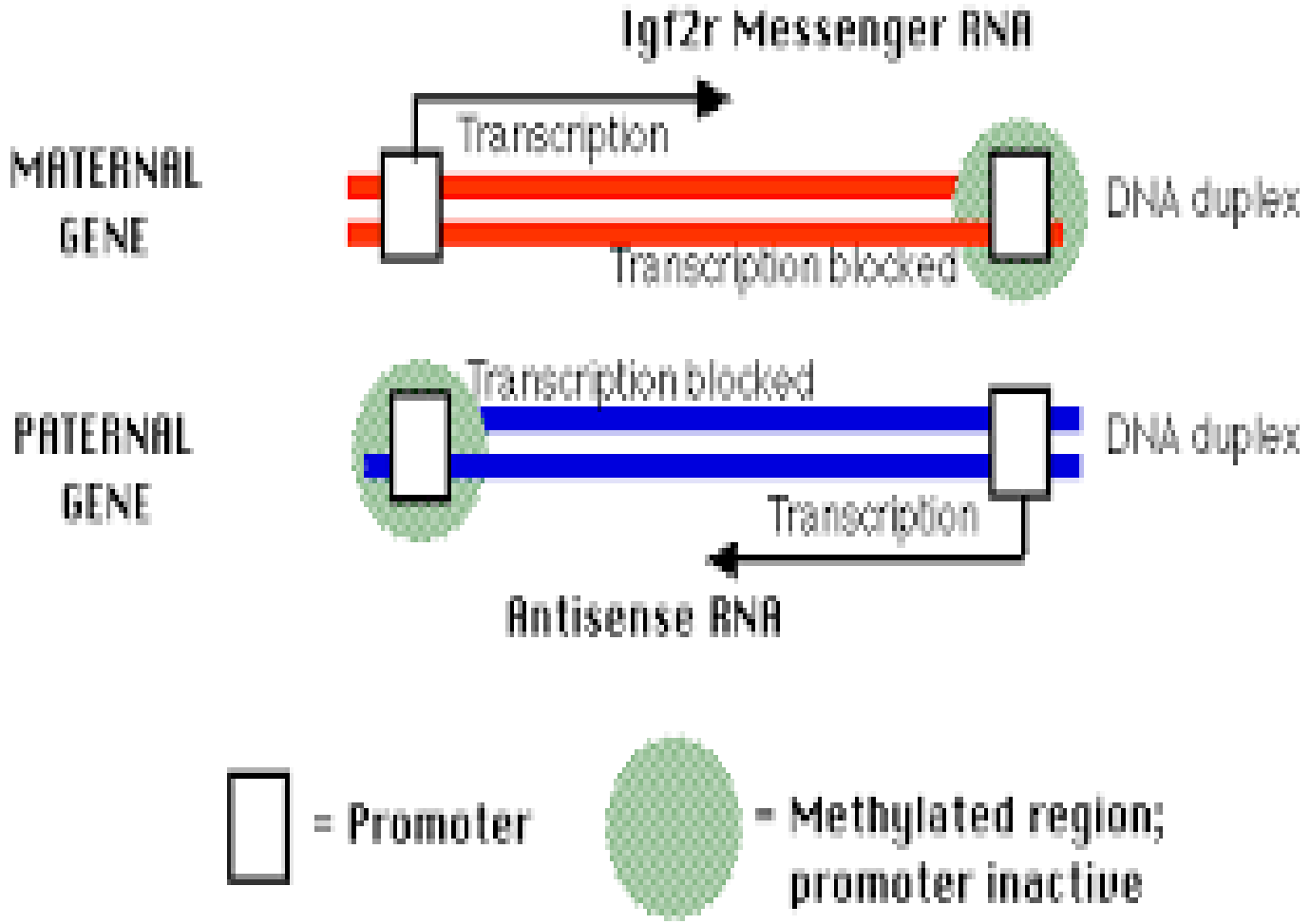
# DNA methylation

- Two of DNA's four bases, cytosine and adenine, can be methylated. Cytosine methylation is widespread in both eukaryotes and prokaryotes. Adenine methylation has been observed in bacterial, plant, and recently in mammalian DNA.
- In humans, three enzymes called DNA methyltransferases, 1, 3a and 3b are responsible for the DNA methylation process (DNMT1, DNMT3a, DNMT3b)
- With the age, occurs decrease in the level of DNA methylation. The DNA that was taken from embryos and newborns contains the largest amount of methylated cytosine bases. It turns out that some genes that were silent in childhood and young age, begin to show activity in old age. The other, smaller part of the genes, on the contrary, "falls silent" with methylation with age.

# Imprinted genes

- In diploid organisms (like humans), the somatic cells possess two copies of the genome, one inherited from the father and one from the mother. Each autosomal gene is therefore represented by two copies, or alleles, with one copy inherited from each parent at fertilization. For the vast majority of autosomal genes, expression occurs from both alleles simultaneously. In mammals, however, a small proportion (<1%) of genes are **imprinted**, meaning that gene expression occurs from only one allele. The expressed allele is dependent upon its parental origin. For example, the gene encoding insulin-like growth factor 2 (IGF2/Igf2) is only expressed from the allele inherited from the father.
- A distinctive feature of imprinted genes is their expression which determined by the parent that contributed them (mono-allelic expression). Most (about 70%) imprinted genes are expressed from the father allele and about 30% from the mother. It should be noted that the expression of some imprinted genes may be mono-allelic on one stage of ontogenesis and bi-allelic on the other.

# Imprinted genes



# Imprinted genes

- **In the mother's (maternal) copy of the gene:**

- there is an upstream (left) promoter that is unmethylated and active
- binding of transcription factors to this upstream promoter enables transcription of the sense strand of the gene to produce Igf2r messenger RNA.
- There is also a downstream set of CpG sites that are methylated

- **In the father's (paternal) copy of the IGF2r gene (the imprinted version):**

- the promoter for IGF2r transcription is methylated (and inactive),
- but the downstream promoter is unmethylated and active.
- Transcription of the antisense strand from the downstream promoter produces an antisense RNA (a long noncoding RNA) that participates in shutting his gene down.

# THANK YOU FOR ATTENTION