MOLECULAR BIOLOGY

بسم الله الرحمن الرحيم

FINAL – Lecture 8

Transcription (Pt.1)

﴿ وَإِن تَتَوَلَّوْاْ يَسْتَبْدِلْ قَوْمًا غَيْرَكُمْ ثُمَّ لَا يَكُونُوٓاْ أَمْثَٰلَكُم ﴾

اللهم استعملنا ولا تستبدلنا

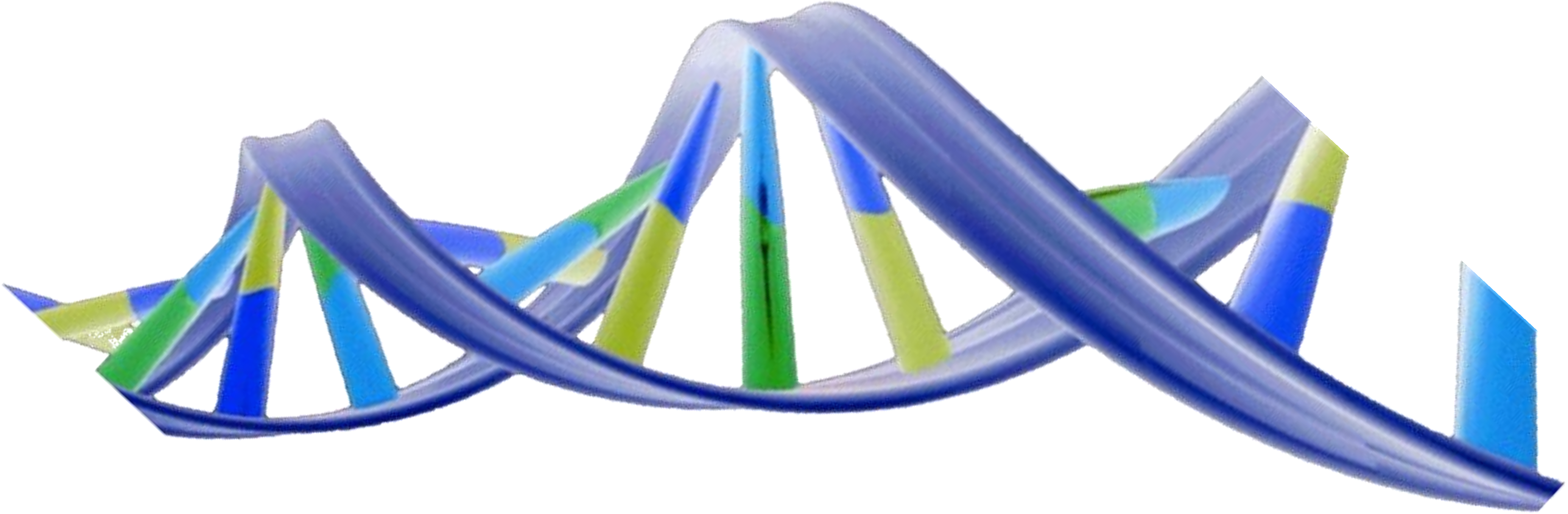
كتابة وتدقيق:

- معاوية الزغول
- المثنى خليل

فريق دوبامين العلمي - THE SCIENTIFIC TEAM OF DOPAMINE -

APPROVED 0501220

Quiz on the last lecture:



Definition of a gene

- The entire DNA sequence that is necessary for the synthesis of a functional RNA (mRNA, rRNA, tRNA, lncRNA, microRNA, etc.) or a polypeptide, which may become a protein or a functional peptide(s).
  - ➢ **the initial product of a gene is a polypeptide to be folded and coiled to make a functional protein, or sometimes to be cleaved into smaller functional peptides without possessing a 3D structure.**
    - The DNA sequence encompasses the coding region (that makes the protein), other regulatory sequences like a promoter, an enhancer, etc., or a non-coding region like introns.
  - ➢ **Almost 75% of human genome is transcribed, but not translated, whereas only relatively extremely small proportion of the entire genome (2%) constitutes protein-coding genes.**
- A cistron: an alternative term for a gene.
  - If it encodes one polypeptide from one mRNA, it is monocistronic.
  - If it encodes several or different polypeptides from ONE mRNA molecule, it is polycistronic.

The general mechanism of transcription

General description

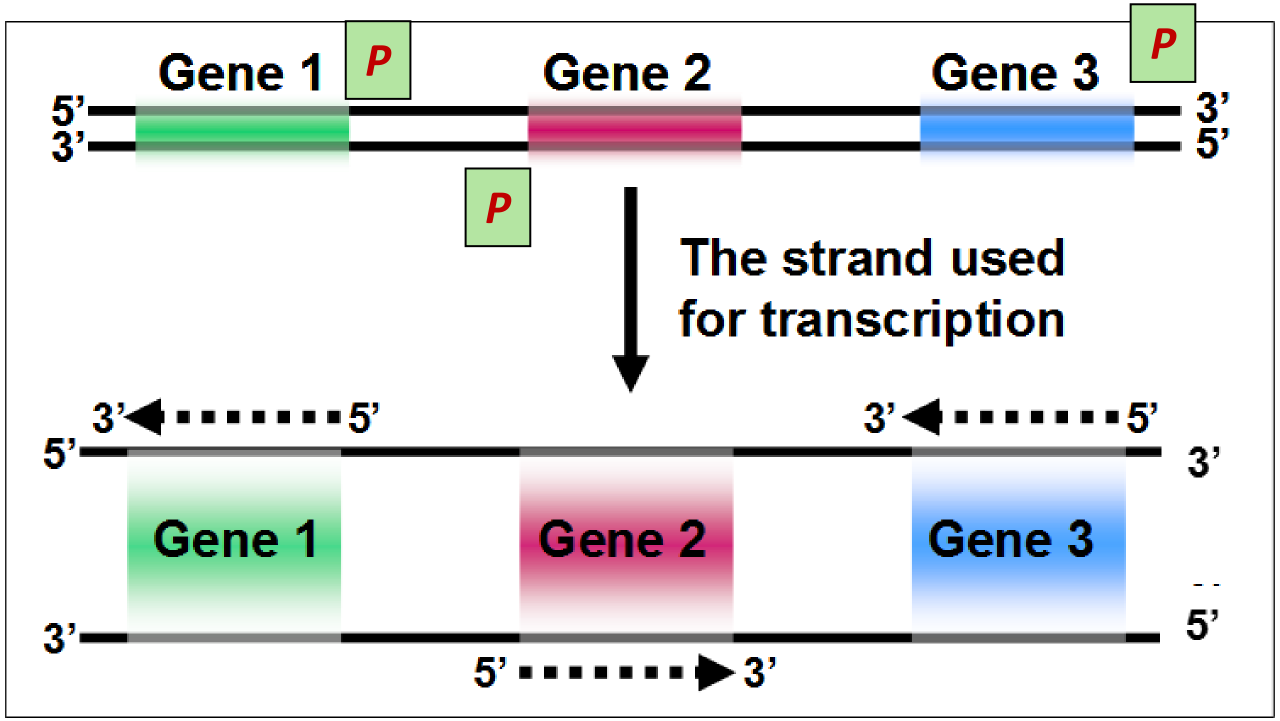
- Transcription is the process of making RNA from DNA.

- **<u>One</u>** of the two strands of the DNA double helix acts as a template for the expression of a particular gene (that is, synthesis of a RNA molecule).
  - Remember? In DNA replication, both strands are the template of the daughter strands.

➢ **DNA strands serve as a template for the synthesis of RNA. In replication, both strands act as templates, however, in the transcription of a particular gene, only one strand does.**

Using DNA strands

- Although RNA polymerase can read both DNA strands, it uses one strand for any particular gene in order to make RNA.



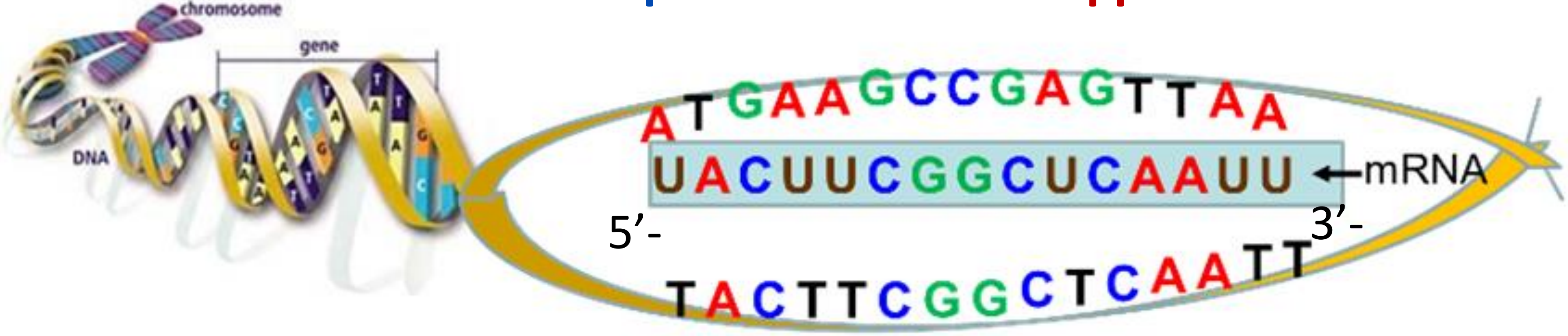
> ➢ **Notice in the figure that each gene is transcribed utilizing either one of the two strands for the synthesis of RNA from 5′ to 3′ while the template is read in the opposite direction.**
>
> ➢ **Promotor region in the beginning of a gene (to be discussed) determines which strand to use for a particular gene.**

What determines which strand is used for transcription?

Complementary sequences

- RNA is complementary to its DNA template.
- The RNA chain produced by transcription is also known as the transcript.

➢ **Again, RNA synthesis proceeds from 5′ to 3′ while the template is read in the opposite direction.**



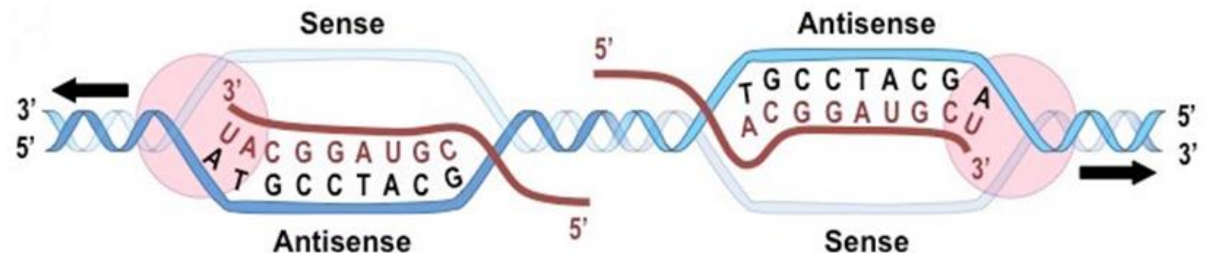
The growing RNA chain is extended in the 5’ to 3’ direction.

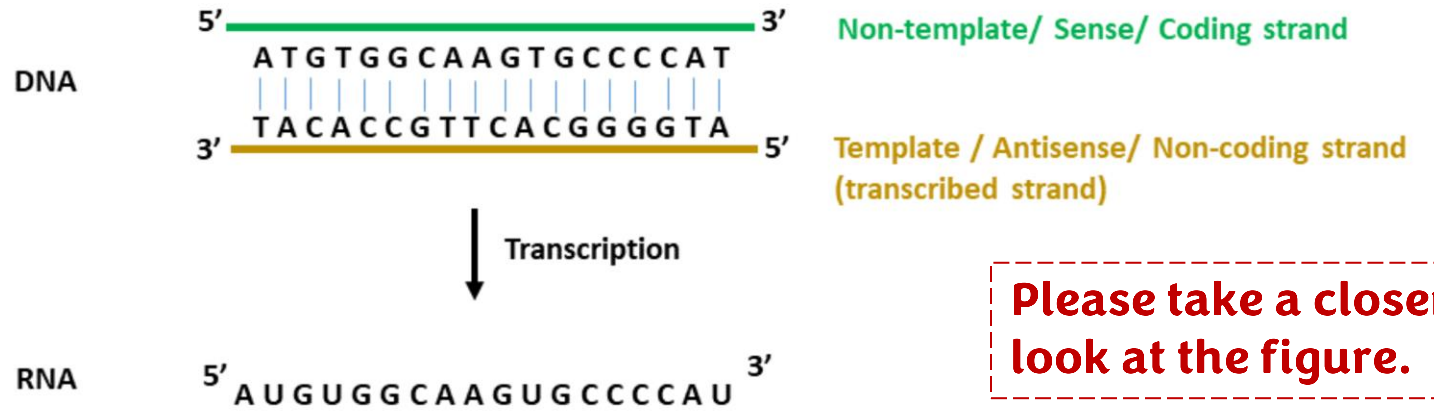
➢ **Remember that one of the differences between DNA and RNA molecules is that the latter possesses uracil U instead of thymine T.**

Enzyme and substrate

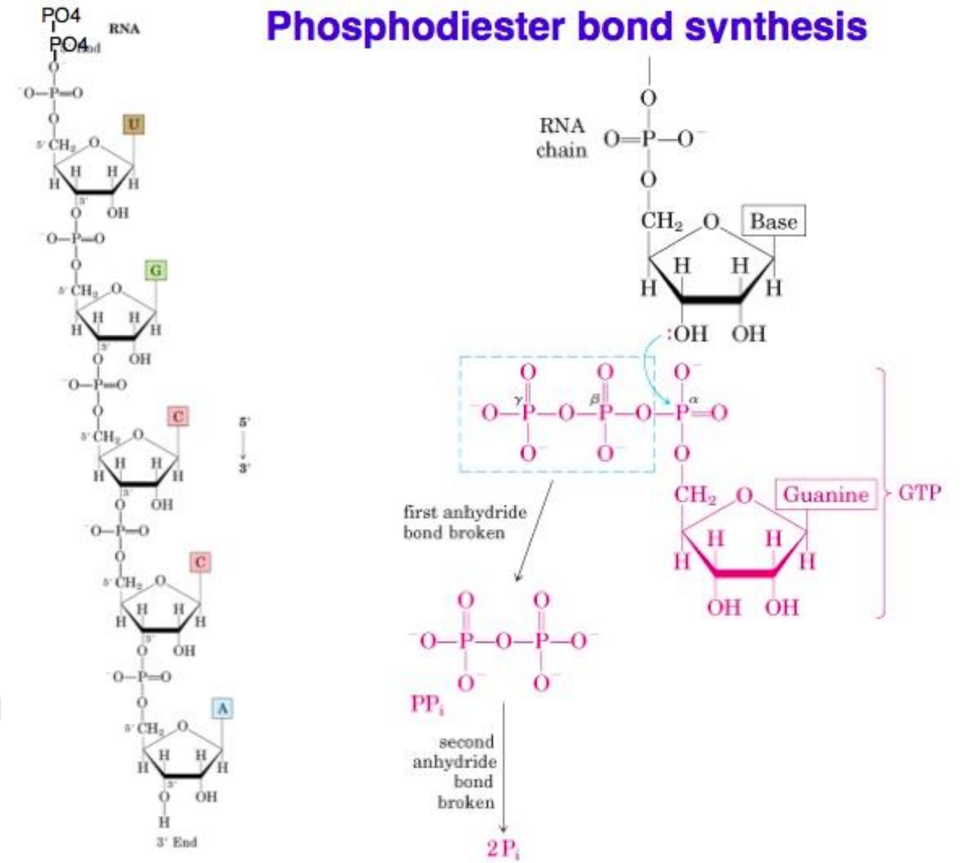
- The enzymes that perform transcription are called RNA polymerases.
- They catalyze the formation of the phosphodiester bonds between two nucleotides.
- RNA polymerase does not require a preformed primer to initiate the synthesis of RNA.
    - Transcription initiates de novo at specific sites at the beginning of genes.
- The substrates are nucleoside triphosphates (ATP, CTP, UTP, and GTP).
    - What are substrates for DNA polymerases?
- Hydrolysis of high-energy bonds in NTPs provides the energy needed to drive the reaction forward.

More clarification and some extra terms





Please take a closer look at the figure.



- The strand used as a template is called **non-coding** or **antisense**.
- Whereas the other strand is called **coding**, **sense** or **non-template** strand.
- RNA transcript sequence is similar to that of the non-template strand except that RNA has **uracil** instead of **thymine**, hence, that is why non-template strand is called sense or coding strand.
- **Ribonucleoside triphosphate** is hydrolysed via cleavage of the anhydride bond yielding pyrophosphate which is further cleaved into free phosphate groups, and the nucleotide is thereupon incorporated into the **growing 3′ end**, by the formation of phosphodiester bond.

DNA replication vs. transcription

- The newly synthesized portion of the RNA is bound to the DNA template but is released as RNA extends further.
- RNA polymerase reads the A in DNA and inserts U in the growing chain of RNA rather than T.
- RNA molecules are much shorter than DNA molecules.
- Unlike DNA, RNA does not store genetic information in cells.

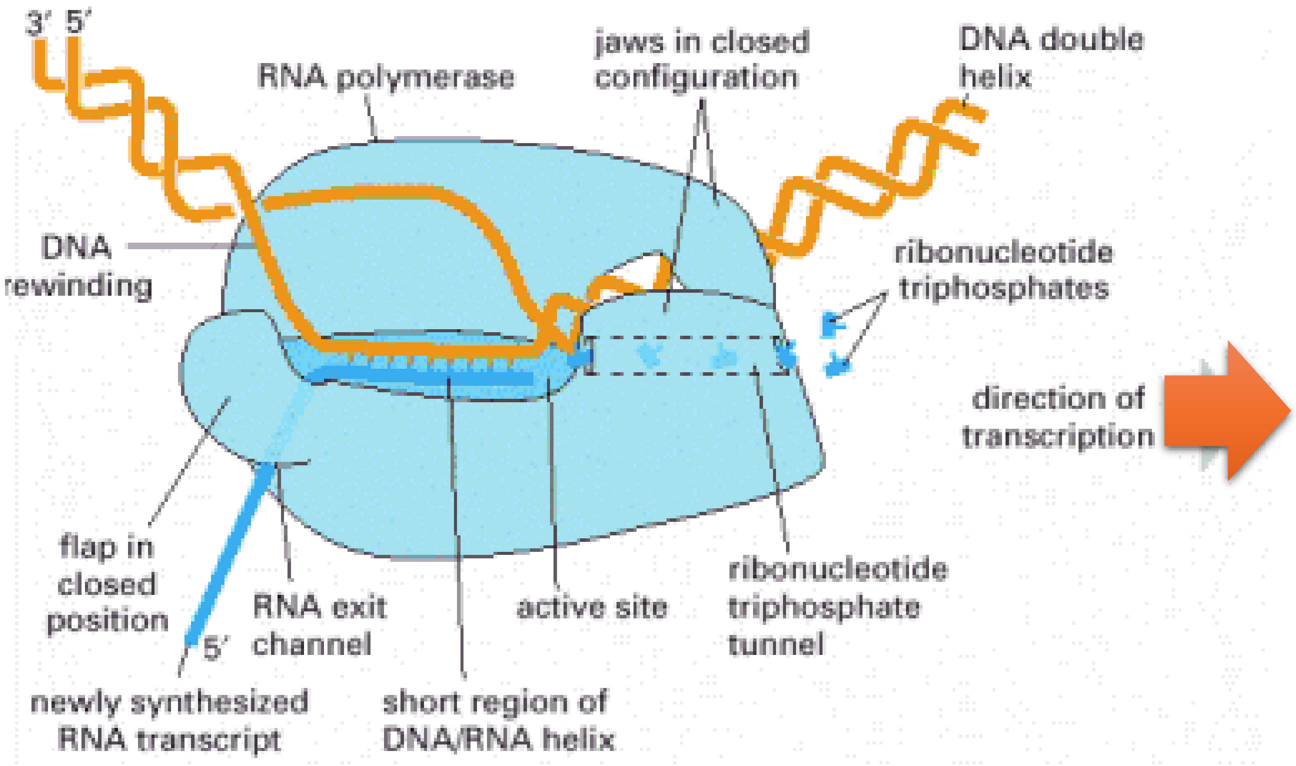
DNA polymerase vs. RNA polymerase

- RNA polymerase catalyzes the linkage of ribonucleotides, not deoxyribonucleotides.
- Unlike DNA polymerases, RNA polymerases can start an RNA chain without a primer.
- RNA polymerases make about one mistake for every 10^4 nucleotides.
  - the consequences of an error in RNA transcription are much less significant than that in DNA replication.
- Although RNA polymerases are not as accurate as DNA polymerases, they have a modest proofreading mechanism.

> ➢ **DNA polymerase must be highly accurate since DNA is the storage of the genetic instructions, therefore, this entails the preservation of DNA. Whereas, RNA polymerase must be accurate as well, but not as much as DNA polymerase.**

RNA binding to DNA is temporary

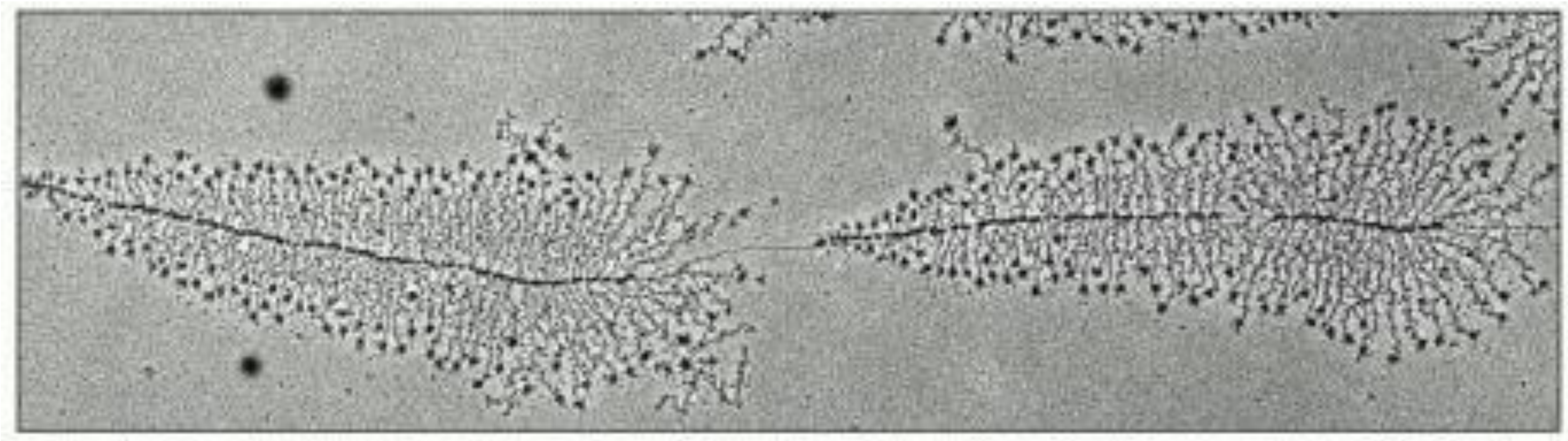
- As RNA is synthesized, it is initially bonded to DNA, but after a short distance, the older polymerized RNA nucleotides are separated, and the newer ones become bonded.



- **RNA polymerase is relatively larger than DNA polymerase. During transcription, DNA double helix opens up and RNA synthesis is initiated.**
- **As the subunits are condensed one after another by the enzyme and the growing chain extends, the newly-synthesized RNA is initially and temporarily hydrogen-bonded to the DNA template (remember that RNA is complementary to the DNA template).**
- **As the process proceeds, the already-polymerised (synthesized) part dissociates from the template.**
- **This temporary initial binding is essential for holding RNA polymerase bound to the DNA.**

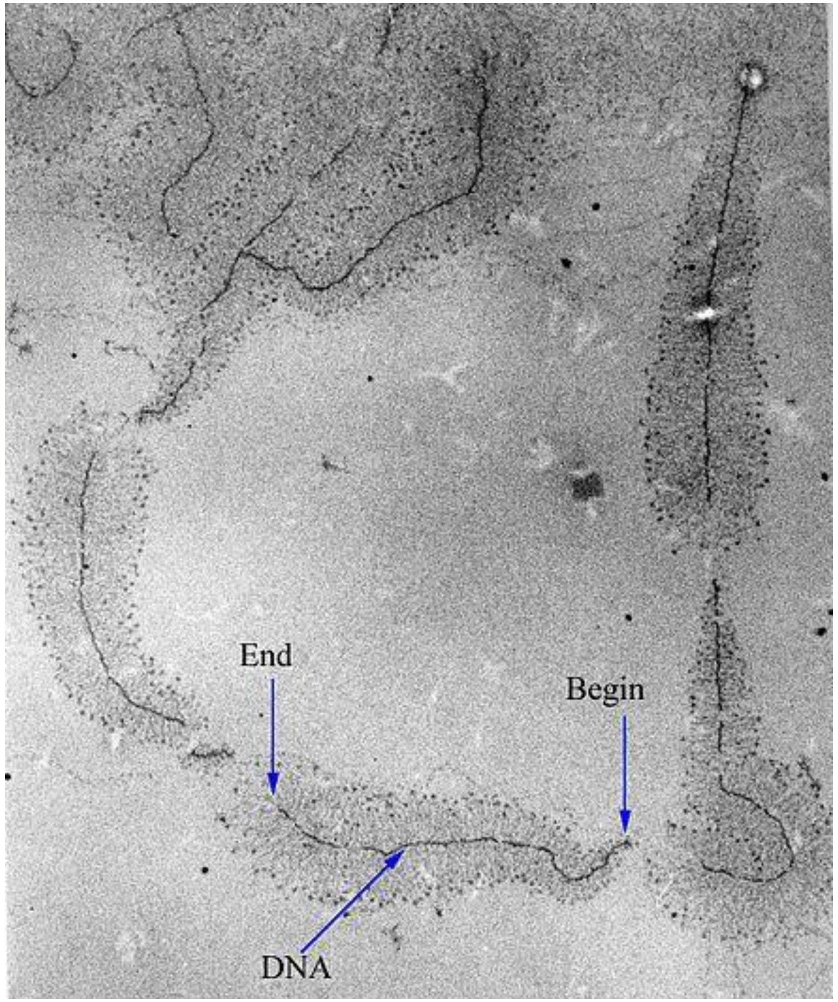
Polysomes.

- This allows the simultaneous synthesis of many RNA chains from the same gene forming structures known as polysomes.



Where is the starting point of transcription? Where is the beginning of the genes?

- **By looking at the images, these delicate outward-extended threads actually represent RNA chains while they are being synthesized on the same gene.**
- **Shorter chains are near the starting point of gene, whereas longer ones are toward the end.**



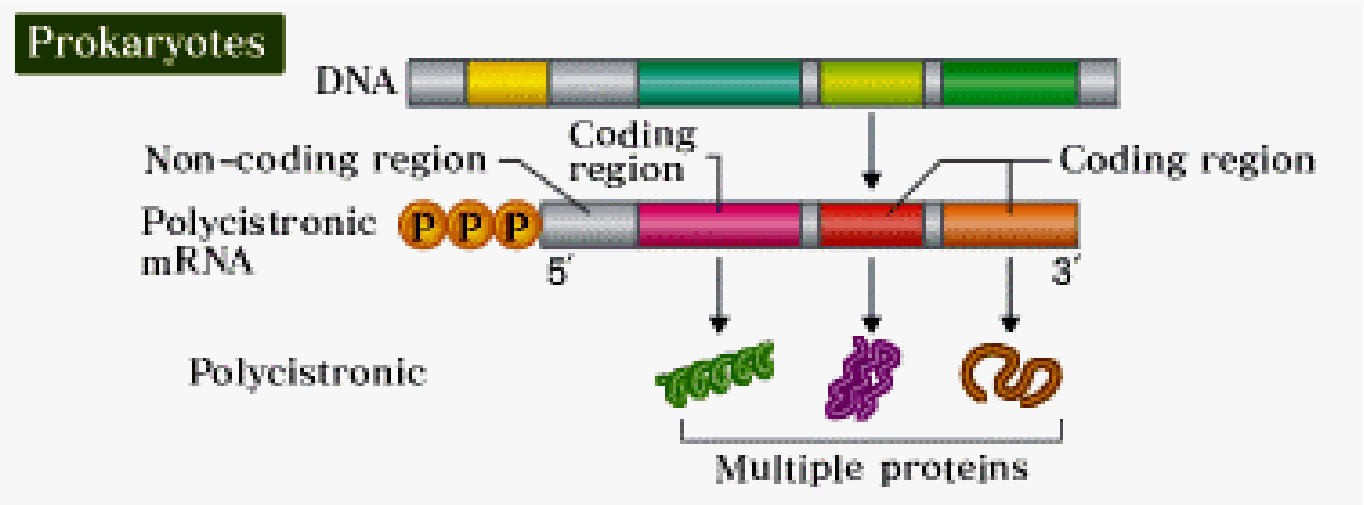
Transcription in prokaryotes

Prokaryotic genes (operons)

- In bacteria, genes can be **polycistronic** (define!).
- Genes that encode enzymes that are involved in related functions, are often transcribed as one unit from one cistron.
  - Example: the genes encoding the enzymes required to synthesize the amino acid tryptophan are contiguous.
- This cluster of genes comprises a single transcriptional unit referred to as an **operon**.

Please Take a closer look at this figure.

Please see next slide for more clarification.



Further elucidation

- Many Genes are **monocistronic**, that is, these genetic units make single mRNA transcript that in turn produces one polypeptide. This is common in both prokaryotes and eukaryotes.

- However, prokaryotes also have genes which are **polycistronic**, that is, these genetic units make a single mRNA transcript, different regions of which are translated into multiple polypeptides which ultimately become distinct but functionally related proteins that, for example, participate together in the same metabolic pathway.

- These unique transcriptional units allow coordinated and simultaneous gene expression of the **functionally-related** proteins.

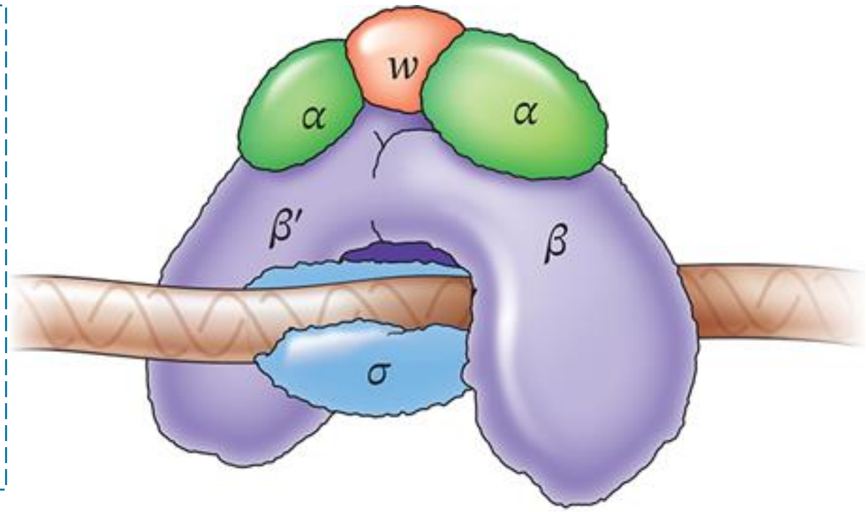
- **Trp operon** contains adjacent genes encoding all the proteins and enzymes necessary for **tryptophan synthesis**.

- **Lac operon** encompasses several contiguous genes encoding all the proteins and enzymes for **lactose metabolism**.

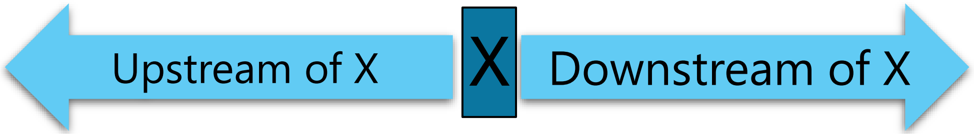
The RNA polymerase

- E. coli RNA polymerase is made up of multiple polypeptide chains or subunits.
- The core polymerase consists of two α, one β, one β', and one ω subunits.
  - The core polymerase is fully capable of catalyzing the polymerization of NTPs into RNA.
- The enzyme also contains a σ subunit, but it is not required for the basic catalytic activity of the enzyme.

➢ **RNA polymerase is a huge protein relative to DNA polymerase. It has a quaternary structure composed of multiple subunits, two α, one β, one β', and one ω subunits forming the core polymerase. This enzyme as well has additional subunit known as σ, without which, the enzyme can still function and carry out the polymerization reaction. However, this subunit has a significant role in the transcription initiation process (to be further discussed).**

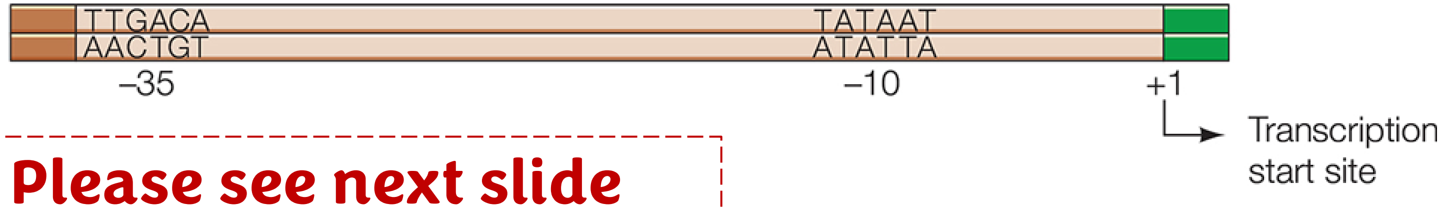


Consensus sequences (the promoter)



- The DNA sequence to which the RNA polymerase binds to to initiate transcription of a gene is called the **promoter.**
  - **A promoter is "upstream" of the transcription initiation site.**
- The promoter region upstream of the transcription initiation site contains two sets of sequences that are similar in a variety of genes.
  - **Consensus**!
- They are called the (-10) and (-35) **elements** **(each encompass 6 base pairs)** because they are located approximately 10 and 35 base pairs upstream of the transcription start site.
- The transcription initiation site is defined as the +1 position.

> **RNA polymerase binds to these elements in the promotor region, and the synthesis of RNA chain commences at the initiation site.**



Please see next slide for more clarification.

Further elucidation

- **Consensus** denotes that these unique sequences, upstream of initiation site, are most frequently and commonly found in the beginning of a variety of genes. Current evidence demonstrates that they are not identical among various genes, but similar enough to establish the concept of consensus.

- **Transcription initiation site** is the site where RNA polymerase commences the synthesis of RNA chain, first base pair of which is designated as **+1**, this site is the center, any sequence **upstream** is designated with **negative numbers** and any sequence **downstream** is designated with **positive numbers**.

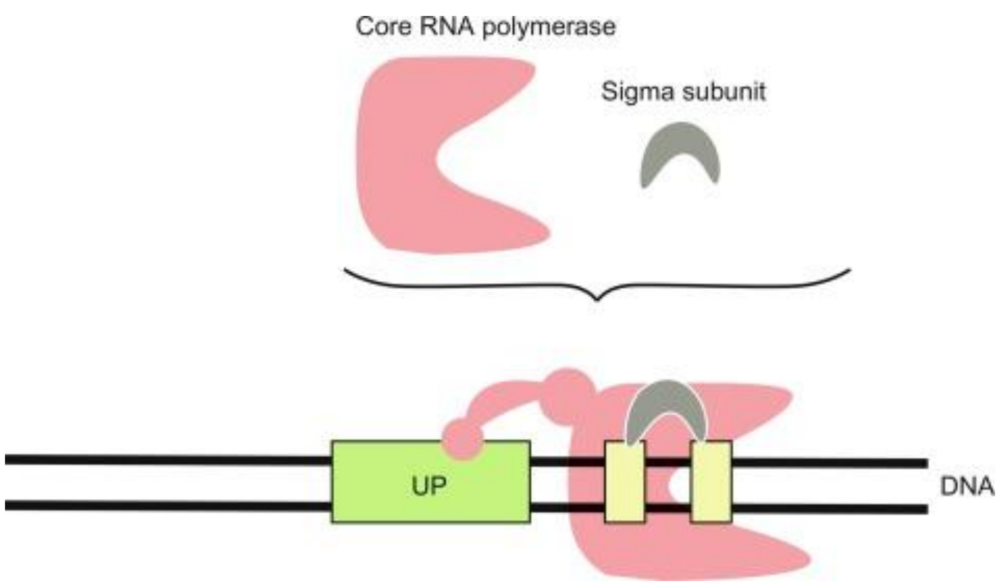
- Upstream of point X, means before or toward **5′** end of either the RNA chain or DNA sense strand **(non-template)**

- Downstream of point X, means after or toward **3′** of either the RNA chain or DNA sense strand **(non-template)**.

Role of the σ subunit

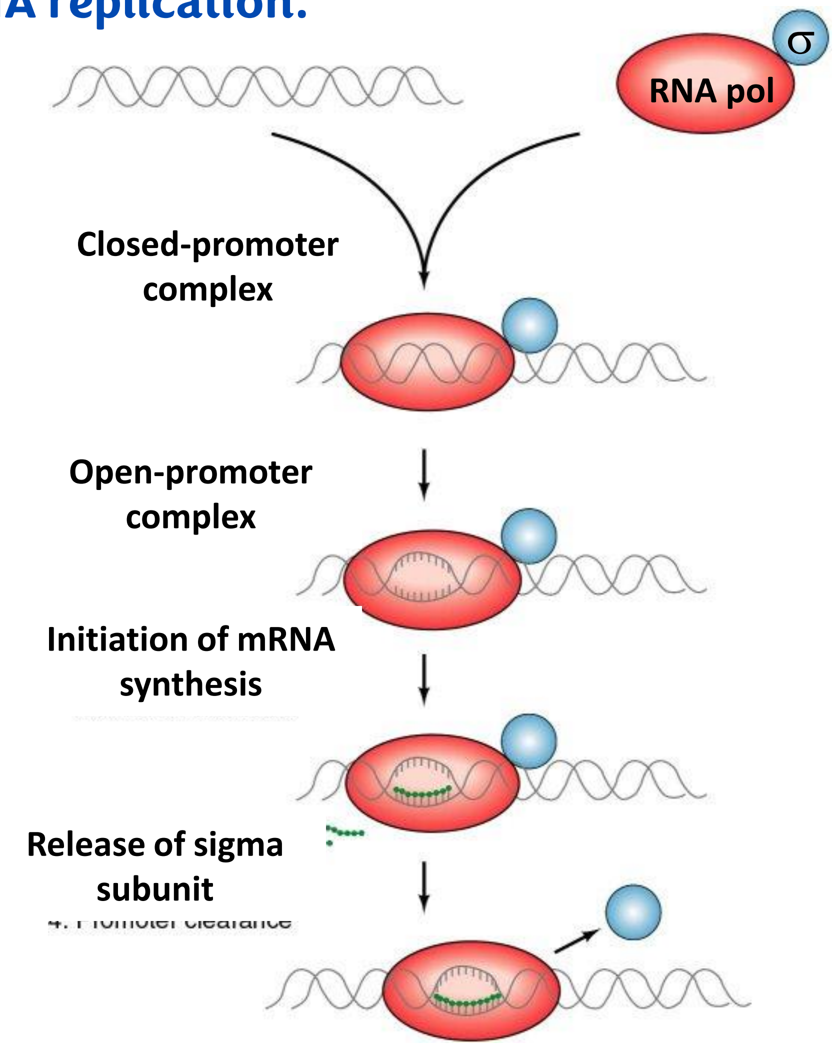
- In the absence of σ, the RNA polymerase binds to DNA with low affinity and nonspecifically.
- The role of σ is to identify and guide the polymerase to the -35 and -10 sequences.

> **RNA polymerase can still function and carry out the polymerization reaction without σ, since it is not required for the catalytic activity.**
> **σ subunit (as a part of the enzyme) guides the enzyme and binds specifically to these elements.**
> **Once RNA polymerase binds to the promotor facilitated by σ, and the transcription starts, σ is released so that it can bind to another RNA polymerase and the cycle repeats until a polysome complex is formed.**



Mechanism of transcription (initiation)

- The RNA polymerase binds to the promoter and opens it (like what?).
  - **Resembling the activity of helicase in DNA replication.**
- The single-stranded DNA is now available as a template.
- Transcription is initiated by the joining of two NTPs **(nucleoside triphosphates)**.
- After addition of about 10 nucleotides, σ is released from the polymerase.
- What do you think happens to it?

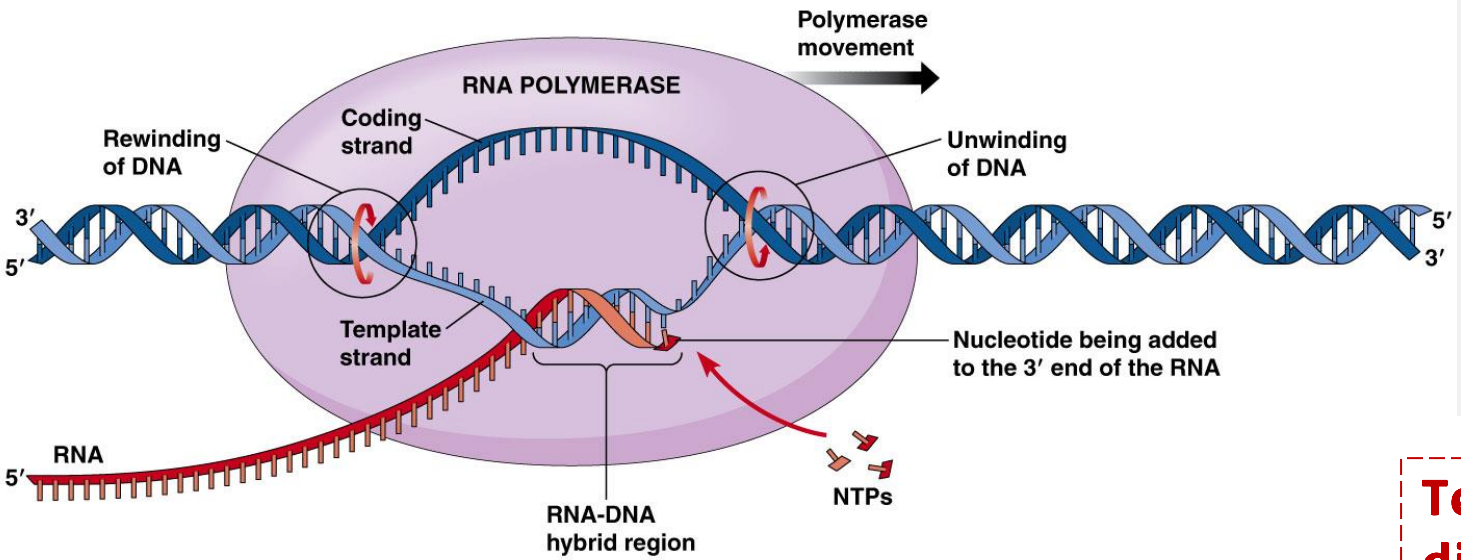


Mechanism of transcription (elongation)

- As the polymerase moves forward, it
  - unwinds the template DNA ahead of it (like what?)
  - elongates the RNA
  - rewinds the DNA behind it

> **As previously denoted, the newly-polymerized chain is bound to DNA within the unwound region, this association stabilizes polymerase on the DNA template. As it moves forward and the synthesis proceeds, the older-polymerized chain dissociates.**

> **The process continues Until RNA polymerase reads termination sequence in the end of the gene Where transcription ceases. This sequence is also consensus.**



RNA synthesis continues until the polymerase encounters a termination signal where the RNA is released from the polymerase, and the enzyme dissociates from its DNA template.

Termination process will be further discussed in the coming lecture.

Last but not least (;

If you are willing to assess your understanding and comprehension, come to challenge yourself with these questions; scan (or click) here:

Transcription MCQ

For any feedback, scan the code or click on

Corrections from previous versions:

Versions	Slide # and Place of Error	Before Correction	After Correction
V0 → V1			
V1 → V2			

Additional Resources: رسالة من الفريق العلمي:

M.Z.

لا يَظهَرُ العَجزُ مِنّا دونَ نَيلِ مُنىً

وَلَو رَأَينا المَنايا في أَمانيـــنا

تُدافِعُ الكَسَلَ المَشؤومَ هِمَّتُنا

عَنّا وَنَخصِمُ مُرَّ الجَهْلِ إِذْ شـــينا

عَزائِمٌ كَالنُّجومِ الشُهبِ ثاقِبَةً

ما زالَ يُحرِقُ مِنهُنَّ الشَـياطينا