VIEW ACADEMY
YOUR BEST WAY

Molecular Med – Lecture 5

Transcription

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Original Slides for Prof .Sameer mahjoub

- خلاصة المحاضرة الخلية لما بدها تنتج بروتين معين ، هاذ البروتين بكون في اله جين موجود في الdna والdna موجود في النواة ، ولكن عملية تصنيع البروتين تكون في السيتوبلازم، فلازم تطلع نسخة من هذا الجين تسمى mRNA من النواة للسيتوبلازم من خلال عملية تسمى النسخ Transcription ووقتها بتصير عملية الترجمة (تصنيع البروتين)، محاضرة اليوم هو كيف رح تتصنع الmRNA منذ البداية كيف تعرف الانزيم على هذا الجين ووين ارتبط وشو احتاج لحتى ارتبط وايش الانزيم المسؤول عن هاي العملية وانواع الانزيمات سواء في خلايا حقيقية النواة او بدائية النواة والناتج رح يكون MRNA غير فعال 100% وشو التعديلات الي بتصير عليه لحتى يكون فعال 100% لحتى تكون الخلية قادرة على ترجمته على شكل بروتين
- معلومة مهمة : عملية النسخ بتصنع Mrna وانواع اخرى من RNA فلازم ننتبه انه لما نحكي انه بدنا ننسخ جين لحتى نصنع بروتين فقط وقتها نوع RNA بتكون MRNA عدا ذلك بتكون الناتج انواع اخرى رح نتكلم عنها بالمحاضرة

Record

The Central Dogma of life

لون بني : نوت من د حمزة

Central dogma : Theory involves all the processes that happened in the nucleus and process after .

The processes that happened inside the nucleus are: **replication and transcription, Translation occur in cytoplasm**

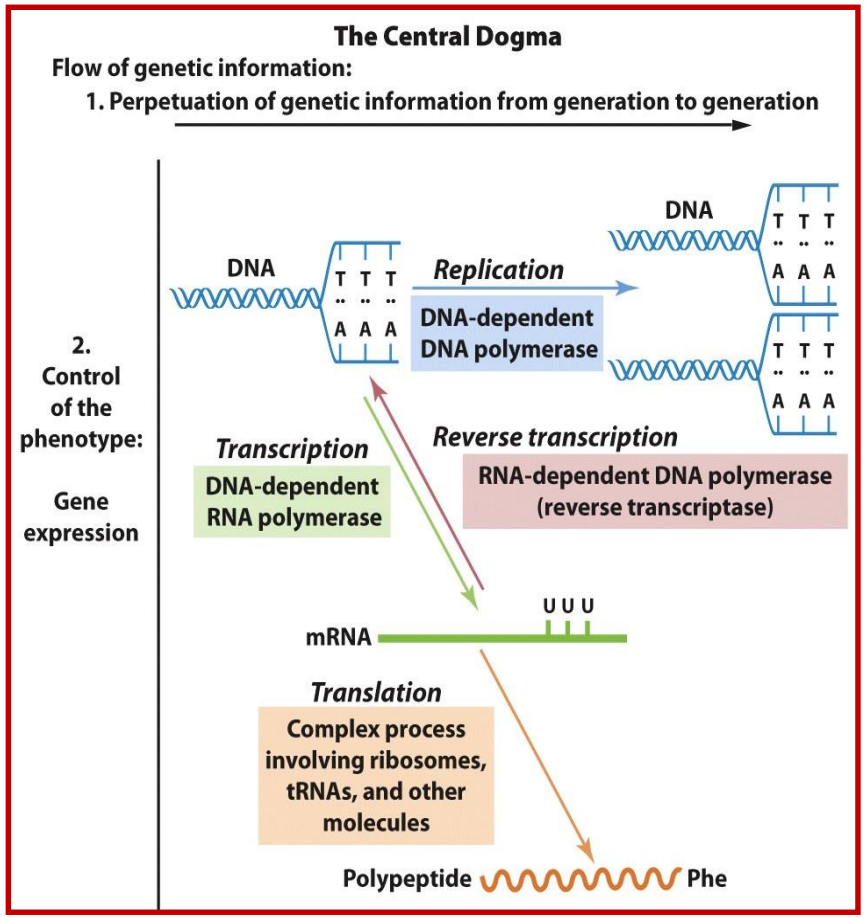
Replication is "DNA-dependent DNA polymerase" process,
by enzyme **DNA polymerase enzyme**

Transcription is "DNA dependent RNA polymerase" process,
by enzyme **RNA polymerase enzyme**

RNA polymerase enzyme is the enzyme which responsible
for RNA transcription, Will synthesize Single-Stranded molecule (mRNA)

Translation: is the process of producing a protein initially taking place on ribosome which will produce non-functional protein, then modifications occur to become mature, the whole process of producing functional mature protein taking place on protein synthesizing machinery which composed of ribosome, ER, Golgi apparatus

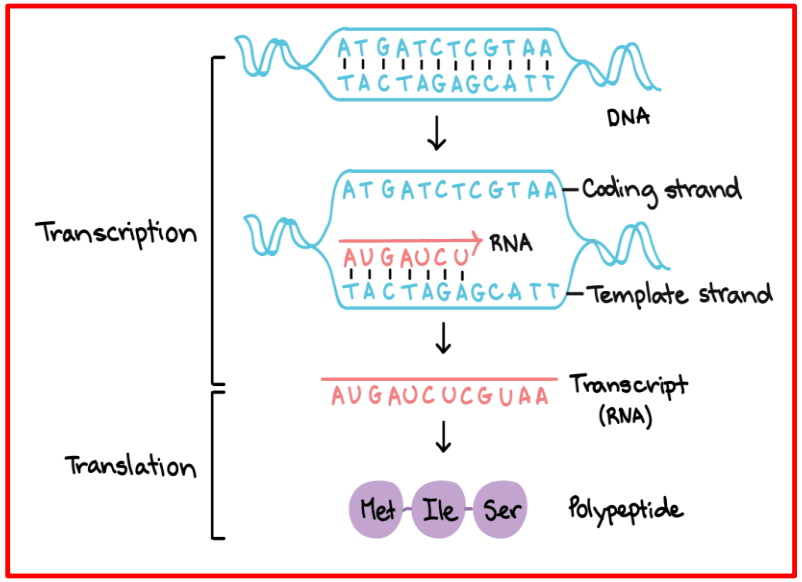
Reverse Transcription : Synthesize DNA from an RNA template by **RNA-Dependent DNA polymerase**



Template in Replication and transcription : Strand of DNA Consist of deoxyribonucleotide sequence, used by

DNA polymerase for synthesize new strand of DNA (in Replication) **or** used by **RNA polymerase for synthesize new strand of RNA**

DNA Polymerase + RNA polymerase read from 3 to 5 , and produce from 5 to 3 , If There is no DNA in cell like RBC , no replication will occur



Now the differences between Replication and Transcription are?

1- Replication is **Semi-conservative** but transcription is **Conservative**
2- Replication is Bidirectional process going to make two strand , transcription is UNI-directional make one strand
3- Replication happened for all DNA , but transcription nobody said that all our gene transcribe at the same time !

Something similar in Replication and transcription that they never act at double strand molecule .

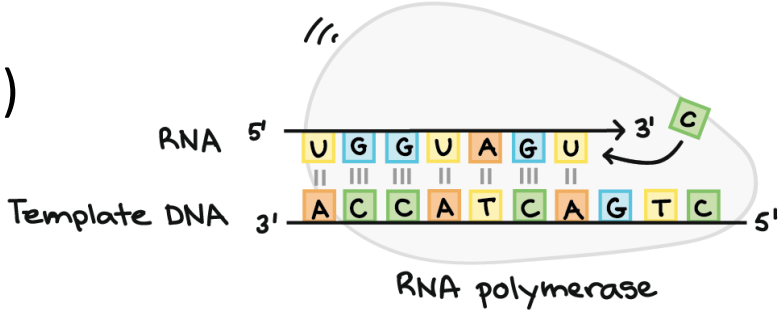
Overview of transcription

لسا بنحكي بشكل عام عن النسخ

- The first stage in the expression of genetic information is transcription of the information in the DNA deoxyribonucleotides sequence into RNA ribonucleotides sequence.
- For any gene, only one strand of the DNA molecule, called the **Template strand**, is transcribed by **RNA polymerase**.

- Because RNA polymerase moves in the 3' to 5' direction along the template strand of DNA, the RNA product is antiparallel and complementary to the template and identical to the second strand with replacement each T with U.

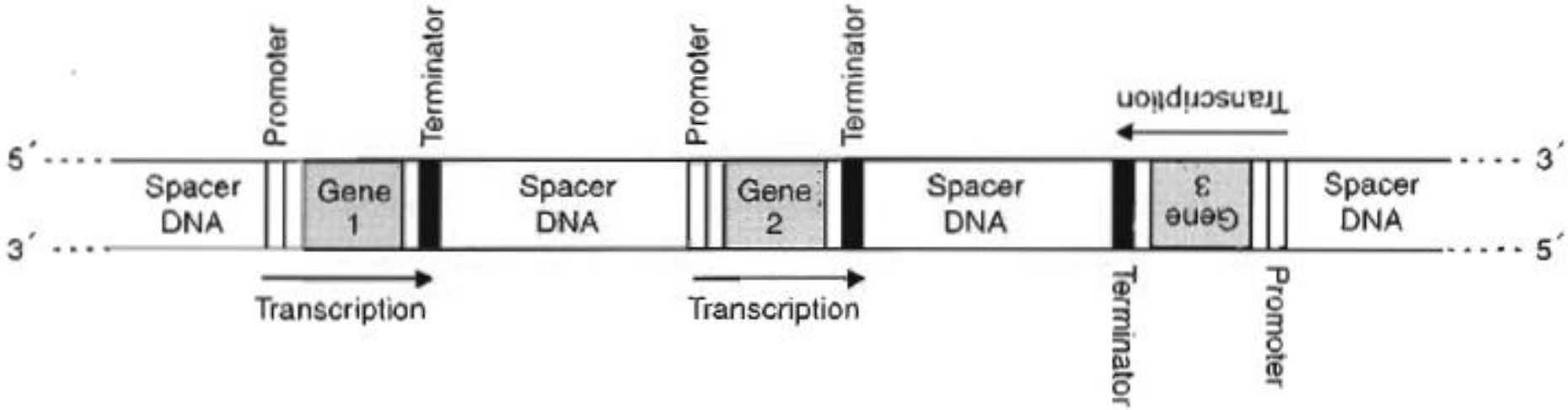
- RNA polymerase **blind so must** recognizes start signals (**Promoters**) and the first area to bind is operator and stop signals (**Terminators**) for each of the thousands of transcription units in the genome of an organism.



Promoter: is deoxyribonucleotide sequence , to allow RNA Polymerase recognizing the starting point , placed before the gene (that we want to make transcription for) .
- Promoter is the binding site for transcription factors and RNA polymerase.

Terminator: is deoxyribonucleotide sequence , to allow RNA Polymerase recognizing the stopping point, So If we don't have promoter = RNA polymerase will not work , and if we don't have terminator RNA Polymerase will not Stop!

لسا بنحكي بشكل عام عن النسخ



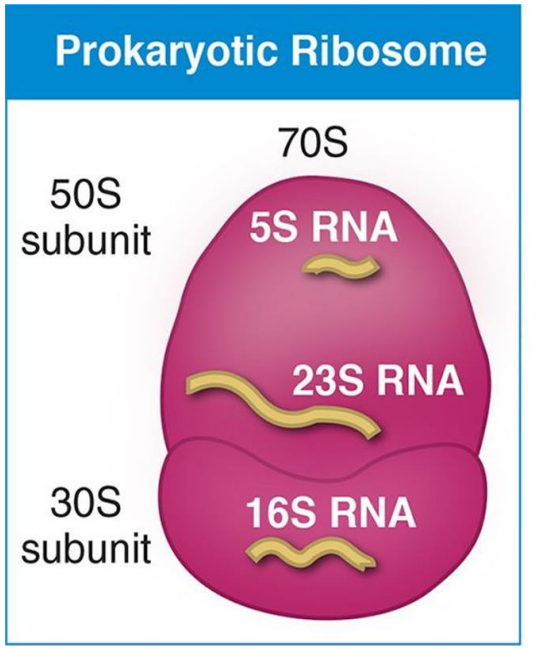
Transcription of several genes on a chromosome

انواع الRNA وكل واحد شو وظيفته

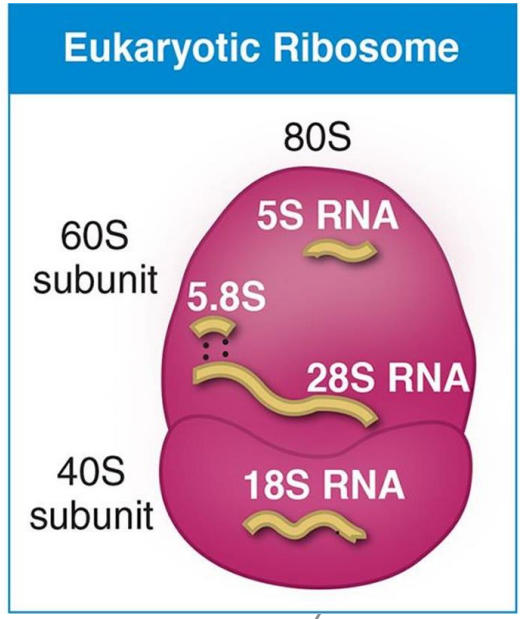
Types of RNA

- RNA molecules play a variety of roles in the cell,
- 3 types of them processing RNA are snRNA, scRNA , snoRNA
- 2 types of them regulating the transcription are siRNA +MicroRNA
- The types of RNA are:

1- Ribosomal RNA (rRNA), which is the most abundant type of RNA in the cell.



- Resemble the structural and functional core for protein synthesis,
- ❖ The difference between Prokaryote and Eukaryote ribosomes is not the size or molecular weight but it is for **Sedimentation Rate**
- ❖ Ribosomes in Prokaryotic cell contain two Sub-Unit = **30S and 50S** , sum of them **70S** , "S" Unit is Svedberg Unit , its not weight unit , but it is sedimentation rate unit.
  30s Means this subunit will precipitate in the test tube at specific speed in a unit of Svedberg .
- ❖ Ribosomes in **Eukaryotic cell** contain two subunit **= 60s and 40s ,** Sum of the **80s**
- ❖ We have **3 rRNA (5s,5.8s,28s)** in the large sub-unit **(60s)** and **1 rRNA(18s)** in the small subunit **(40s)** in the large subunit .

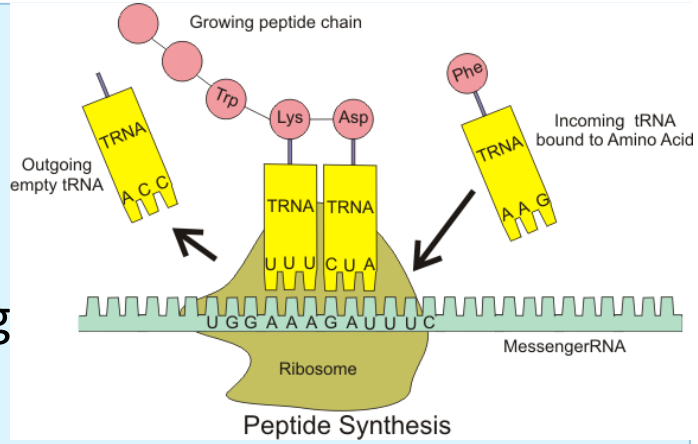


Types of RNA

انواع الRNA وكل واحد شو وظيفته

2- Transfer RNA (tRNA), which is the second most abundant type of RNA.
Produced by : RNA Polymerase III
- The most one heterogenous in size and in base sequence.

Function: Transfer Amino acids from Cytosol to the site of protein synthesizing machinery (ribosomes), carries the anticodon to ribosomes



- 20 Amino acids entering in protein synthesize and there is specificity in carrying an Amino acids!
So,I must have at least 20 different tRNA .

3- Messenger RNA (mRNA):
- the only type of RNA that is translated, which carries the information specifying the amino acid sequence of a protein to the ribosome.
- The mRNA population in a cell is very heterogeneous in size and base sequence, as the cell has essentially a different mRNA molecule for each of the thousands of different proteins made by that cell, (Number of mRNA depends on the cell needs of proteins)

Types of RNA

انواع الRNA وكل واحد شو وظيفته

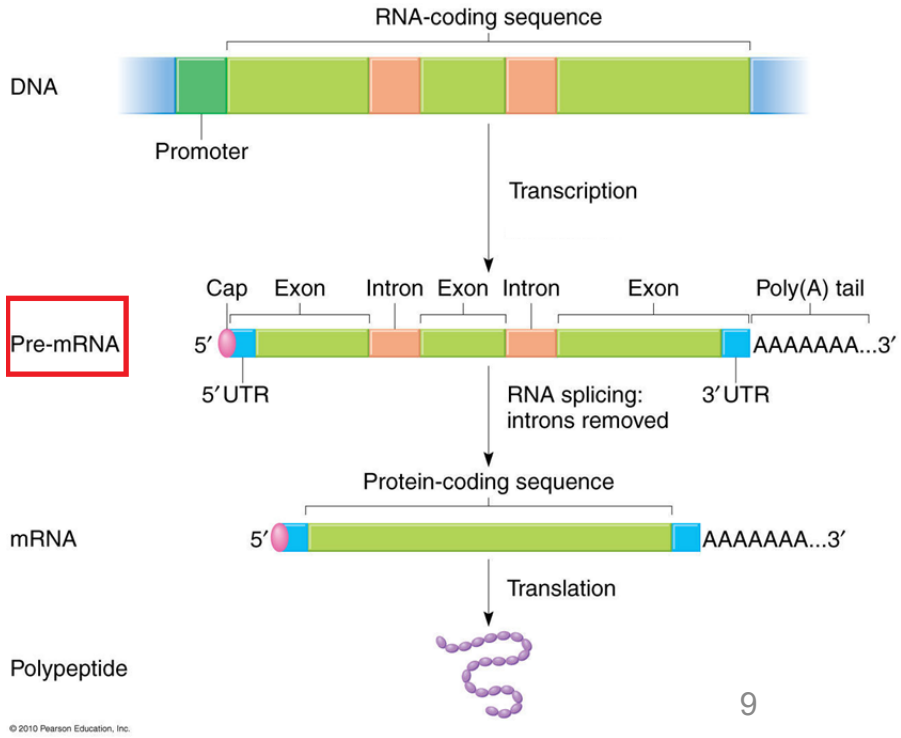
4- Heterogeneous nuclear RNA (hnRNA or pre-mRNA) , precursor for mRNA or the mother of mRNA

Single-stranded ribonucleic acid product synthesized by transcription of DNA consider as immediate product of gene transcription, Which is found only in the nucleus of eukaryotic cells, and it represents precursors of mRNA, **contain introns**

- Not present in Prokaryotic Cells

- 75% of hnRNA is degraded in the nucleus **(introns splicing)** and 25% only is processed to mature RNA.

So, Introns can be transcribed but not translated



Types of RNA

انواع الRNA وكل واحد شو وظيفته

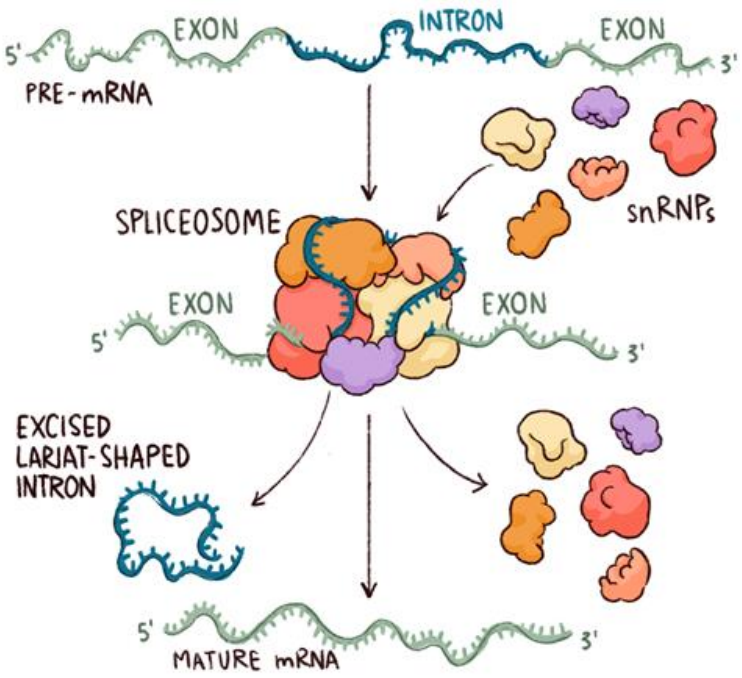
5- Small nuclear RNA (snRNA) (RNA molecules **(90-300 nucleotide)** with enzymatic activity**) also called Ribozymes .**

Which is also only found in the nucleus of eukaryotes, small in size and complexed with proteins (forming ribonucleoproteins), **Not present in Prokaryotic Cells**

SnRNA or Ribozymes have 2 main roles (self-splicing introns):

One of its major functions is to participate in splicing (removal of introns).

Second role is joining of Exons .



6- Small cytoplasmic RNA (scRNA), Second type has catalytic activity **,**

First one is snRNA

Function: tRNA processing and acts as signal recognition particle.

7- Small nucleolar (snoRNA) acts in rRNA processing/maturation/ methylation.

-rRNA Synthesized in Heterogenous long molecule and needs to be converted to mature form .

Types of RNA

انواع الRNA وكل واحد شو وظيفته

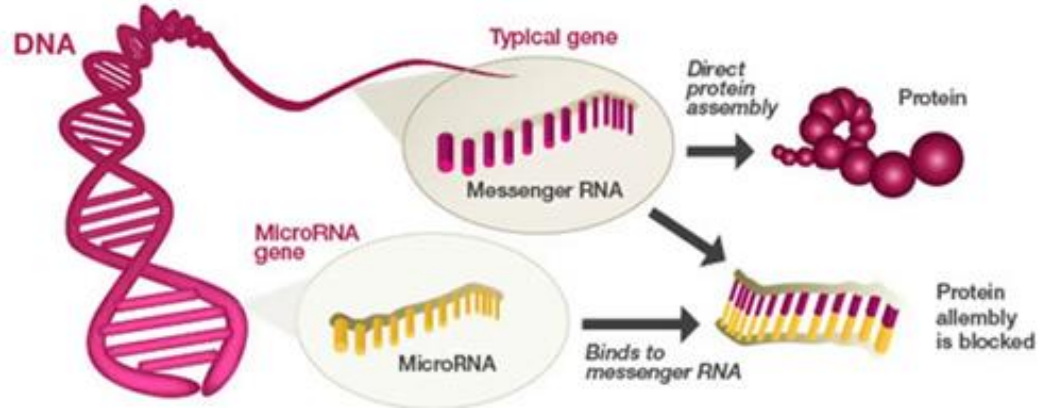
8- Micro-RNA, short, non-coding, ~ 22 nucleotide long, generated by nucleolytic processing of the products of distinct genes or transcription unites (during mRNA producing) at least some of which control the expression of other genes during development, two mature micro-RNA molecules can hybridize (binds) together to **form imperfect RNA- RNA duplex** within the 3' untranslated regions of specific target mRNA causing unexplained gene expression regulation in at least half of the human genes.

Function: Regulation of Gene expression .

Synthesis and working method: some of mRNAs are degraded into small pieces by enzymes, these pieces called Micro- RNA, these micro-rna hybridize with other mRNA and prevent protein formation

- 50% of our genes regulated by miRNA.
- Some of micro-RNA used as biomarker for several diseases

* Regulation occur because RNA Polymerase need terminator to stop Translation , but (RNA –RNA Duplex) function is not giving the chance for RNA Polymerase to recognize terminator .



Types of RNA

انواع الRNA وكل واحد شو وظيفته

9- Small interfering RNA (siRNA) are derived by specific nucleolytic cleavage of larger **double stranded RNAs** to form small 21-25 long products.

Function : Regulation of gene expression

- They form **perfect** RNA-RNA hybrids with their targets anywhere within the length RNA where the complementary sequence exists resulting in reduction of specific protein production because **(siRNA-mRNA**) complexes are degraded by nucleolytic machinery (interferes with the expression of specific gene by hybridizing to its corresponding RNA sequence in the target mRNA, then activates degradation of mRNA which can not be translated into proteins).

So , Any extra mRNA , it will degraded by siRNA.

Synthesis and working method: some of dsRNA are degraded into small pieces called siRNA, These siRNA can hybridize **perfectly** with mRNA , then the product degraded.

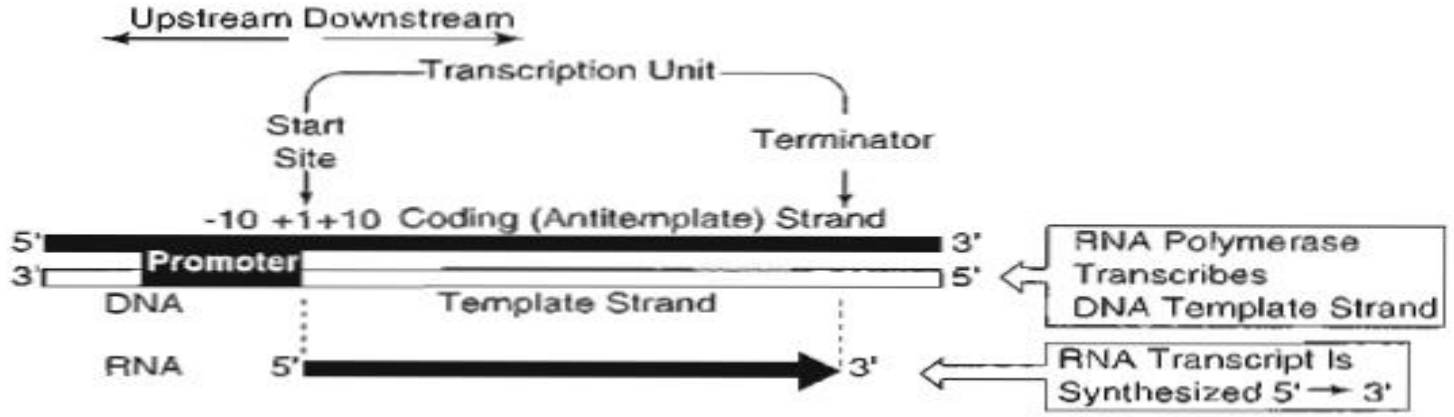
هاذ السلايد ملخص لكل شي حكيناه قبل

Transcription: important concepts and terminology

- RNA polymerase locates genes in DNA by searching for promoter regions.
- The promoter is the binding site for transcription factors and RNA polymerase.
- Binding establishes where transcription begins, which strand of DNA is used as the template, and in which direction transcription proceeds.
- RNA polymerase moves along the template strand in the 3' to 5' direction as it synthesizes the RNA product in the 5' to 3’ direction using NTPs (ATP, GTP, CTP, UTP) as substrates.
- RNA polymerase does not proofread its work.
- The RNA product is **complementary** and **antiparallel** to the **template strand**.
- The coding (**non-template**) strand is not used during transcription.

It is identical in sequence to the RNA molecule, except that RNA contains uracil instead of the thymine found in DNA.

- By convention, the base sequence of a gene is given from the coding strand (5' → 3').
- Transcription ends when RNA polymerase reaches a termination signal



Promoter “Strength” (activity)

- Affects amount of RNA made, so, it affects level of expression for that gene.
- Not all promoters have same “strength” ,
- **Meaning of Strength: Number of initation taking place per second.**
- **Each Promoter is for one type of gene, and produce specific type of mRNA.**
  So, if the cell need specific type of mRNA from another types, the promoter of the needed mRNA should be strong enough (very active)
- RNA polymerase binds differently to different sequences “Strong promoters” initiate transcription more often than “weak promoters”
  - rRNA has strong promoter: ~1 initiation per second
  - lacZ has a weak promoter: ~1 initiation per minute

- Promoters differ in DNA sequences

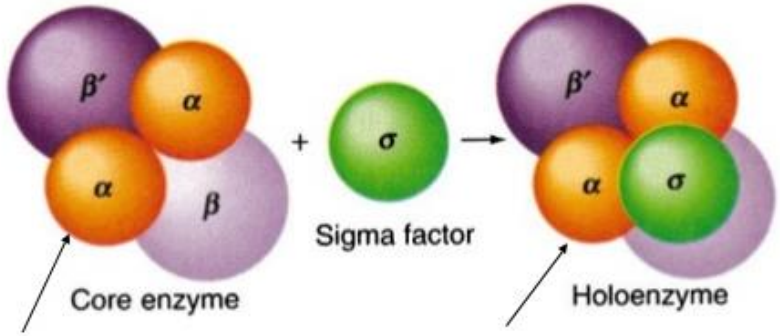
For Example: TATA BOX is promoter , the sequence may TATAAT ,OR TATAAAAT **Extra**

RNA Polymerases in Prokaryotic Cells

عدد الانزيمات المسؤولة عن النسخ
العدد 1 في خلايا بدائية النواة

- There is a single prokaryotic RNA polymerase that synthesizes all types of RNA in the cell.
- The **Core** polymerase has the subunit structure (α2ββ`), **but RNA polymerase is blind enzyme** so it need Factor for the initiation of transcription at the promoter , this protein factor called **sigma (σ)**
- **σ** factor is released immediately after transcription initiation.

-Core enzyme are non-functional , but
(core enzyme + σ) called Holo-enzyme and functional .



Functions of the subunits:

α: assembly of the tetrameric core
β: ribonucleoside triphosphate binding site (link ribonucleotides together)
β’: DNA template binding region
σ: (sigma factor): initiation of transcription

- Termination of transcription **sometimes** requires a protein called rho **(ρ)** factor , **this factor help RNA polymerase to recognize the termination site , Rho (ρ) factor not a part of RNA polymerase**

يعني الخلاصة من الحكي ال sigma factor برتبط مع RNA polymerase ومجرد ما يتعرف على ال initiation site **بفترة** بفك الارتباط وبروح , ال Rho بكون موجود عند نقطة البداية لل mRNA وبعدين بروح على نقطة النهاية وبفك ارتباط ال mRNA من RNA polymerase (رح ينحكى بالتفصيل) factor

- This enzyme is inhibited by rifampin and actinomycin D. **انتبـه هون بحكي عن Prokaryotic cells**

عدد الانزيمات المسؤولة عن النسخ
العدد3 في خلايا حقيقة النواة

RNA Polymerases in Eukaryotic Cells

- Three types which can be distinguished by the particular types of RNA they produce:

1- **RNA polymerase I** is located in the nucleolus and synthesizes **28S**, **18S**, and **5.8S** rRNAs.

2- **RNA polymerase II** is located in the nucleoplasm and synthesizes hnRNA/mRNA and some snRNA.

3- **RNA polymerase III** is located in the nucleoplasm and synthesizes tRNA, some snRNA, and **5S** rRNA.

Types	Product	Location
RNA polymerase I (RNAP I)	28S, 18S and 5.8s **rRNA**	Nucleolus
RNA polymerase II (RNAP II)	**hnRNA/mRNA** + some snRNA	Nucleoplasm
RNA polymerase III (RNAP III)	tRNA + some snRNA + 5S rRNA	Nucleoplasm

So, snRNA produced by RNA Polymerase II + III

عوامل النسخ المسؤولة عن مساعدة
الانزيم على عملية النسخ

RNA Polymerases in Eukaryotic Cells

- Transcription factors (such as **TFIID** for RNA polymerase II) help to initiate transcription.
- The requirements for termination of transcription in eukaryotes are not well understood.
- In addition, RNA polymerase II is inhibited by (**α-amanitin**) a toxin from certain mushrooms, it inactivates RNA pol II and can kill a person, while, RNA pol I and III are less affected by toxin

انتبه حكينا في الprokaryotic احتاج الانزيم الى sigma factor لحتى يبدأ عملية النسخ اما هون بحتاج transcription factor

	Prokaryotic	Eukaryotic
Type of enzymes	Single RNA Polymerase (α2ββ`)	RNAP1 +RNAP 2 + RNAP3
Initiation requirement	Require sigma (σ)	No sigma (σ) required, but transcription factors (TFIID) bind before RNA Polymerase
Termination requirement	Sometimes require Rho **(ρ)**	No Rho **(ρ)** required
Enzyme inhibition	Inhibited by rifampin + actinomycin D	RNAP2 is inhibited by (**α-amanitin**) + actinomycin

Comparison of eukaryotic and prokaryotic RNA polymerases

- The following events occur during the expression of a prokaryotic gene:

- With the help of sigma factor, RNA polymerase recognizes and binds to the **promoter**, region.
  But How the Sigma factor will recognize the promoter?
- The bacterial **promoter** contains two "consensus" sequences,

1- First one called the **Pribnow box also called [TATA box]**:
Consist of 6 ribonucleotide (TATTAT), lies about -10 Ribonucleotide from transcription initiation site
2- Second one lies about 35 Sequence from initiation site and consist of 8 ribonucleotide (TGTTGACA).

After checking TATA Box and 35-Box , RNA polymerase can bind .

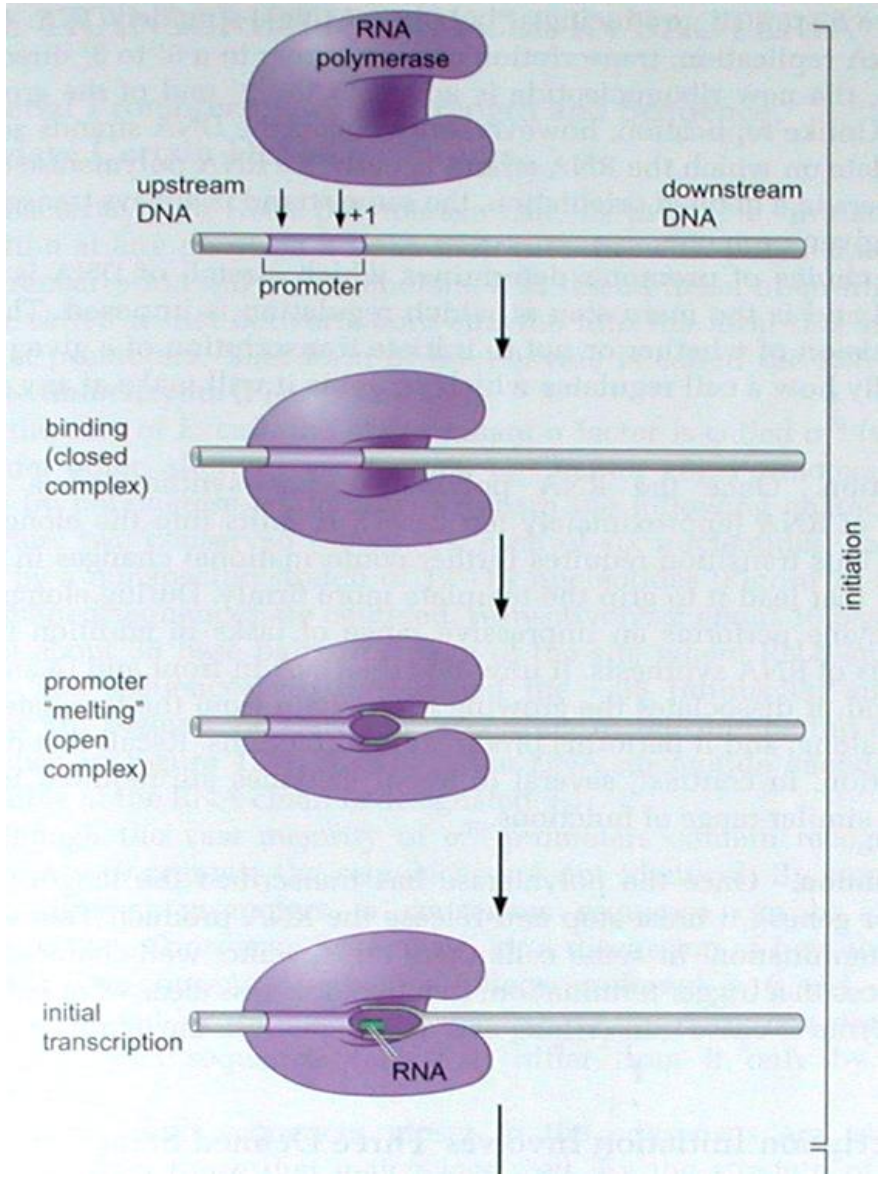
1- The promoter identifies the start site for transcription and orients the enzyme on the template strand.
2- Transcription begins at the + 1 base pair Sigma factor is released as soon as transcription is initiated.
3- The core polymerase continues moving along the template strand in the 3' to 5' direction, synthesizing the mRNA in the 5' to 3' direction.

Steps of Transcription:

1- Initiation

Initiation

Prokaryotic cells النسخ في ال



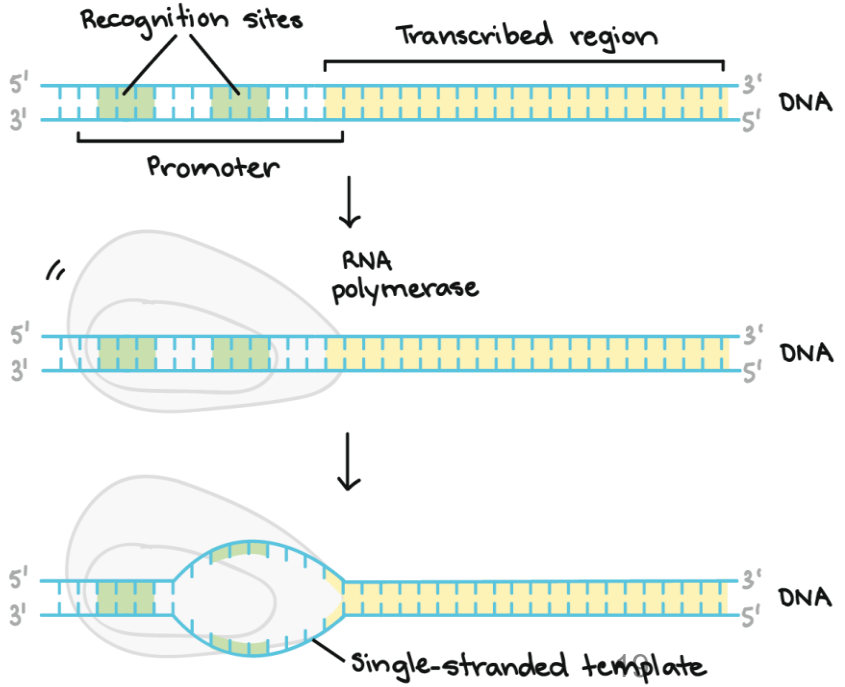
A- Binding (closed complex) :

After check TATA BOX and the 35 box

B- Promoter "melting" (open complex)

-After RNA polymerase binding , the strand must separate from one another to start the transcription , this process called Melting or opening, Done by helicase enzyme

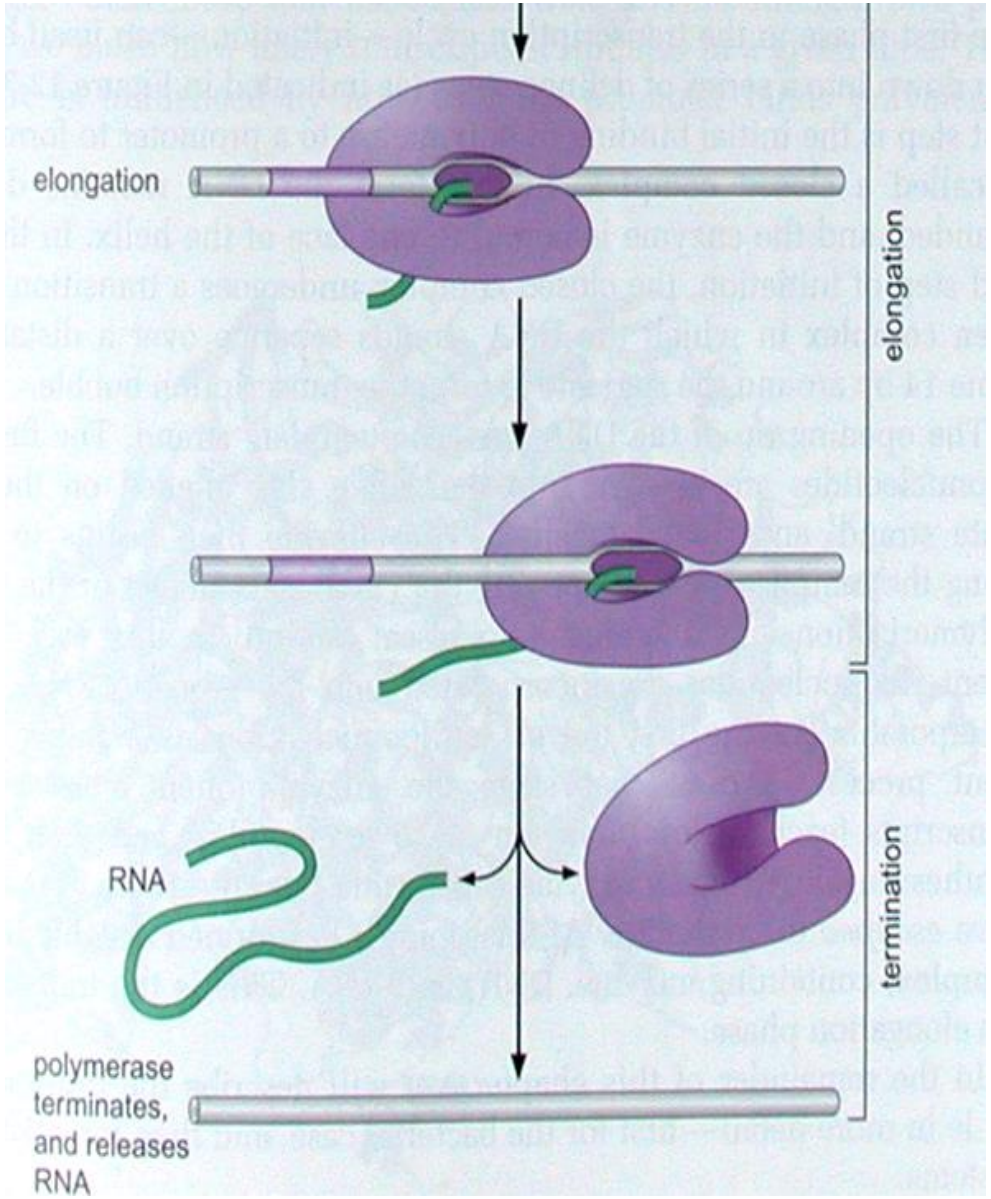
C- Initial transcription



Previous note:

After produce 22 ribonucleotide , the RNA polymerase will recheck the starting point if correct or not , this process called abortive transcription

-about 4 to 5 times the process get aborted



Elongation

Previous note from Dr.Samer:

After checking the starting point , RNA polymerase will produce about 80 ribonucleotide , at this point Sigma factor made sure that the starting point was correct and it will be released from RNA polymerase .

Termination

❖ RNA polymerase eventually reaches a transcription termination signal, at which point it will stop transcription and release the completed **mRNA molecule**.

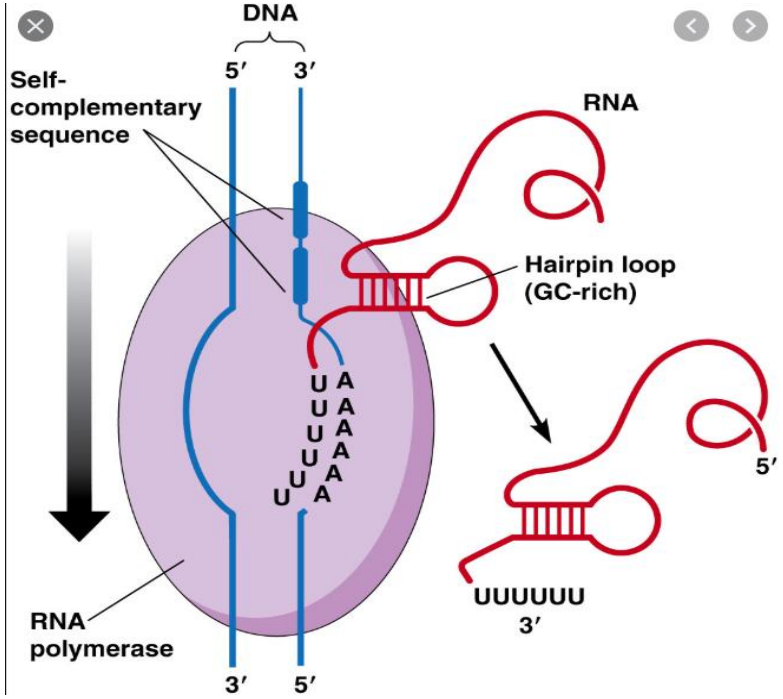
- There are two kinds of transcription terminators commonly found in prokaryotic genes:

A- Rho-independent termination occurs when the newly formed RNA folds back on itself forming A G-C Rich hairpin loop closely followed by **6-8 U** residues.

G-C : because bonded by 3 hydrogen bonds

UA : to allow mechanical stress (WEAK BONDS)

These two structural features of the newly synthesized RNA promote dissociation of the RNA from DNA template.



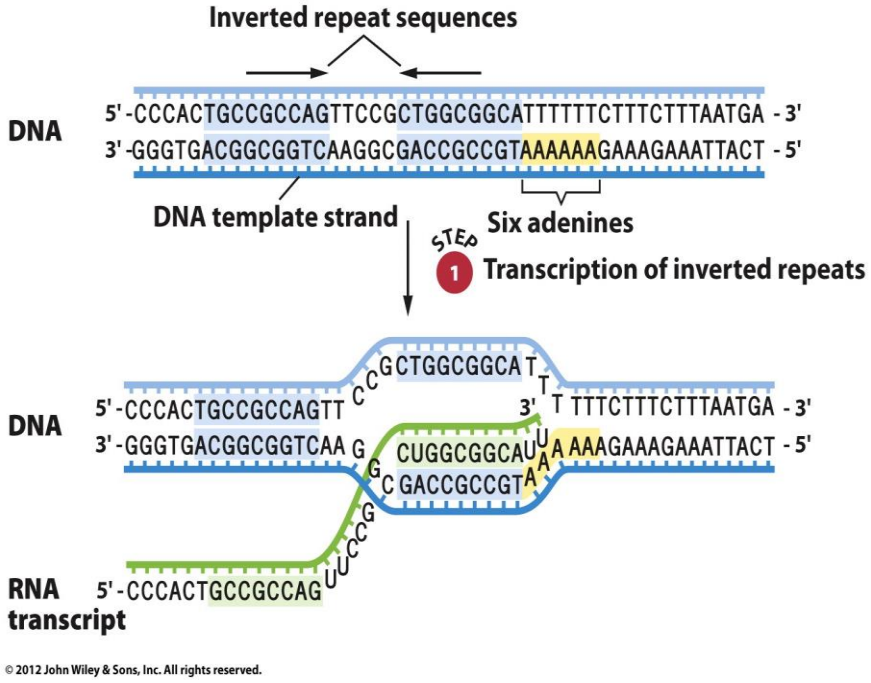
- **B- Rho-dependent termination** requires participation of **rho factor**.

This protein binds to the newly formed RNA (recognition site) and moves toward the RNA polymerase that has paused at a termination site.

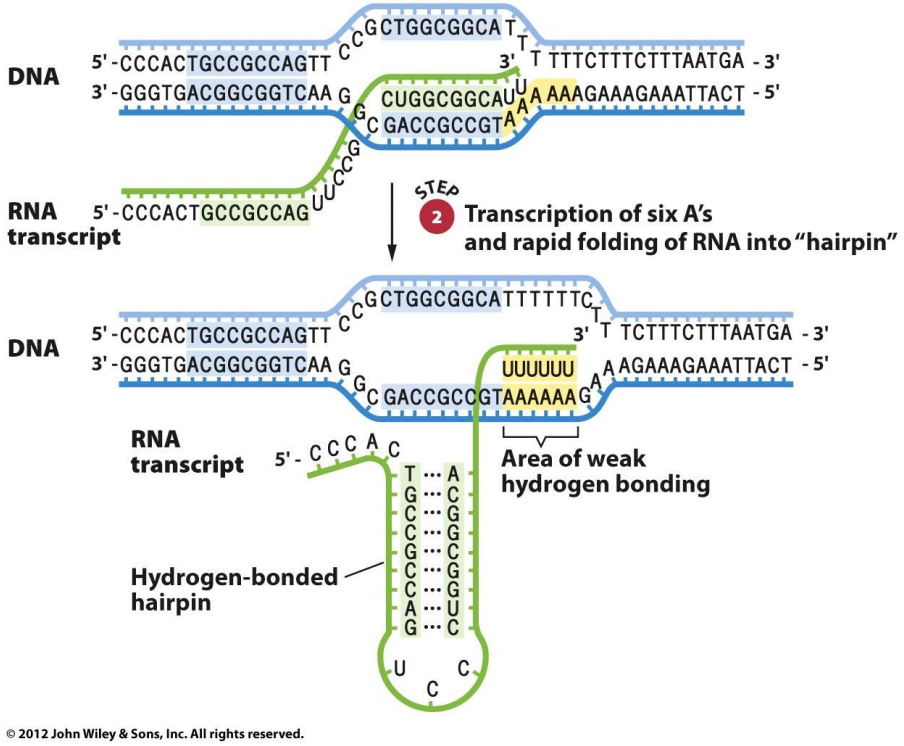
- rho then displaces RNA polymerase from the 3' end of the RNA.

https://www.youtube.com/watch?v=cxhXCsvHVuI

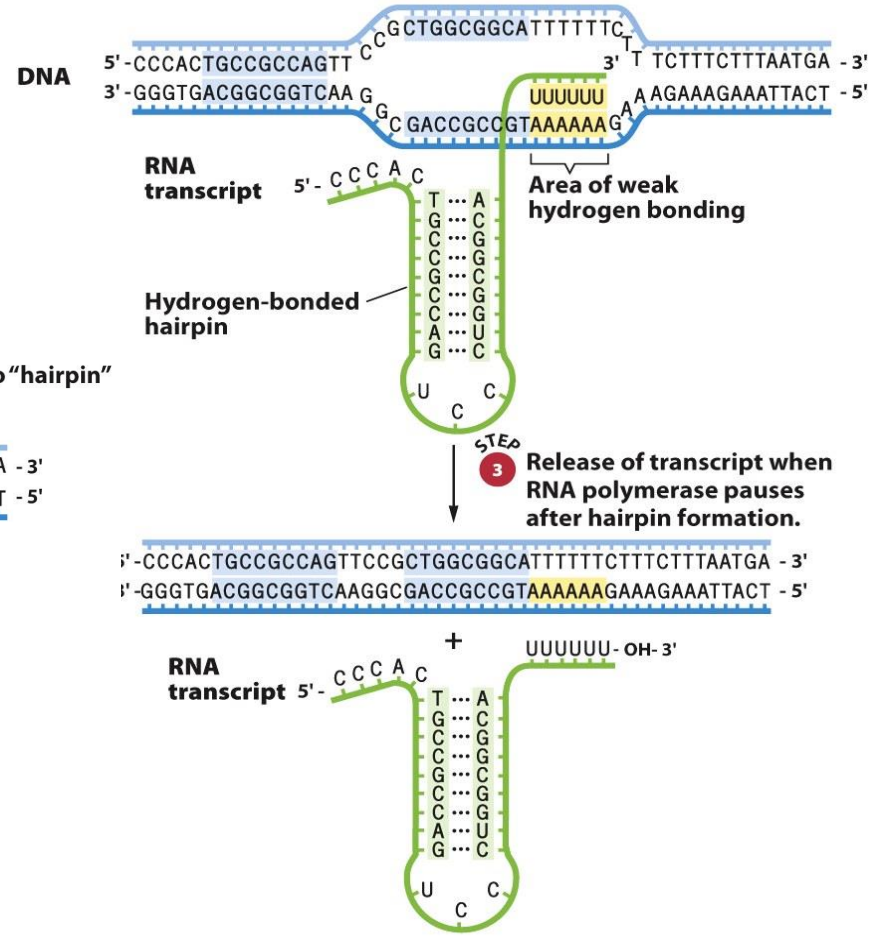
Transcription termination



A



B



C

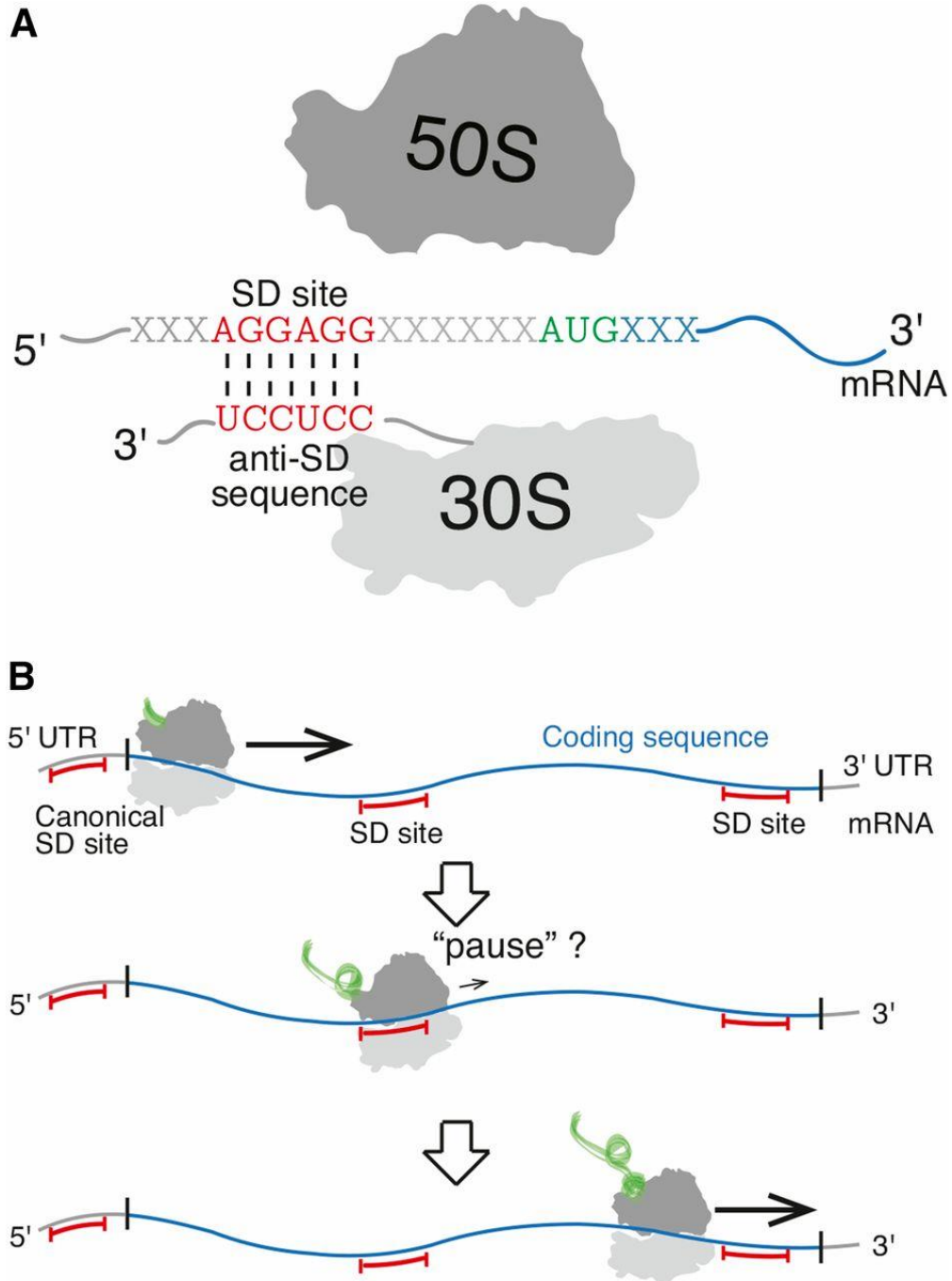
❖ **Transcription** and **translation** can occur **simultaneously** in bacteria because there is no processing of prokaryotic mRNA (generally no introns) **also because no nuclear membrane founds**, ribosomes can begin translating the message even before transcription is complete.

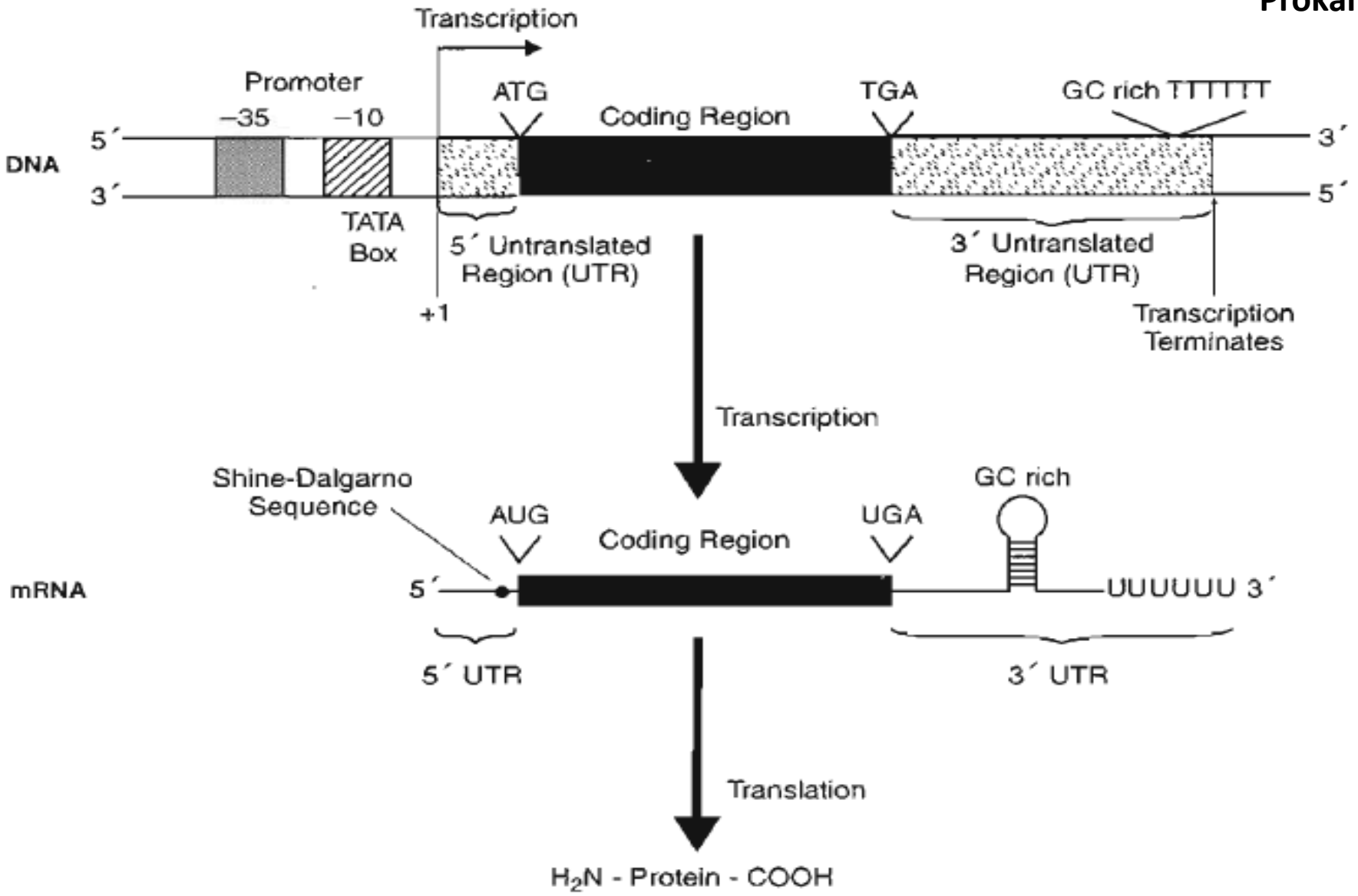
هاذ الكلام كله رح يتاخذ بالمحاضرة القادمة

Ribosomes (small Subunit) bind to a sequence called **Shine-Dalgarno sequence** in the 5’ untranslated region (UTR) of the message

- Protein synthesis begins at an AUG codon at the beginning of the coding region and continues until the ribosome reaches a stop codon at the end of the coding region.

❖ The ribosome translates the message in the 5' to 3' direction, synthesizing the protein from amino terminus to carboxyl terminus.


A
50S
SD site
5'
XXXAGGAGGXXXXXXAUGXXX
3'
mRNA
3'
UCCUCC
anti-SD
sequence
30S
B
5' UTR
Coding sequence
3' UTR
Canonical
SD site
SD site
SD site
mRNA
"pause" ?
5'
3'
5'
3'

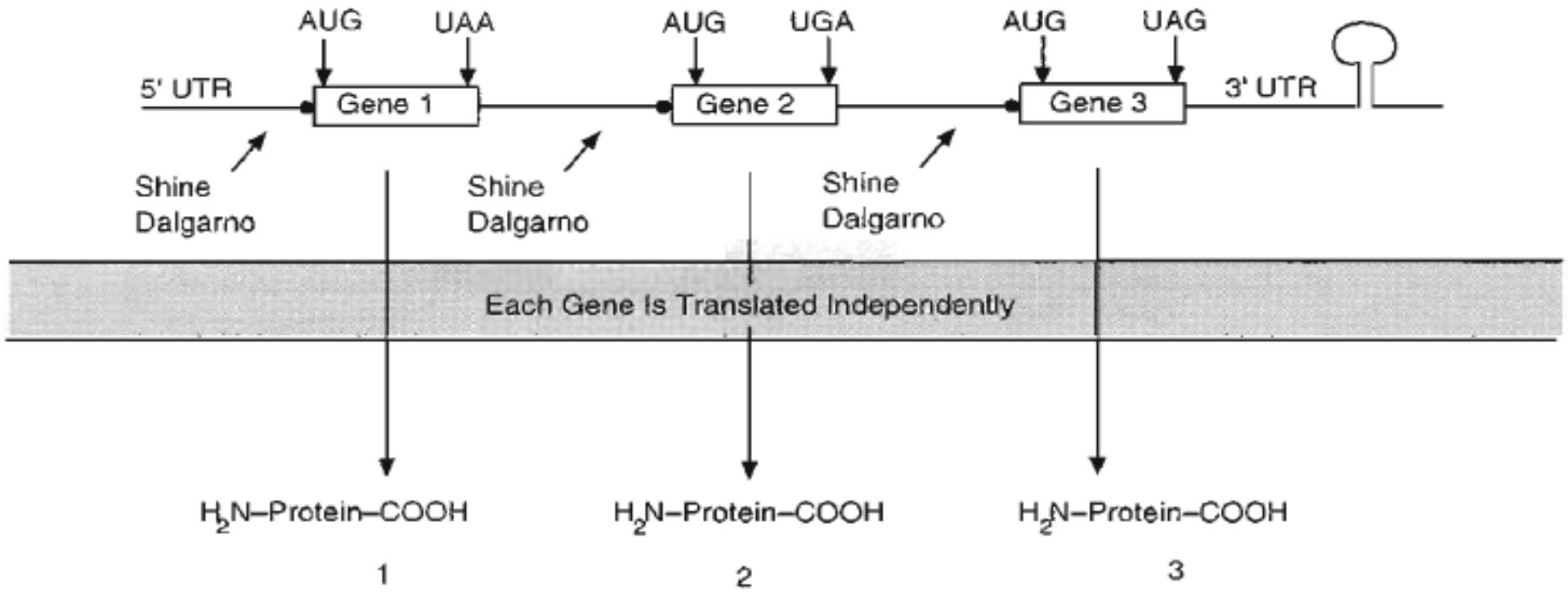


A prokaryotic transcription unit.

- An additional feature of bacterial mRNAs is that most are **polycistronic ,**
  Explanation: Gene in prokaryotic cells are bound in clusters, So some bacterial operons produce **polycistronic** messages (the word **cistron** is another name for a gene)

- In these cases, RNA Polymerase transcribe more than one gene as one unit , which means that multiple polypeptides can be synthesized from a single primary transcript.

- The mRNA in this case contains information from several genes and codes for several different proteins
- In addition, several viruses encode **polycistronic** RNAs.
- - Polycistronic mRNAs are very rare in eukaryotic cells but have been identified.
- So The mRNA produced by eukaryotic cell is a **monocistronic** message.
  That is, it is transcribed from a single gene and codes for only a single protein.



Prokaryotic polycistronic message codes for several different proteins

Production of eukaryotic mRNA

- In eukaryotes, most genes are composed of coding segments (**exons**) interrupted by non-coding segments (**introns**).

- Both exons and introns are transcribed in the nucleus.
- Introns are removed during processing of the RNA molecule in the nucleus.

- In eukaryotes, all mRNA is **monocistronic**.
- The mature mRNA is translated in the cytoplasm.

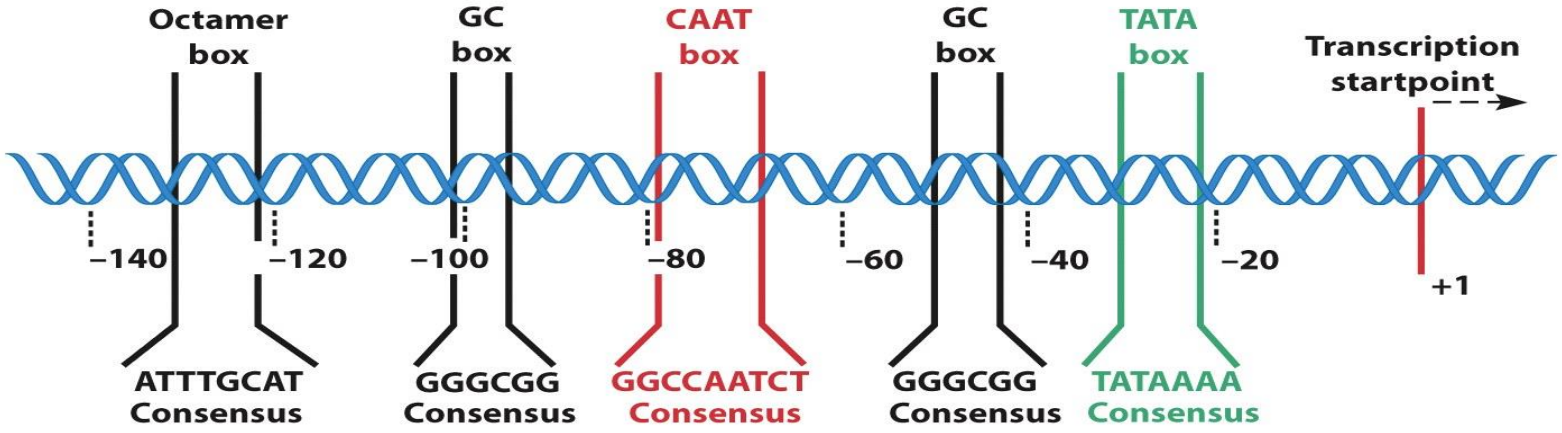
الخلاصة اول شيء يتم تصنيعه هو pre-mrna وبحتوي على intron وطبعاً كونها non-protein coding genes ف لازم تنخذف ويتحول الى mRNA
قبل بدء عملية الترجمة ، لأنه في حال انا عندي مثلاً pre-mrna وفرضًا بدي اعمله ترجمة ، وقتها بدل ما يطلع على شكل polypeptideواحد ، رح يكون على شكل عدة polypeptide

- Transcription of a typical eukaryotic gene occurs as follows:

1. With the help of proteins called transcription factors**(TFIID),** RNA polymerase II recognizes and binds to the promoter region.

- The basal promoter region of eukaryotic genes usually has **two consensus sequences** called the **TATA box** (also called **Hogness box**) **Which lie 25 ribonucleotide from initiation site** and the **CAAT box Which lie 70-90 ribonucleotide from initiation site**
- **there is GC box which present only in some promoter , and its site independent.**

2. RNA polymerase II separates the strands of the DNA over a short region to initiate transcription and read the DNA sequence. The template strand is read in the 3' to 5' direction as the RNA product (the **primary transcript**) is synthesized in the 5' to 3' direction.



A Typical RNA Polymerase II Promoter

Class II promoters (most similar to bacterial promoters)

- Common type of promoter (most genes use this)
- Many variations, but "consensus" has a **"Core"** + **"Upstream" Core** (3 elements):
1. **"TATA box"** (5'-TATA-3')
2. **TFIIB recognition element (BRE)** ; TFIIB recognize this element **.**
3. **Initiator box (Inr)** with an "A" at +1, most common **the site where the transcription is started .**

- **Downstream promoter elements** (DPE, less common, **not always present)** , There are boxes in it that can be recognized by transcription factor for initiation the formation of the preinitiation complex .
  - Preinitiation complex consist of RNA polymerase, transcription factors and DNA template .
  - Core promoter is recognized by general TFs that associate with RNA pol to form a preinitiation complex at great majority of promoters

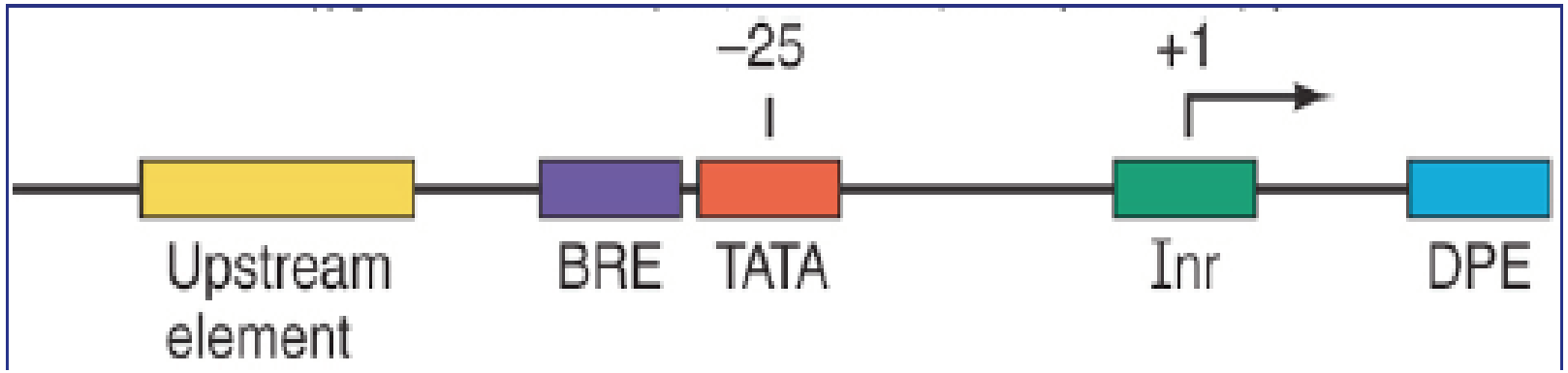
- At least one of these elements is missing in most promoters e.g., highly expressed specialized genes tend to have TATA boxes (may have more than one TATA box), but promoters for housekeeping genes tend to lack them

But Transcription factor + initiator box + RNA polymerase are essential

- Upstream elements: quite varied in number and can be orientation-independent (but relatively position-dependent) & recognized by other TFs (relatively gene-specific) that participate in initiation at smaller sub-sets of promoters.

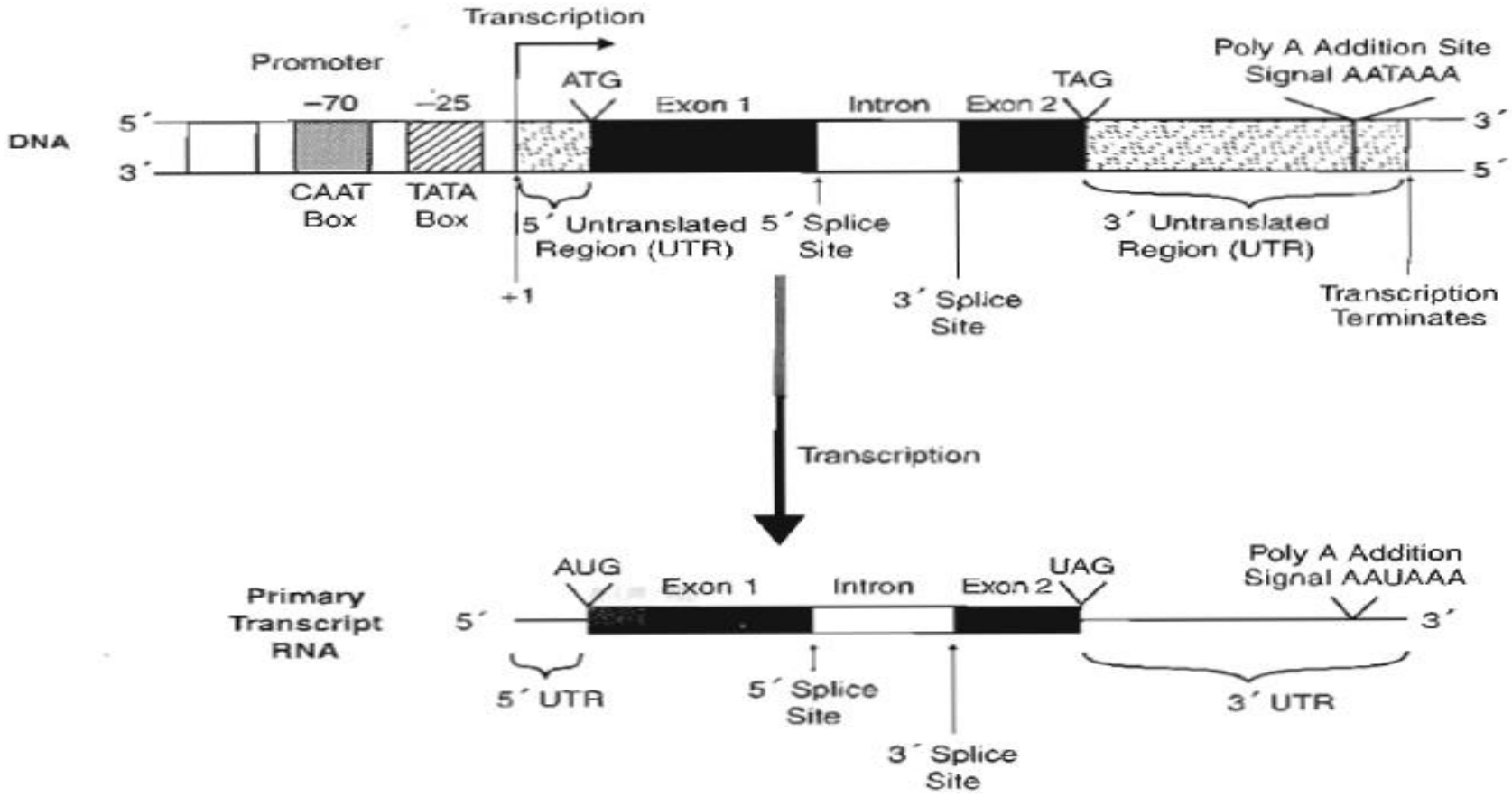
1. GC box (GC rich)
2. CAAT box (5'-CCAAT-3)

Position dependent : means that CAAT + GC at upstream , it will never to be in downstream
Orientation in-dependent : means CAAT may located before GC or Vice versa .
Gene specific: means some genes contains CAAT without GC or GC without CAAT or contain both of them **.**



BRE: TFIIB recognition element; **Inr:** initiator box; **DPE:** (downstream promoter element)

3. RNA polymerase II ends transcription when it reaches a termination signal.
- These signals are not well understood in eukaryotes.



A eukaryotic transcription unit

Table 11.3

General Transcription Initiation Factors

Factor	Subunits	Size (kDa)	Function
TFIID-TBP	1	27	TATA box recognition, positioning of TATA box DNA around TFIIB and Pol II
TFIID-TAF_{II}s	14	15–250	Core promoter recognition (non-TATA elements), positive and negative regulation
TFIIA	3	12, 19, 35	Stabilization of TBP binding; stabilization of TAF–DNA binding
TFIIB	1	38	Recruitment of Pol II and TFIIF; start-site recognition for Pol II
TFIIF	3	156 total	Promoter targeting of Pol II
TFIIE	2	92 total	TFIIH recruitment; modulation of TFIIH helicase ATPase, and kinase activities; promoter melting
TFIIH	9	525 total	Promoter melting; promoter clearance via phosphorylation of CTD

Promoter melting means separating RNA polymerase from promoter.

رح يتعرف على TATA وبخليه يلتف حول TFIIB و Polymerase II

رح يتعرف على CAAT+GC ، ويتميز انه More powerful مقارنة بأول نوع
لأنه عنده القدرة على ربط TAF+ TBP

Eukaryotic Transcription: DNA-Directed RNA Synthesis

consists of

Initiation

requires

Chromatin remodeling, binding of general transcription factors and *RNA polymerase* to core promoter sites up- or downstream of the coding region

which is facilitated by

Specific transcription factors bound to enhancer sequences

Elongation

requires

Local unwinding of the DNA helix

followed by

Synthesis of a 5'→3' RNA transcript coded for by the DNA template read in the 3'→5' direction

Termination

requires

A termination signal sequence

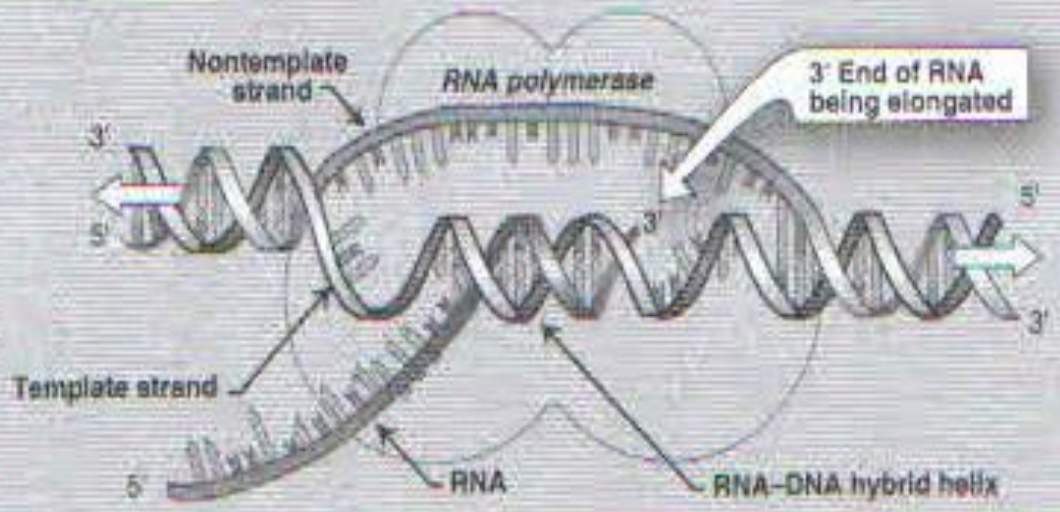
results in

Release of *RNA polymerase* and newly synthesized transcript from DNA

Co- and post-transcriptional modification

for example

- Splicing of pre-mRNA to remove non-coding introns and join exons
- Cleavage, trimming and base/sugar modification in pre-rRNA
- Trimming, addition of 3'-CCA, and base modification in pre-tRNA.
- Addition of a 3' poly-A "tail" and a 5'-7-methyl guanosine "cap" to pre-mRNA.



Transcription enhancers and silencers

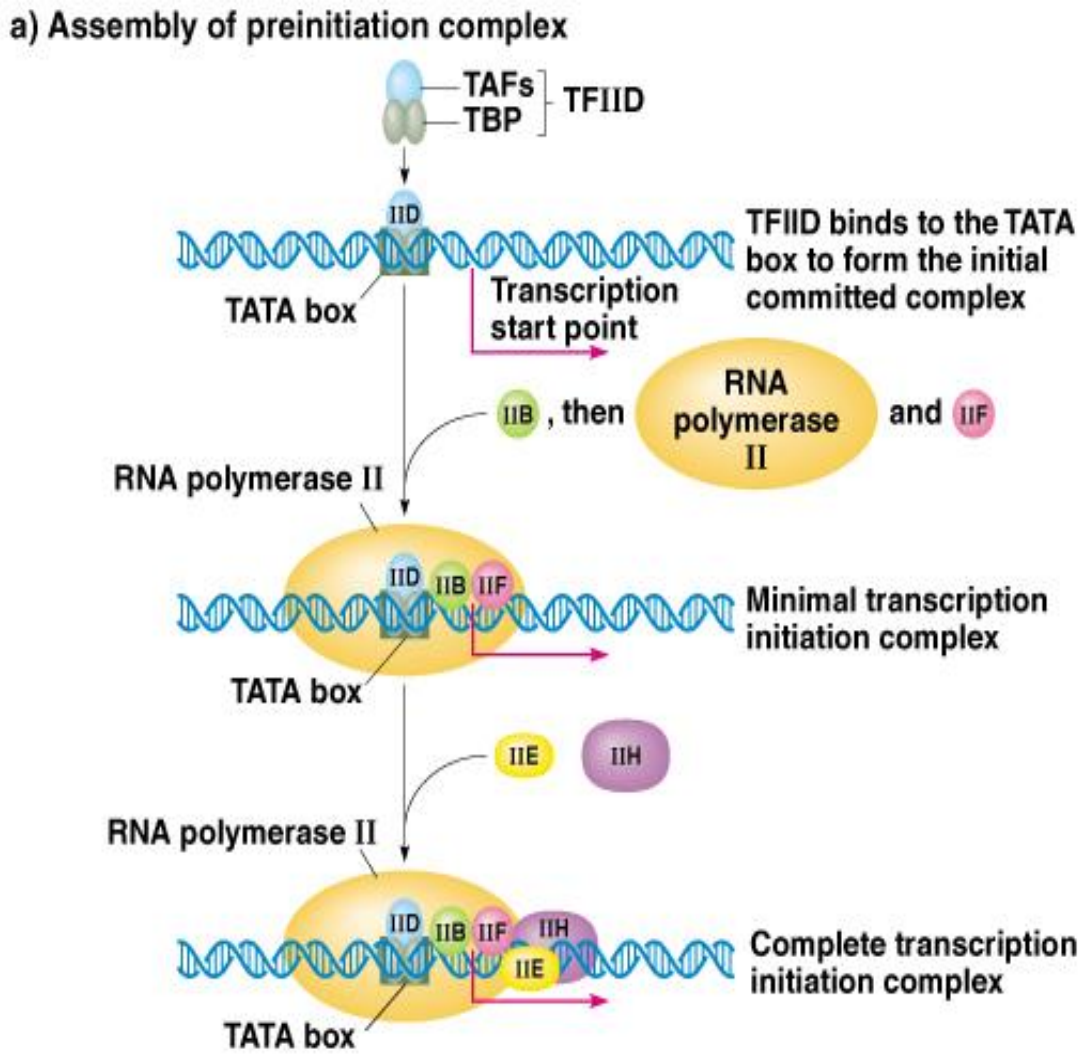
- Both are binding sites for another transcription factors (TF's) **not the previous 7 .**
- **Enhancers**: "non-promoter DNA elements that stimulate transcription"

- They interact with general transcription factors it will promote formation of pre-initiation complex to increase the amount of Transcription from a nearby promoter (core + upstream elements) **, in other word It will increases gene expression as much as 200 fold**

- **Silencers**: Decrease amount of Transcription from nearby promoters, **slowing the formation of PRI**
- **Both not found in promoter,** Initially Defined as being **"Position and orientation independent"**
- **Both** Founds upstream, within **the gene it-self** , or downstream of genes, they function in either orientation (not always true) .
- **both Not active all the time .**
- Sometimes a DNA element can act as an enhancer or a silencer depending on what is bound to it.

THE transcription factors need co-factor to bind to Enhancers or Silencers.

Order of binding is: IID + IIA + IIB + RNA poly. II + IIF +IIE +IIH



1- TBP in TFIID binds to the TATA box
2- TFIIA and TFIIB are recruited with TFIIB binding to the BRE
3- RNA Pol II-TFIIF complex is then recruited
4- TFIIE and TFIIH then bind upstream of Pol II to form the **pre-initiation complex**
- Promoter melting using energy from ATP hydrolysis by TFIIH)
- Promoter escapes after the phosphorylation of the C-terminal domain tail

ترتيب الارتباط+ هاي الخطوات عبارة عن نوتات قديمة للدكتور ، ما حكى الخطوات بالزبط بالترتيب

Post-transcriptional processing of RNAs only in eukaryotic cells:

- In contrast to bacterial transcripts, eukaryotic RNAs (all 3 classes) undergo post-transcriptional processing.
- All 3 classes of RNA are transcribed from genes that contain introns.
  - 3 Types of modifications occur : Capping, polyadenylation and intron splicing
  - Bacterial **rRNAs** and **tRNAs** undergoes no additional processing, after being transcribed they are immediately ready for use in translation.

- Translation of bacterial **mRNAs** can begin even before transcription is completed due to the lack of the nuclear-cytoplasmic separation that exists in eukaryotes and to afford a unique opportunity for regulating the transcription of certain genes.

Post-transcriptional Modification

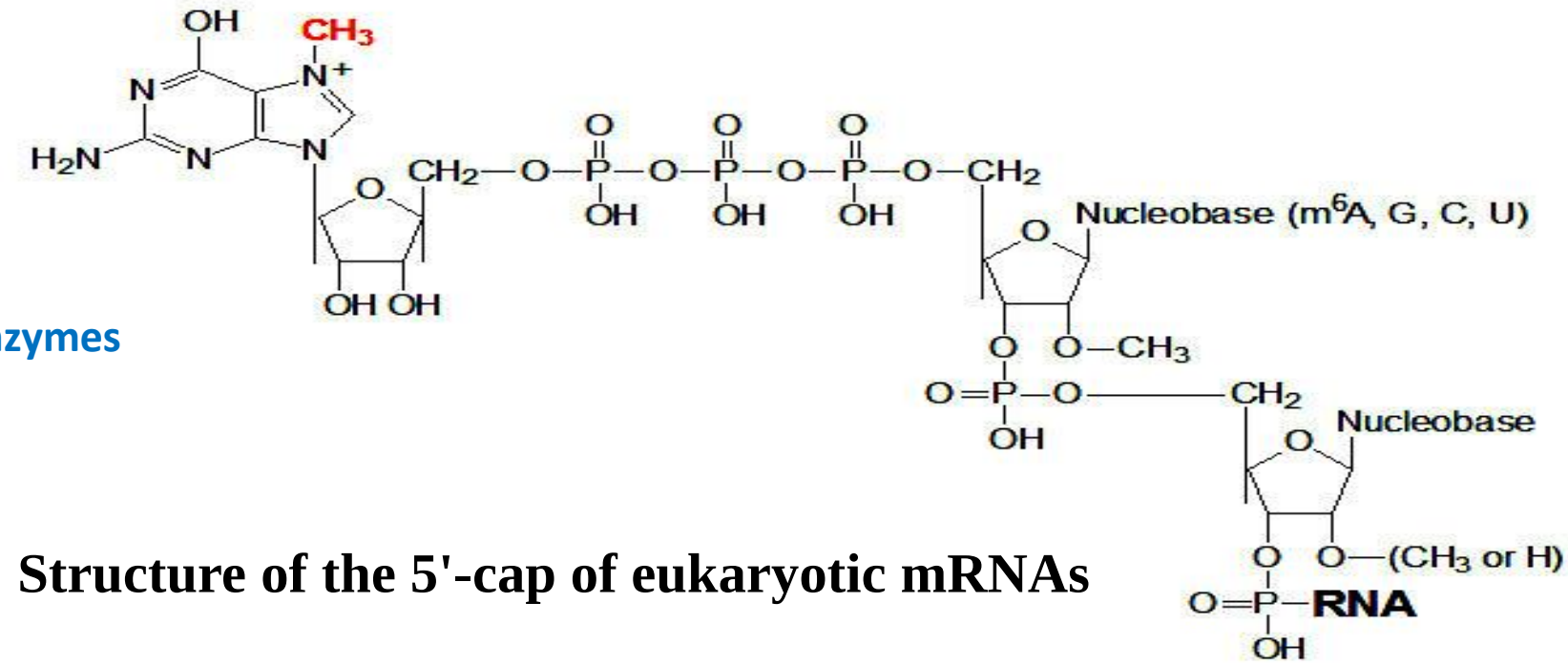
1- Capping: the 5' end of all eukaryotic mRNAs are capped with a unique 5'→5' linkage to a **7-methyl GTP, Why?**

A- The capped end of the mRNA is thus, protected from **exonucleases.**

B- and more importantly is recognized 5` end by specific proteins of the translational machinery.

(Ribosomal Subunit can easily recognize mRNA (differentiate 5' end from 3' end)

- The capping process occurs after the newly synthesizing mRNA is around **20–30** bases long.



Structure of the 5'-cap of eukaryotic mRNAs

This process done by 3 enzymes

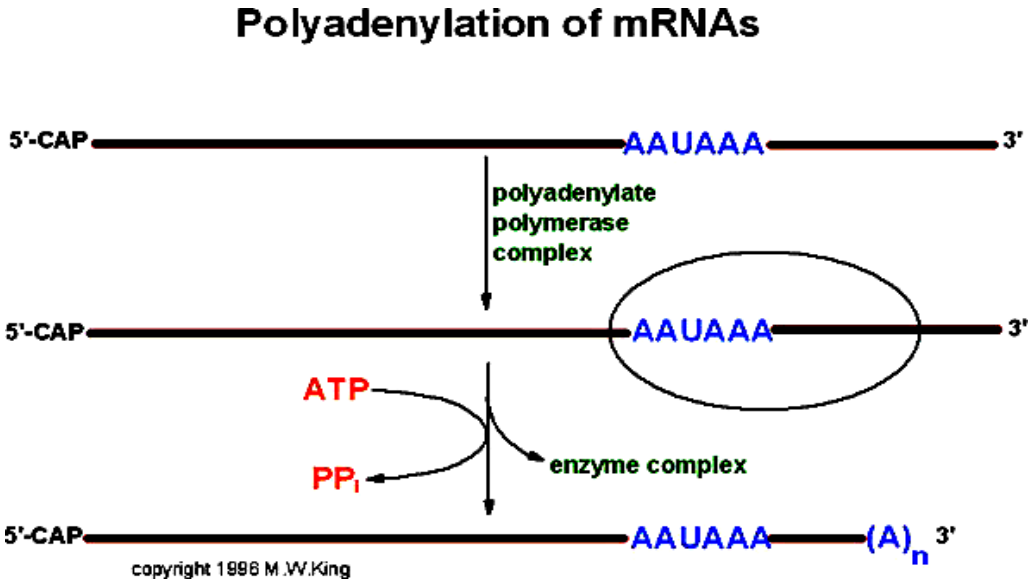
2- Polyadenylation:

Messenger RNAs also are polyadenylated at the 3' end **by polyadenylate polymerase , HOW?**

A specific sequence, AAUAAA, is recognized by the endonuclease activity of by **polyadenylate polymerase** which cleaves the primary transcript approximately 11–30 bases 3' of the sequence element.

- A stretch of 20–250 A residues is then added to the 3' end by the **polyadenylate polymerase** activity.

- **In other words, Polyadenylate polymerase will recognize the polyadenylate segment (**AAUAAA**) at the** 3'end of mRNA after recognition it will make two things :
  A- Work as endonuclease : it will remove 11–30 bases 3' of the sequence element
  B- Polymerase : Add 20–250 AA at 3' end of the sequence element to protect mRNA from the attack of exonuclease



Splicing of RNAs

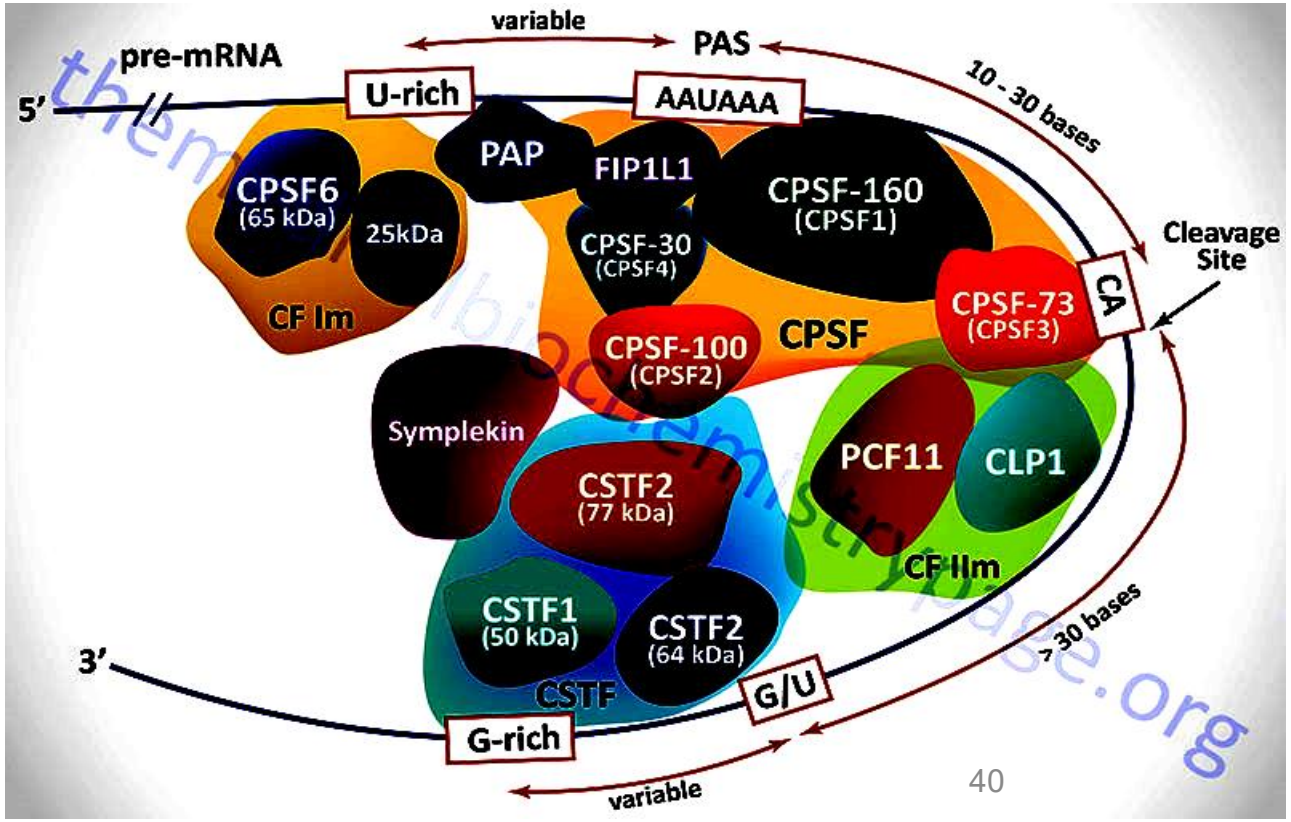
- The sequences encoded by the intronic DNA must be removed from the primary transcript prior to the RNAs being biologically active, this process of intron removal is called **RNA splicing** **which done in nucleus**
- There are several different classes of reactions involved in intron removal.
- The 2 most common are the group I and group II introns.

Group I introns are found in nuclear, mitochondrial and chloroplast rRNA genes,

Group II in mitochondrial and chloroplast mRNA genes.

- Many of the group I and group II introns are self-splicing.

- Group I introns require an external guanosine as a cofactor.



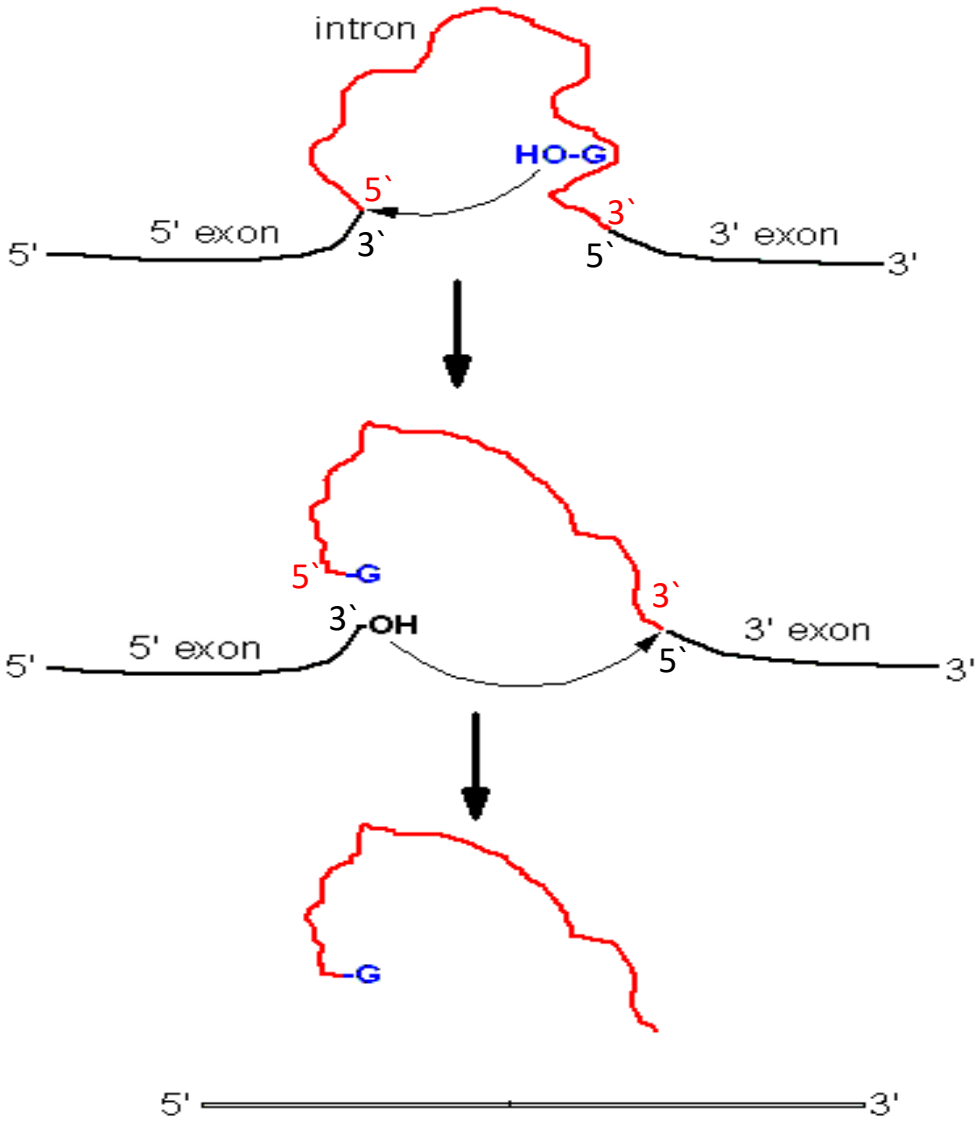
- The removement of intron depends on external guanosine , and this guanosine has a hydroxyl group at position 3

- The 3'–OH of the guanosine nucleotide acts as a nucleophile to **break the phosphodiester bond between 5` exon and intron by** attacking 5'–phosphate of the 5' nucleotide of the intron

- The resultant 3'–OH at the 3' end of the 5' exon then attacks the 5' nucleotide of the 3' exon releasing the intron and covalently attaching the two exons together.

- In this process 2 OH used (one from guanosine and the other from the 3` end of 5` exon)

- The 3' end of the 5' exon is termed the **splice donor site** and the 5' end of the 3' exon is termed the **splice acceptor site**.

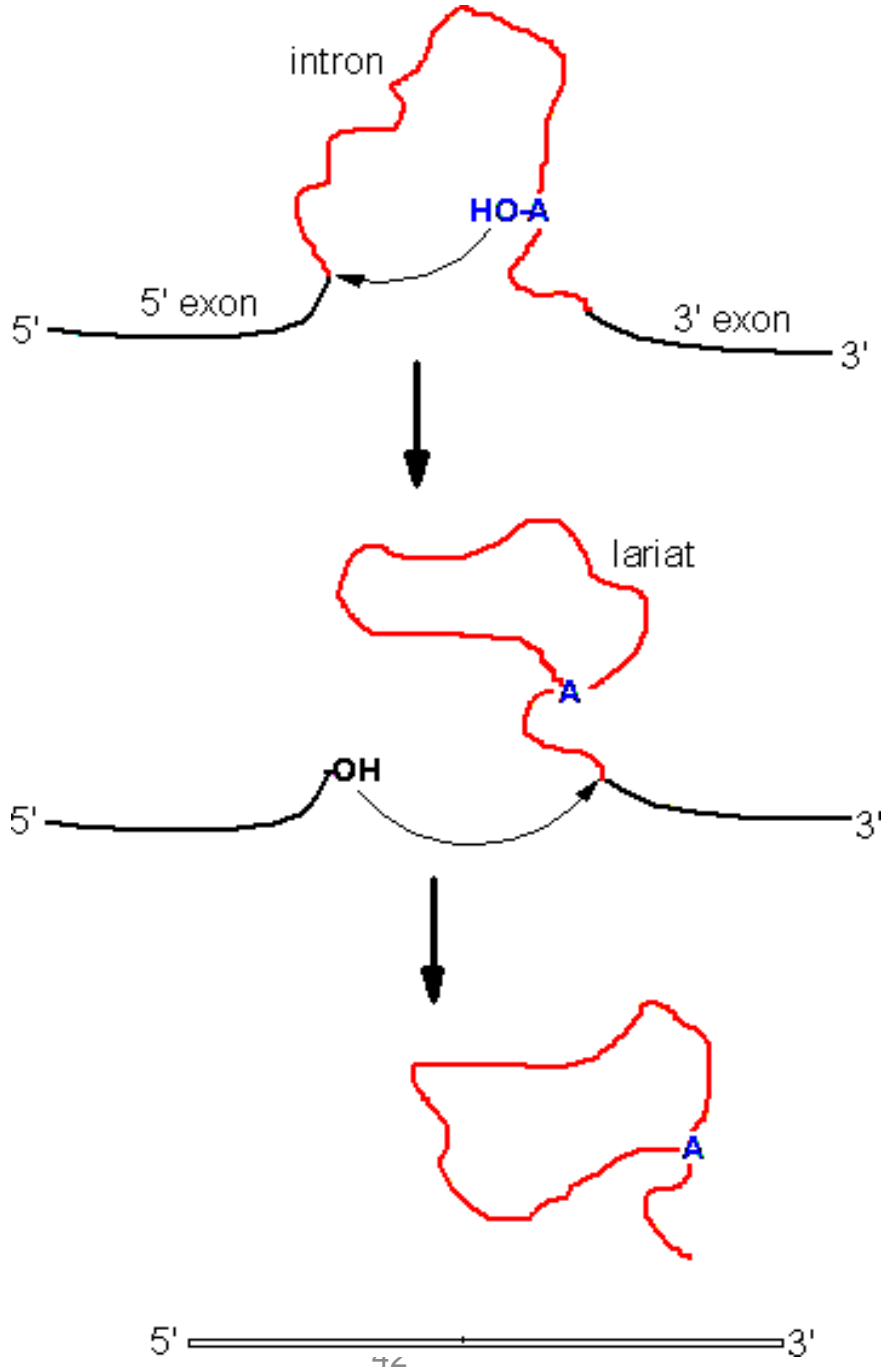


Splicing by group 1 introns

- Group II introns are spliced similarly except that instead of an external nucleophile, the 2'–OH of an adenine residue within the intron is the nucleophile.

- This residue attacks the 3' nucleotide of the 5' exon forming an internal loop called a **lariat structure.**

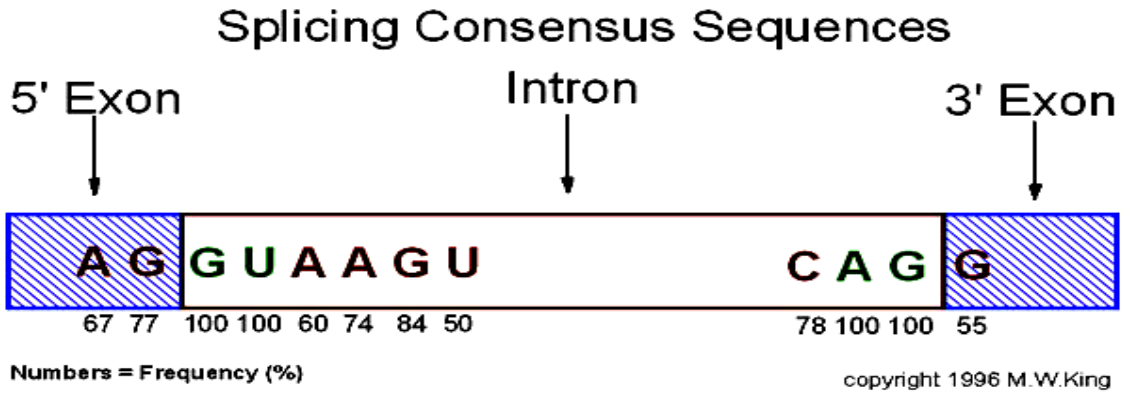
- The 3' end of the 5' exon then attacks the 5' end of the 3’ exon as in group I splicing releasing the intron and covalently attaching the two exons together.

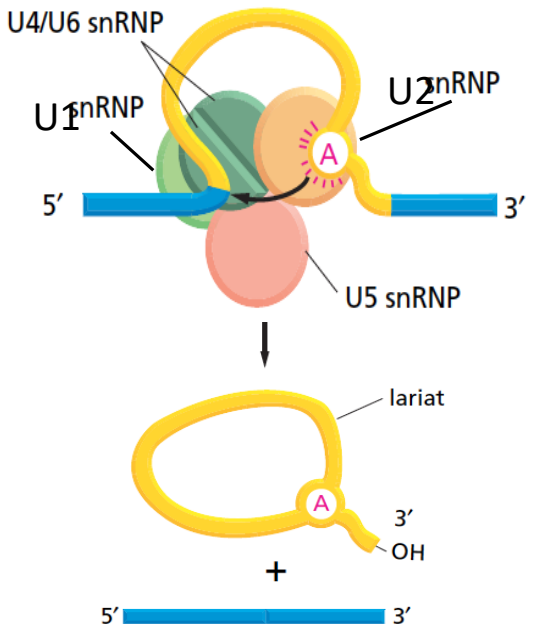


Splicing by group 2 introns

- Group III introns are also the largest class found in nuclear mRNAs, that undergoes a splicing reaction similar to group II introns in that an internal lariat structure is formed.
- However, the splicing depends on specialized RNA–protein complexes called **small nuclear ribonucleoprotein particles (snRNPs).**
-5 types : U1, U2, U4,U5 and U6.
Analysis of a large number of mRNA genes has led to the identification of highly conserved consensus sequences at the 5' and 3' ends of essentially all mRNA introns.

- The U1 RNA has sequences that are complimentary to sequences near the 5' end of the intron, its binding allows distinguishing the GU at the 5' end of the intron from other randomly placed GU sequences in mRNAs.
- The U2 RNA also recognizes sequences in the intron, in this case near the 3' end.
- The addition of **U4, U5 and U6** RNAs forms a complex identified as the **spliceosome** (snRNA plus ~40 proteins) that removes the intron and joins the two exons together.
- U7 is involved in the production of the correct 3‘ ends of histone mRNA which lacks poly (A) tail.



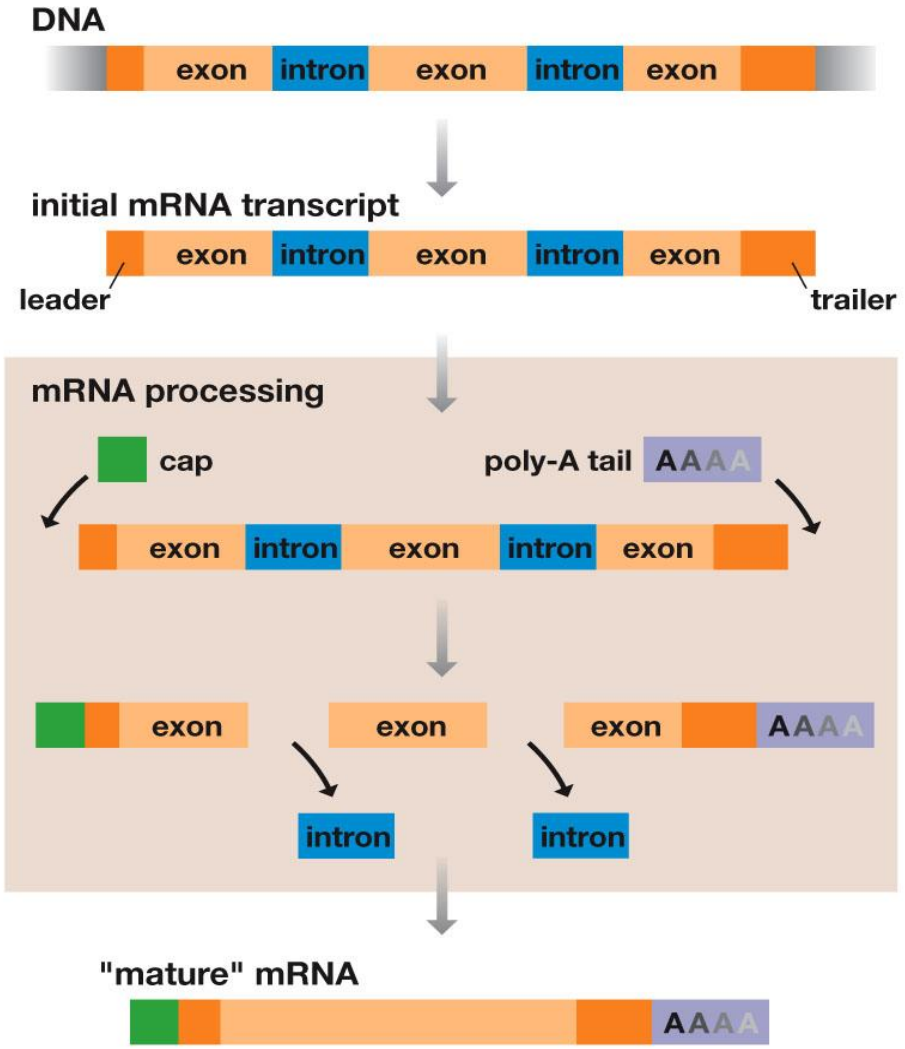


- An additional mechanism of intron removal is the process of tRNA splicing.
- These introns are spliced by a specific splicing endonuclease that involves a cut-and-paste mechanism.

- These introns are spliced by a specific splicing endonuclease that involves a cut-and-paste mechanism.

- In order for tRNA intron removal to occur the tRNA must first be properly folded into its characteristic cloverleaf shape.

- Misfolded precursor tRNAs are not processed which allows the splicing reaction to serve as a control step in the generation of mature tRNAs.



RNA processing: pre-mRNA → mRNA

Modifications of tRNA

1- Removal of 5' extra sequence **by endonuclease enzyme**

2- Addition of:

- CCA at 3' end **because the CCA is a site of attachment of AAs**
- Anticodon loop **remove of introns at this site by endonuclease**

3- Methylation of some bases **like uracil result in Thymine**

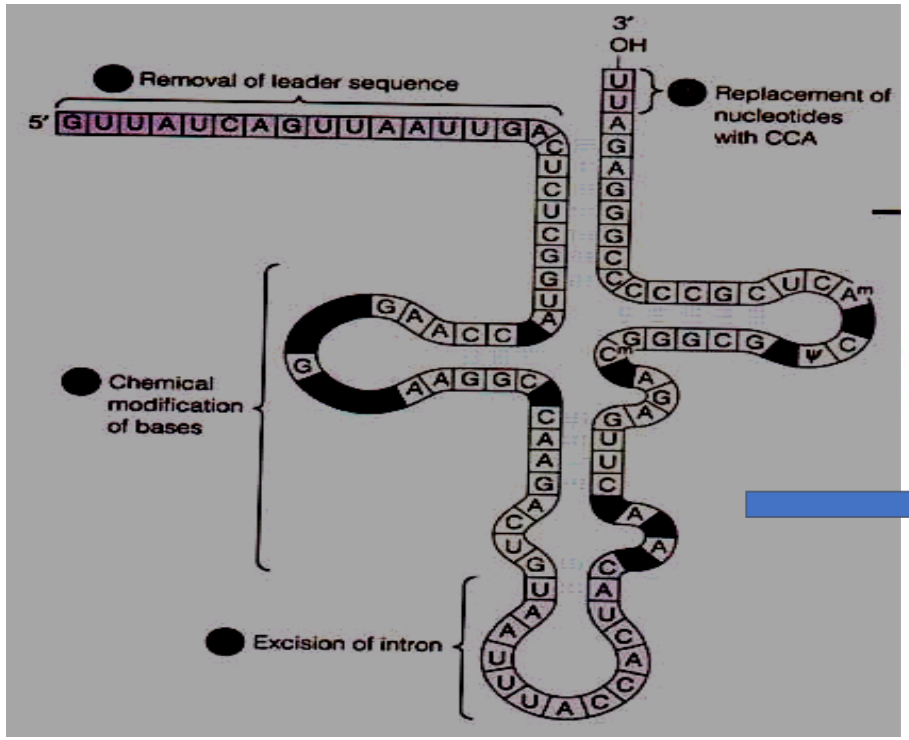
tRNA contain unusual ribonucleotide:

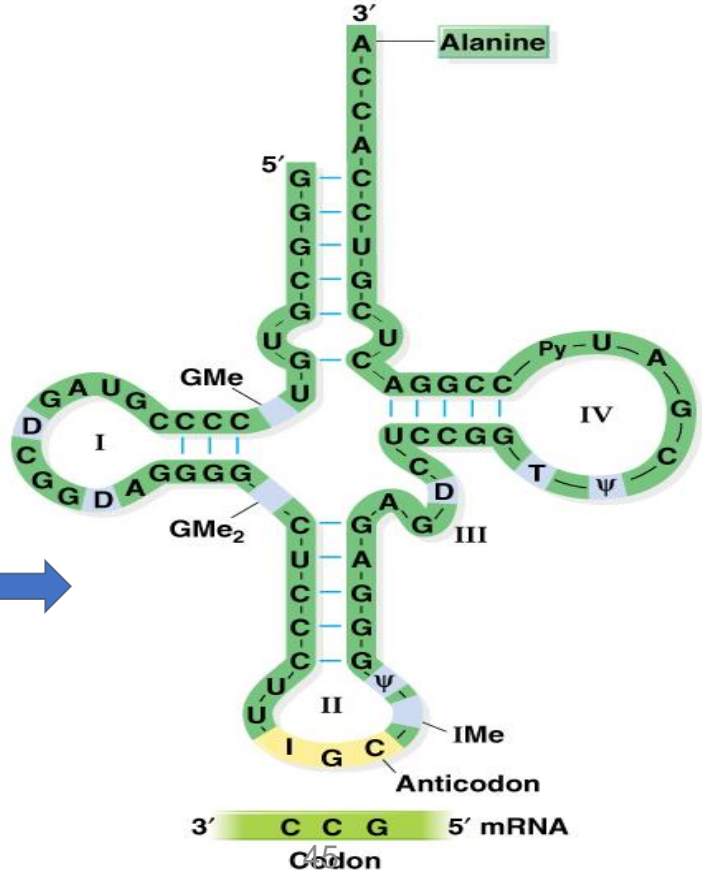
1- Thymine

2- Pseudouridines

3- Dihydrouridine

4- Inosine





rRNA is used to construct ribosomes

- Eukaryotic ribosomal RNA is transcribed in the nucleolus by **RNA polymerase I** as a single piece of **45S RNA**, which is subsequently cleaved to yield **28S rRNA**, **18S rRNA, and 5.8S rRNA.**

- **RNA polymerase III** produce **5S rRNA** unit from a separate gene.

- The ribosomal subunits assemble in the nucleolus as the rRNA pieces combine with ribosomal proteins.

- Eukaryotic ribosomal subunits are 60S and 40S.

- They join during protein synthesis to form the whole 80S ribosome.

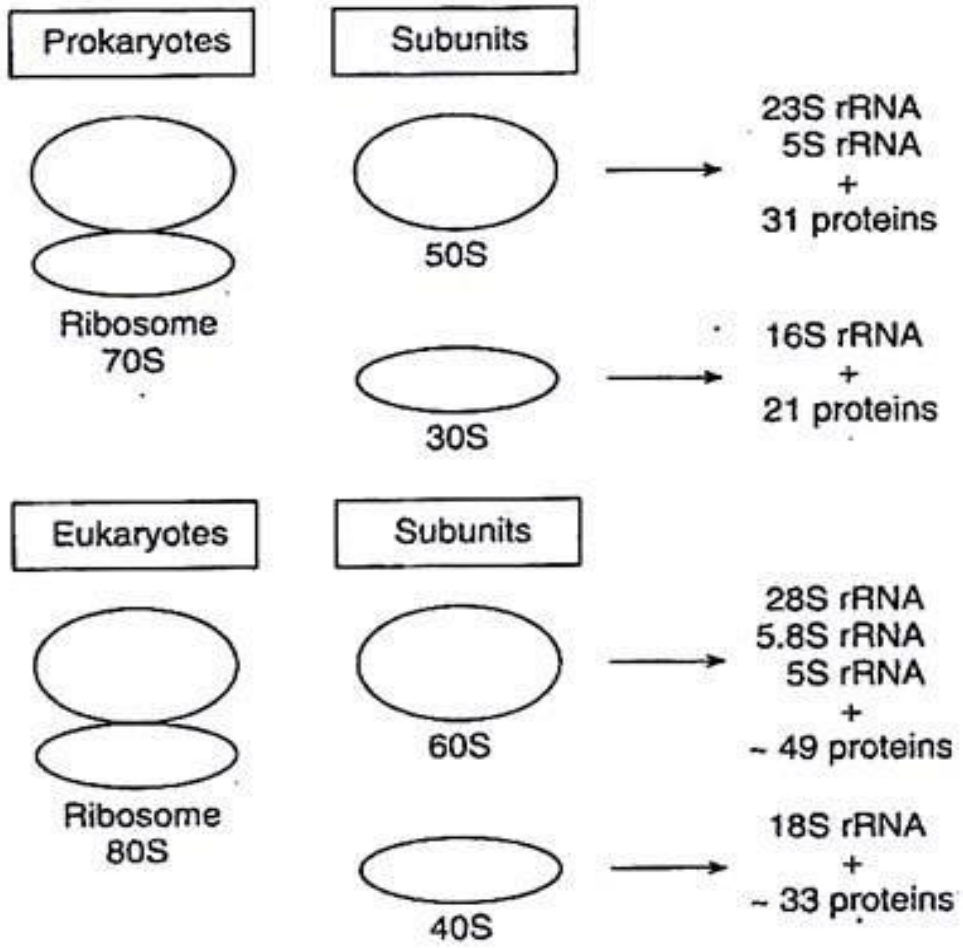


Fig. 2.47: Composition of typical prokaryotic and eukaryotic ribosomes