Chapter 17 : From Gene to Protein

Over View

- **Genetic material** : -is the information content of DNA
  Or – it is specific sequences of nucleotides along strands of the DNA .

- The DNA inherited by an organism leads to specific traits by dictating the synthesis of proteins .
  -Proteins are the links between genotype and phenotype (this is the main point of this chapter**).**

- **Gene expression**: the process by which DNA directs protein synthesis, includes two stages: Transcription and Translation

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Concept 17.1 : *Genes specify proteins via transcription and Translation*.

-How was the fundamental relationship between genes and proteins discovered?

- **Evidence from the Study of Metabolic Defects**
  - In 1909, British physician Archibald **Garrod** first suggested that genes dictate phenotypes through enzymes that catalyze specific chemical reactions in the cell .
    So , genes dictate the production of a specific enzyme , and the inability to synthesize a certain enzyme leads to inherited disease.
    - Biochemists accumulated much evidence that, cells synthesize and degrade molecules via metabolic pathways , in which each chemical reaction is catalyzed by a specific enzyme .(such metabolic pathway leads to synthesis of pigments that give the color of eyes of fruit fly)

➲ ***Nutritional Mutants in* Neurospora**
- Scientific Inquiry

- George Beadle and Edward Tatum exposed bread mold called (neurospora crassa)to X-rays, for creating mutants (they wanted mutants which are unable to survive in minimal medium .

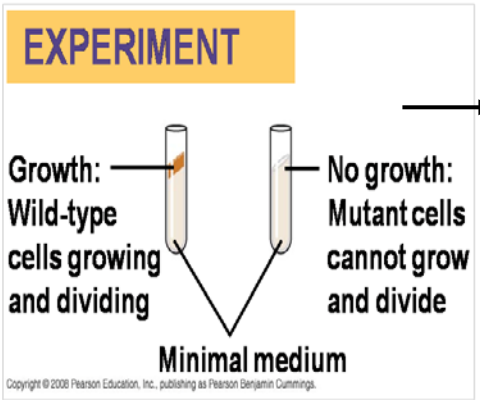
Note : in minimal medium wild-type bread mold can survive ; by their metabolic pathways they produce all the other molecules they need , while mutants couldn't because they were unable to synthesize certain essential molecules from the minimal ingredients , so they need COMPLETE MEDIUM or minimal medium with additional nutrients , to grow .

what did Beadle & Edward do ?

- they want to charecterize the metabolic defect in each mutant , they took samples from grown mutant and distributed them in vials (vials contained minimal mediums+single additional nutrient) ; **the particular additional nutrient that allowed the growth indicated the metabolic defect** .

- for example if the suplemented vial that supported growth of the mutant was the one contain amino acid (argenine) they will conclude that mutant was defictive in synthesise of (argenine) .
Note : Argenine biosynthesis involved precursor nutrient + intermediate molecules (ornithine & citruline) together. And that needs many enzymes .

their expierment below is about (**Do individual genes specifiy the enzymes that function in abiochemical pathway)**



- mutant cell cannot grow because it needs areginine (it cant produce it)

- mutants that required argenine in their growth are fell in 3 classes , each lacking a different necessary enzyme for synthesizing argenine .

-they grew each one of these 3 classes into 4 conditions to know what the differences between these 3 classes of mutants .

the results are in the figure >>

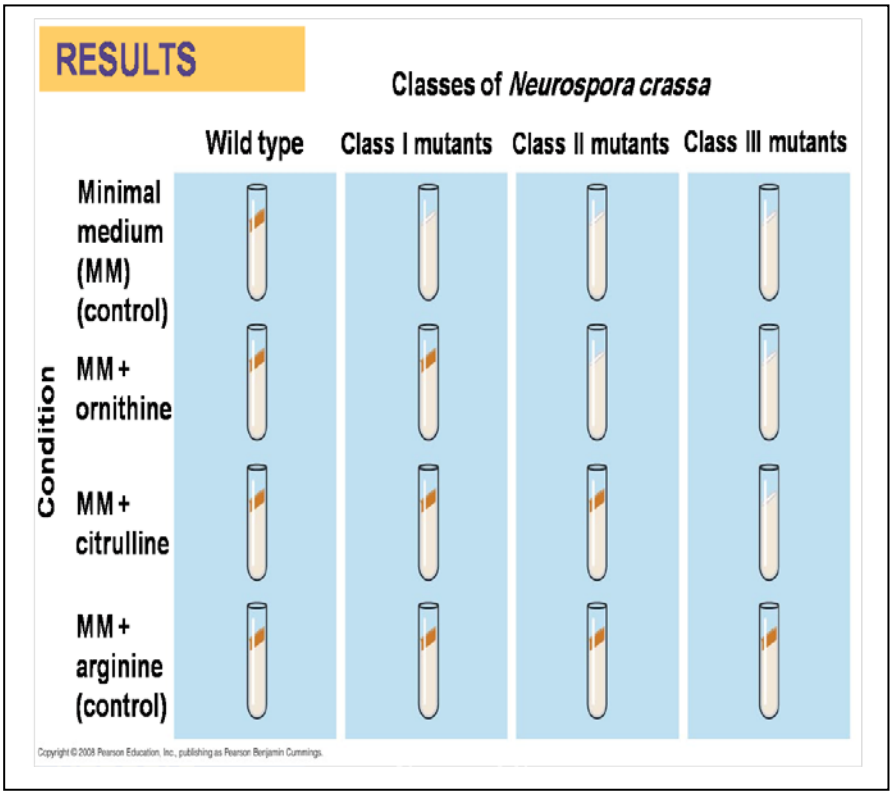
(MM) means (minimal medium)

1st column : wild type was capable to growth in all conditions , requiring only the minimal medium .

1st row : no nutrient was added to the minimal medium (it's pure precursor nutrient) so mutant cell wasn't grown in any one .

4th row : argenine was added directly so the mutant cell was grown in the 3 classes .

-**the three classes of mutants each had a specific s growth requirements** , for example (class ll) mutants couldn't grow when ornithine alone but could grow when either citrulline or argenine was added .



التجربة معقدة قليلا لكن الاستنتاج النهائي بوضّح كل فكرتها ، أكمّل للنهاية ... ☺

Conclusion : - Each class of mutant was unable to carry out one step in the pathway for synthesizing argenine , because it lacked the necessary enzyme which catalyze that blocked step.

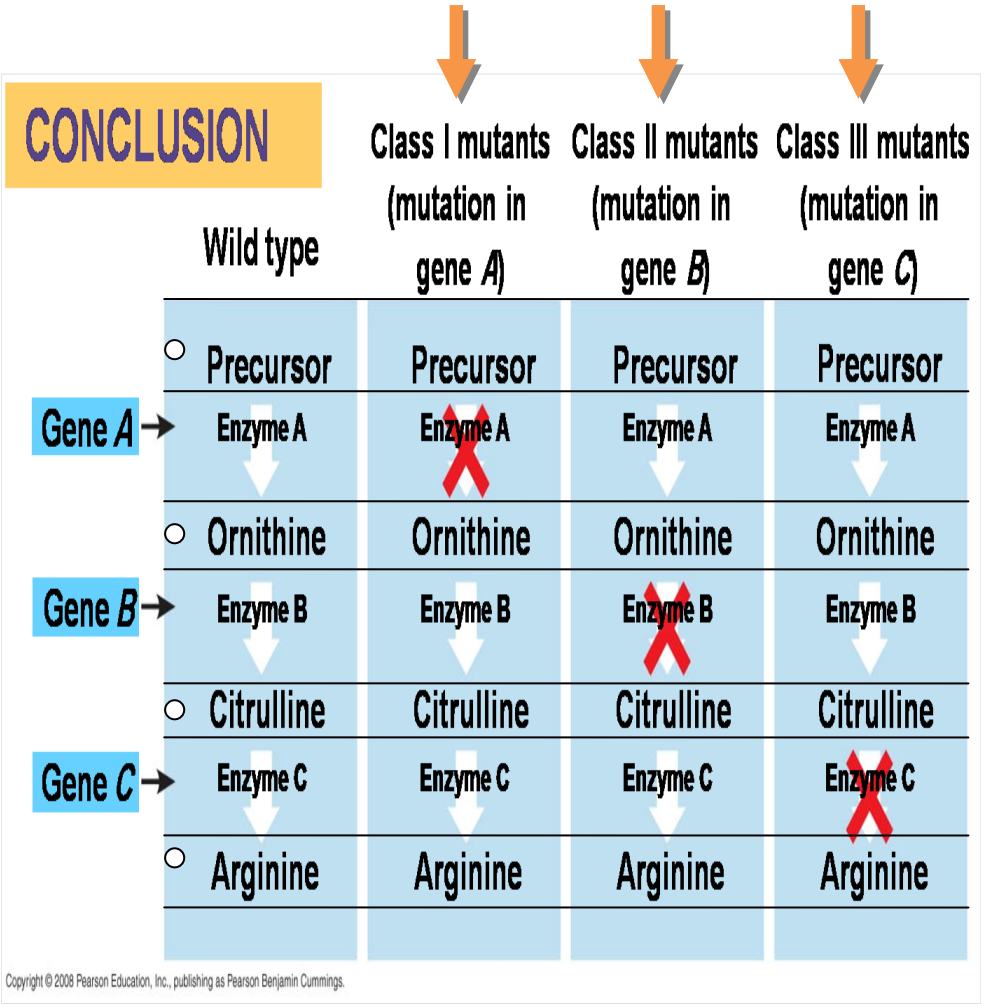
Steps of argenine synthesis :

1.synthesis (**ornithine**) needs (**precursor nutrients**) and (**enzyme A**)

2. synthesis (**Citrulline**) needs (**Ornithine**) and (***enzyme B***) .

3. synthesis (**Argenine**) needs (**Citrulline**) and (***enzyme C***).

In another word >>
No enzyme A >it needs a ready ornithine to begin the synthesis way of argenine …



From this inquiry >>

- **they concluded that each mutated gene must normally dictate the production of one enzyme .**

-They developed a(***one gene–one enzyme hypothesis***), which says that the function of the gene is to dictate production of a specific enzyme .

- **But ,** researchers later revised the hypothesis: *one gene–one protein* because Some proteins aren't enzymes and Many proteins are composed of several polypeptides, each one has its own gene .

Beadle and Tatum's hypothesis is now restated as the ***one gene–one polypeptide hypothesis*** , but it is common to refer to gene products as proteins rather than polypeptides ☺

• Basic Principles of Transcription and Translation

- Genes provide instructions for making specific proteins but not directly , the bridge between DNA and Protein is the nucleic acid RNA .

  • **Transcription** is the synthesis of RNA under the direction of DNA

- Both nucleic acids **(DNA & RNA)** use the same language , and information is simply transcribed or copied from one molecule to another .
  1. DNA molecule serves as a template for assembling a complementary sequence of RNA nucleotides .
  2. Then the RNA molecule will carry the genetic massage from the DNA to Protein synthesizing machinery of the cell . This type of RNA called **Messenger RNA (mRNA)** .

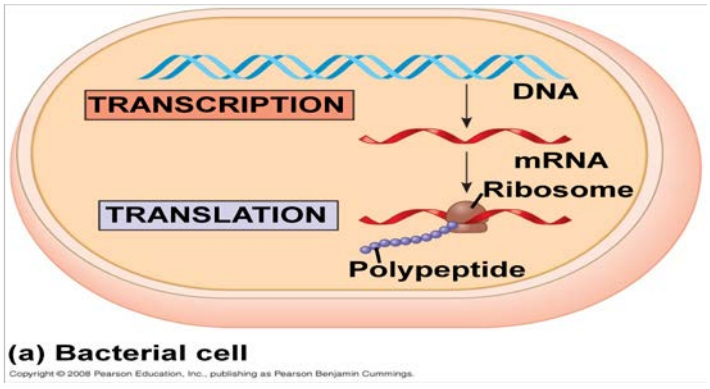
Note transcription is the general term for the synthesis of any kind of RNA on a DNA template , not only for mRNA synthesizing .

• **Translation** is the synthesis of a polypeptide, which occurs under the direction of mRNA

- **During this stag there is a change in language :** the cell must translate the base sequence of an mRNA molecule into **the amino acid sequence of a polypeptide** .
- the sites of translation are **Ribosomes** (complex particles that facilitate the orderly linking of amino acids into polypeptide)

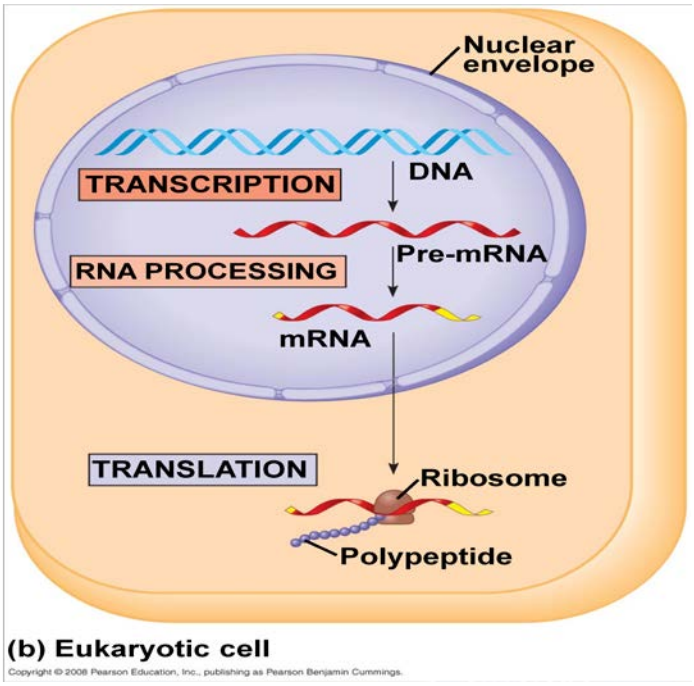
➢ Transcription and Translation occur in all organisms , the basic mechanics are similar for bacteria and eukaryotes , but there is an difference in the flow of genetic information within the cell.

-In prokaryotes, mRNA produced by transcription is immediately translated without additional processing .



- Because of lacking for nuclei ; DNA is not segregated (separated) from ribosomes that allows translation of an mRNA to begin while its transcription is still in progress.

-in eukaryotes , the nuclear envelope separate transcription from translation in space and time . Transcription occur in nucleus and mRNA is transported to the cytoplasm where translation occurs.
-before the mRNA leave nucleus , it modified in various way to produce the final functional mRNA .



1. The primary transcript : The initial RNA transcript from any eukaryotic gene (in protein coding) which results in **Pre-mRNA.**

2. Pre-mRNA will modified in various ways to produce the final functional mRNA.

- We concludes that , cells are governed by a cellular chain of command: **DNA → RNA → protein** (this concept was dubbed **The central dogma**).

Revision from chapter 5 :

What are the differences between DNA & RNA ?

RNA contains ribose while DNA contains deoxyribose
RNA has the nitrogenous base uracil , while DNA has nitrogenous base Thymine.
RNA molecule consists usually of a single strand .

• The Genetic Code

- When biologists guessed that the instructions for protein synthesis were encoded in DNA they recognized a problem : **There are 20 types of amino acids , but there are only four types of nucleotide bases in DNA ! How many bases correspond to an amino acid?!**

1- *The flow of information from gene to protein is based on a* ***triplet code*** *a series of (non-overlapping, three-nucleotide)words .*

triplet code *= the genetic instructions which are written in the DNA for the polypeptide chain.*

- These **triplets** are the smallest units of uniform length that can code for all the amino acid . (هي أصغر وحدة يمكن ان تكون شيفرة لأحماض امينية)

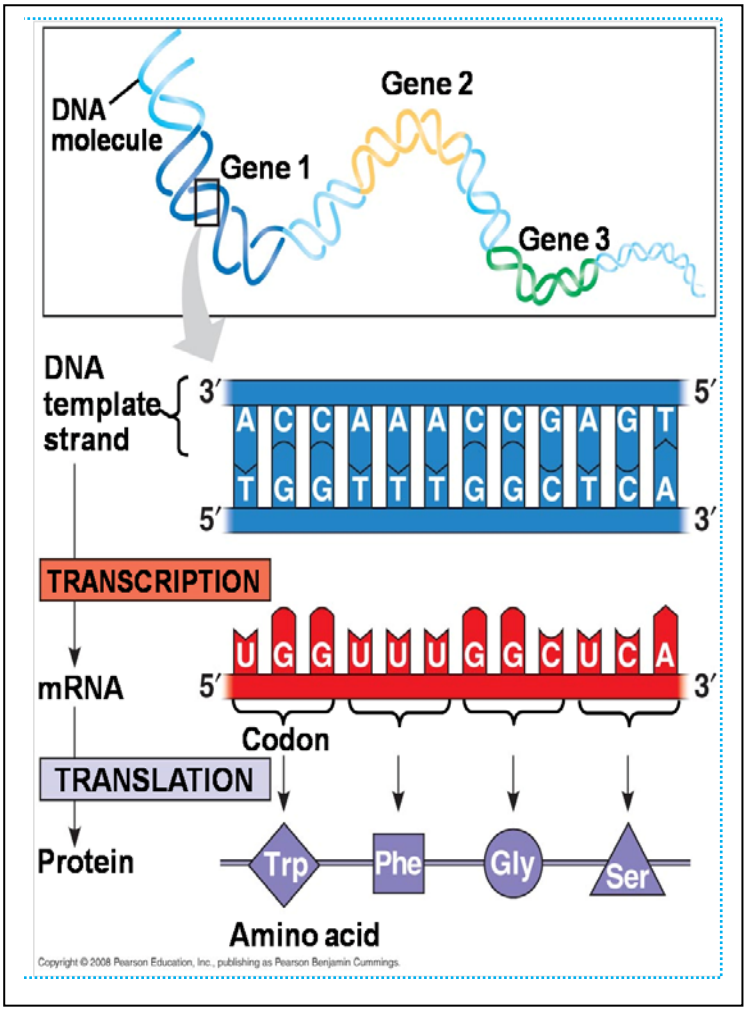
Example: this triplet code (AGT) at a particular position on a DNA strand results the amino acid (serine) at the corresponding position of the polypeptide chain .

Figure17.4 : The Triplet Code

1)During Transcription, one of the two DNA strands called the **template strand** works as a template for ordering the sequence of nucleotides in an RNA transcript.

Note the RNA molecule is synthesized in an anti-parallel direction to the template strand of DNA.

2) During **Translation**, the mRNA base triplets, called **codons**, they are translated (decoded) by translation machinery in the 5′ to 3′ direction ,each codon specifies the addition of one of 20 amino acids .



Notes :

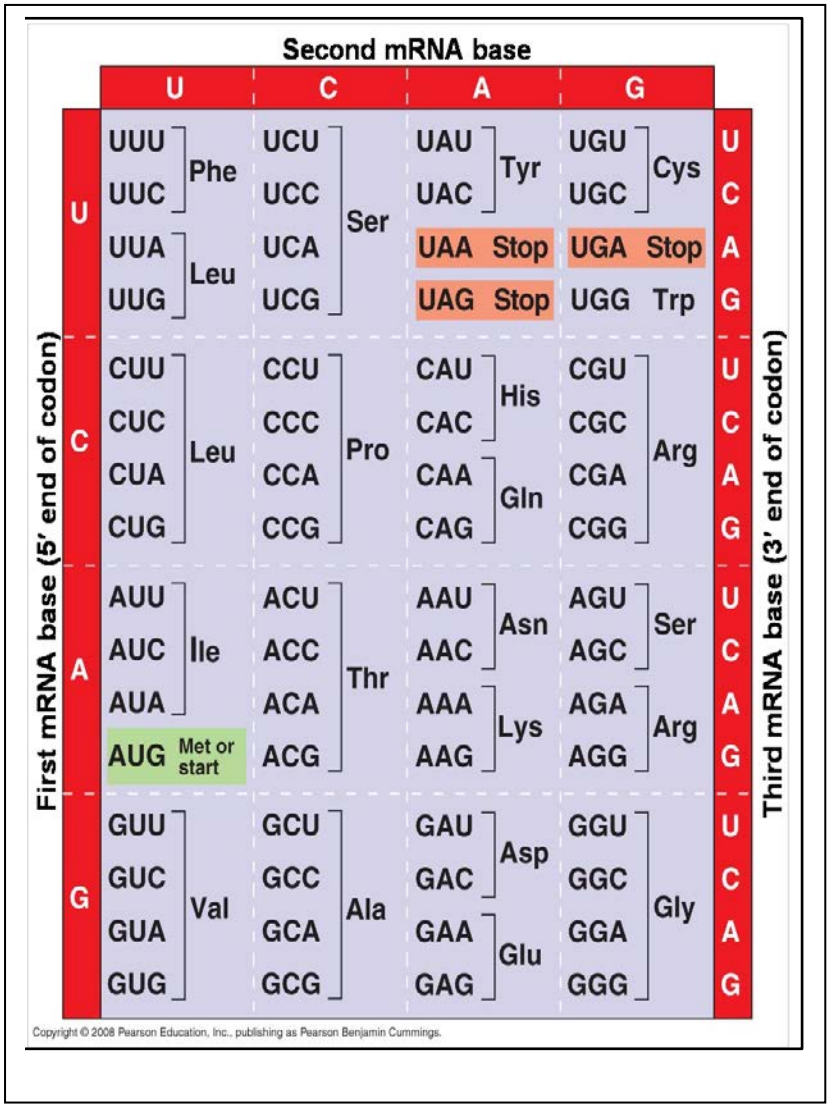
1. mRNA molecule is complementary ,not identical to its DNA template ☺

2.the term codon is also used for the DNA base triplets along non-template strand , and thus identical in sequence to the mRNA except they have (T) instead of (U) .for this reason the non-template DNA strand called the coding strand ;)

3. the number of nucleotides in mRNA must be 3 times more than the number of the resulted amino acids . (12 nucleotide on mRNA = 4 amino acids)

☒ Cracking the Code

- All 64 codons were deciphered by the mid-1960s . look to figure below ☺



- **Of the 64 triplets, 61 code for amino acids ; and 3 triplets are "stop codons"** signals to end translation (UAA , UAG , UGA).

- **the codon(AUG) has a dual function** >

1. it codes for the amino acids methenine (Met)

2. functions as a "start " signal or initiation codon .

So , genetic messages must begin with the mRNA codon (AUG) which signals the protein –synthesizing machinery to begin translating mRNA . And because this codon stands for the amino acid (methonine) , polypeptide chains also must begin with (methonine) . however, an enzyme may then remove this starter amino acid from the chain .

- The genetic code is **redundant** but **not ambiguous** ;
  no codon specifies more than one amino acid(no ambiguity) , but more than one codons may specify the same amino acid (redundancy)
  Example : the amino acid (Pro)is specified by 4 codons . look to the figure ↑

- Codons must be read in the correct **reading frame** (correct groupings) in order producing a specified polypeptide.
  ** Although a genetic message is written with no space between the codons , the cells protein- synthesizing machinery reads the message as a series of non overlapping three letters words (the message isn't read in this way UGGUUU >> it is read in this way : UGGUUUGGC) .

❖ *Evolution of the Genetic Code*

- The genetic code is nearly universal, shared by the simplest bacteria to the most complex animals .

there are exceptions to this universality (as example there are a slight variations *in the genetic code exist in certain unicellular eukaryotes . and in* translating stop codons into one of two amino acids not found in all organisms) but it's a small variations!

- Genes can be (transcribed and translated after being transplanted) from one species to another.

Example : bacteria can be programmed by the insertion of human genes to synthesize certain human proteins for medical use , such as Insulin.

Concept Check ^_^

Q1 : what polypeptide product would you expect from a poly-G mRNA that is 30 nucleotides long ? you can use the last table ☺

Q2 : the template strand of the gene contains the sequence (3'-TTCAGTCGT-5') ,

a) drawn **1 –** the non-template sequence **2- the** mRNA sequence indicating 3 and 5 ends of each ..
b) imagine that the non template sequence is transcribed instead of the template sequence , draw the mRNA sequence and translate it . you can use the last table.

Answers :

Q1 :

the poly peptide made up of 10 Gly (glycine) amino acid .

Q2 :

a) 1- non template sequence : 5'-AAGTCAGCA-3'
2- mRNA sequence : 5'-AAGUCAGCA-3 '

b) **1-** you have to write the non template sequence again conversely (3'-5') before transcription

non template sequence ⟶ template sequence
5'-AAGTCAGCA-3' ⟶ **3'-ACGACTGAA-5'**
2- mRNA sequence = 5'-UGCUGACUU-3'

3- Translated polypeptide = Cys-STOP- Leu (from the table in page 7)

Concept 17.2 : Transcription is the DNA-directed synthesis of RNA (*a closer look*)

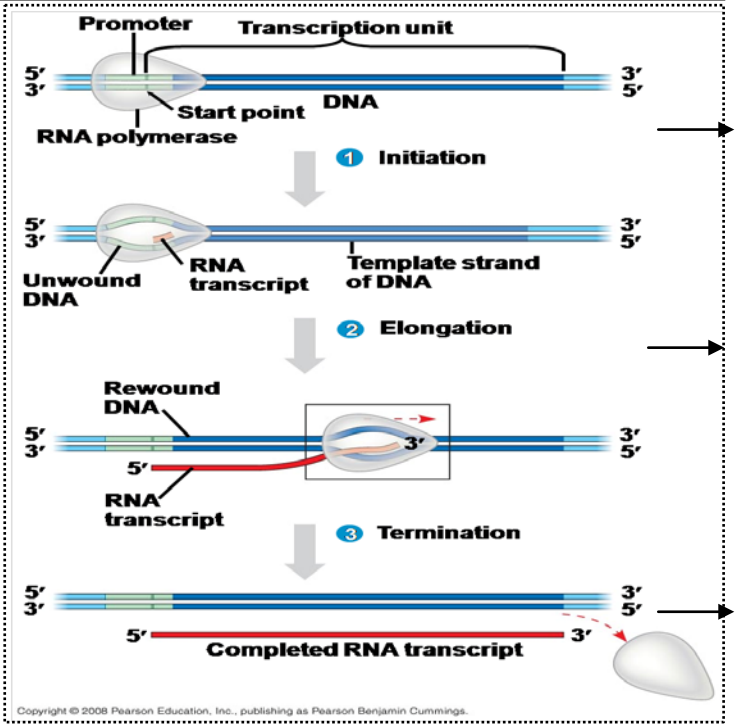
Transcription, the first stage of gene expression, can be examined in more details >>

a) **Molecular Components of Transcription** :

- An enzyme called an **RNA Polymerase** pries the two strands of DNA apart and joins the RNA nucleotides as they base-pair along the DNA template.

- This enzyme (like the DNA polymerase that functions in DNA replication) can assemble the nucleotides only in its (5'→ 3') direction , and (unlike DNA polymerase) it is able to start a chain from scratch (من الصفر) ; it doesn't need a primer .
- RNA synthesis follows the same base-pairing rules as DNA, except (Uracil) substitutes for (Thymine) and the type of nucleotides.
- Specific sequence of nucleotides along the DNA mark where transcription of a gene begins and ends :
  - **Promoter** : The DNA sequence where RNA polymerase attaches .
  - **Terminator** : the sequence signaling the end of transcription .
- biologists refer to the direction of transcription as "downstream" and the other direction as "upstream" , these terms are also used to describe the positions of nucleotide sequences within DNA and RNA. So the promoter sequence is up – stream in DNA from the terminator .
- The stretch of DNA that is transcribed into an RNA molecule is called a **transcription unit** .

Figure 17.7 : the stages of Transcription



Initiation : after RNA polymerase binds to the promoter , The DNA strands unwind , and the polymerase initiates RNA synthesis at the start point on the template strand .

Elongation : The polymerase moves downstream , unwinding the DNA and elongating the RNA transcript (5→ 3). in the wake of transcription , the DNA strands re-form a double helix .

Termination : eventually , the RNA transcript is released , and the polymerase detaches from the DNA.

Note while bacteria has a single type of RNA polymerase that synthesize not only mRNA but also another types of RNA , eukaryotes have at least 3 types of RNA polymerase in their nuclei , **the one that used for mRNA synthesis is called (RNA polymerase II) .**

b) **Synthesis of an RNA Transcript consists of 3 stages :**

- Initiation
- Elongation
- Termination

- ***stage 1 RNA Polymerase Binding and Initiation of Transcription***

- Promoter's roles are : serving as a binding site for RNA polymerase , determining the initiation of RNA synthesis and determining which one of the two strands of the DNA helix is used as a template .
  - In bacteria RNA polymerase itself recognizes the promoter and binds to it, but in eukaryotes there is a collection of proteins called "**Transcription factors**" mediate the binding of RNA polymerase and the initiation of transcription (only after certain transcription factors are attached to the promoter RNA polymerase II bind to it) .

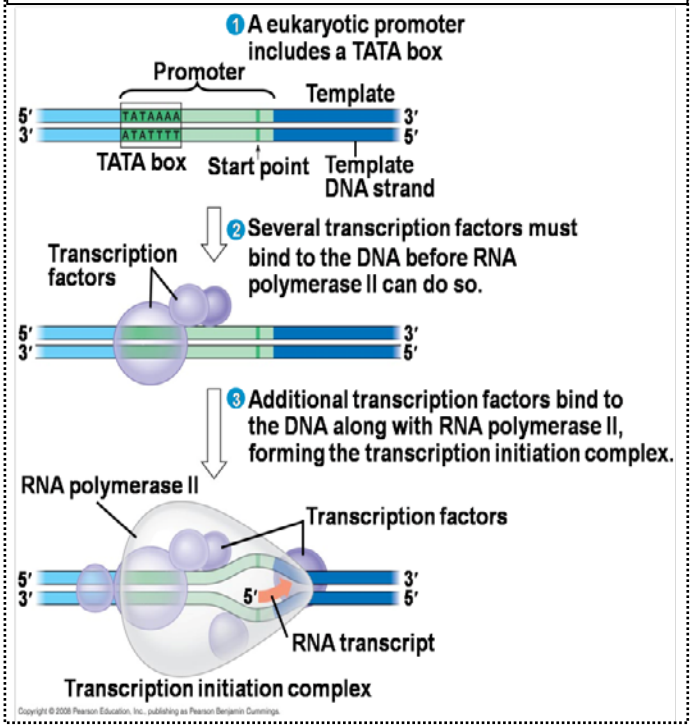
in eukaryotes

- transcription factors and RNA polymerase II biund to a promoter to form a completed assembly called a" **Transcription initiation complex**" .
- a section of promoter (a promoter DNA sequence) called "**TATA box**" is crucial in forming the initiation complex at the eukaryotic promoter . (without it transcription factors will not bind to DNA promoter and so polymerase will not binds too !) look to next figure >>

Note : the interaction between eukaryotic polymerase II and the transcription factors is an example of the importance of protein – protein interactions in controlling eukaryotic transcription.

Once the polymerase is firmly attached to the promoter DNA , the two DNA strands unwind there and the enzyme starts transcribing the template strand.

Figure 17.8 : the initiation of transcription at eukaryotic promoter



Stage2 : ***Elongation of the RNA Strand***

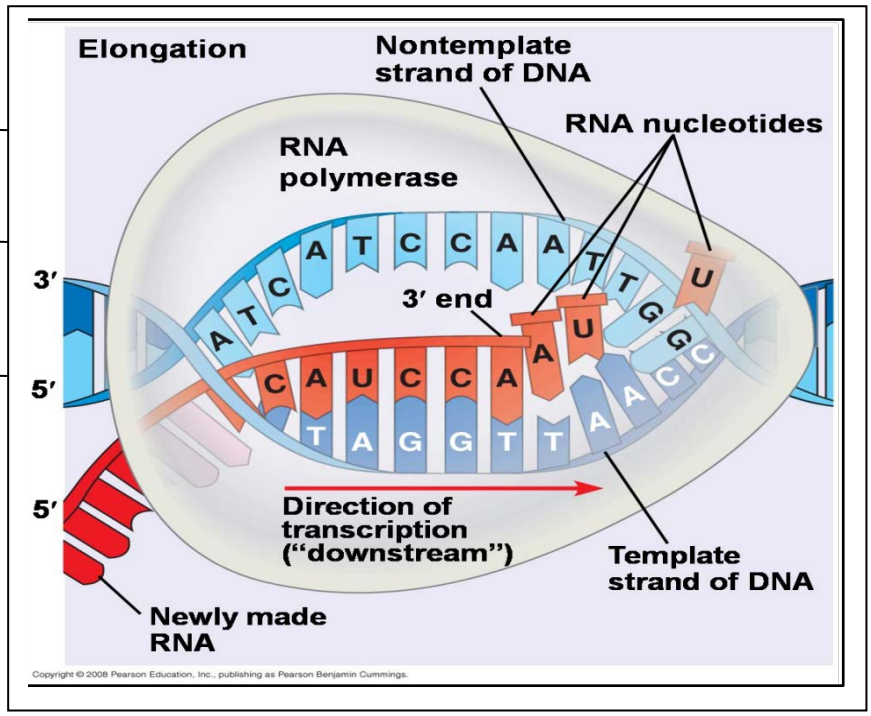
- RNA polymerase moves along the DNA, it untwists the double helix, 10 to 20 bases at a time.

-Transcription progresses at a rate of 40 nucleotides per second in eukaryotes

- A gene can be transcribed simultaneously by several RNA polymerases following each other, Which helps the cell make the encoded protein in large amounts .

1.the enzyme adds nucleotides to the **3‘ end** of the growing RNA molecule .

2.in the wake of RNA synthesis , The new RNA molecule peels away from its DNA template and **the DNA double helix reform .**



Stage 3 : ***Termination of Transcription***

- The mechanisms of termination are different in bacteria and eukaryotes

- **In bacteria**, the polymerase stops transcription at the end of the terminator (polymerase detach from the DNA and release the transcript , which is available for use as mRNA)
- **In eukaryotes**,

  1. RNA polymerase II transcribes a sequence on the DNA called the polyadenylation signal sequence , which codes for a **polyadenylation signal** (AAUAAA) in the pre-mRNA ..
  2. at a point about 10 to 35 nucleotides downstream from the **AAUAAA signal** , the proteins cut it free from the polymerase forming (pre-mRNA) , but polymerase continues transcribing .

  in another word : in eukaryotes , (polymerase doesn't release from DNA template at the same time with pre-mRNA transcript). ☺

  (recent research suggests that the RNA produced by the continued transcription (after pre-mRNA released) is digested by enzyme that moves along the RNA ; when this enzyme reach the polymerase , transcription is terminated and the polymerase falls off the DNA .

Concept 17.3 : Eukaryotic cells modify RNA after transcription

- Enzymes in the eukaryotic nucleus modify pre-mRNA before the genetic messages are dispatched to the cytoplasm

  - During RNA processing, both ends of the primary transcript are usually altered.
  - Also, usually some interior parts of the RNA molecule are cut out, and the other parts spliced together .

These modifications produce an mRNA molecule ready for translation …

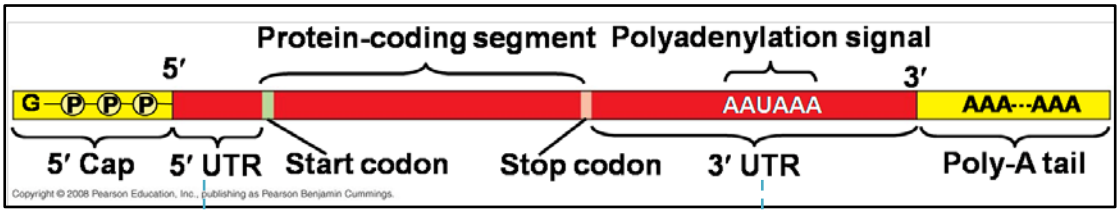
- **Alteration of mRNA Ends**
  - Each end of a pre-mRNA molecule is modified in a particular way:
    - The 5′ end receives a modified nucleotide (**5′ cap**)
    - The 3′ end gets a **poly-A tail**

    - **5′ cap** = a modified form of (**G**uanine) nucleotide added onto the 5′ end , after transcription of the first 20 to 40 nucleotides . **(not translated into protein)**
    - **Poly A-tail** = (50 – 250) (**A**denine) nucleotides added to the 3′ end . **(not translated into protein)**

  - **These modifications share several functions:**
  - **They seem to facilitate and promote the export of mRNA from nucleus .**
  - **They protect mRNA from degradation from hydrolytic enzymes .**
  - **They help ribosomes attach to the 5′ end (in conjunction with cytoplasmic proteins) when mRNA arrive to cytoplasm .**

Look to the figure >>

Figure 17.9 : **RNA processing : addition of the 5 cap and poly-A tail**



UTR (**U**n **T**ranslated **R**egion): parts of the mRNA that will not be translated into protein , but they have other functions such as ribosome binding .

- **Split Genes and RNA Splicing**

  - Most eukaryotic genes and their RNA transcripts have long noncoding stretches of nucleotides that lie between coding regions .that means the sequence of DNA nucleotides that codes for a eukaryotic polypeptide is usually not continuous , its split into segments .

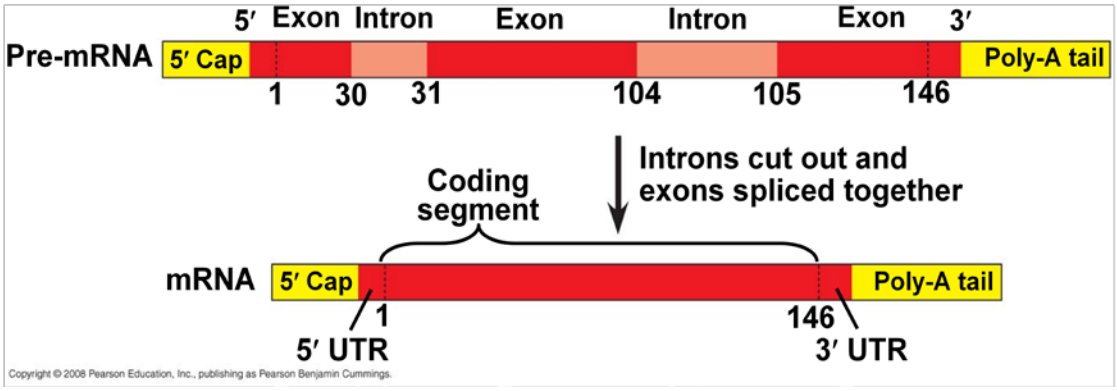
- These non-coding regions are called intervening sequences, or **introns**
- The other regions are called **exons** because they are eventually expressed (usually translated into amino acid sequences).

  But , there is an exception; UTR codes of the exons at the ends of the RNA , which make up part of the mRNA aren't translated into protein !!
  because of this exceptions ,you can say Exons are "sequences of RNA that exit the nucleous " , with this definition UTR are part of Exons ☺

  - **RNA splicing** removes introns and joins exons, this process creating an mRNA molecule with a continuous coding sequence .

Note : the term exons and introns are used for both RNA sequence and the DNA sequence that encodes them .
Look to next figure

Figure : 17.10 "RNA processing : RNA Splicing"



➢ figure (17.10) shows RNA molecule codes for B-globin ..

- The number under the RNA refers to codons , in this figure > it consists of 164 codon .
- There are 3 exons , those will form the sequence of mRNA ..
- During RNA processing the introns will cut out and exons spliced together.

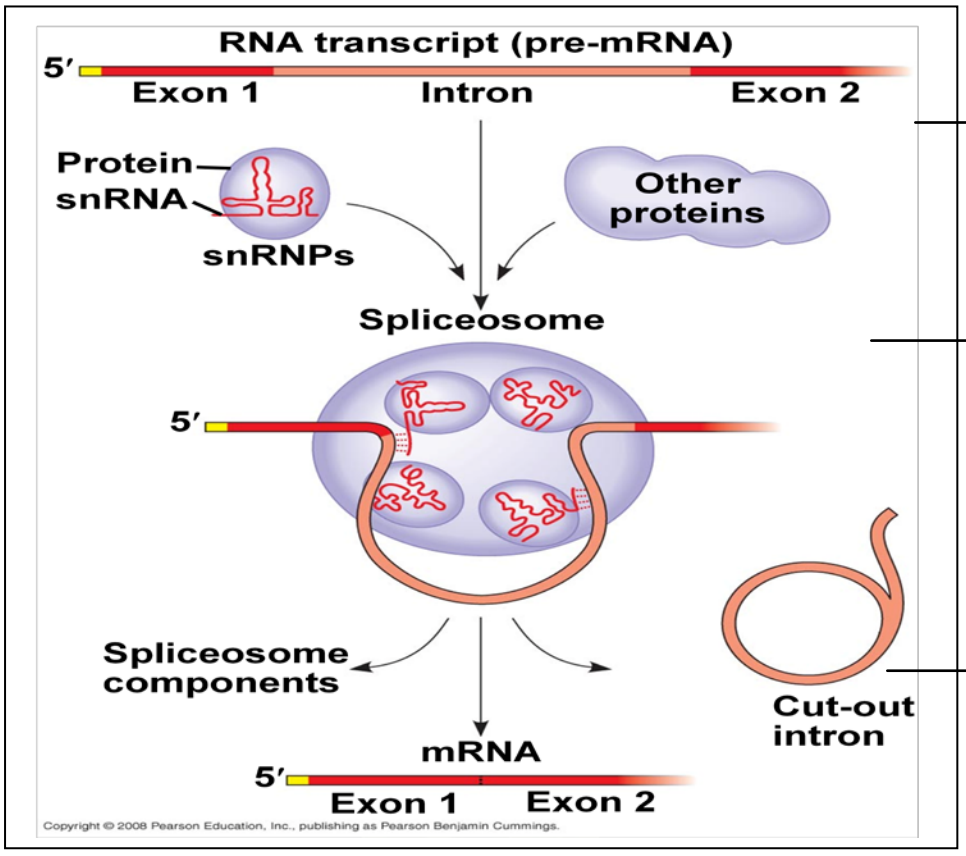
How is Pre-mRNA splicing carried out ?

Researchers have concluded that >>

1) There is a short nucleotide sequence at each end of an intron works as a signal for RNA splicing.
2) particles called **s**mall **n**uclear **R**ibo**N**ucleo**P**roteins (snRNP) recognize these splice sites.
3) Several different snRNPs join with additional proteins to form **Spliceosome** (which is almost as big as Ribosome) .
   Splicesome interacts with certain sites along an intron , releasing the intron and joining together the two exons ..

small **n**uclear **R**ibo**N**ucleo**P**roteins (snRNP) pronounced "*snurps*"
-they are located in the cell nucleus
-they are composed of RNA and protein molecules
**RNA in those particles called small nuclear RNA .
Its Role :
- Catalyze the previous process.
- Participate in spliceosome .
- Recognize Splice sites .

Figure 17.11 : The Role of snRNP and spliceosomes in pre-mRNA splicing.



Forming Splicesome on a pre-mRNA molecule

Within the splicesome snRNA base-pairs with nucleotides at a specific sites of along the intron.

Splicesome **releases** the intron and at the same time **splices** the exons together .. and the result mRNA contains exons only

<<Ribozymes >>

- **Ribozymes** are catalytic RNA molecules that function as enzymes and can splice RNA, that splicing can be called self-splicing.
- For example in *Protozoan Tetrahymena self splicing occurs in the production of ribosomal RNA* (rRNA) "a component of the organisms ribosomes". The pre rRNA actually removes its own introns .
- The discovery of ribozymes disagreed the belief that all biological catalysts were proteins
- Three properties of RNA enable it to function as an enzyme (aribozymes)

  1- It can form a three-dimensional structure because of its ability to base pair with itself .
  2- Some bases in RNA contain functional groups .
  3- RNA may hydrogen-bond with other nucleic acid molecules .

Concept 17.4: **Translation is the RNA-directed synthesis of a polypeptide**

❖ **Molecular Components of Translation**

- the cell keeps its cytoplasm stocked with all 20 amino acids in the (cytoplasmic pool of amino acids) ; by synthesizing them from other compounds or by taking them up from the surrounding solution .
- A cell translates an mRNA message into protein in ribosomes with the help of **transfer RNA "**tRNA**" .**

  - The mRNA message is a series of codons.
  - tRNA molecules transfer the amino acids from cytoplasm to a ribosome.
  - Ribosomes adds each amino acid brought to it by tRNA to the growing end of a polypeptide chain (join the amino acids into a chain)

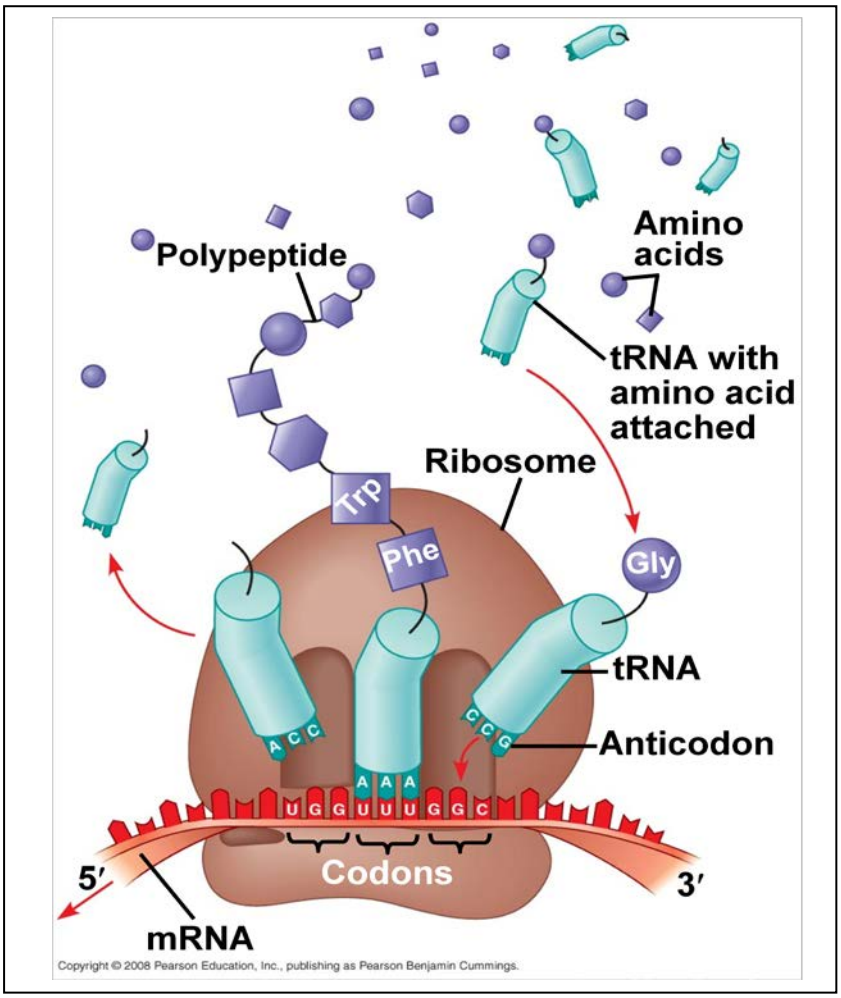
Note :

Molecules of tRNA are not all identical :

- Each tRNA molecule translates a particular mRNA codon into a particular amino acid , how ?

Each tRNA carries a specific amino acid on one end . and has an **anticodon (nucleotide triplet)**on the other end ; the anticodon base-pairs with a complementary codon on mRNA (by hydrogen bonds).

look to figure 17.13 " Translation :the basic concept"



This figure shows that >>

1) As the mRNA is moved through a ribosome , codons are translated into amino acids , one by one .
2) tRNA adds its amino acid to a growing polypeptide chain when the anticodon hydrogen bonds to a complementary codon on the mRNA .

Q : tRNA is a translator , how ?
because it can read a nucleic acid word (mRNA codon) and interpret it as a protein word (the amino acid) .

a) The Structure and Function of Transfer RNA

- tRNA Like other types of cellular RNA ; it is transcribed from DNA templates . and in eukaryotic cells its made in nucleus like mRNA and then travel to the cytoplasm ,where translation occurs .
- tRNA in both bacterial and eukaryotic cells is used repeatedly ; after transferring an amino acid to ribosome goes to cytoplasm and pick another one and transfer it , and so on ..
- A tRNA molecule consists of a single RNA strand that is only about 80 nucleotides long (it is shorter than mRNA)
- tRNA , this single strand can fold back upon itself and form a molecule with a three-dimensional structure by hydrogen bonds between its bases .
- when tRNA is Flattened into one plane it looks like a clover leaf, and when it is twisted and folds in 3-D structure it is roughly has L-shape .

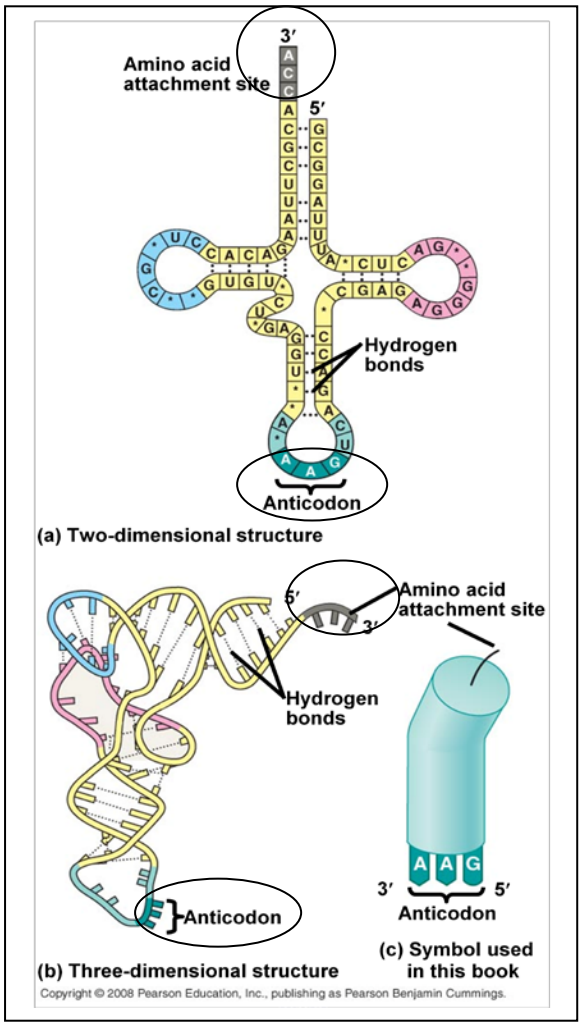


Figure 17.14 The structure of transfer RNA (tRNA)

a) In Two dimensional structure :

- In all tRNA , there are 4 base-paired regions with 3 loops , and base sequence of the amino acid attachment site at the 3‘ end .
- The colored sequences in these 3 loops are unique to each tRNA type. so anti-codon is unique too.

b) In three dimensional structure :

-The loop extending from one end of the (L -shaped tRNA) includes the anti-codon .

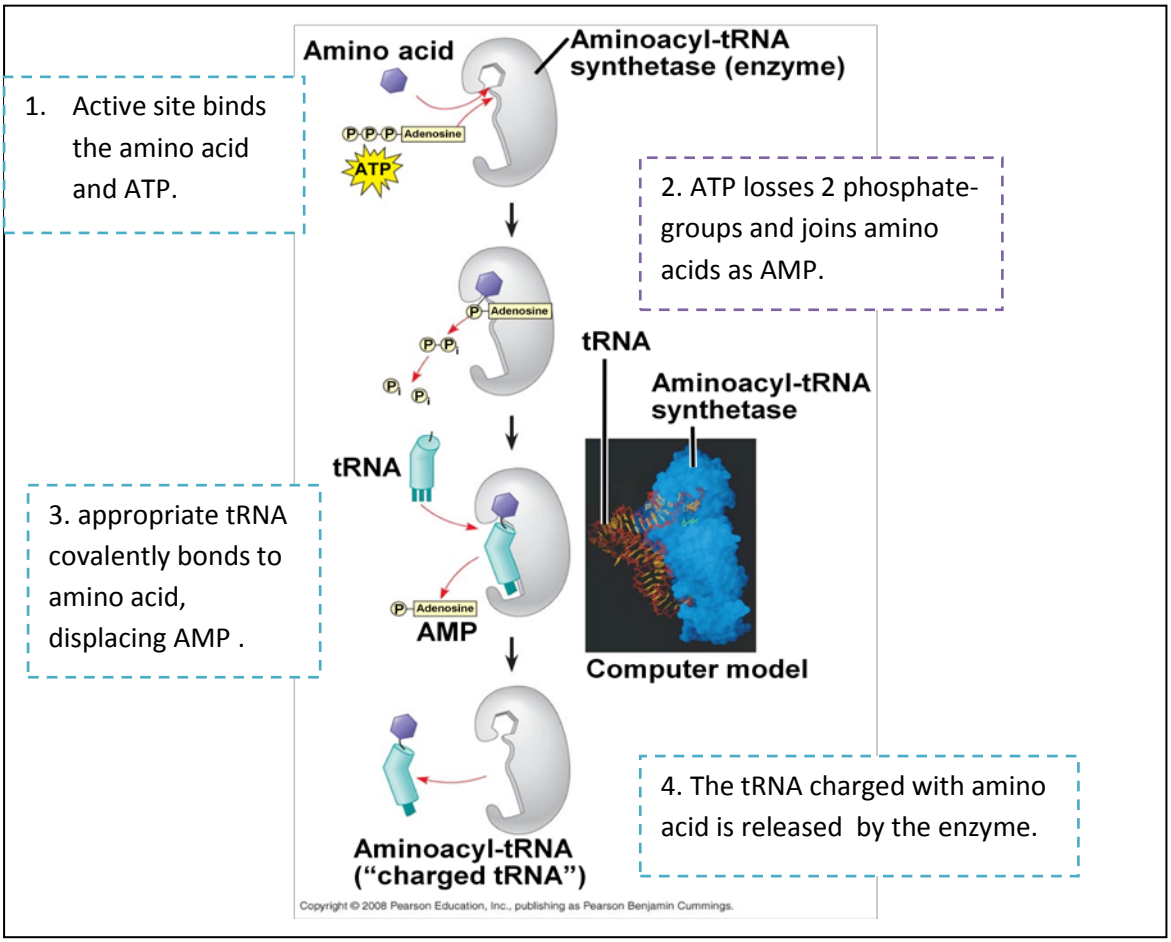
- the other end of (L-shaped tRNA) protrudes its (3‘ end) which is the attachment site for amino acid.

c) look to anti-codon its written as 3‘ to 5‘ sequence for aligning properly with codon which is 5‘ to 3‘ sequence .

➲ Accurate translation requires two processes of molecular recognition :

- **First** : a correct match between a tRNA and an amino acid, done by a family of related enzymes called **aminoacyl-tRNA synthetases**

- The active site of each type of aminoacyl-tRNA synthetase fits only a specific combination of amino acid and tRNA . there are 20 different synthetases .
- Synthetase catalyses the covalent attachment of the amino acid to its tRNA in a process driven by the hydrolysis of ATP .and the resulted **aminoacyl tRNA** also called **charged tRNA** which is ready to go ribosome.



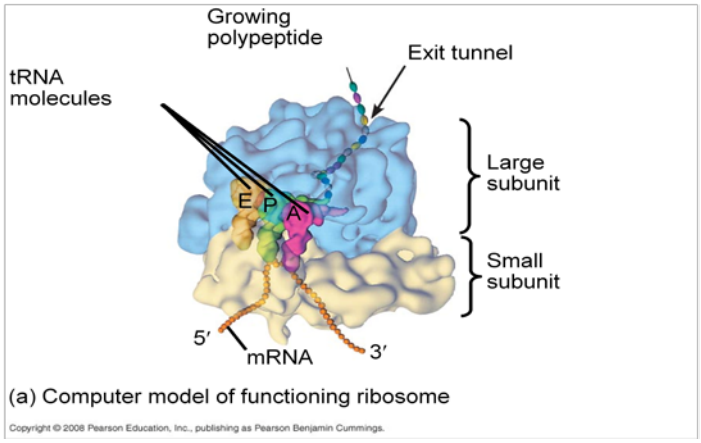
- **Second**: a correct match between the tRNA anticodon and an mRNA codon
  There are 61 types of mRNA codons while only 45 types of tRNA anti-codons .. because of that , some of tRNA s must bind to more than one codon .
  For example : the base U at the 5-end of the anti-codon can bind with A or G in the third position(at the 3-end) of an mRNA codon ...

- Flexible pairing at the third base of a codon is called **wobble**
- Wobble explains why one amino acid can be presented by more than one codon differs in their third bases only ☺

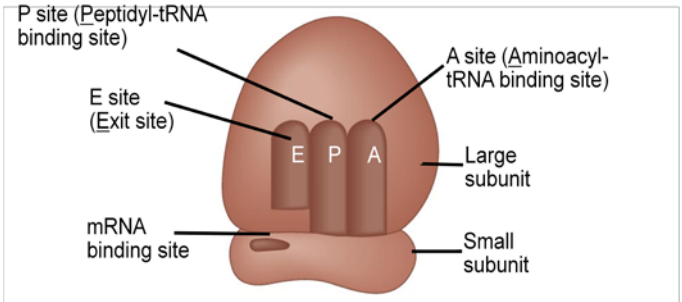
b) Ribosomes

- Ribosomes facilitate specific coupling of tRNA anti-codons with mRNA codons in protein synthesis

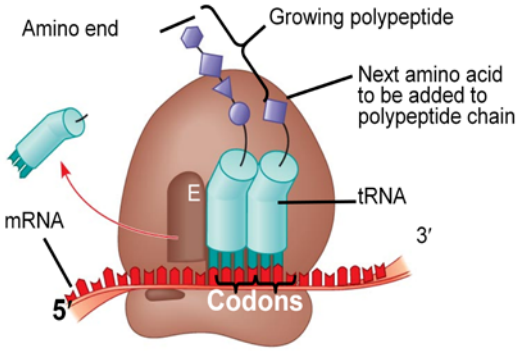
- The two ribosomal subunits (large and small) are made of **proteins** and **ribosomal RNA "rRNA" (**rRNA form 2/3 of ribosome mass , and it's the most abundant type of RNA in the cell)
- In eukaryotes the subunits are made in the nucleolus . when the rRNA is transcribed , it will be processed and assembled in nucleus with imported protein from cytoplasm to form the subunits , which will exported to cytoplasm via nuclear pores.
- **In bacteria and eukaryotes , large and small subunits join to form a functional ribosome only when they attach to an mRNA molecule .**



(a) Computer model of functioning ribosome



(b) Schematic model showing binding sites



(c) Schematic model with mRNA and tRNA

Picture (a) :

When the polypeptide becomes longer it passes through "exit tunnel" in the large subunit , and when its become complete it leaves to cytosole.

picture (b) & (c)

- A ribosome has three binding sites for tRNA:
  - The **P site** : holds the tRNA that carries the growing polypeptide chain
  - The **A site :** holds the tRNA that carries the next amino acid to be added to the chain
  - The **E site : it** is the exit site, where discharged tRNAs leave the ribosome.

- After addition the new amino acid to the carboxyl end of the growing polypeptide . ribosome catalyze the formation of peptide bond .

Note :

Recent researches support the hypothesis that the rRNA is responsible for both structure and function of ribosome ; it's the main constituent of the interface between the 2 subunits and(A and P sites) and it's the catalyst of peptide bond formation .

What about protein ? it supports the shape changes of the rRNA molecules during translation.

❖ Building a Polypeptide

- we can divide translation (synthesis of polypeptide) into three stages :
  - **Initiation**
  - **Elongation**
  - **Termination**

- All three stages require **proteins "factors"** that aid the translation process

- **energy** is provided by the hydrolysis of **GTP**(**G**uanosine **T**riphos**p**hate) .

- The polypeptide is synthesized always in one direction from **N-terminus** (initial amino acid end ,**met**) toward the **C-terminus** (carboxyl end).

---

a) ***Ribosome Association and Initiation of Translation***

- The initiation stage of translation brings together mRNA, tRNA with the first amino acid, and the two ribosomal subunits : figure(17.17)

1. the small ribosomal subunit binds with mRNA and a special initiator tRNA which carried the amino acid **(met**hionine) and anti-codon (UAC).

2. the small subunit moves along the mRNA until it reaches the start codon (AUG) .
   - In bacteria : small subunit binds to mRNA at a specific nucleotide sequence

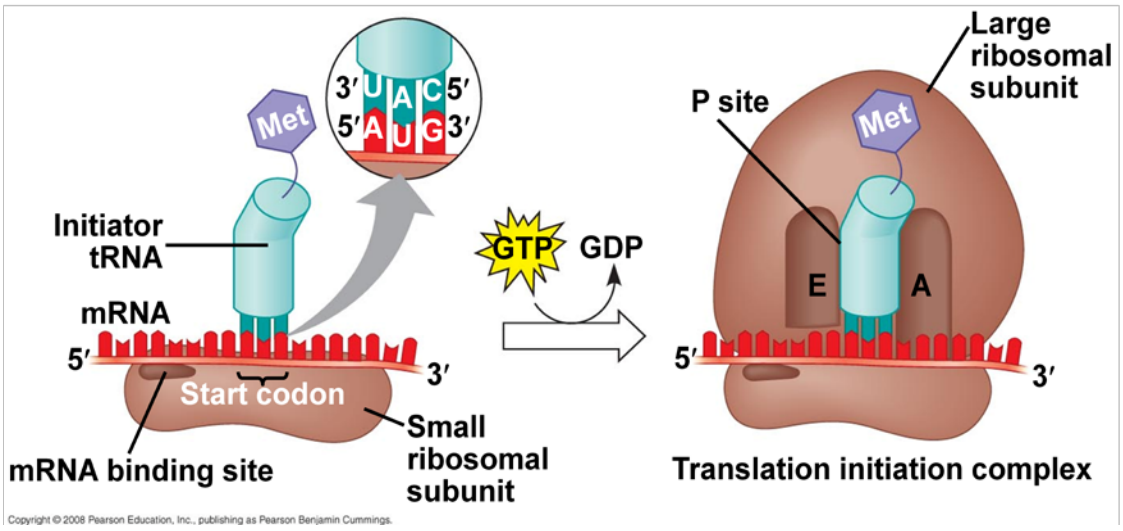
     in eukaryotes : small subunit with the initiator tRNA are already bound and then **bind to the 5'cap of the mRNA** (which is upstream to the start codon) . then they move downstream along the mRNA until they reach the start codon , and tRNA hydrogen bonds to it .

   - **Start codon** : it signals the start of translation ; it establishes the codon reading frame for the mRNA .

3. The union of mRNA & initiator tRNA& small ribosomal subunit is followed by attachment of large ribosomal subunit and forming **Translation Initiation Complex .** and that requires **:**
   - Proteins called **initiation factors** are required to bring all these components together .
   - **GTP molecule is required** to form the **initiation complex .**

- When the initiation process is completed , the initiator tRNA sits in the P site of the ribosome and A site will be ready for the next aminoacyl tRNA .

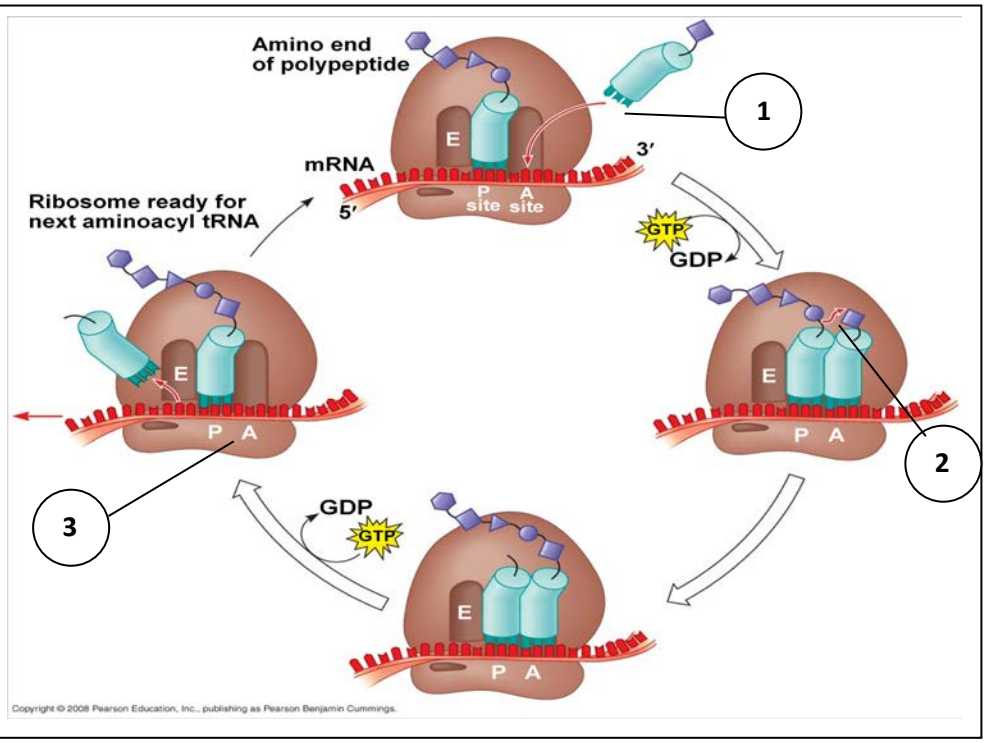
Figure 17.17 The initiation of translation



b) ***Elongation of the Polypeptide Chain***

- During the elongation stage, amino acids are added one by one to the preceding amino acid
- Each addition involves proteins (elongation factors) and occurs in three steps cycle : **codon recognition**, **peptide bond formation**, and **translocation.**
- Energy consuming occurs in the first and third steps . <u>each one requires hydrolysis of one GTP .</u>
- This cycle takes less than a tenth of second (0.1 sec)in bacteria , and is repeated at each amino acid is added to the chain until the polypeptide is completed.

Figure 17.18 The elongation cycle of translation



Figure(17.18) Illustration :

1.codon recognition : the anticodon of an incoming aminoacyl tRNA base-pairs with complementary mRNA codon in the A site . hydrolysis of the GTP increases the accuracy and efficiency of this step .

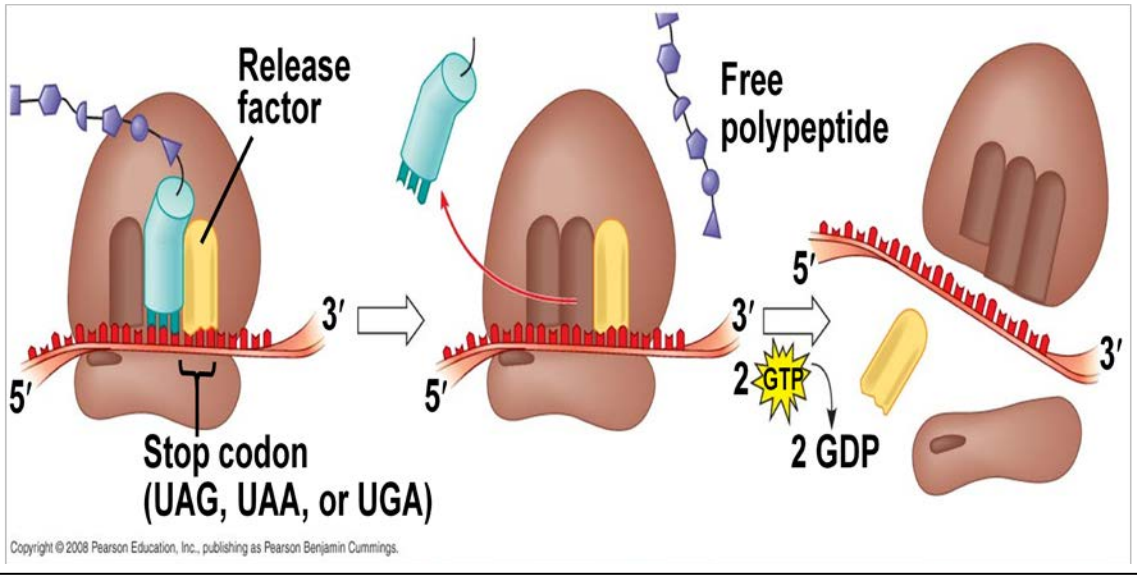
2. **peptide bond formation :** an rRNA molecule of the large ribosomal subunit catalyzes the formation of a peptide bond between the new amino acid in the A site and the carboxyl end of the growing polypeptide in the P site .. this step removes the polypeptide from the tRNA in the P site and attaches it to the amino acid on the tRNA in the A site .

3. **Translocation** : The ribosome translocates the tRNA in the A site to the P site . and the empty tRNA in the P site is moved to the E site , where its released . the mRNA moves along with its bound tRNAs , bringing the next codon to be translated into the A site .
-mRNA moves in the ribosome in one direction only (from 5‘ end) . and it moves relatively to each other (unidirectionally) , codon by codon .

c) Termination of Translation

- Termination occurs when a stop codon (UAG, UAA,UGA) in the mRNA reaches the A site of the ribosome .
- a protein called a **release factor** (shaped like tRNA) binds directly to the stop codon in A site, and that causes the addition of a water molecule instead of an amino acid to the poly peptide chain .
- This reaction breaks (hydrolyzes)the bond between the completed polypeptide and the tRNA in the P site. releasing the polypeptide through the exit tunnel .
- translation assembly then comes apart (dissociate) in a multistep process , **aided by other protein factors and requires hydrolysis of 2 more GTP molecules .**

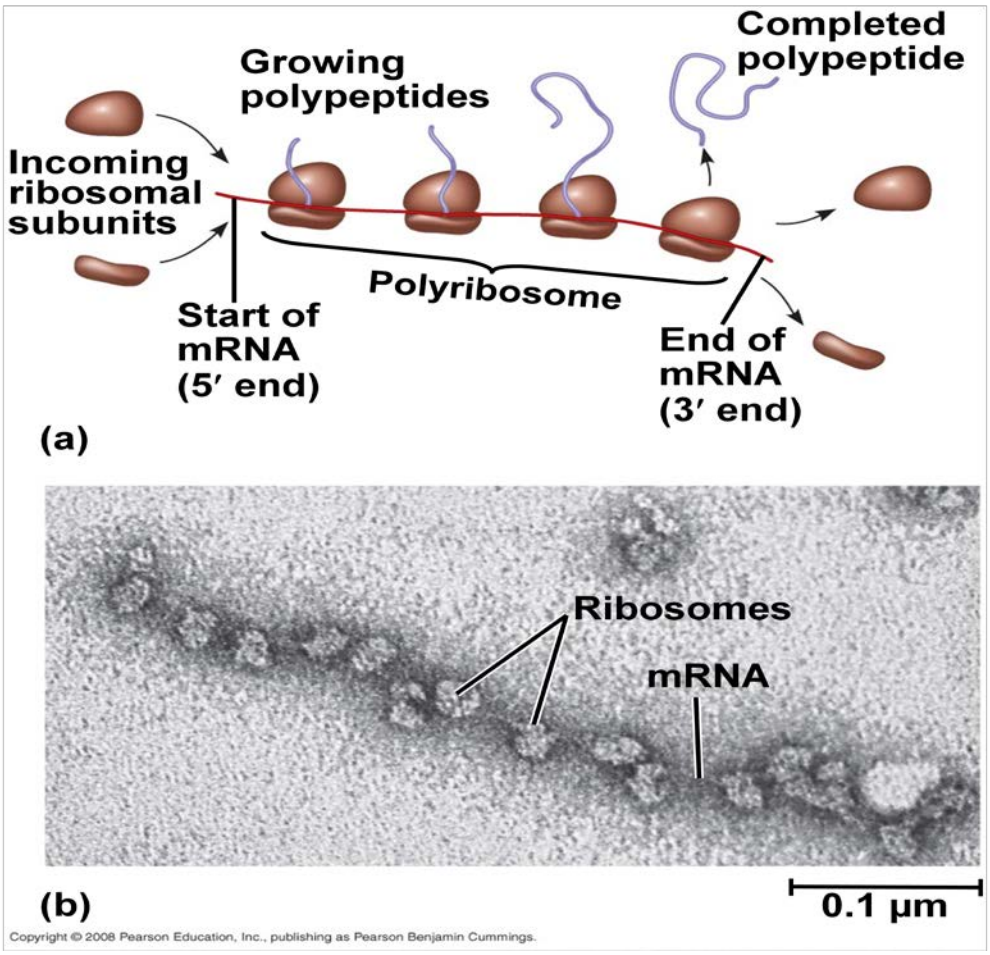
Figure 17.19 The termination of translation



➢ ***Polyribosomes***

- A number of ribosomes can translate a single mRNA simultaneously, forming a **polyribosome** (or **polysome**)
- Polyribosomes enable a cell to make many copies of a polypeptide very quickly .
- Poly ribosome are found in bacteria and eukaryotes .

Figure 17.20 Polyribosomes



❖ **Completing and Targeting the Functional Protein**

-Often translation is not sufficient to make a functional protein ,Polypeptide chains are modified after translation and Completed proteins are targeted to specific sites in the cell .

a) ***Protein Folding and Post-Translational Modifications***

- During and after synthesis, a polypeptide chain spontaneously coils and folds into its three-dimensional shape (secondary or tertiary structure) .
  Thus , the gene determines the primary structure , and primary structure in turn determines shape.
  ** in many cases , a chaperone protein helps the polypeptide fold correctly .
- Proteins may also require post-translational modifications before doing their job, for example :
  1- Certain amino acids may be chemically modified by the attachment of sugars, lipids, phosphate groups or other addition .
  2- Enzymes may remove one or more amino acids from the leading (amino) end of the polypeptide chain . or cleaved polypeptide chain into 2 or more pieces .
  3- 2 or more polypeptides that are subunits of a protein that has quaternary structure , a familiar example is hemoglobin.

b) ***Targeting Polypeptides to Specific Locations***

- Two populations of ribosomes are evident in cells: free ribsomes (in the cytosol) and bound ribosomes (attached to the ER) , both they are identical and can switch their status from free to bound .
- Remember : free ribosomes synthesize proteins that stays in the cytosol and function in it. While bound ribosomes synthesize protein of the endo-membrane system and proteins secreted from the cell .
- Polypeptide synthesis always begins in the cytosol , and finishes in the cytosol *unless* the polypeptide signals the ribosome to attach to the ER
- Polypeptides destined for the ER or for secretion are marked by a sequence of amino acids(about 20 amino acid)near the leading (amino) end called **signal peptide**
- a protein- RNA complex called **signal-recognition particle (SRP)** binds to the signal peptide . it brings the signal peptide and its ribosome to the ER (to receptor protein built into ER membrane this receptor is part of a multi-protein translocation complex).

Figure 17.21 The signal mechanism for targeting proteins to the ER

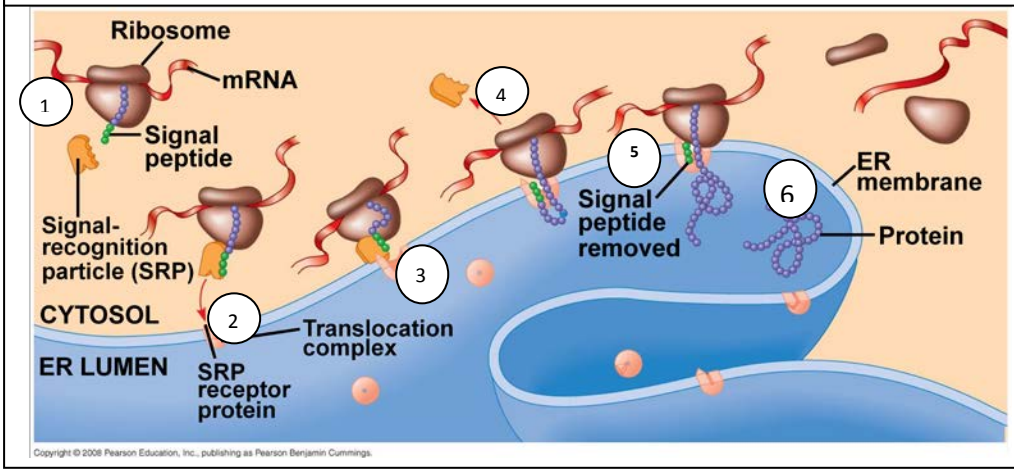


Figure 17.21 Illustration :
1- polypeptide synthesis begins on a free ribosome in the cytosol.

2-An SRP binds to the signal peptide , halting synthesis momentarily .

3-The SRP bind to a receptor protein in the ER membrane . This receptor is part of a protein complex (a translocation complex) that has a membrane pore and a signal-cleaving enzyme.

4-The SRP leaves , and polypeptide synthesis resumes , with simultaneous translocation across the membrane (The signal peptide stays attached to the translocation complex) .

5-the signal-cleaving enzyme cuts off the signal peptide .

6-the rest of the completed polypeptide leaves the ribosome and folds into its final conformation . if it's for secretion it will released within the ER lumen , and if it is membrane protein it remains partially embedded in the ER membrane .

Note 1 : There are another Kinds of signal peptide that target the protein to organelles which aren't of endo-membrane system . and in these cases translation will be completed in cytosol before the polypeptide is embedded in the organelle .

Note 2 : bacteria also employ signal peptides to target proteins for secretion .

Concept check :

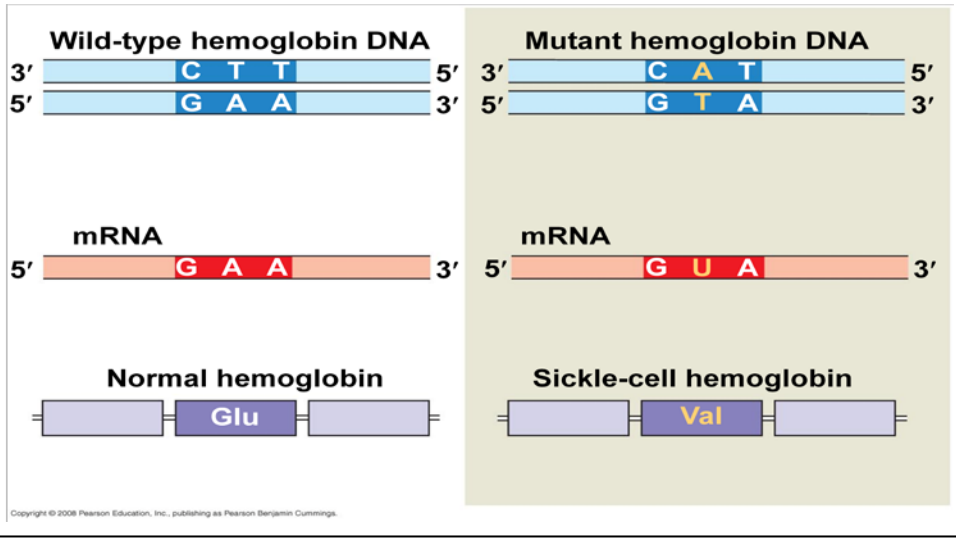
1. what two processes ensure that the correct amino acid is added to a growing polypeptide chain ?

   first , each aminoacyl-tRNA synthetase specifically recognize a single amino acid and attaches it only to an appropriate tRNA .
   second, tRNA charged with its specific amino acid binds only to an mRNA codon for that amino acid .

Concept 17.5: Point mutations can affect protein structure and function

- **Mutations** are changes in the genetic material of a cell or virus
- **Point mutations** are chemical changes in just one base pair of a gene
  - The change of a single nucleotide in a DNA template strand can lead to the production of an abnormal protein
  - Point mutation may lead to many disorders , such as heart condition that are responsible for sudden death in young athletes and Sickle –cell disease ..

Figure 17.22 The molecular basis of sickle-cell disease: a point mutation



❖ **Types of Point Mutations**

- Point mutations within a gene can be divided into two general categories

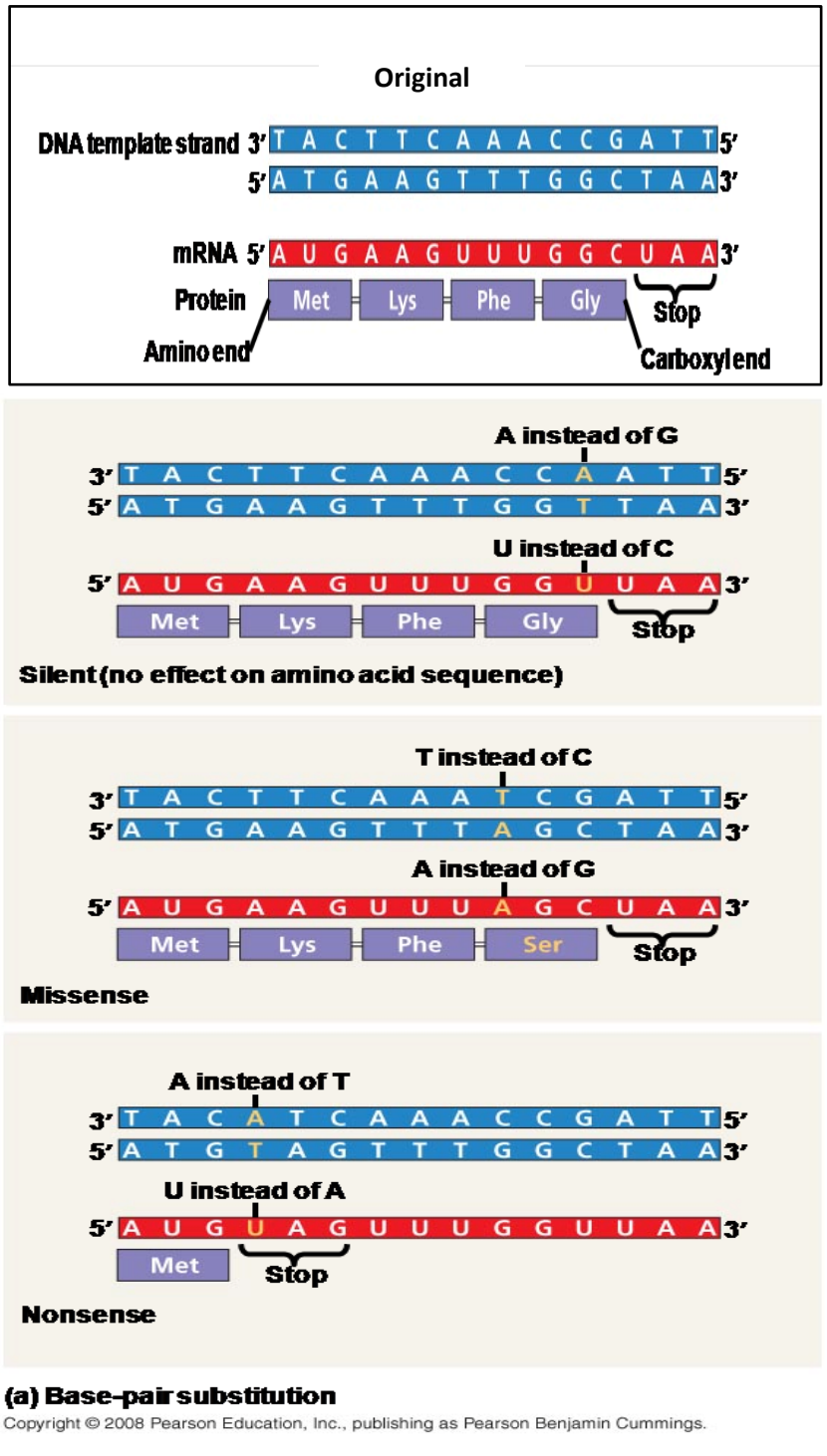
1- Base-pair substitutions
2- Base-pair insertions or deletions

1- Base-pair substitutions

- base-pair substitution replaces one nucleotide and its partner with another pair of nucleotides .
- depending on its effect , we can categorize it to : look to figure 17.23
  - **Silent mutations** : have no effect on the amino acid produced by a codon because of redundancy in the genetic code (change in codon but no change in amino acid)
  - **Missense mutations (most common substitution mutation)** still code for an amino acid, but not necessarily the right amino acid and it has a little effect on the protein ; the amino acid may have properties similar to those of the amino acid it replaces , or the new amino acid is in a non essential region to protein function .

- **Nonsense mutations** change an amino acid codon into a stop codon, nearly always leading to a nonfunctional protein because the resulting polypeptide will be shorter than the polypeptide encoded by the normal gene .

Figure 17.23 Types of point mutations

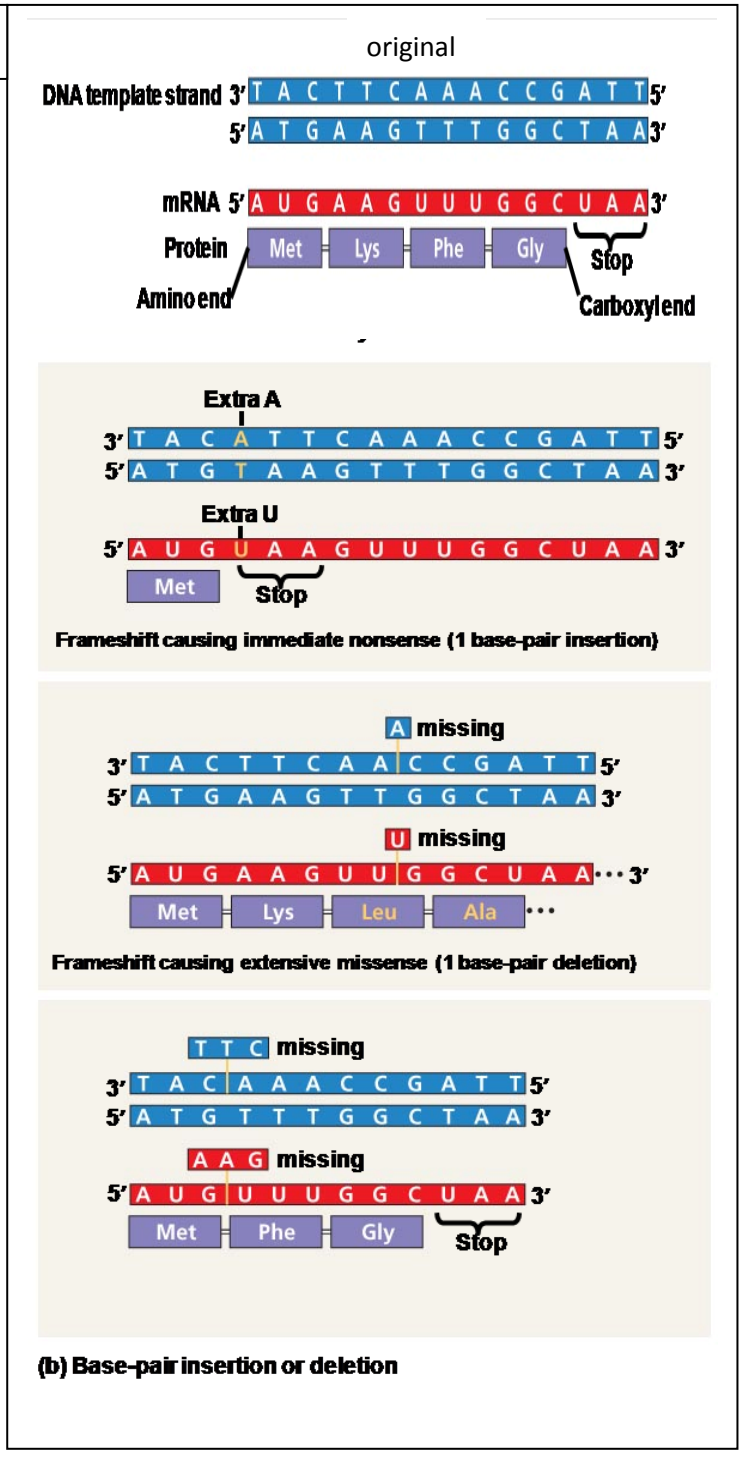


(a) Base-pair substitution

2- ***Insertions and Deletions***

- **Insertions** and **deletions** are additions or losses of nucleotide pairs in a gene
- These mutations have a disastrous effect on the resulting protein more often than substitutions do
- Insertion or deletion of nucleotides may alter the reading frame, producing a **frame shift mutation (**when the number of nucleotides inserted or deleted isn't multiple or three**)** .

Figure 17.23 Types of point mutations



- All the nucleotides downstream of the deletion or insertion will be improperly grouped into codons , and the result will be , extensive missense and usually ending sooner or later in nonsense .

- Unless the frame shift is very near the end of the gene , the protein is almost certain to be nonfunctional .

❖ **Mutagens**

- We studied that , When there is an error in replication it will be corrected by a system that we learned about in chapter #16 . otherwise the incorrect base will be used as a template in the next round of replication , resulting in a mutations .
- The rate of mutation is about (1 nucleotide in every 10^{10} is altered).
- **Mutagens** are physical or chemical agents that can cause mutations , such as :
  Physical mutagens > X-rays , ultraviolet light (which can cause disruptive Thymine diamers in DNA).
  Chemical mutagens > they affect the structure of DNA and they are carcinogenic >> examples of types
  -Base analogs : Chemical mutagens that are similar to normal DNA bases , but they pair incorrectly during DNA replication .
  - some Chemical mutagens insert themselves into the DNA and distorting the double helix .

Concept check :

- What happens when one nucleotide pair is lost from the middle of the coding sequence of a gene ?

  The answer is in (insertion and deletion) part ☺

- What if a gene whose template strand contains the sequence (3'-TACTTGTCCGATATC-5') is mutated to (3'-TACTTGTCCAATATC-5')
  For both normal and mutant genes draw
  1- the double stranded DNA
  2- resulting mRNA
  3- amino-acid sequence (depend on the table page 7).

	Normal	Mutant
1- double DNA strand	3'- TACTTGTCCGATATC-5' 5'-ATGAACAGGCTATAG-3'	3'-TACTTGTCCGATATC -5' 5'-ATGAACAGGTTATAG-3'
2- mRNA sequence	5'-AUGAACAGGCUAUAG-3'	5'- AUGAACAGGUUAUAG -3'
3- amino acid sequence	Met-Asn-Arg-Leu-stop	Met-Asn-Arg-Leu-stop

- The mutation has no effect ☺ because the amino acid sequence still as it was ..

Concept 17.6: While gene expression differs among the domains of life, the concept of a gene is universal

- before 40 years organisms were divided into 3 categories : bacteria , archea, eukaryotes.

- Archaea are prokaryotes, but share many features of gene expression with eukaryotes

❖ **Comparing Gene Expression in Bacteria, Archaea, and Eukarya**

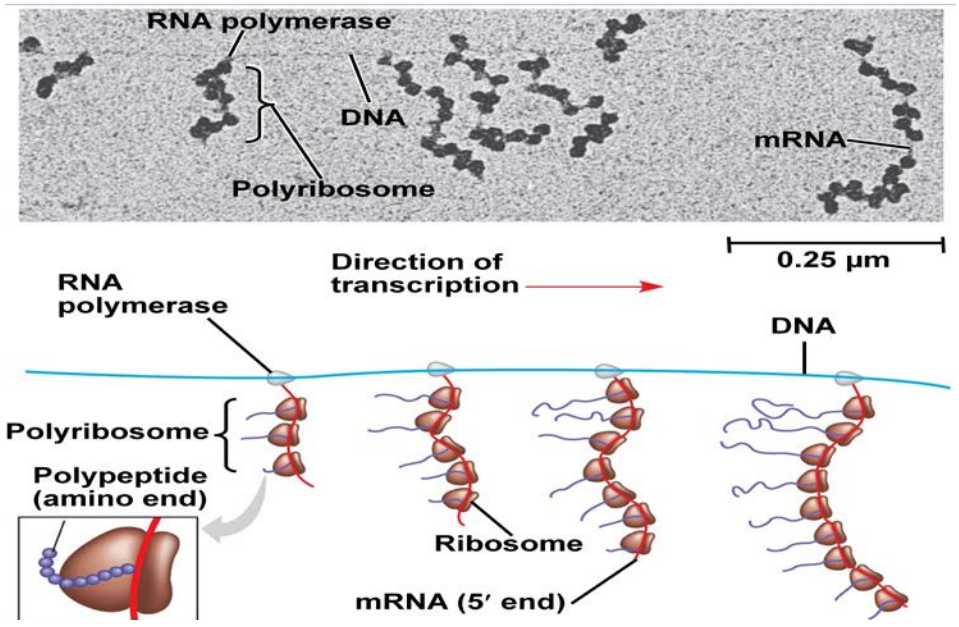
- Bacteria and eukarya differ in their RNA polymerases, termination of transcription ; archaea tend to resemble eukarya in these respects .

- bacterial and eukaryotic differ in ribosomes and initiation of translation, archea ribosome are the same size with bacterial ribosome , but their sensitivity to chemical inhibitors is most closely to eukaryotic ribosomes , and in initiation it is more like bacterial process .

- Bacteria (with absence of nucleus) can simultaneously transcribe and translate the same gene . and the resulted protein quickly diffuse to its site of function .
- In eukarya, nuclear envelope separates transcription and translation . it provides a compartment of extensive RNA processing that leads to have additional steps in the process
- In archaea, since it lack nuclear envelope ,transcription and translation are likely coupled .

The Idea of this figure >>
In bacterial cells the translation of mRNA can begin as soon as the leading (5‘) end of mRNA molecule peels away from the DNA template .

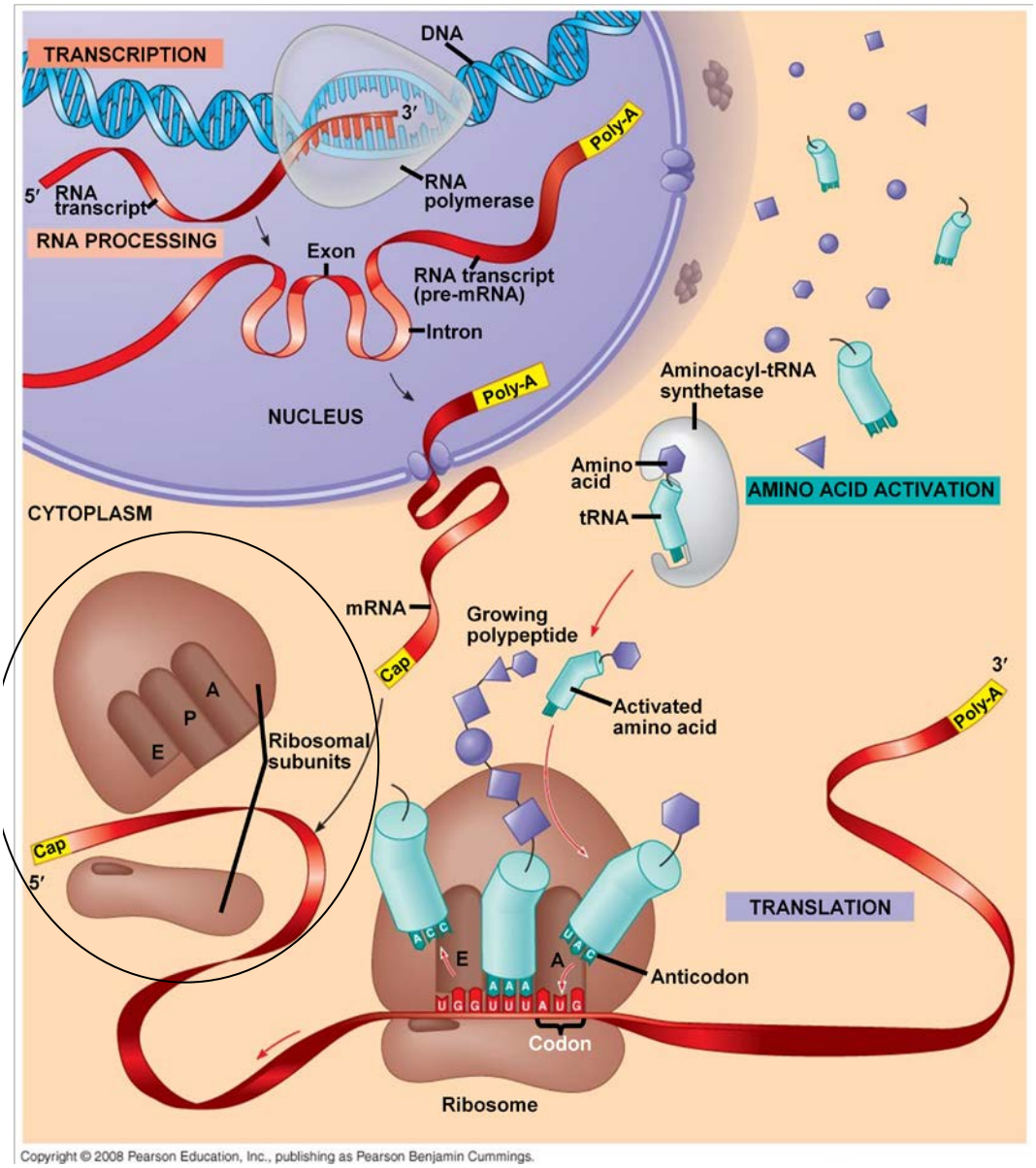
Figure 17.24 Coupled transcription and translation in bacteria



❖ **What Is a Gene?** ***Revisiting the Question***

- The idea of the gene itself is a unifying concept of life
  - We have considered a gene as:
- A discrete unit of inheritance
- A region of specific nucleotide sequence along the length of a DNA molecule in a chromosome (chapter 16)
- A DNA sequence that codes for a specific polypeptide chain (chapter 17)

Figure 17.25 A summary of transcription and translation in a eukaryotic cell



The 2 subunits dissociate after each translation .

Finally we can define gene as >>

a region of DNA that can be expressed to produce a final functional product that is either a polypeptide or an RNA molecule .

Quick Summary :
types of RNA >>

Type of RNA	Functions
Messenger RNA (mRNA)	Carries information specifying amino acid sequences of proteins from DNA to ribosomes.
Transfer RNA (tRNA)	Serves as adapter molecule in protein synthesis; translates mRNA codons into amino acids.
Ribosomal RNA (rRNA)	Plays catalytic (ribozyme) roles and structural roles in ribosomes.
Primary transcript	Is a precursor to mRNA, rRNA, or tRNA, before being processed. Some intron RNA acts as a ribozyme, catalyzing its own splicing.
Small nuclear RNA (snRNA)	Plays structural and catalytic roles in spliceosomes, the complexes of protein and RNA that splice pre-mRNA.

This chapter has many Terms , we try to make it easier but all of them are necessary ..!

if there is any mistakes we are sorry and please tell us by a message to our account on face book " lijan Rehabilitation"

best wishes for You ☺