NO.9

Molecular Biology

Doctor 2019 | Medicine | JU

Sheet Slides

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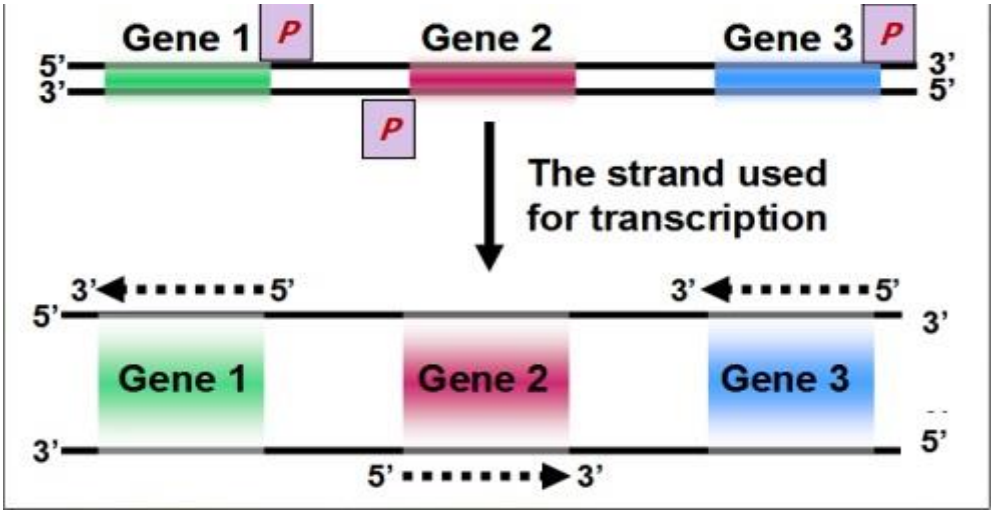
Doctor

Walhan alshair

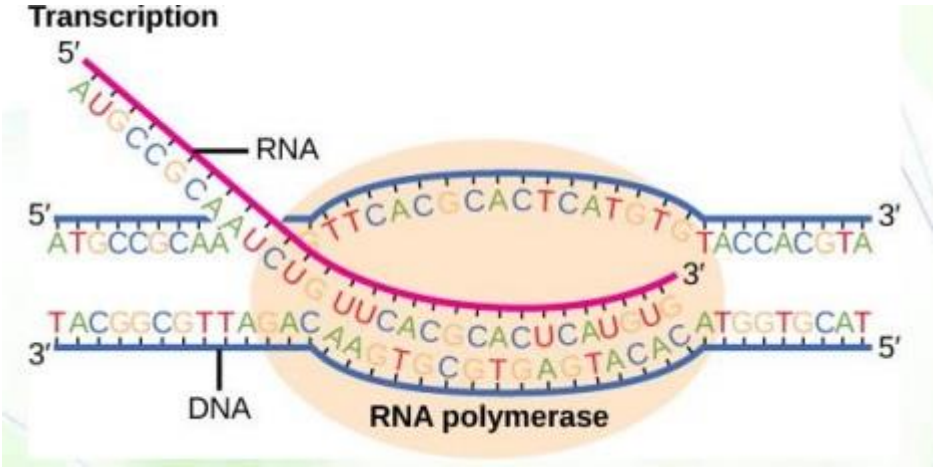
- What is a gene? The entire DNA sequence that is necessary for the synthesis of a functional RNA (mRNA, rRNA, tRNA, lncRNA, microRNA, etc.) or a polypeptide, which may become a protein or functional peptides
- mRNA for coding the protein, rRNA and tRNA for the synthesis , also lncRNA and microRNA plays a role in regulation of different mechanisms inside the cell ,and we mentioned Every protein is a polypeptide but not every polypeptide is a protien so the polypeptide later can be a protein or a functional peptide.(شرح الدكتور للجملة الاولى)
- So. The DNA sequence encompasses the coding region (that makes the protein), other regulatory sequences like a promoter, an enhancer (we will know about them more in the coming slides and lectures), etc., or a non-coding region like introns
- **A cistron**: an alternative term of 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**, so you should know the difference between mono and polycistronic and we will talk about them in the coming slides

The general mechanism of transcription

- As you remember that Transcription is the process of making RNA from DNA, so as in DNA replication DNA polymerases uses origin strand As template to produce daughter strand so we have the same mechanism in transcription: **One of the two strands of the DNA double helix acts as a template for the synthesis of an RNA molecule.**
- So what can decide which strand can be used by RNA polymerase to synthesize RNA, to answer this question lets take a look at this figure (page 2).
  - we have a sequence of three genes, also you need to know that transcription and the synthesis of RNA should be from 5' to 3' direction, RNA produced from transcription will be synthesized from 5' to 3' and will be complementary to the template strand.
  - So as you can see here we have gene 1 2 3, so if you take a look at the transcription direction … gene 1 and 3 goes from 5' to 3' and complementary to the origin strand, while in gene 2 it's the opposite direction and it will be complementary to the second strand, so what can determine the direction of transcription? actually it's the promoter (**P in the figure**)
  - We will talk about the promoter in the coming slides, it is the region that initiate transcription



So this figure bellow explains the complementarity concept of the RNA transcription, so the RNA is complementary to DNA template, you see here we have the double stranded DNA and the transcription of RNA, so RNA polymerase uses this strand as a template so it synthesize RNA from 5' to 3' and it will be equivalent to the other template (DNA template but we have U instead of T in the RNA)



Now what about the enzymes and substrate that are used in the transcription?

- The enzymes that perform transcription are called RNA polymerases
- They catalyze the formation of the phosphodiester bonds between two nucleotides, you remember we also have phosphodiester bonds between DNA, so both DNA and RNA polymerase synthesize phosphodiester bonds between nucleotides.
- The substrates are nucleoside triphosphates (ATP, CTP, UTP (Uracil instead of thymine in the DNA), and GTP).
- So, it can get the energy from the **Hydrolysis of high-energy bonds in NTPs provides the energy needed to drive the reaction forward, so simply the hydrolysis of NTPs provide the energy required for the synthesize of RNA**

nucleoside triphosphate

Lets compare between DNA replication vs transcription

- ✓ The RNA strand does not remain hydrogen-bonded to the DNA template strand.(it is released free as a single stranded RNA not like DNA which remain hydrogen bonded to the original strand)
- ✓ RNA polymerase read the A in DNA and inserts U in the growing chain of RNA rather than T
- ✓ RNA molecules are much shorter than DNA molecules. (because not all DNA sequences are transcripted into RNA)
- ✓ **Unlike DNA, RNA does not store genetic information in cells** (RNA will end in the cytoplasm and its function mainly to produce proteins so after doing its job will be degraded so it can not be stored as a source of genetic information in the cell but DNA does)

- ➢ RNA polymerase catalyzes the linkage of ribonucleotides, not deoxyribonucleotides(at DNA on the c-2' sugar we have h while in ribonucleotide we have OH)

- ➢ Unlike DNA polymerases, RNA polymerases can start an RNA chain without a primer, in DNA polymerase we need primase to synthesize the strand from 5' to 3'.

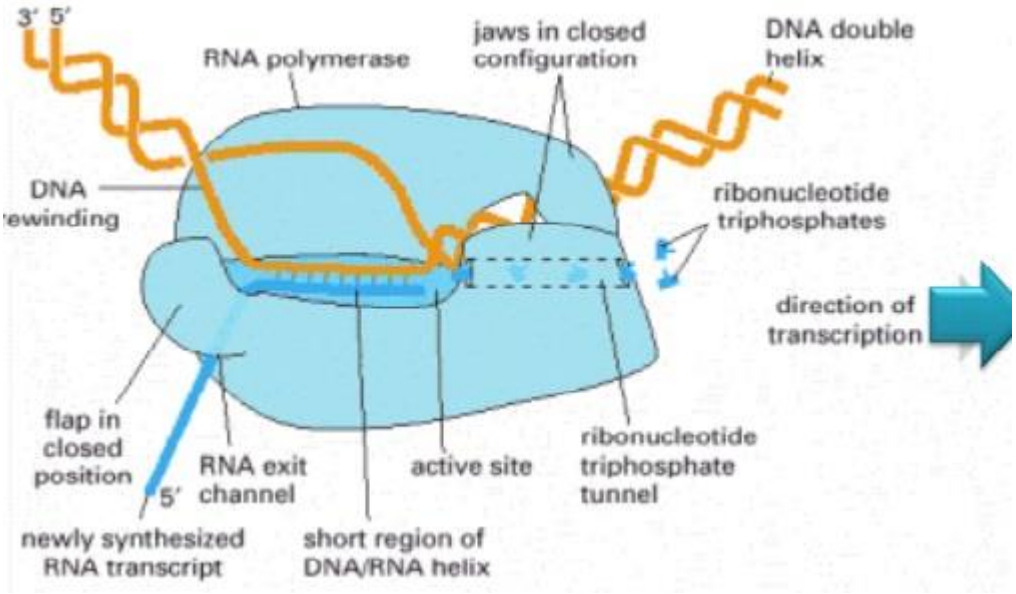
- ➢ RNA polymerases make about one mistake for every 10000 nucleotides.

- ➢ the consequences of an error in RNA transcription are much less significant than that in DNA replication, why? Simply if you have the mutation occurs in the DNA so it will stay in the DNA it will be transferred form cell to cell and will be stored in genetic information but in case of RNA, the RNA will be removed later on and it will not stay in the cell, so it will be for a short moment and wont hurt the function of the cell.

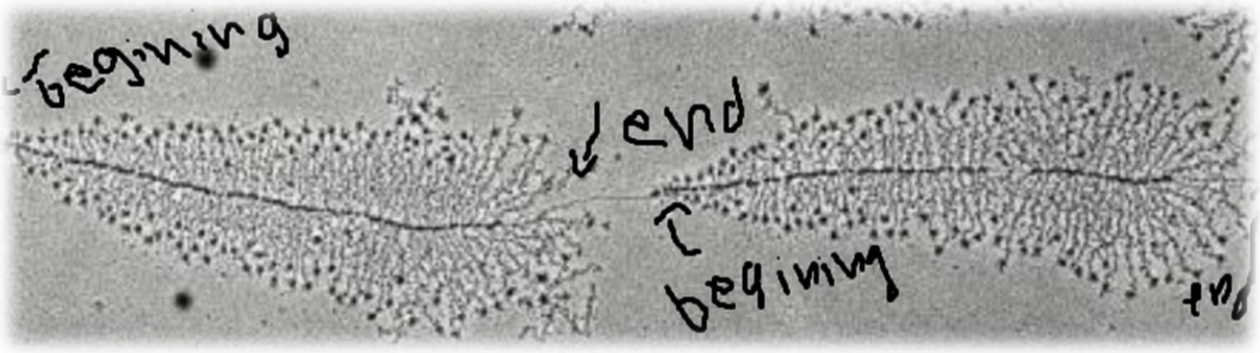
- ➢ Although RNA polymerases are not as accurate as the DNA polymerases, they have a modest proofreading mechanism. If there are any mismatch nucleotides it can read it and cut it then adding the correct base to the RNA sequence.

- ❑ RNA binding to DNA is temporary, what do we mean by that? The transcriptional complex has an eight base pair DNA RNA hybrid, as you can see here in this figure, so

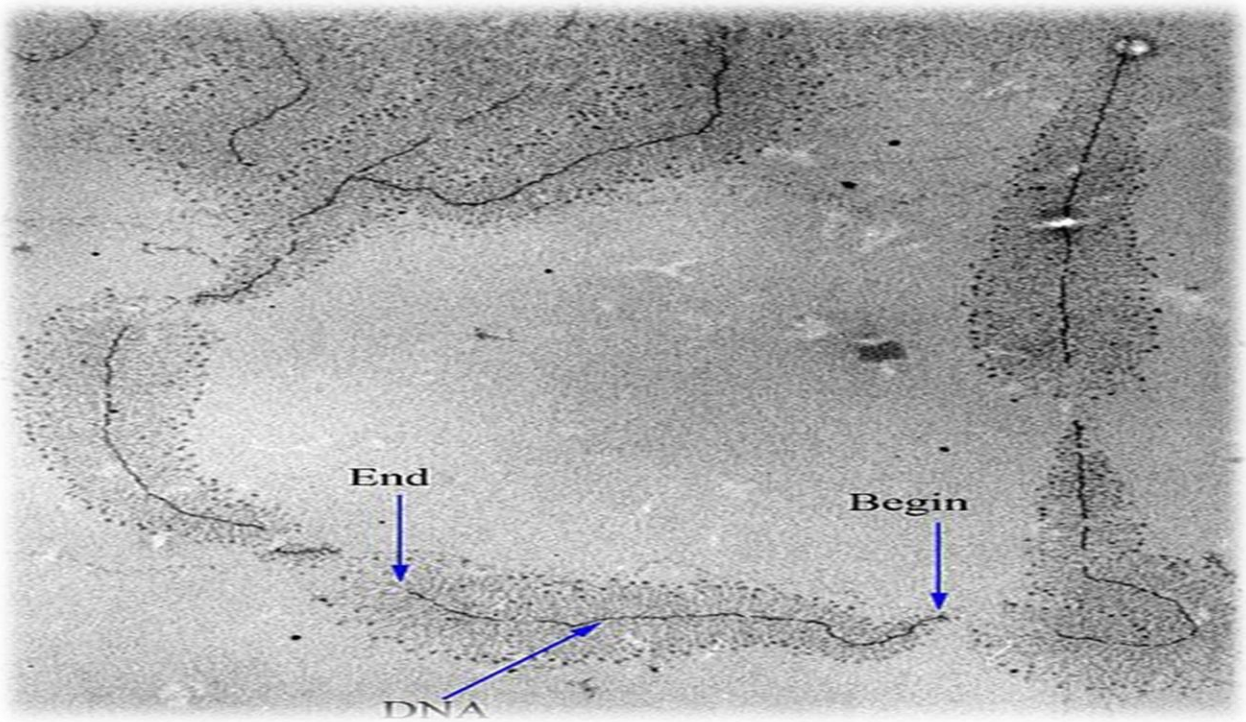
when RNA is synthesized the initial RNA bounded to DNA template will be separated and moved away allows the new RNA nucleotides to bond to DNA and continuing the synthesize of RNA sequence, that's why its temporary and few bases are bound o DNA each moment and will be moved in order to continues RNA synthesis.



- Now lets talk about another important bond in the RNA transcription, maybe you think that transcription produce single strand of RNA **,so you imagine we have one mRNA , rRNA , tRNA produced from one DNA template**, **So that's not true simply the possibility for producing many RNA molecules from the same gene at one time, and these structure known as polyribosomes.**
- Maybe this figure explains more, so this is an EM for the transcription mechanism , you see that we have here one line in the middle and branches going out of the line ,this the gene actually and this is the RNA and every branch here is a RNA molecule, so you can see that we have many RNA sequences produced on following the other ,so my question now Where is the beginning of the gene and where is the end?
- This is the beginning because rna molecule will continoue growing while the transcription continoues and the beginning will be short and it will be longer by transcription more and more



- This figure bellow describe the same idea of the previous one (the idea of the polyribosomes)….you can see that we have several genes and you can notice the beginning and the end of the DNA sequence bellow (**arrows**) and it contains several branches each one represents mRNA strand so we have several copies of the mRNA . **How many genes can you see in the figure?**



- Why we need many copies of the mRNA? Why it is transcripted as a polyribosomes?

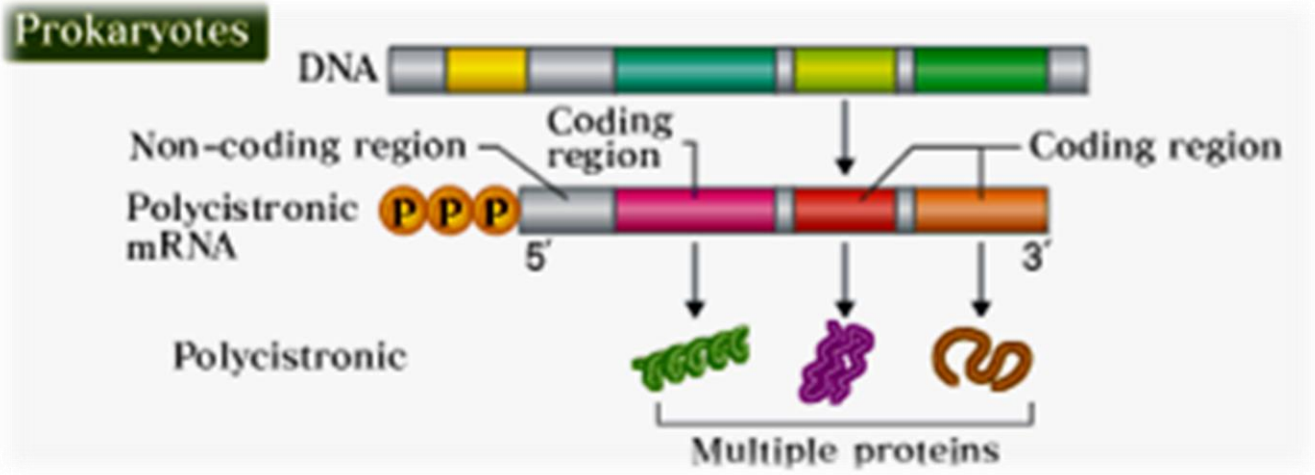
Sometimes we need large quantities of the same protein or polypeptide so the transcription will be very active and you don't have the time to transcript the gene one single time every transcription process , also remember that we have two copies of the each gene (because we are diploid), imagine that we need a large of insulin for example so there is a need for a large number of copies for the insulin protein , that's why several copies of the mRNA are synthesized at the same time.

Transcription in Prokaryotes

➢ Prokaryotic genes (operons)

- In bacteria, genes can be polycistronic (produces several or different polypeptides from one mRNA).

- Genes that encode enzymes that are involved in related functions, are often transcribed as one unit from one mRNA (these genes go transcription as one unit).
  - Example: the genes encoding the enzymes required to synthesize the amino acid tryptophan are contiguous, here we have multiple enzymes involved in the synthesis of that amine acid and all of these genes transcriped as one unit by one mRNA.
- This cluster of genes comprises a single transcriptional unit referred to as an operon.
- Operon: is cluster of genes tha Co- transcriped as a single transcriptional unit (They will be transcripted as single mRNA) ... واحد mRNA أكثر من جين تنسخ سوية في جزيء ...and this cluster of genes will be under the control of a DNA sequence called promoter (it is a DNA sequence that control the initiation of transcription ...we will talk about it in more details later on)

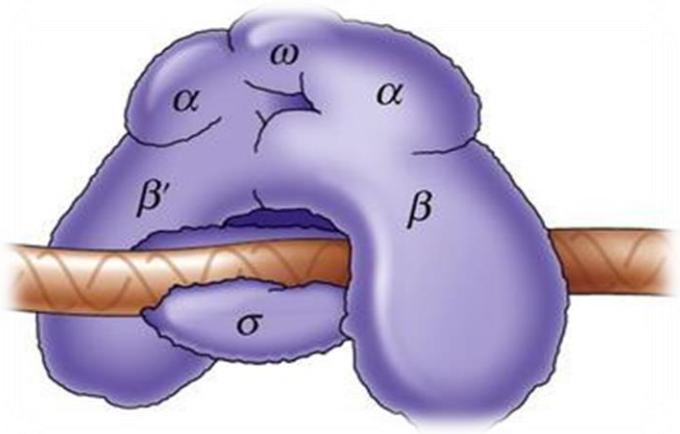


The RNA Polymerase:

- We have differences between RNA polymerases between Eukaryotes and prokaryotes

- 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 omiga subunits.

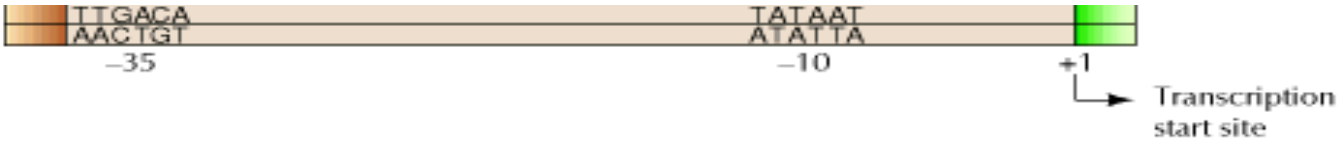
- The core polymerase is fully capable of catalyzing the polymerization of NTPs into RNA.



- The σ subunit is not required for the basic catalytic activity of the enzyme.
- another subunit called sigma subunit sometimes we call it sigma factor specificity factor and it is part of the catalytic core of the enzyme, so it do other functions with RNA polymerase…we will talk more details about it.

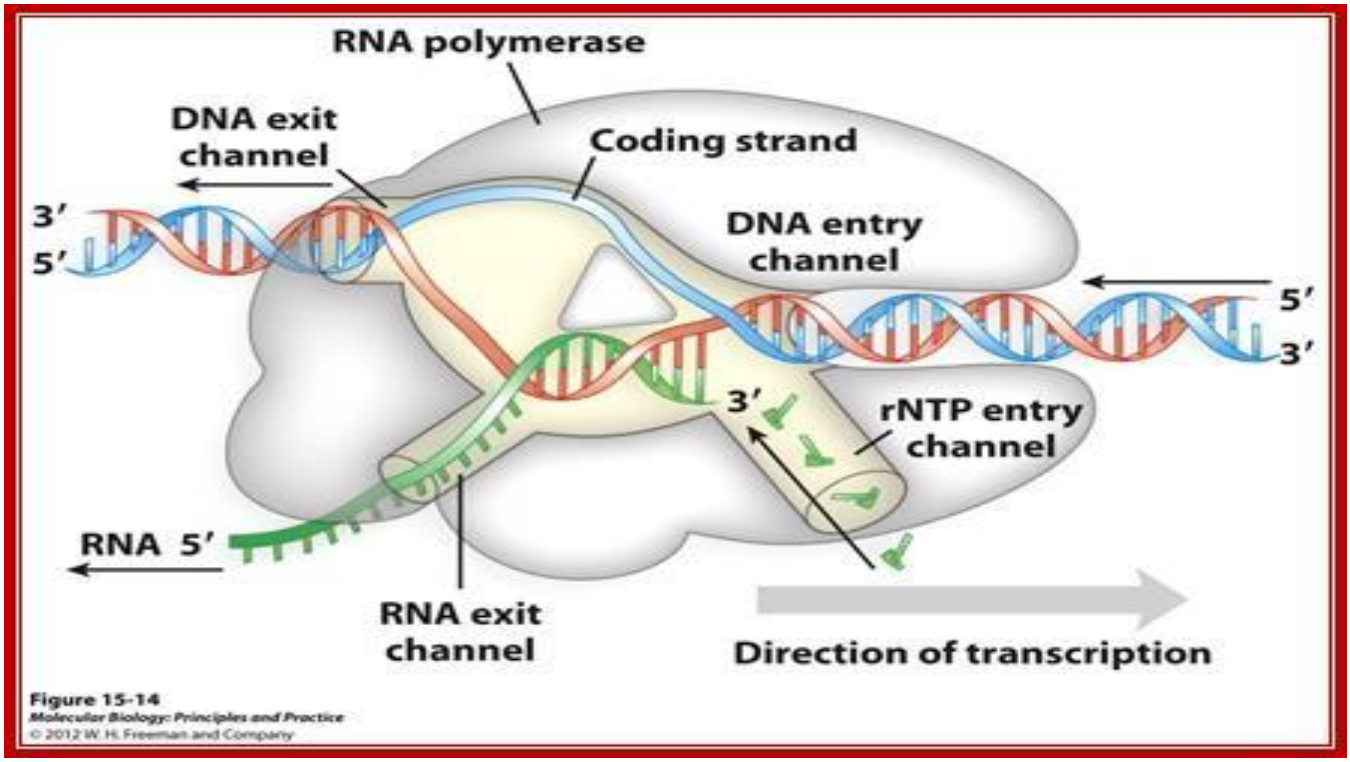
Consensus Sequences (the promoter):

- The DNA sequence to which a RNA polymerase binds to initiate transcription of a gene is called the promoter.
  - A promoter is "upstream" of the transcription initiation site.
  - Upstream is used to describe the region before the transcription initiation **while** we use the term of downstream to describe the region after the transcription site (the transcription start).
- The region upstream (the promoter region) of the transcription initiation site contains two sets of sequences that are similar in a variety of genes.
  - Consensus! ... they are fixed or similar sequences that can be found at different places and do similar function
- In bacteria there are two sits of consensus sequences that are similar in variety of genes called the (-10) and (-35) elements because they are located approximately 10 and 35 base pairs upstream of the transcription start site (within the promoter site).
- The transcription initiation site is defined as the +1 position.
- Note that : we use negative sing to represent upstream and positive sign to represent down stream positions and we don't have a zero position.



Role of The σ Subunit:

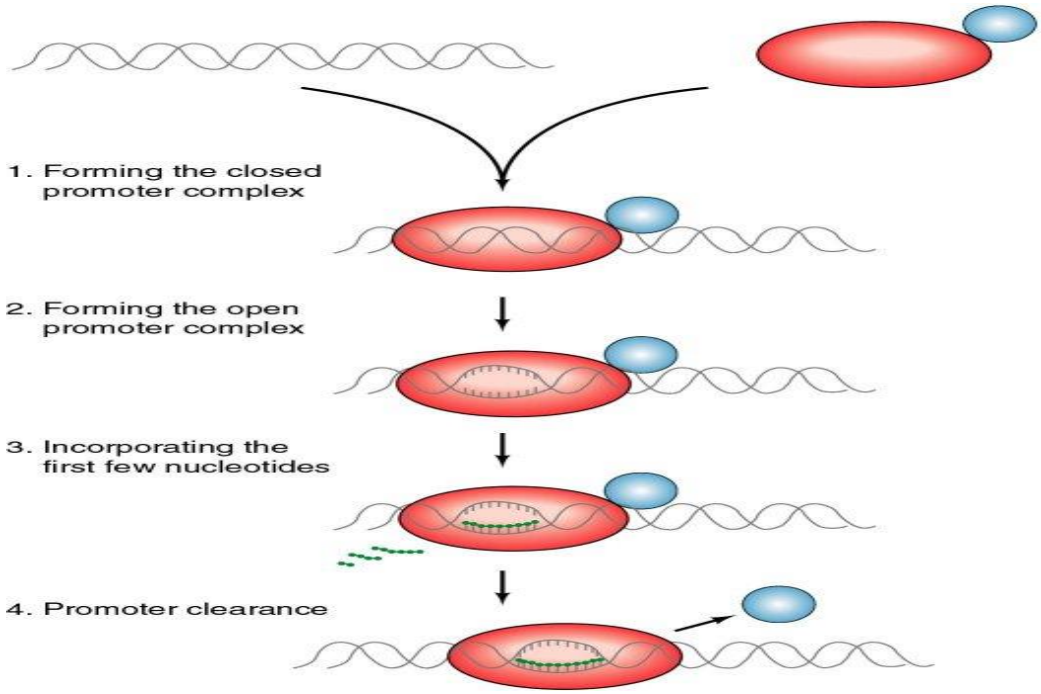
- In the absence of σ , a RNA polymerase binds to DNA with low affinity and nonspecifically.
- The role of σ is to identify and guide the polymerase to the -35 and -10 sequences.



❖ Mechanism of transcription:

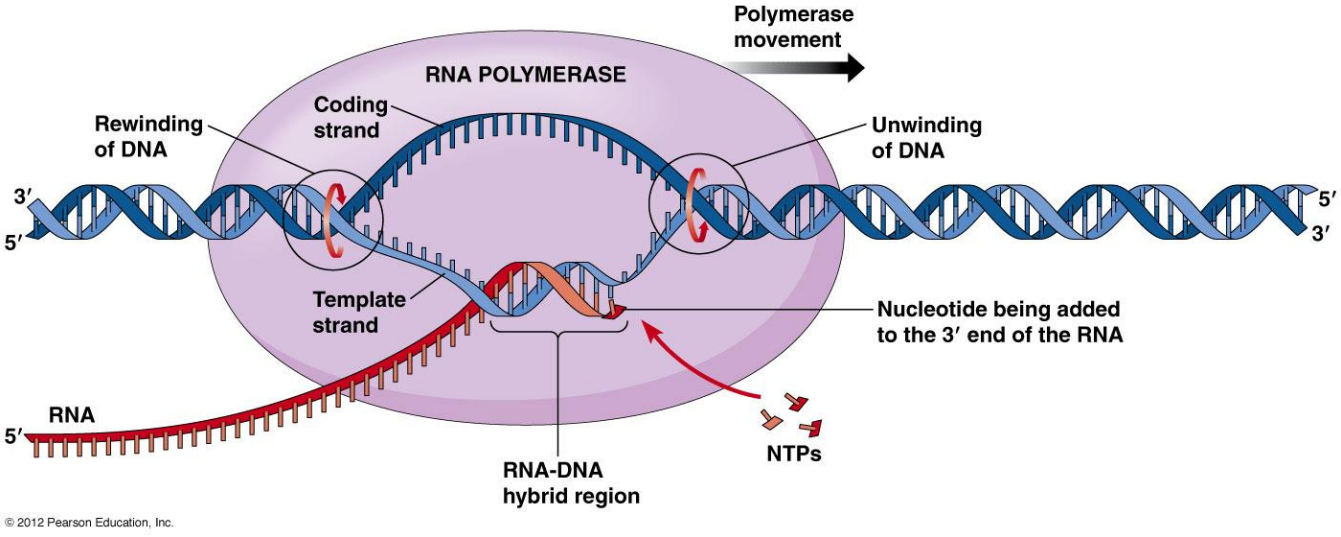
Initiation …

- ➢ The RNA polymerase binds to the promoter and opens it (It opens the DNA double helix), *(like what? Like the DNA A protein).*
- ❖ The single-stranded DNA is now available as a template.
- ❖ Transcription is initiated by the joining of two NTPs.
- ❖ After addition of about 10 nucleotides, σ is released from the polymerase.
- ❖ *What do you think happens to it?* It goes to another DNA polymerase and do the same function again.



(elongation)

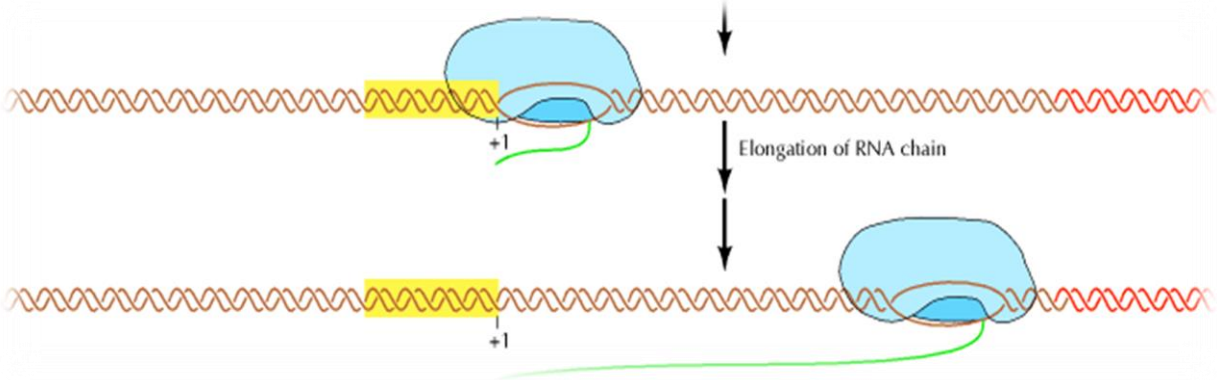
- As the polymerase moves forward, it
  - unwinds the template DNA ahead of it.
  - elongates the RNA
  - rewinds the DNA behind it



(termination)

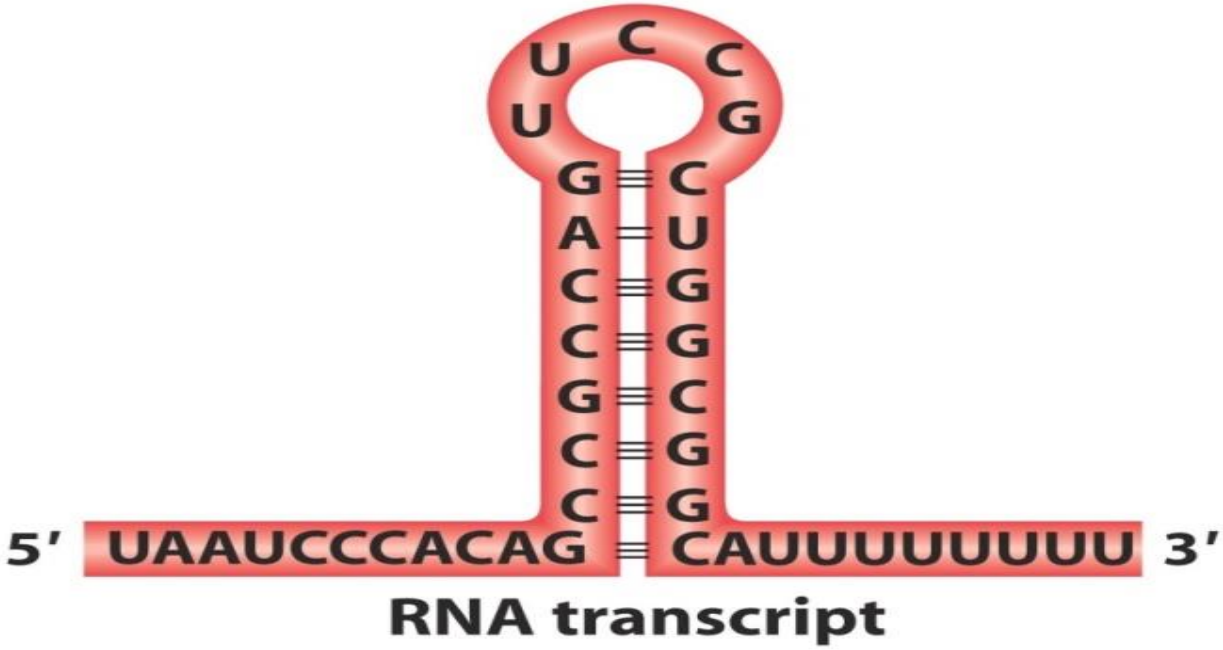
- The stopping of the transcription is not random and the transcription will not continue for ever

- 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.



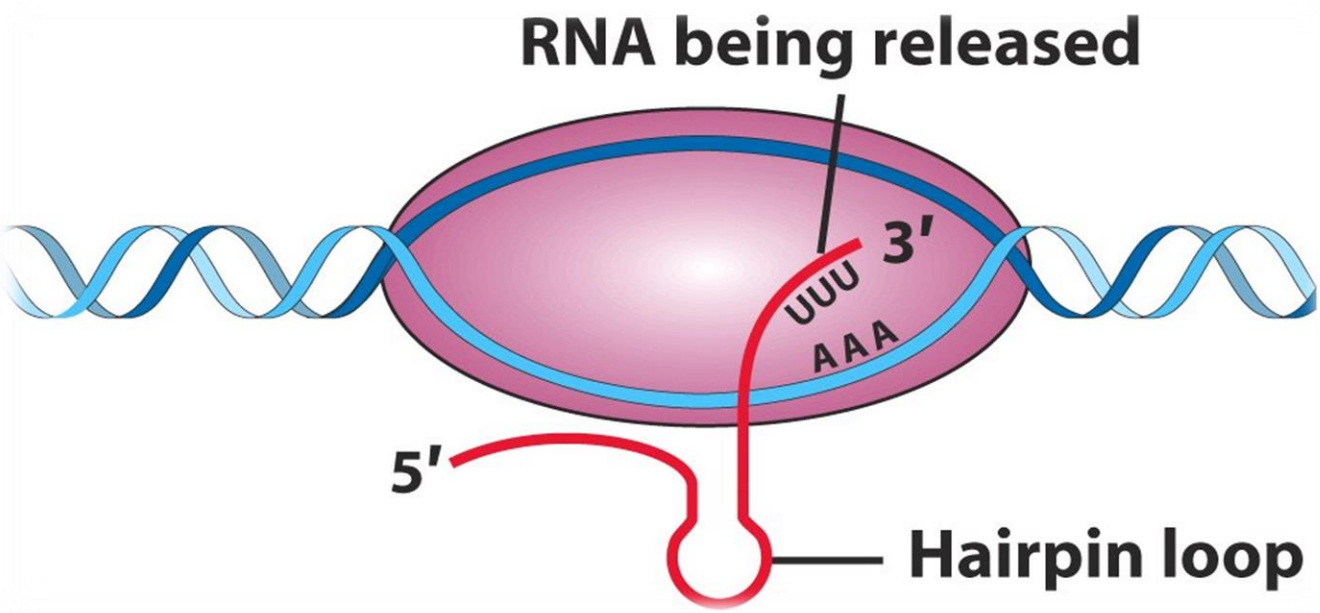
Termination sequences:

- The simplest and most common type of termination signal among genes (we called them stem loops or hairpin loop and they are intermolecular base pairing) in E. coli consists of a symmetrical inverted repeat of a GC-rich sequence followed by A residues (why it is rich with G AND C ? because the interaction between C and G occur via a three hydrogen bond while the interaction between T and A occur via two hydrogen bonds and the high number of GC in the loop make the interaction between its strands stronger and establish a stable loop).
- Transcription of the GC-rich inverted repeat results in the formation of a stable stem-loop structure



The effect of the stem loop structure:

- The formation of this structure breaks RNA association with the DNA template, destabilizes the RNA polymerase binding to DNA, and terminates transcription.
- There this figure shows the idea of the stem loop and the poly-A signal, the formation of the stem loop followed by poly-A sequence induce the dissociation of RNA polymerase
- The poly-A sequence will be very weak in the inter action with poly-U and that will easily allow the release of the RNA and the dissociation of the RNA.



في ظل الوباء المتفشي في العالم اليوم تفضل بقراءة هذا الموقف واستنبط العبرة و الموعظة و كلنا أمل أن تعمل بمكنونها

في مرة من المرات كان عنترة بن شداد العبسي أشهر فرسان العرب و شاعر معلقاتها وعشيق عبلة المغوار يتجول في جنبات المدينة و في اثناء تجواله اعترض طريقه ثور هائج.... من يتخيل هذه اللحظة يجزم بأن عنترة بفروسيته و شجاعته سيواجه بطش هذا الثور الهائج و يعقره... لكن فارسنا ما كان منه الا و أن جعل الغبار يتناثر من وراءه و لاذ بالفرار ليأتيه بعدها جمع من رجال مدينته فيقولون أنت عنترة العبسي فارس فرسان العرب ذو السيط المدوي الذي شاع في الأرجاء فأصبح يهابك القاصي و الداني تفر من ثور هائج!!!!!

كان جواب عنترة يحمل في طياته مدلولا جوهريا أن الشجاعة والفروسية ليست قوة في الجسد ... لكنها قوة في العقل ...قوة في التدبير... فقال : " وما أدى الثور أني عنترة "

الزم بيتك حفظنا الله وإياك