Sheet no.7

Slides
Doctor`s comments
Writers`notes

Molcular biology

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Definition of a gene

The entire DNA sequence(the specific region in DNA in chromosome) that is necessary for the synthesis of a functional RNA (mRNA, rRNA, tRNA, lncRNA, microRNA, etc.)(they have regions for transcription and producing RNA and for regions to regulate transcription 'promoters,enhancers, silencers;don't transcripe but necessary for transcription)

or a polypeptide, which may become a protein or functional peptides.

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.

there are differences between polypeptides and proteins

proteins:3 dimensional structure and has a function

polypeptide: stretch of amino acids, no particular structure,may not have function

can be cleaved into smaller peptides that have functions

*production of MRNA →synsethize polypeptides

* entire DNA sequence is necessary for functional RNA

- **A cistron**: an alternative term of a gene,old term for gene

*If it encodes one polypeptide from one mRNA, it is monocistronic.

1mRNA-→1 polypeptide not necessarily proteins

If it encodes several or different polypeptides from ONE mRNA molecule, it is polycistronic

1 MRNA→ different of polypeptides from different region

*to catch up:

DNA : regions in chromosomes necessary for function of RNA (translated like MRNA Or non translated like lncRNA

The general mechanism of transcription

*this sheet for transcription in prokaryotic systems!

General description

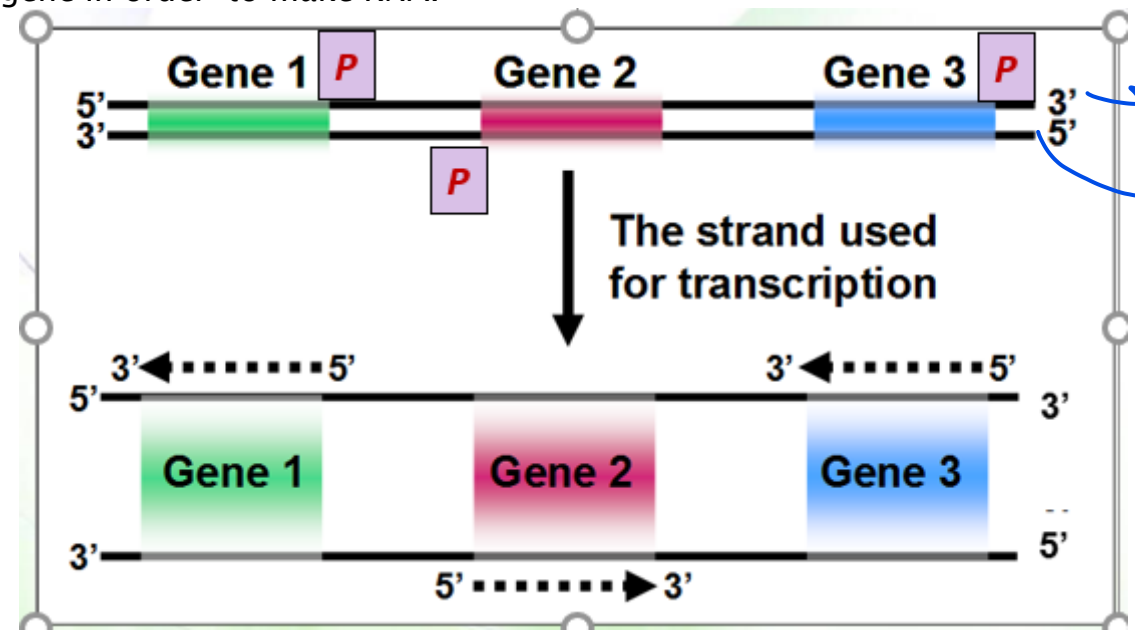
Transcription is the process of making RNA from DNA.

One of the two strands of the DNA double helix acts as a template for the synthesis of an RNA molecule.(RNA transcription uses 1 strand for any particular cistron

Remember? In DNA replication, both strands are the template of the daughter strands.

Using DNA strands

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



3 genes on the region in chromosome

Gene 1: uses first strand as a template

Gene3: also uses the first

Gene2:uses the second as a template

It indicates that both strands can be used as a template but for a particular gene only one is used as a template

What does determine which strand is used for transcription?

The promoter: P 

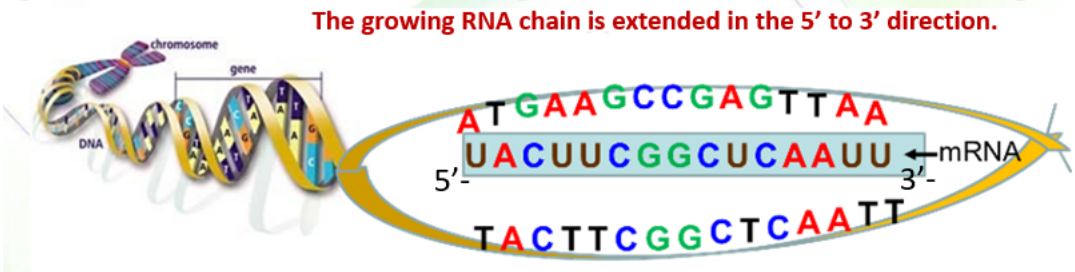
: that leads the synthesis 5` to 3`

** notice that the template is read from 3` to 5`

Complementary sequences

RNA is complementary to its DNA template.

* The RNA chain produced by transcription is also known as the transcript



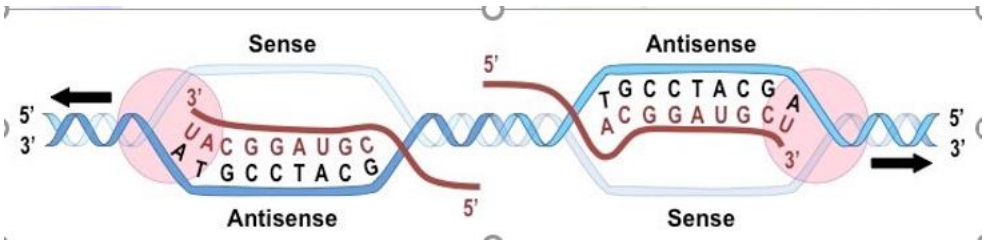
Notes on image:

The synthesis of mRNA: 5` to 3`

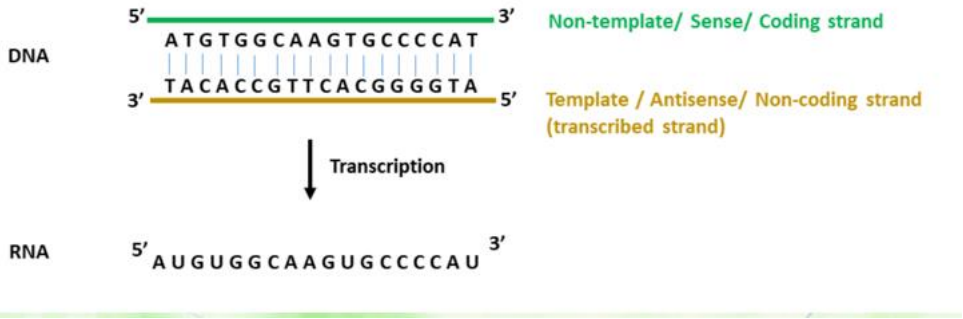
DNA template is read from 3` to 5`

*there is U instead of T in RNA

More clarification and some extra terms



2 genes are transcribed here from 5` to 3`



The strand that is transcribed(is read 3` to 5`) is the template=antisense (means it is complementary to RNA and noncoding strand

the coding strand is the sense cause it is identical to RNA except T= NON template

Enzyme and substrate

* The enzymes that perform transcription are called RNA polymerases.(with different types)

They catalyze the formation of the phosphodiester bonds between two nucleotides.

The substrates are ribonucleoside triphosphates (ATP, CTP, UTP, and GTP).

What are substrates for DNA polymerases?deoxy ribonucleoside triphosphate

Hydrolysis of high-energy bonds in NTPs provides the energy needed to drive the reaction forward(energy is needed like DNA pol, energy comes from substrate

pyrophosphate groups are removed to form phosphodiester bonds that needs energy

DNA replication vs. Transcription

The RNA strand does not remain hydrogen-bonded to the DNAtemplate strand. (synthesis DNA strand from DNA template , both of them remains boned with H bond)))RNA is synthesized but H bonds are released)

RNA polymerase reads the A in DNA and inserts U in the growing chain of RNA Rather thanT

RNA molecules are much shorter than DNA molecules.
(DNA is large and huge for genome, transcription,...)
RNAs that are produced from DNA regions are usually small regions of DNA That is read to synthesise RNA molecules)

Unlike DNA, RNA does not store genetic information in cells.(if there are Mistakes in RNA it is not a big problem , it could be degredaded)but in DNA it Has to be accurate.

DNA polymerase vs. RNA polymerase

They have differences in terms of substrates,enitiating Synthesis

*RNA polymerase catalyzes the linkage of ribonucleotides, no deoxyribonucleotides.

*Unlike DNA polymerases, RNA polymerases can start an RNA chain without a primer.(RNA is de nova can start from scratch , RNA pol dont need primers while DNA pol need)

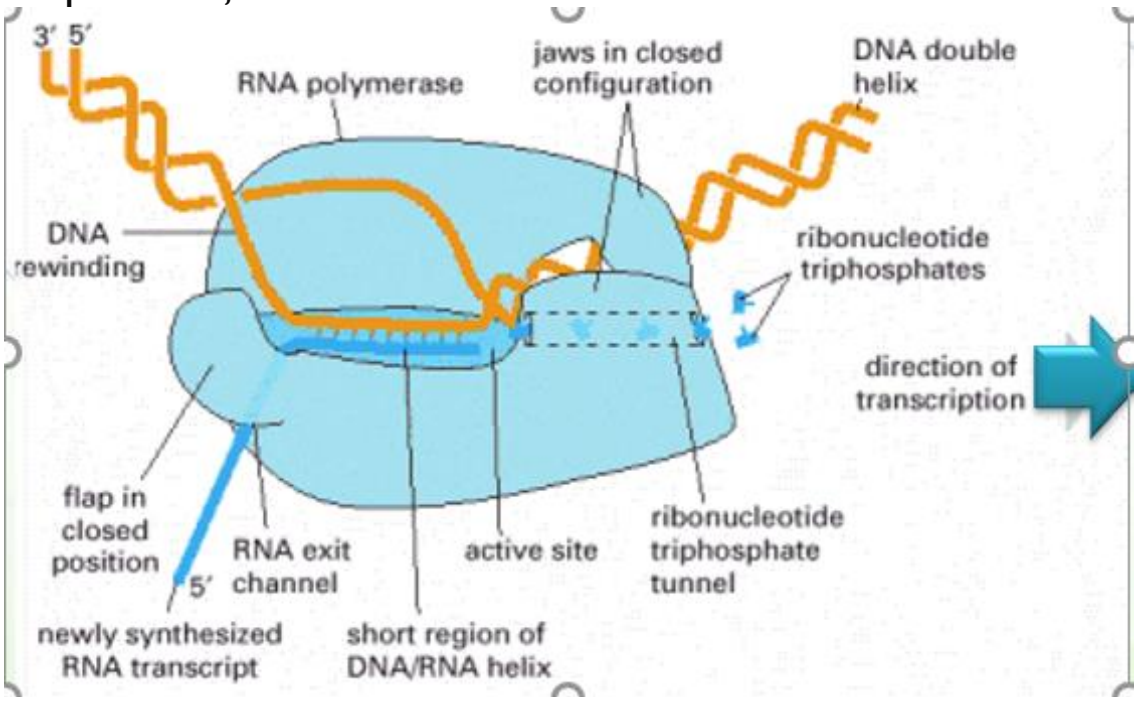
* RNA polymerases make about one mistake for every 10^4 nucleotides.
(RNA pol are accurate even though they do mistakes , they do have proof Reading mechanism, they are less accurate than DNA pol)
(DNA pol are very very accurate they have proof reading mechanism)

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 the DNA polymerases, they have a modest proofreading mechanism.

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.



Notice from the image:

Binding of RNA pol to DNA template using one of strands to synthesize RNA molecule

As RNA pol moves ➡ it starts forming phosphodiester bonds Between nucleotides(mRNA is complementary to DNA strand)

The newly synsethized nucleotides are bound to DNA template By H bonding that is necessary for stabilizing the whole complex (interactions between RNA pol ,mRNA and DNA)

Older nucleotides are released and the newly synthesized form H bonds

Notice the bubble that is form as a result of RNA pol moving forward Causing separation of 2 strands

لتوضيح الصورة: زي الطلاب اللي بنقلوا من بعض
الواجب أول واحد بنسخ أول صفحة بعدين بعطي التاني
بعدين التالت بعدين الأول برجع ينسخ تاني صفحة

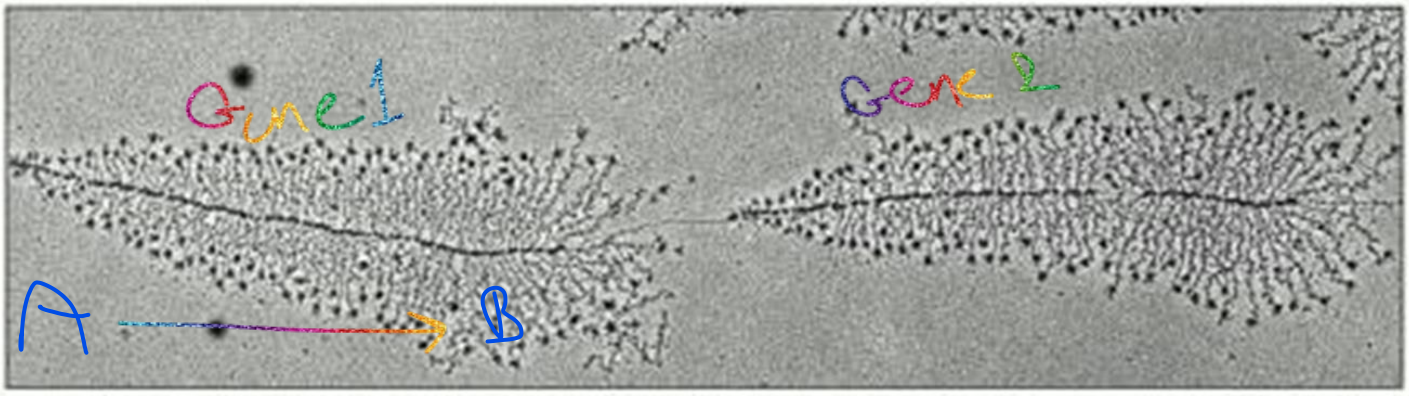
Polysomes

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

*poly = multiple ,, some: body

It is RNA synthesis from 2 genes so giving multiple RNA molecule

From the same gene at the same time saving time for organisms

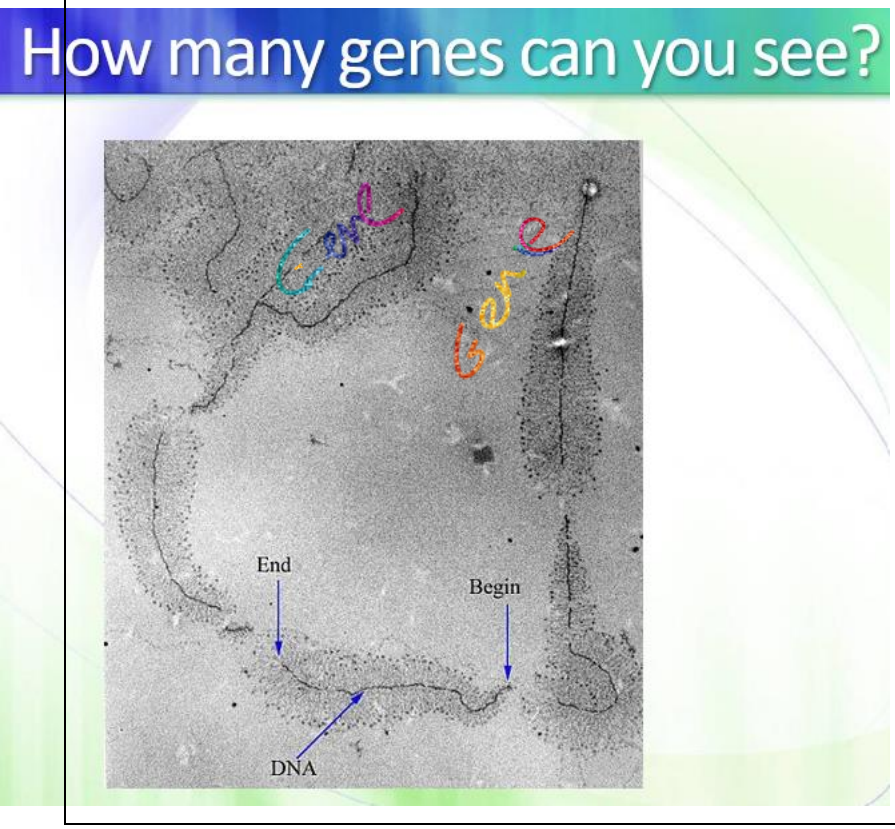


Where is the starting point of transcription? Where is the beginning of the genes?

The beginning is point A , the lenghth of RNA molecule is the shortest at A then it becomes Longer and longer reaching to B

→ RNA pol moves together sequentially one afer another

After RNA pol passes through DNA another will bind then another so getting multiple RNA pol binding to gene synsethizing RNA molecules



Here we have more than one gene

RNA molecules are extending outside to RNA pol

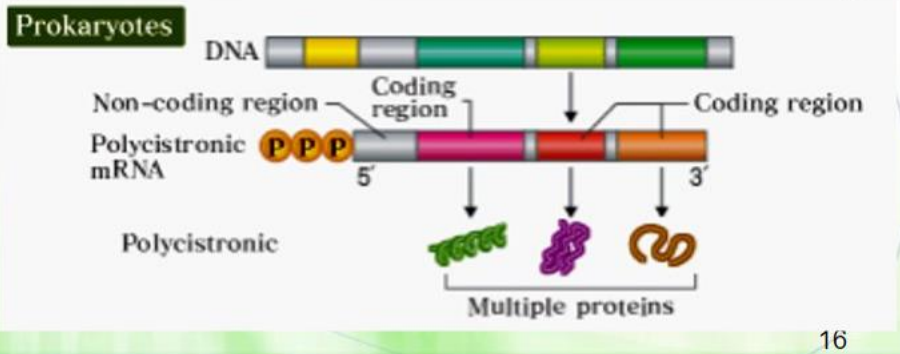
DNA molecules are in the centre where RNA pol pass through

Forming polysomes

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.



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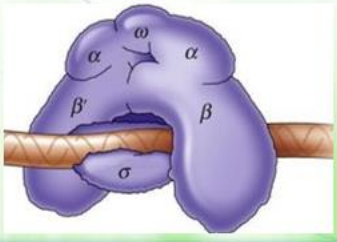
-In bacteria, some genes are monocystronic, others are polycistronic.
-Monocystronic: 1 gene --→ 1 mRNA --→ 1 polypeptide.
-Polycistronic: 1 gene --→ 1 mRNA --→ more than one different polypeptide from different regions of THIS SINGLE mRNA.
-Polycistronic then seems an efficient system,why? Because multiple proteins(from the same mRNA which came from the same cistron) participate in a particular function such as: 1-Metabolism of lactose
2- Synthesis of tryptophan.
-mRNA is transcribed from the genetic unit known as cistron.
-In conclusion, operons are polycistronic.

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 σ subunit is not required for the basic catalytic activity of the enzyme.



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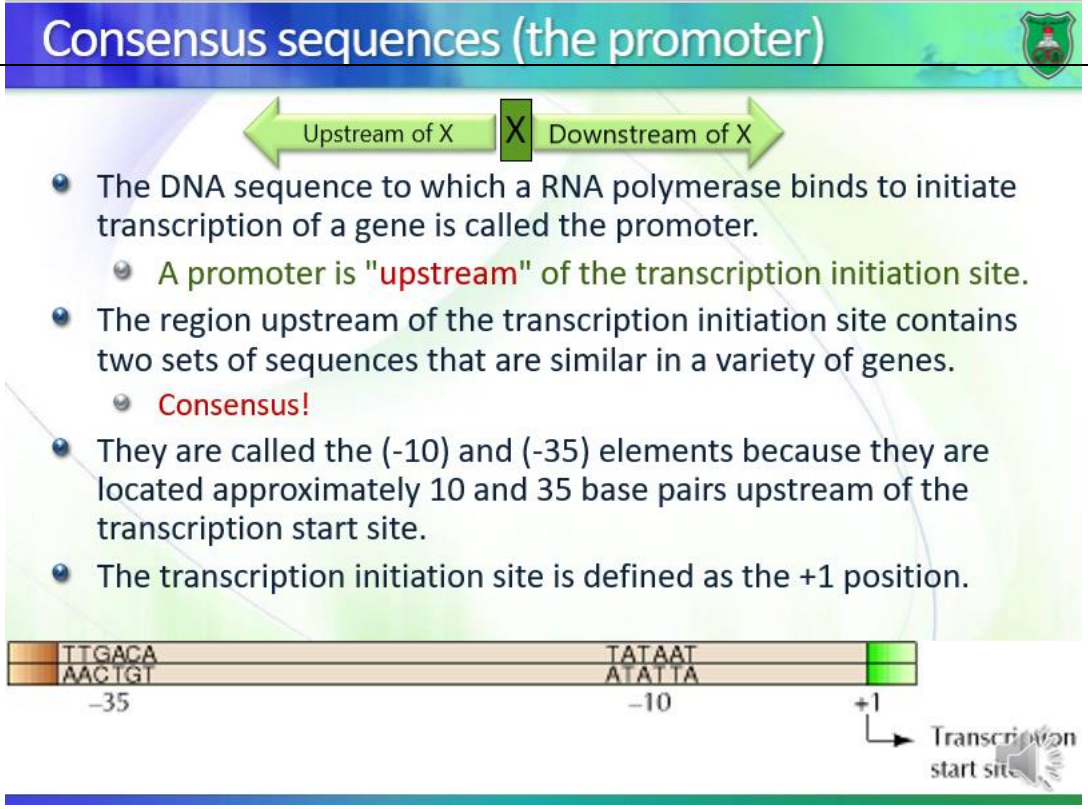
-Generally speaking, RNA polymerase is responsible for transcription.

- omiga (w), 2a, B, B', all these 4 subunits make up the core enzyme(functional enzyme).

Sigma subunit is an additinal subunit (not required for catalytic activities of B)

-NOTE: In E.Coli bacteria, there is 1 single RNA polymerase.



Look at the image again, notice that -35 region is also UPSTREAM of the -10 region

-Upstream: Before

-Downstream: After

-Transcription start site includes the first nucleotide that is read by the RNA polymerase which is for sure the first nucleotide of the RNA molecule (+1 site) .

-It uses ribonucleoside triphosphate to attach the second nucleotide(+2) forming phosphodiester bond, then, the 2nd nucleotide becomes ribonucleoside monophosphate. The same process takes place to +3 +4 +5 etc.

-Promoter is RNA-polymerase binding site.

- (-10) & (-35) are 2 important CONSENSUS sequences found WITHIN a promoter.

-NOTE: A consensus sequence exists in different bacterial species which means that it has functional significance (meaning it's important).

-What is the meaning of (-10) region & (-35) region?

(-10): includes nucleotide #10 upstream of transcription start site

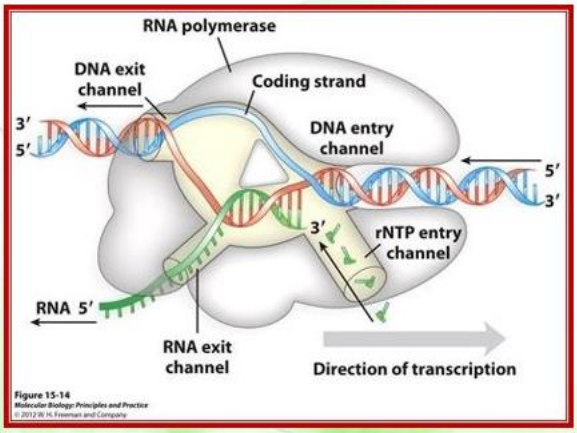
(-35): includes nucleotide #35 upstream of transcription start site.

-Keep in mind that these 2 regions (-35 and -10) are actually where an RNA polymerase binds to, which in turn is the region of interaction between DNA and RNA polymerase

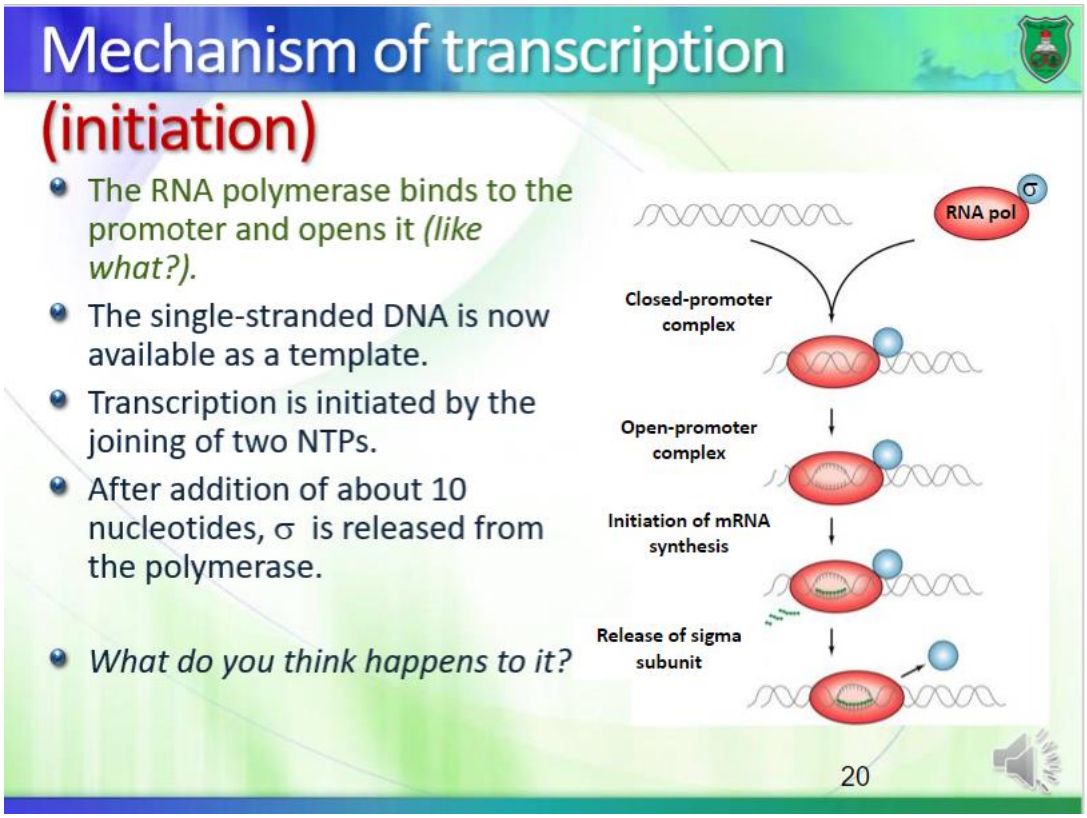
-NOTE: Changing any of the nucleotides in (-35) & (-10) regions would compromise the interaction of RNA polymerase with promoter which in turn affects the efficiency of transcription.

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.



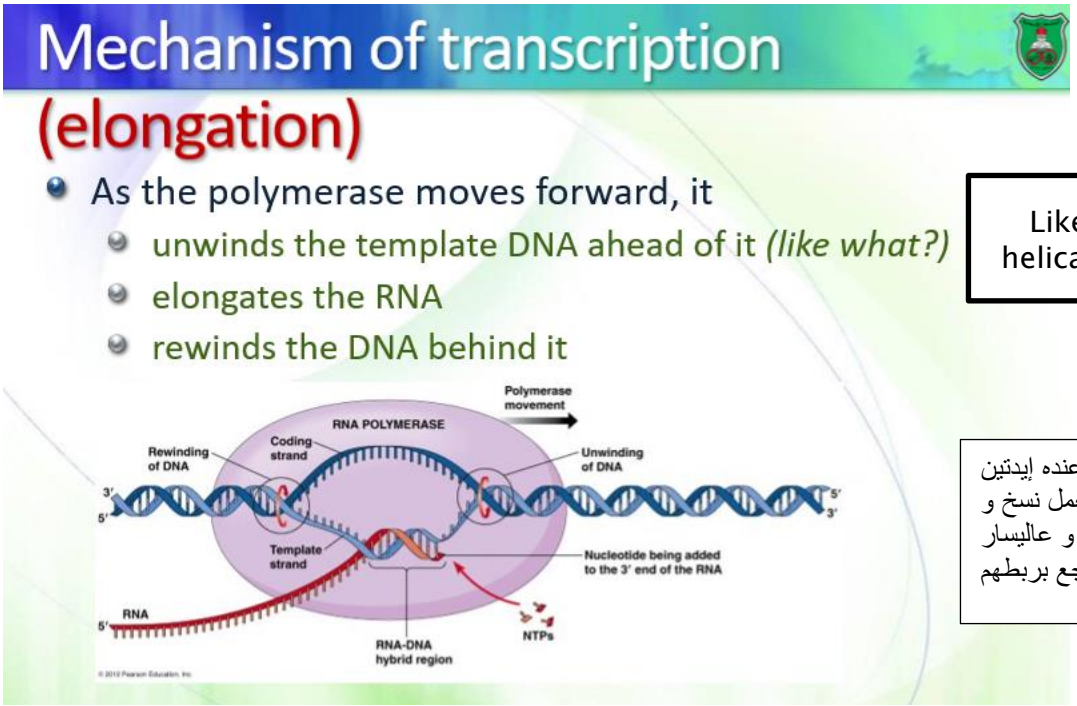
sigma subunit strengthens the interaction of RNA polymerase with the promoter.



-The RNA polymerase ITSELF pulls apart the 2 strands.

-Once the bubble forms, RNA polymerase initiates transcription.

-What do you think happens to the sigma subunit? It binds to another RNA polymerase so that more than 1 RNA polymerase are synthesizing RNA from the same gene at the same time!

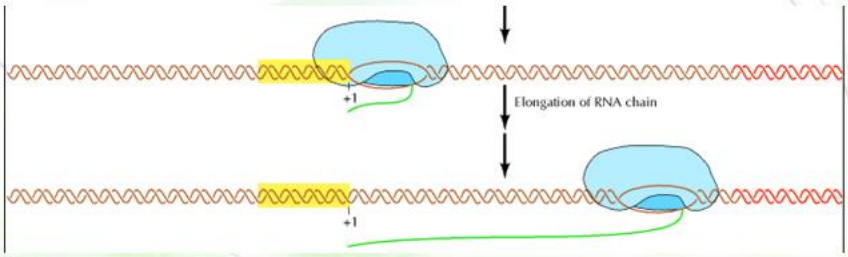


-RNA&DNA hybrid stabilizes the interaction between the 3 units (1.RNA Polymerase, 2.DNA, 3.RNA).

-NOTE: Concerning the image (in the rewinding part), we may have RNA polymerase before, but between them the DNA is double stranded so the DNA is not fully separated.

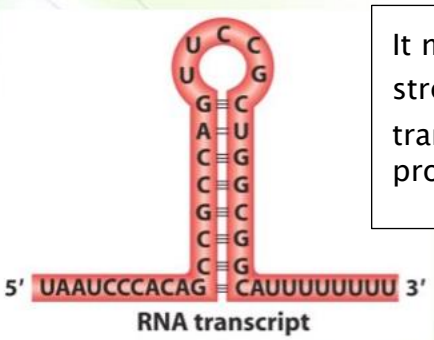
Mechanism of transcription (termination)

- 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 (*what do we call it?*) in E. coli 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.



It must be loop and stretch of U to stop transcription in prokaryotes

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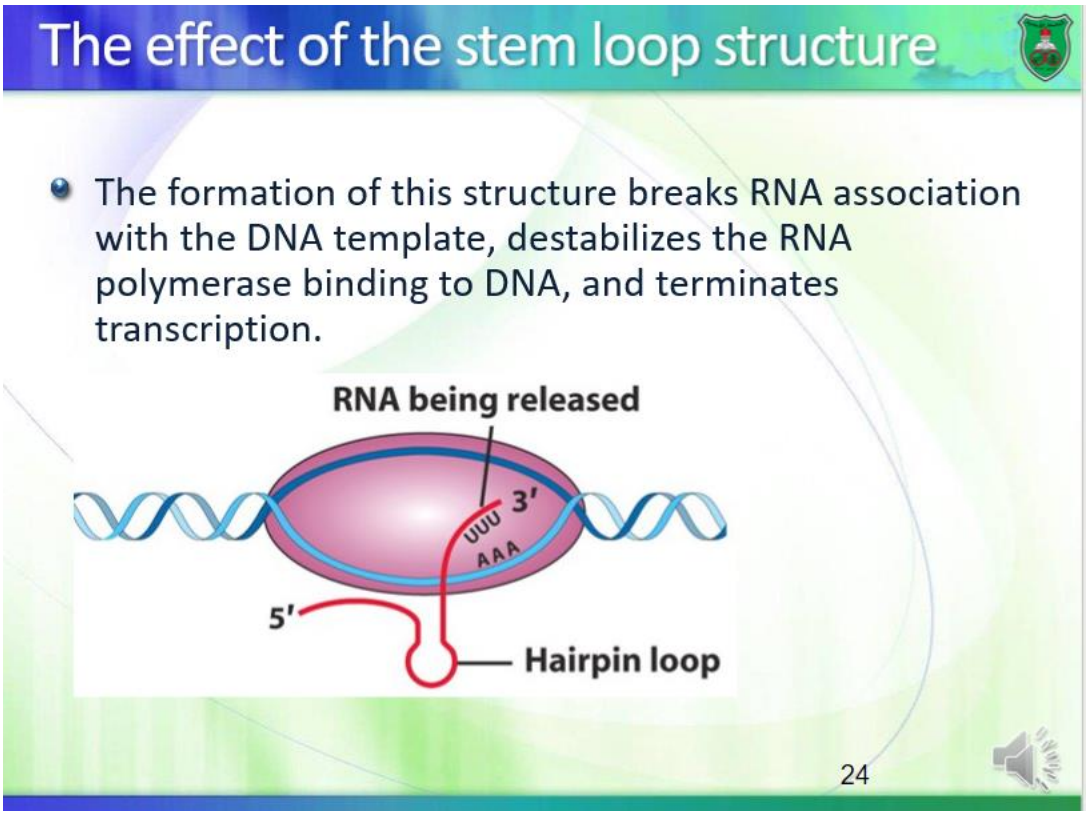
-NOTE: Termination sequence is CONSENSE, due to 2 reasons:

1-You can find this sequence at the end of different genes

2-You can find this sequence in different bacterial species.

-In the GC region, there is stronger interaction between the trio (mRNA, DNA, RNA polymerase) due to the 3 hydrogen bonds between G & C.

-In the stretch of Us region, there is weaker interaction between the trio due to the 2 hydrogen bonds between A & U.



-TIME TO IMAGINE: Imagine a hero running away from a monster, suddenly, a monster comes out of of the ground (Stem loop structure)! The ground becomes slippery which causes the hero to fall, the interaction between the hero and the ground is weak (weak interaction between U&A). The doctor showed us a clip from a film to illustrate what's written.

-The mRNA dissociates from DNA which leads to RNA polymerase dissociating as well.

Past paper:

38.Which of the following is considered, correct?

A) RNA error rate is less than DNA error rate.

B) NTP nucleotides are added during synthesis of mRNA using the energy gained by hydrolysing of all of the phosphate groups.

C) A mutation in a certain gene on the homologous chromosome might cause a decrease in the number of proteins synthesized from the cell

Answer: C

39. In bacteria, which of the following RNA polymerase enzyme subunits is responsible for promoter recognition?

A) The beta (13) subunit.

B) The gamma (y) subunit.

C) The delta (6) subunit.

D) The sigma (a) subunit.

E) The epsilon (E) subunit.

Answer: D

40. What happens to the sigma subunit in RNA polymerase (in prokaryotes) after it attaches the RNA polymerase to the DNA?

A) It stays on the DNA until transcription ends.

B) It leaves the RNA polymerase after about 10 base pairs then it is recycled.

C) It is degraded after it attaches the RNA polymerase.

D) None of the above

Answer: B

41. Which of the following is correct?

A) Two phosphate groups are removed from nucleoside triphosphate that are added to the growing RNA sequence because they are too long.

B) Transcription requires a primer.

C) DNA replication is more accurate than RNA transcription.

D) All of the above are correct.

Answer: C