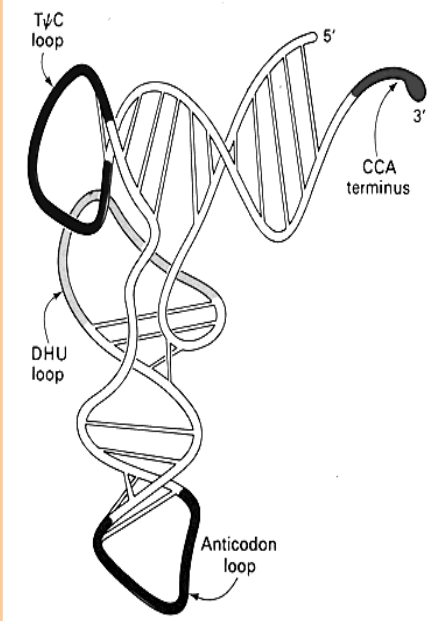
بسم الله الرحمن الرحيم



ليه التعقيد .. ركز معنا ☺

- الطريقة أصبحت معروفة للجميع ☺
- هذه المذكرة اهداء الى جدي الغالي – رحمه الله فلا تنسوه من دعائكم ،،،

RNA Structure & synthesis

Team leader : مجهول

والشكر لجميع من ساهم في اخراجها بالصورة التي هي عليه وأخص بالشكر :

أبو يسرا

عبدالعزيز التركي

Ocean

وجنودنا المجهولين ☺

RNA STRUCTURE & SYNTHESIS

OVERVIEW :

- ✓ Deoxyribonucleotides (DNA) are the *genetic* master plan of an organism
- ✓ DNA serves as template … thru RNA(working copies)
  (this process is called **transcription**)

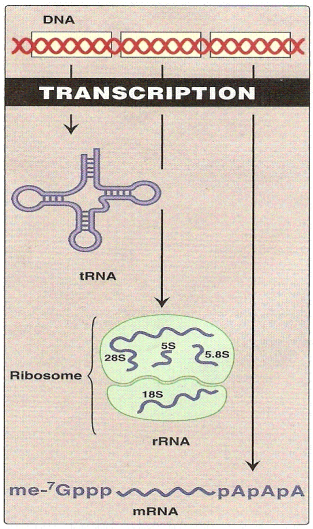


Figure 30.1
Expression of genetic information by transcription. [Note: RNAs shown are eukaryotic.] me-^{7}Gppp = 7-methylguanosine triphosphate "cap," described on p. 414. AAA = poly-A tail, described on p. 414.

✓ ***AND then bigining of thr synthesis of :***

a) messenger RNAs are translated into sequence of amino acids ,
b) ribosomal RNA ,
c) transfer RNA .

☑ **SO the RNA are three types**

- the transcription is highly selective (MCQ)
✓ This selectivity is the reason of biochemical differentiation

◙ *highly selective*
:

- **Explanation** : many transcripts , are made of some region of DNA . In other region , Few or no transcripts are made .

- **Reasons** :

  1. signals sequence in DNA instruct RNA polymerase where & How to Start & stop transcription .

  2. regulatory proteins .

- **ultimate result : Biochemical differentiation .**

the RNA transcripts undergo various modifications (terminal additions, base modification , trimming, internal segmental removal)

then by splicing convert the inactive primary transcripts into a functional molecule . (MCQ)

Q- splicing can make the transcript into functional form (T)

STRUCTURE OF RNA :

- 3 types (participate in protein synthesis):
  1. ribosomal RNA(rRNA)
  2. transfer RNA(tRNA)
  3. messenger RNA(mRNA)

- differ from each other in size, function, and structural modification .

- Like DNA they are **Unbranched polymeric** molecules composed of **mononucleotie** joined together by **phosphodiester bonds** . (MCQ)(IMP)

DNA	RNA(MCQ)
bigger	smaller
deoxyribose	ribose
thymine	uracil
double	Single strand (capable to folding into complex structure)
More stable	Less stable

- NOTE :
  - in eukaryotes small RNA molecules found in the nucleus (snRNAs)

A. Ribosomal RNA :

- rRNA make up **80%** of total RNA in the cell (most)(MCQ)

- Found in **ribosomes** (sites for protein synthesis) in association with several proteins (not histones)

- In prokaryotes there are three rRNA species : (23S , 16S , 5S)
- In eukaryotes there are four rRNA species : (28S , 18S , 5.8S , 5S) (there is question on them) (MCQ)

Q- 5S are found only in eukaryotes (F)

*Note: S=Svedberg unit which is related to MW & shape of the compound . (MCQ)

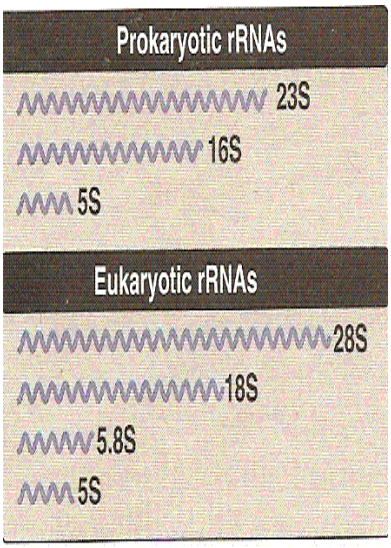


Figure 30.2
Prokaryotic and eukaryotic rRNAs.

B. Transfer RNA :

- tRNA make up **15%** of total RNA in the cell .

- is the **smallest RNA molecules** (4S) (always come and he said smallest in amount but the true is smallest in size) , & have (74-95) nucleotides residues .

- at least one specific tRNA molecule for one amino acid ➔ so, at least there are 20 species of tRNA .

- **tRNA molecules contain :**
  a)unusual bases (e.g. pseudouracil TΨ C loop {not uracil }) b)extensive intrachain base pairing (not interchain) (MCQ)

- each tRNA serve as **adaptor molecule** (MCQ) that carries its specific amino acid that attached by covalent bond to 3′-end (CCA) to the site of protein synthesis (NOT from nucleus to cytoplasm) . there it recognizes the genetic code word on an mRNA , which specifies the addition of its amino acid to the growing peptide chain

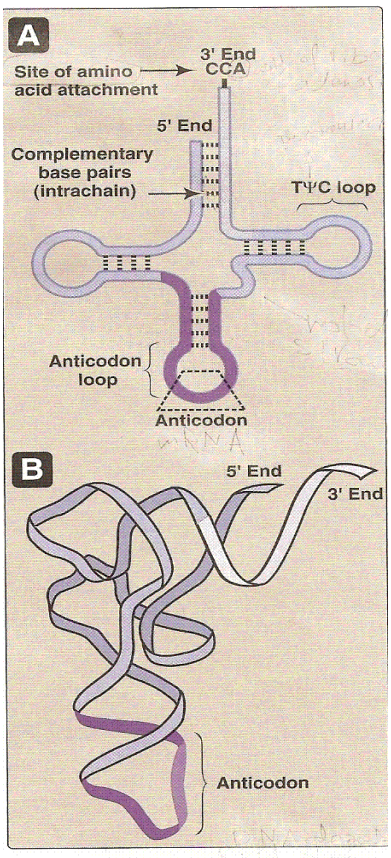


Figure 30.3
A. Characteristic tRNA structure.
B. Folded tRNA structure found in cells.

C . Messenger RNA :

- mRNA comprises only about **5%** of RNA in the cell .(smallest in amount not size)
- Most heterogenous & base sequence type (500 to 6000 nucleotide).
- carries information from nuclear DNA to the cytosol where it used as template for protein synthesis . (not like tRNA) .

- Eukaryotic mRNA (NOT prokaryotes) contains :(always come in exams)

a. **long sequence of Adenine (poly-A tail) on the 3 –end of RNA (only one P)**

b. **one molecule of 7-methyguanosine "cap" on the 5-end attatched " backword " (5' → 5') (here we have triphosphate linkage)**

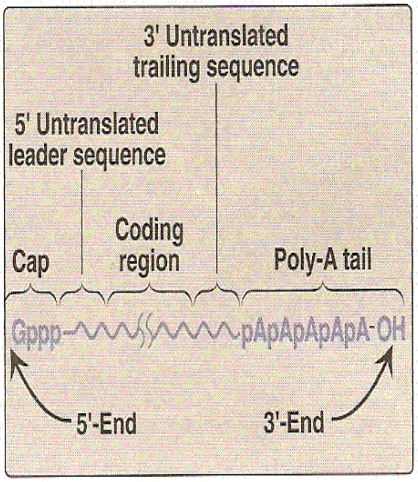


Figure 30.4
Structure of eukaryotic messenger RNA.

TRANSCRIPTION OF PROKARYOTIC GENES :

- eukaryotes & prokaryotes differ in;

1) RNA polymerase .

2) signals that control trancription

3) varieties of modification that RNA transcripts .

A - Properties of prokaryotic RNA polymerase :

◙ In bacteria, one species of RNA polymerase synthesize all of the RNA **except** for the short RNA primers which synthesize by specialized enzyme ,**primease**. (MCQ) (very important)

(RNA primer needed for DNA replication)

◙ **RNA polymerase** – multisubunit enzyme(MCQ) that is:

1- recognize a nucleotide sequence (the promoter region) at the beginning of the length of DNA that is to be transcribed.

2- next , makes a complementary RNA copy of DNA template strand.

3- then , recognize the end of DNA sequence to be transcribed (the termination region).

- RNA is synthesized from its **5̀-end to its 3̀-end antiparallel** to its DNA template strand. (MCQ)
- **the template is copied as in DNA synthesize :**

DNA		RNA
G	specifies	C
C	specifies	G
T	specifies	A

But

A	specifies	U

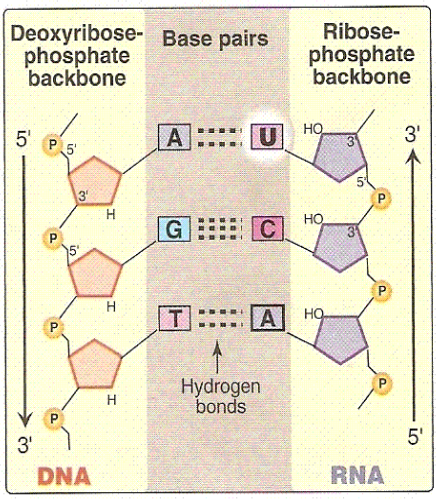


Figure 30.5
Antiparallel, complementary base pairs between DNA and RNA.

- **transcription unit:** extend from the promoter to termination region
- **primary transcript:** product of the process of transcription by RNA polymerase.

Q- Promoter >>> initiation of transcription.

* transcription by RNA polymerase involves a core enzyme and several auxillary proteins:

1-core enzyme :

- Four enzyme's peptide subunits, 2α, 1β, and 1β̀ .

- responsible for 5̀➔ 3̀ RNA polymerase activity

- lacks specificity, that is, it cannot recognize the promoter region on DNA template (MCQ)

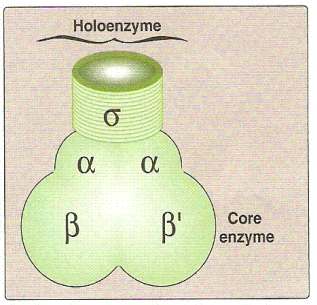


Figure 30.6
Prokaryotic *RNA polymerase.*

2-holoenzyme :

- holoenzyme = σ (sigma) subunit +core enzyme

- **σ subunit (sigma factor) function :**

✓ Enable RNA polymerase to recognize the promoter region on the DNA

Note : (Different σ factor recognize different groups of genes)

Q- Concerning prokaryotic transcription :

>Sigma (σ) factor enables RNA polymerase to recognize the promoter region.

Q- Holoenzyme (except)
> Its Rho-factor recognizes the termination signal.

3-termination factor :

- Some regions on DNA that signal the termination of transcription are recognize by **RNA polymerase** itself

- others are recognized by specific termination factors e.g. rho (ρ) factor of E.coli .

B . Steps in RNA synthesis :

- Three phases :

1) initiation . 2) elongation . 3) termination .

◙ within DNA molecule : both strand serve as template for RNA .
◙ **within a specific stretch of double helix : only one strand serve as template .**

1) Initiation :

- binding of **RNA polymerase holoenzyme** (MCQ) to the promoter region .
- **consensus sequence** (MCQ) of the promoter region are **highly conserved** (MCQ) . { so , different promoters contain very similar or identical Sequences }

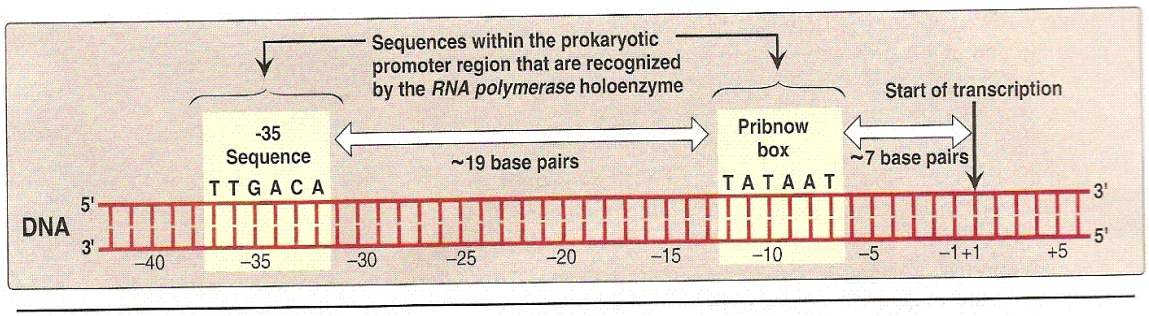


Figure 30.7
Structure of the prokaryotic promoter region.

- **Those that are recognized by prokaryotic RNA polymerase σ factor :**

a) Pribnow box :

- stretch of **six nucleotides (5 – TATAAT- 3)(MCQ)** centered about **eight to ten nucleotide** (in the figure he said 7 nucleotide) to the **left** of the Transcription start site that code for the **initial** base of the mRNA

✓ The regulatory sequence are in 3 to 5 in template strand & 5 to 3 in nontemplate strand .

◙ **NOTE :** **(Regarding the transcription site):**

- **(up stream) (prior) assigned a negative number .** e.g : pribnow box -9 .
- **at & after (downstream) assigned a positive number .** e.g : the first base at the transcription site is +1 .

i.e : (there is no base designated "0") (MCQ)

B) – 35 sequence :

- **A consensus sequence (5- TTGACA – 3) ,** which is centered about 35 bases To the **left** of the transcription start site . (MCQ)

◙ Note :

✓ A mutation in either Pribnow box or the – 35 sequence can affect the transcription of the gene controlled by mutant promoter .

2) Elongation:

- Once the promoter region has been recognized by the holoenzyme, *RNA polymerase* begins to synthesize a transcript of the DNA sequence (usually beginning with purine), & the **σ subunit** is released. (MCQ) .

- Unlike *DNA polymerase, RNA polymerase:* .(always come in exams)

  a. **does(not)** require a primer

  b. **has(no) known endonuclease or exonuclease activity**. It, therefore, **has(no) ability to repair** mistakes in the RNA, as does *DNA polymerase* during DNA synthesis.(always come in exams)

- *RNA polymerase* uses **ribonucleoside triphosphats,** & releases pyrophosphate (Tor F) each time a nucleotide is added to the growing chain.

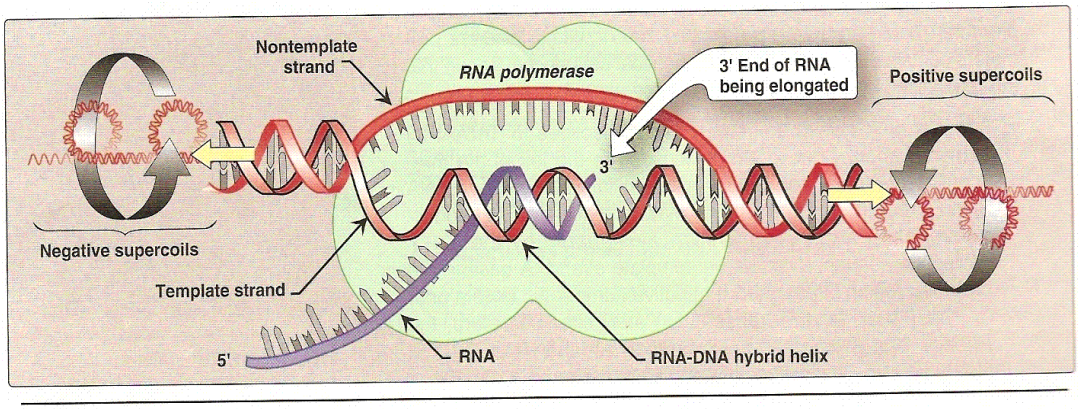


Figure 30.8
Local unwinding of DNA caused by *RNA polymerase*.

◙ Note :

- As in DNA synthesis, two high-energy bonds are thus used for the addition of each nucleotide.

- The binding of the enzyme to the DNA template result in local unwinding of the DNA helix. (MCQ)

- This process can generate supercoils that can be relaxed by *DNA topoisomerases I & II.*.

Q- Regarding to Prokaryotic transcription
>>> Supercoils that relaxed by DNA topoisomerase I & II.

3) Termination:

- The process of elongation of the RNA chain continues until a termination signal is reached.

- An additional protein, ρ **(rho) factor**, may be required for the release of the RNA product (ρ- **dependent termination**). Alternatively, the tetrameric *RNA polymerase* can, in some instance, recognize termination regions on the DNA template (**ρ- independent termination**).

a. Rho-dependent termination:

✓ protein not enzyme (☹MCQ)
✓ have some enzymatic activity
✓ requires the participation of an additional protein, ρ **factor**. (MCQ)

- ρ **factor** binds to **a C-rich region near the 3'-end** of the newly synthesized RNA, & migrates along behind the *RNA polymerase* in the **5'→3' direction** until the termination site is reached.

◙ **Note :**

- Rho factor has ***ATP- dependent RNA-DNA helicase activity*** (MCQ) (very important).that : hydolyzes ATP,& uses the **energy to unwind the 3'-end of the transcript** from the template.

  ✓ This facilitates the movement of the protein along the RNA/DNA duplex.

- At the termination site, ρ factor displace the DNA template strand, facilitating the dissociation of the RNA molecule.

So, what you should know it : ☺
1) protein not enzyme .
2) bind to **a C-rich region near the 3'-end .**
3) Rho factor has ***ATP- dependent RNA-DNA helicase*** activity

b. Rho- independent termination :

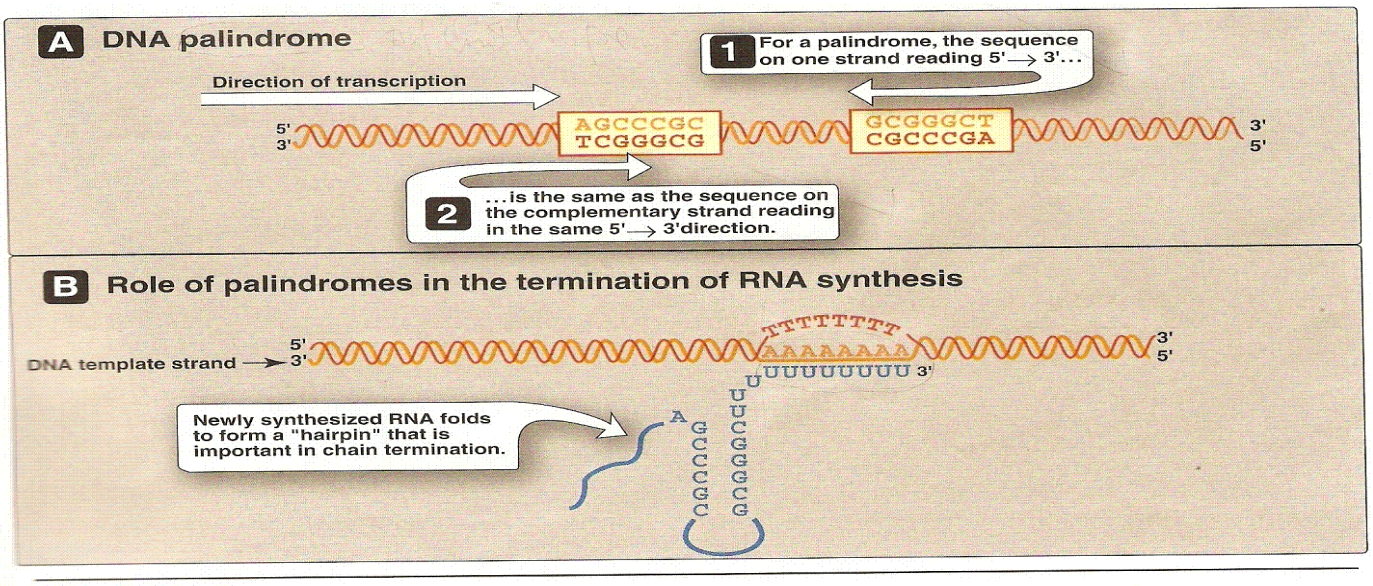


Figure 30.9
Rho-independent termination of transcription. A. An example of a palindrome in double-stranded DNA. B. A transcribed DNA palindrome codes for RNA that can form a hairpin turn.

*** requires that the newly synthesized RNA have two important structural features:**

First,

- **the RNA transcript must be able to form a staple hairpin turn** .(MCQ)

Q- Regarding to termination Prokaryotic transcription
>>> RNA form a stable hairpin turn.

◙ **when we ask what is the function of a stable hairpin turn ?**
Answer : it slows down the progress of *RNA polymerase* & causes it to pause temporarily.

◙ **Note :** **A palindrome** is a region of **a double-stranded DNA** in which each of the two strand contain stretches that have the same nucleotide sequence when read **in the same** (for example, 5'→ 3') direction

◙ **Note :** **as a result of the presence of a palindrome**
The hairpin turn of the RNA is complementary to a region of the DNA template near the termination region exhibits **two-fold symmetry(MCQ)**

Secondary structure of RNA is stabilizes by a lot of G &C in the base of the stem Of hairpin . (MCQ) (always come in exams) (IMP)

Second ,

beyond the hairpin turn,

- the RNA transcript contains a string of **U's**. The bonding of **U's** to the corresponding DNA template **A's** is **weak** .This facilitates the separation of the newly synthesized RNA from its DNA template, as the double helix "zips up" behind the *RNA polymerase*

◙ **Rivision** : (**Rho- independent termination)☹->☺**
** **staple hairpin turn** ** **two-fold symmetry**
** ***Secondary structure of RNA is stabilizes by a lot of G &C in the base of the stem Of hairpin**
****beyond the hairpin turn,**
- the RNA transcript contains a string of **U's**. The bonding of **U's** to the corresponding DNA template **A's** is **weak**

4) Action of antibiotics:

- **some antibiotics prevent bacterial cell growth by inhibiting RNA synthesis.**

◙ Note : **Dactinomycin:**

- known to biochemists as actinomycin D .

- It bind to the DNA template & interferes with the movement of the *RNA polymerase* along the DNA.

- was the first antibiotics to find **therapeutic** application in tumor chemotherapy (MCQ)

◙ Note : **rifampin :(MCQ)**

ركز عليه كثير دائما يجي وركز انه ما يأثر علىeukaryotes

- inhibits the initiation of transcription by binding to the **β-subunit** of **prokaryotic** *RNA polymerase* (Not eukaryotes) (MCQ) (always come), thus interfering with the formation of the **first phosphodiester bond** .

- Is useful in the treatment of tuberculosis

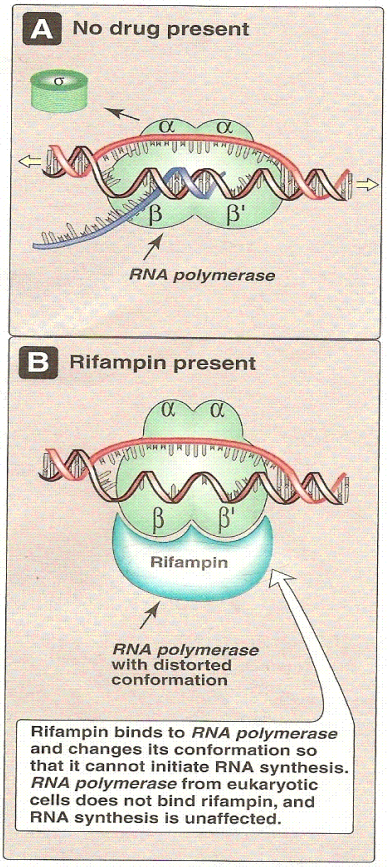


Figure 30.10
Inactivation of *RNA polymerase* by rifampin.

ما أشبه الليلة بالبارحة

أتذكر في هذه اللحظة أول مذكرة كتبت سبحان الله كيف تمر الأيام سراعا

لا تنسونا من دعائكم

Done < --------- المحاضرة الأولى في هذا الباب