بسم الله الرحمـٰن الرحيم

(وَفَوْقَ كُلِّ ذِي عِلْمٍ عَلِيمٌ)

Cytology & Molecular Biology | FINAL 10

Transcription pt.1

Written by : DST

Reviewed by : NST members

وَلِلَّهِ الْأَسْمَاءُ الْحُسْنَى فَادْعُوهُ بِهَا

المعنى: الذي ينفذ ما يريد إيجاده على الصفة التي يريدها، الذي جعل خلقه على الصور التي شاءها بمقتضى حكمته.

الورود: ورد مرة واحدة في القرآن.

الشاهد: ﴿ هُوَ ٱللَّهُ ٱلْخَٰلِقُ ٱلْبَارِئُ ٱلْمُصَوِّرُ ﴾ [الحشر:٢٤].

٧٣

اضغط هنا لشرحٍ أكثر تفصيلًا

Transcription (8)

Prof. Mamoun Ahram
School of Medicine
Second year, First semester, 2024-2025

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.

➢ RNA types other than mRNA are considered non-coding.

  - 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 from different regions, it is polycistronic.

The general mechanism of transcription

General description

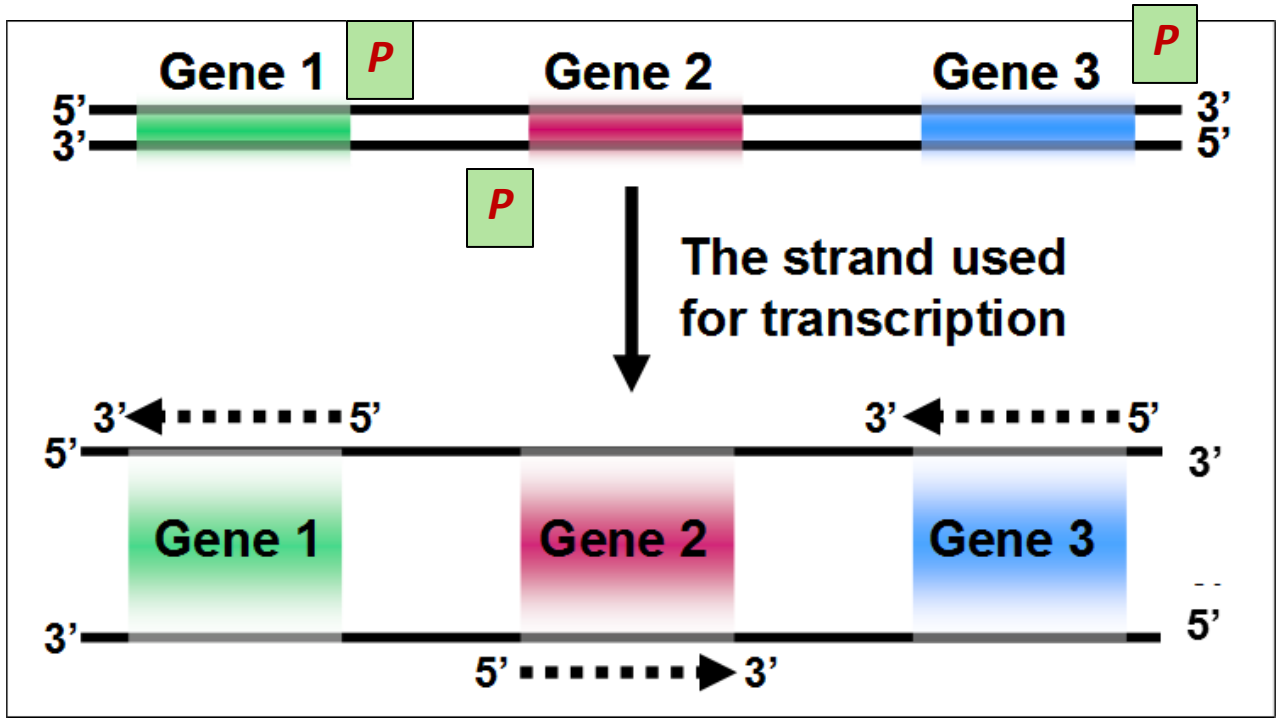
- Transcription is the process of making RNA from DNA.

- **One** 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**.

What determines which strand is used for transcription?

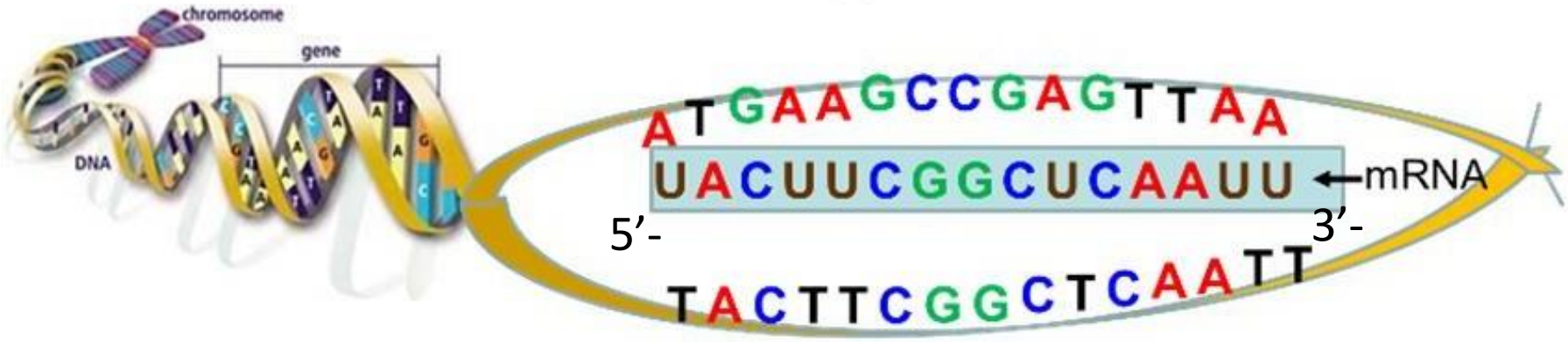
- Promotor region in the beginning of a gene **(to be discussed)** determines which strand to use for a particular gene.

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.**

➢ **Because RNA is complementary and antiparallel to the DNA.**



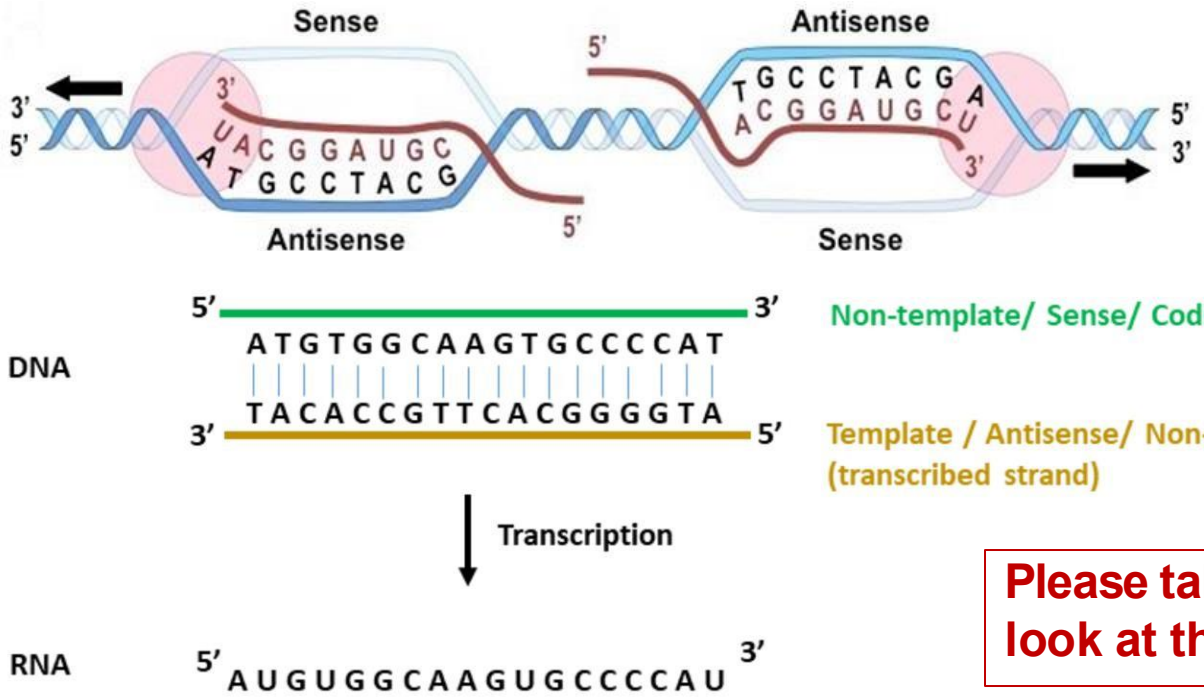
The growing RNA chain is extended in the 5’ to 3’ direction.

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

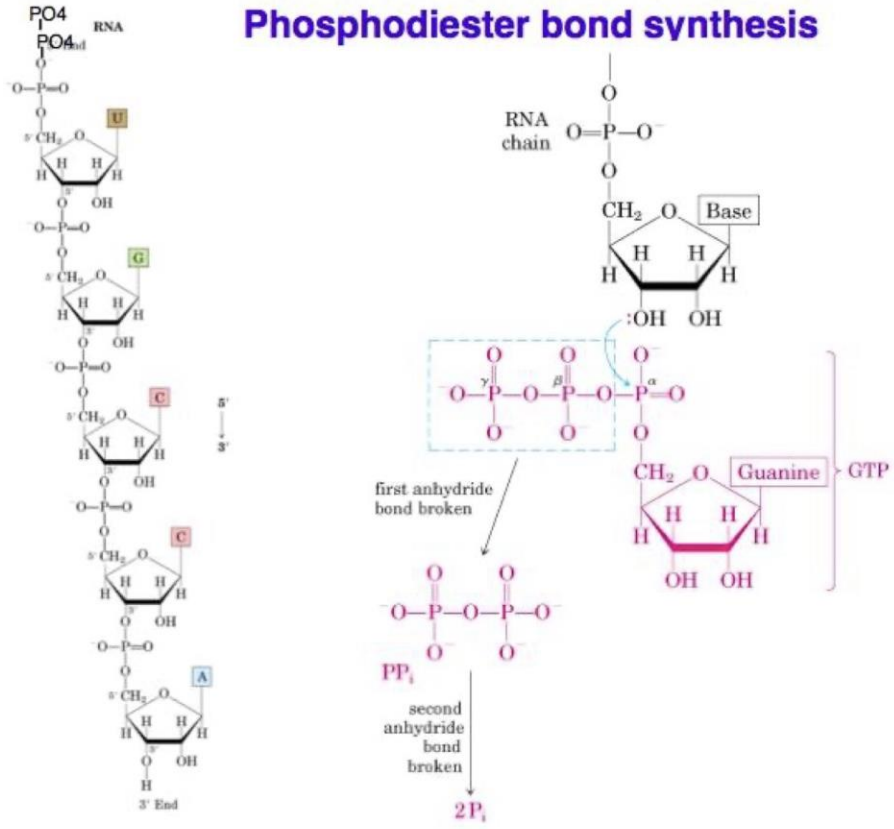
Enzyme and substrate

- 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 ribonucloside triphosphates (ATP, CTP, UTP, and GTP).
  - What are substrates for DNA polymerases? deoxyribonucloside triphosphates (dATP, dGTP, dTTP and dCTP)
- Hydrolysis of high-energy bonds in NTPs provides the energy needed to drive the reaction forward. NTPs released as NMPs, because two phosphate groups are needed to provide the energy needed for the ligation process.

More clarification and some extra terms



Please take a closer look at the figure.



- The strand used as a template is called **non-coding, antisense or template.**
- 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

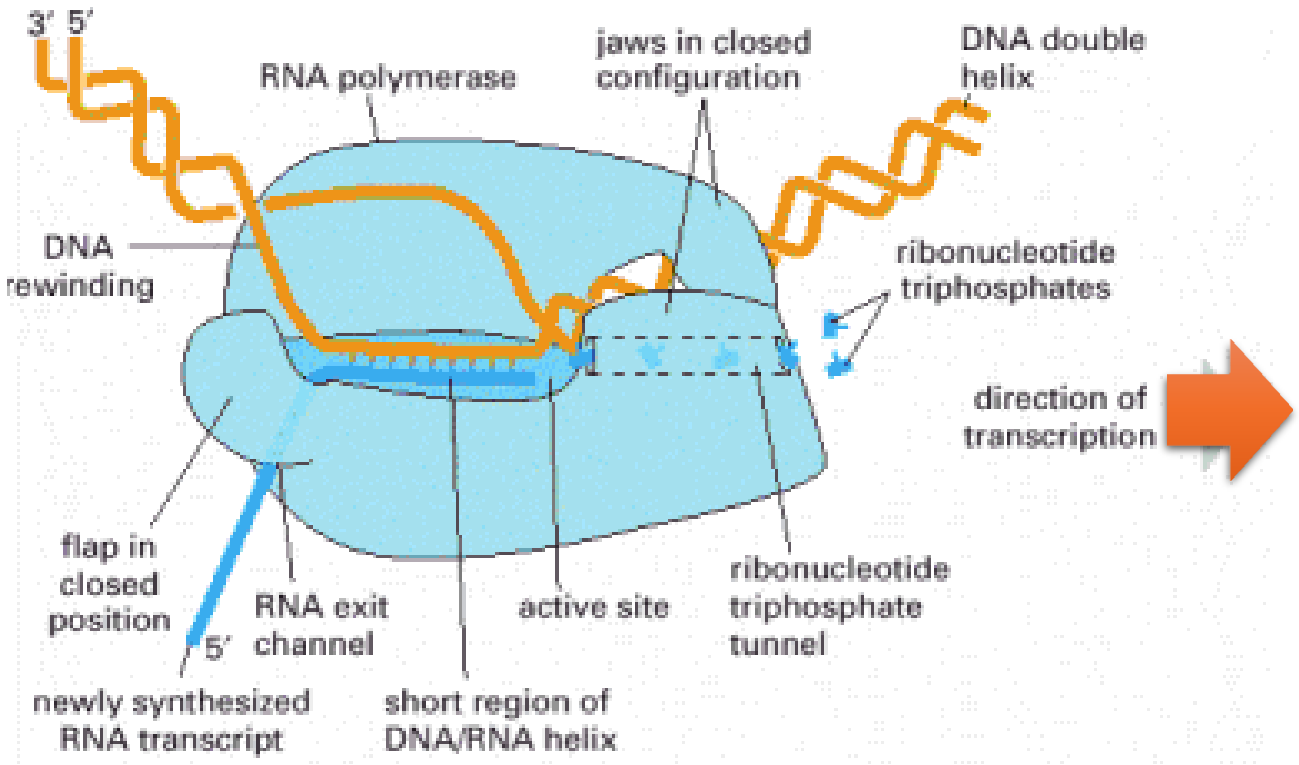
- RNA polymerase catalyzes the linkage of ribonucleotides, not deoxyribonucleotides.
- Unlike DNA polymerases, RNA polymerases can start an RNA chain without a primer.
- RNA polymerases make about one mistake for every 10^4 nucleotides.
  - the consequences of an error in RNA transcription are much less 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

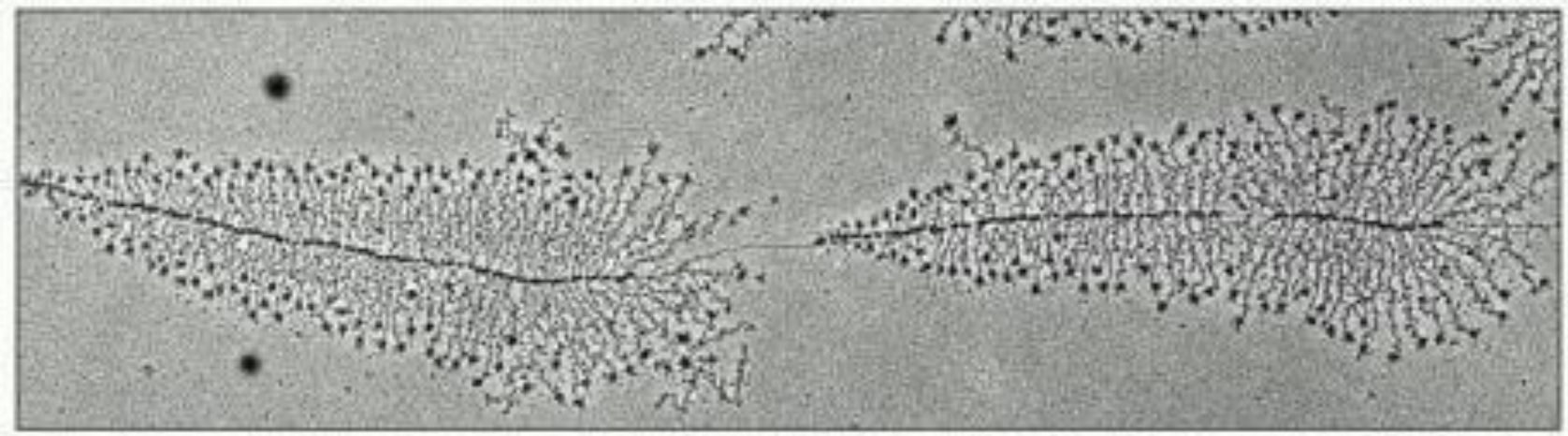
- As RNA is synthesized, it is initially bonded to DNA, but after a short distance, the older polymerized RNA nucleotides are separated, and the newer ones become bonded.

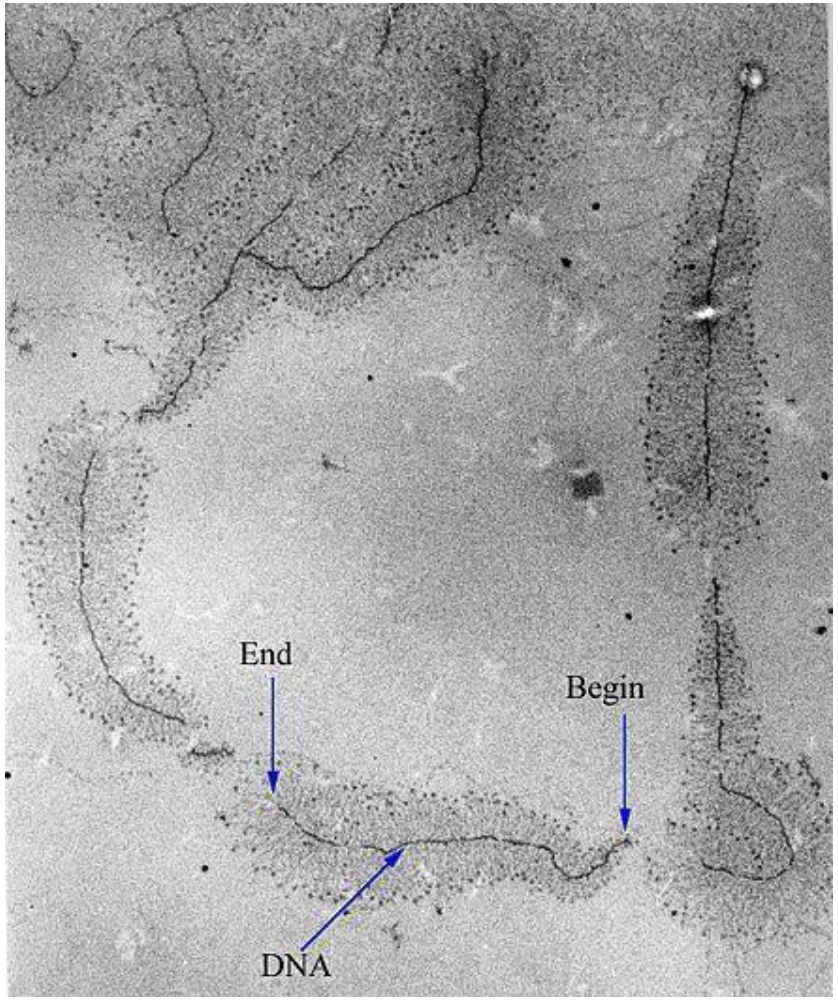


- **RNA polymerase** is relatively larger than **DNA polymerase**. During transcription, DNA double helix opens up and RNA synthesis is initiated.
- As the subunits are condensed one after another by the enzyme and the growing chain extends, the newly-synthesized RNA is initially and temporarily hydrogen-bonded 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.

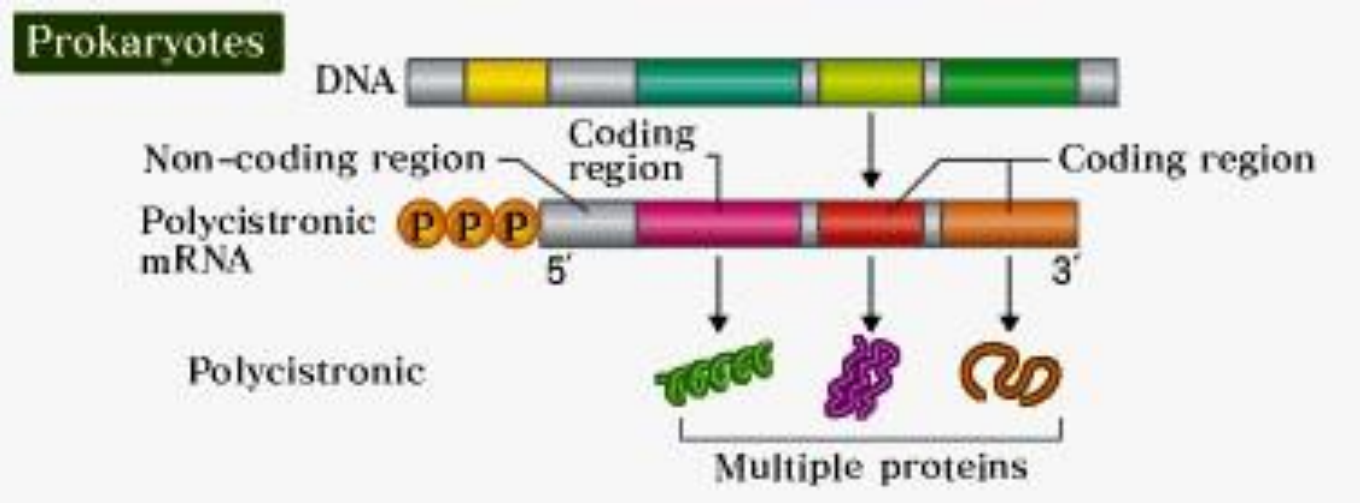
- **Shorter** chains are near the starting point of gene, whereas **longer** ones are toward the end.

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.

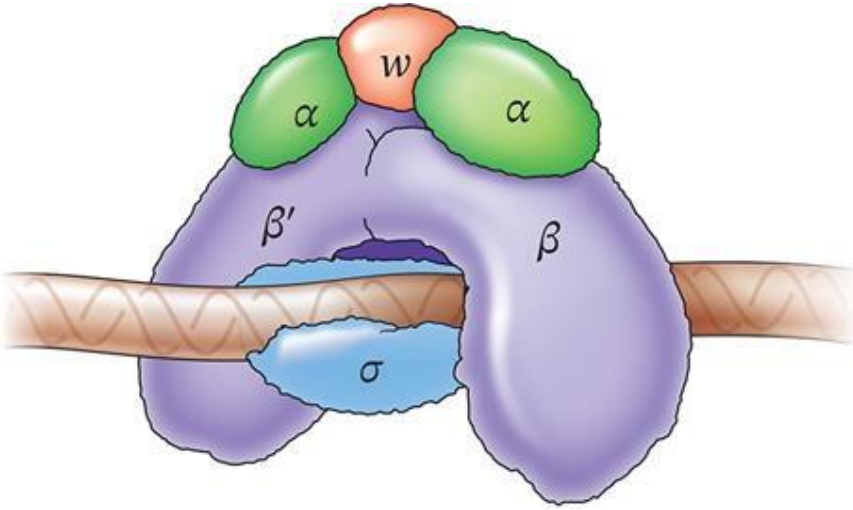
Further elucidation

- 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 (only one in bacteria)

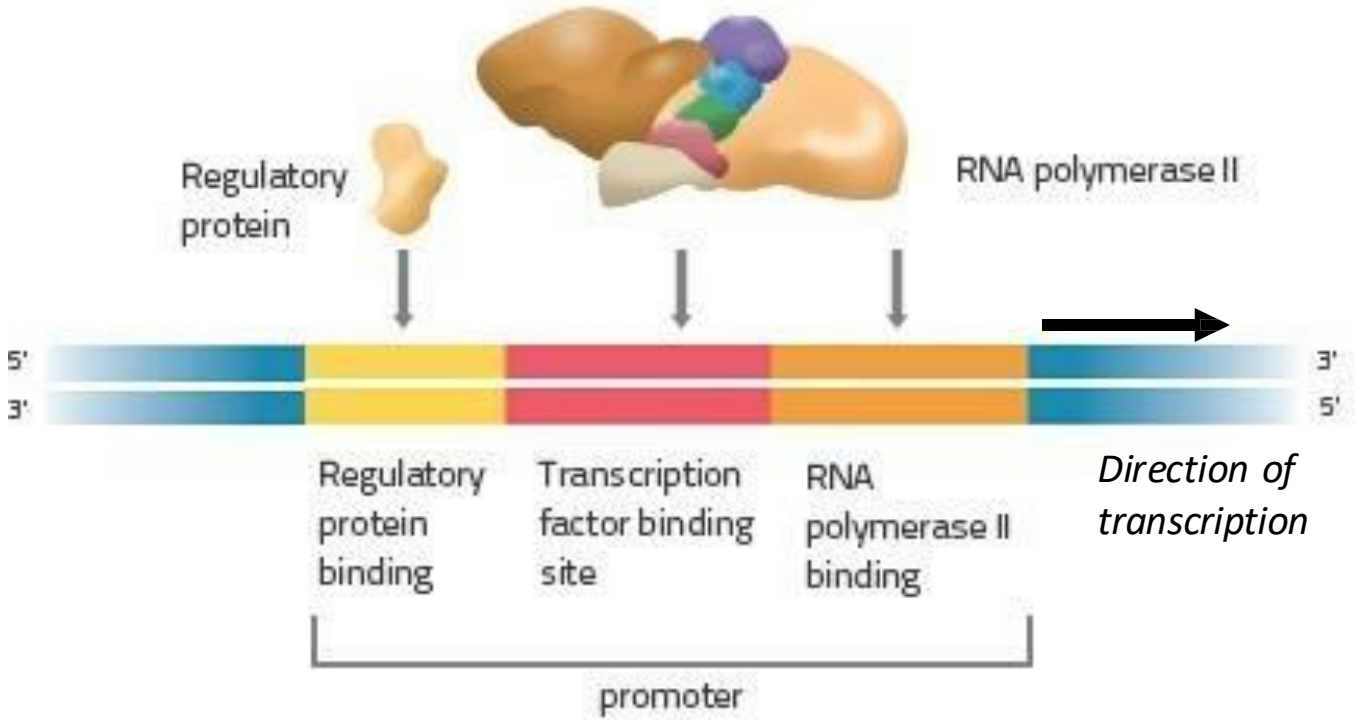
- E. coli RNA polymerase is made up of multiple polypeptide chains or subunits.
- The core polymerase consists of two α, one β, one β', and one ω subunits.
  - The core polymerase is fully capable of catalyzing the polymerization of NTPs into RNA.
- The enzyme also contains a σ subunit, but it is not 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 , one , one ', and one subunits forming the core polymerase and all of them are responsible for the catalytic activity of the enzyme. This enzyme as well has additional subunit known as , 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).**

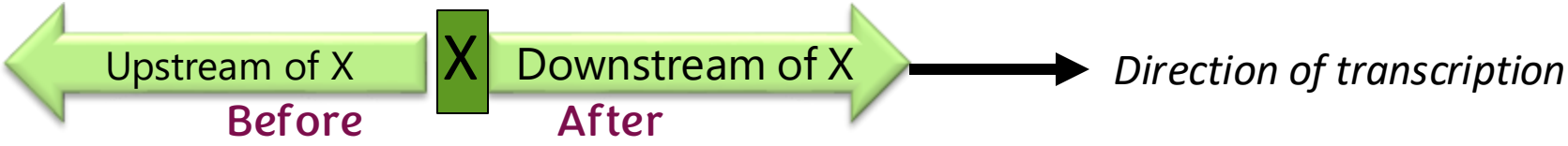


The promoter

- The DNA sequence to which the RNA polymerase and other transcriptional regulatory proteins bind to initiate transcription of a gene is called the **promoter.**



A promoter is the region of DNA where the RNA polymerase sit on and initiate the transcription process, also the regulatory proteins. The promotor essentially is the RNA polymerase binding site.



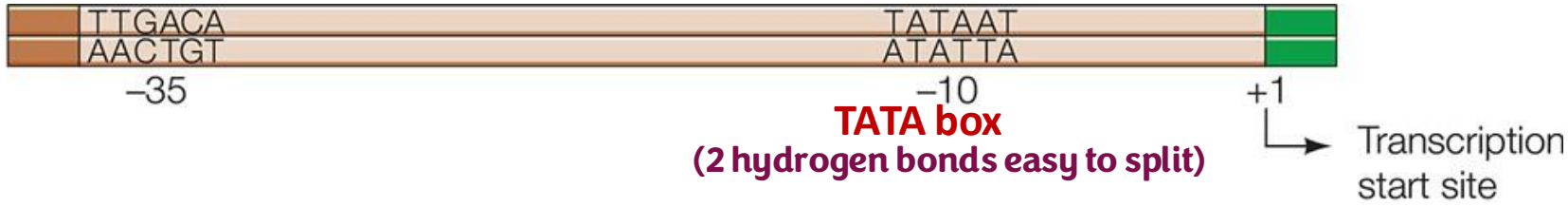
A promoter is usually "upstream" of the transcription initiation site.

A promoter contains consensus sequences مُتَّفَق عَلَيْه

- The prokaryotic promoter region contains two sets of sequences that are similar in a variety of genes.
  - **Consensus sequences!** In molecular biology, a **consensus sequence** is a DNA sequence that is **shared and conserved among different organisms.**
    When we compare **human DNA** with the DNA of **monkeys, yeast, and mice**, we find that they all share the **same sequence**, indicating that this consensus sequence is **functionally important** and **essential**
- They are called the (-10 TATA BOX) and (-35) **elements** = DNA sequence **(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.

When I say element means DNA sequence, but when I say factor it means protein

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



A DNA element is a specific and functional sequence in DNA.

Please see next slide for more clarification

Further elucidation

next slides

يفنى العباد ولا تفنى صنائعهم ..
فاختر لنفسك ما يحلو به الأثر .

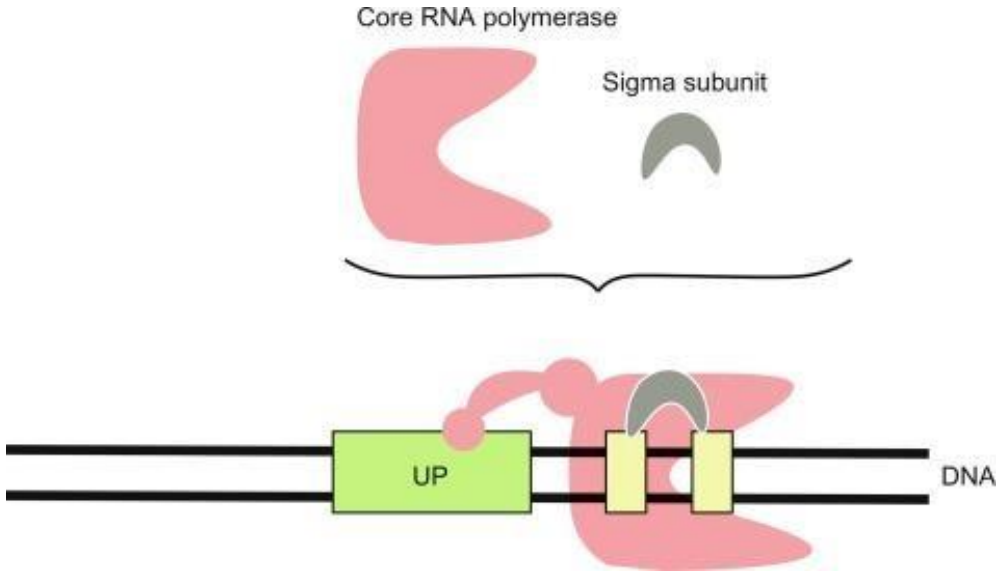
- The term **consensus** denotes that these promoter sequences are the **most frequently and commonly found sequences** located upstream of the transcription initiation site in a wide variety of genes.
  Although these sequences are **not identical** among different genes, they are **similar enough** to define a common pattern, which establishes the concept of a **consensus sequence**. This similarity reflects their **functional importance** in transcription.
- The **transcription initiation site** is the exact position where **RNA polymerase begins the synthesis of the RNA chain**. The first nucleotide incorporated into the RNA molecule is designated as **+1**. This site serves as the reference point for numbering DNA sequences.
- Any DNA sequence located **upstream of the +1 site** is assigned **negative numbers**, such as −10 and −35, while any sequence located **downstream of the +1 site** is assigned **positive numbers**.
- The **−35 and −10 promoter regions** are essential binding sites for RNA polymerase. RNA polymerase recognizes these regions, binds to them, and then **opens the DNA double helix**, converting it into a **single-stranded template** that can be read for RNA synthesis. Transcription then proceeds starting from the +1 site.

- The **-10 region (TATA box)** is particularly important because it is rich in adenine and thymine bases, which are held together by **two hydrogen bonds**, making this region easier to separate during the initiation of transcription.
- The terms **upstream** and **downstream** are defined relative to the direction of transcription:
- **Upstream of a point X** refers to sequences located toward the **5′ end** of the DNA **sense (non-template) strand** or the RNA chain.
- **Downstream of a point X** refers to sequences located toward the **3′ end** of the DNA **sense (non-template) strand** or the RNA chain.
- If mutations occur in the **-10 or -35 promoter elements**, RNA polymerase may not bind efficiently or correctly, which can lead to **reduced transcription efficiency** or complete failure to transcribe the gene

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 , since it is not required for the catalytic activity.**
- **σ subunit (as a part of the enzyme) guides the enzyme (RNA polymerase) to the promoter and binds specifically to these elements.**
- **Once RNA polymerase binds to the promotor facilitated by , and the transcription starts, is released so that it can bind to another RNA polymerase and the cycle repeats until a polysome complex is formed.**

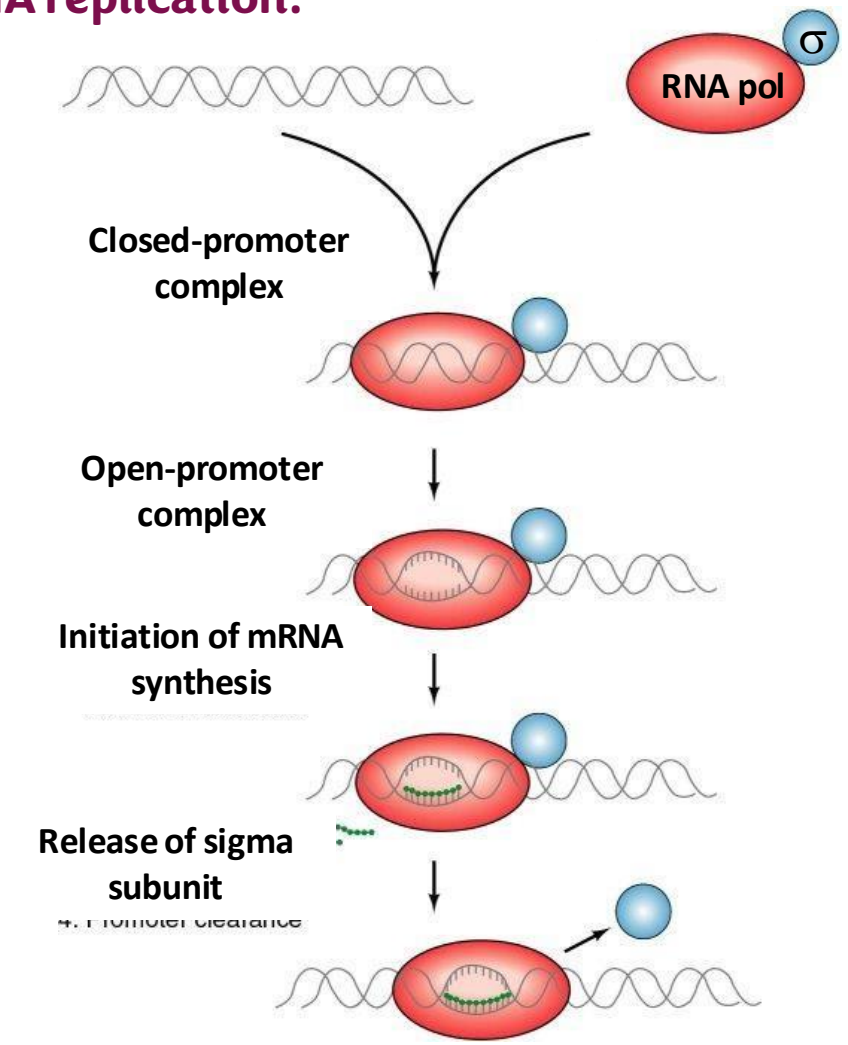


Mechanism of transcription (initiation)

اللَّهُمَّ إِنَّكَ عَفُوٌّ تُحِبُّ
الْعَفْوَ فَاعْفُ عَنَّا

- The RNA polymerase binds to the promoter and opens it (like what?).
- The single-stranded DNA is now available as a template.

➢ **Resembling the activity of helicase in DNA replication.**

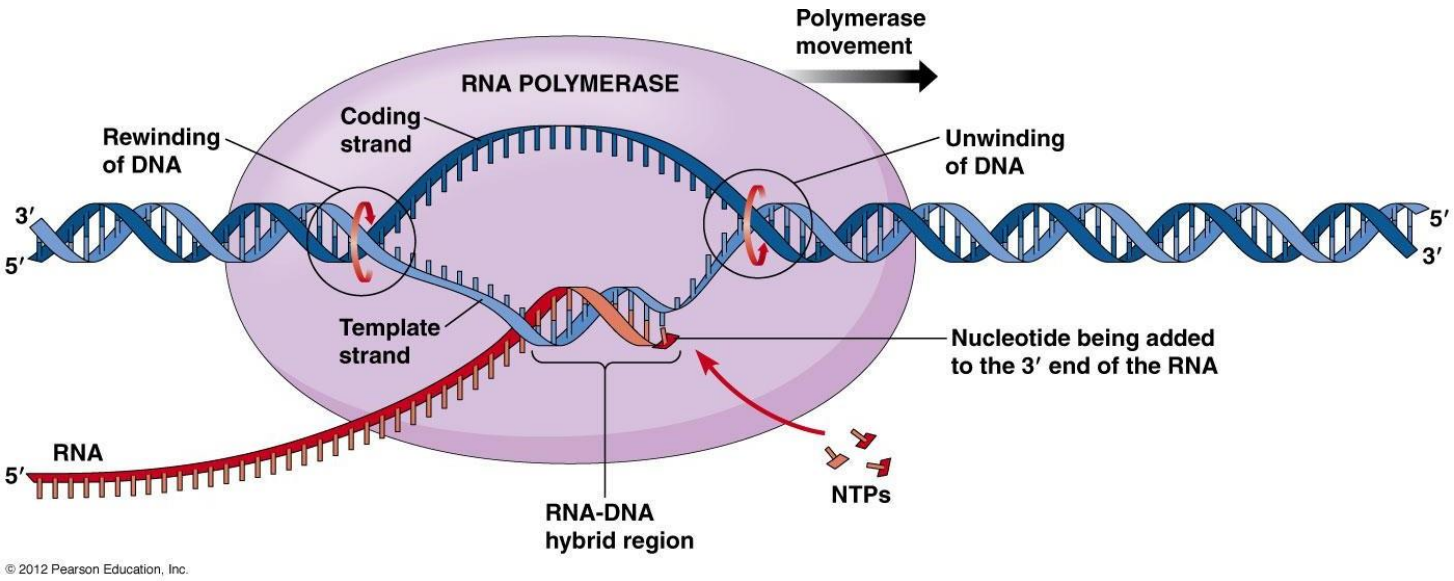


- Transcription is initiated by the joining of two NTPs **(nucleoside triphosphates).**
- After addition of about 10 nucleotides, σ is released from the polymerase.
- What do you think happens to it? **Reuse**

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.

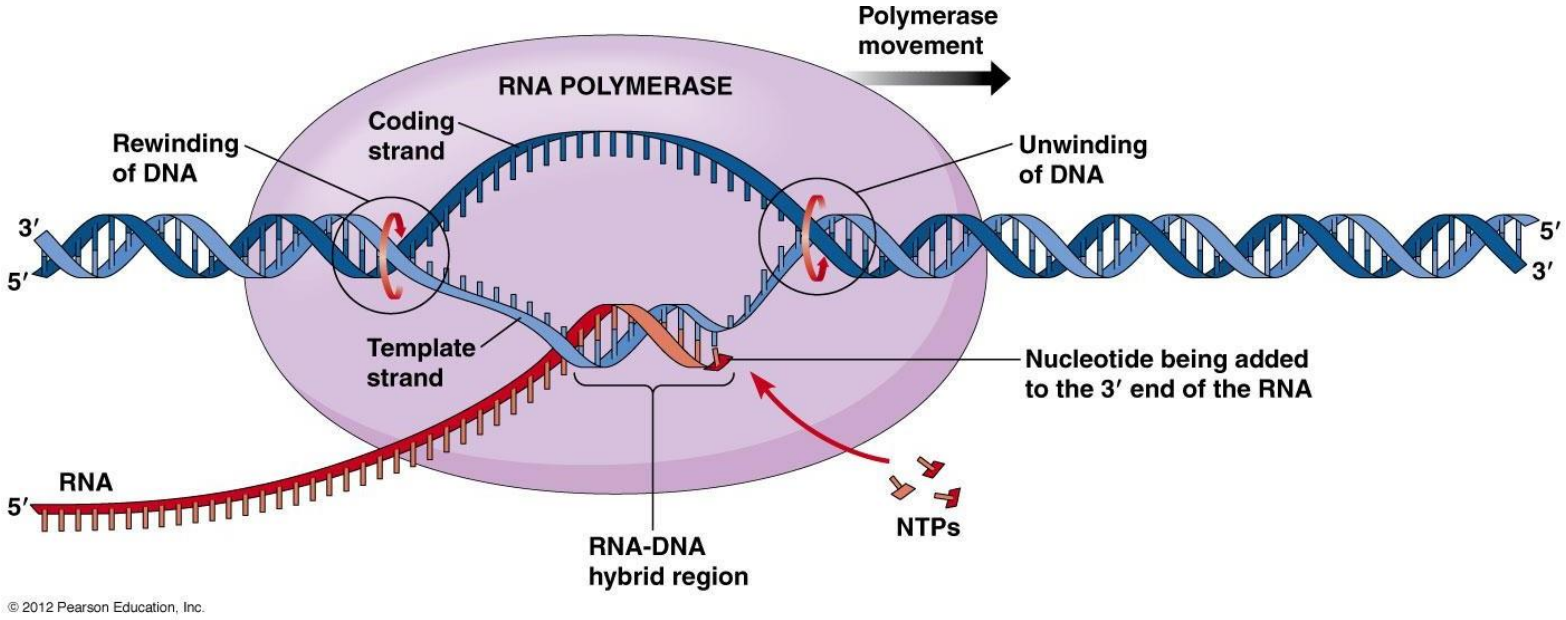


RNA synthesis continues until the polymerase encounters a termination signal where the RNA is released from the polymerase, and the enzyme dissociates from its DNA template.

Mechanism of transcription (elongation)

- As the polymerase moves forward, it:
  1. Unwinds the template DNA ahead of it (like what?)
  2. Elongates the RNA
  3. Rewinds the DNA behind it

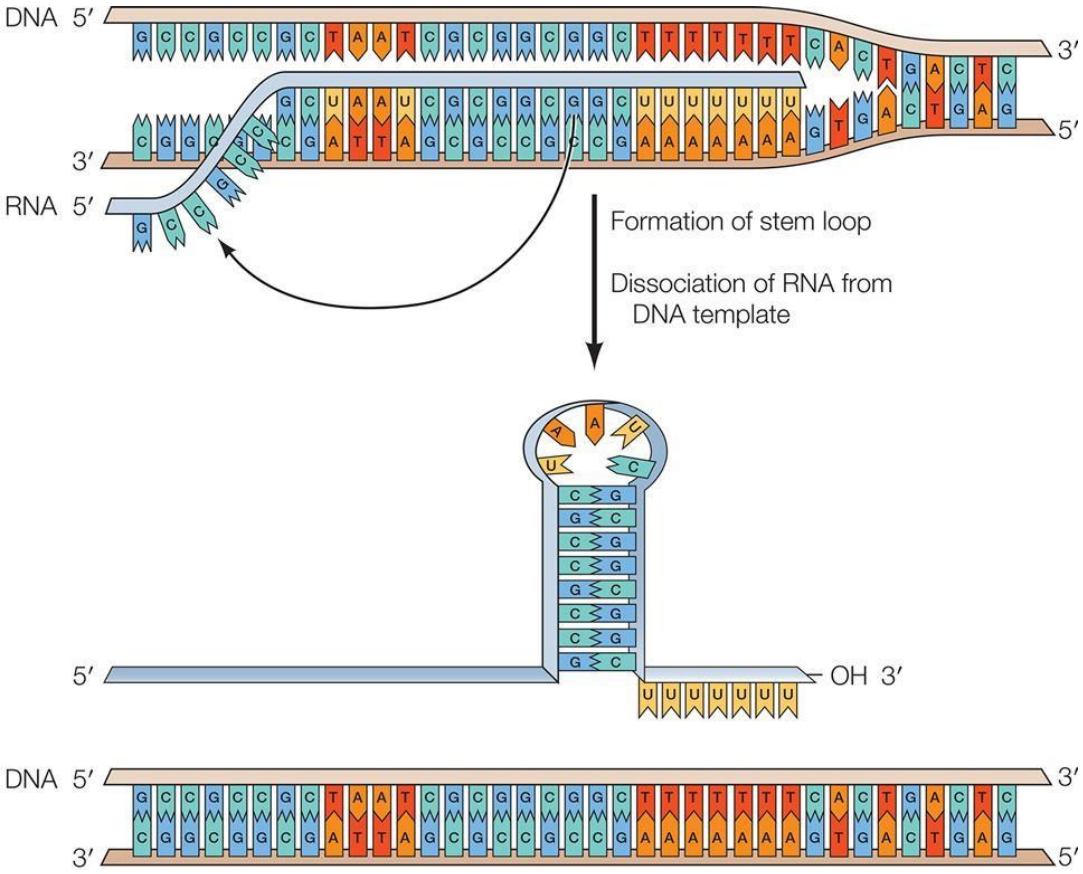
- Imagine the RNA polymerase as having six arms, two arms in front of it unwinding the DNA strand, two on the sides adding nucleotides, and two behind its back rewinding the DNA.

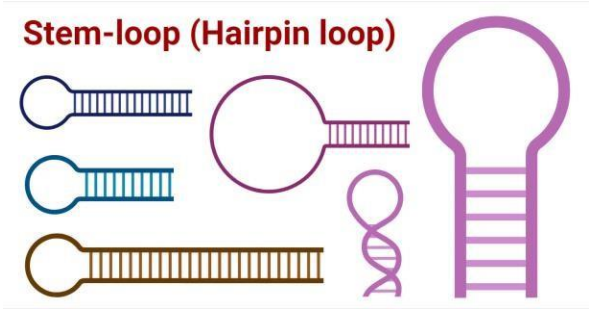


RNA synthesis continues until the polymerase encounters a termination signal (consensus sequence) where the RNA is released from the polymerase, and the enzyme dissociates from its DNA template.

Termination sequences

- The most common termination signal among genes in E. coli is a stem-loop structure that consists of a symmetrical inverted repeat of a GC-rich sequence followed by A residues (why?).
- Transcription of the GC-rich inverted repeat results in the formation of a stable stem-loop structure.
- The GC-rich sequence (a palindromic sequence) forms a stable stem-loop structure, also called a hairpin loop, in the RNA. This structure disrupts the transcription complex.
- The A-rich region in the DNA template produces a U-rich region in the RNA. These A-U base pairs are weak due to their two hydrogen bonds, causing RNA polymerase & RNA to dissociate from the DNA template.



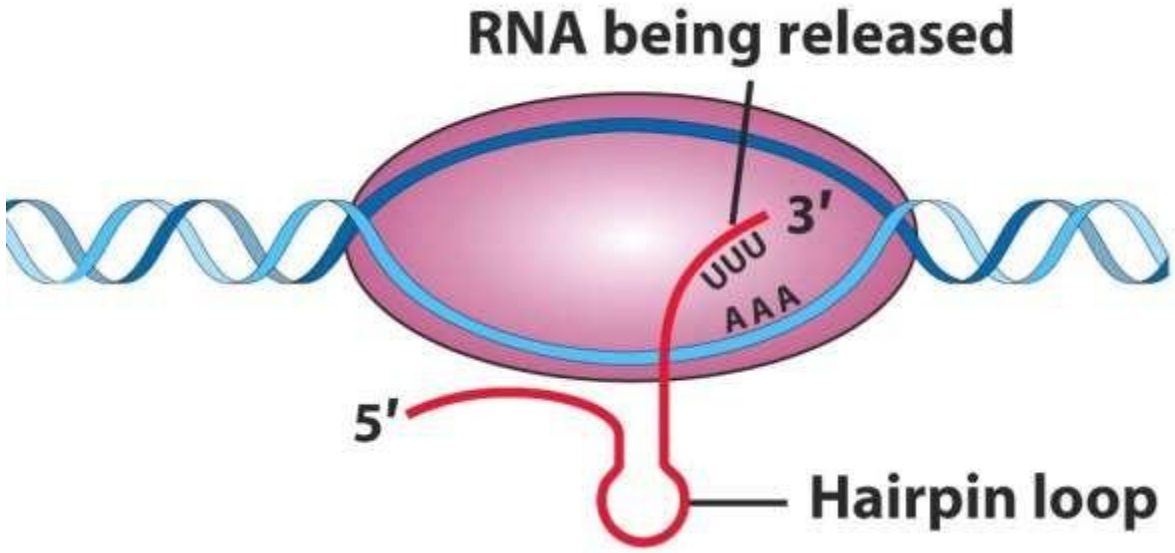


Click here to view a video explaining the termination sequence

The effect of the stem loop structure

وكان فضل الله عليك عظيما

- The formation of this structure breaks RNA association with the DNA template, destabilizes the RNA polymerase binding to DNA, and terminates transcription.



رسالة من الفريق العلمي:

في بداية المحاضرة تم طرح موضوع ان تروج لنفسك كما هو في الثقافة الامريكية sell yourself ، وذُكر كلام قد يُفهم منه أن الزهد ليس أمرًا ذا أهمية. ولا أقصد بذلك انه كان من المتعمد طرحه بتلك الطريقة من قبل الدكتور، إلا أن طرح هذا الموضوع قد يختلط على البعض ويفهم بطريقة خاطئة، لذلك أحببت هذا التوضيح حرصًا على الفهم الصحيح.

فالزهد في الإسلام **لا يعني ترك الدنيا أو تعطيل السعي أو عدم السعي للمكانة الاجتماعية...** ، وإنما **عدم تعلّق القلب بها**، مع الأخذ بالأسباب والعمل والإحسان فيها.

وقد حثّ الإسلام على القوة والسعي لما ينفع، كما في قوله ﷺ:

«المؤمن القوي خيرٌ وأحبُّ إلى الله من المؤمن الضعيف... احرص على ما ينفعك، واستعن بالله، ولا تعجز» (رواه مسلم).

وفي الوقت نفسه، قرن ذلك بالتواضع وعدم الفخر والبغي، فقال ﷺ:

«إن الله أوحى إليَّ أن تواضعوا، حتى لا يبغي أحدٌ على أحد، ولا يفخر أحدٌ على أحد» (رواه مسلم).

فالميزان هو أن يكون المال والمكانة **وسيلة لا غاية**، وأن يكون **السعي في الدنيا مع توجيه القلب للآخرة**، كما قال تعالى:

﴿وَابْتَغِ فِيمَا آتَاكَ اللَّهُ الدَّارَ الْآخِرَةَ وَلَا تَنْسَ نَصِيبَكَ مِنَ الدُّنْيَا﴾ سورة القصص - الآية 77.

لا بأس بالسعي حتى يعرف الناس اسمك او ترتفع مكانتك **ولكن إياك والكبر إياك والكبر إياك والكبر**; فقد ورد عن النبيّ ﷺ قَالَ: لا يَدْخُل الجَنَّةَ مَنْ كَانَ في قَلْبِهِ مِثْقَالُ ذَرَّةٍ مَنْ كِبرٍ.

For any feedback, scan the code or click on it.

Corrections from previous versions:

Versions	Slide # and Place of Error	Before Correction	After Correction
V0 → V1	Slide 9	What are substrates for DNA polymerases? deoxyribonuclosi de triphosphates (ATP, GTP, TTP and CTP)	What are substrates for DNA polymerases? deoxyribonuclosid e triphosphates (dATP, dGTP, dTTP and dCTP)
V1 → V2			