MOLECULAR BIOLOGY

بسم الله الرحمن الرحيم

DOPAMINE SCIENTIFIC TEAM

بحران

﴿ وَإِن تَتَوَلَّوْا يَسْتَبْدِلْ قَوْمًا غَيْرَكُمْ ثُمَّ لَا يَكُونُوا أَمْثَالَكُم ﴾

اللهم استعملنا ولا تستبدلنا

FINAL – Lecture 17

Translation pt.2

Written by :

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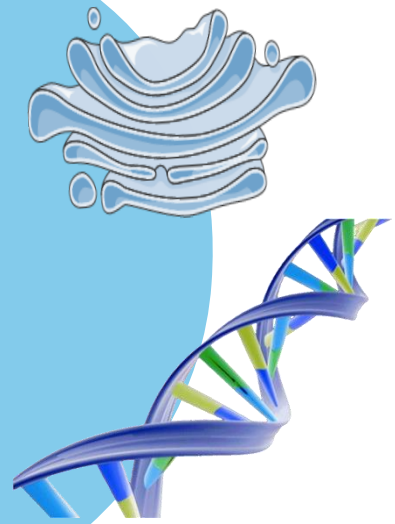
- **Hala Al-Turman**



~ Let's review! ~

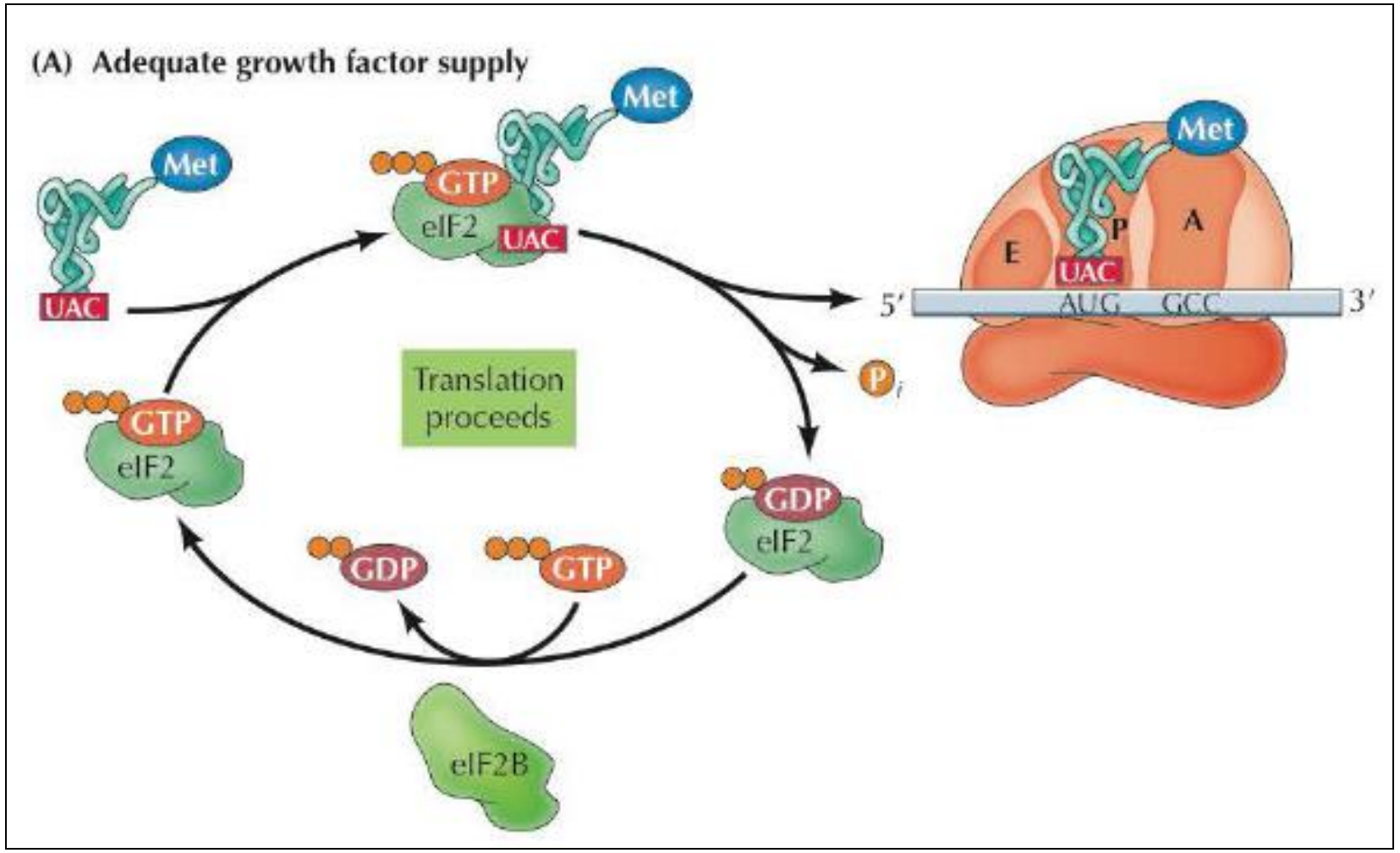
- Translation is the process of synthesizing proteins from mRNA.
- Mechanism: mRNA is read as triplets (3 nucleotides make a codon and each one gives a certain specific amino acid molecule)
- For the mRNA to be translated and the protein to be synthesized, the small ribosomal subunit recognizes the beginning of mRNA (in prokaryotes, this involves the Shine-Dalgarno sequence before every START codon. In eukaryotes, it involves binding to the 7 methylguanosine cap located in mRNA 5' terminus)

SEE NEXT SLIDE

- And then the first tRNA that carries “Met” binds to the mRNA START codon (AUG), and finally the large subunit joins locating the initiator tRNA at the P-site. This is followed by another tRNA that comes in at the A-site of the ribosome while carrying the next amino acid.

- “Met” jumps to the neighboring tRNA (by forming a peptide bond with the amino acid of the tRNA in A-site) and the ribosome translocates to release the initiator tRNA through E-site. This process continues until a STOP codon (UAA or UAG or UGA) is reached, then a release factor binds to the ribosome to release the polypeptide.

Regeneration of eIF2

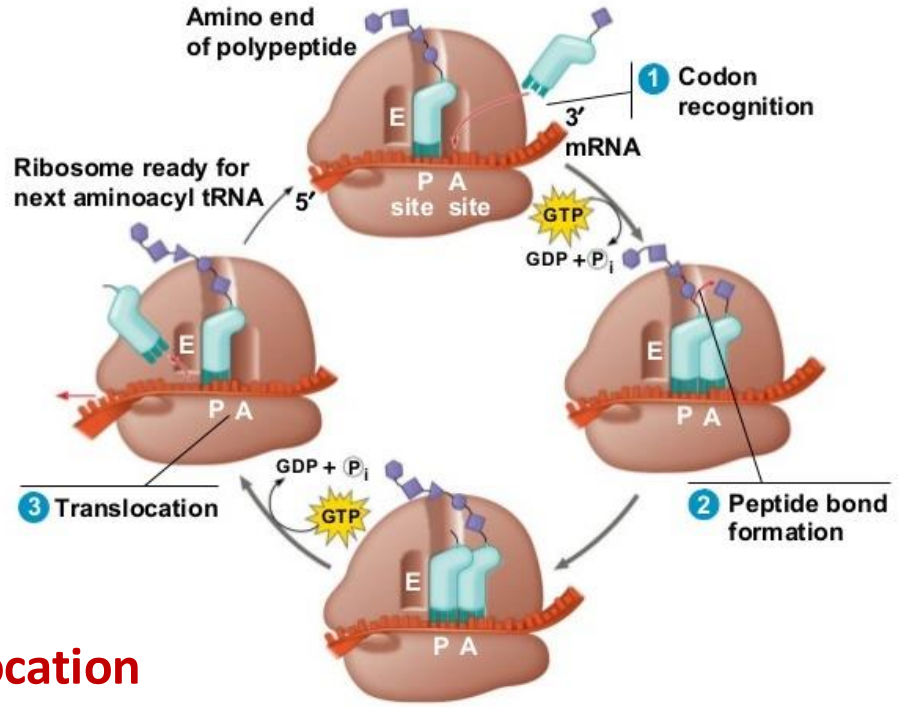


- eIF2 is complexed to GTP to be active. When the correct tRNA is inserted, GTP is hydrolyzed to GDP.
- The active eIF2/GTP complex must be regenerated by exchanging of the GDP for GTP.

Translation elongation

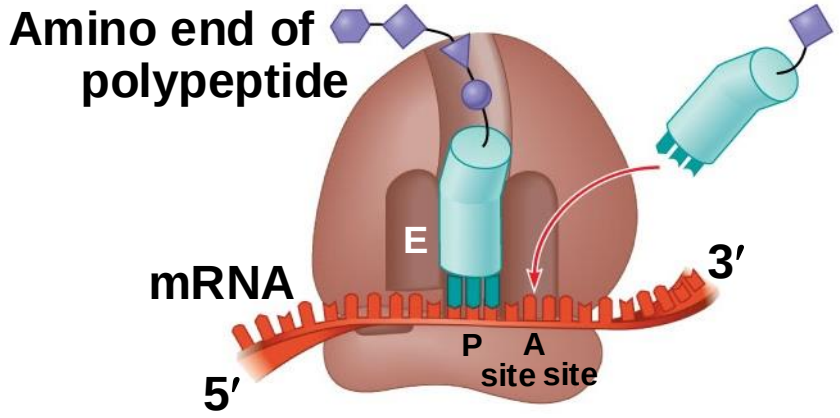
Three steps:

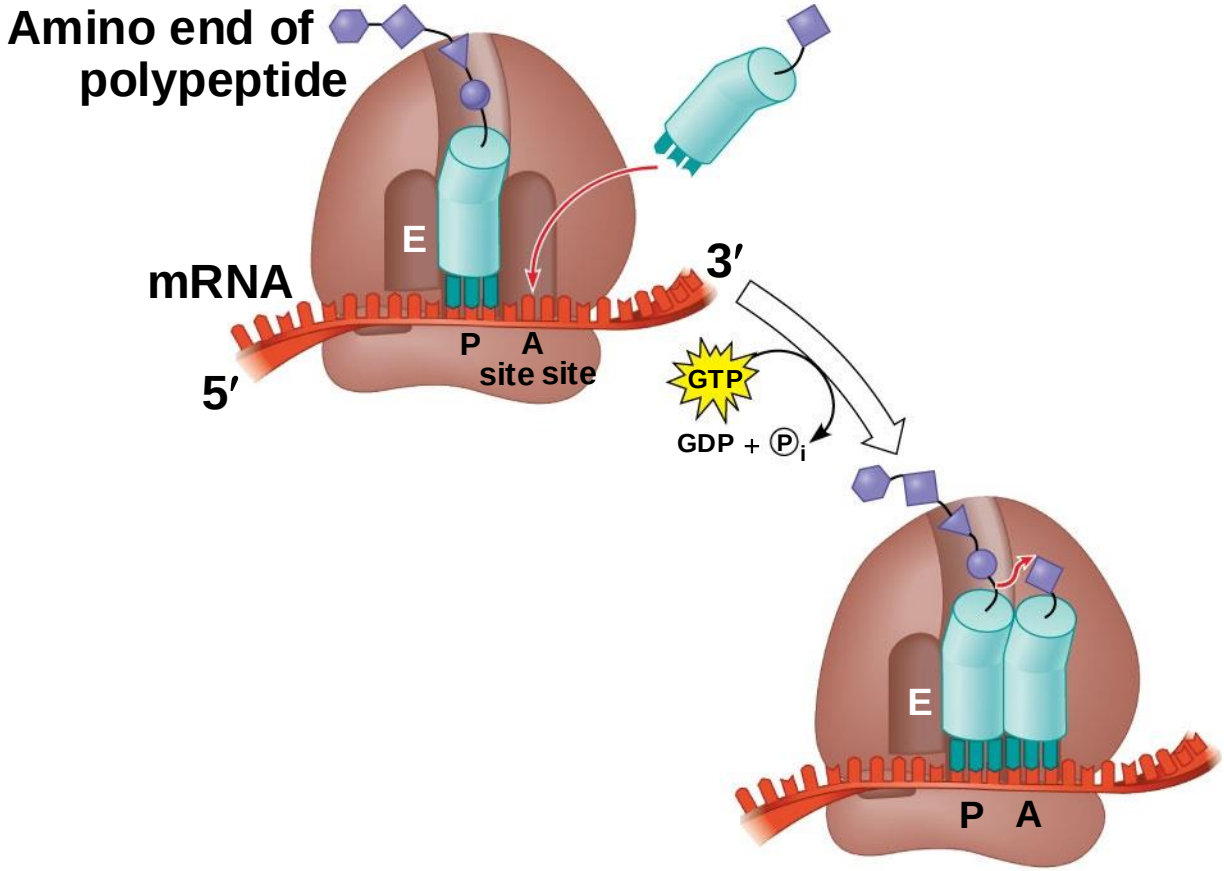
1. **aminoacyl-tRNA binding**
2. **peptide bond formation**
3. **translocation with the help of elongation factors (eEF).**

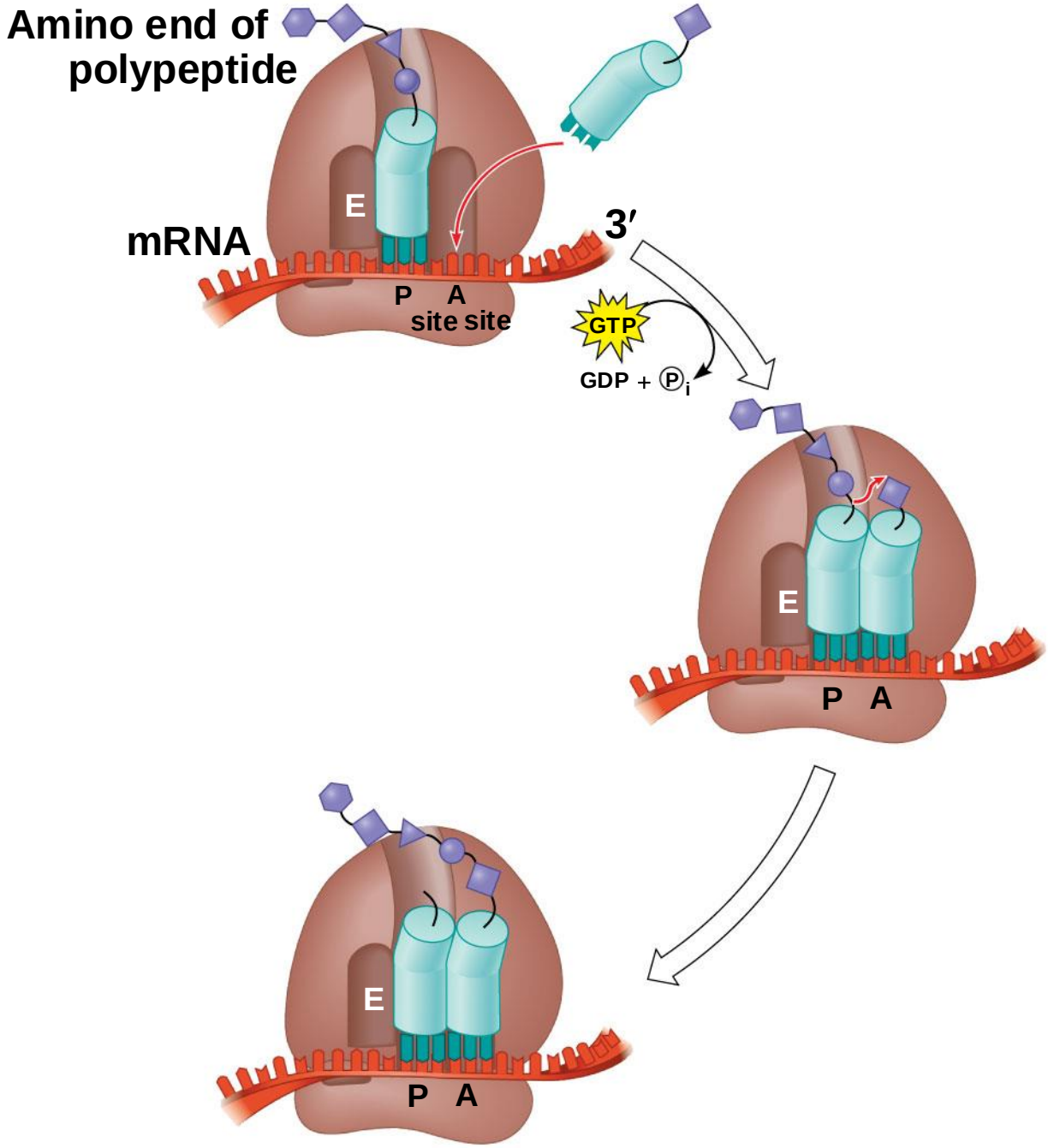


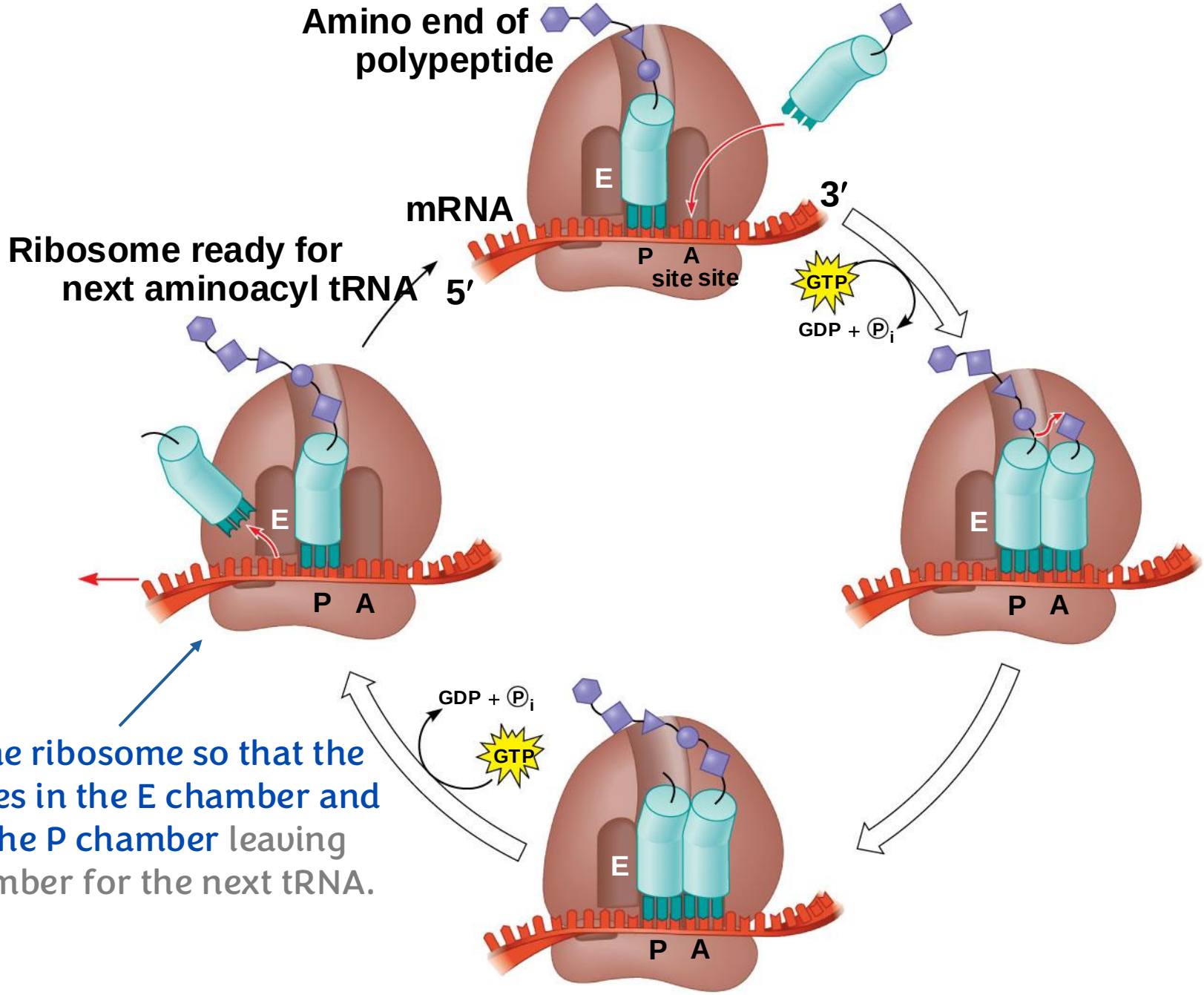
eEF1α brings next aminoacyl-tRNA to the A chamber

eEF2 is critical in ribosomal translocation


Amino end of
polypeptide
E
mRNA
3′
P
site
A
site
5′

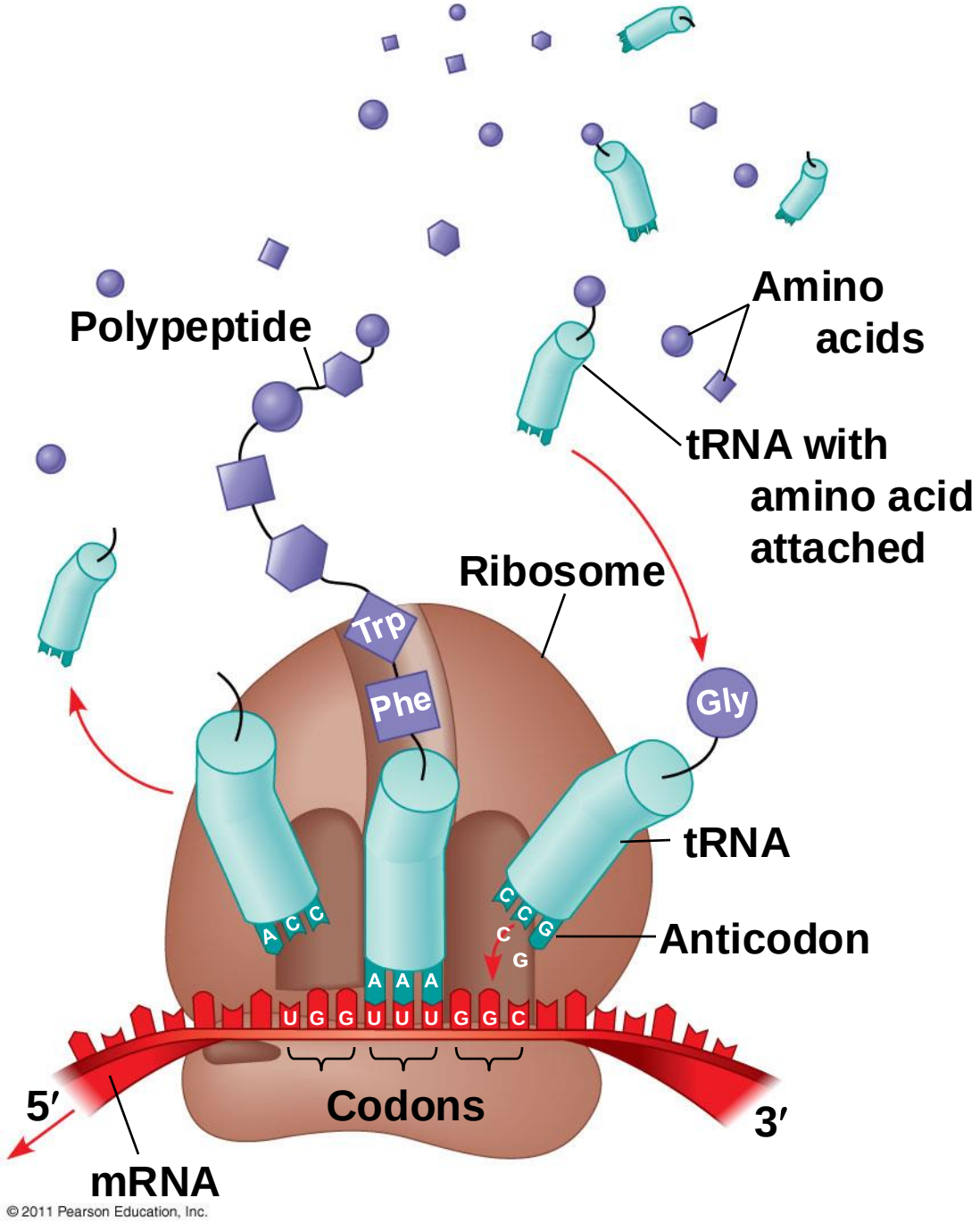

Amino end of polypeptide
E
mRNA
3′
P site
A site
5′
GTP
GDP + Ⓟi
E
P
A

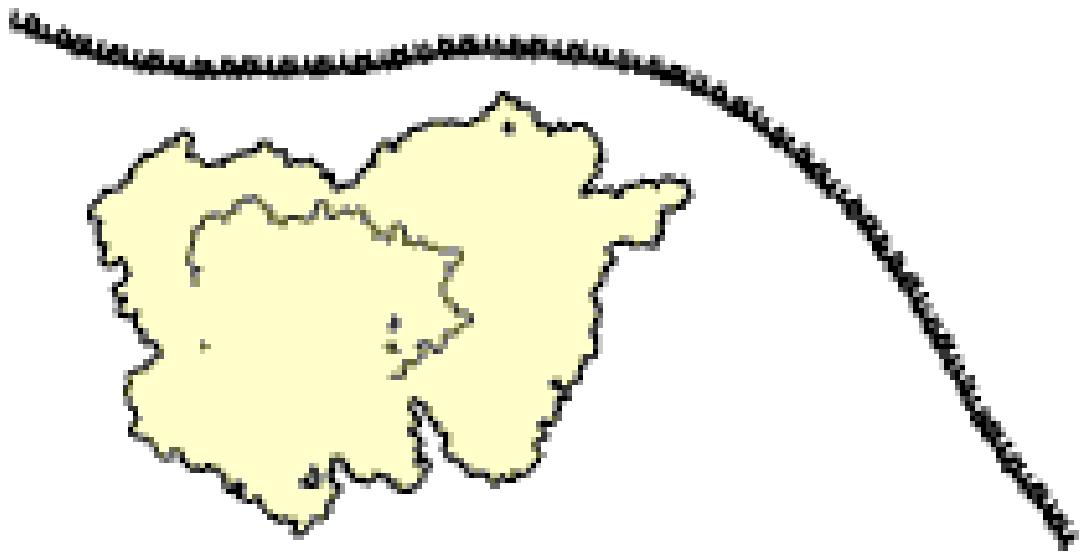




Translocation of the ribosome so that the "old" tRNA becomes in the E chamber and the other tRNA in the P chamber leaving space in the A chamber for the next tRNA.

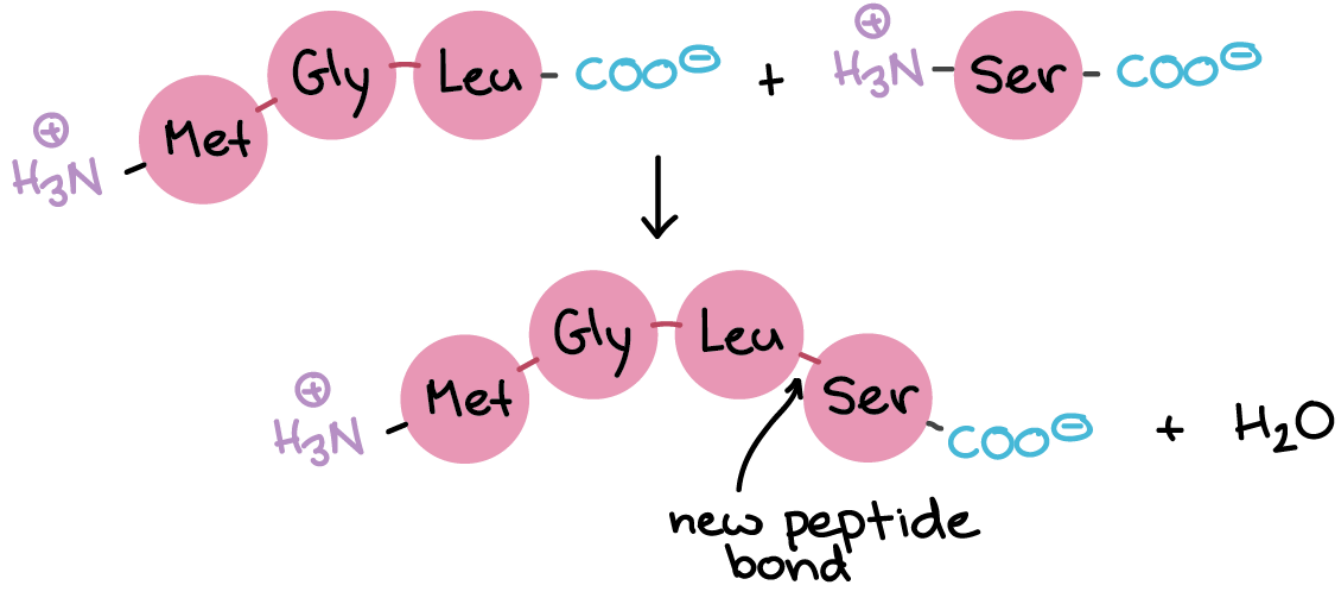
The process continues





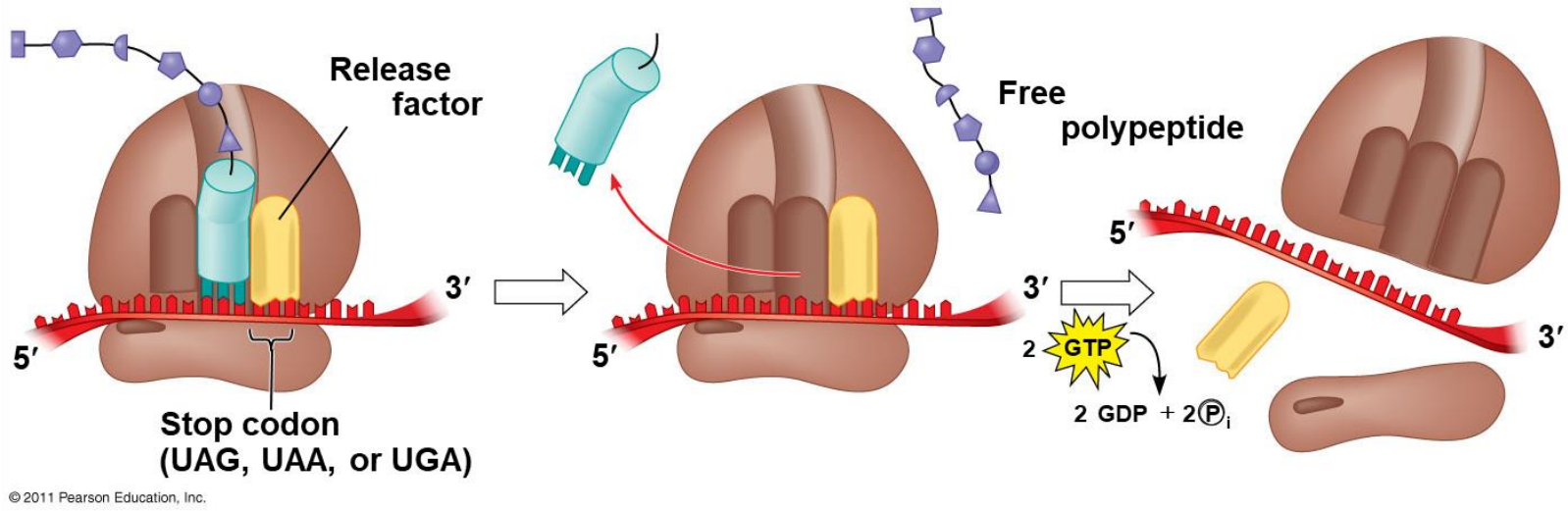
Elongation of the Polypeptide Chain

- 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

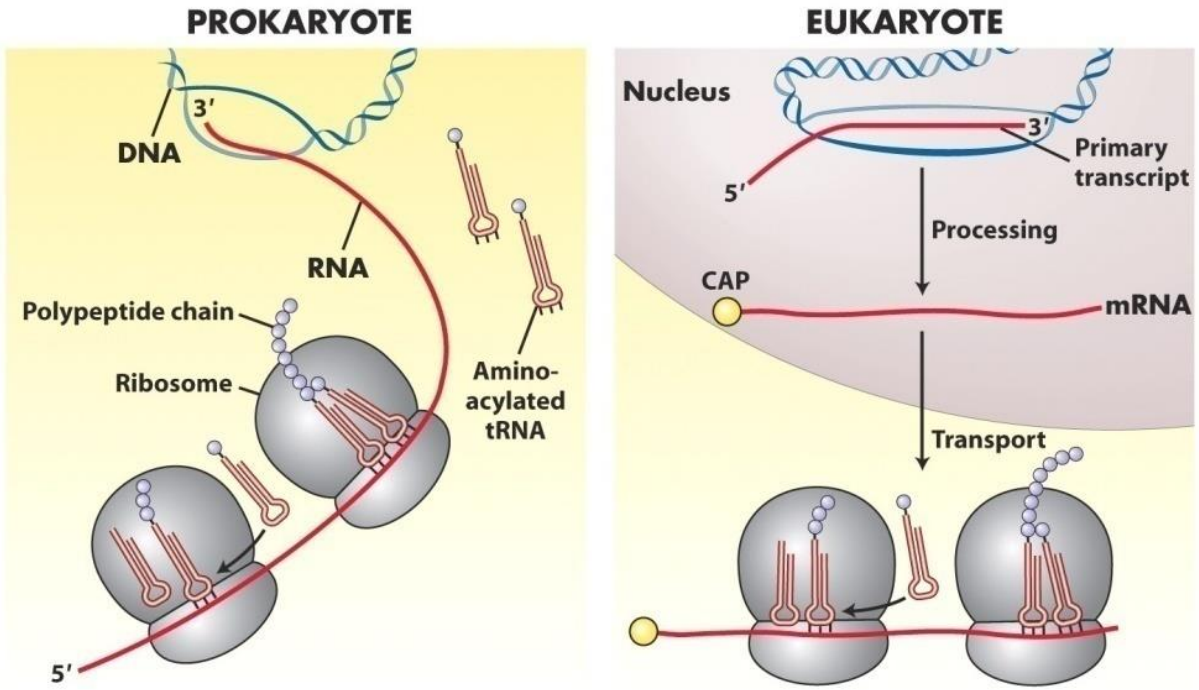
- The codons UAA, UAG, and UGA are the stop signals. They are not recognized by any tRNAs, but a release factor protein.
- The empty A site accepts release factors, which cause the release of the polypeptide, and the translation assembly then comes apart.



The polypeptide –after release– undergoes protein folding in the cytosol, glycosylation, protein localization (to go to different compartments) and so on.

Transcription/translation Coupling

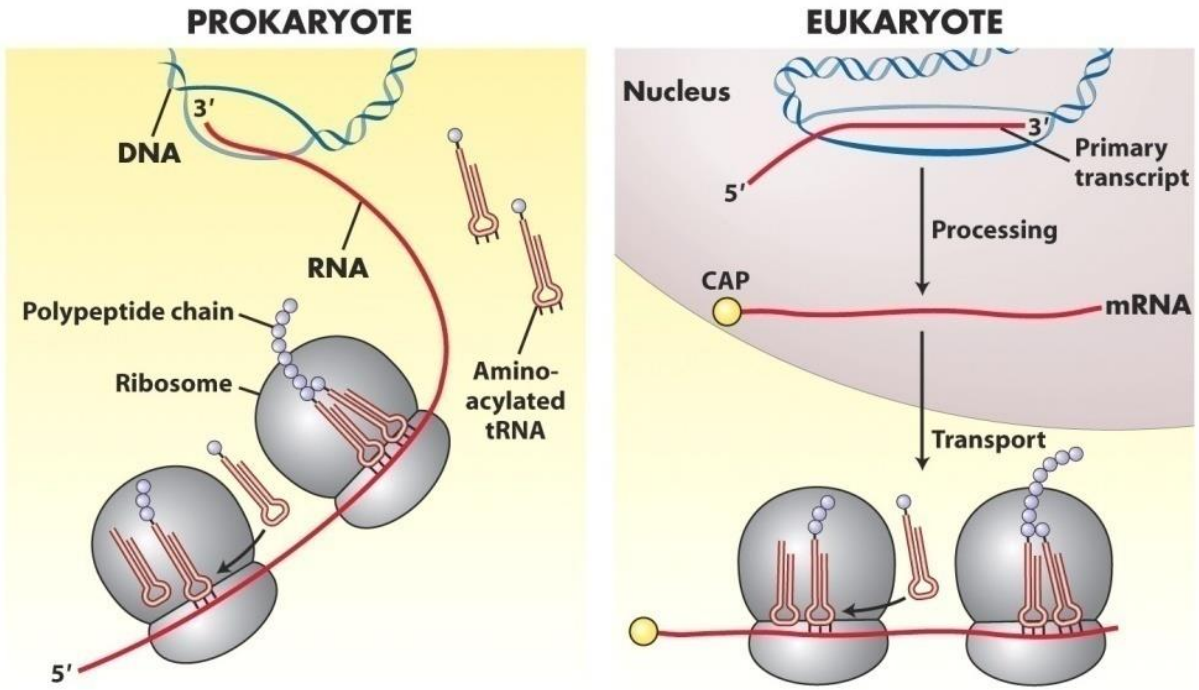
- Translation and transcription are coupled in space and time in prokaryotes.



For example, in Rbcs, to produce Hemoglobin molecules and insure the efficiency of transcription and translation, one single gene gets transcribed multiple times resulting in a lot of mRNA sequences.
Each mRNA sequence gets translated to many polypeptides until eventually we are left with multiple copies of the same polypeptide that fold in the same way to produce multiple copies of the same protein.

Transcription/translation Coupling

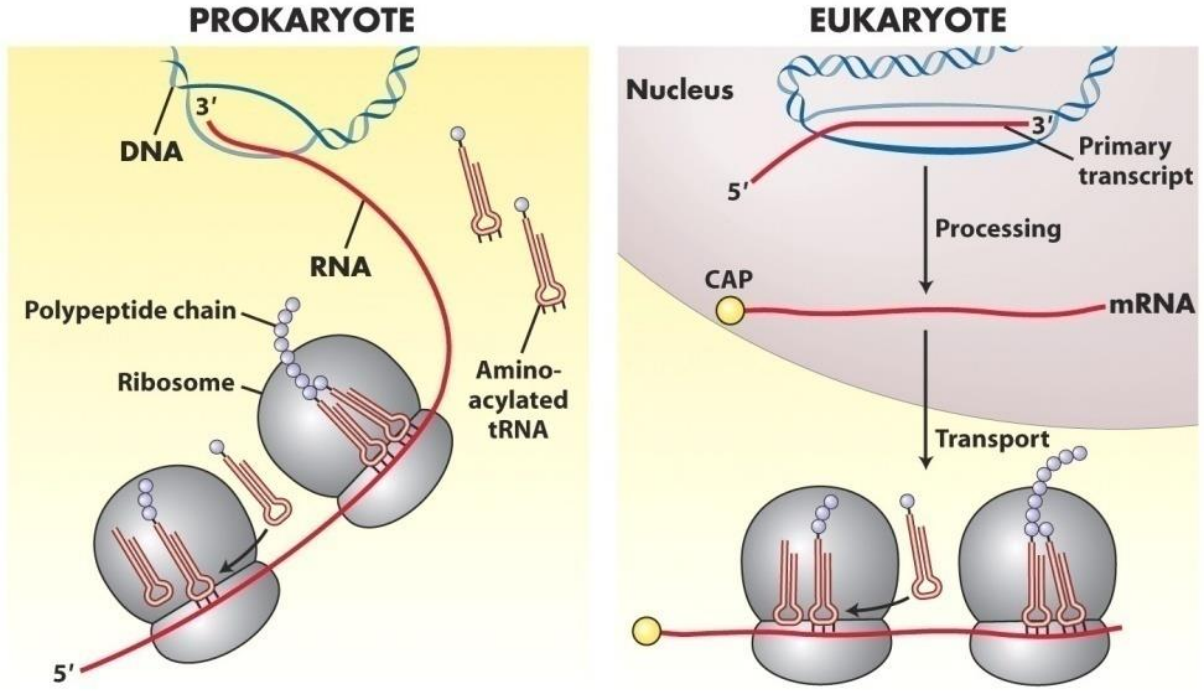
In prokaryotes, transcription and translation happens at the same time & place by **polysomes**, which transcribe multiple mRNA sequences from the same gene, and **polyribosomes**, which synthesizes multiple polypeptides from the same mRNA sequence.



Transcription/translation Coupling

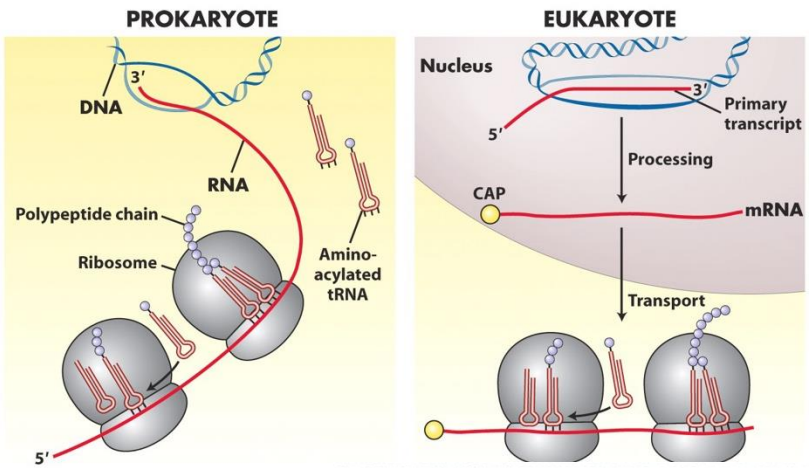
In eukaryotes, however, there's no transcription/translation coupling. Instead, the eukaryotic cell undergoes transcription (DNA to mRNA), and then multiple translational processes at the same time by polyribosomes.

Why? This is because the eukaryotic cell is compartmentalized, therefore we have a nuclear membrane which works as a barrier separating the two mechanisms. As well as the pre-mRNA that undergoes RNA processing.



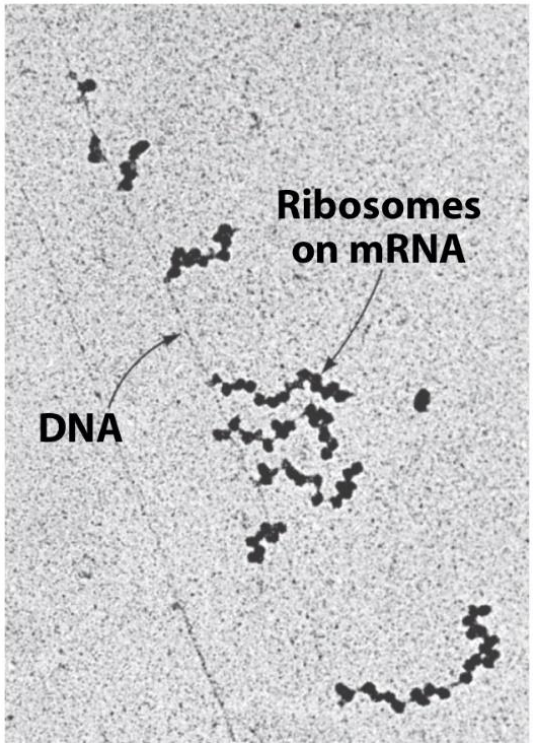
Polyribosomes (polysomes)

- 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.

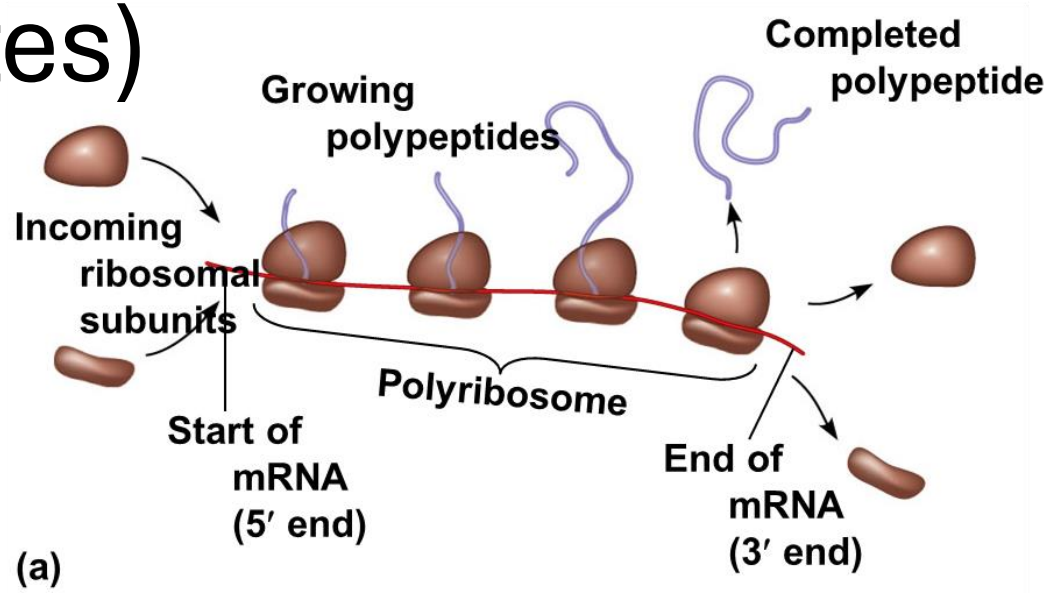
However, that's not what we see in eukaryotes.

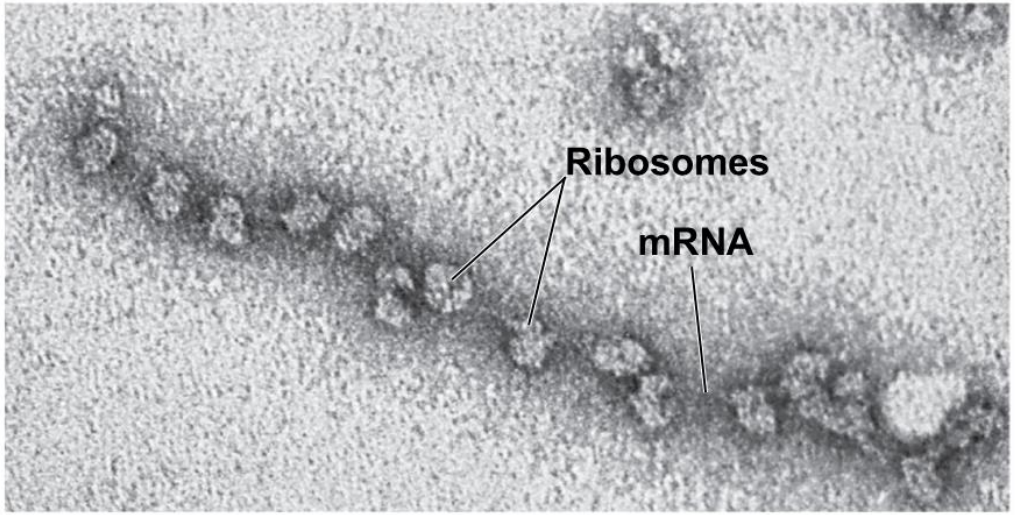


Polysomes (in eukaryotes)

- 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.



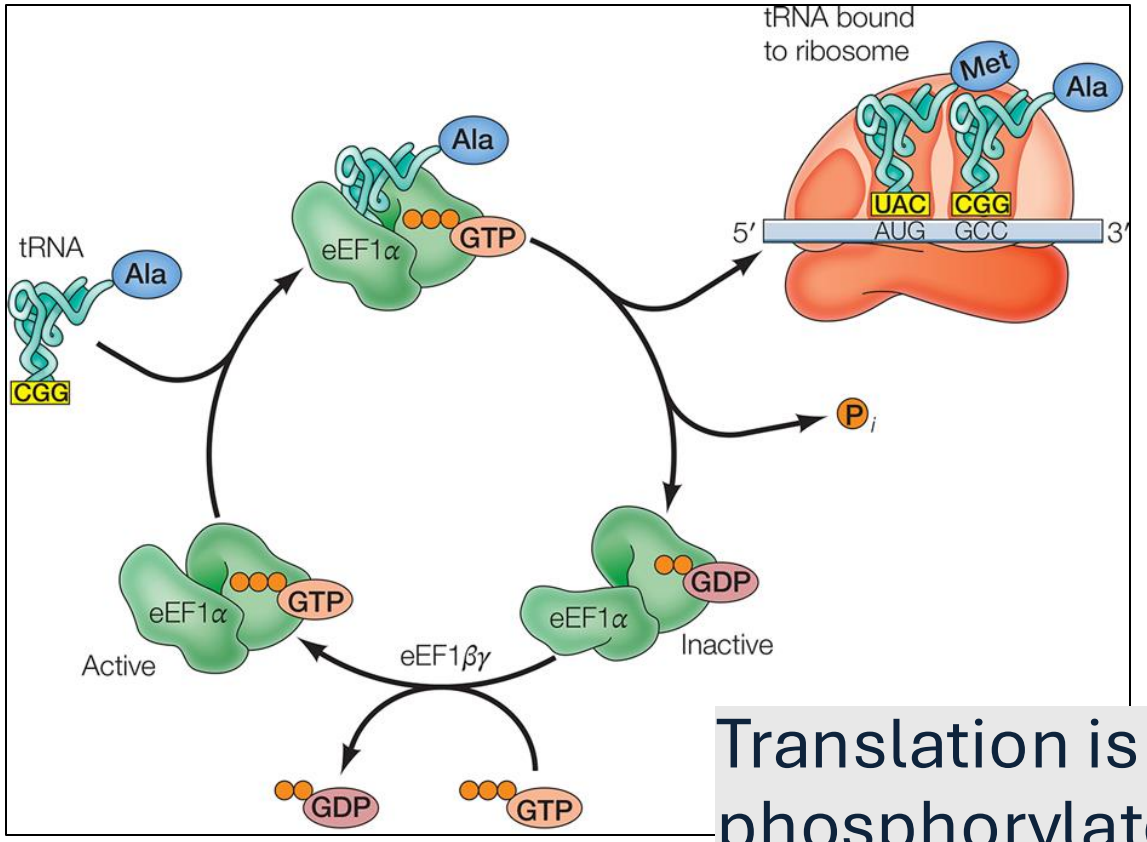


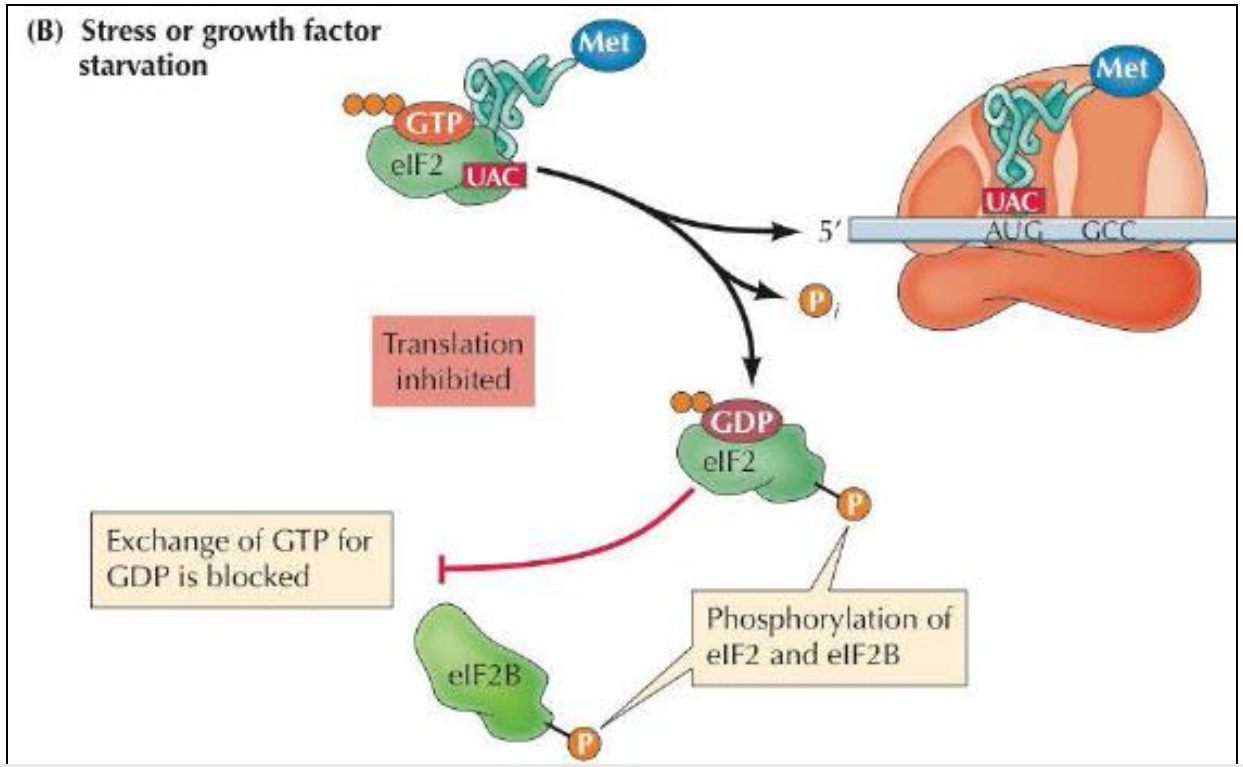
Regulation of translation

mRNA can be synthesized but may not be transcribed or get transcribed at a low efficiency/speed depending on the cell needs.

Global regulation

- To continue translation, eIF2 must be reactivated by GTP/GDP exchange.





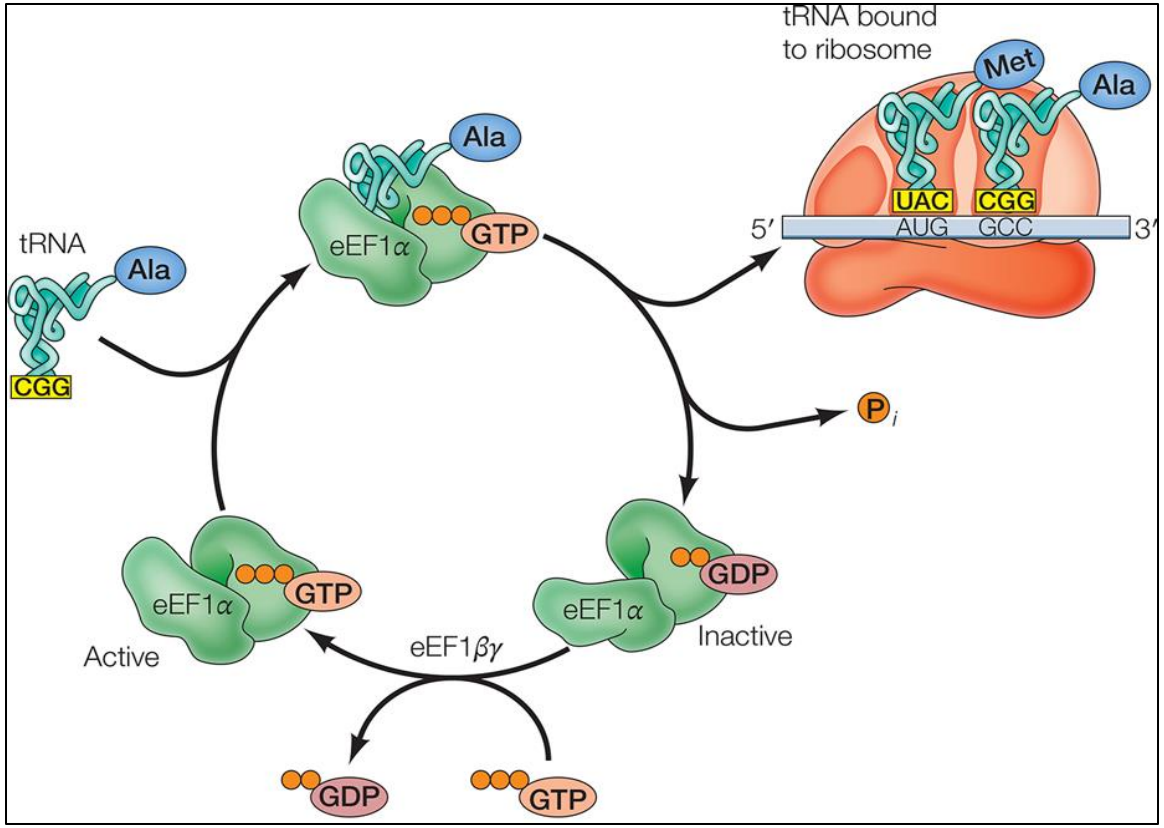
Translation is inhibited by regulatory kinases that phosphorylate eIF2 blocking the GTP/GDP exchange.

Global regulation

- To continue translation, eIF2 must be reactivated by GTP/GDP exchange.

~ Let's Refresh! ~

- eIF2 main function: when bound to GTP, it brings initiator tRNA (first tRNA) to the small ribosomal subunit, and GTP gets exchanged by GDP.
- In order to reuse the same tRNA, GDP must be exchanged to GTP and can bind to tRNA + "Met" complex.

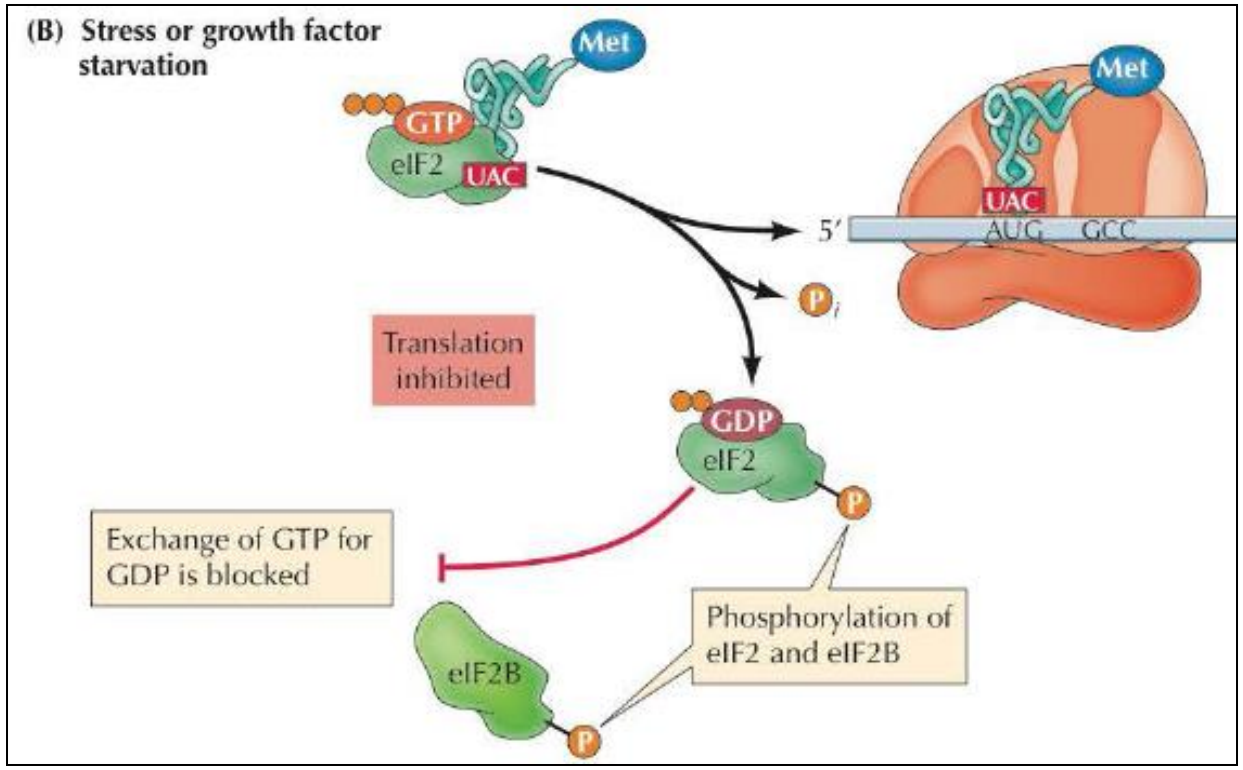


eIF1: brings next tRNA + amino acid complex to A chamber in ribosome.

eIF4: brings mRNA to the tRNA + small ribosomal subunit complex (by binding to the 5' CAP and to the 3' poly-A tail via poly-A binding protein "PABP")

Global regulation

Translation is inhibited by regulatory kinases that phosphorylate eIF2 blocking the GTP/GDP exchange.



When eIF2 is phosphorylated by a kinase, it CANNOT exchange GDP for GTP.

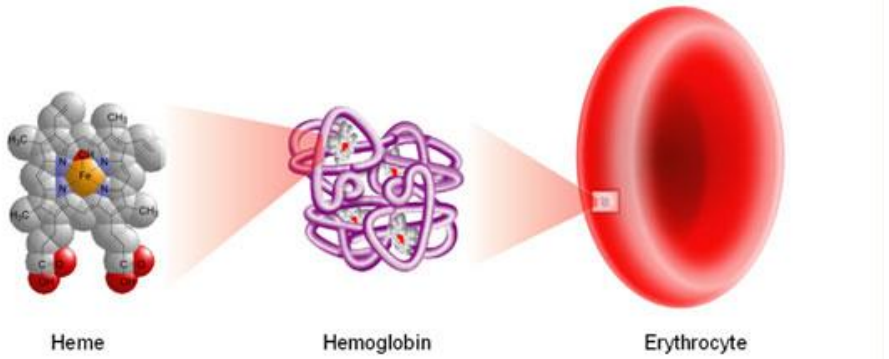
-> inhibition of the translation process.

This is exactly what happens to reticulocytes.

SEE NEXT SLIDE

Heme and protein synthesis

- In reticulocytes (immature erythrocytes), if adequate heme is available, heme stimulates overall protein synthesis.
- Reticulocytes undergo maturation to turn into Rbcs.
- Rbcs MUST have a lot of Hemoglobin that carry O2.
- The hemoglobin molecule consists of a heme group as well as a globin group, If the heme is insufficient, no globin molecules are synthesized.



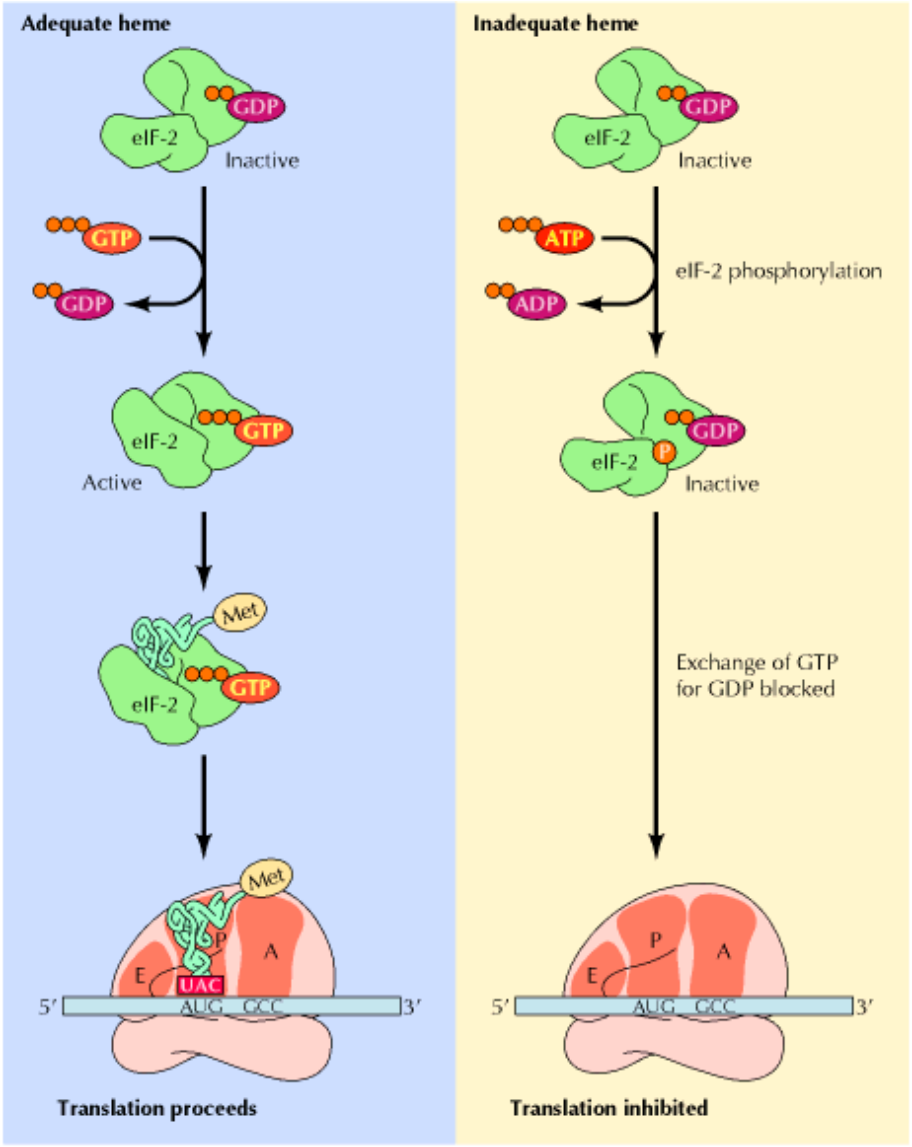
Heme and protein synthesis

- If adequate heme is available, GDP-GTP exchange occurs and translation can proceed.

  eIF2 is active all the time.

- If heme supplies are inadequate, a protein kinase phosphorylates eIF2.

  No GTP/GDP exchange -> no translation occurs.



Regulation of mRNA stability

This regulatory mechanism is specific for mRNA
And it deals with iron.

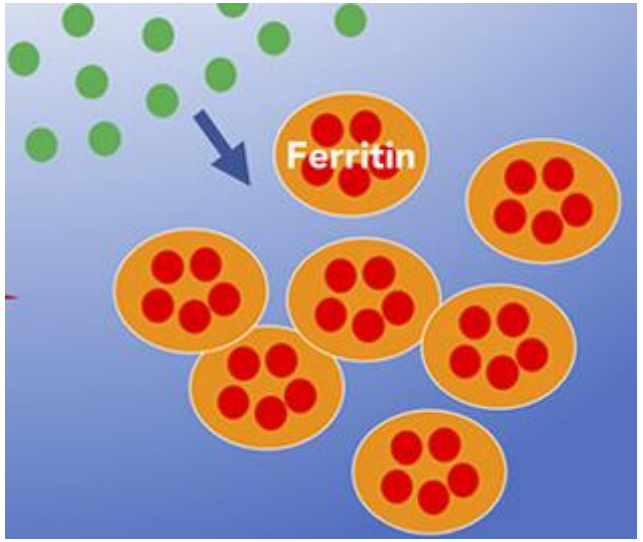
Physiology of iron

- Iron is mostly found in RBCs in our body.
- Although billions of RBCs are killed everyday, upon which these RBCs release iron, we don't actually lose it, but store it to reuse.

But in the same time, iron level should be maintained, that is shouldn't be high nor low.

- Iron is an essential metal for the human body.
  - Oxygen transport
  - Enzyme function
- Too much iron can be toxic.
  - Organ failure — By damaging cells
  - Bacterial infection — Because it allows the growth of bacteria
- The level of iron is intricately maintained.

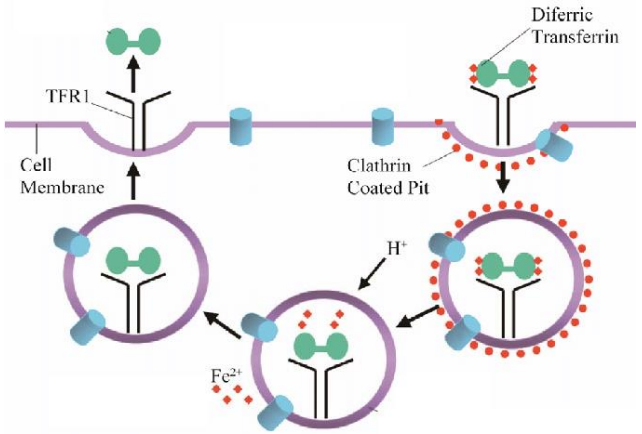
The players : 2 proteins



- 1- Liver ferritin protein stores 4000 iron **atoms** when abundant (in the liver).

- 2- Transferrin receptor mediates iron entry via receptor-mediated endocytosis into peripheral cells when needed.

These two proteins won't be expressed at the same time, it depends on iron level, see the up coming slides for explanation.



Let's talk about transferrin and transferrin receptor first

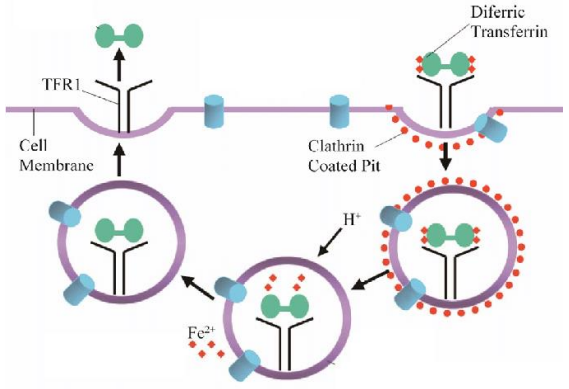
Transferrin receptor

- When we eat something that contains iron, it's absorbed by intestinal cells, then these intestinal cells transport iron out to bind to a protein that is called **transferrin**.
- steps:

1- Transferrin carries iron throughout the body.
2- **Transferrin** binds to **transferrin receptor** (which exists on the surface of cells)
3- binding of transferrin to transferrin receptor stimulates **receptor mediated endocytosis.**
4- this complex enters the cell then iron is released to be used.

- Usage of iron:

Cytochrome p450 enzymes.
Electron transport chain proteins.

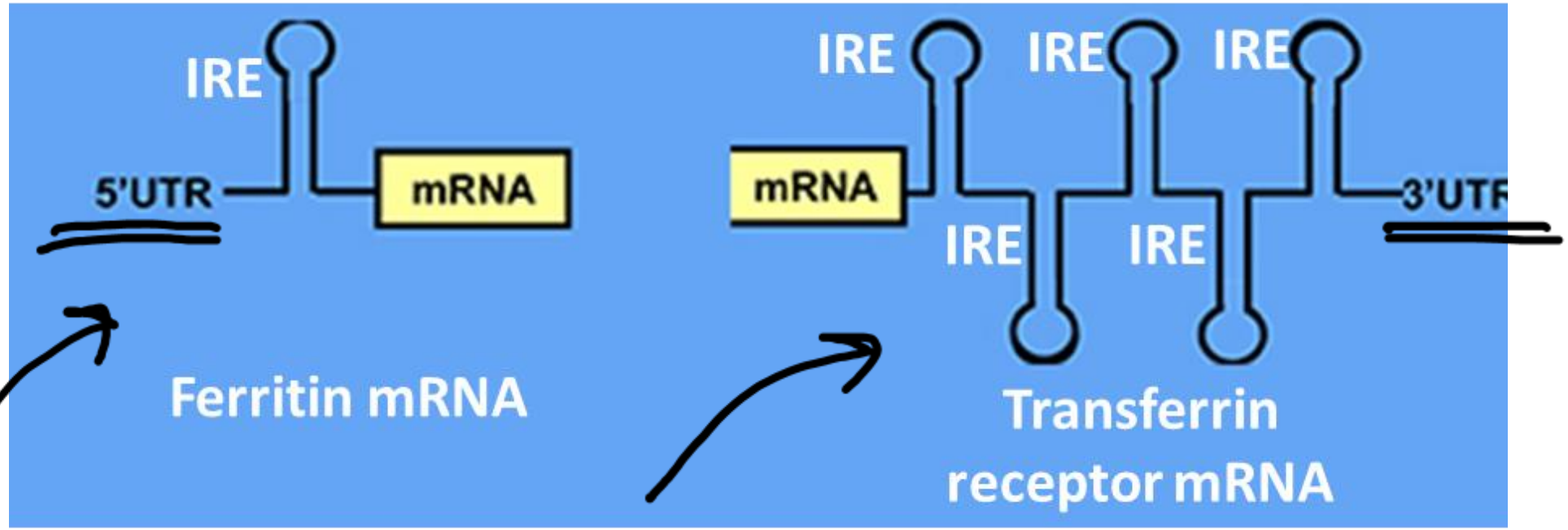


- When iron is high, expression of ferritin should be up-regulated and expression of transferrin receptor should be down-regulated, and vice versa.

- Cells have limited capacity to store iron, because high levels of iron inside the cell would kill it and, hence it down regulates transferrin receptor (stops expressing transferrin receptor on its surface), which in turn, iron will be stored in **ferritin** in the liver (which is up regulated in this case) and vice versa.

Iron-response elements (Regulation of ferritin and transferrin receptor)

- In human iron-regulatory genes, there are genetic regions (of mRNAs, as well) called iron response elements (IREs).
- These regions also exist within the mRNAs of ferritin and transferrin receptor but at different sides.



Iron response element exists at 5' UTR of ferritin mRNA and 3' UTR of transferrin receptor mRNA

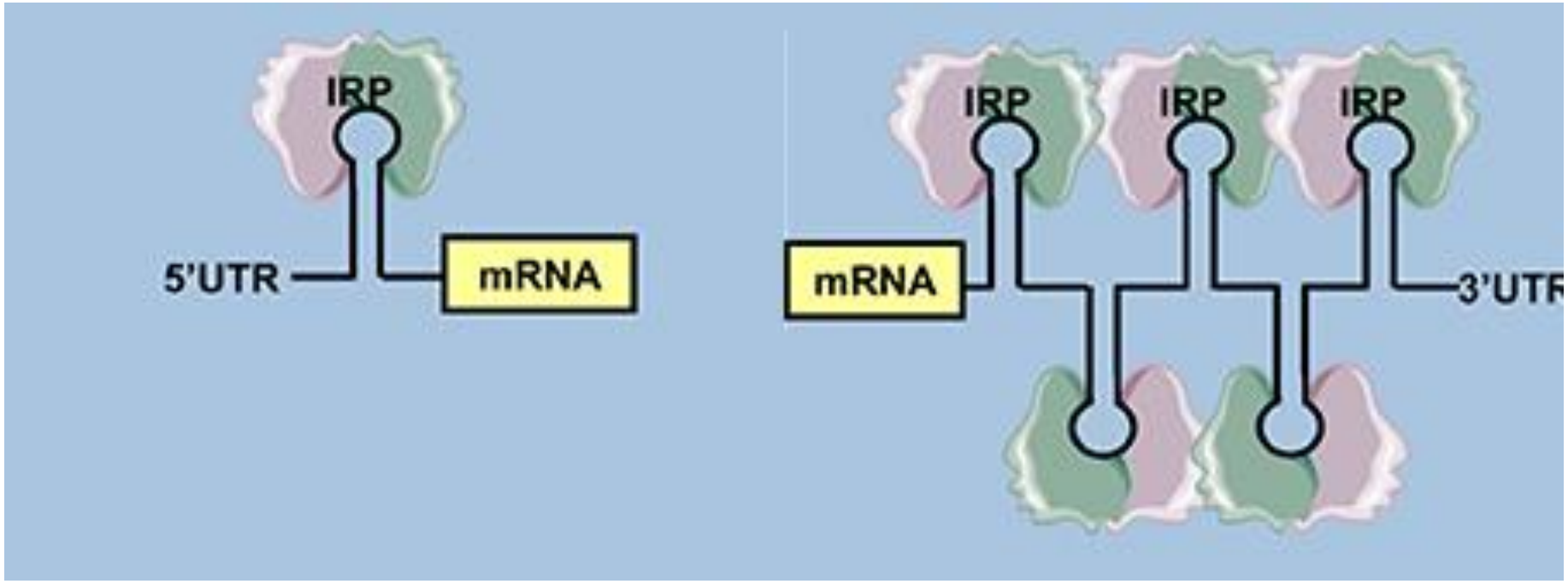
UTR: untranslated region

- Iron response element is in both DNA and mRNA

Slide 31+32 explanation in slide 33

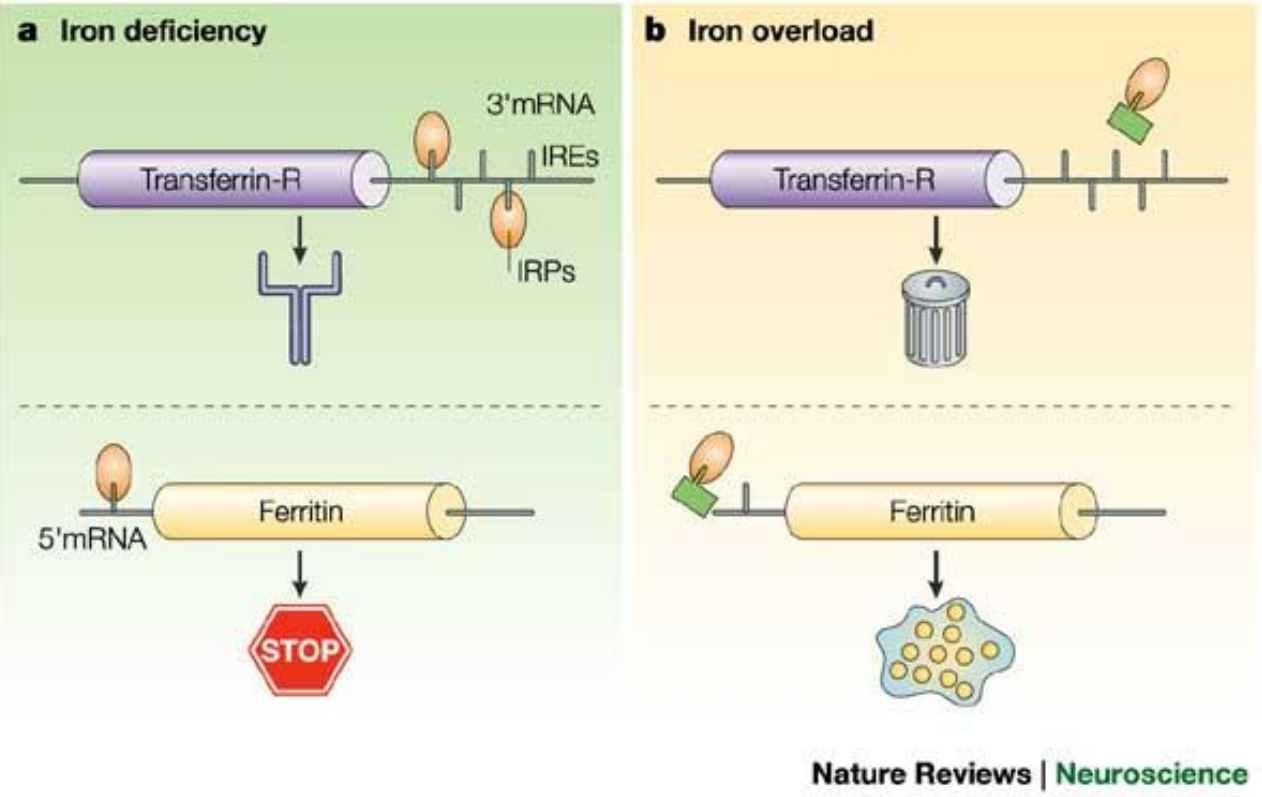
Iron regulatory protein

- When iron is low, the iron regulatory protein (IRP) binds to IREs influencing protein expression.
  - Remember, this binding happens when iron is low.
- When iron is high, iron binds to IRP preventing its binding to the IRE.



Effect on expression

- When iron is abundant (high) in the cells, it binds to IRP, disabling the binding of IRP to the mRNAs of transferrin receptor and ferritin.
  - Transferrin receptor: mRNA is destabilized and is degraded, lowering protein level, and, hence, iron uptake.
  - Ferritin: Translation is activated and storage increases.
- When iron is low, the IRP is iron-free and can bind to the mRNAs of transferrin receptor and ferritin.
  - Transferrin receptor: mRNA is stabilized, more protein is made, and, hence, iron uptake into the cells increases.
  - Ferritin: Translation (protein synthesis) is blocked, and less protein is available for storage.



IRP: iron response protein
IRE: iron response element

1

- When iron level is low, IRP binds to IRE of both mRNA, but at different sites.

1- it binds to 3' UTR of transferrin receptor mRNA stabilizing it (3' end won't prevent ribosomes from translation).

2- it binds to 5' UTR of ferritin mRNA, preventing ribosomes from translating it.

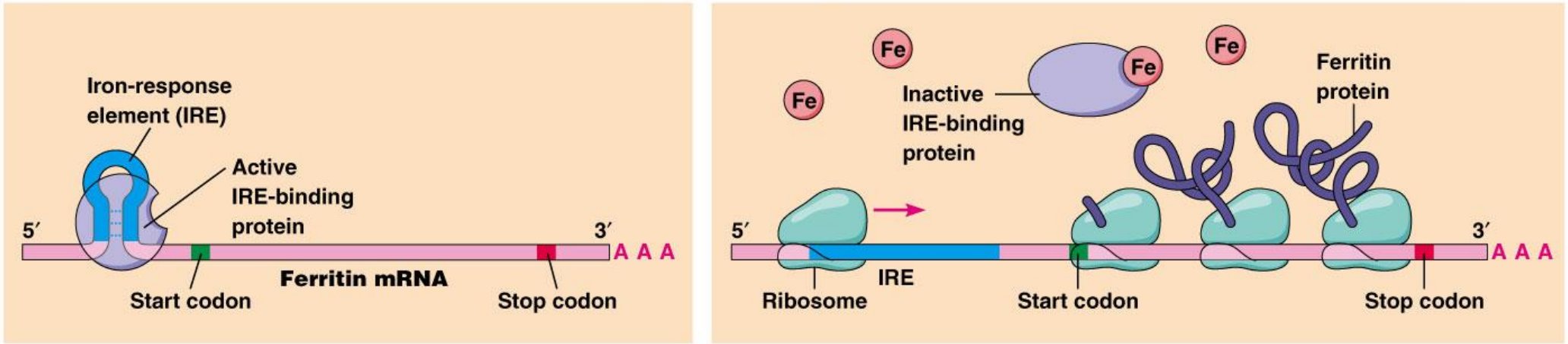
2

- But when iron level is high, iron itself binds to IRP releasing it from IRE and, hence, allows for ribosomes to translate ferritin not transferrin receptor (because it's destabilized).

The next 2 slides are talking about the same concept

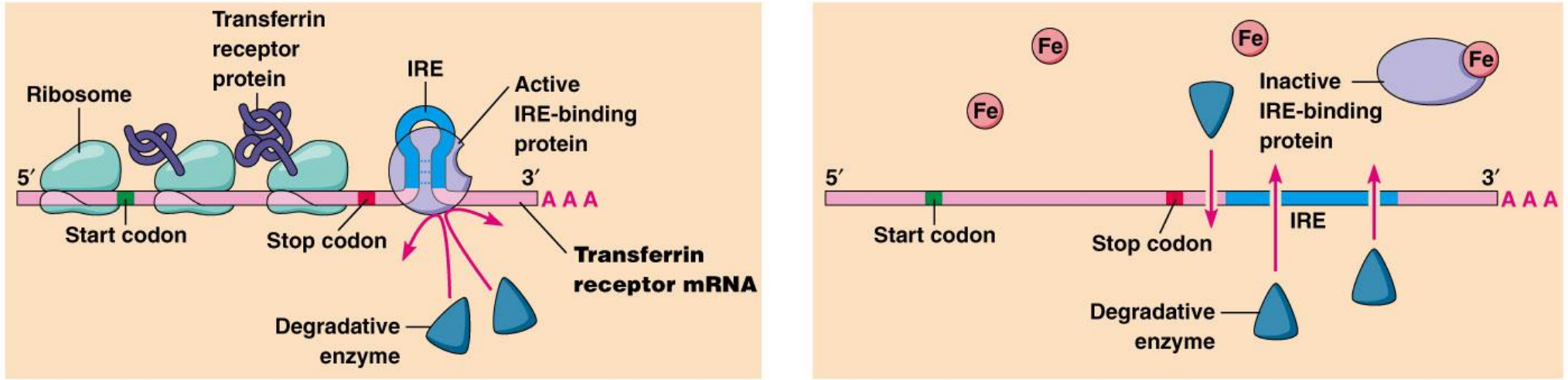
(a) Low iron concentration. IRE-binding protein binds to IRE, so translation of ferritin mRNA is inhibited.

(b) High iron concentration. IRE-binding protein cannot bind to IRE, so translation of ferritin mRNA proceeds.



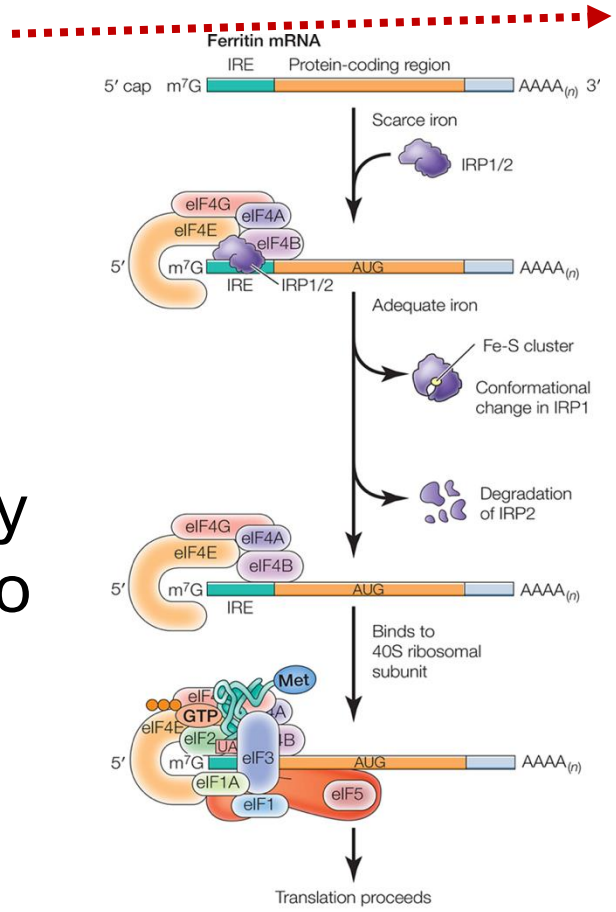
(a) Low iron concentration. IRE-binding protein binds to the IRE of transferrin receptor mRNA, thereby protecting the mRNA from degradation. Synthesis of transferrin receptor therefore proceeds.

(b) High iron concentration. IRE-binding protein cannot bind to IRE, so mRNA is degraded and synthesis of transferrin receptor is thereby inhibited.



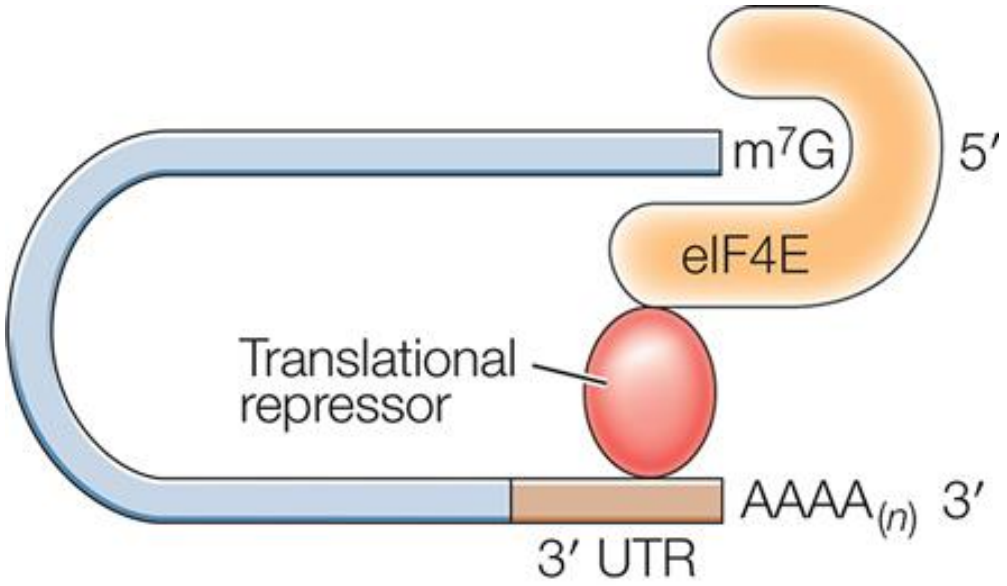
Regulation of ferritin synthesis

- The mRNA contains an iron response element (IRE) near its 5' cap.
- If iron is scarce, proteins called iron regulatory proteins 1 and 2 (IRP1/2) bind to the IRE, blocking translation by interfering with binding of the mRNA to the 40S ribosomal subunit.
- In the presence of iron, cells produce IRP1 is inhibited and IRP2 is degraded enabling translation of the mRNA.



Prof. said Forget about point 3 as well as IRP1/2, just know that there's IRP.

Regulation of eIF4E



- Translational repressors can bind to regulatory sequences in the 3' untranslated region (UTR) and inhibit translation by binding to the initiation factor eIF4E, bound to the 5' cap.
- This interferes with translation by blocking the formation of a normal initiation complex

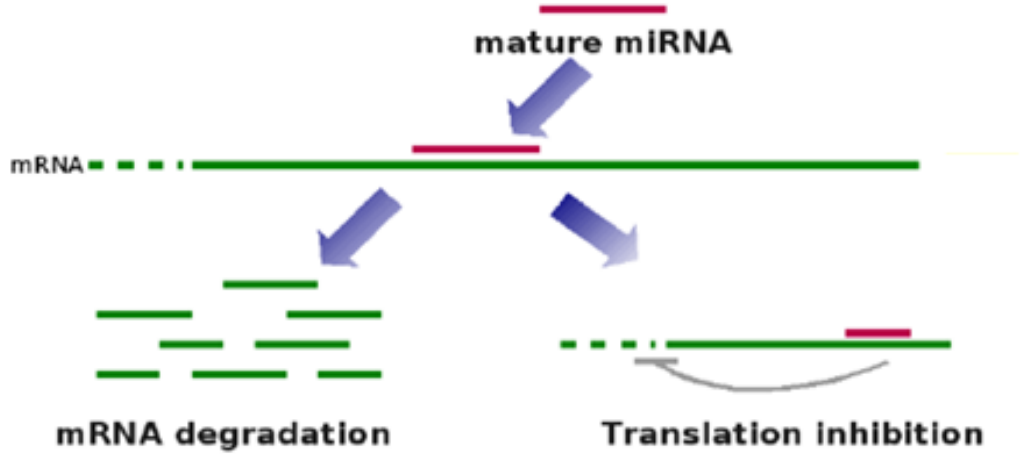
Regulation by microRNA (miRNA)

and siRNA

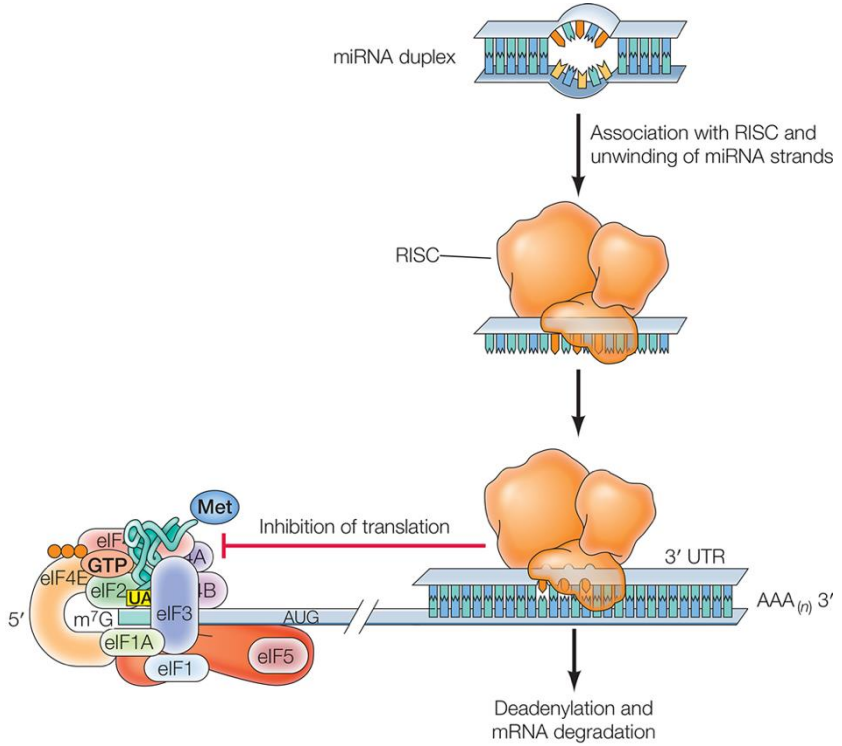
Human cells make microRNA, about 4000 molecule in each, and that's a lot!!.

Regulation by microRNA (miRNA)

- MicroRNA is synthesized by RNA polymerase II into single-stranded, primary miRNA (pri-miRNA) transcript.
- It (miRNA) gets processed into double-stranded molecules but only one strand is loaded onto the RNA-induced silencing complex (RISC), a complex where miRNA targets and binds to the 3'-UTR of mRNA.
- The end-result is:



Further explanation Next slide

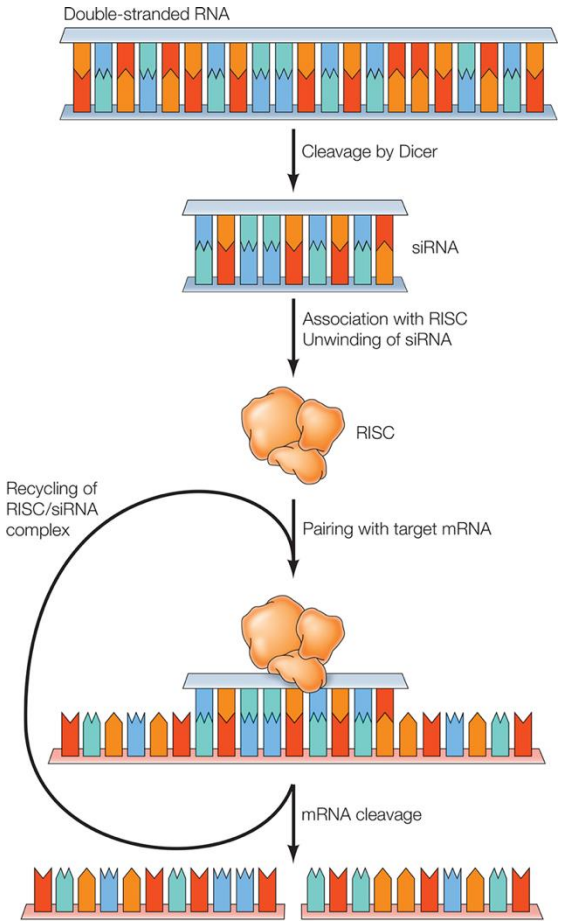


- miRNA is synthesized by RNA polymerase II, the same polymerase that makes mRNA. And it's about 20 nucleotides, that's why it's called miRNA, because it's micro compared to mRNA that contains hundreds or thousands nucleotides.

- miRNA molecules are synthesized as **double-stranded RNA**. A protein called **RISC** (RNA induced silencing complex) binds to **<u>one</u> of miRNA strands,** and transport it to hybridize to a sequence in 3' UTR (that is complementary to it) on mRNA molecules.
- Binding of miRNA to mRNA causes either degradation of mRNA or inhibition of translation. And, hence, protein level decreases.

RNA interference and short interfering RNA (siRNA)

- Targeting mRNA by synthetic siRNA can be used for experimental and therapeutic purposes.
- The double-stranded siRNA associates with the RISC complex, which targets one strand onto a homologous mRNA.
- The mRNA is cleaved and, as a result, the protein level decreases or the protein is not expressed at all.



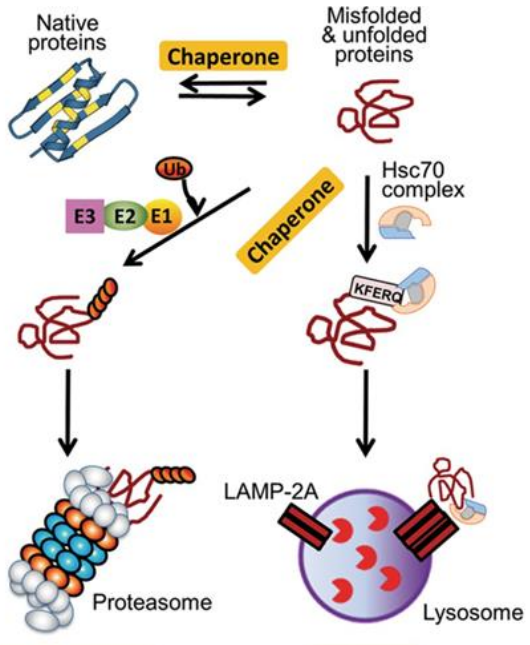
Prof. mentioned therapy that is related to immunology, and said he knows that we haven't studied it yet, you can watch the lecture if you want to.

- siRNA works by the same mechanism of miRNA, but it's synthetic, that's, it's not normally synthesized in our cells, and we've taken advantage of it in therapy. →
- Treatment nowadays is either immunotherapy, monoclonal antibody (against important proteins to cells) or siRNA (by blocking the production of certain proteins).

What else?

Fate of (mis)- and (un)-folded proteins

- Proteins are degraded either in degradative subcellular organelles like lysosomes or by the macromolecular proteasomes when they are uniquitinylated.



➢ Protein level is maintained by regulating transcription, translation and degradation of proteins such as mis-folded and un-folded proteins.

The multi-levels of regulation

Levels of regulation

Prof. mentioned the highlights of these mechanisms that we've already studied, check out the lecture.

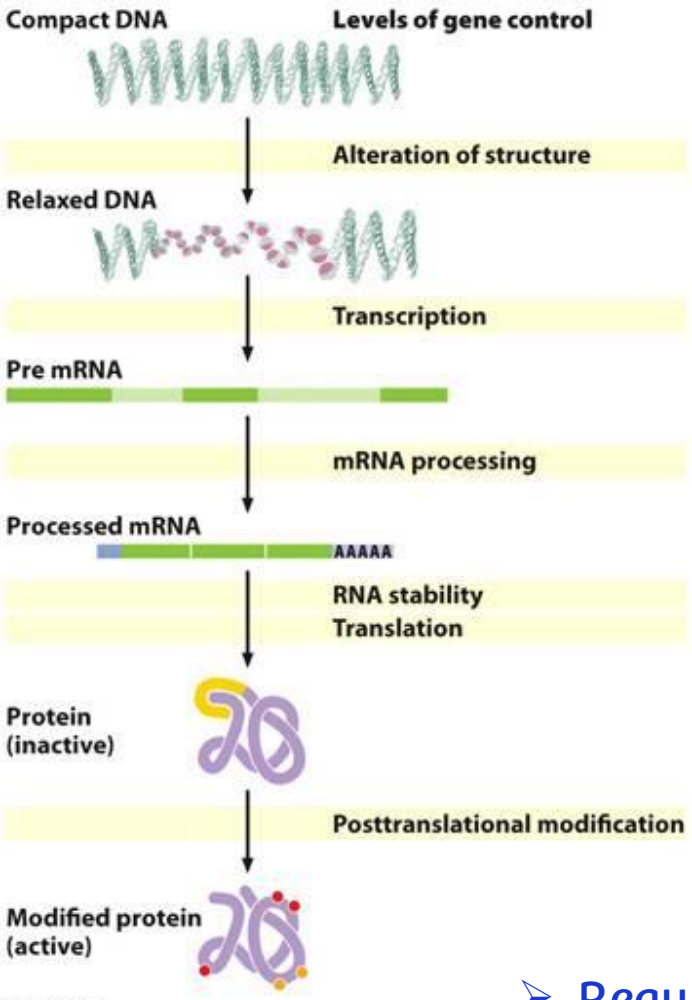


Figure 16.1
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- Transcription
- RNA processing
- RNA transport
- mRNA stability
- Translation
- Post-translational modification
- Protein activity
- Protein degradation

➢ Regulation at different levels makes human cells different than animal cells. This what makes human human and mouse mouse. It's not always about on and off, it's about regulation.

For any feedback, scan the code or click on

Corrections from previous versions:

Versions	Slide # and Place of Error	Before Correction	After Correction
V0 → V1			Slide 31 was added
V1 → V2			

Additional Resources:

رسالة من الفريق العلمي:

بناءً على طلبكم، حاولنا نختصر قدر الإمكان بطرح الفكرة دون كتابة الملاحظات الموجودة بالسلايدات، بالتوفيق جميعًا.