CYTOLOGY

Premed 2018 - JU

◉ Sheet ○ Slides

Number

13

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*In this sheet we are going to talk about **Transcription**.

❖ ***First of all, we must know what is a gene ??***

Gene is the entire DNA sequence that is necessary for the synthesis of a functional RNA (rRNA, tRNA, or miRNA) or a polypeptide, which may become a protein or functional peptides.

When we have one complete piece of DNA that represents a chromosome -Recall that chromosomes are made up of DNA- , certain regions on this DNA have the code which is the GENE on them , and we use this Gene to make RNA molecules (By **Transcription**).

Remember that we said that the human genome is made up of coding sequences -Sequences that can be transcribed into RNA-.

There is a difference between Proteins and Polypeptides :

-**Polypeptides** are stretches of amino acids , whereas **proteins** are present when you have polypeptides that can fold forming a 3D structure known as Protein.

So, we can use the gene to synthesize mRNA and then using this mRNA we can synthesize a polypeptide → then we can cut this polypeptide into smaller pieces forming functional **Peptides** -around 10 amino acids only- , or this polypeptide may fold to form a **Protein.**

- Keep in mind that not all the DNA sequence will be used to make those molecules , Some regions on DNA are Regulatory -They are important for the process of transcription-.
- Example on these Regulatory regions : **Promoter** & **Enhancer.**

Another name for Gene is : **Cistron.**

- In bacteria genes could be **Polycistronic** , which means that we can make different polypeptides from the same mRNA molecule.

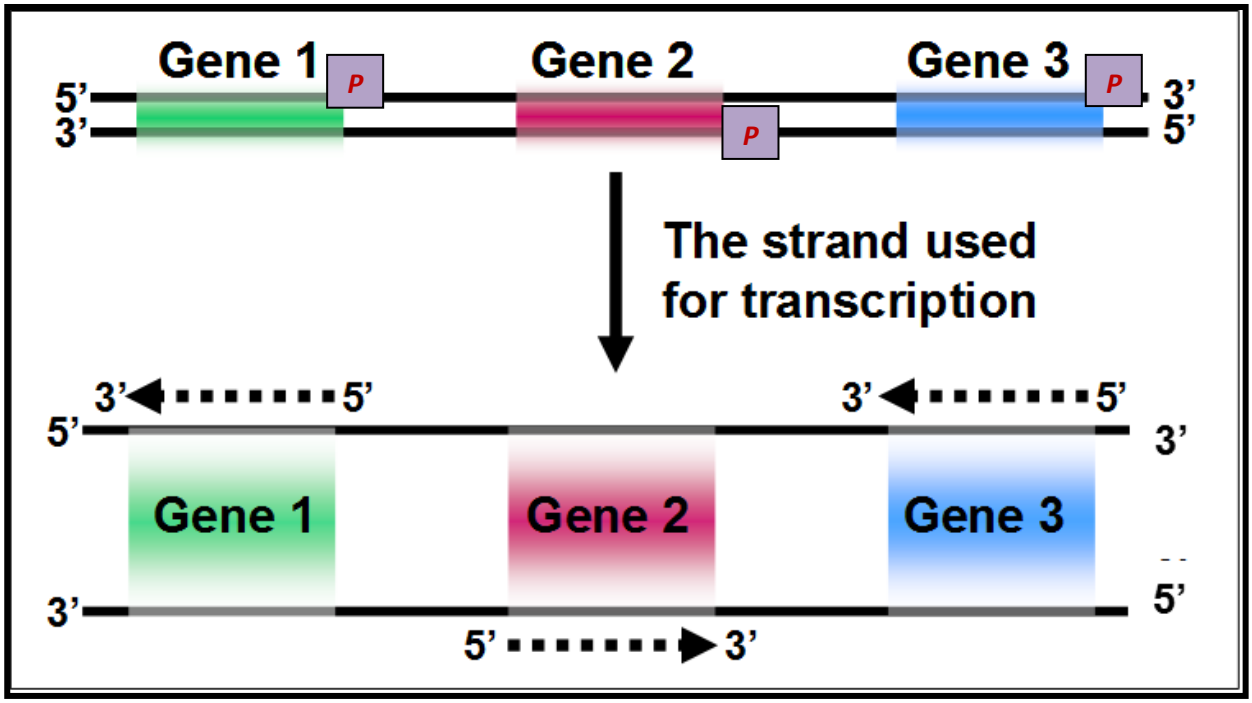
- In Eukaryotic system it's **Monocistronic** , Which means we make just one polypeptide from the same mRNA molecule.

❖ *The General Mechanism of Transcription:*

-Transcription is the process of making RNA out of DNA using DNA as a template.

Notice that in DNA replication we use the 2 DNA strands as a template -DNA polymerase reads both strands-.

RNA polymerase -The enzyme that responsible for the transcription process- also reads the 2 strands but it uses one strand for any particular gene in order to make RNA.



For further clarification:

Look at **GENE 1** for example , the RNA polymerase reads only the Upper strand.

For **GENE 2** , RNA polymerase reads the Opposite strand.

For **GENE 3** , it reads the Upper strand.

*Now the question is what does determine which strand the RNA polymerase read ?

-Well actually it is the presence of a sequence called **Promoter** -which is part of the gene itself-.

-Once the promoter is present it means that here we have a gene and we need to start transcription here -Using this strand-.

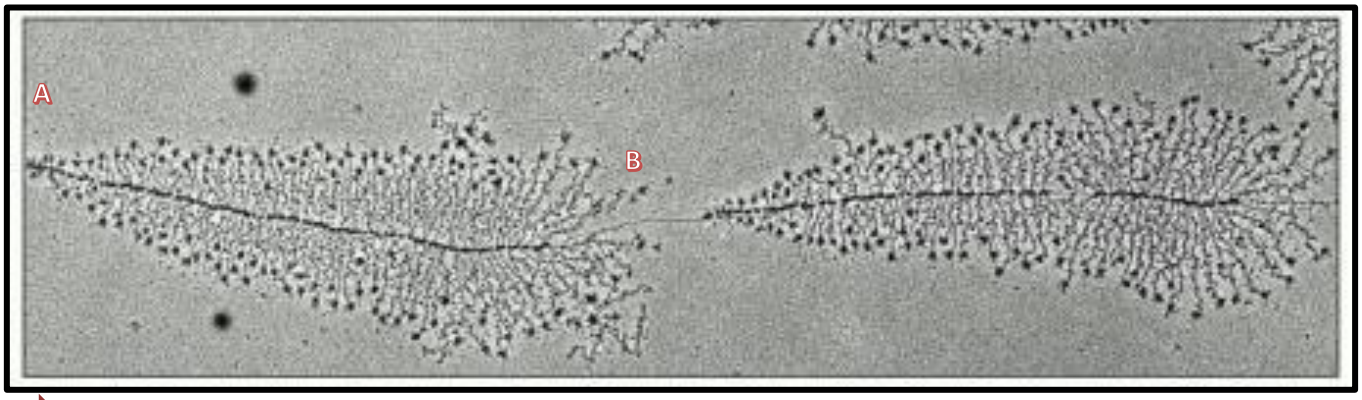
-This promoter region is a specific sequence that is not present on the other strand except for another gene -In the example above for Gene 2-.

-This promoter region isn't read by RNA polymerase.

➡ Keep in mind those points:

1- RNA synthesis takes place from the **5' to the 3'** , and the DNA template is read from the 3' to the 5'.
2- mRNA molecule has the nitrogenous base **Uracil (U)** instead of **Thymine (T).**
3- The sequence of mRNA is complementary to the template strand and almost identical to the opposite strand except that it has **U** instead of **T**.

- The RNA polymerase substrates are **Nucleoside Triphosphates** : Adenine, Uridine, Guanine, Cytosine **Triphosphate** , but what is added to the RNA molecule is **Nucleoside Monophosphate** : Adenine, Uridine, Guanine, Cytosine **Monophosphate** -The 2 phosphates are hydrolyzed (removed) , because that is what provides energy for the RNA polymerase to synthesize RNA-.



➡ Look at this electron microscopic image that is focused on the mechanism of transcription in bacteria.

Here we have 2 genes and the transcription is going on , The middle black line is DNA , and the narrower lines extending from it is actually RNA , so RNA polymerase is actually reading the DNA .

Notice that what is happening here is that we have multiple RNA polymerases reading the DNA all at the same time one after the other → This structure is called **Polysome**.

Look at the image again , where do you think transcription starts ? Point A or B?

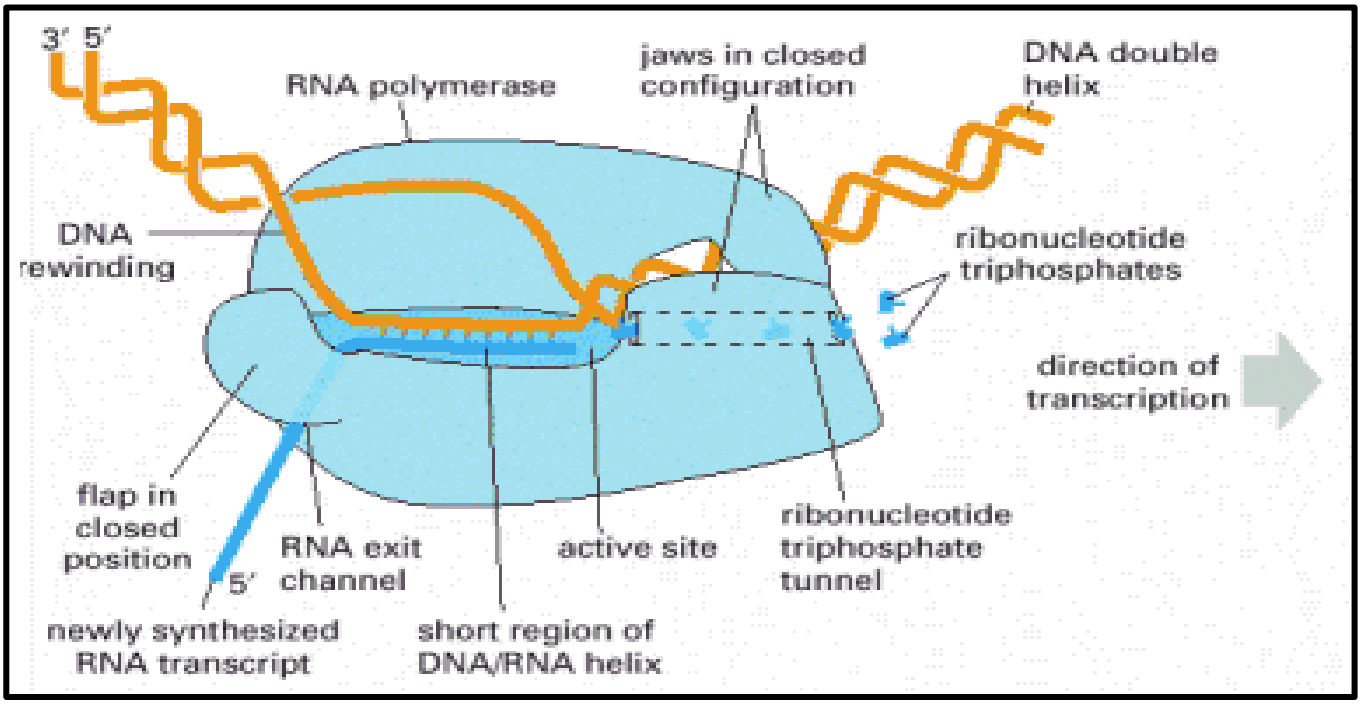
Actually, it is at point A , because it's shorter.

- ***The differences between DNA replication and Transcription :***

1- The RNA strand doesn't remain Hydrogen-bonded to the DNA template strand.
2- RNA polymerase reads the A in DNA and inserts U in the growing chain of RNA rather than T.
3- RNA molecules are much shorter than DNA molecules.
4- Unlike DNA , RNA doesn't store genetic information in cells.

- ***The differences between DNA polymerase and RNA polymerase :***

1- RNA polymerase catalyzes the linkage of **Ribonucleotides** not **Deoxyribonucleotides**.
2- Unlike DNA polymerase , RNA polymerase can starts an RNA chain **without** a primer.
3- RNA polymerases make about one mistake for every 10^4 nucleotides , but the consequences of an error in RNA transcription are much less significant than that in DNA replication.
4- Although RNA polymerases aren't as accurate as the DNA polymerases , they have a modest proofreading mechanism.



In the image above , the blue structure is actually RNA polymerase -It is a large molecule that covers a large portion of the DNA-.

Notice that the DNA molecule where it is read it is single stranded -It opens up- , so the RNA polymerase can read one strand.

Also notice that part of the RNA molecule is hydrogen bonded to the DNA template.

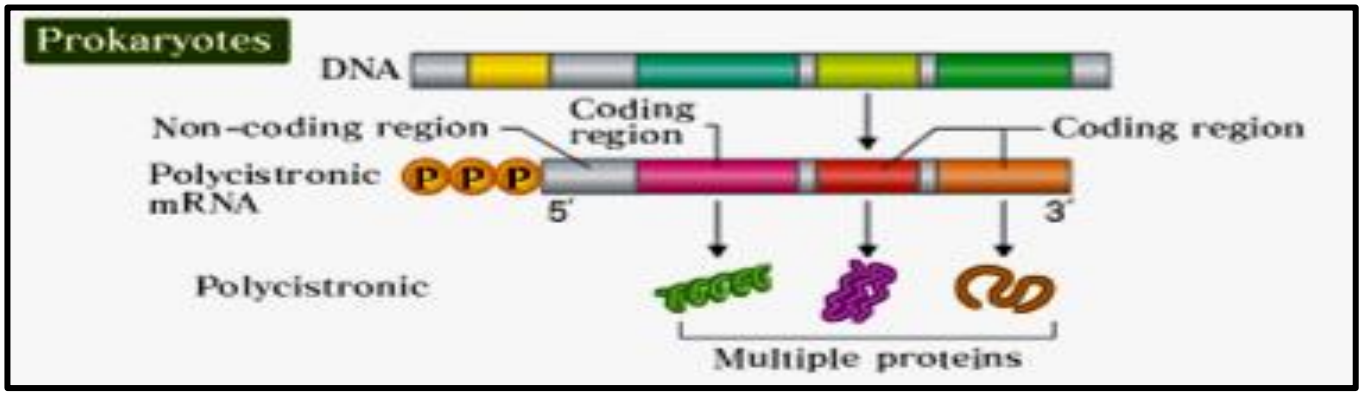
❖ *Transcription In Prokaryotes :*

-We said previously that in Prokaryotic system some genes are **Monocistronic** -One mRNA molecule that I can use to synthesize one Polypeptide- while other genes are **Polycistronic** -Where we have different parts of the mRNA that can be used to synthesize different polypeptides-.

-Those polycistronic arises from genetic units -From cistrons- known as **Operons**.

-So, Operons are genetic units that can make one mRNA with different regions that can be used to synthesize different polypeptides and those polypeptides can form different Proteins -with different functions-.

-In bacteria genes that encode enzymes which are involved in related functions often are located next to each other , For example: the genes encoding the enzyme required to synthesize **Tryptophan** are located in one contiguous stretch.

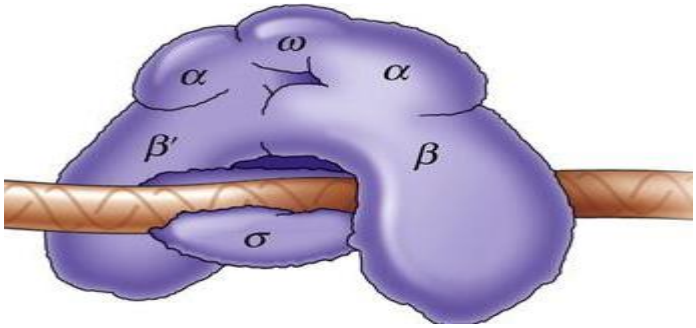


➡RNA polymerase is a very large molecule and it isn't made up of one polypeptide only , it consists of several polypeptides (2α , β , β' , ω , σ)

-The core polymerase (2α , β , β' , ω) is fully capable of catalyzing the polymerization of NTPs into RNA

-One of these polypeptide is called σ

-Sigma- , and this polypeptide is not very important for the function of the RNA polymerase , so if we remove it RNA polymerase can still function well.



➡ We said that RNA polymerase knows that it will use a specific strand to start transcription at by the presence of a **Promoter**.

-A promoter is basically a region that comes right before the beginning of the gene and it tells RNA polymerase that here you have a gene that you need to transcribe.

-When scientists studied the gene region they founded that it has **Consensus sequence** -A sequence that exists in all genes , not only within the same cell but also within different bacterial cells-.

-This sequence is very important, and it didn't change throughout evolution.

Look at this image , the green region is the beginning of the gene , and the first nucleotide read there is called **The Transcription start site** , so the first read nucleotide-downstream- is defined as **+1** , the second one is defined as **+2** , and so on.

-The promoter is ***Upstream*** of the transcription initiation site.

-The nucleotides that is located before the transcription start site -Upstream- are defined as -1 , -2 , and so on.

-Look at the sequences that are defined as -10 & -35 → those sequences are actually **Consensus sequences** → which means that there are genes right there.

-If we change the -10 and -35 sites the RNA polymerase **cannot** function.

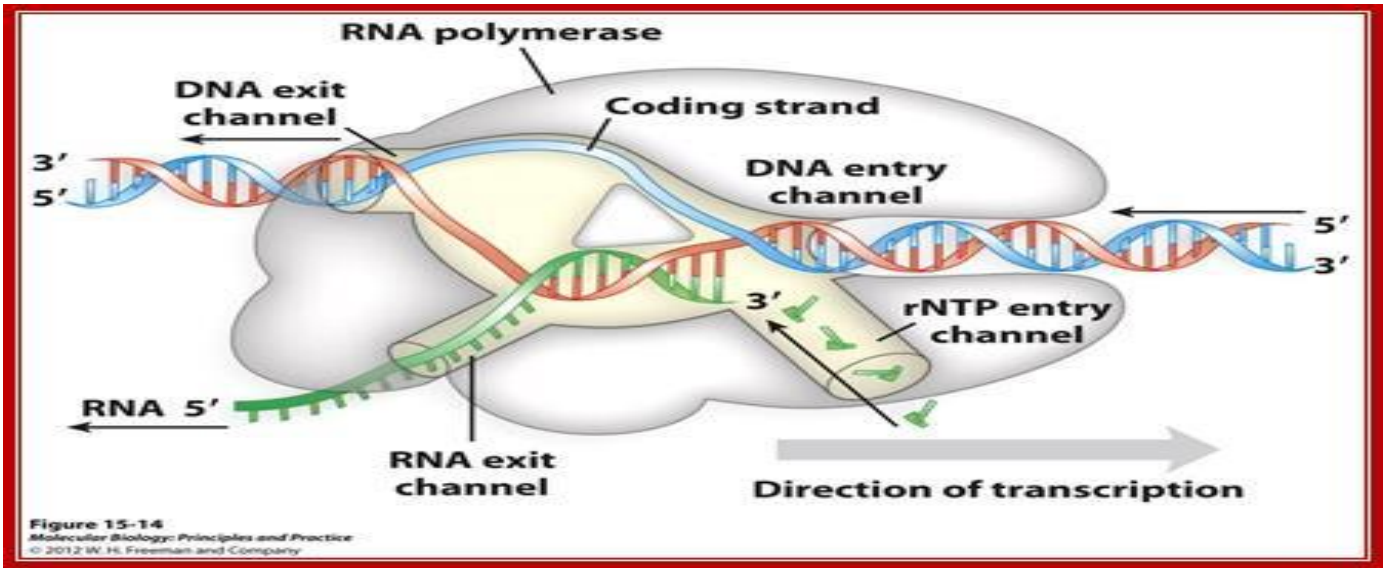
-- The -10 and -35 sequences are defined like that because they are located approximately 10 and 35 base pairs upstream of the transcription start site.

- ***Role of the σ subunit in RNA polymerase*** :

-We said that the σ subunit is not necessary for the catalytic activity of the enzyme.

-The importance of σ subunit is that it guides the RNA polymerase to the promoter region to stabilize the interaction between RNA polymerase with the DNA and thus allow RNA polymerase to start transcription and then it detaches.

-So , if I removed the σ subunit , RNA polymerase can still transcribe genes , but it is not efficient → Because it will have hard time finding promoters.

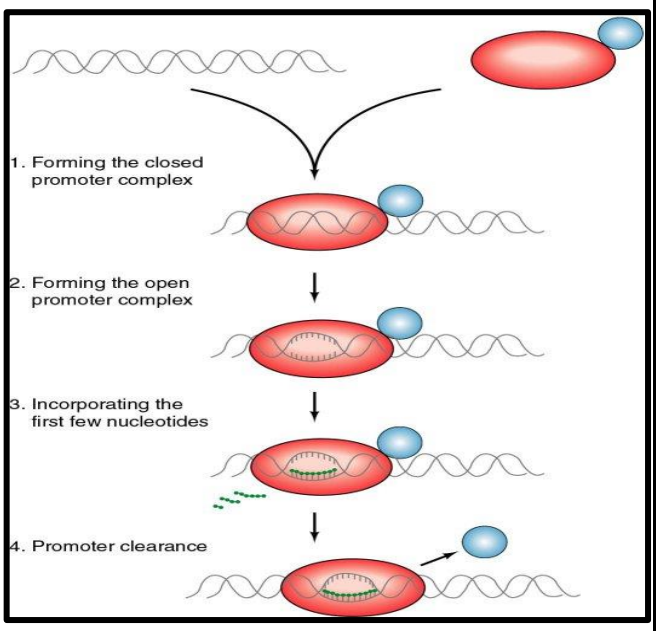


- ***Mechanism of transcription:***

1- Initiation:

- We said that the σ subunit directs RNA polymerase to the promoter region ,then since the RNA polymerase is attached , it opens up the promoter and then it reads the strand -Synthesizes RNA from the 5' to the 3'-.

-Then after the addition of about the first 10 nucleotides , σ is released from the polymerase.



2- Elongation:

-After the σ subunit detaches from the RNA polymerase it goes and binds to another RNA polymerase and guide it to the promoter region so we will have the formation of the structure known as **Polysomes** , and this process is called **Elongation**.

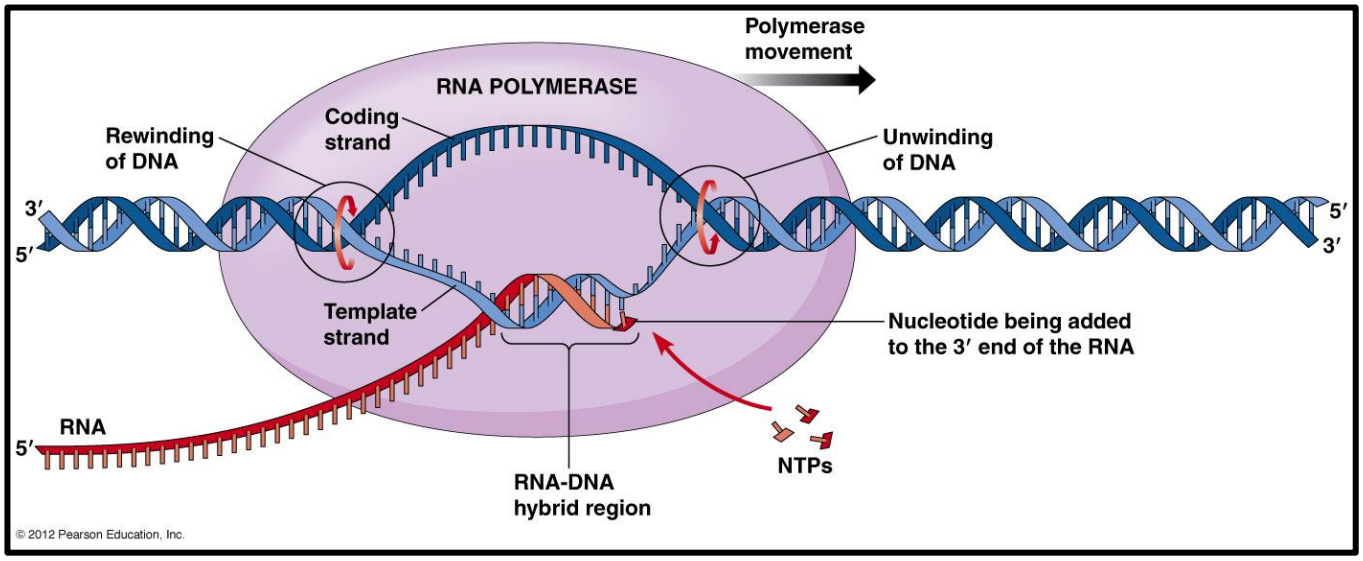
-Notice that part of the RNA is bonded with the DNA and that is important in stabilizing the RNA polymerase with the DNA -The RNA polymerase is attached to both the DNA and the RNA- , so this complex (DNA + RNA + RNA polymerase) is constant.

-As the polymerase moves forward it :

a- Unwinds the template DNA ahead of it

b- Elongates the RNA

c- Rewinds the DNA behind it.

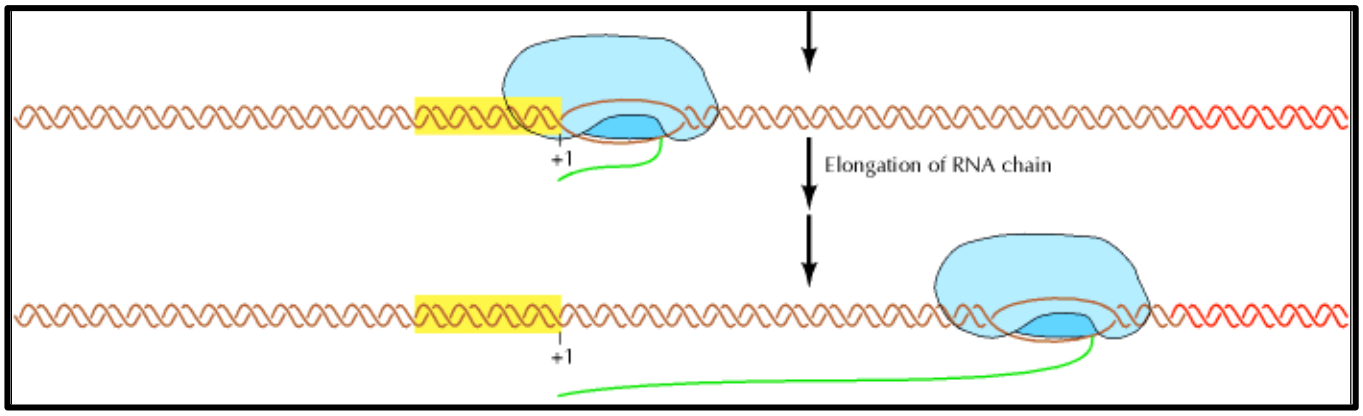


3- Termination :

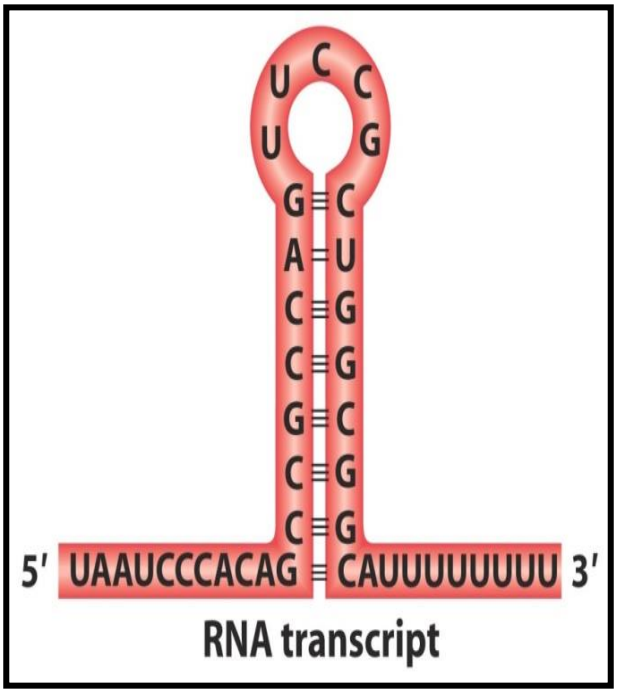
-The process of Elongation continues until we reach a specific sequence -which is basically a **consensus sequence**- and it is called **Termination Sequence.**

-The termination sequence tells us that here in this region the transcription stops.

-Once it reaches the termination sequence the RNA will be released from the polymerase and the enzyme dissociate from its' DNA template.



-The **Termination Sequence** consist mainly of a symmetrical inverted repeat of GC-rich sequence followed by A residues.



-Transcription of the GC-rich inverted repeats results in the Formation of a stable **Stem-Loop Structure**.

-The interactions between G and C is very strong because we have 3 hydrogen bonds but recall that those GC-rich inverted repeats are followed by A stretches , so once this Stem-loop structure is formed, the interaction between RNA and DNA and RNA polymerase will be very weak so the RNA polymerase will detaches.

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