بسم الله الرحمـٰن الرحيم

(وَفَوْقَ كُلِّ ذِي عِلْمٍ عَلِيمٌ)

Cytology & Molecular Biology | FINAL 18

Translation pt.2

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Let's review!

- Translation is the process of synthesizing proteins from mRNA.

- Mechanism: mRNA is read as triplets (3 nucleotides make a codon and each one gives a certain specific amino acid molecule)

REMEMBER :

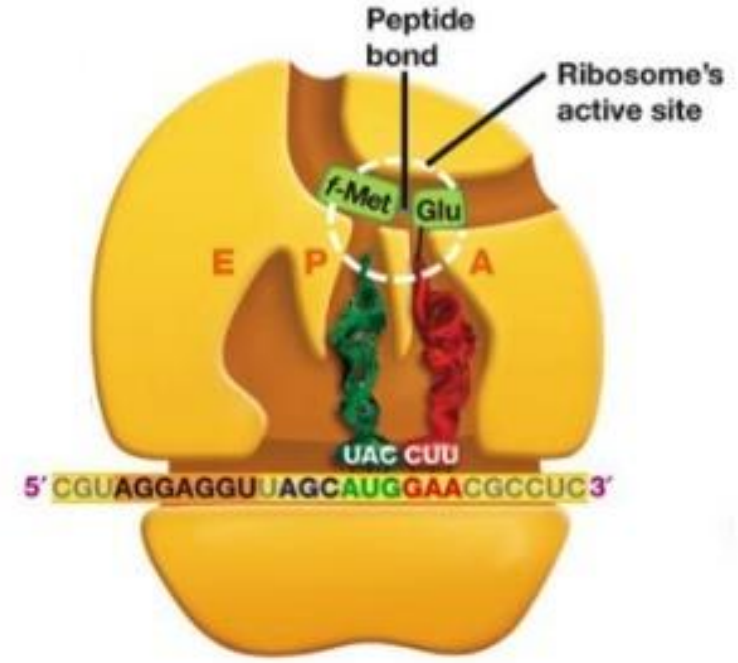
- mRNA is read from 5'→3'

- The protein synthesis from N terminus to C terminus and the start codon is AUG .

The general mechanism of translation

Our focus on human cell or eukaryotic cell. the same process occurs in bacteria

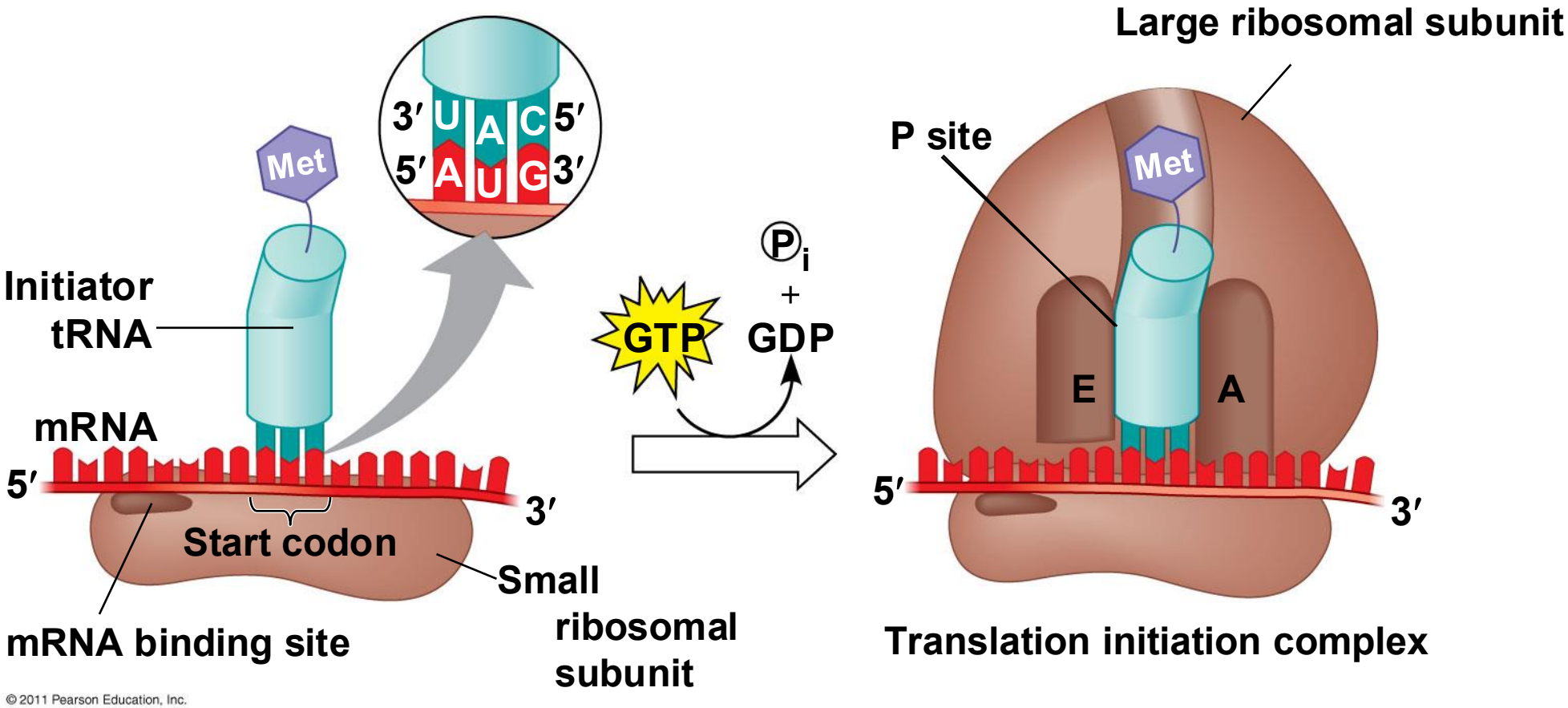
Three stages: initiation, elongation, and termination.
The direction is 5’ → 3’.(the mRNA is read from 5’→3’)
Protein synthesis begins at the amino terminus and extends toward the carboxyl terminus.



Start of translation

In both prokaryotes and eukaryotes, translation starts at specific initiation sites, which is AUG (methionine), and not from the first codon of the mRNA.

See the next slide for explanation



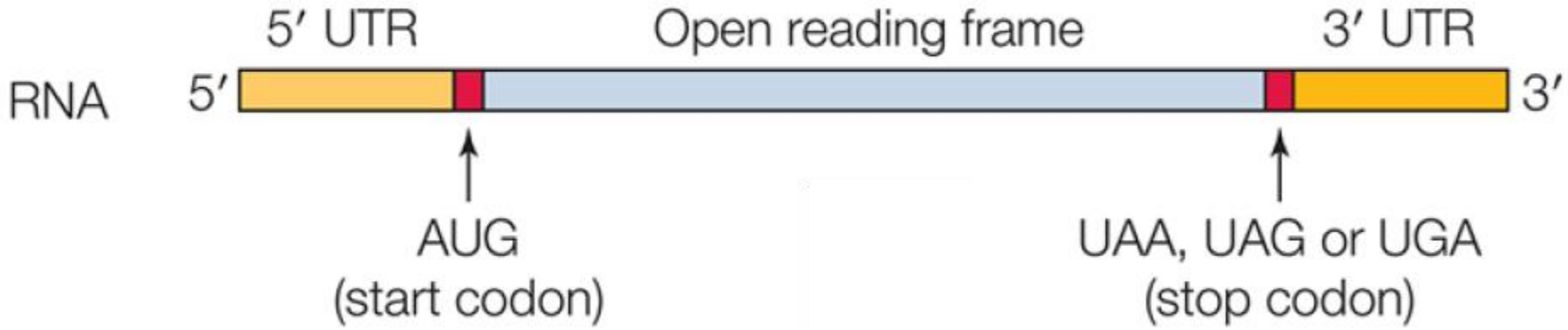
- ✓ What happens that you have the formation of this complex (smaller ribosomal subunit with the mRNA and the tRNA)

1. The first tRNA is the one that has a methionine associated with it.
2. The start codon is AUG (you should know what the anti-codon should be, from 5'→3' always).
3. Then the large ribosomal subunit comes and it places the tRNA in the P chamber while the A chamber stays empty.

- ✓ So, that's initiation.

Untranslated regions

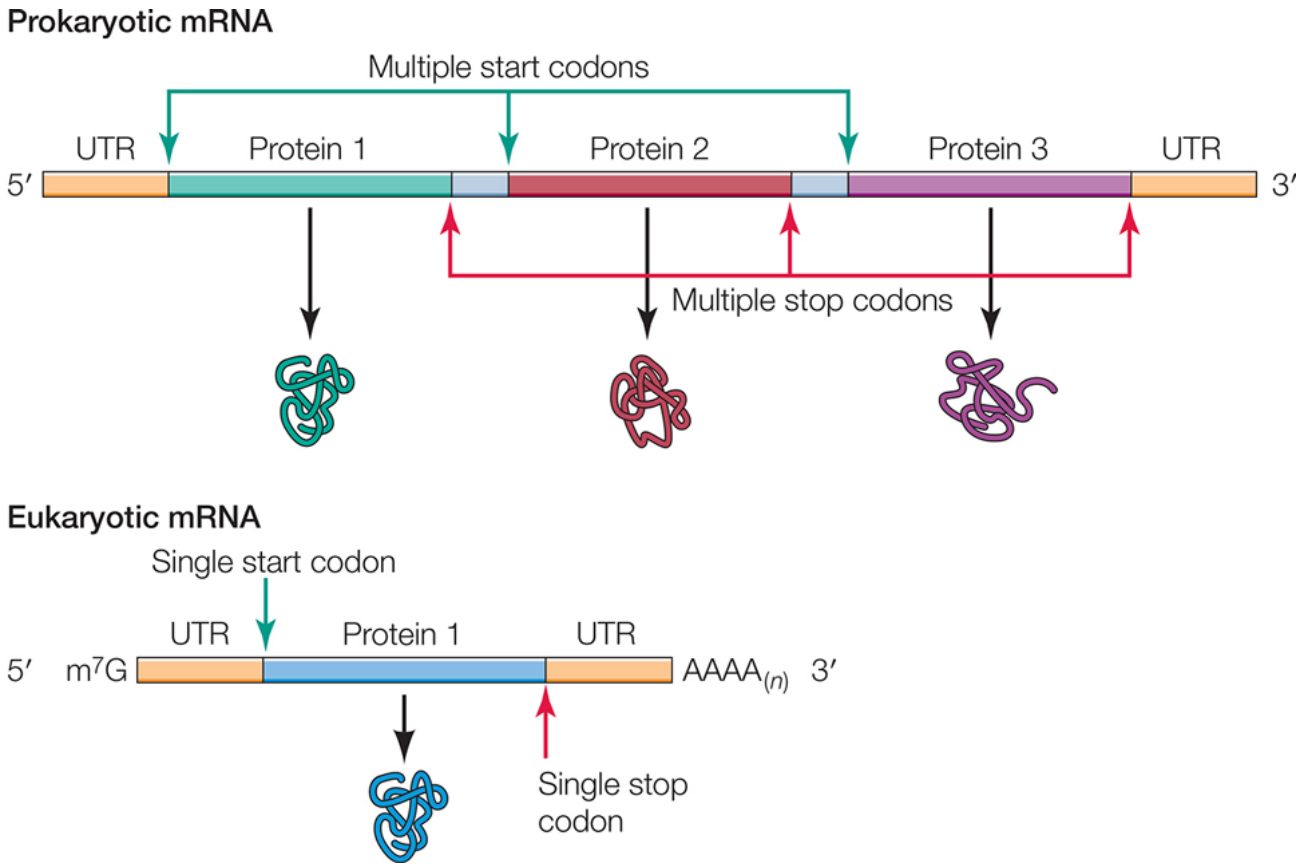
The 5´ terminal portions upstream of the initiation sites of both prokaryotic and eukaryotic mRNAs contain noncoding sequences, referred to as 5´ untranslated regions (UTRs).
There is also a 3'-untranslated region, which follows any of he three stop codons.



- Translation does **not necessarily begin at the first AUG codon present on the mRNA.**
  There must be a specific sequence or signal that allows the ribosomal subunit to recognize **which AUG should be used as the start codon.**
  For this reason, mRNA contains a **5′ untranslated region (5′ UTR)**, which is located upstream of the start codon and is not translated into protein.
  Translation then proceeds until a **stop codon** is reached(one of the following three: UAA, UAG, or UGA).
  After the stop codon, there is another non-translated region known as the **3′ untranslated region (3′ UTR).**
  The untranslated regions are important when discussing regulation in certain examples.

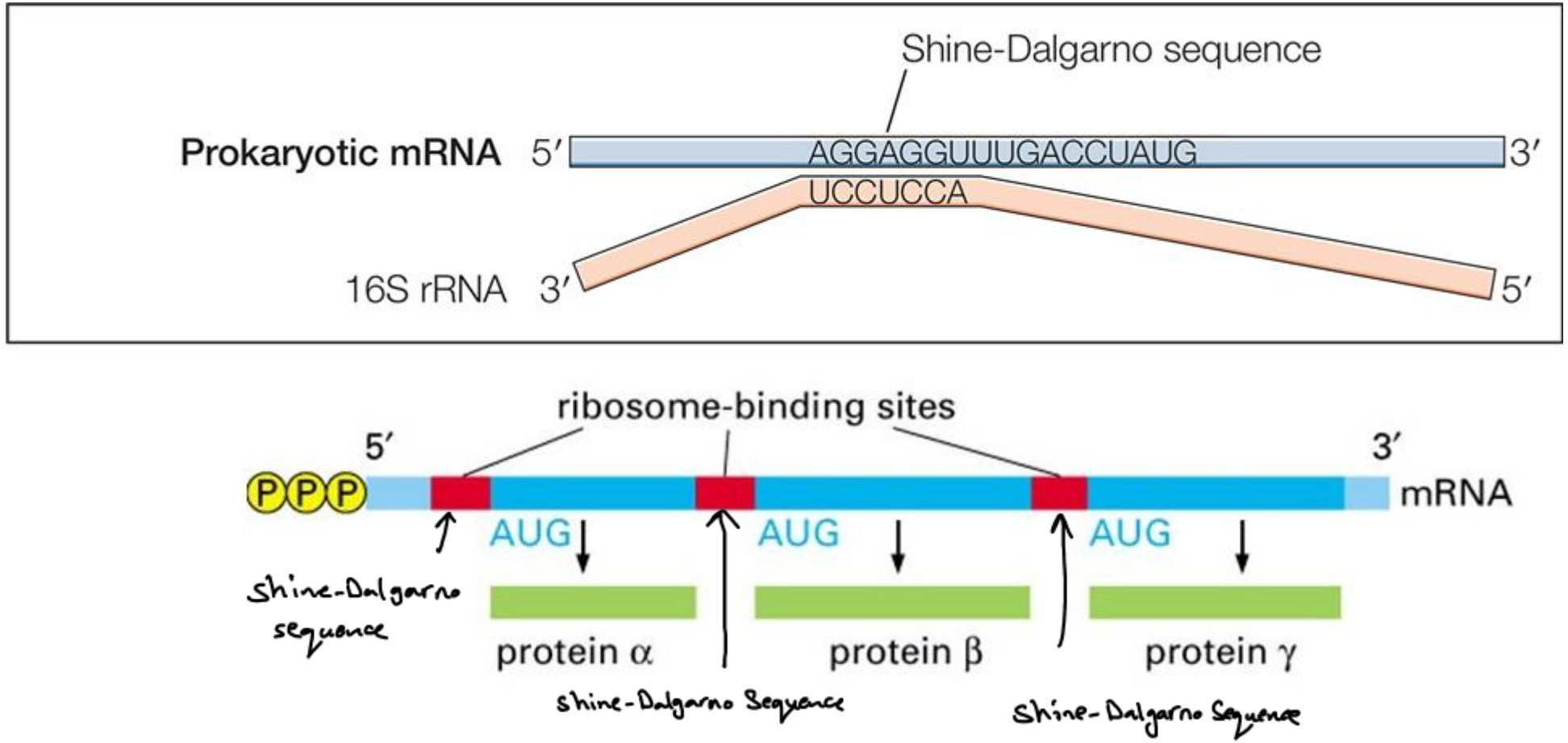
Remember…

Bacterial mRNA is polycistronic
Eukaryotic mRNA is monocistronic



This is why we previously mentioned that in bacterial cells, mRNA can be polycistronic, meaning that a single mRNA molecule can give rise to multiple polypeptides, each translated from a different region of the same mRNA, such as in the lac operon.
Although bacteria do have monocistronic genes, they also contain polycistronic genes.
In contrast, in **eukaryotic cells**, one **mature mRNA** (the ready to use mRNA for translation) generates **one polypeptide**.
This single polypeptide may form one functional protein if it folds properly, or it may undergo degradation or cleavage into smaller peptides, which can result in different peptide products.
Therefore, **the general concept in eukaryotes is:**
one mRNA → one polypeptide.
A question then arises in bacteria:
If a polycistronic mRNA contains multiple AUG codons, how does the cell know **where to start translation, and how does it know to start translation from one AUG and then from another AUG** in order to produce correctly structured polypeptides?

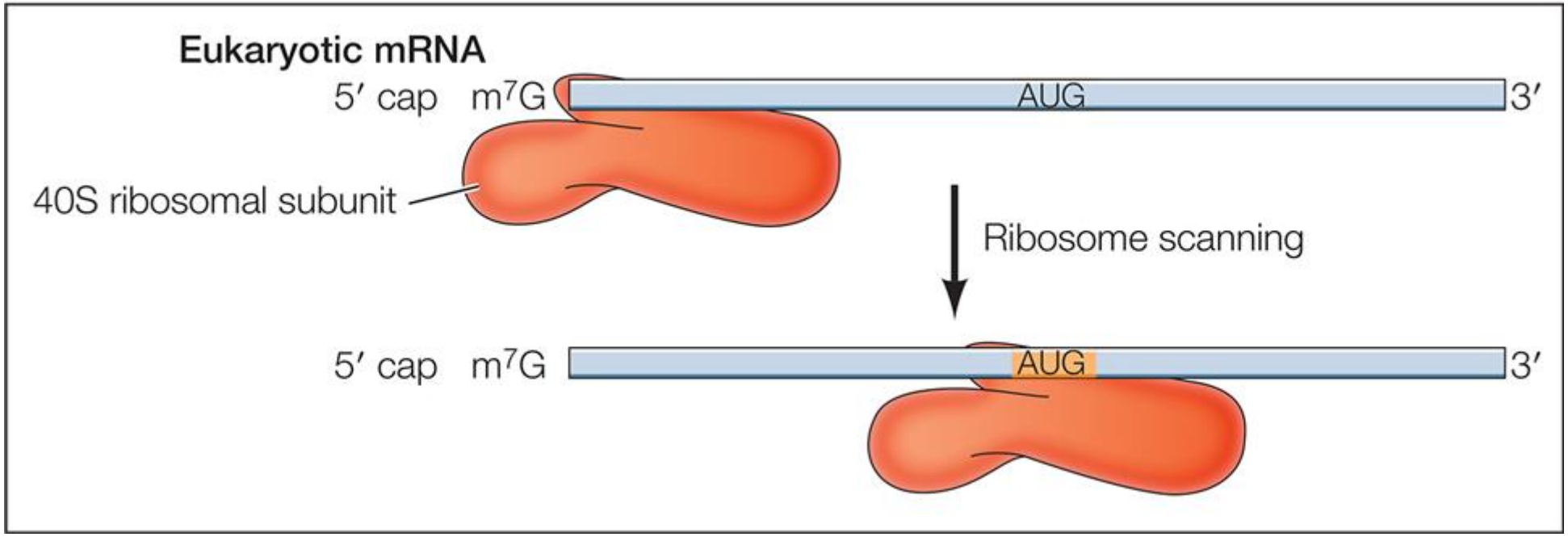
Shine-Dalgarno sequence



We know this because, in bacteria only, there is a sequence called the Shine-Dalgarno sequence, named after the scientists who discovered it.
This sequence is present on the mRNA and is located **upstream of the AUG start codon.**
The small ribosomal subunit scans the mRNA starting from the 5′ end. It was found that the Shine-Dalgarno sequence is **complementary to a sequence in the small ribosomal subunit.**
As a result, the small ribosomal subunit binds to this sequence, **stops scanning the mRNA**, and recognizes that this is the correct site to initiate translation.
Translation then begins from the AUG codon that comes immediately after the Shine-Dalgarno sequence.
The small ribosomal subunit initially associates with the mRNA, but translation does not begin until the ribosome encounters the Shine-Dalgarno sequence and stops.
If there is another AUG elsewhere on the mRNA that is not preceded by a Shine-Dalgarno sequence, translation will not start from that AUG. Instead, translation starts only from the AUG that directly follows the Shine-Dalgarno sequence.

But in eukaryotes…

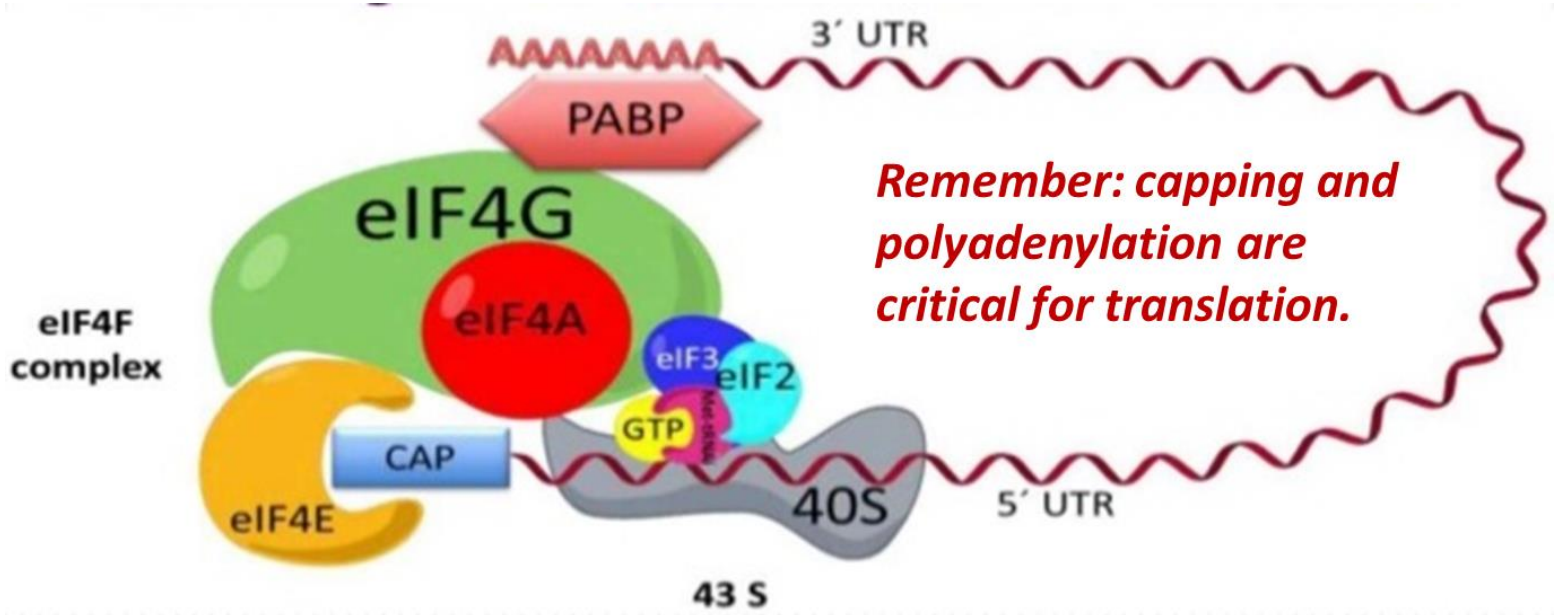
Eukaryotic ribosomes recognize mRNAs by binding to the 7-methylguanosine cap at their 5´ terminus.



Do we have the same mechanism in human cells, the human genome, or human mRNA?
Do we have specific sequences that determine where translation starts?
In eukaryotic cells, there are **two mechanisms** for the initiation of translation, meaning there are two different marks that help initiate the process.
The first mechanism occurs in some mRNA molecules and involves the 5′ cap, (which is a modification at 5′ end of the mRNA when methylguanosine attached in an inverted manner).
One function of the cap is to protect the mRNA, allowing the cell to recognize it as a proper mRNA molecule and preventing it from being damaged.
Another important function of the cap is its role in translation initiation.
In this mechanism, the **small ribosomal subunit** binds to the cap and then **scans the mRNA** until it reaches an AUG codon.
Translation starts from the **AUG that comes after the cap.**

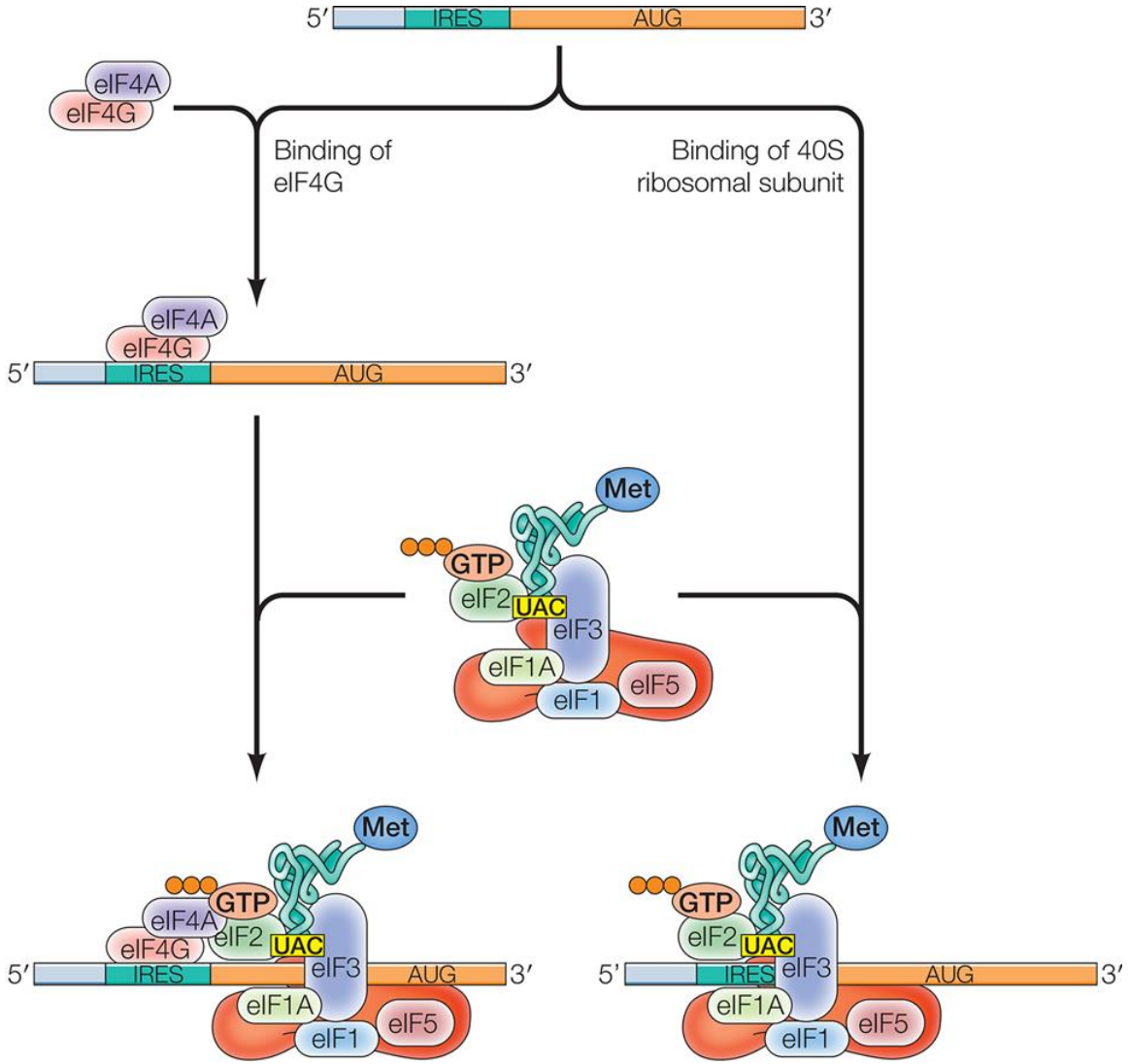
Translation initiation in eukaryotes

The eIF4 initiation factors form a complex that links the poly-A tail to the CAP via poly-A binding protein (PABP).
The eIF4 initiation factors then bring the mRNA to the small ribosomal subunit.



The question now is: how does the small ribosomal subunit find the cap, and how does it know that this AUG is the correct one to initiate translation?
This process requires help from proteins called **eukaryotic initiation factors 4 (eIF4)**.
The small "e" indicates that these are **eukaryotic** initiation factors, distinguishing them from bacterial initiation factors.
The eIF4 is **not a single protein**, but rather a group of proteins, such as eIF4A, eIF4B, eIF4G, and others. Together, they are referred to as the **eIF4 complex**.
The eIF4 complex mediates an association between the **5′ cap** and the **poly-A tail**.
(although the poly-A tail is located at the 3′ end of the mRNA and translation starts at the 5′ end, the poly-A tail contributes to translation by forming an **indirect** association with the cap through the eIF4 complex and the poly-A binding protein.)
This large complex stabilizes the binding of the small ribosomal subunit to the mRNA and signals that translation can begin.
This describes the **first mechanism of translation initiation in eukaryotes.**

Internal ribosome entry site (IRES)



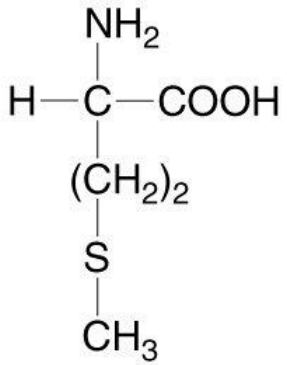
Alternatively, internal ribosome entry site (IRES) exist in some other mRNAs and is recognized by the eIF4G protein followed by recruitment of the first tRNA and the small ribosome.

IRESs in other mRNAs may also be recognized directly by the 40S ribosomal subunit.

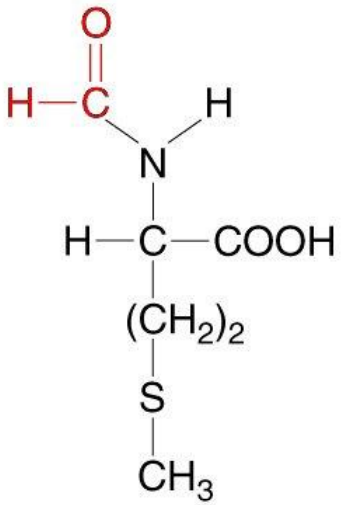
The **second mechanism** of translation initiation occurs in some mRNA molecules that contain special **consensus sequences**, known as **Internal Ribosome Entry Sites (IRES).**
These IRES sequences are similar in concept to the Shine-Dalgarno sequence in bacteria.
They are located **immediately upstream of the AUG start codon** and serve as **binding sites for eIF4 proteins**.
The eIF4 proteins bind to the IRES, which leads to the recruitment of the **small ribosomal subunit together with the initiator tRNA carrying methionine.**
The entire initiation complex is assembled directly at the IRES, and translation begins from the **AUG codon that follows the IRES.**
This represents the **second mechanism of translation initiation in eukaryotes.**
It is important to note that **both mechanisms require eIF4 proteins**, making them essential for translation initiation.

The first amino acid

Translation always initiates with the amino acid methionine, usually encoded by AUG.
In bacteria, it is N-formylmethionine.



Methionine



N-Formylmethionine

There are important differences between prokaryotes and eukaryotes in terms of translation.
In both systems, the start codon is AUG, which encodes for methionine.
However, in prokaryotic cells, specifically in bacteria such as E. coli, the first methionine is not a regular methionine. Instead, it is a chemically modified methionine known as formylmethionine (fMet), which contains a formyl group.
Therefore, in E. coli, the first amino acid of the protein is formylmethionine, and the initiator tRNA carries formylmethionine.
In contrast, in eukaryotic cells, translation initiates with a regular methionine molecule.
It is important to note that throughout molecular biology course , differences between prokaryotic and eukaryotic systems must always be considered, whether in transcription, translation, mRNA processing, or replication.
One key difference between prokaryotes and eukaryotes is that prokaryotic cells initiate translation with formylmethionine, whereas eukaryotic cells initiate translation with methionine.

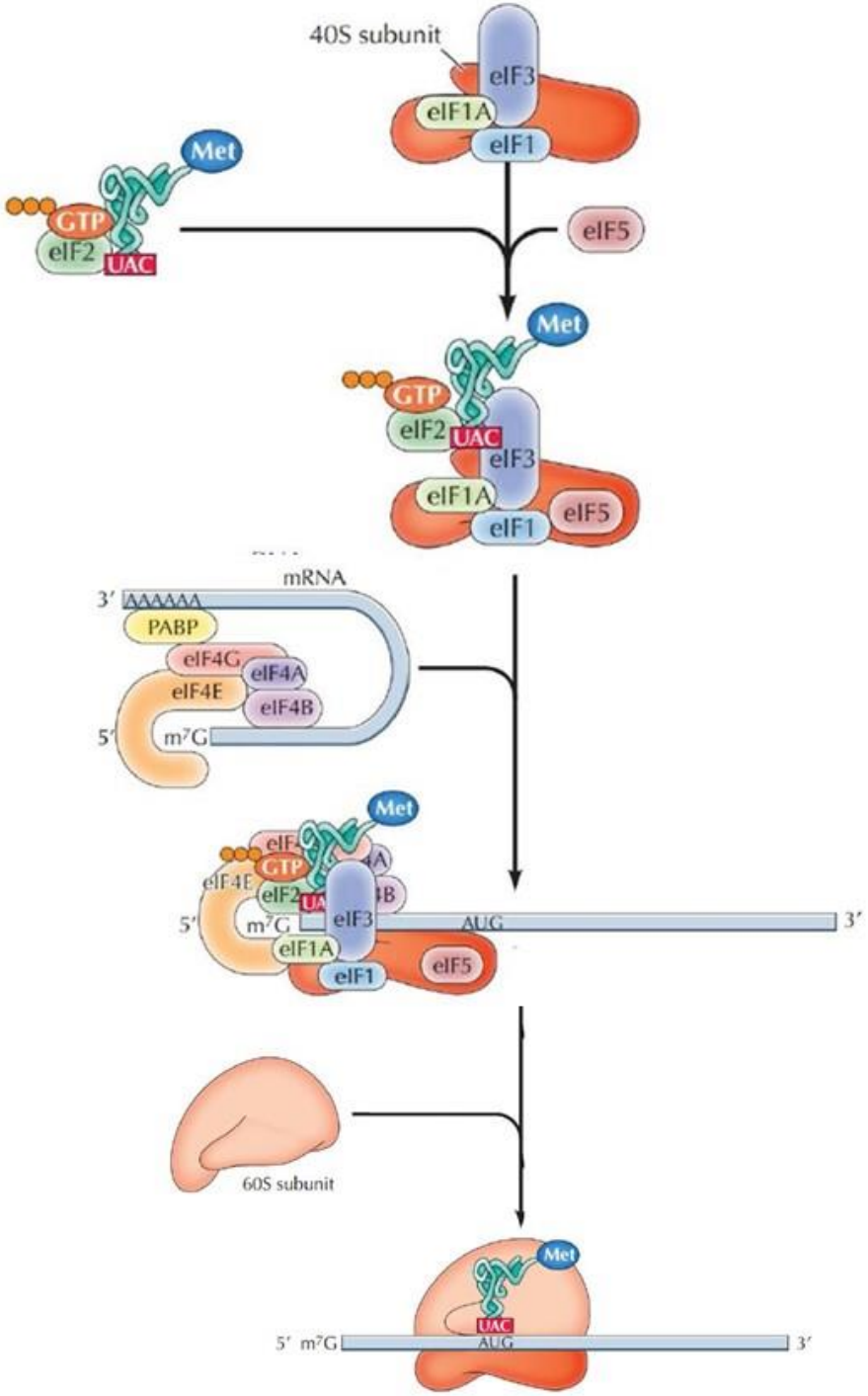
Building a polypeptide

The three stages of translation

- Initiation
- Elongation
- Termination

All three stages require protein “factors” that aid in the translation process.

Translation initiation



- tRNA forms a complex with the small ribosomal subunit with the help of eIF2.
- mRNA joins the complex with the help of eIF4.
- The small ribosomal subunit scans for the first AUG.
- The large ribosomal subunit joins them all.

In translation initiation, we already discussed the role of eIF4, but there is **another** important **initiation factor**, which is **eIF2**.

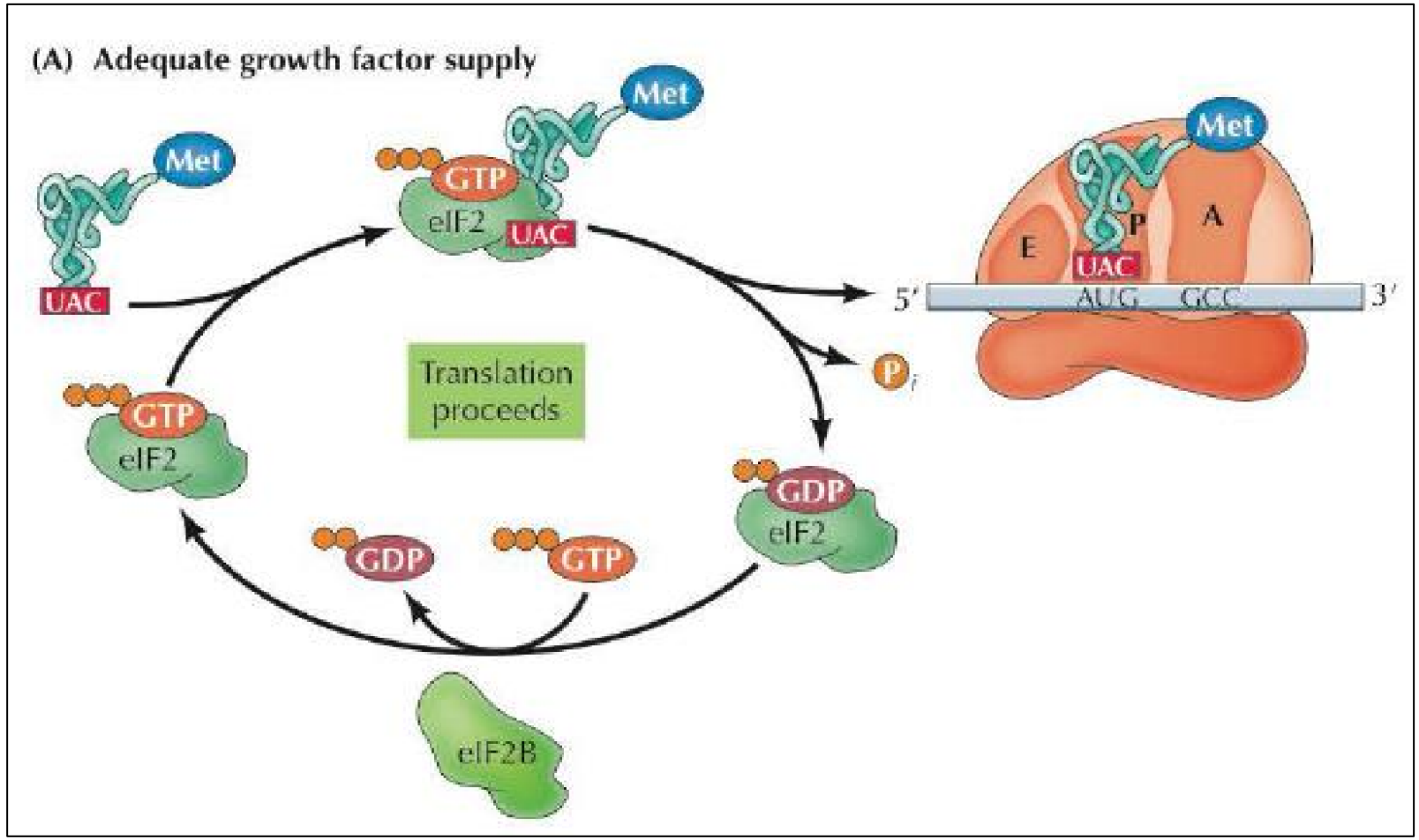
The function of **eIF2** is to **bring the initiator tRNA carrying methionine** and form a complex with the small ribosomal subunit.
This results in a **binary complex consisting of the small ribosomal subunit and the initiator tRNA.**

This complex is then brought to the mRNA with the help of the **eIF4** complex, leading to the formation of the **complete initiation complex** on the mRNA.

Once this large initiation complex is formed, the **large ribosomal subunit** joins the complex and positions the initiator tRNA, which carries methionine, in the **P site.**

Regeneration of eIF2

At this point, initiation has occurred, and the focus shifts to eIF2. For eIF2 to function, it must be bound to GTP.



- eIF2 is complexed to GTP to be active. When the correct tRNA is inserted, GTP is hydrolyzed to GDP.
- The active eIF2/GTP complex must be regenerated by exchanging of the GDP for GTP.

We previously discussed in metabolism that:

- ATP is the general energy currency of the cell,
- UTP is involved in carbohydrate metabolism,
- CTP is involved in lipid metabolism,
- GTP is commonly involved in protein-related functions.

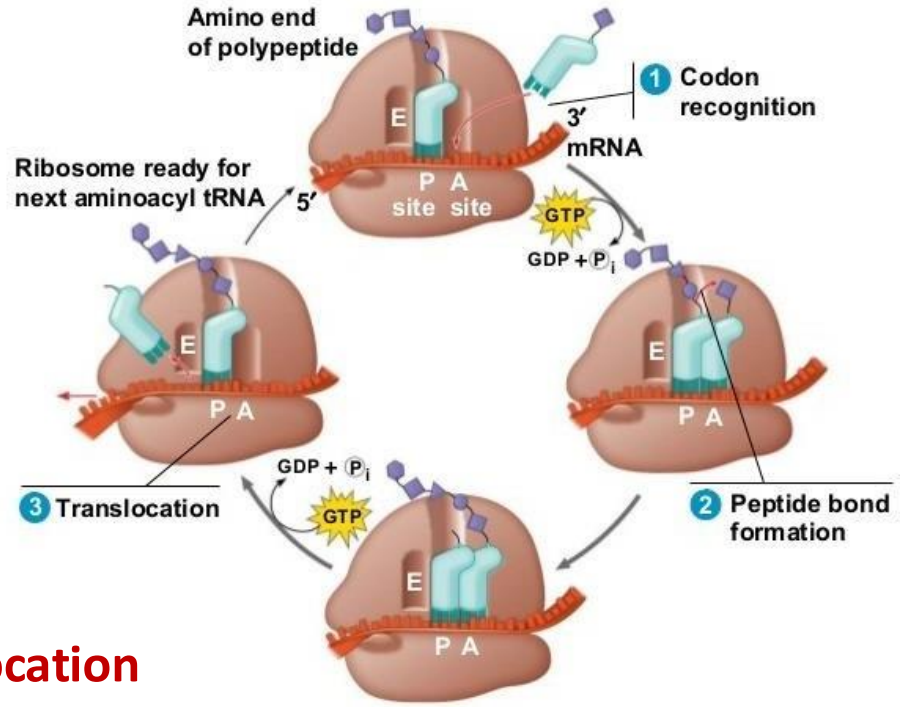
When eIF2 successfully delivers the initiator tRNA to the small ribosomal subunit, **GDP is hydrolysed and released from the complex.**
For eIF2 to function again, GDP must be exchanged for GTP, allowing eIF2 to be reactivated.
This regeneration process is regulated by a number of proteins.

Translation elongation

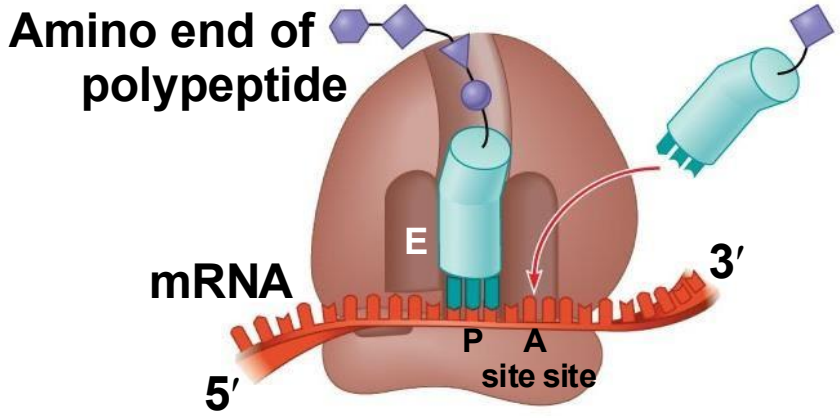
Three steps:

1. **aminoacyl-tRNA binding**
2. **peptide bond formation**
3. **translocation with the help of elongation factors (eEF).**

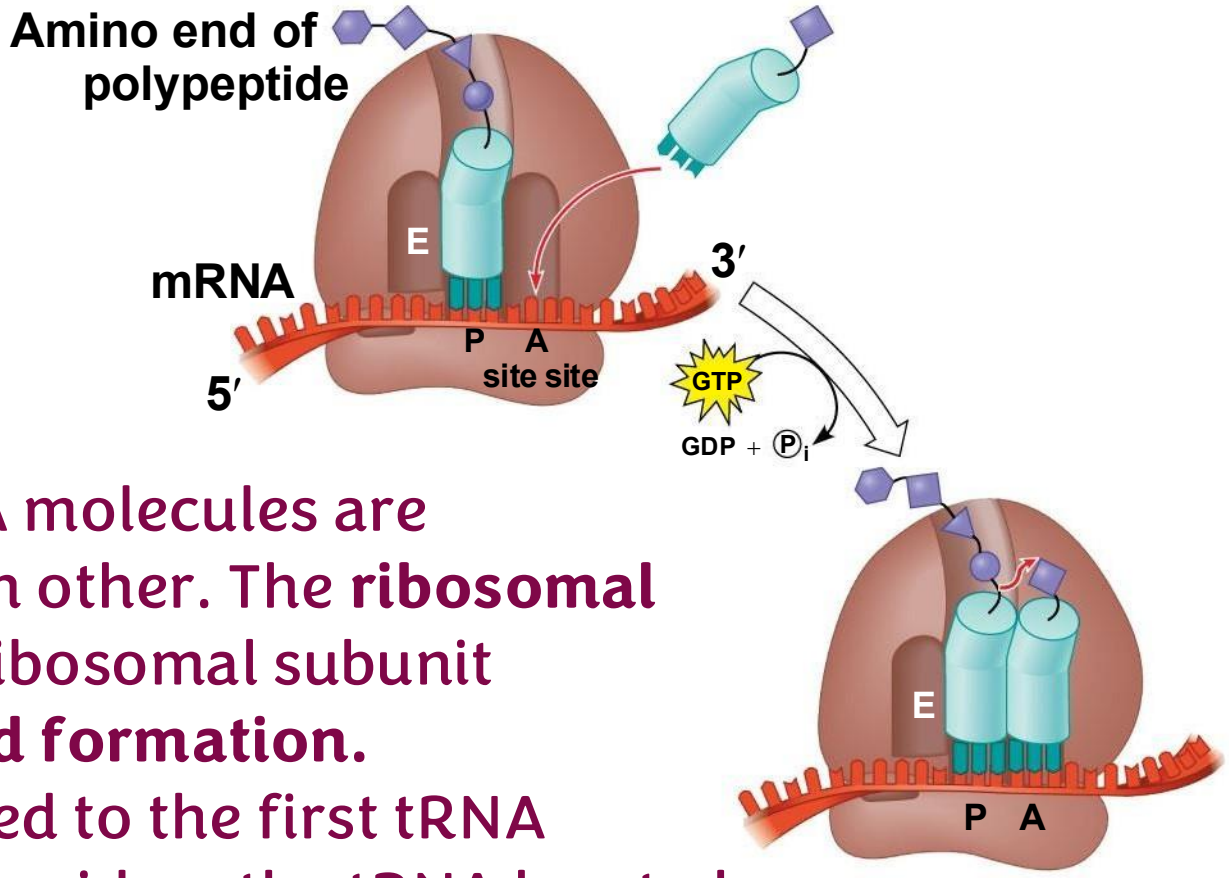
eEF1α brings next aminoacyl-tRNA to the A chamber



eEF2 is critical in ribosomal translocation

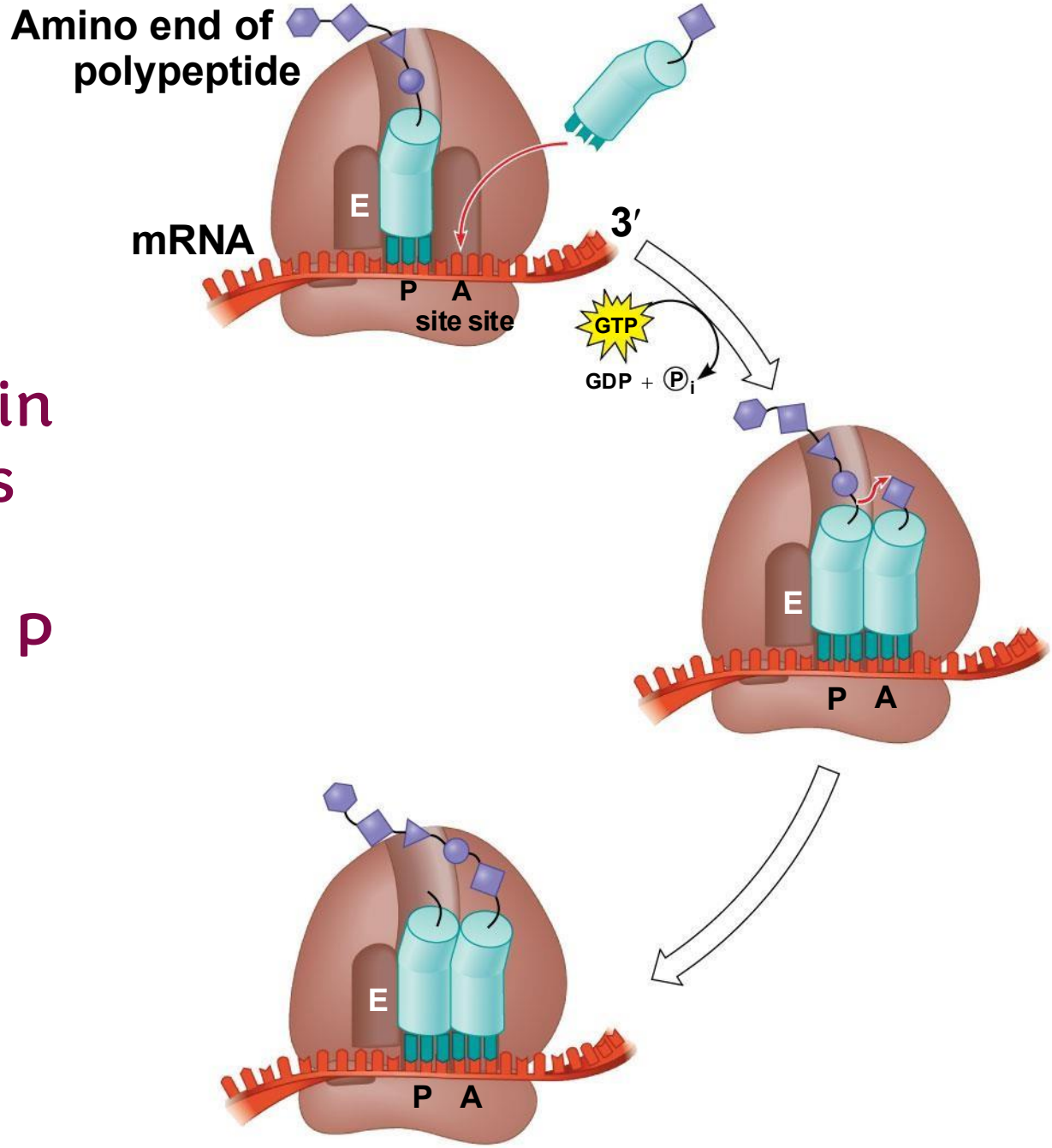


Translation elongation begins with a **tRNA occupying the P site**, while the **A site is empty.**
A second tRNA, whose anticodon is **complementary to the codon on the mRNA**, enters the A site with the help of another protein called **eEF1**.
eEF1 delivers the second tRNA to the **large ribosomal subunit.**



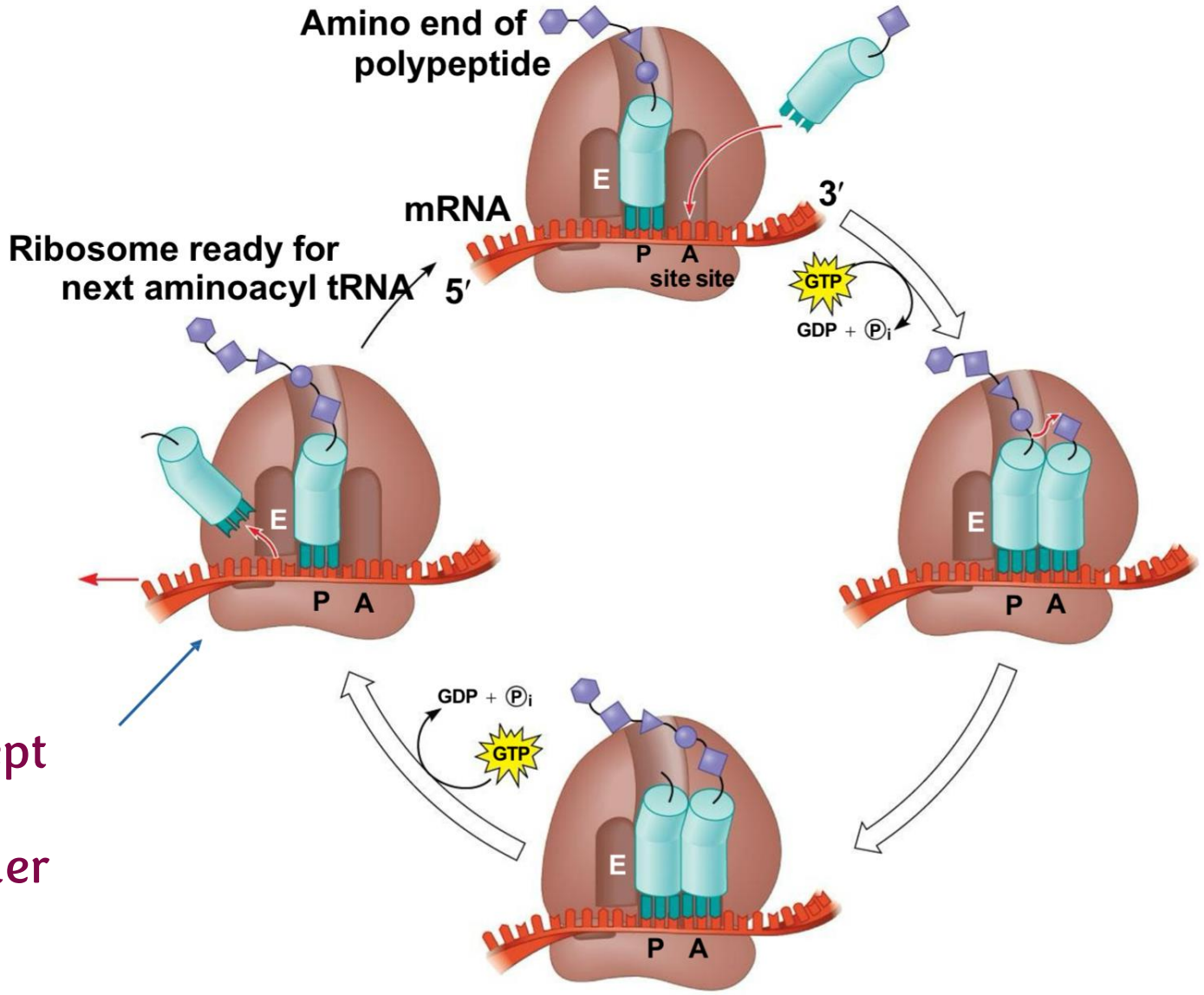
At this point, two tRNA molecules are positioned next to each other. The **ribosomal RNA** within the large ribosomal subunit catalyzes **peptide bond formation.**
The amino acid attached to the first tRNA transfers to the amino acid on the tRNA located in the **A site.**

As a result, the tRNA in the A site now carries **both amino acids**, while the tRNA in the P site becomes **unchanged** .



❑ What happens next?

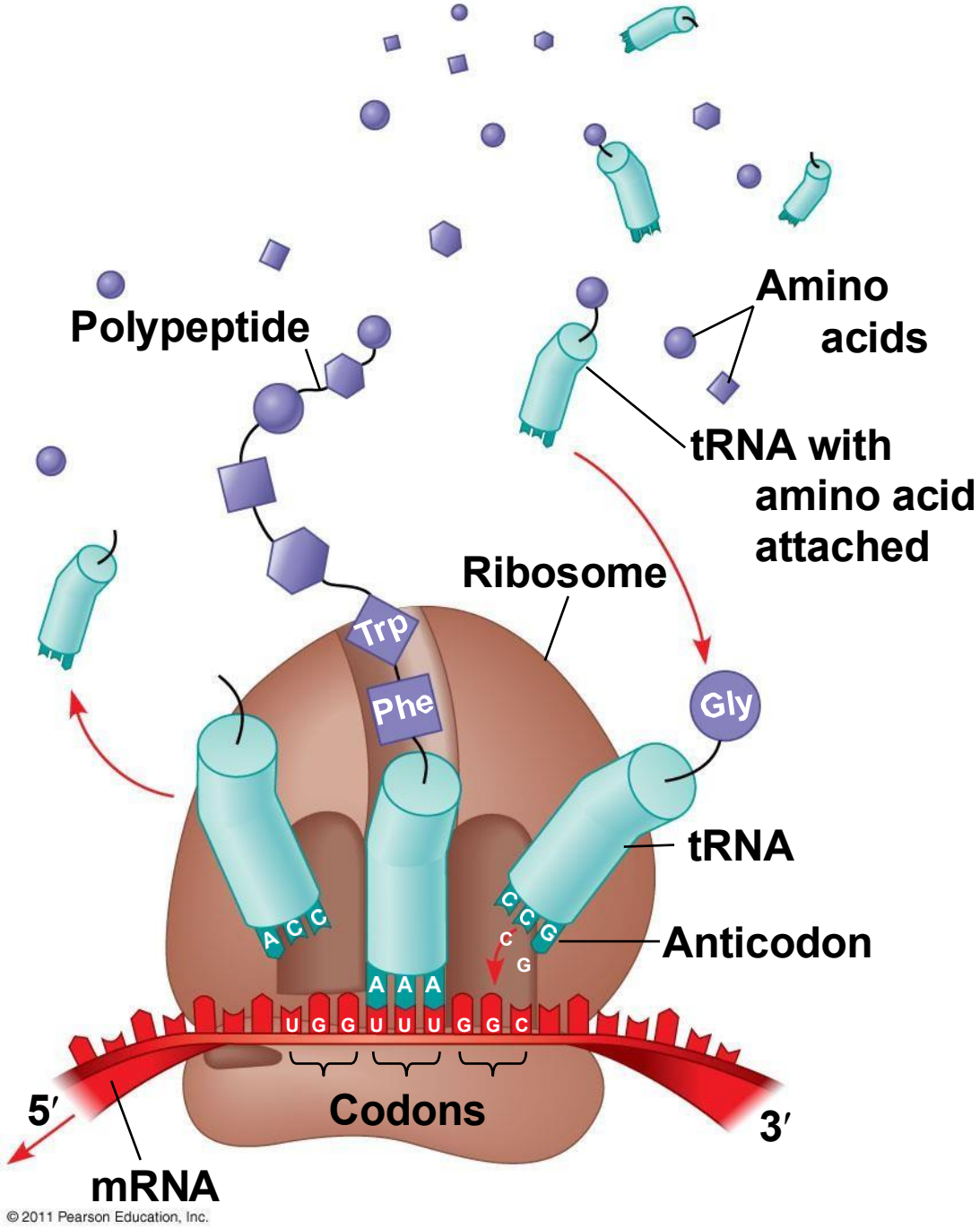
Another protein, **eEF2,** mediates **ribosomal translocation**, causing the ribosome to move **one codon forward** along the mRNA. This movement shifts the ribosome by **three nucleotides**, and the cycle continues.



❑ During translocation:

- The uncharged tRNA moves into the **E site** and exits the ribosome,
- The tRNA that was in the A site moves to the **P site,**
- The **A site becomes empty,** ready to accept the next tRNA.
- This cycle continues as eEF1 brings another tRNA, peptide bonds are formed by rRNA, and eEF2 moves the ribosome forward.

The process continues



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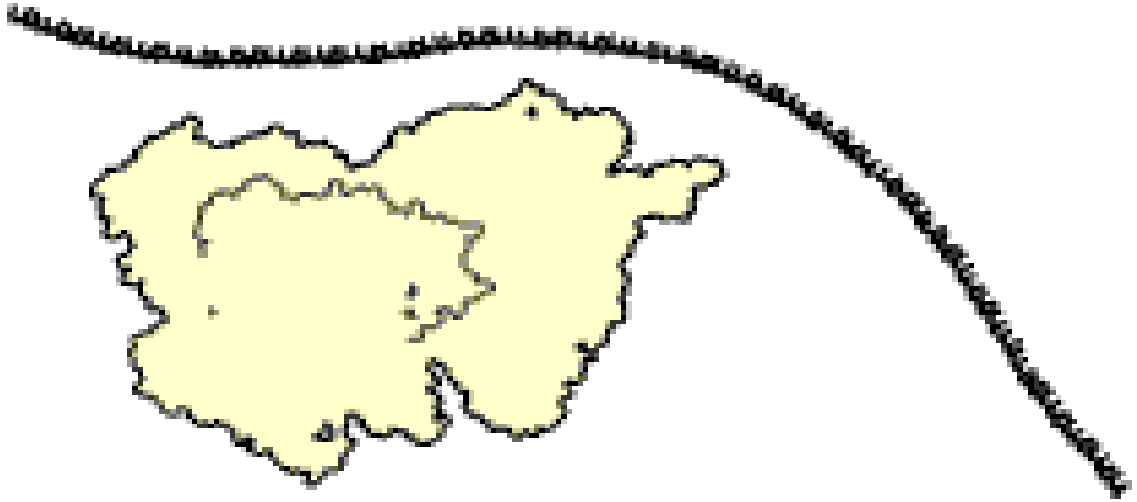
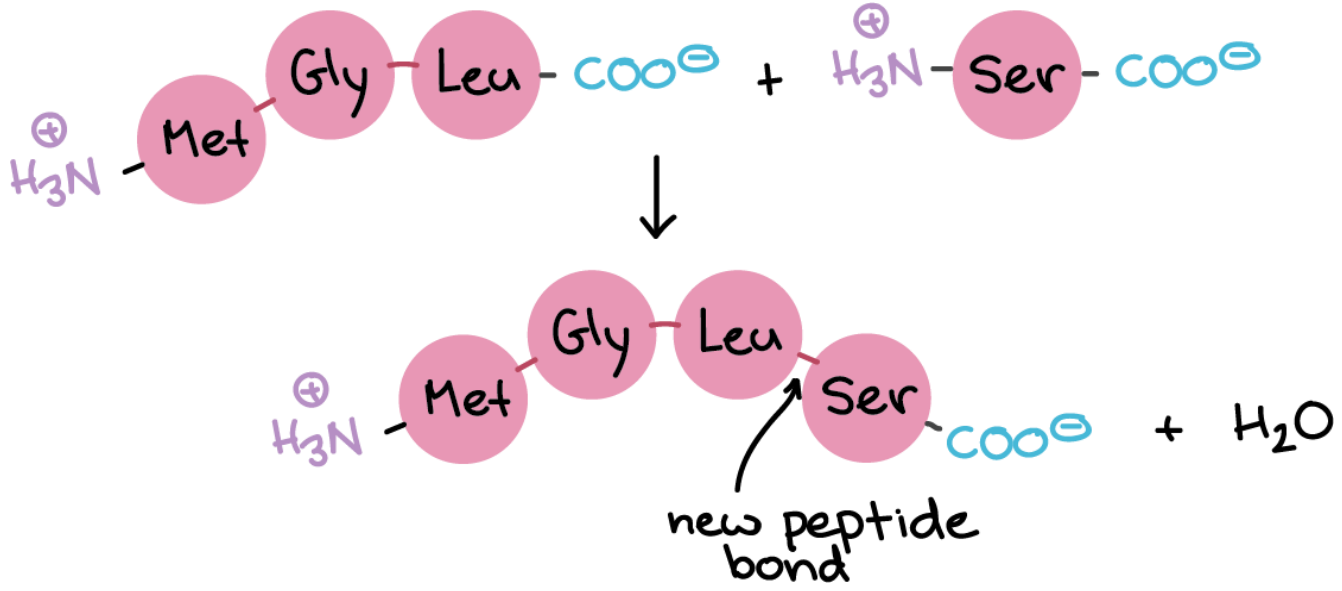


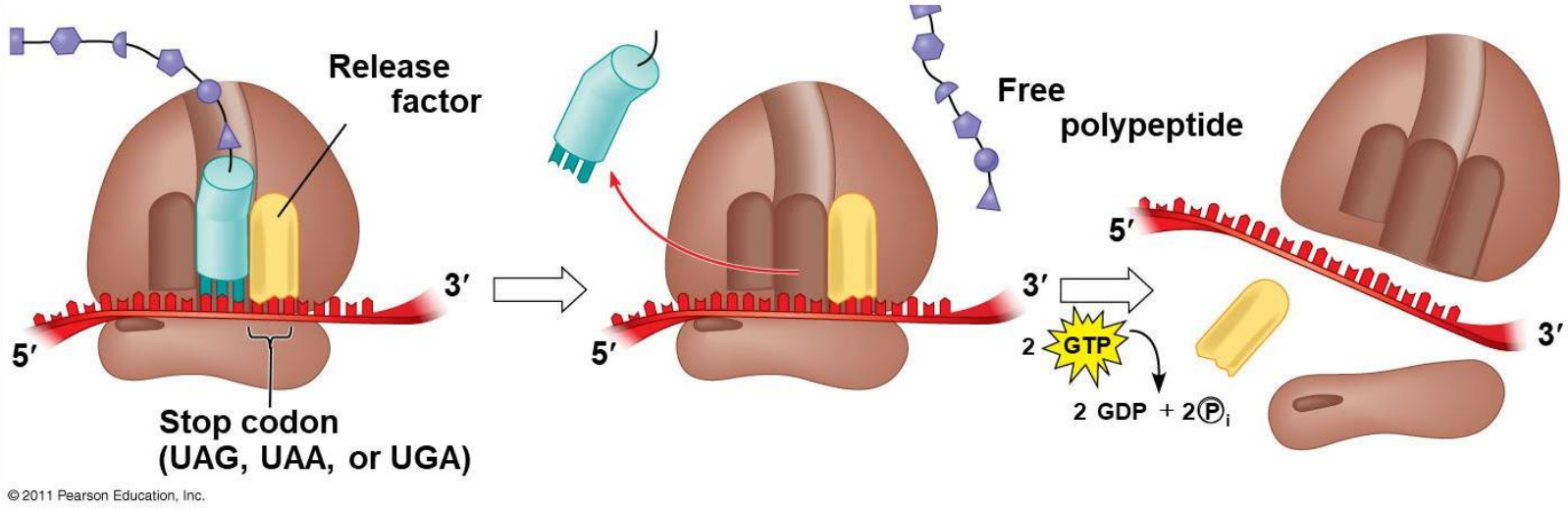
Elongation of the Polypeptide Chain

- During the elongation stage, amino acids are added one by one to the preceding amino (N)-terminus to the carboxy (C)-terminus of the growing chain.



Termination of Translation

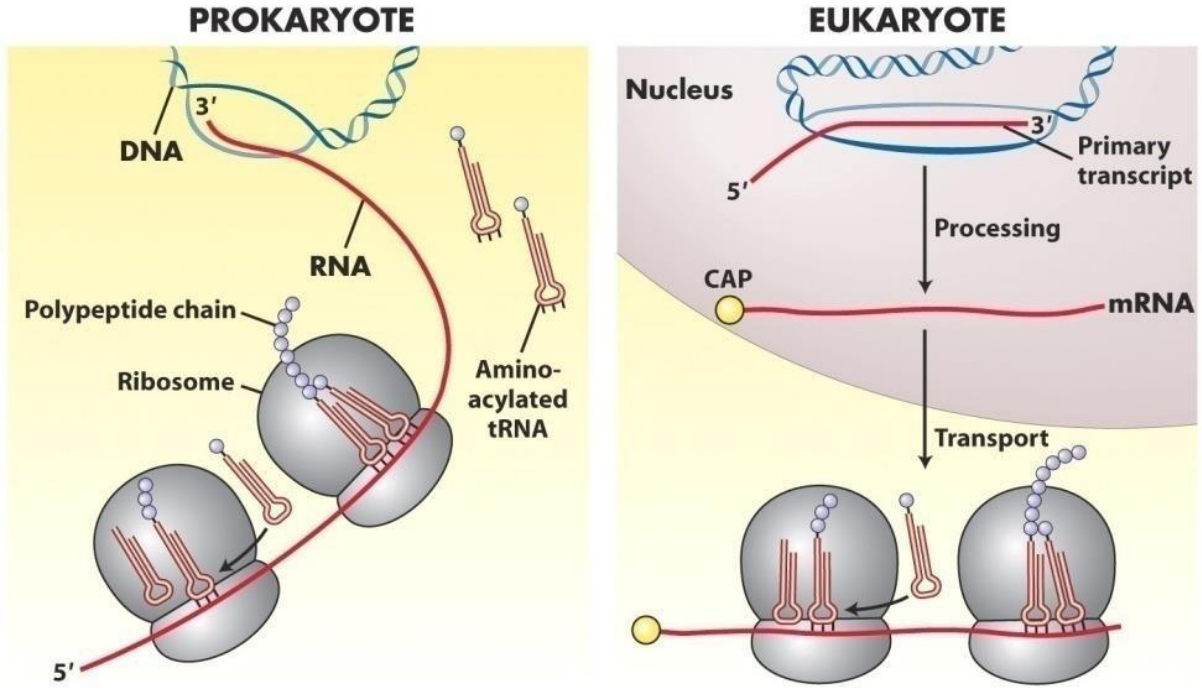
- The codons UAA, UAG, and UGA are the stop signals. They are not recognized by any tRNAs, but a release factor protein.
- The empty A site accepts release factors, which cause the release of the polypeptide, and the translation assembly then comes apart.



Elongation continues until the ribosome reaches a **stop codon**, which can be **UAA, UAG, or UGA.**
There are **no tRNAs** with anticodons complementary to these stop codons, so the **A site remains empty**.
Instead, proteins known as **release factors** recognize the stop codon.
A release factor binds to the A site and causes **dissociation of the entire translation complex**.
The large ribosomal subunit, the small ribosomal subunit, the mRNA, and the tRNA molecules all separate.
This marks the **end of translation**.
Thus, **release factors** are responsible for the **termination of translation** by dissociation of the whole complex.

Transcription/translation Coupling

- Translation and transcription are coupled in space and time in prokaryotes.



Can transcription and translation take place at the same time and in the same place?

The answer is **yes**, but **only in prokaryotic cells.**

In prokaryotes, there is **no nuclear membrane** and **no nucleus**. As a result, the mRNA begins to emerge from the RNA polymerase during transcription, and there is nothing preventing ribosomes from binding to the **5′ end** of the mRNA and starting translation.

The ribosome can recognize the **AUG start codon** and the **Shine-Dalgarno sequence**, allowing translation to begin while transcription is still ongoing.

Therefore, transcription and translation can occur **simultaneously** in prokaryotic cells.

This is possible because prokaryotic cells lack:

•a nuclear membrane,

•mRNA processing steps such as **capping, splicing, and poly-A tail addition**.

Additionally, the first part of the mRNA that exits the RNA polymerase is the 5′ end, which is the starting point for translation.

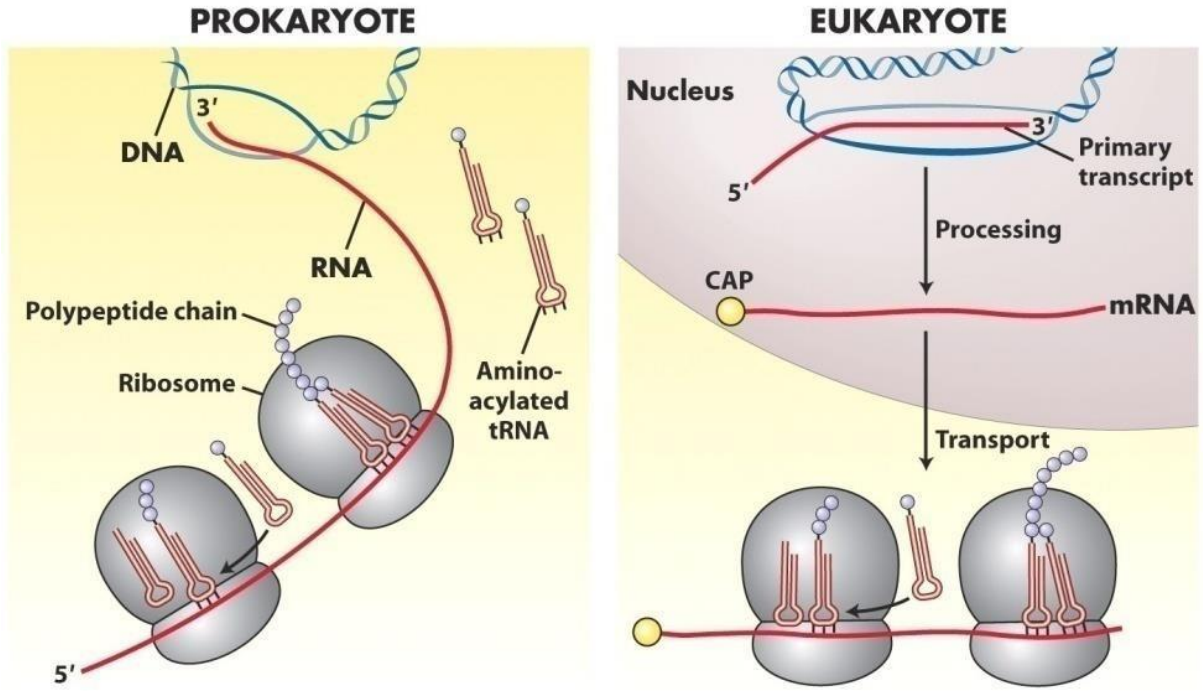
As a result, ribosomes can bind to the mRNA and synthesize proteins at the same time that transcription is taking place.

In eukaryotic cells, however, this process is not straightforward.
1. transcription occurs in the **nucleus**, while translation takes place in the **cytosol**. These two processes are **compartmentalized** by the **nuclear envelope**, meaning they are separated in **space** and also in **time.**

2. eukaryotic mRNA requires **processing** before it can be translated.
Although the cap is added early during transcription, as the mRNA is synthesized, proteins associated with RNA polymerase bind to the mRNA and mark the locations of **exons and introns.**
Once transcription is complete and termination occurs, the mRNA undergoes cleavage, a poly-A tail is added, and splicing takes place, during which introns are removed and the mRNA becomes mature.
During splicing, the cell may select different combinations of exons, such as exon 1, 2, 3, and 4, or undergo **alternative splicing**, where certain exons are included or excluded.
As a result, translation in eukaryotic cells **cannot begin until the mRNA is fully processed and mature**.
Therefore, there are two main reasons why transcription and translation do not occur simultaneously in eukaryotic cells:
1. The presence of a nuclear membrane that separates the two processes.
2. The requirement for mRNA processing before translation can begin.

Transcription/translation Coupling

In prokaryotes, transcription and translation happens at the same time & place by **polysomes**, which transcribe multiple mRNA sequences from the same gene, and **polyribosomes**, which synthesizes multiple polypeptides from the same mRNA sequence.



Transcription/translation Coupling

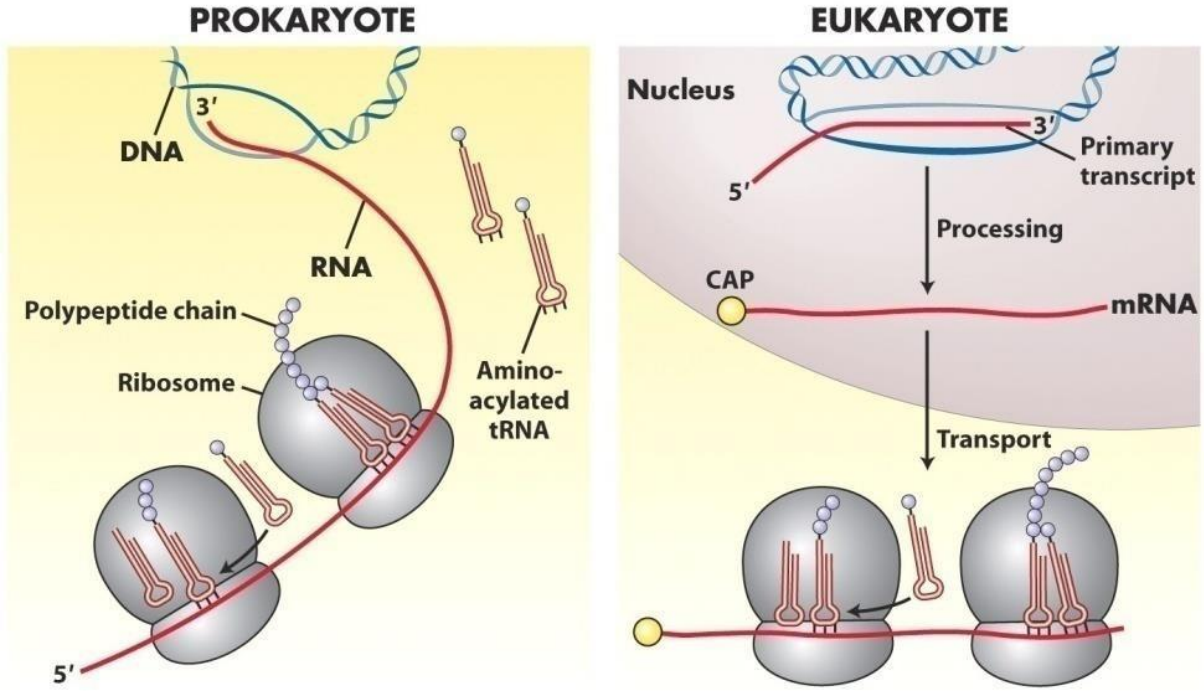
In bacteria, we mentioned that there can be multiple transcriptional activities occurring at the same time from the **same gene.**
(Some textbooks refer to this situation as **polysomes**.
However, in other textbooks, the term polysome is used to describe a different concept, where one mRNA molecule is translated by multiple ribosomes at the same time. In these books, this may also be referred to as multiple ribosomes , polyribosomes.)

According to the Dr's definitions:

•Polysomes refer to the presence of multiple RNA polymerases transcribing the same gene simultaneously, resulting in the production of multiple mRNA molecules.

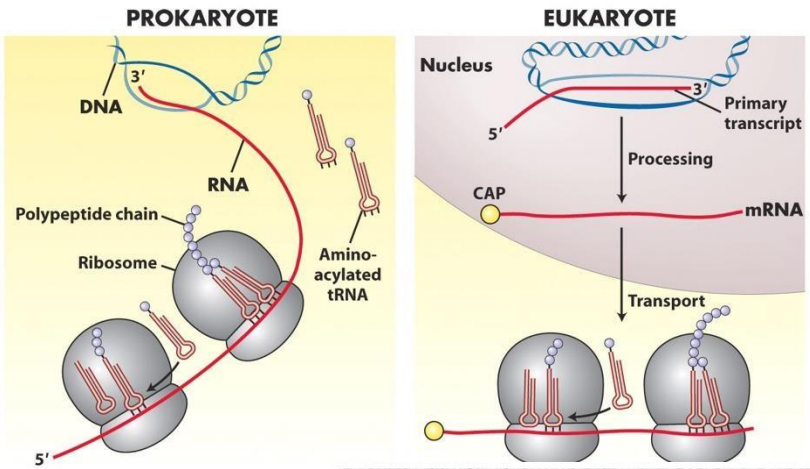
•Polyribosomes (polyribosomes) refer to multiple translational activities happening from the same mRNA .

Thus, multiple transcription events from the same gene are described as polysomes, while multiple translation events occurring on the same mRNA are described as polyribosomes.



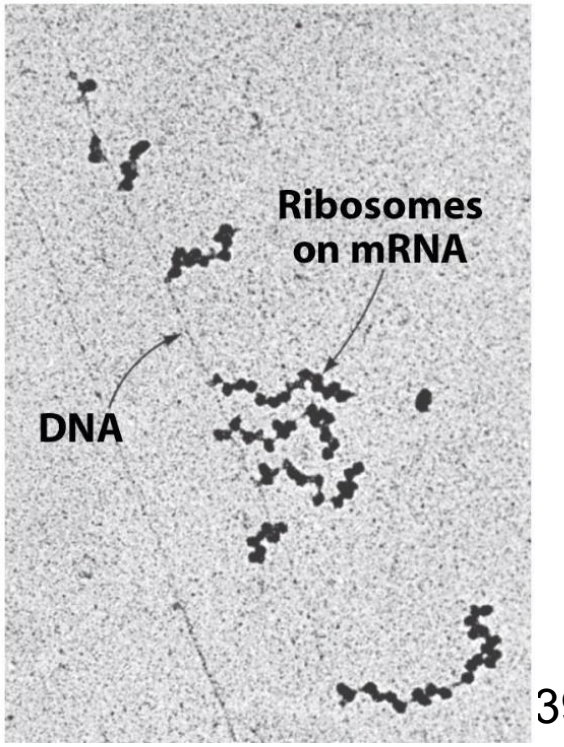
Polyribosomes (polysomes)

- A single mRNA molecule is translated by several ribosomes simultaneously. Each ribosome produces one copy of the polypeptide chain specified by the mRNA. When the protein has been completed, the ribosome dissociates into subunits that are used in further rounds of protein synthesis.



In prokaryotes, we find polysomes & polyribosomes in the same time & place.

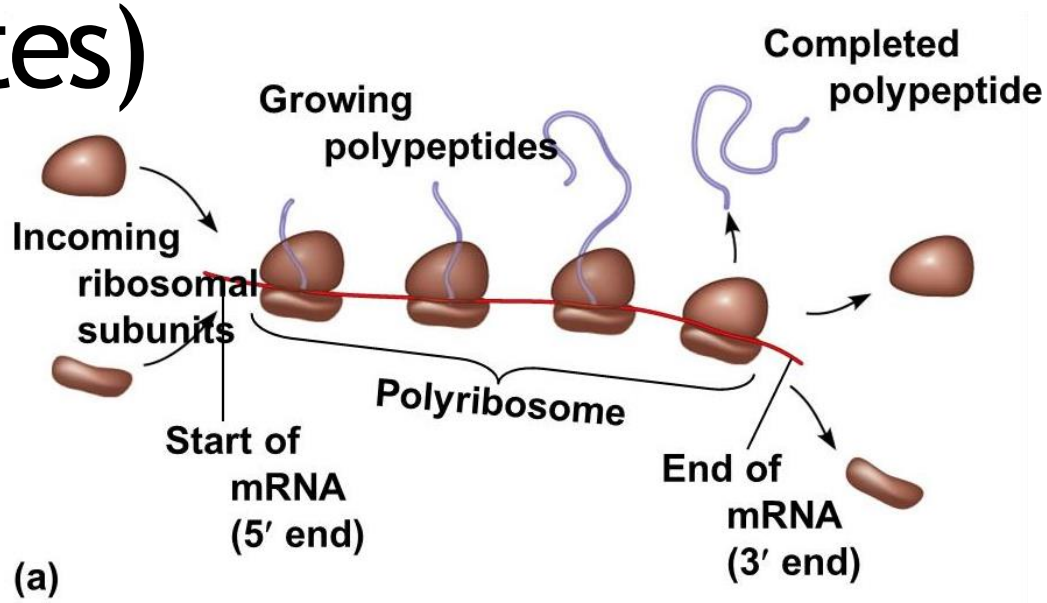
However, that's not what we see in eukaryotes.

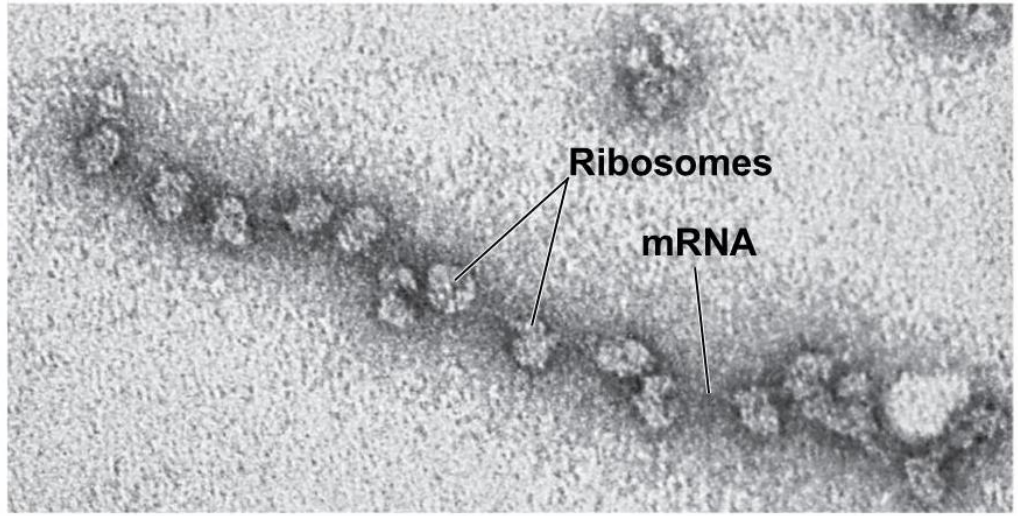


Polysomes (in eukaryotes)

- 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.





رسالة من الفريق العلمي:

استغفرالله العظيم وأتوب إليه

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Corrections from previous versions:

Versions	Slide # and Place of Error	Before Correction	After Correction
V0 → V1			
V1 → V2			