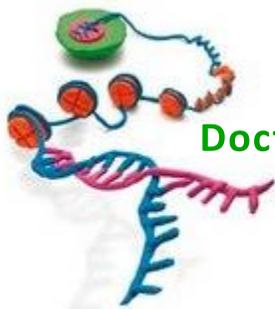




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Molecular biology sheet



Doctor 2021 -mercy- | medicine | MU

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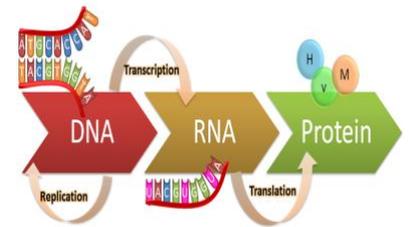
DOCTOR

Dr. Nesrin Mwafi

Transcription of Genes

The first and foremost fundamental idea of molecular biology is called central dogma.

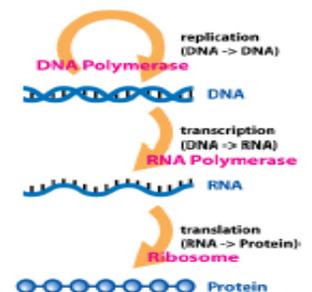
For cells to produce and divide, there must be DNA replication. DNA is transcribed to RNA; RNA is translated to protein. DNA replication must occur before cell division, even in zygote (meiosis). Why? To maintain the same number of chromosomes in somatic cells. DNA replication occurs with high fidelity (اخلاص) with no error. Why does DNA replication occur with no errors? Because DNA polymerase enzyme has proof-reading activity. From where do instructions for making proteins come from? From genes. We have 25,000 genes. Messenger RNA (mRNA) is translated to protein



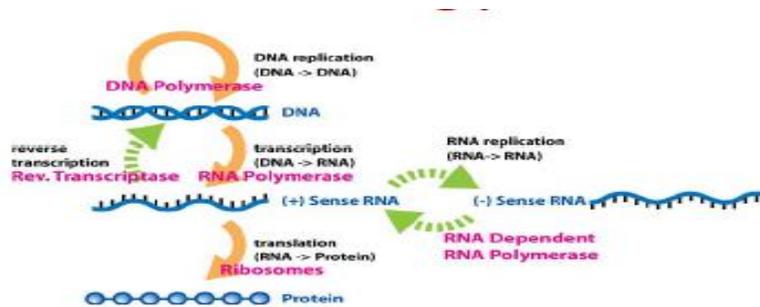
The Central Dogma of Molecular Biology

- Central dogma describes the flow of genetic information in living organisms
- Gene expression: is the process by which information from a gene is used in the synthesis of functional gene products (proteins or functional RNAs)
- Transcription is the first step in gene expression

Amino acid sequence in proteins is derived from mRNA sequence from DNA sequence. Gene expresses itself by: synthesis of proteins OR sometimes functional RNAs other than mRNA. Of the 25,000 genes, there is a large portion called protein-coding genes (Meaning that they convert to mRNA, and mRNA converts to protein.) And there is a small portion of genes called non-protein-coding genes (Meaning that they only convert to functional RNAs other than mRNA, such as transfer RNA and ribosomal RNA, microRNA, small nuclear RNA.



Gene expression = transcription + translation OR Gene expression = transcription



The first one who used the term Central Dogma is the British molecular biologist **“Crick, 1957”** and he said that: DNA makes RNA which makes protein but once protein is synthesized, it can't flow back to nucleic acids. We took with Dr. Jehad the double-helical structure which Crick and Watson discovered in 1953 and awarded Nobel Prize.

So far, this theory holds true; no scientific research has been known to refute this theory.

According to the theory, translation is irreversible, there is no way to convert protein to mRNA. The theory also describes directionality.

Also, scientists discovered another two biological processes, becoming updated (recent, modern) central dogma.

1. Reverse transcription: RNA to DNA. Specific viruses convert RNA to DNA sequence, it is catalyzed by reverse transcriptase enzyme. It is both in vivo (microorganisms) and in vitro. However, transcription is catalyzed by RNA polymerase enzyme. In lab, ssDNA (single-stranded DNA) is synthesized from RNA by RT Kit (Reverse Transcription Kit).

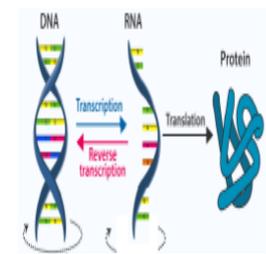
2. RNA Replication: RNA is copied from RNA (it is in some viruses).

There is no such thing called protein replication (protein is not replicated or converted to mRNA or DNA).

General	Special	Unknown
DNA → DNA	RNA → DNA	protein → DNA
DNA → RNA	RNA → RNA	protein → RNA
RNA → protein	DNA → protein	protein → protein

According to probability, we have 3 elements (DNA, RNA, and protein) there are 9 probabilities. 3 of these 9 are general transfer, and another 3 is special (or specific) transfer. The last 3 are impossible to happen and are unknown

- General transfer Occurs normally and naturally inside our cells
- Specific transfer Occurs under specific conditions (either in some viruses or in lab experiments)
- Unknown transfer.



Function of different RNA Molecules

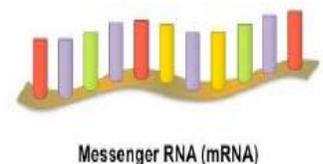
- There are different types of RNA molecules:

1. **Messenger RNA (mRNA):** it is produced from the transcription of protein-coding genes.

In eukaryotic (It occurs inside nucleus and mature RNA goes to cytoplasm to begin translation) cells, pre-mRNA (primary transcript, or precursor RNA, or pre messenger RNA) is modified to mature mRNA

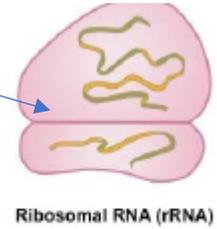
. In prokaryotes (It occurs in cytoplasm) transcript means RNA. Primary transcript means pre-mRNA. Pre-mRNA undergoes post-transcriptional modification to become mature RNA and then translation. However, in prokaryotes there is no post-transcriptional modification; because prokaryotes are simple and have less chromosome number.

single mRNA (bicistronic (making two related proteins from a single messenger RNA molecule) / polycistronic transcript) (More than two) codes for different proteins



2. **Ribosomal RNA (rRNA):** are specialized RNA molecules synthesized in the nucleolus (from the transcription of non-protein-coding genes).

In the cytoplasm, they bind with protein, the pink here is protein to form ribosomes (the machinery to synthesize proteins) When messenger RNA exits the nucleus, it goes to ribosomes and translation starts. Ribosomes are protein synthesis factors.

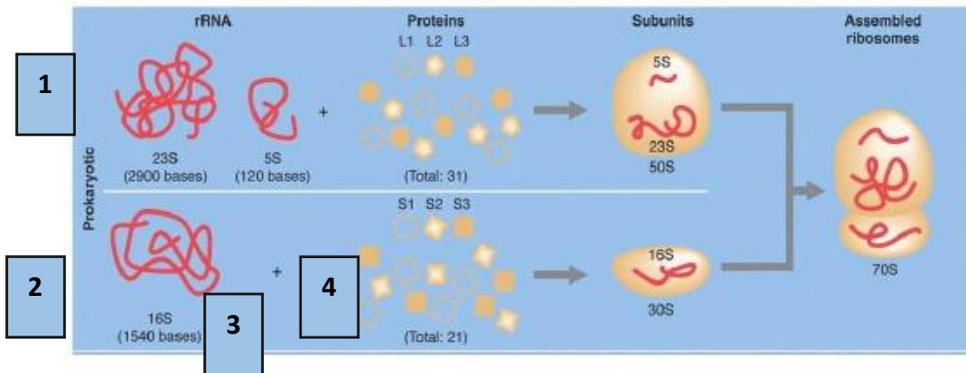
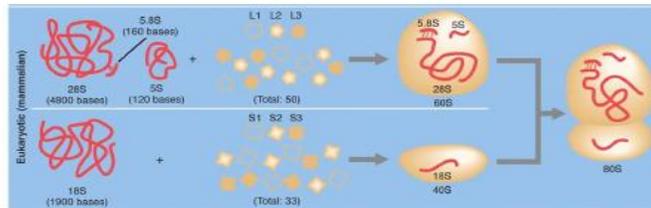


Function of different RNA Molecules

Ribosome consists of small and large subunits. In eukaryotes (80S) the small subunit (40S) consists of 18S rRNA and 33 proteins while the large subunit (60S) contains: 5S rRNA, 5.8S rRNA, 28S rRNA and 50 proteins In eukaryotic cells, we call the ribosome 80S, it consists of small subunit (40S) → has one type of rRNA (18S) and 33 proteins large subunit → has 3 types of rRNA (5S rRNA, 5.8S rRNA, and 28S rRNA and 50 proteins

...S is a non-additive unit used to measure sedimentation rate.

S stands for **Svedberg unit** (a measure of sedimentation rate depending on particle size)



In prokaryotes like bacteria, the ribosome (70S) consists of the small subunit (30S) and the large subunit (50S)

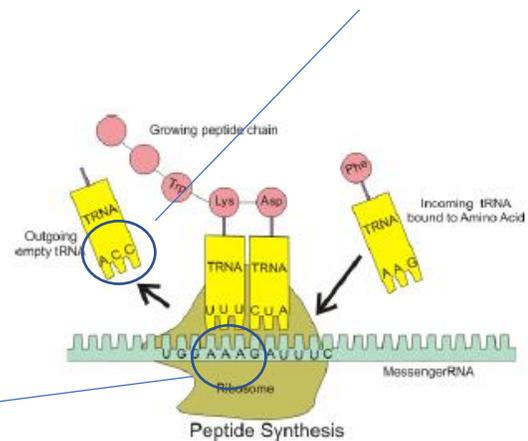
They can be determined in gel electrophoresis

3. Transfer RNA (tRNA): are specialized

molecules (adaptors) that collect the proper amino acid, bring it to the ribosome and attach it to the growing polypeptide chain

It is involved in translation, a type of functional RNAs, transcribed from non-protein-coding genes. They are called adaptors, because they read codons one time each 3 nitrogenous bases

Codon (nitrogenous base)



So far, there are 3 RNA types involved in translation:

1. mRNA: contains the codons
2. rRNA: makes ribosomes which are sites for protein synthesis
3. tRNA: is adaptor that brings amino acids according to the codon

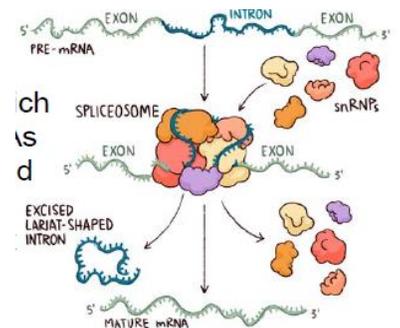
4. Small nuclear RNA (snRNA): is a class of small

RNA molecules (150 nucleotides) which play an important role in pre-mRNA processing by removing introns (RNA splicing).

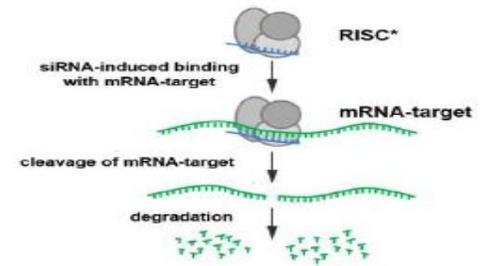
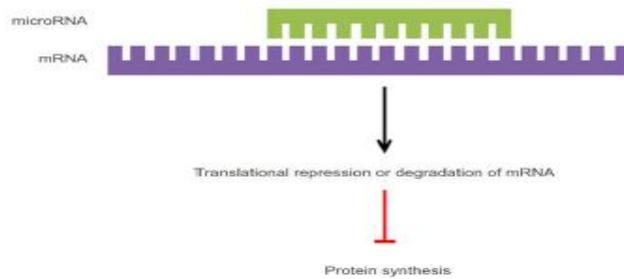
snRNAs are main components of spliceosome (a large protein-RNA complex) which consists of 5 types snRNAs (U1,U2,U4,U5 and U6) and over 150 proteins.

The RNA-protein complex is known as snRNPs (small nuclear ribonucleoproteins) or “snurps”.

They have a role in post-transcriptional modification, particularly in a process called RNA splicing, they cut a portion in RNA called “introns,” and assemble exons with each other. Spliceosome does this. It consists of 5 types of snRNAs (U, 1,2,4,5,6)



Small regulatory RNAs: short RNAs with average size of 21-27 including small interfering RNA (siRNA) and microRNA (miRNA) have a role in gene silencing (prevent translation of mRNA) and regulation of gene expression by basepairing with complementary sequence of target mRNA molecules.



siRNA goes to target mRNA and binds to complementary sequence on it and makes degrades it each time the mRNA is transcribed from the gene (specific gene) as if the gene were not existing.
 miRNA either degrades the target mRNA OR blocks binding site of ribosome in translation process. Both inhibit protein synthesis (translation)

- **RNA polymerase:** is an enzyme which synthesizes RNA from DNA template
- Three classes of RNA polymerases in Eukaryotic (In prokaryotes only one) cells:

Form	Product	
RNA Polymerase I	All rRNAs except 5S rRNA	No mRNA
RNA Polymerase II	All mRNAs, miRNAs, some snRNAs	There is mRNA, that's what we'll take about
RNA Polymerase III	All tRNAs, 5S rRNA, other small RNAs	No mRNA

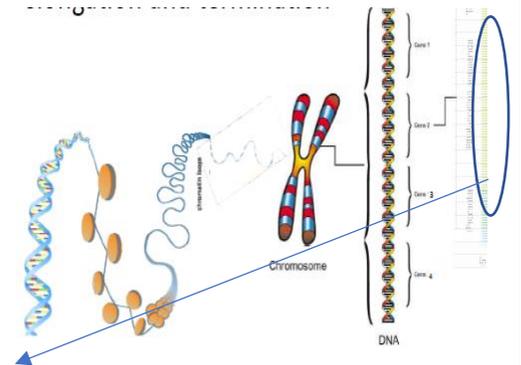
This enzyme is responsible for catalysis in transcription.
 In replication, DNA polymerase enzyme catalyzes.

- Gene transcription in eukaryotes is more complex compared to prokaryotes
 - Stages of transcription: initiation, elongation and termination (In both eukaryotes and prokaryotes, stages are three)
- Site in eukaryotes: nucleus
 Site in prokaryotes: cytoplasm

Each gene will undergo transcription. RNA polymerase will bind to transcription initiation site of the gene and reads it and transcribes it to mRNA. When it reaches termination site, it stops and goes to another gene

Is polymerase RNA smart and can distinguish initiation and termination sites of gene? Yes!
Because gene has the same composition(it has promotor)

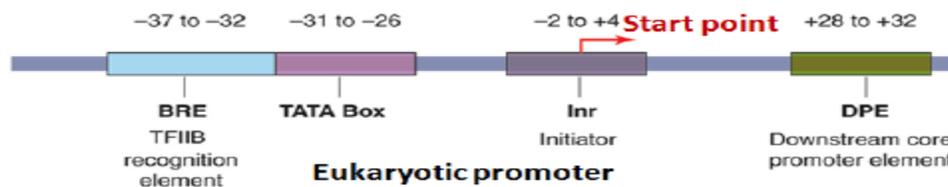
Coding region (+1), RNA polymerase starts here.
If in coding region A,G,C RNA polymerase will add



U,C,G

Transcription Initiation

- **Promoter:** is a stretch of DNA sequence (non-coding DNA) (meaning that codons don't convert to mRNA) where RNA polymerase can bind to start the transcription. Promoters are located upstream the genes that they regulate
- Promoters are found in Eukaryotes and Prokaryotes
- Eukaryotic promoter is a short DNA sequence (~100bp) which consists of (4 types of) consensus sequences (sequences with specific order) such as TATA box, BRE, INR and DPE



Start point (TSS) is numbered +1 (the first nucleotide to be read by RNA polymerase and transcribed to mRNA. Any nucleotides following the TSS are given positive numbers and assigned as downstream, and any nucleotides preceding TSS are given negative numbers and assigned as upstream. TATA Box contains Thymine and Adenine. BRE where the protein TFIIB (recognition element). Initiator is similar to TATA Box. In most genes, the promoter is TATA Box, if it does not exist, the promoter is the Inr (Initiator)

All promoters have 2 or maximum 3 out of 4 consensus sequences. DPE does not always exist.

- General transcription factors (GTFs): are DNA-binding proteins which recognise specific regions in promoter and correctly position the RNA polymerase at the transcription start site (TSS)
- There are five types of GTFs: TFIIB, TFIID (TBP and TAFs subunits), TFIIE, TFIIIF and TFIIH

They are used by RNA polymerase II, and called general because they are needed by all genes. They have DNA-binding domain, they bind with specific region in promoter. That's why they are called DNA binding proteins.

Table 6-3 The General Transcription Factors Needed for Transcription Initiation by Eucaryotic RNA Polymerase II

NAME	NUMBER OF SUBUNITS	ROLES IN TRANSCRIPTION INITIATION
TFIID		
TBP subunit	1	recognizes TATA box
TAF subunits	~11	recognizes other DNA sequences near the transcription start point; regulates DNA-binding by TBP
TFIIB	1	recognizes BRE element in promoters; accurately positions RNA polymerase at the start site of transcription
TFIIIF	3	stabilizes RNA polymerase interaction with TBP and TFIIB; helps attract TFIIE and TFIIH
TFIIE	2	attracts and regulates TFIIH
TFIIH	9	unwinds DNA at the transcription start point, phosphorylates Ser5 of the RNA polymerase CTD; releases RNA polymerase from the promoter

حفظ

TFIID is composed of TBP and ~11 additional subunits called TAFs (TBP-associated factors); CTD, C-terminal domain.

Subunit=polypeptide. TFIID has two parts:

1. TBP → TATA-binding protein has 1 subunit.
2. TAF → TBP-Associated Factors bind to other sites in promoter, and regulate DNA-binding by TBP

TFIIB consists of 1 subunit and binds to BRE.

TFIIIF consists of 3 subunits, it holds RNA polymerase to go to promoter and attracts TFIIE and TFIIE attracts TFIIH. (TFIIIF attracts TFIIE and TFIIE attracts TFIIH)

TFIIH works as an enzyme with two functions:

1. Helicase activity
2. Kinase activity

- Pre-initiation complex

(PIC) formation: is the assembly of GTFs along with RNA polymerase II at the promoter region. It is an important step in the transcription initiation process

- TFIID initially binds TATA box region via its TBP subunit. TFIIB subsequently binds BRE region of the promoter

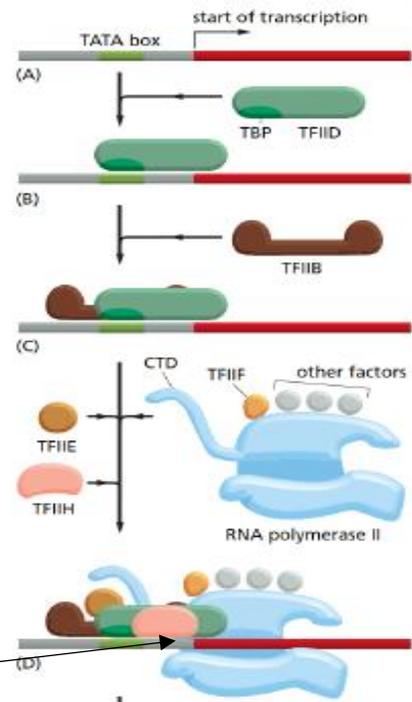
For initiation, we have promoter + 5 general transcription factors + RNA polymerase II. General transcription factors guide RNA polymerase II to the promoter.

First GTF to bind is TFIID by TBP to TATA Box.

Then, TFIIB binds to BRE region in promoter.

Then, TFIIF goes to RNA polymerase II and attracts TFII E and TFII H to go to promoter.

+1 Start point



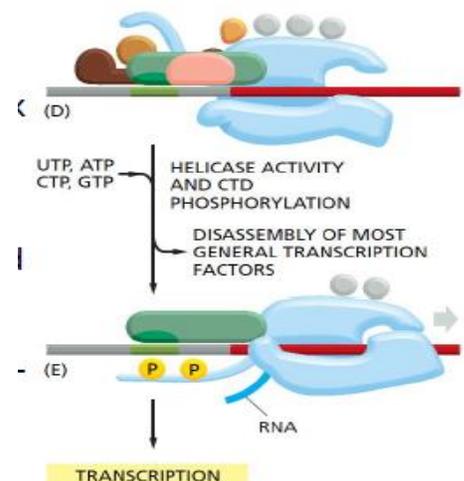
TFIIH can perform several enzymatic steps required for the transcription elongation.

1. It unwinds (unzips) the double helix at the transcription start site (TSS) via its helicase activity (Separates two strands from each other) (Breaks the first bond in start site).
2. It phosphorylates (adds phosphate group at C-terminus) Ser5 located at the tail of RNA polymerase II called CTD (C-terminal domain) via its kinase activity. The CTD tail consists of 52 tandem repeats of 7-amino acids sequence (Ser2,Ser5). This event is important for RNA polymerase II to escape the promoter and start elongation

TFIIH is the last GTF to bind to polymerase II

Phosphatase = dephosphorylation

Kinase = phosphorylation



- Elongation: the extension of the newly synthesized transcript
- The template strand (antisense strand, -): 3'-5' strand which is used to synthesize the RNA molecule. It is complementary to RNA or transcript strand
- The coding strand (sense strand, +): 5'-3' strand which resembles the mRNA strand except T is replaced with U and deoxyribose is changed to ribose

The strand that polymerase II reads is antisense strand (negative) mRNA is sense. Coding strands resembles it

- RNA polymerase II can only read 3'-5' DNA strand and elongate the growing RNA strand in the 5'-3' direction (add only to free 3' end) It also has helicase activity like TFIIH
- A polymerase II unwinds/ unzips the helical DNA and creates a bubble that exposes the single strands

of DNA during the elongation process

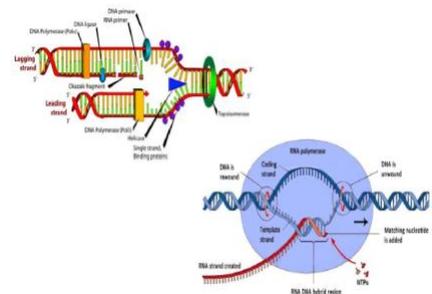
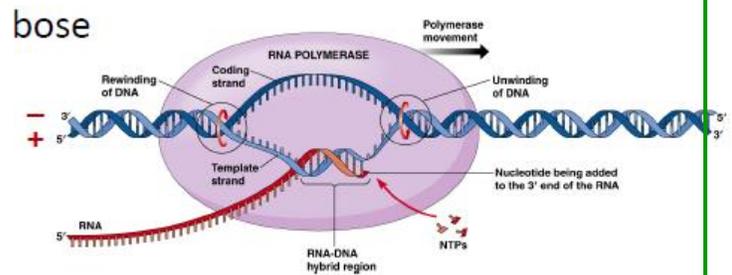
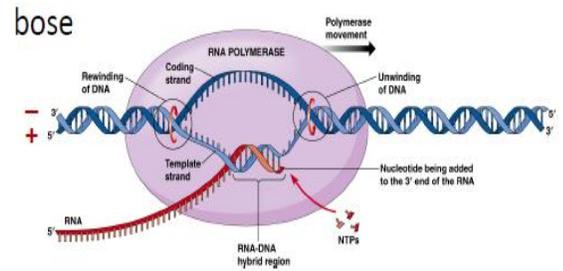
- Transcription bubble: is a region (10-20 nucleotides) where the two single strands of DNA are separated and exposed

Transcription bubble moves along the coding region with RNA polymerase II. Why does RNA polymerase II read template strand? Because it reads from 3' to 5' and adds nucleotides to free 3' end. And elongates 5' to 3' end in RNA.

DNA & RNA polymerases

- What are the differences between DNA polymerase and RNA polymerase enzymes?

- 1- DNA polymerase is responsible for replication; RNA polymerase is responsible for transcription
- 2- Replication occurs with high fidelity due to proof-reading activity.
- 3- RNA polymerase has no proof-reading, it has helicase activity; it opens transcription bubble (10-20 nucleotides)



How does termination occur? So far, it is not well known, but once RNA polymerase reaches termination site, it detaches and begins another transcription process. It is also dephosphorylated by phosphatase

Transcription Termination

- **Transcription termination: in eukaryotes is not well understood. RNA polymerase II meets a termination signal and knows that it is the end of transcription so will stop at that point.**
 - RNA polymerase II detaches from the template strand to initiate another round of transcription.
 - To reinitiate transcription, soluble phosphatases remove the phosphates on CTD tail of RNA polymerase II

The mature mRNA will leave to cytoplasm to start the second step in gene expression: **Translation**

كم يرفع العلم أشخاصًا الى رتب
ويخفض الجهل أشخاصًا بلا أدب
ليس اليتيم يتيم المال والأب
إن اليتيم يتيم العلم والأدب