



Genetics

***Subject* : Genetics**

***Lec no* : 8(part1)**

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وَقُلْ رَبِّ زِدْنِي عِلْمًا

Gene expression

By

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Gene expression → The process where a gene is used to synthesize some sort products.

proteins : \rightarrow ←
expression = (transcription + translation)

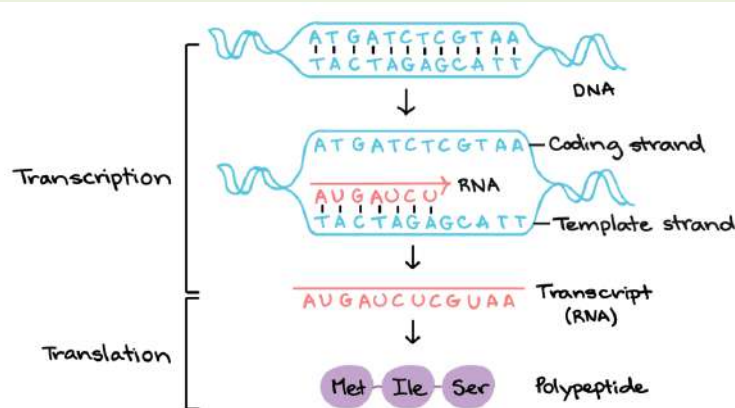
- **Definition:** Gene expression can be defined as the gene (DNA) undergoes transcription into mRNA that can translate the encoded genetic information into protein.

mRNA ← عندنا يحصل (translation) ويعطي proteins اننا نكون عرضة genetic information على mRNA طلعت ابي بروتين .

Transcription (RNA synthesis)

Transcription (gene) strand of DNA
ويطلع mRNA عن طريق DNA-dependent RNA polymerase (RNAP)

- **Definition:** Transcription is the synthesis of RNA using DNA as a template by an enzyme DNA –dependent RNA polymerase or RNA polymerase (**RNAP**)



Features of transcription:

- One strand of the two DNA strands is transcribed only, this strand is called template strand (anti-sense), because it provides template for ordering the sequence of nucleotides in an RNA transcript, *sequence of nucleotides in DNA* *هي والي رح تعرفني sequence في RNA strand*
- The other strand (non-transcribed) is called coding strand (sense strand), because its sequence is the same as the newly synthesized RNA transcript (except for thymine is substituted by uracil)

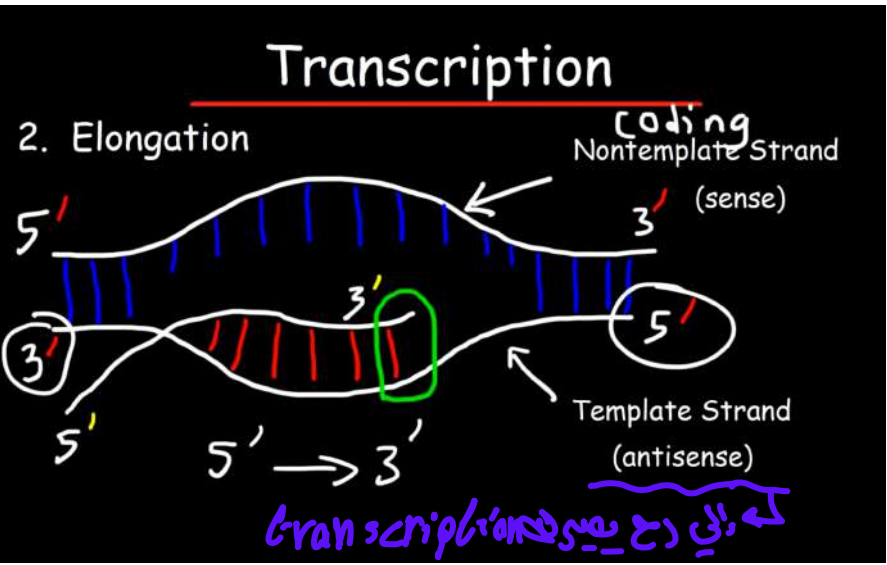
DNA transcription هذه العملية تتم على only one strand of DNA وتكون according to gene (أنا بدى انتج بروتين معين وهذا البروتين مسؤول عن انتاجه gene معين

في strand التي دح يصير transcription هي التي بتعمل هذا الجين .

لأنه يكون أمه template strand (anti sense) لأنه يعملي template. For ordering the sequence of nucleotide in an RNA transcript.

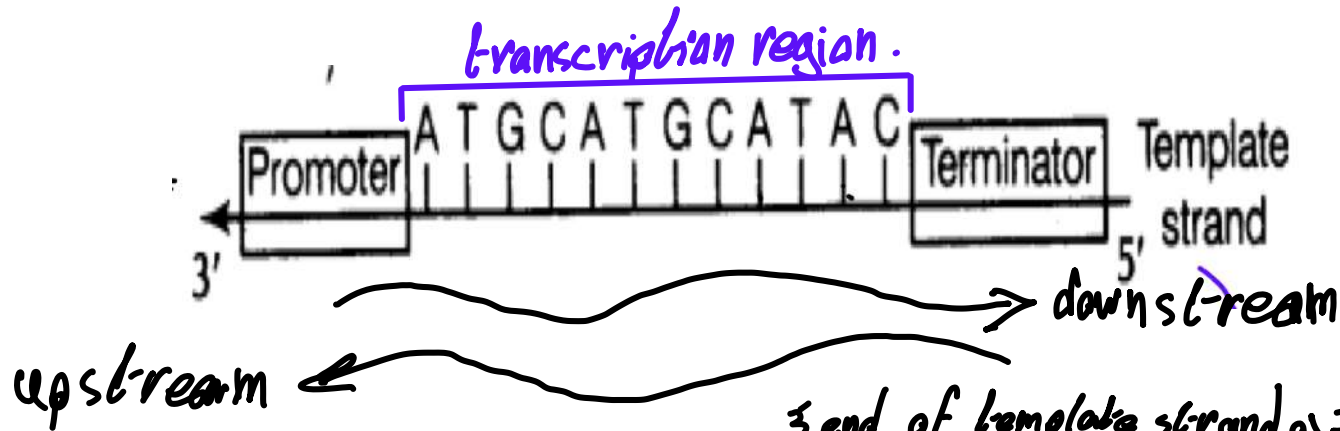
نucleotides التي عليه هي التي عرفتي ال nucleotides التي على RNA

← DNA strand الآخر التي ما صار transcription في coding strand (sense strand)



- The DNA template strand is read in 3' to 5' by RNA polymerase enzyme and the new RNA is synthesized in the direction of 5' to 3'.
- Upstream means in the 3' direction of the template strand.
- Downstream means in the 5' direction of the template strand.
- A transcription region is the nucleotide sequence transcribed by the enzyme RNAP. It is the region between the promoter and the terminator.

RNA polymerase يبدأ عملية البناء من 3' → 5' ، إذن عملية transcription تبدأ من 3' of template strand



upstream ← تكون باتجاه 3' end of template strand

downstream ← تكون باتجاه 5' end of template strand

transcription region ← المنطقة من template strand ، أي دح يصير transcription وتكون محدودة بين 2 regions

promoter Terminator

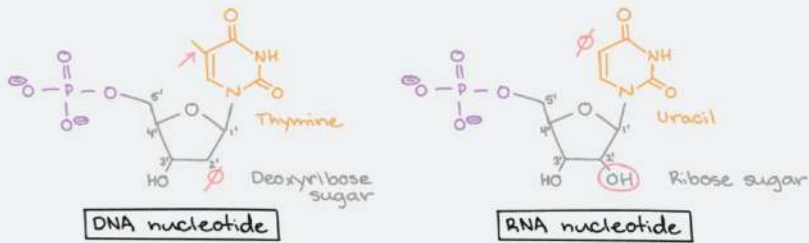
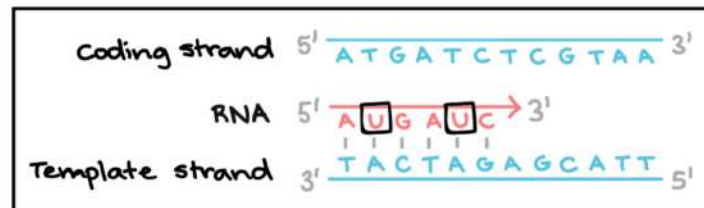


Image based on similar image from CyberBridge³.

RNA nucleotides are similar to DNA nucleotides, but not identical. They have a ribose sugar rather than deoxyribose, so they have a hydroxyl group on the 2' carbon of the sugar ring. Also, in RNA, there is no T (thymine). Instead, RNA nucleotides carry the base uracil (U), which is structurally similar to thymine and forms complementary base pairs with adenine (A).

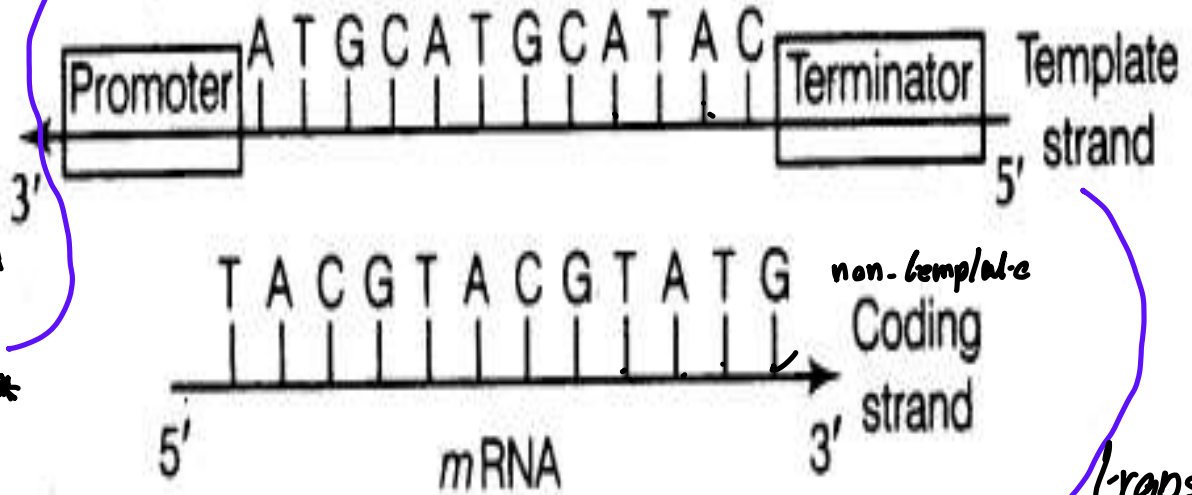


The RNA transcript is nearly identical to the **non-template**, or **coding**, strand of DNA. However, RNA strands have the base uracil (U) in place of thymine (T), as well as a slightly different sugar in the nucleotide. So, as we can see in the diagram above, each T of the coding strand is replaced with a U in the RNA transcript.

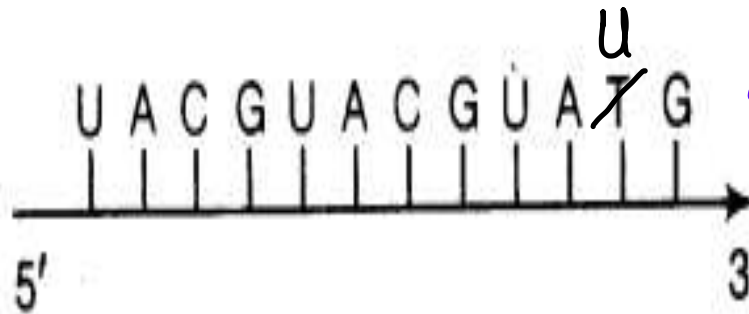
(i) Transcription unit

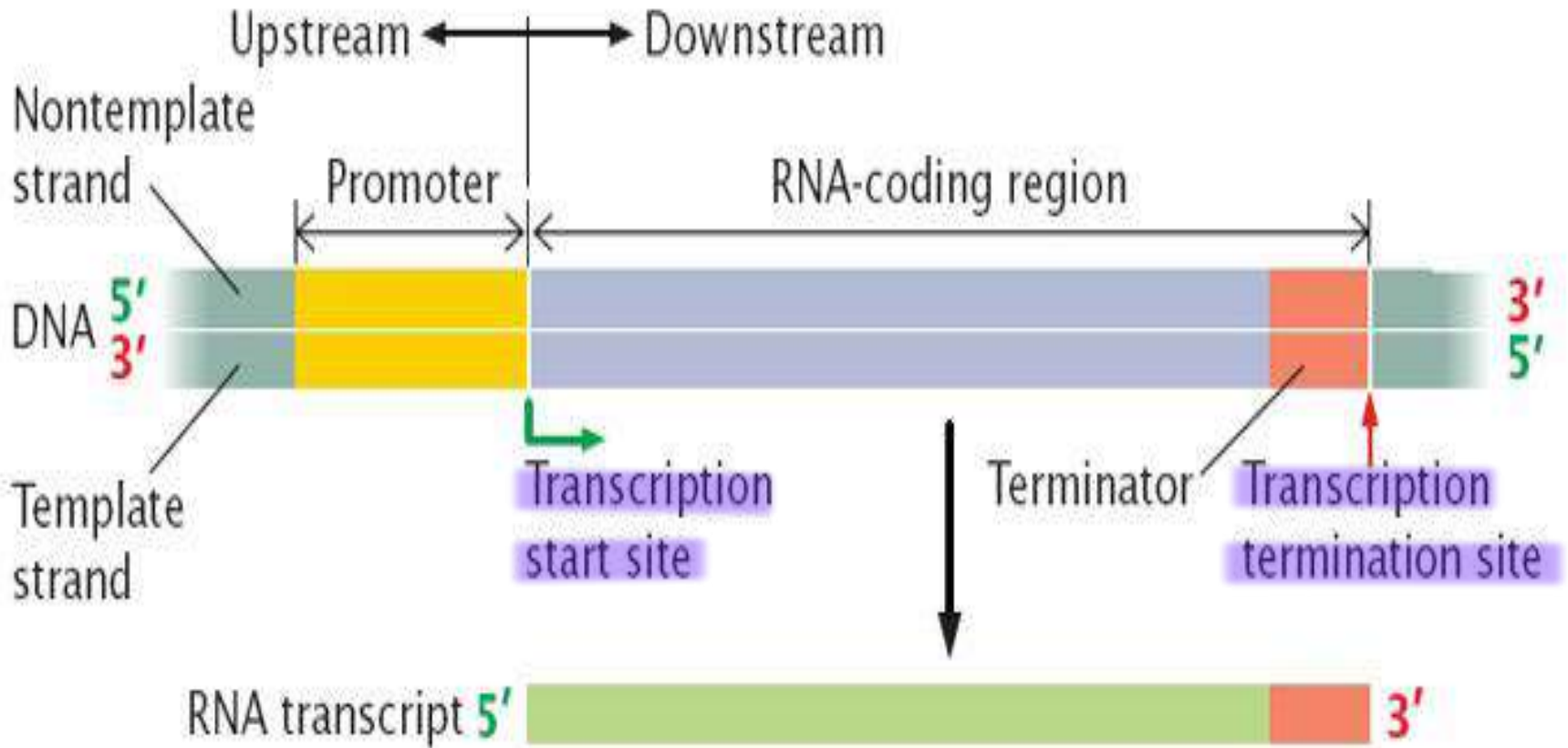
* لماذا تسمى تسمى non-template coding strands
 لأنها يجب أن يكون RNA transcript
 لا اختلاف فقط يكون فقط في U, T

الـ template strand هي anti sense strand
 sense strand → تكون RNA transcript.



(ii) RNA transcribed





transcription ← بينا لا promoter ←
 ← بينا Terminator بينا

- **Promoter**: A promoter is the DNA sequence that initially binds the RNA polymerase (together with any initiation factors required). i.e. Nucleotide sequence in DNA to which RNA polymerase binds to begin transcription.
- **Terminator**: Sequences trigger the elongating polymerase to **dissociate** from the DNA and **release** the RNA chain it has made.
- **The start point** is the nucleotide at the 3` end of the transcription region "that codes for the initial base of the mRNA". It is designated +1. Adjacent nucleotides are given positive numbers that increase as we go downstream the transcription unit.

promoter هي DNA sequence التي يرتبط فيها RNA polymerase حتى يبدأ transcription

DNA sequence to which RNA polymerase binds to begin the transcription

Terminator هي DNA sequence التي عندها دع توقف عملية transcription of transcription region حيث أنه عند الوصول إليها ينقطع RNA polymerase عن template strand و RNA strand release التي تم تكوينها.

start point هي أول nucleotide من transcription region الذي يحصل له transcription

وهو ذلك دع ينتج عنها أول nucleotide يقع ومنها في RNA transcript

بشميتها (+1) nucleotides التي بعدها باتجاه downstream بمعنى positive numbers nucleotides التي قبلها باتجاه upstream بمعنى negative numbers

negative numbers
in promoter

- The nucleotide in the promoter adjacent to the +1 nucleotide is designated -1 and adjacent nucleotides are given negative numbers that increase as we go upstream the promoter.

*Terminator and promoter ← Transkription region
Terminator + promoter + Transkription region ← Transkription unit*

- The transcription unit Sequence of nucleotides in DNA that codes for a single RNA molecule, along with the sequences necessary for its transcription; normally contains a promoter, an RNA-coding sequence, and a terminator. (i.e. includes the promoter, the transcription region, and the terminator)

هذا السلايد نفس تعريف transcription start site الذي تعلمنا من سابقه ولكن بصيغه مختلفه من اجل التدريب على بعض الامثله التي تأتي بصيغ مختلفه .

- ① • The DNA nucleotide encoding the beginning of the RNA chain is called the transcription start site and is designated the “+1” position.
- ② • Sequences in the direction in which transcription proceeds are referred to as downstream from the start site. Likewise, sequences preceding the start site are referred to as upstream sequences.
- ③ • When referring to a specific position in the upstream sequence, this is given a negative value. Downstream sequences are allotted positive values.

نقطة ② ← اتجاه transcription هو نفس اتجاه downstream → 3' → 5'

← upstream يسبق start point

upstream → negative values

downstream → positive values

Transcription in prokaryotes:

- All types of RNA is synthesized by a specific enzyme called RNA polymerase **except** for the short RNA primers needed for DNA replication are synthesized by a primase enzyme.

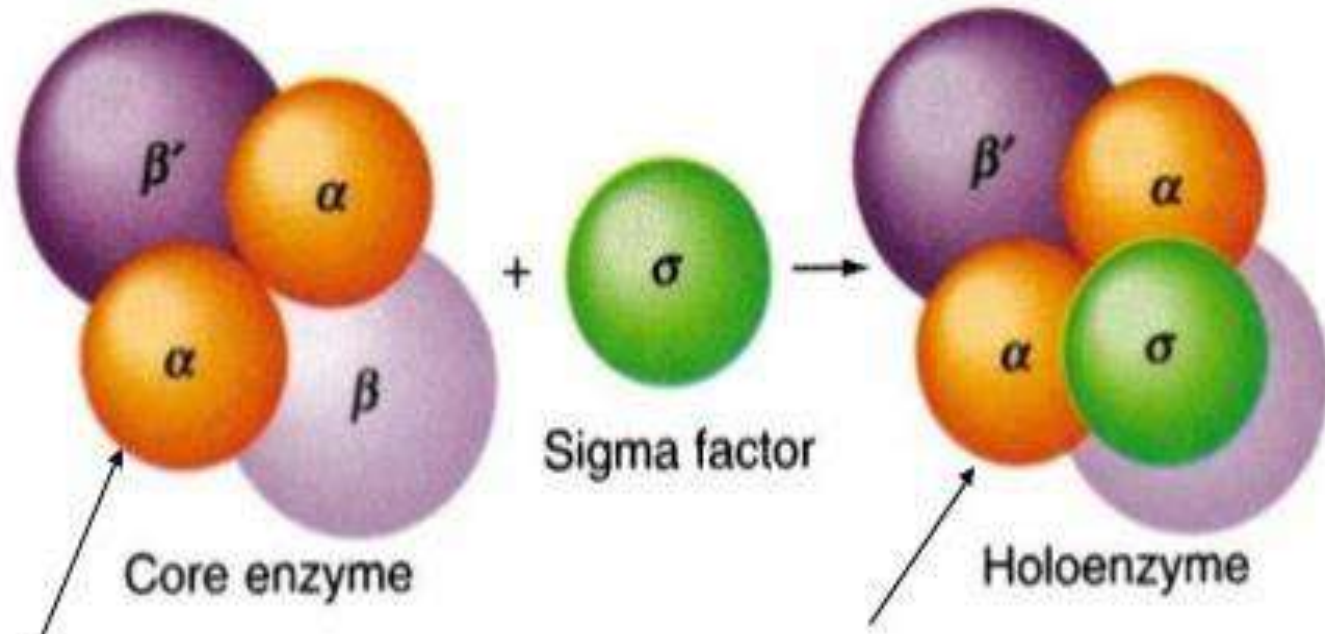
* Structure of prokaryotic RNA polymerase:

- It is a multi-subunit enzyme formed of core enzyme and sigma factor

* RNA انواع rRNA, tRNA, mRNA في prokaryotes تنتج عن طريق نفس الإنزيم RNA polymerase

لما عدا primer التي يمتنع primase enzyme

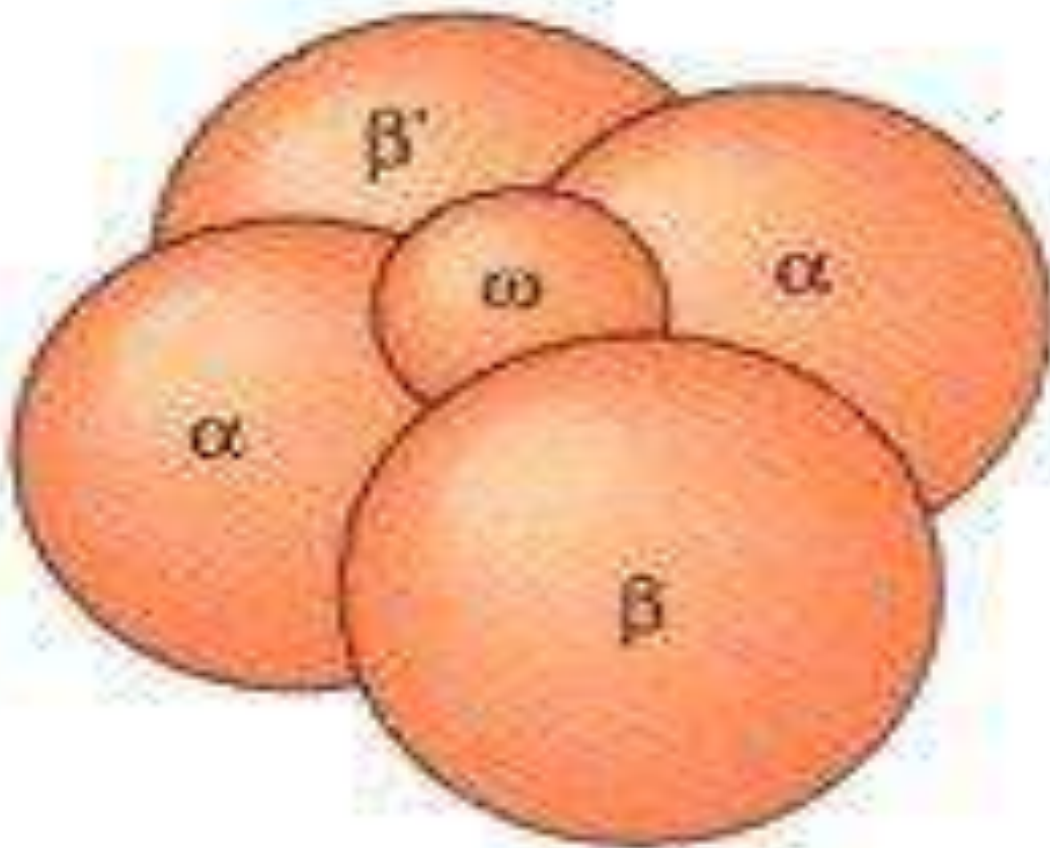
* RNA polymerase يتكون من polypeptide chains مرتبطة مع بعض ويكونوا core enzyme و sigma factor مرتب في هذا multi subunit enzyme



Required for polymerization activity

Required for correct initiation of transcription: ***binding to promoter***

2 α , 2 β , 1 ω



Core enzyme



Subunit

- **Core enzyme:** two identical α subunits (regulatory subunits) and two β not identical (β & β') and one ω chain. One of the β subunits (β) binds to the DNA and the other (β') is responsible for the formation of phosphodiester bond. *DNA template في enzyme نسخ في β subunit*
RNA transcript في RNA nucleotides بين phosphodiester bond تتكون β' subunit

● RNA polymerase enzyme lacks specificity, that is, it cannot recognize the promoter region on the DNA template.

RNA polymerase lacks specificity لا يستطيع التعرف على promoter region، التي دح ترتبط فيها نسخ يبدأ replication

- **The σ subunit ("sigma factor"):** It enables RNA polymerase to recognize promoter regions on the DNA. The σ subunit plus the core enzyme make up the holoenzyme. [Note: Different σ factors recognize different groups of genes.]

sigma factor ترتبط RNA polymerase بـ promoter region (نسخي امم RNA) فتعرف على region
core enzyme + σ subunit = holoenzyme

☀️ N.B.: The antibiotic rifampicin binds to the β subunits of RNA polymerase and inhibits RNA synthesis in prokaryotes as it interferes with the formation of the first phosphodiester bond. Rifampicin is useful in the treatment of tuberculosis.

استخدم في علاج tuberculosis (TB) ! يمنع في β subunits
antibiotic rifampicin
وبالتالي يوقف function of β subunits وبالتالي نكت أوقفنا
تصنيع RNA في prokaryotes وبالتالي نمنع تصنيع proteins في
prokaryotes وهكذا نكون قد دمرناها.

Steps of RNA synthesis in prokaryotes:

- It is divided into three phases: initiation, elongation and termination.

* تبدأ عملية transcription من ارتباط RNA polymerase (بمساعدة σ) في promoter region (بالتحديد في TATA box) وبعد ذلك يبدأ RNA polymerase عملية transcription من عند (+1) start point.

RNA polymerase σ ربح يرتبط
TATA box σ

Initiation:

RNA nucleotide يترجم بناؤها في RNA

- It involves the binding of RNA polymerase to a specific region on the DNA known as the promoter region formed of specific base sequence. It needs a specific protein factor called sigma factor (σ) that recognizes and **binds to the promoter region at the TATA box** then RNA polymerase starts transcription at the start point (+1) it is the first base transcribed as RNA.

- The characteristic nucleotide sequences of the prokaryotic promoter region (as indicated in the coding strand in the 5` to 3` direction) include:

☀ **TATA box**: It is formed of six nucleotides (TATAAT) and is located 10 bases upstream (i.e. usually occurs around base-10)to the start point (+1 point).It determines where transcription starts.

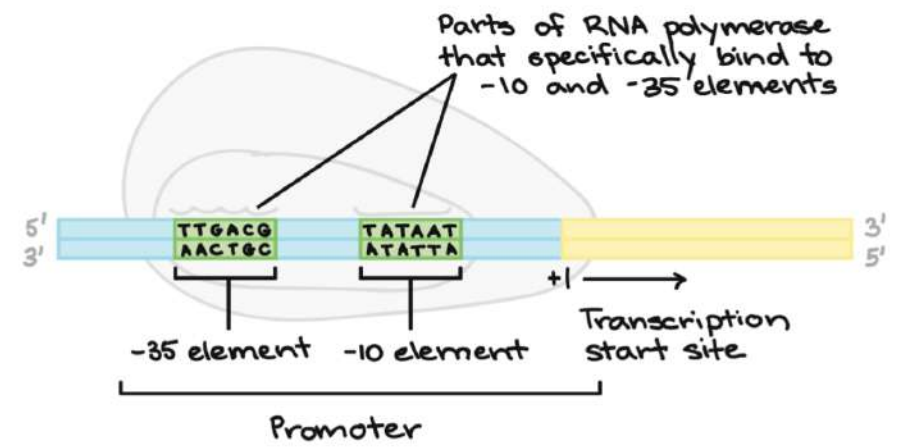
☀ **The (TTGACA) box**: this sequence is 35 bases upstream to the start point (located at -35 base i.e. centered about 35 bases to the left of the transcription start site) .It determines the frequency of transcription

تكون من 6 nucleotides TATAAT
 وتلك TATA دائماً موجود ، (around base -10)
 أول ما توقف عنده رخ يتحدد مكان بداية transcription
 (start point)

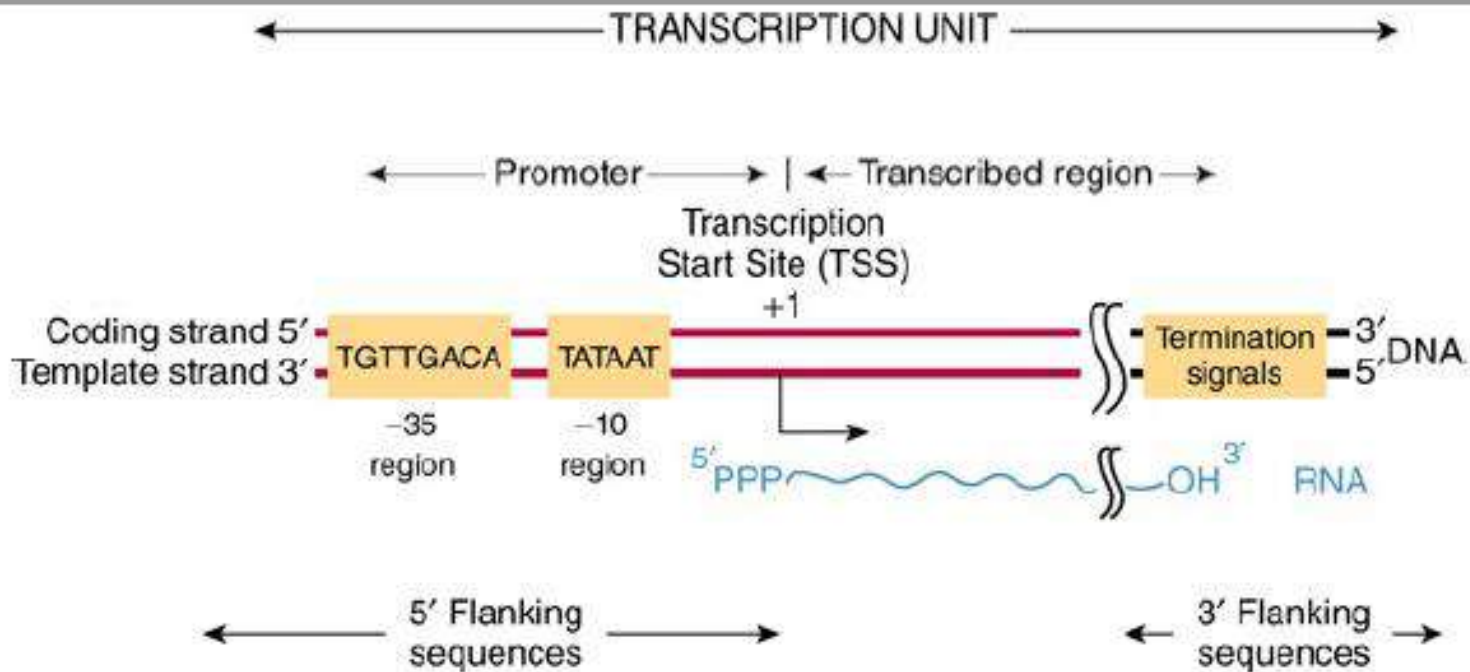
(located at -35 base) TTGACA box
 يحدد قوة transcription

nucleotide sequence of the prokaryotic promoter region

القراءة لانه يكون according to there presence in coding strand from 5' → 3'



The -10 and the -35 elements get their names because they come 35 and 10 nucleotides before the initiation site (+1 in the DNA). The minus signs just mean that they are before, not after, the initiation site.

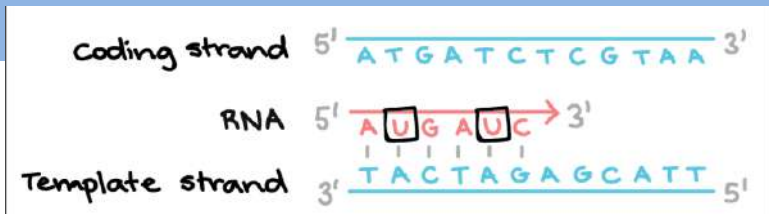


Source: Murray RK, Bender DA, Botham KM, Kennelly PJ, Rodwell VW, Weil PA: *Harper's Illustrated Biochemistry, 29th Edition*: www.accessmedicine.com

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Elongation:

- In prokaryotes only **one** type of RNA polymerase synthesizes the three types of prokaryotic RNA.
- The binding of RNA polymerase to DNA template produce local unwinding of the DNA double helix to expose the bases.
- The enzyme begins to synthesize RNA in the direction of 5' to 3' with the base sequence complementary to that of the DNA template strand. Sigma factor is released after initiation of transcription.
- The core enzyme moves along the DNA template uses ribonucleoside triphosphate (ATP, GTP, CTP & UTP) and releases pyrophosphate



DNA هو double strands فلازم نفضلهم عن بعض حتى نبدأ في replication . فعند ارتباط RNA polymerase يقوم بعمل local unwinding لـ double helix وبينما template strand ← to expose the nucleobases ← transcription coding strand

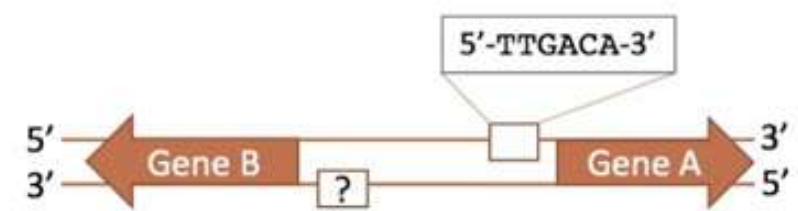
RNA polymerase يبدأ العمل من 3' → 5' core enzyme - بإضافة ribonucleotide triphosphate وعند الارتباط ترتبط على nucleobase nucleotides لإخراج pyrophosphate بعد مناعه على ملاقة ترتبط nucleotides

RNA polymerase يستطيع أن يبني de novo DNA polymerase ← ما يعرف يبني de novo (محتاج primer / محتاج يسوف OH على 3' لـ nucleotide إلى ببسبقة) RNA polymerase عنده helicase activity يعني بيقد يفتح 2 strands من بعضها عن طريق تكسير hydrogen bonds بين nucleobases لـ على عكس DNA polymerase إلى ما بيقد يفتح 2 strands حيث هنالك DNA helicase يفتح 2 strands RNA polymerase عنده proofreading activity (كفاءة أقل من DNA polymerase) ← بيقد detect incorrect bases لـ ويصحح (يعمل replacement لـ) ويطرد correct bases

RNA polymerase عنده termination recognition activity يعني بيقد يتعرف على terminatore region وينهي replication

- Unlike DNA polymerase, RNA polymerase does not require a primer and has intrinsic helicase activity, therefore no separate enzyme is needed to unwind the DNA (in contrast to DNA polymerase).
- RNAP not only initiates RNA transcription, it also guides the nucleotides into position, facilitates attachment and elongation, has intrinsic proofreading (It doesn't not possess a proof reading feature as efficient as the DNA polymerase but it possesses the capability of correct some misadded nucleotide as well) and replacement capabilities, and termination recognition capability.

C18. Two genes on a bacterial chromosome are transcribed in opposite directions as shown. Transcription of both genes is initiated by RNA polymerase coupled with sigma protein 70. The sequence of a -35 promoter element for gene A exactly matches the consensus sense strand sequence: 5'-TTGACA-3'. Which of these sequences would you be most likely to find on the indicated strand (?) at the -35 position of gene B?



- A. 5'-TGTCAA-3'
- B. 5'-TTGACA-3'
- C. 5'-ACAGTT-3'
- D. 5'-AACTGT-3'

What is the consensus sequence of the bacterial promoter?

- TATAAT ----- 17±1 ----- TTGACA
- TTGACA ----- 17±1 ----- TATAAT
- TAATAT ----- 17±1 ----- ACAGTT
- ACAGTT ----- 17±1 ----- TAATAT