



Molecular Biology (8)

Translation

Don't forget transcription → translation

Mamoun Ahram, PhD

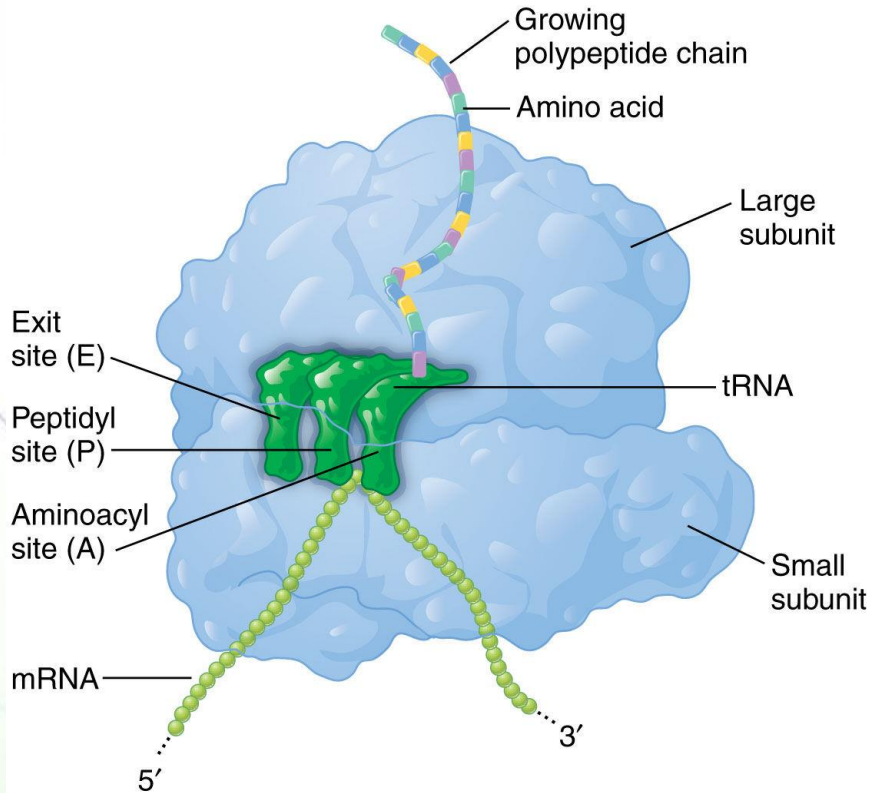
Resources



- This lecture
- Cooper, Ch. 9

General information

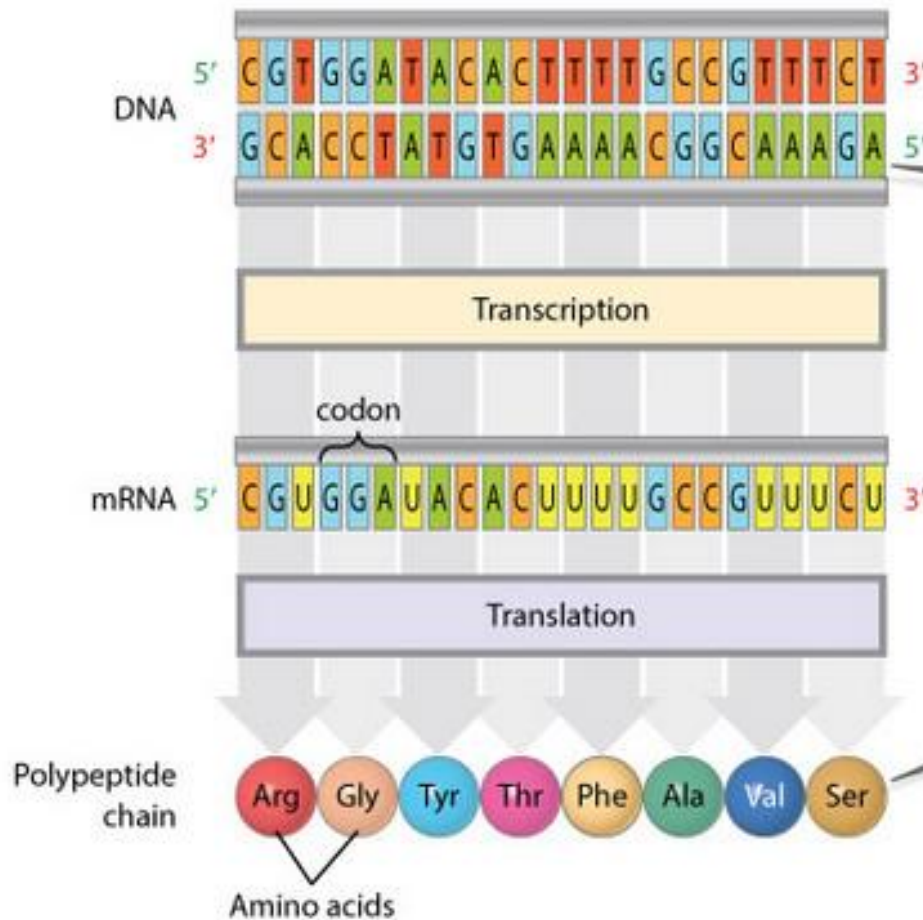
Ribosome is the factory of protein + 3 RNA molecules



© 2010 Pearson Education, Inc.

- Protein synthesis involves interactions between ribosomes with three types of RNA molecules:
 - tRNAs → have amino acid on it (محمل حمض)
 - rRNAs,
 - mRNA templates

Colinearity of genes, mRNAs, & proteins



they found linear relationship between genes on DNA and mRNA with protein

1 A continuous sequence of nucleotides in the DNA...

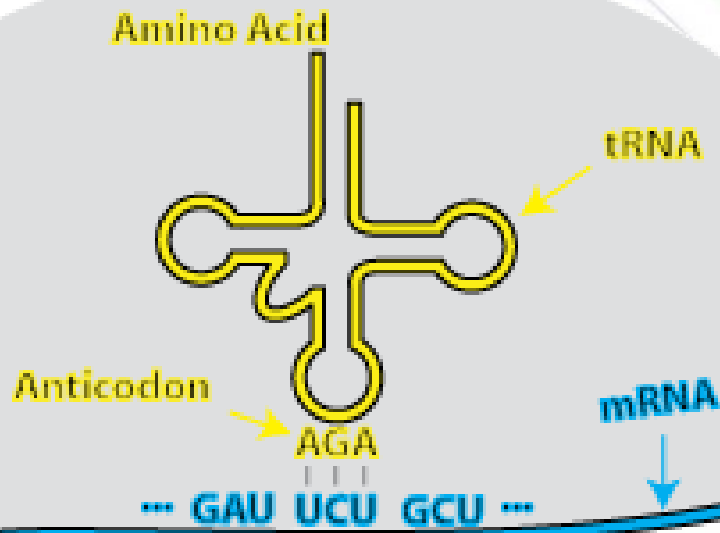
So if mutation happened on first part of DNA the mutation will be on the first part on RNA also and continue to beginning of protein. and if the mutation on the end of DNA it will be on the end of mRNA and so on.

2 ...codes for a continuous sequence of amino acids in the protein.

So I can conclude that the same direction the DNA was read is the same for RNA

With colinearity, the number of nucleotides in the gene is proportional to the number of amino acids in the protein.

mRNA is read by tRNA in triplets



MCAT-Review.org

The mRNA is read in triplets and tRNA is helping that



Second letter we have 64 codon

		Second letter					
		U	C	A	G		
First letter	U	UUU } Phe UUC } UUA } Leu UUG }	UCU } UCC } Ser UCA } UCG }	UAU } Tyr UAC } UAA Stop UAG Stop	UGU } Cys UGC } UGA Stop UGG Trp	U C A G	
	C	CUU } CUC } Leu CUA } CUG }	CCU } CCC } Pro CCA } CCG }	CAU } His CAC } CAA } Gln CAG }	CGU } CGC } Arg CGA } CGG }	U C A G	
	A	AUU } AUC } Ile AUA } AUG Met	ACU } ACC } Thr ACA } ACG }	AAU } Asn AAC } AAA } Lys AAG }	AGU } Ser AGC } AGA } Arg AGG }	U C A G	
	G	GUU } GUC } Val GUA } GUG }	GCU } GCC } Ala GCA } GCG }	GAU } Asp GAC } GAA } Glu GAG }	GGU } GGC } Gly GGA } GGG }	U C A G	

Not for memorizing except that selected

it is Universal but have exceptions as mitochondria (some of them is different)

for initiation translation

methayonine

So maybe more than codon translated to one amino acid

Features of the genetic codon



61 translated to codons and 3 → stop codon

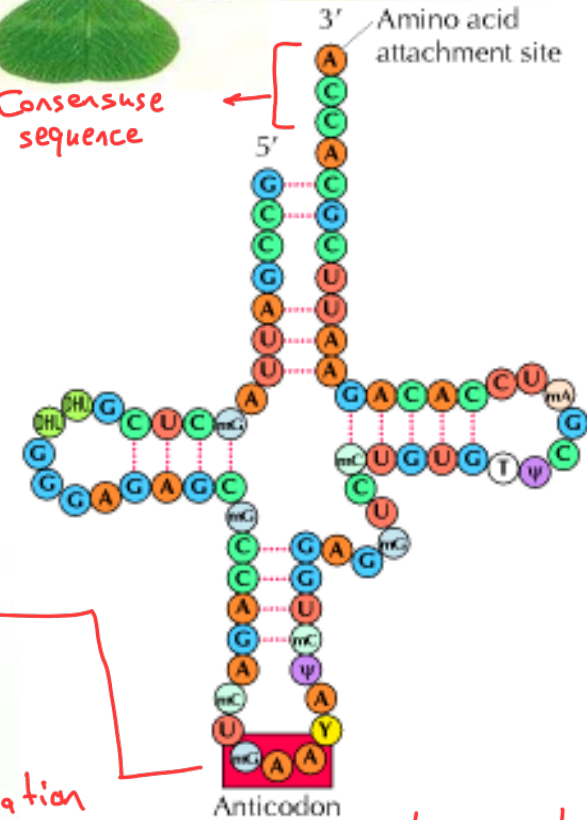
- All 64 possible codons of the genetic code and the amino acid specified by each, as read in the 5'→3' direction from the mRNA sequence.
- Sixty-one codons specify an amino acid.
 - Three STOP codons (UAA, UAG, and UGA) do not encode any amino acid.
- The genetic code for mitochondrial mRNA (mtDNA) conforms to the universal code except for a few variants.

tRNA structure



Consensus sequence

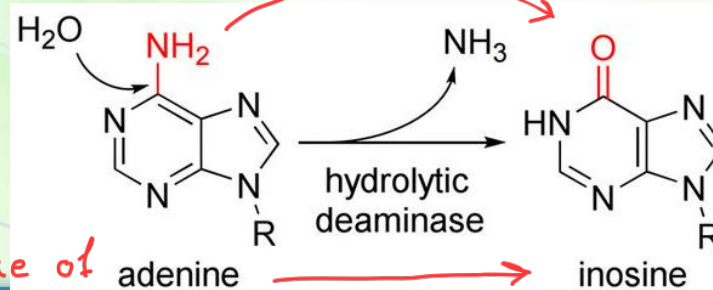
- ▶ tRNAs are short single-stranded RNA molecules (80 bases long).
- ▶ “Charged” or “activated” tRNA carries one amino acid, which is attached by **aminoacyl-tRNA synthetases**.
- ▶ An amino acid is covalently attached to the ribose of the terminal adenosine at CCA.
 - ▶ The amino acid attached to tRNA is specified not only by the anticodon, but also identifier sequences within the tRNA.
- ▶ tRNAs contain stem loop structures, modified bases, and unusual bases (example: inosine).



↳ most important sequence

not all of them ←

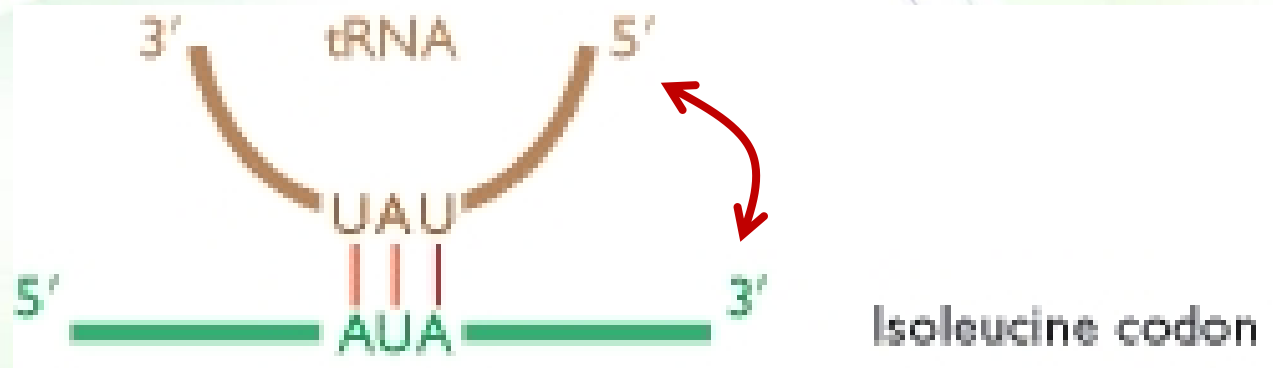
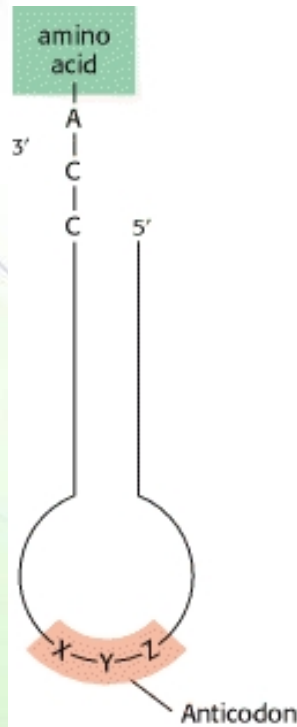
Some of



Codon vs. anticodon



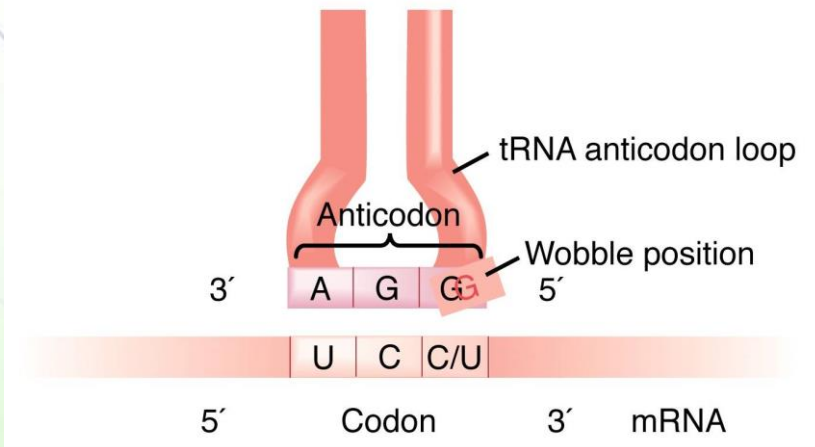
- tRNAs contain a three-nucleotide sequence known as “anticodon” that pairs with the “codon” or “triplet” mRNA molecules (note the anti-parallel alignment of mRNA-tRNA complex)



Fidelity of translation



- Accurate translation requires two steps
 - First: accurate association of amino acid to tRNA by **aminoacyl-tRNA synthetase** *which is very accurate*
 - Second: a correct match between the tRNA anticodon and an mRNA codon *↳ By complementary base pairing*



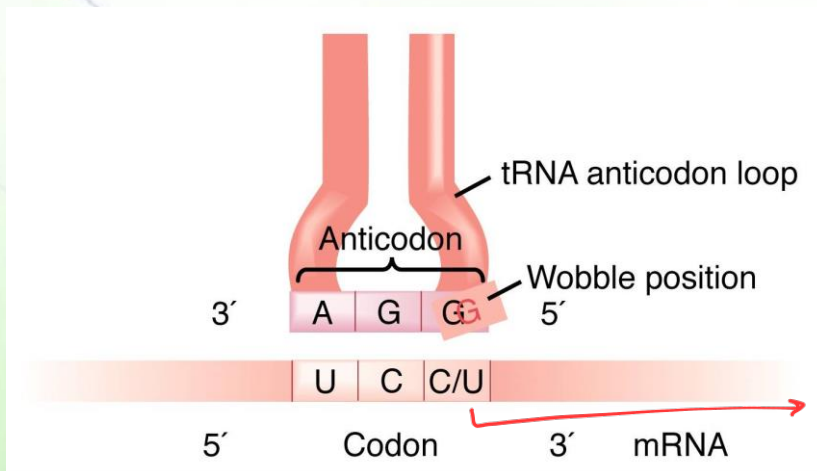
Wobble base pairing



There is flexible pairing at the **third base** of a codon to the **anticodon** allowing some tRNAs to bind to more than one codon.

It is called **wobble** base pairing.

The bases that are common to several codons are usually the first and second bases, with more room for variation in the third base, The degeneracy of the genetic codons
It acts as a buffer against deleterious mutations.



so maybe C or U has base pairing with G instead of A why? so if mutation occur it does not affect the translation so it act as buffer

Examples of wobble base pairing



- Relaxed base pairing results from the formation of G-U base pairs.

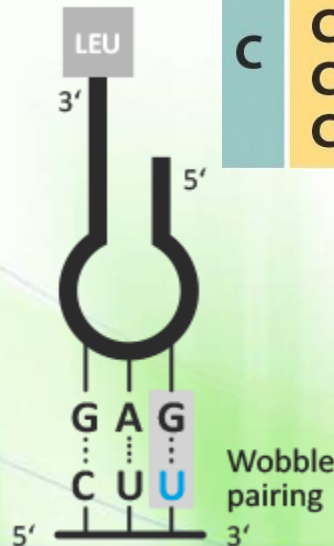
Both are hydrophobic amino acids

So if I have G or A it is the same amino acid and even if mutation changed G to U the new amino acid will be from same family

	U	C	A	G	
U	UUU } Phe UUC } UUA } Leu UUG }	UCU } UCC } Ser UCA } UCG }	UAU } Tyr UAC } UAA Stop UAG Stop	UGU } Cys UGC } UGA Stop UGG Trp	U C A G
C	CUU } CUC } Leu CUA } CUG }	CCU } CCC } Pro CCA } CCG }	CAU } His CAC } CAA } Gln CAG }	CGU } CGC } Arg CGA } CGG }	U C A G



Identical leucine tRNAs

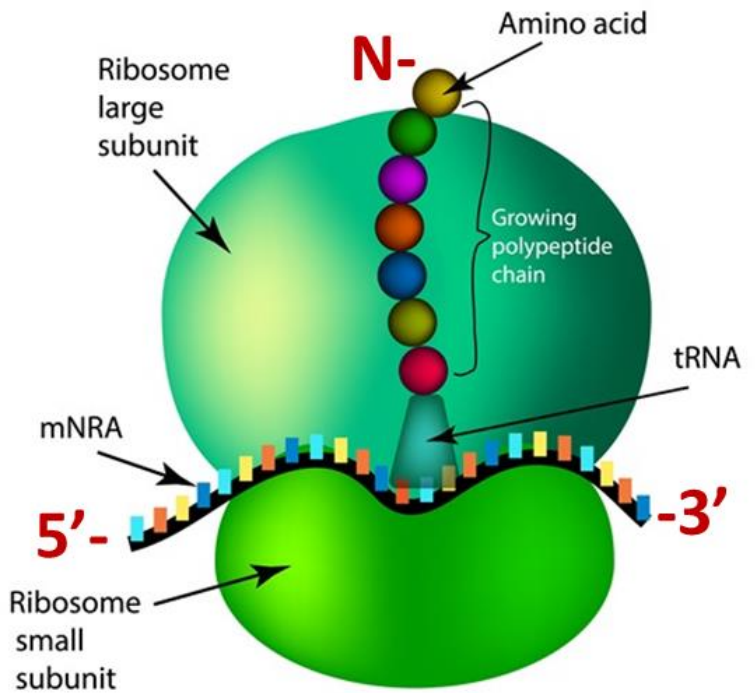


So if you focused the third nucleotids is not important.

Ribosomes is the factors



- Ribosomes are the sites of protein synthesis.
- *E. coli* contain about 20,000 ribosomes (~25% of the dry weight of the cell).
- Rapidly growing mammalian cells contain about 10 million ribosomes.



The peptidyl transferase reaction of a peptide bond is catalyzed by the rRNA of the large ribosomal subunit. → act as enzyme

two subunits } → large
 } → small

Ribosome structure

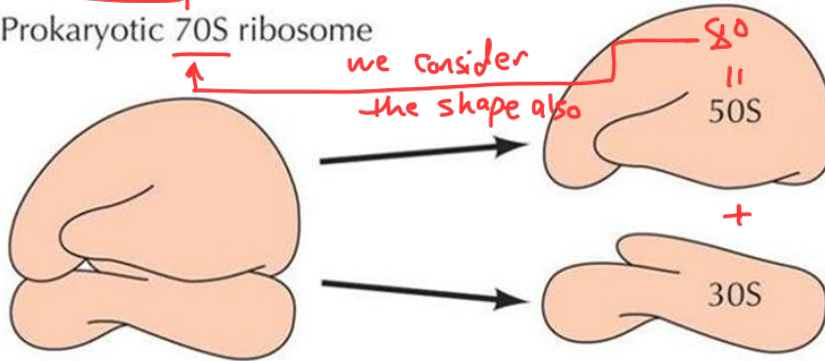


→ indication of weight and the S is a Unit which depend on weight and shape

the ribosomes subunits is almost same in eukaryote and prokaryote so it indicate the importance of it as histons also is similar in almost all living cells

(A)

Prokaryotic 70S ribosome

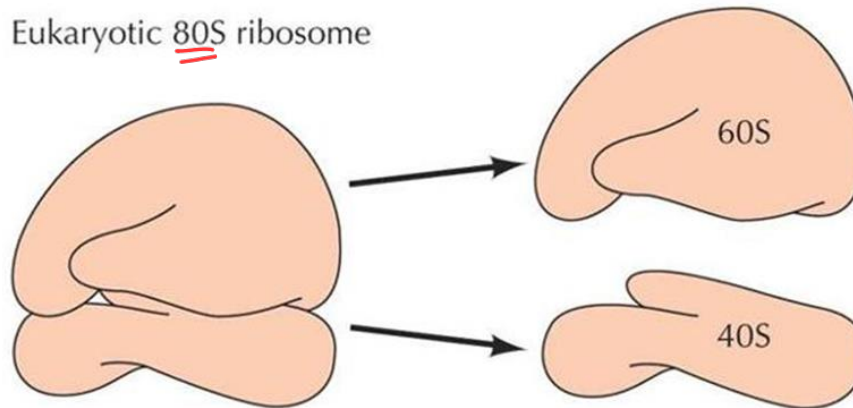


23S and 5S rRNAs
(34 proteins)

16S rRNA
(21 proteins)

we don't care about Number of ribosomal subunit in prokaryote

Eukaryotic 80S ribosome



28S, 5.8S, and 5S rRNAs
(~45 proteins)

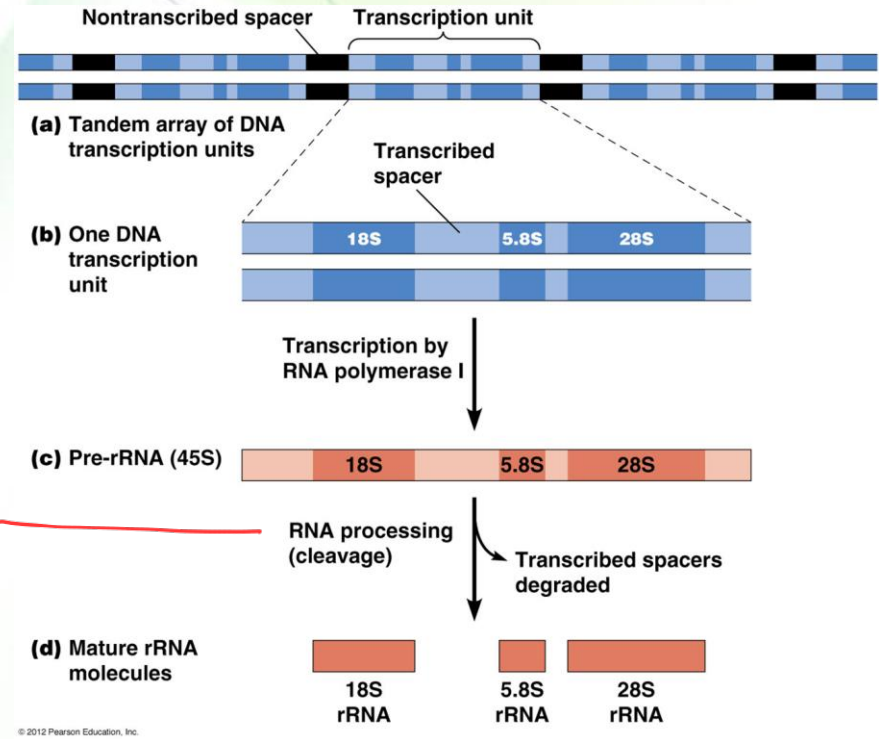
18S rRNA
(~30 proteins)

→ important

rRNA synthesis and processing



- The 18S, 5.8S, and 28S rRNAs are encoded by a single gene (13 kb long). *which is repeated*
- Transcription by RNA polymerase I produces a primary transcript (45S rRNA) that then undergoes post-transcriptional cleavages producing individual 18S, 28S, and 5.8S rRNA molecules.
- The 18S rRNA associates with the small ribosomal subunit.
- The 5.8S and 28S rRNAs associate with the large ribosomal subunit.
- The large ribosomal subunit also contains 5S rRNA, which is encoded by different genes transcribed by RNA polymerase III. *→ 5S rRNA + tRNA*



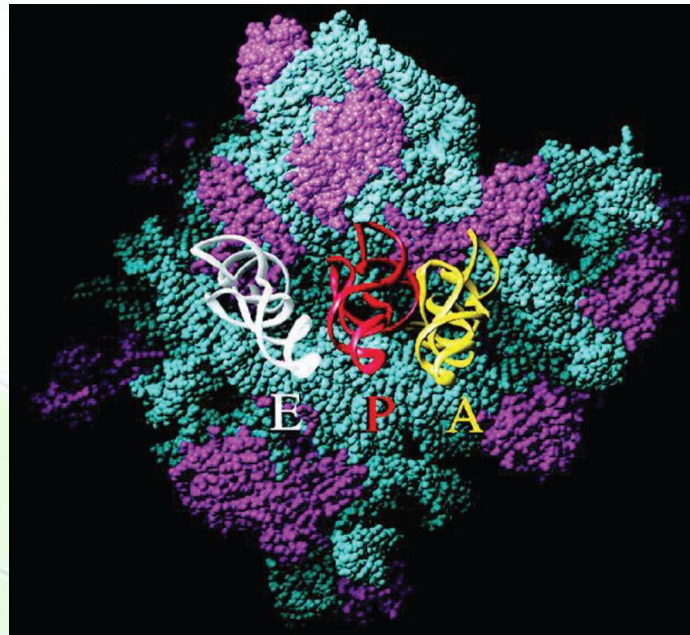
don't forget RNA pol II is for mRNA and micro RNA

Functional and structural components of ribosomes



So if we remove 90% of protein that make ribosomes rRNA will stay make proteins synthesis

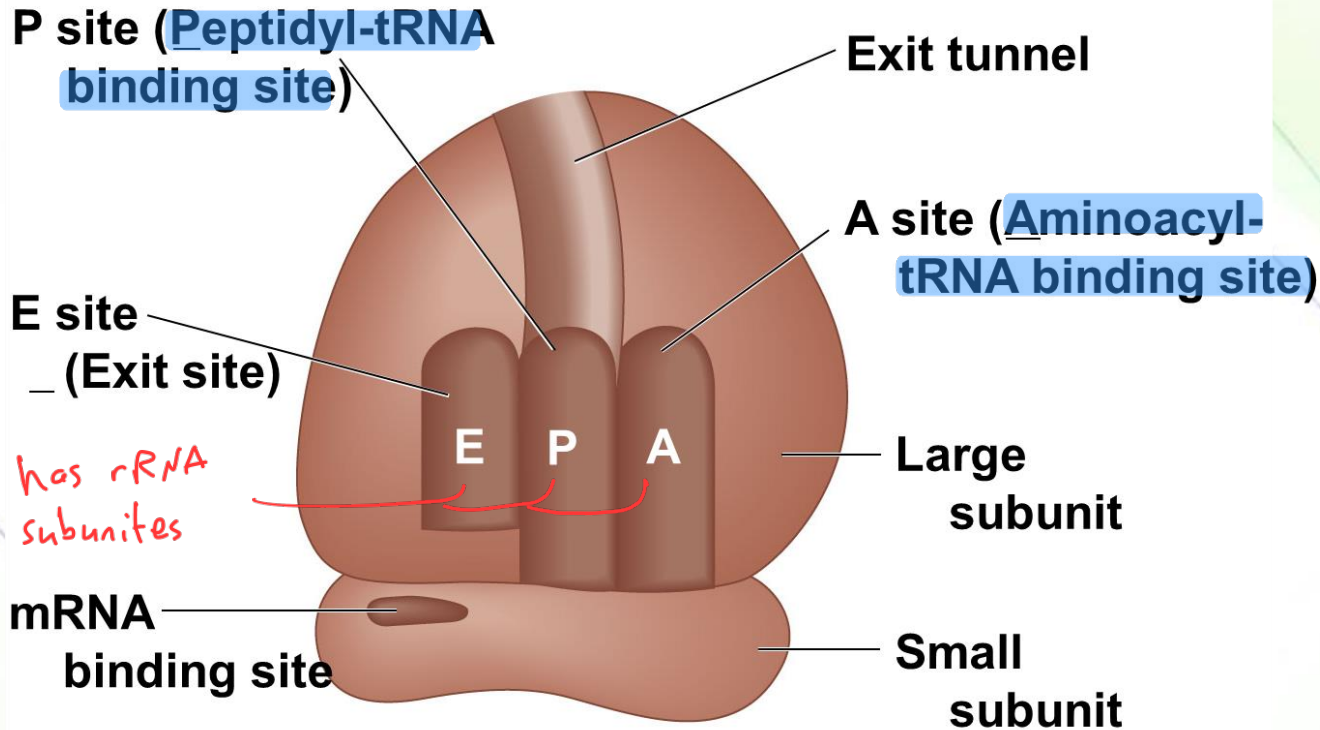
- Ribosomes facilitate specific coupling of tRNA anticodons with mRNA codons in protein synthesis.
- The RNA components are predominantly responsible for the catalytic function of the ribosome, the protein components enhance the function of the rRNA molecules.



and rRNA location is very important (the same location of formation of peptid bonds)

The chambers of secret

HARRY POTTER AND THE
CHAMBER OF
SECRETS
J. K. ROWLING



tRNA binding sites on a ribosome:

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

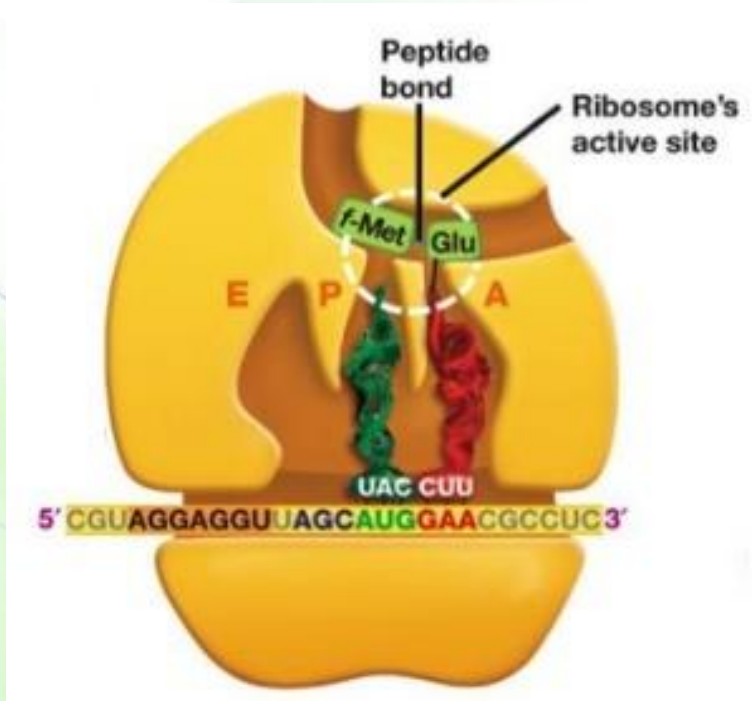
The **E site** is the exit site, where discharged tRNAs leave the ribosome

The general mechanism of translation



- Three stages: initiation, elongation, and termination.
- The direction is $5' \rightarrow 3'$. \Rightarrow reading direction
- Protein synthesis begins at the amino terminus and extends toward the carboxyl terminus. So adding on

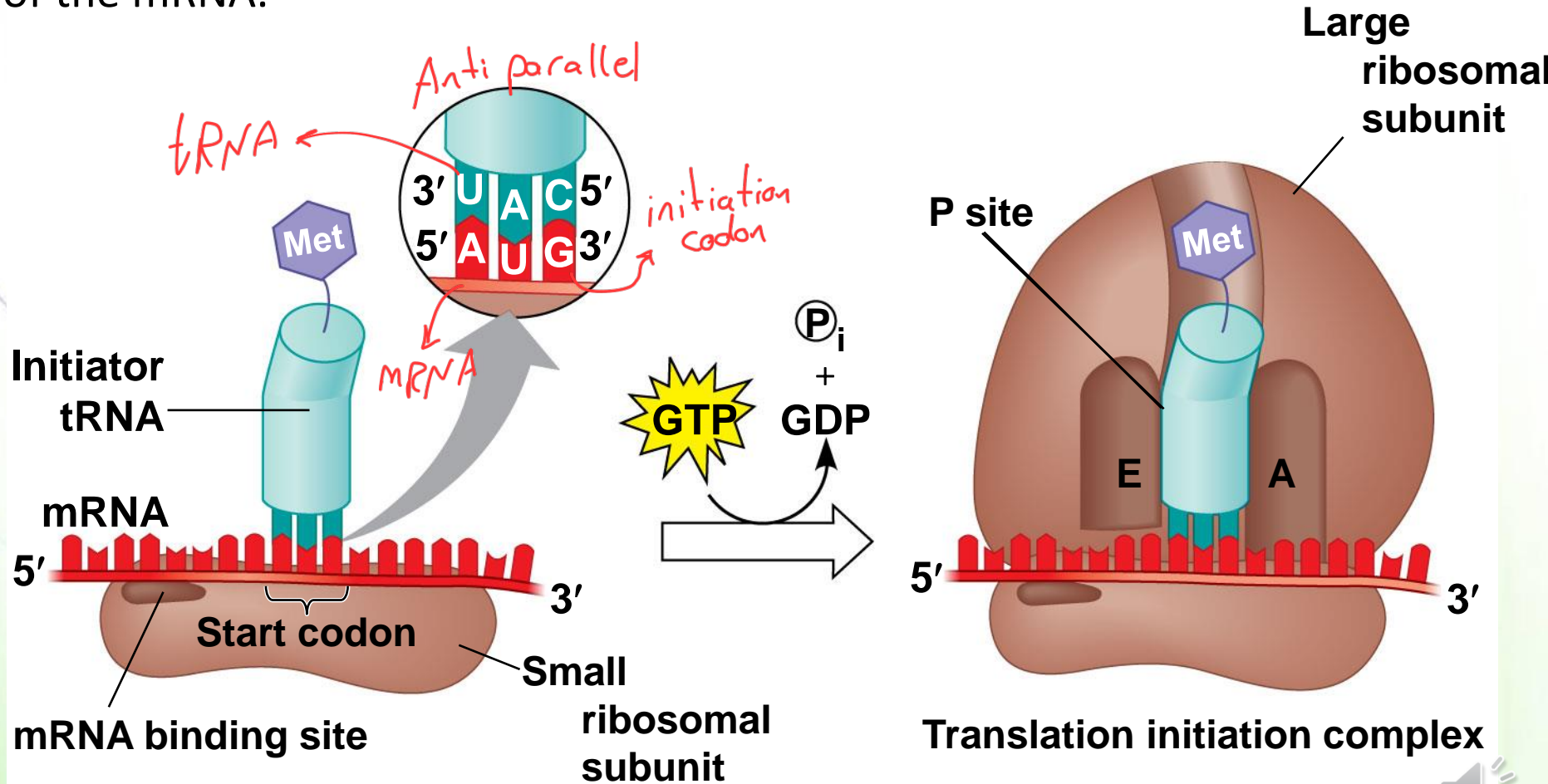
Carboxyl group



Start of translation



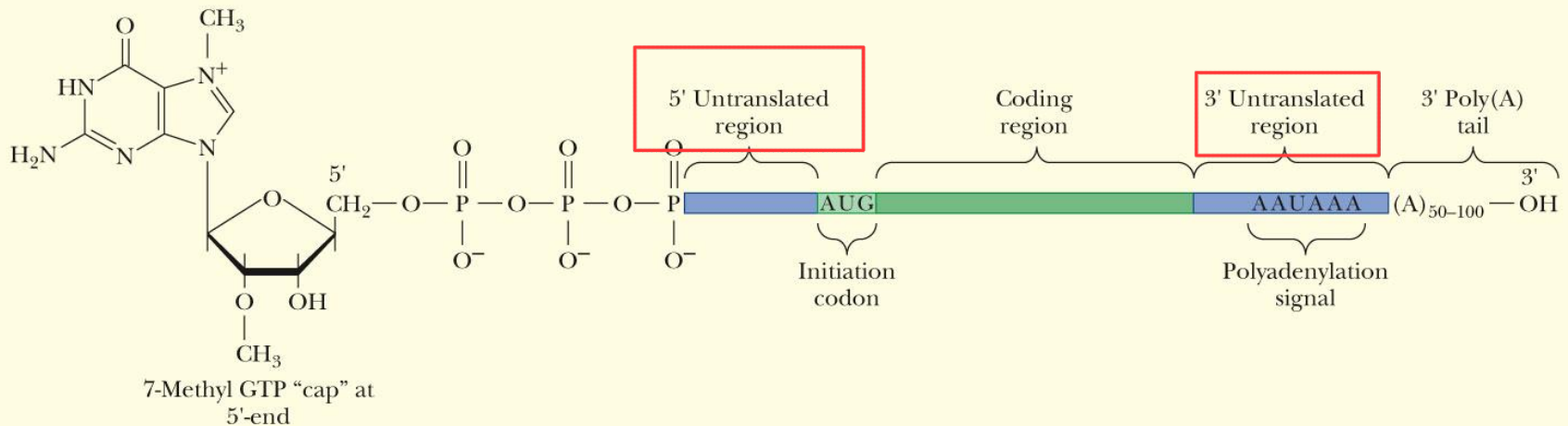
In both prokaryotes and eukaryotes, translation starts at specific initiation sites, which is AUG (methionine), and not from the first codon of the mRNA.



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 the three stop codons.

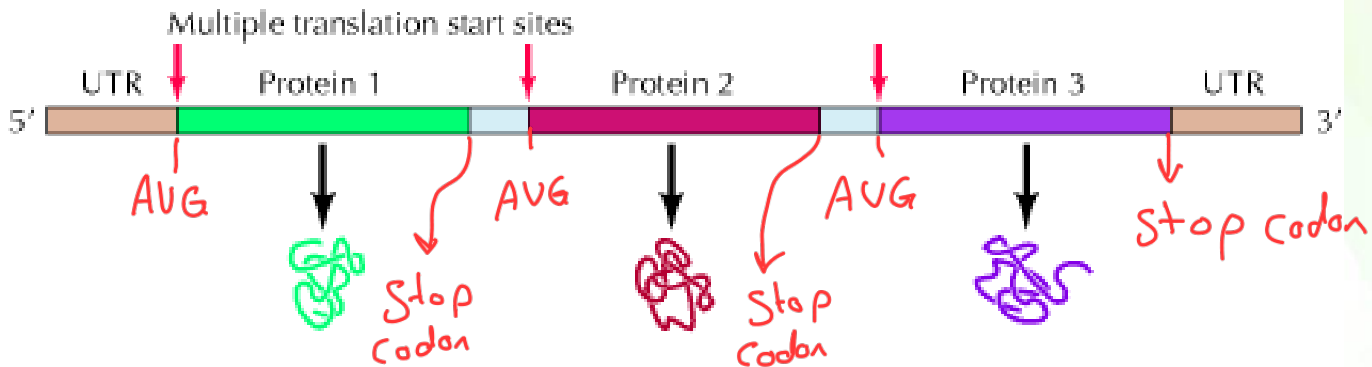


Remember...

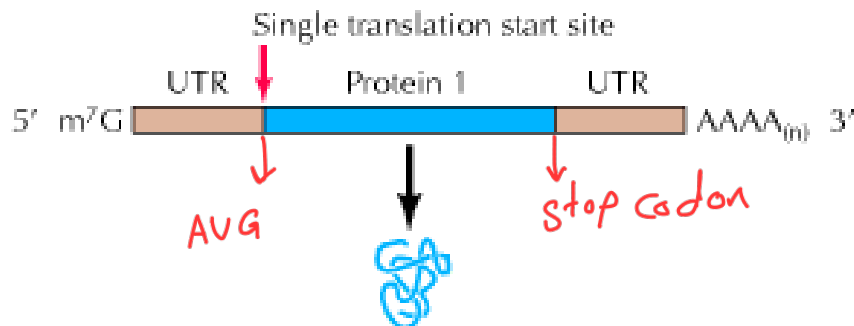


- Bacterial mRNA is polycistronic
- Eukaryotic mRNA is monocistronic

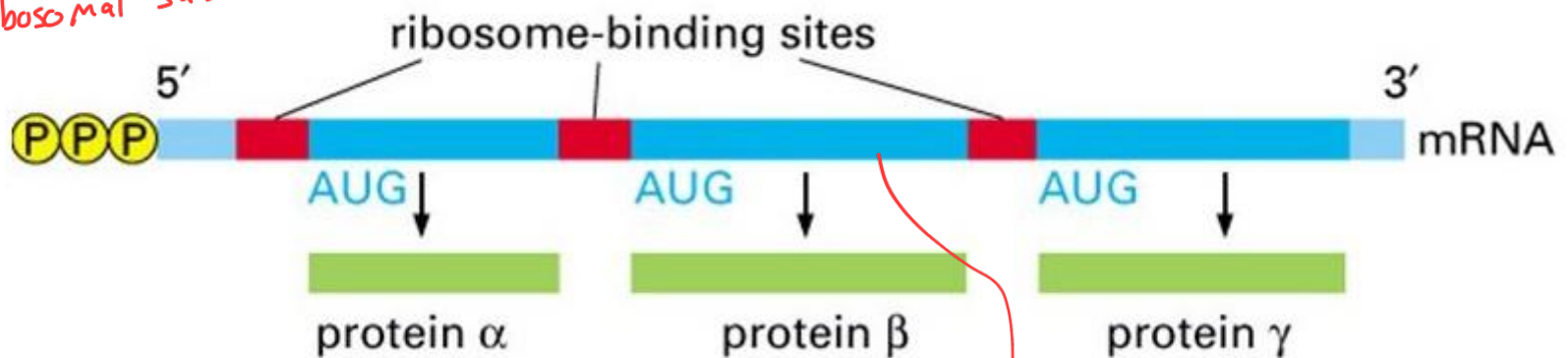
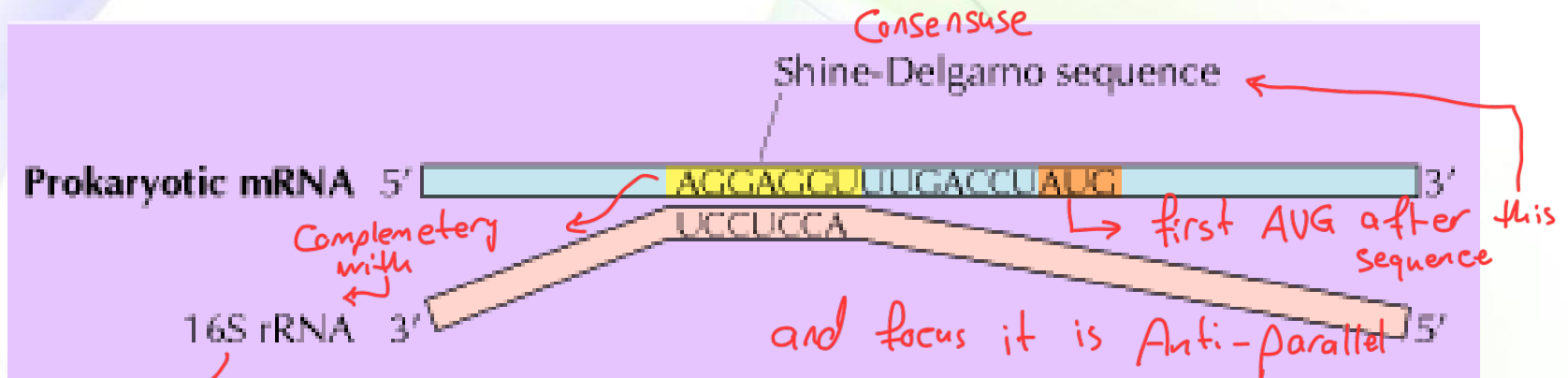
Prokaryotic mRNA



Eukaryotic mRNA



Shine-Dalgarno sequence

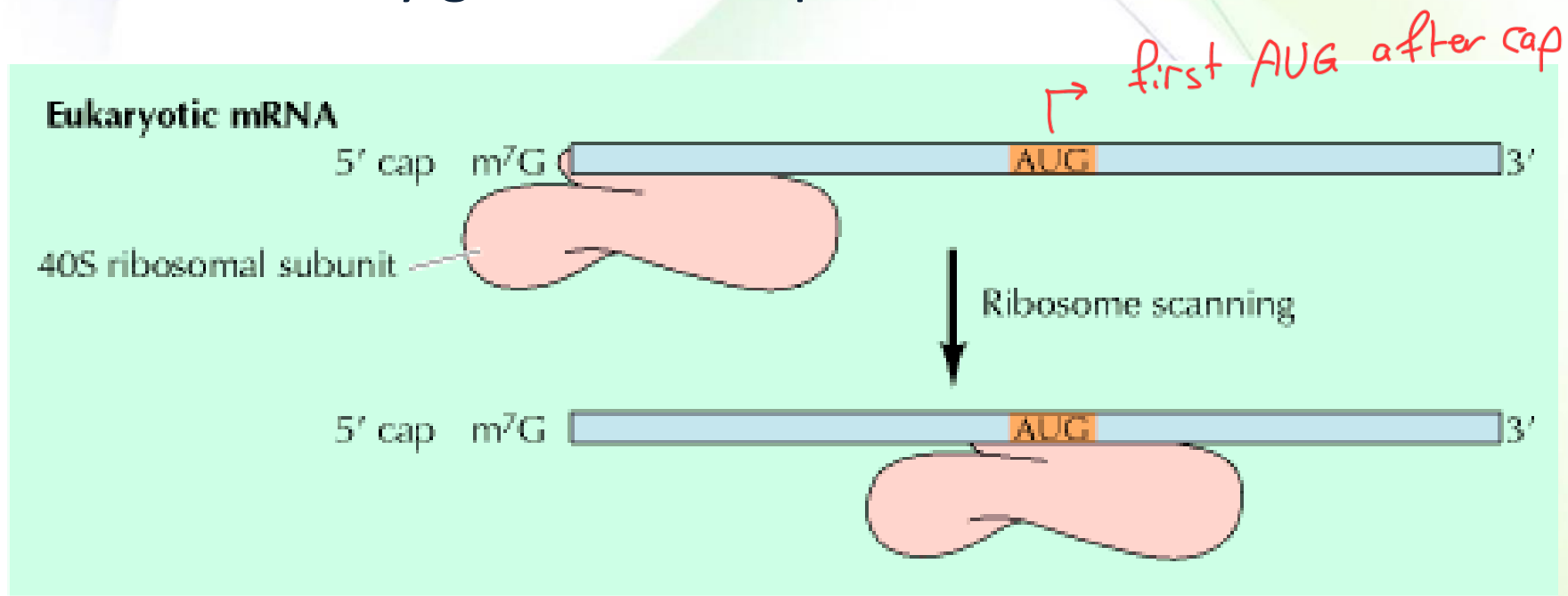


So even if I have AUG here it does not matter because it does not have sequence before it

But in eukaryotes... *does not have same sequence*



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

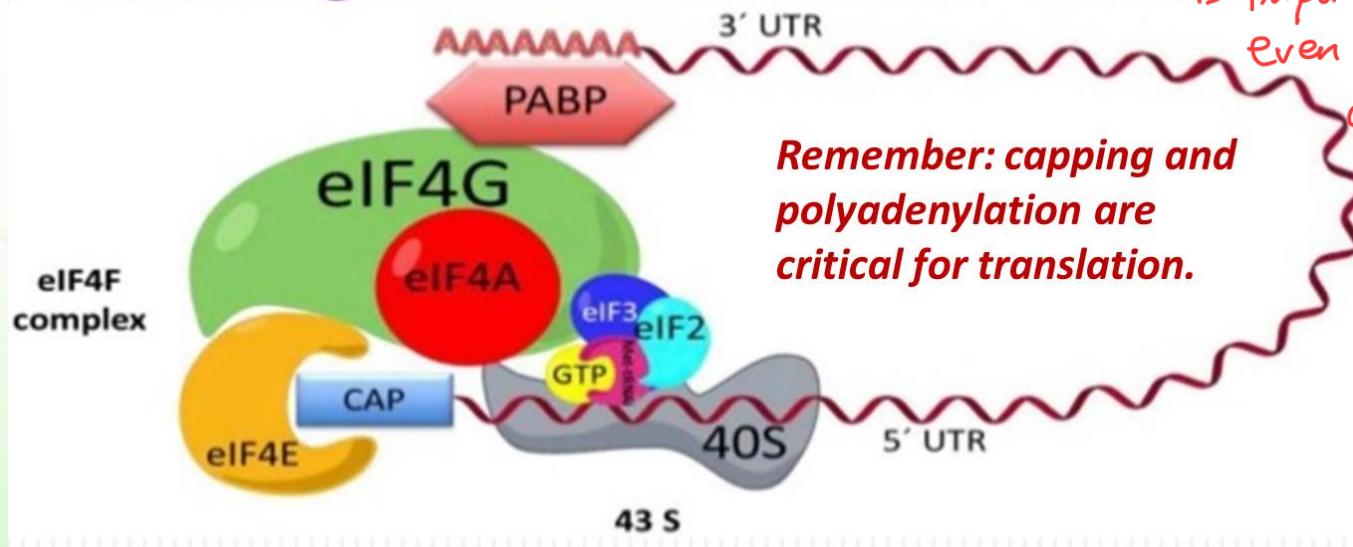


Translation initiation in eukaryotes



has lot of proteins such as eIF4A, eIF4E, ...
proteins

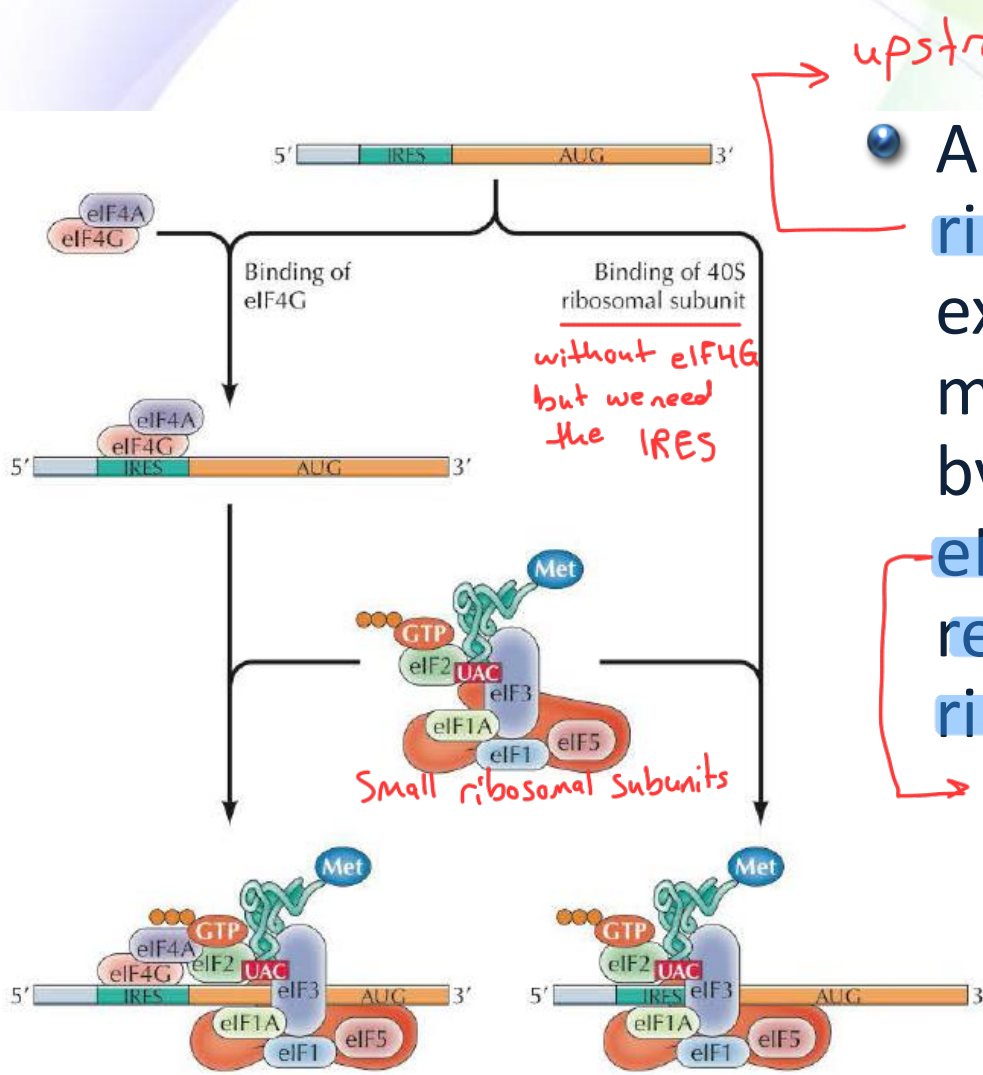
- The eIF4 initiation factors form a complex that links the **poly-A tail** to the **CAP** via poly-A binding protein (PABP) to the cap.
- The eIF4 initiation factors then bring the mRNA to the 40S ribosomal subunit.



So the poly-A tail
is important in translation
even if it is in the end
of RNA

**Remember: capping and
polyadenylation are
critical for translation.**

Internal ribosome entry site (IRES)



upstream of AUG

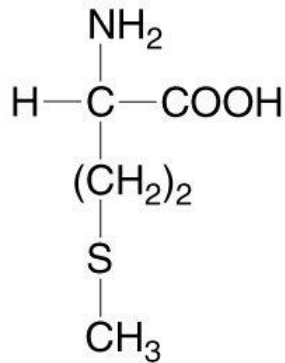
- Alternatively, **internal ribosome entry site (IRES)** exist in some other mRNAs and is recognized by the 40S ribosome or **eIF4G** protein followed by **recruitment of the 40S ribosome.**

and eIF4A

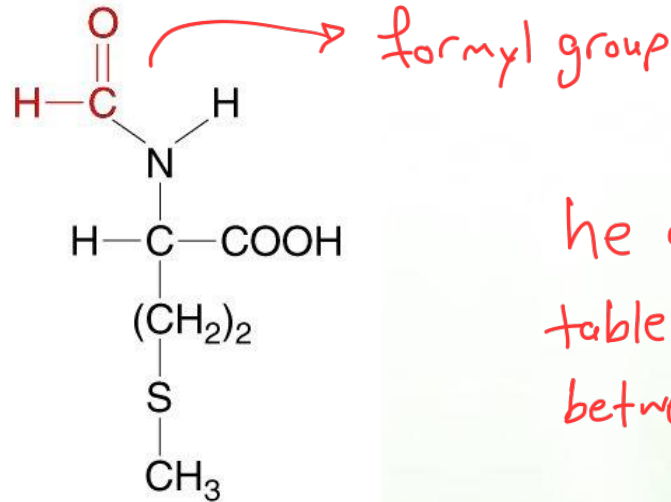
The first amino acid



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



Methionine



N-Formylmethionine

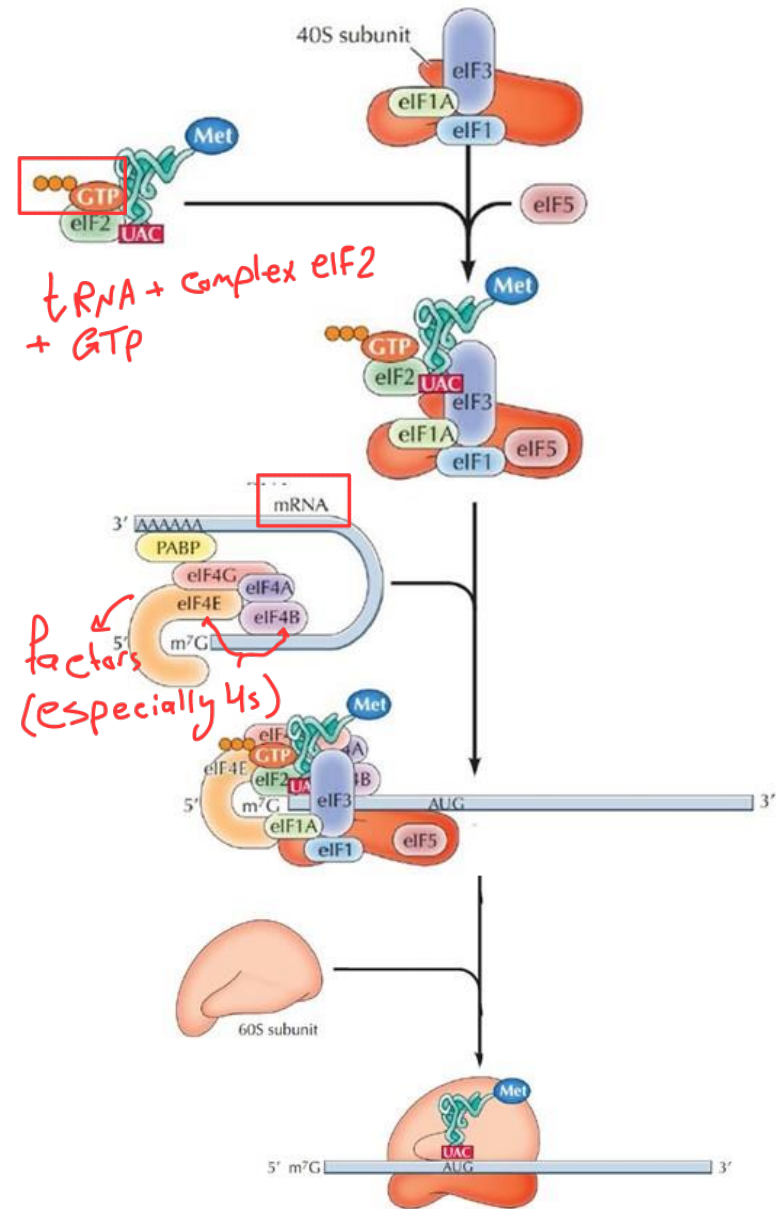
he advise us to make table about the differents between eukaryote and prokaryote

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

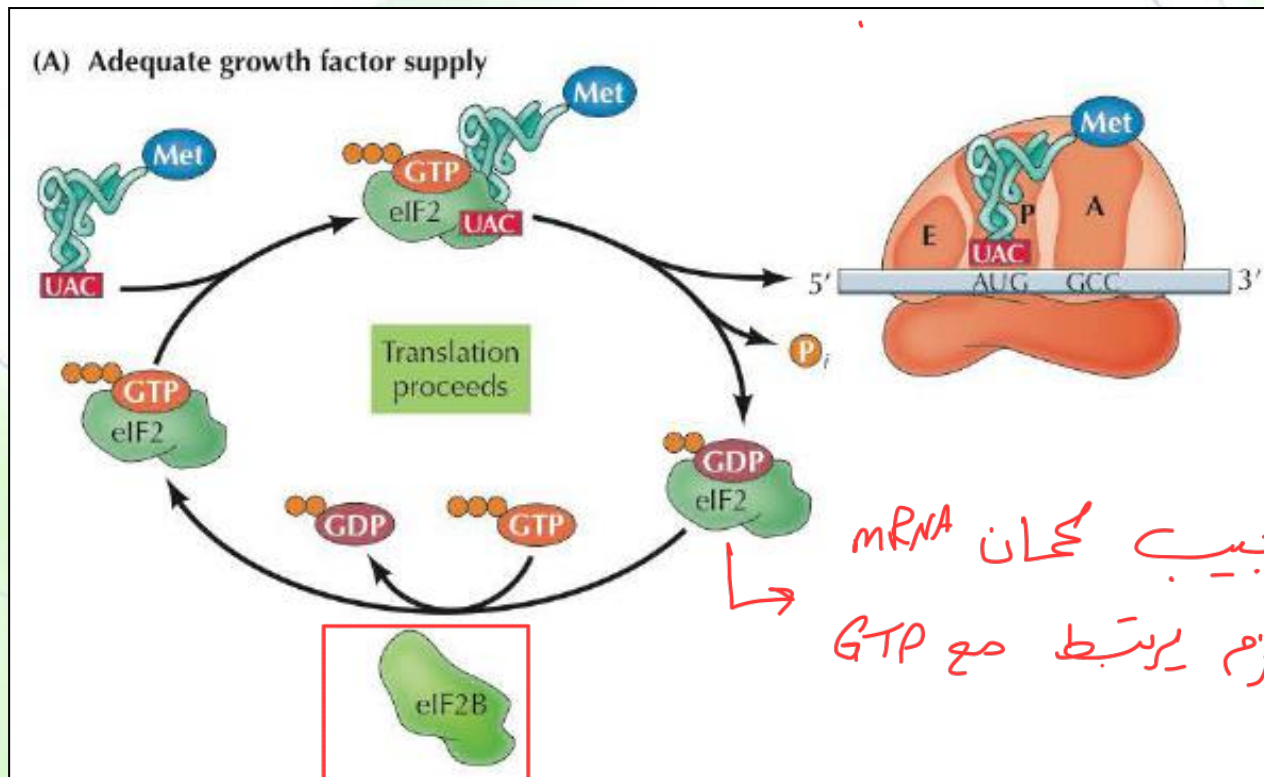


- tRNA forms a complex with 40S ribosomal subunit.
- mRNA joins the complex.
- The 40S ribosomal subunit scans for the first AUG.
- The large ribosomal subunit joins them all.
- A large group of initiation factors facilitate every step.
 - eIF2 brings tRNA to small ribosomal subunit.
 - eIF4 complex brings mRNA to tRNA/40S ribosomal subunit.

Regeneration of eIF2



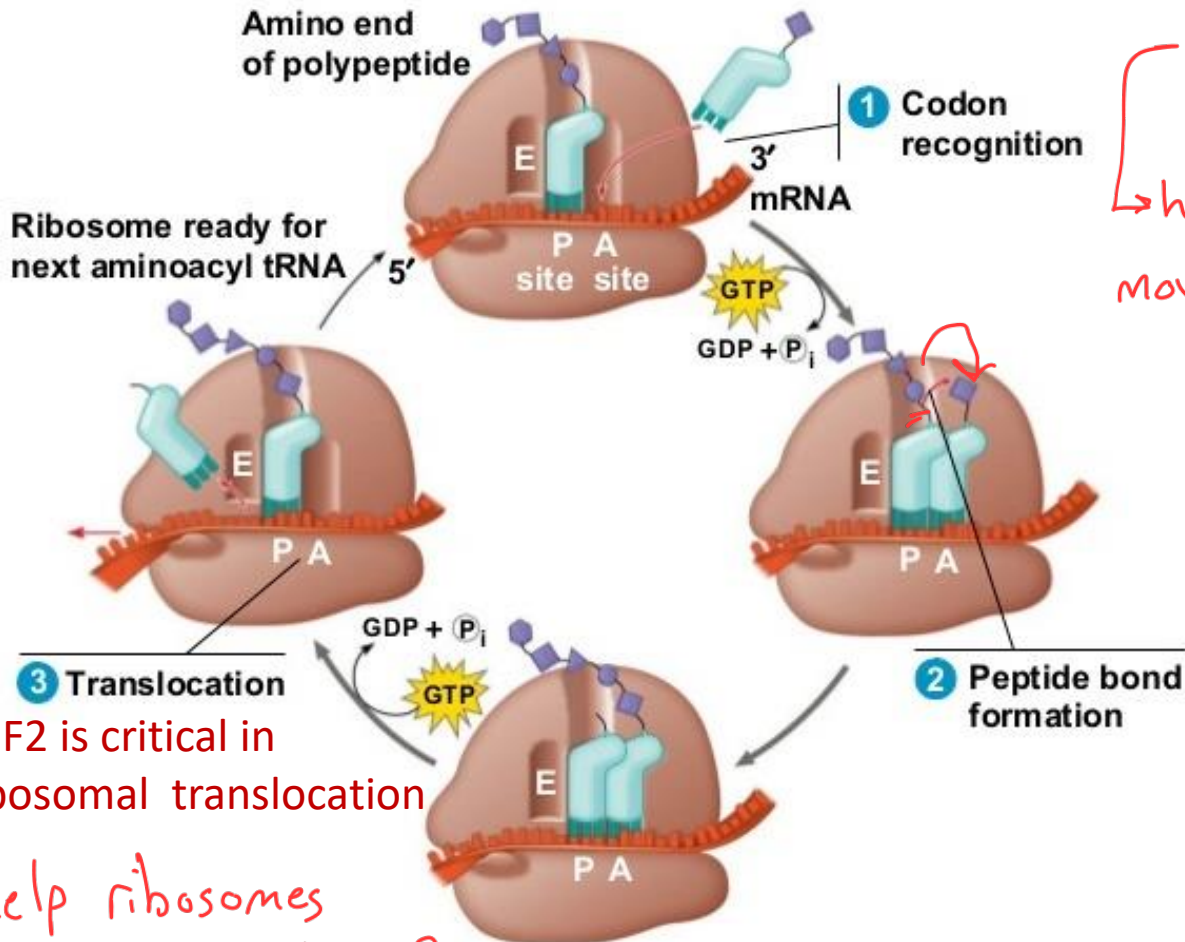
- eIF2 is complexed to GTP to be active. When the correct tRNA is inserted, GTP is hydrolyzed to GDP.
- The active eIF2/GTP complex must be regenerated by exchanging of the bound GDP for GTP.



Translation elongation I



Three steps: aminoacyl-tRNA binding, peptide bond formation, and translocation with the help of elongation factors (eEF).

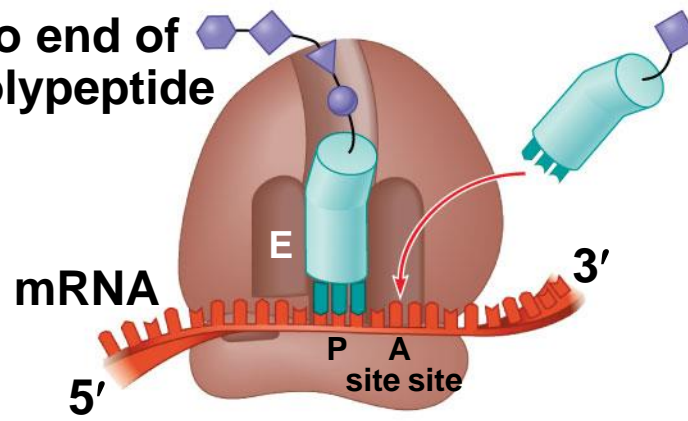


elongation factor 1
↑
eEF1α brings next aminoacyl-tRNA to the A chamber
→ help tRNA to move to A site

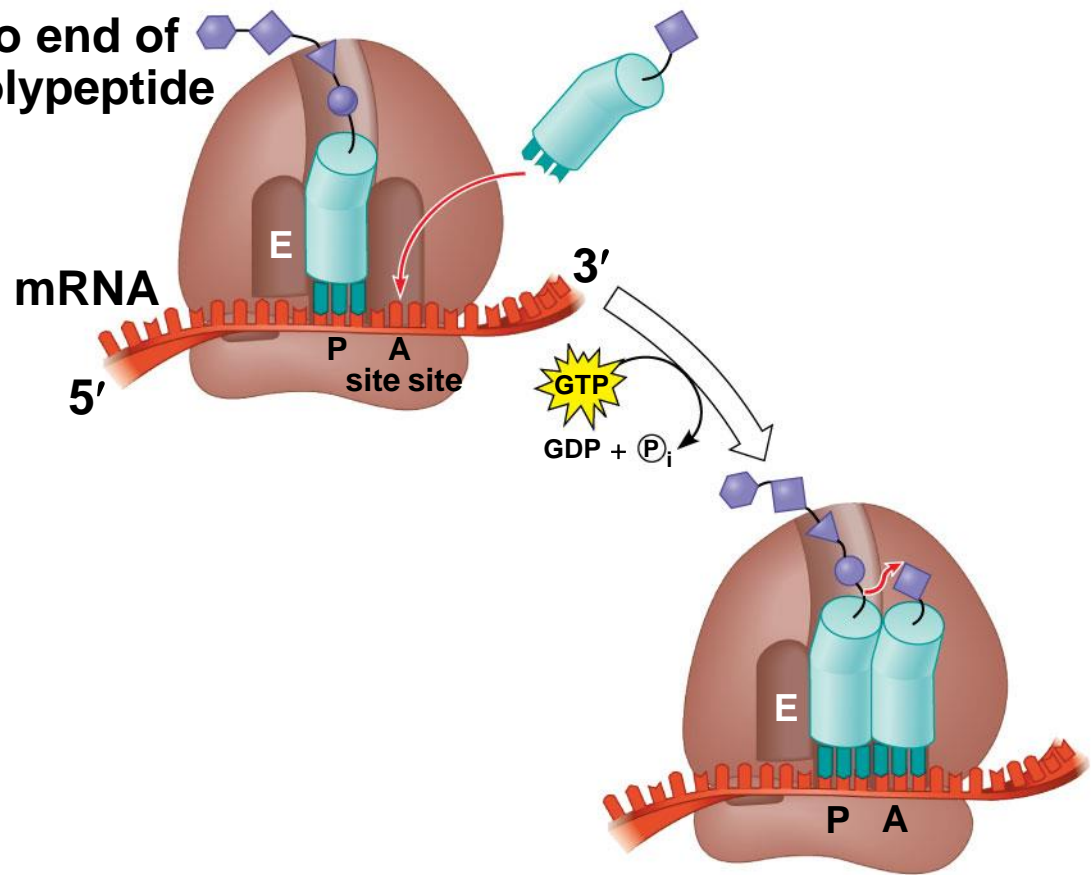
eEF2 is critical in ribosomal translocation
→ help ribosomes to move codon farther



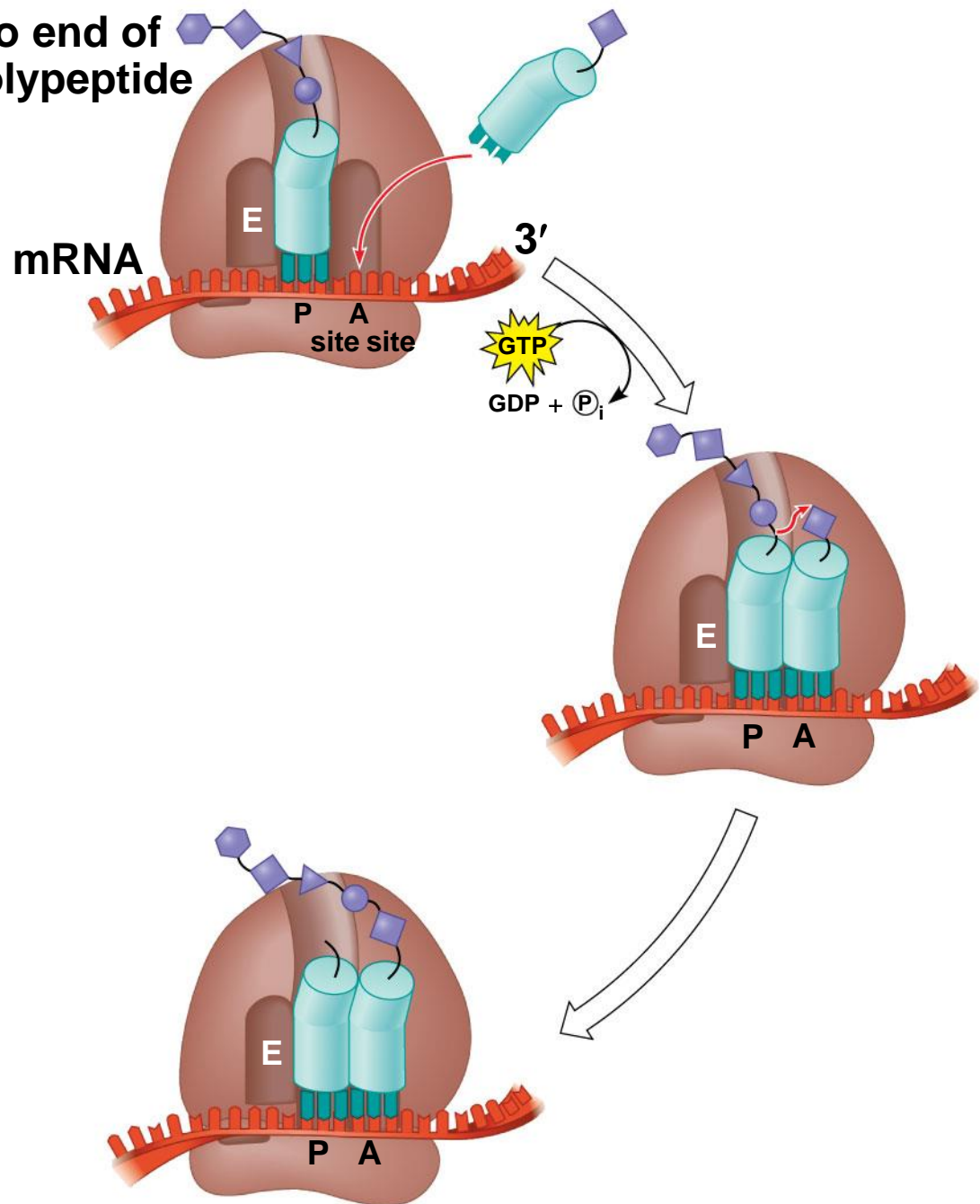
Amino end of polypeptide

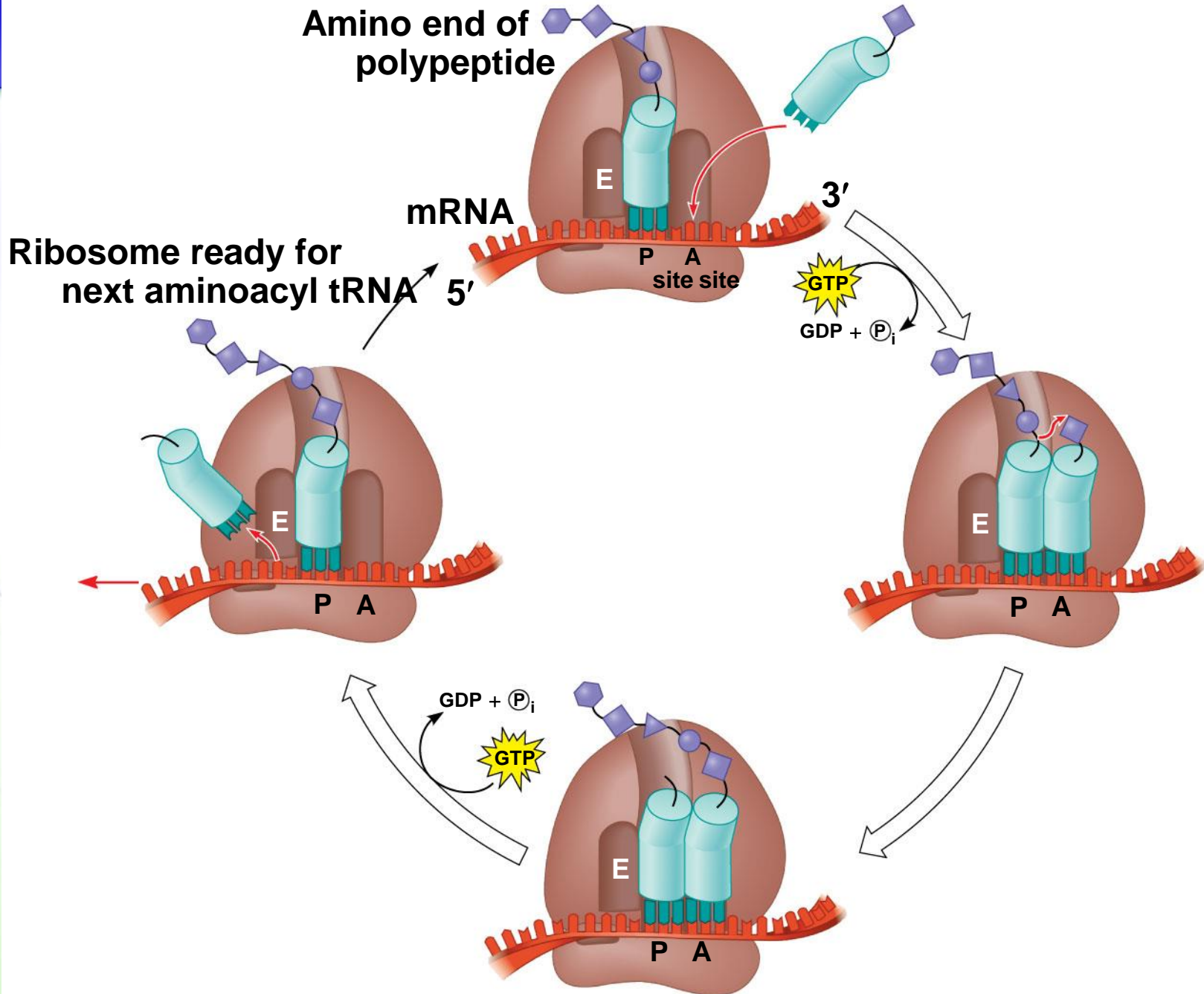


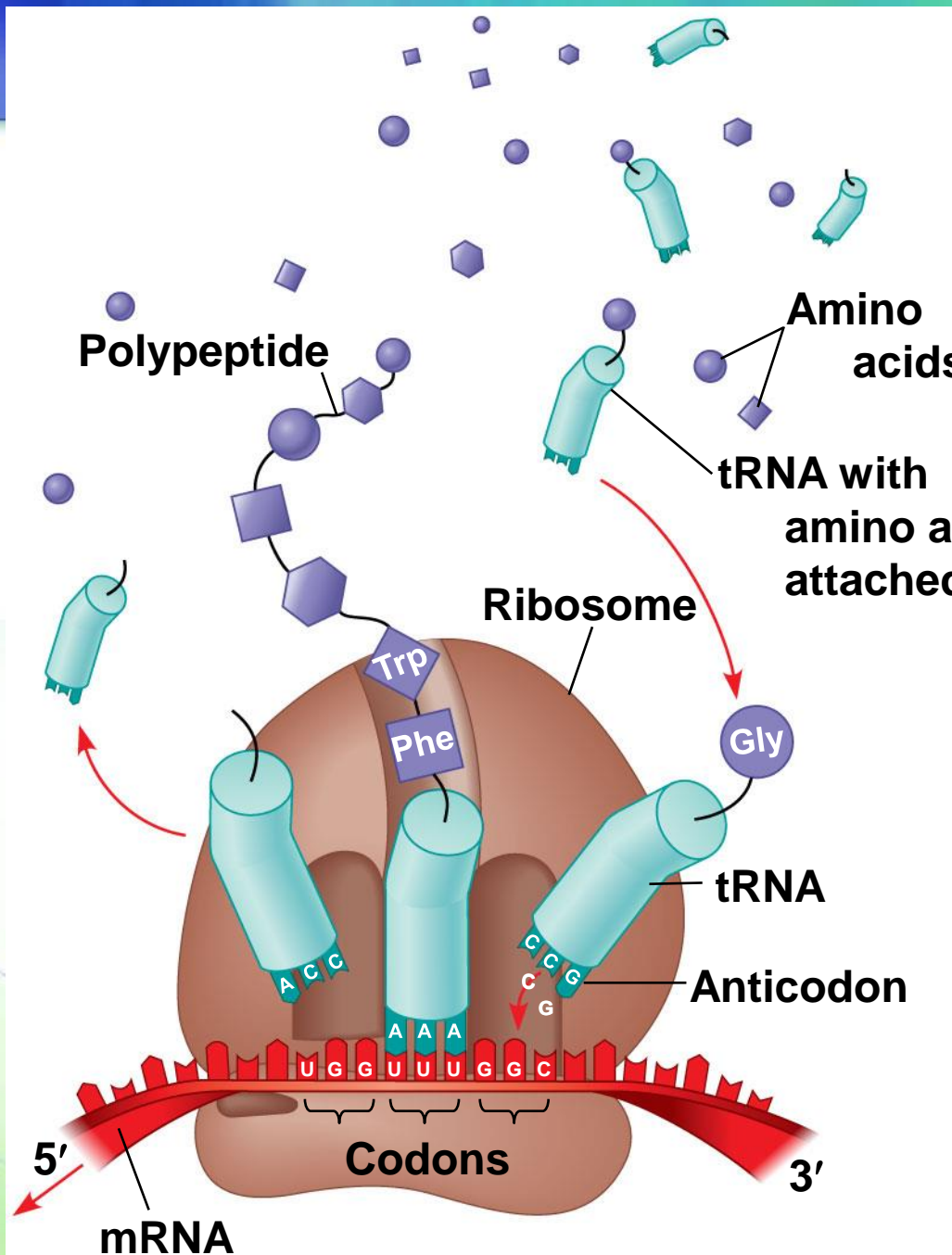
Amino end of polypeptide

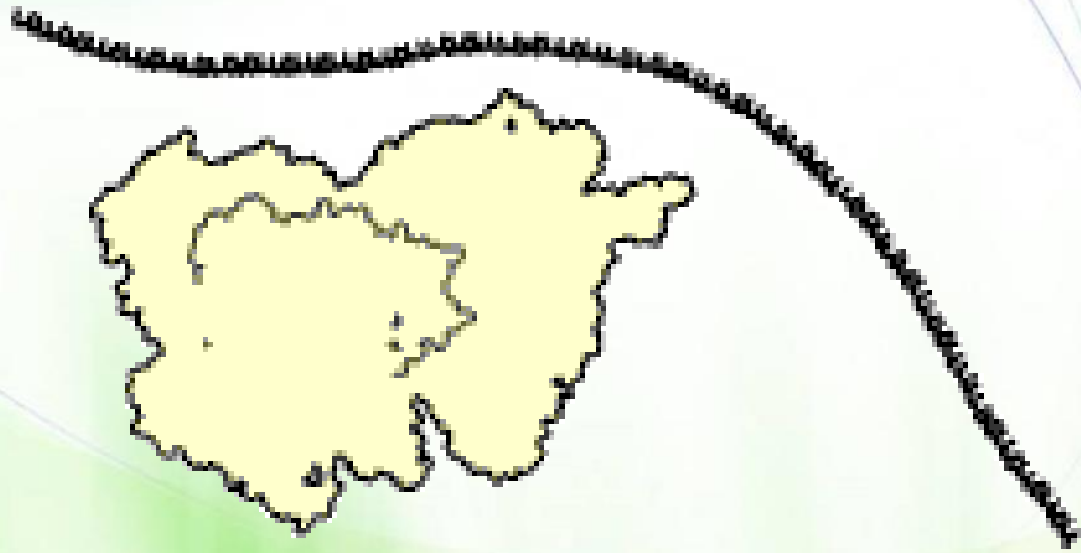


Amino end of polypeptide









Elongation of the Polypeptide Chain



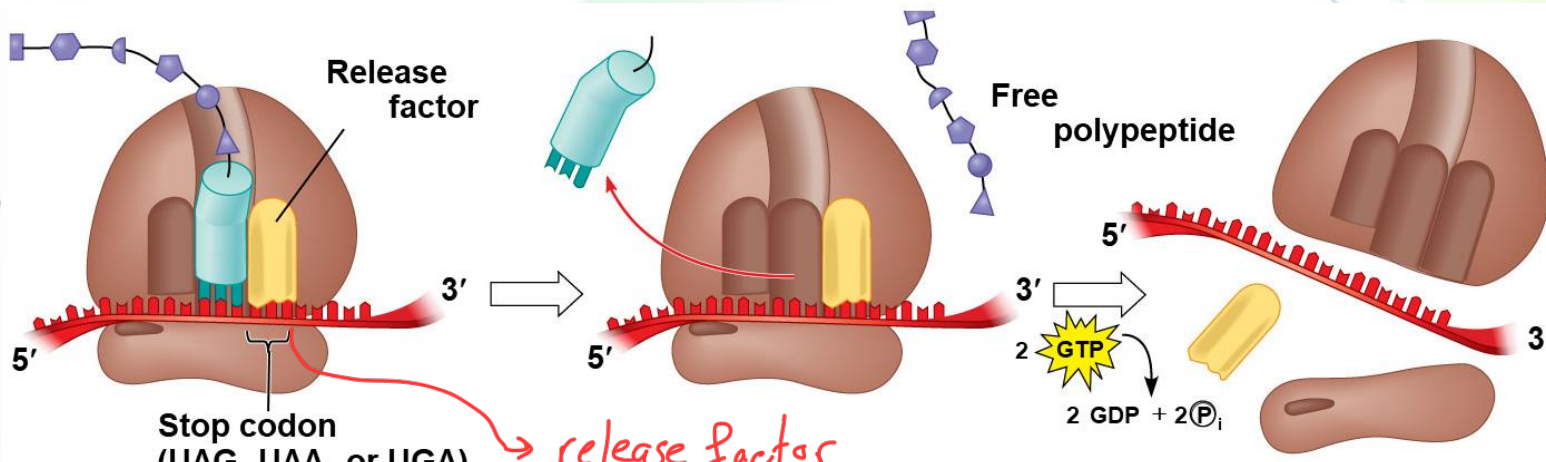
- During the elongation stage, amino acids are **added** one by one to the preceding amino (N)-terminus to the **carboxy (C)-terminus** of the growing chain.



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



© 2011 Pearson Education, Inc.

release factor put themselves in A site

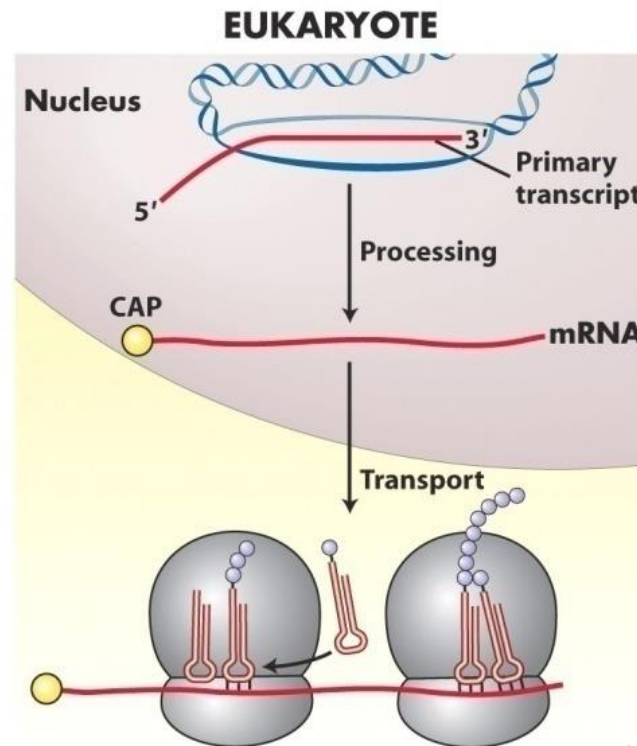
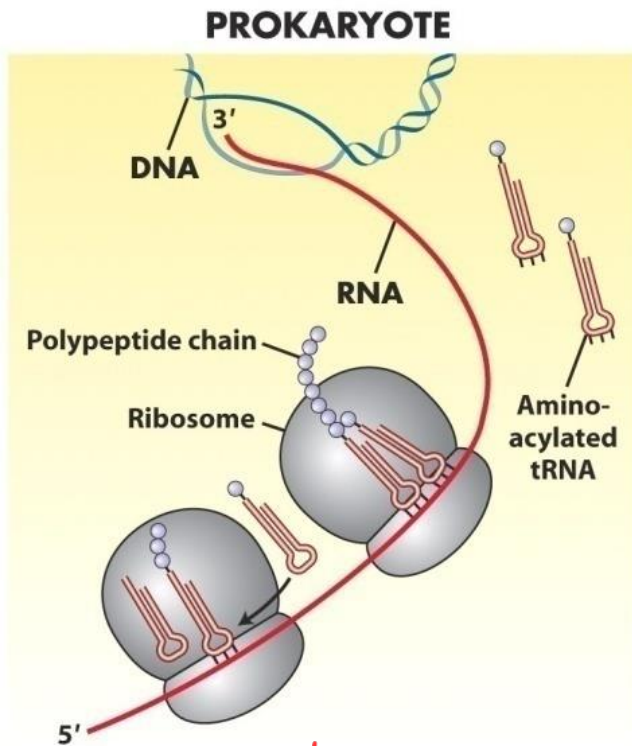
you should know the differences of termination of transcription and translation

Transcription/translation Coupling



- Transcription and translation are coupled in space and time in prokaryotes.

Same

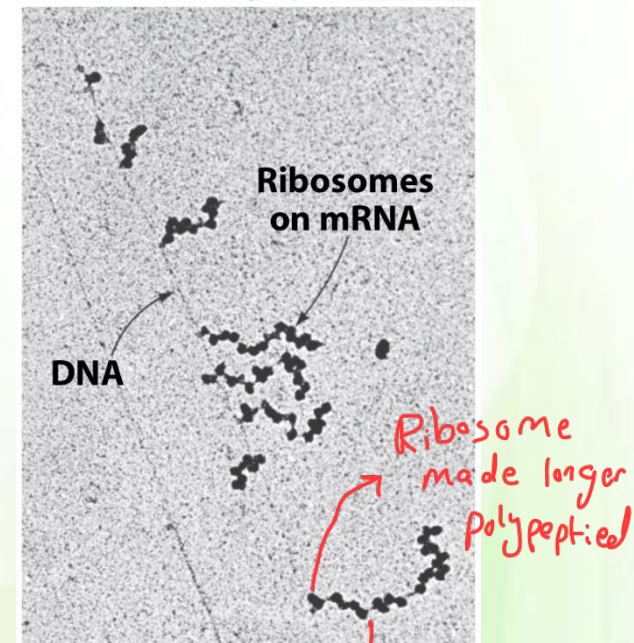
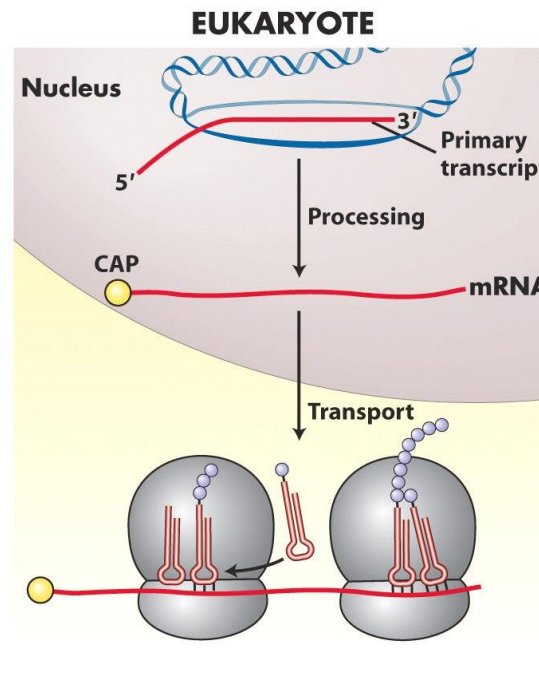
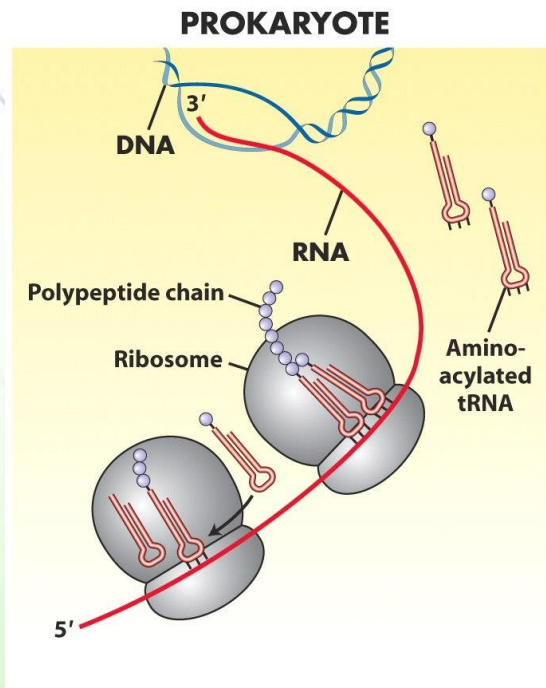


but in eukaryote we have nuclear envelop and mRNA have to be processed

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.

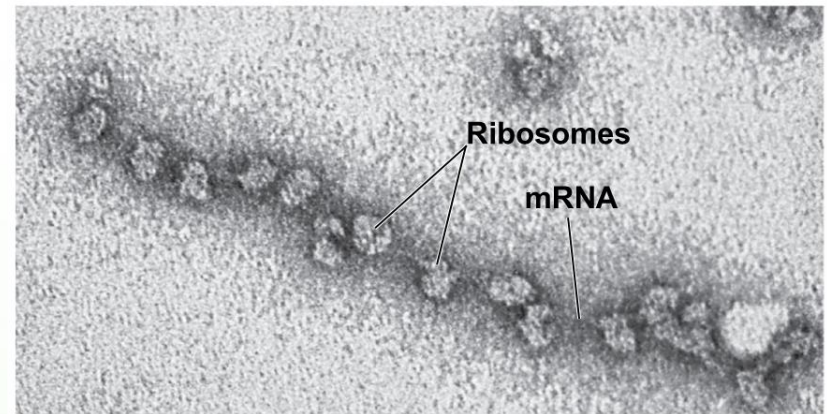
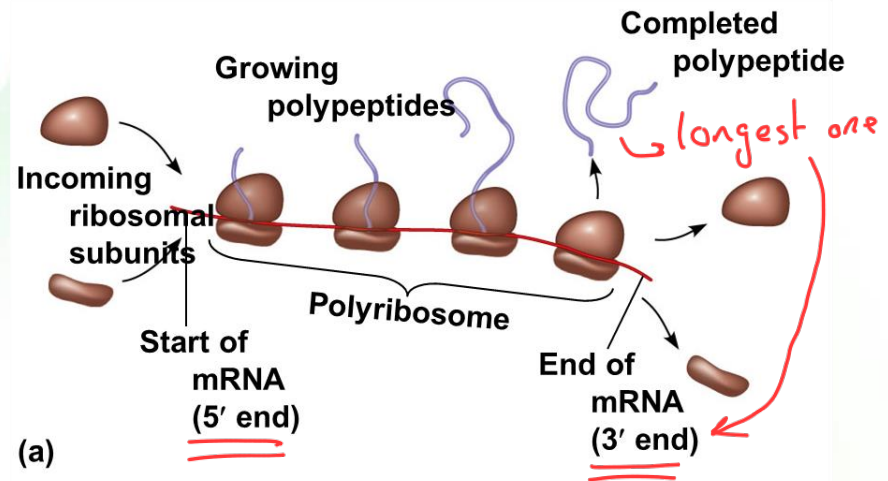


40 This is longest mRNA

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.



(b)

0.1 μm



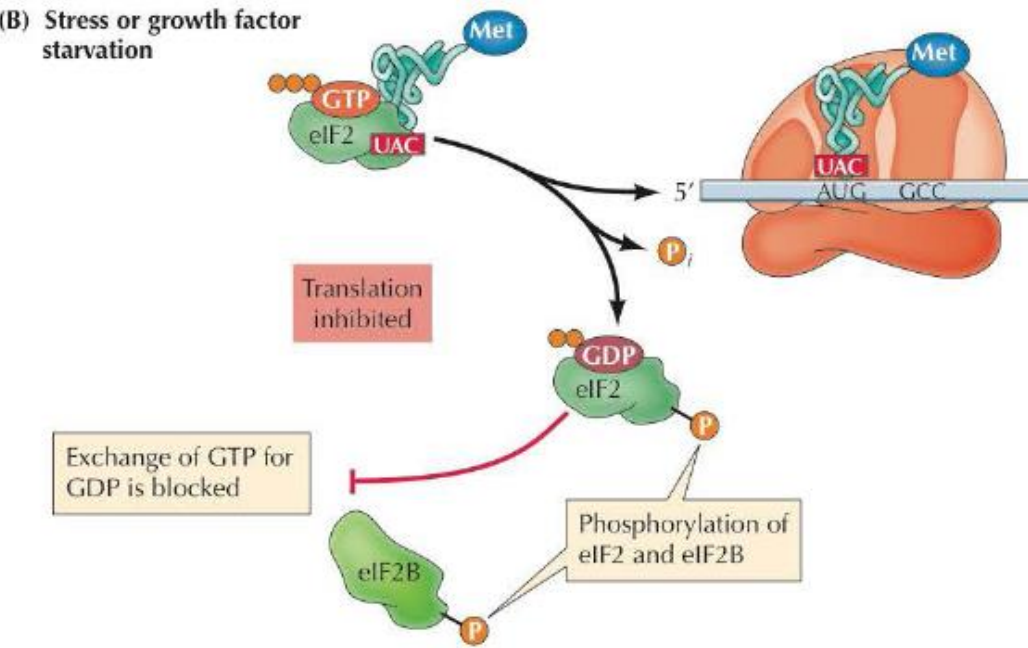
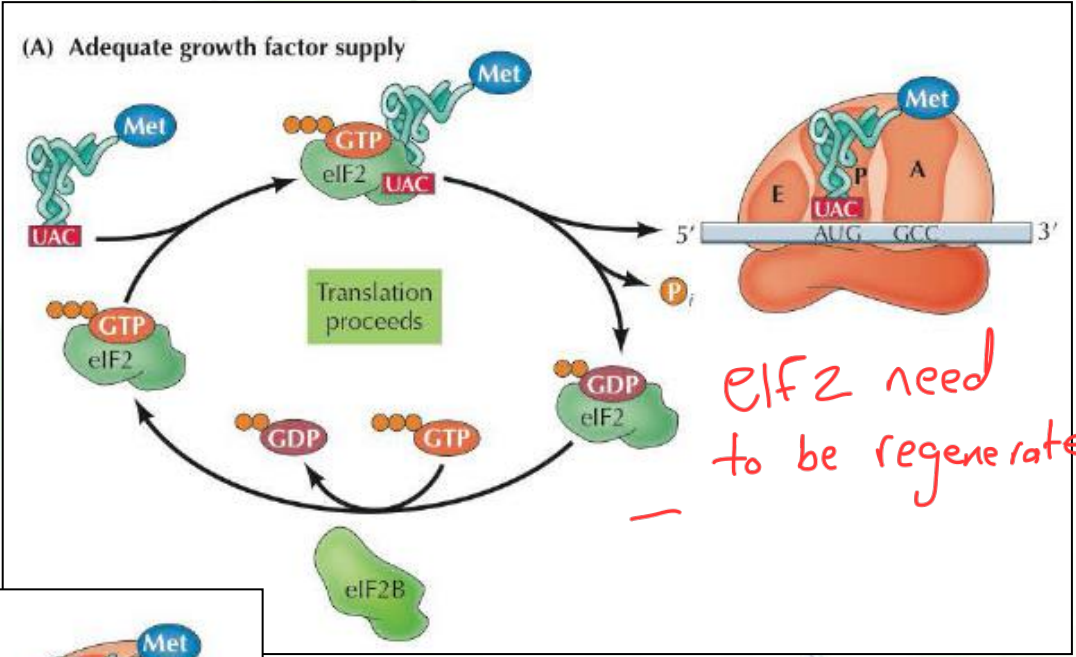
Regulation of translation

Global regulation

so I regulate all translation process



- In order to continue translation, eIF2 must be reactivated by eIF2B, which stimulates the GTP/GDP exchange.
- Translation is inhibited by **regulatory protein kinases** that **phosphorylate either eIF2 or eIF2B**, which cannot undergo GTP/GDP exchange when phosphorylated.



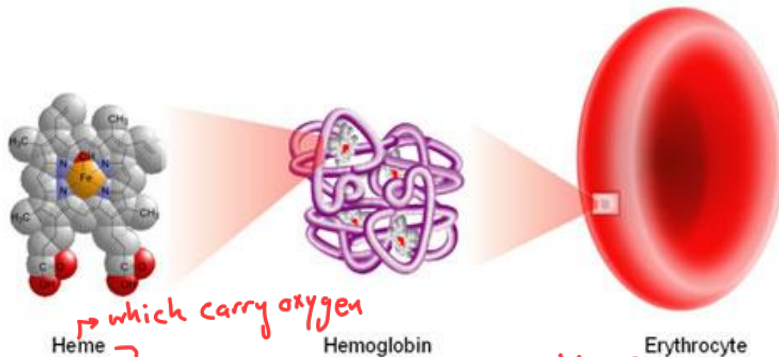
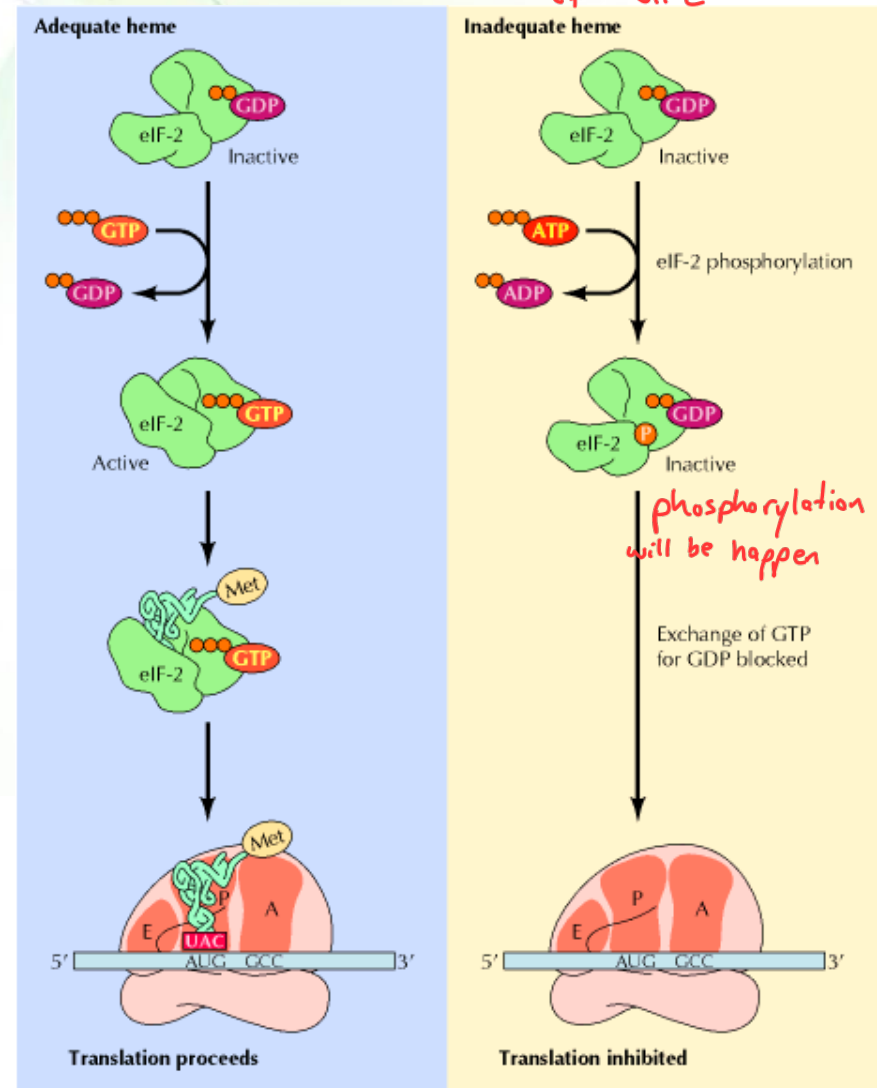
Heme and protein synthesis



to make big amount of hemoglobin

- In reticulocytes (immature erythrocytes), heme stimulates protein synthesis. *because if we don't have heme we can not use globin*
- The mRNA is translated only if adequate heme is available to form functional hemoglobin molecules.
- If adequate heme is available, GDP-GTP exchange occurs and translation can proceed.
- If heme supplies are inadequate, a protein kinase phosphorylates eIF-2.

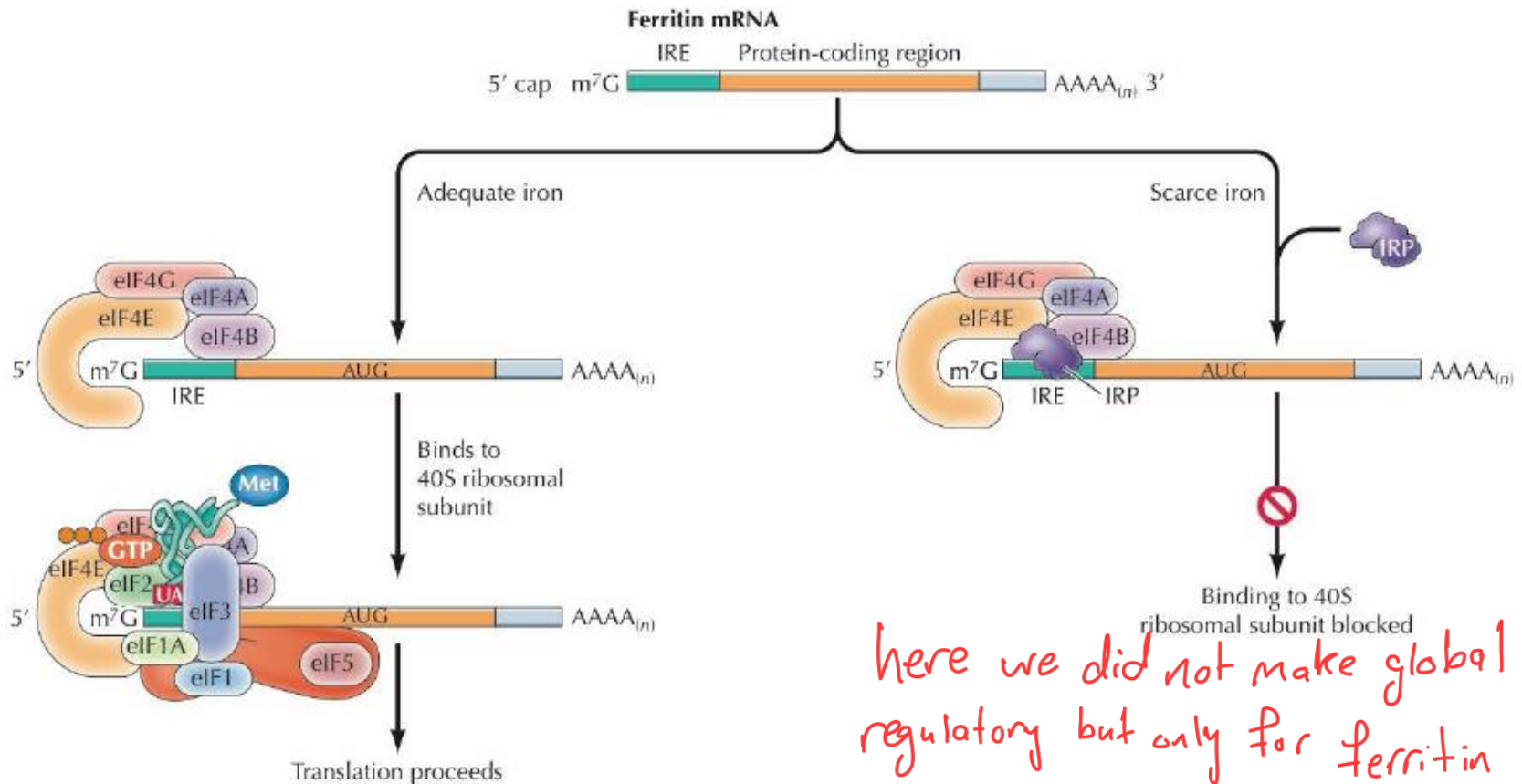
High amount of heme → high regeneration of eIF2



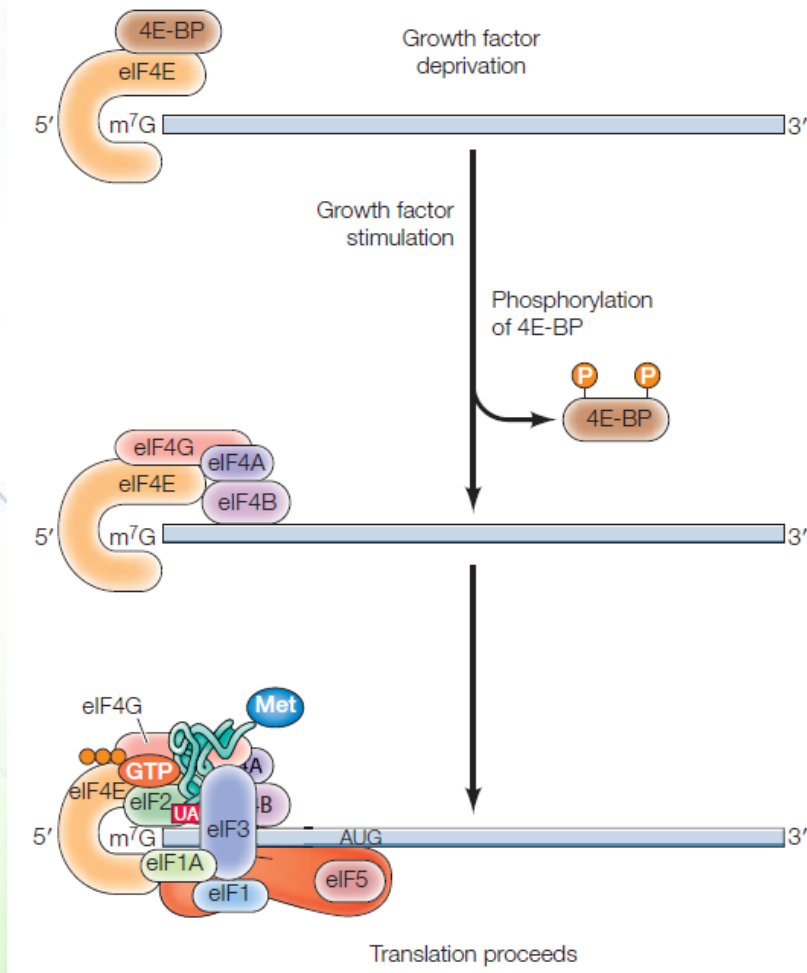
which carry oxygen

responsible for transporting oxygen from lung to peripheral parts

Also, remember...ferritin



Regulation of eIF4E



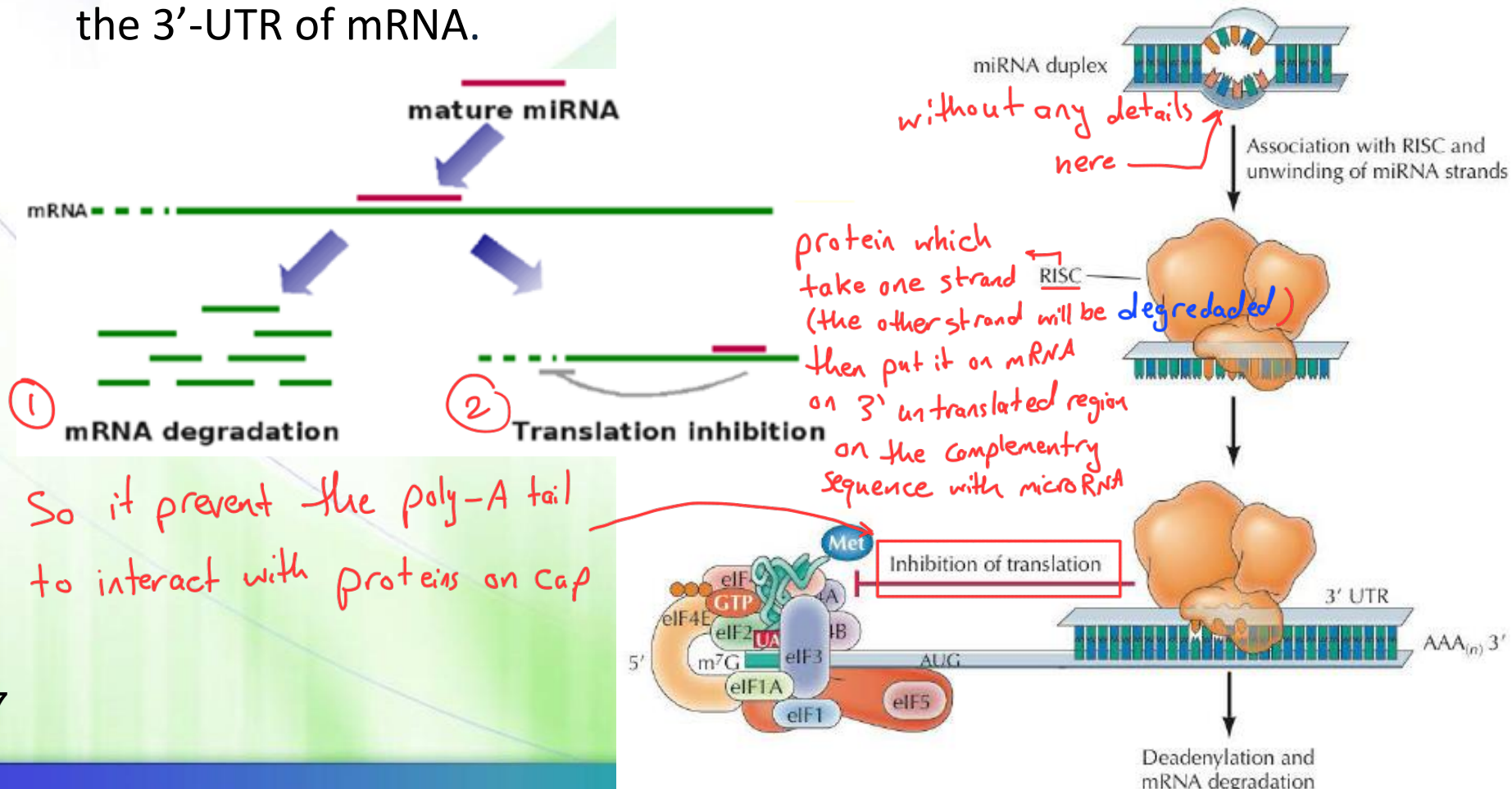
- In the absence of growth factors, translation is inhibited by eIF4E binding proteins (4E-BPs), which bind to eIF4E and **block its interaction with eIF4G**. *4e Binding protein*
- Growth factor stimulation leads to the **phosphorylation of 4E-BPs**, which **then dissociate from eIF4E**, allowing translation to proceed.

Regulation by microRNA (miRNA)



↳ short RNA molecule

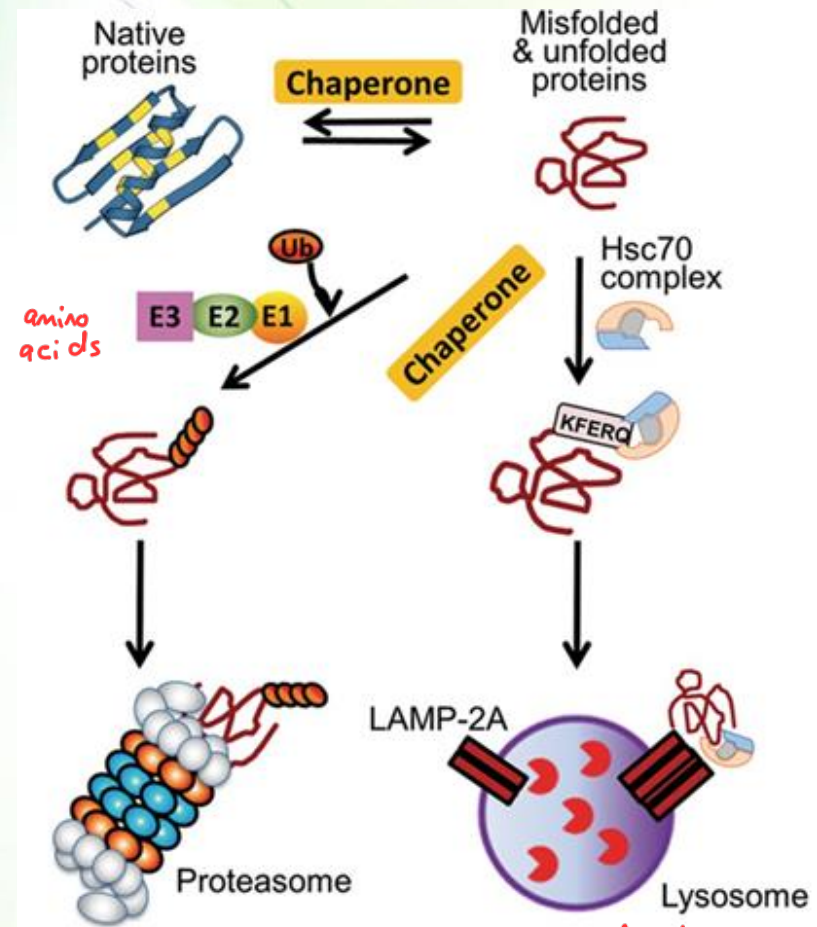
- MicroRNA is synthesized by RNA polymerase II into single-stranded, primary miRNA (pri-miRNA) transcript.
- It gets processed into double-stranded molecules but only one strand is loaded onto a RISC complex where miRNA is targeted to the 3'-UTR of mRNA.



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



- Proteins are degraded either in degradative subcellular organelles like lysosomes or by the macromolecular proteasomes. → turning down protein to amino acids
- Proteins are targeted for destruction in either a proteasome by ubiquitylation (also known as ubiquitination or ubiquitylation), which involves labeling by small polypeptides known as ubiquitin, or inside lysosomes.



So ubiquitens label the protein → proteasome degraded it

Levels of regulation

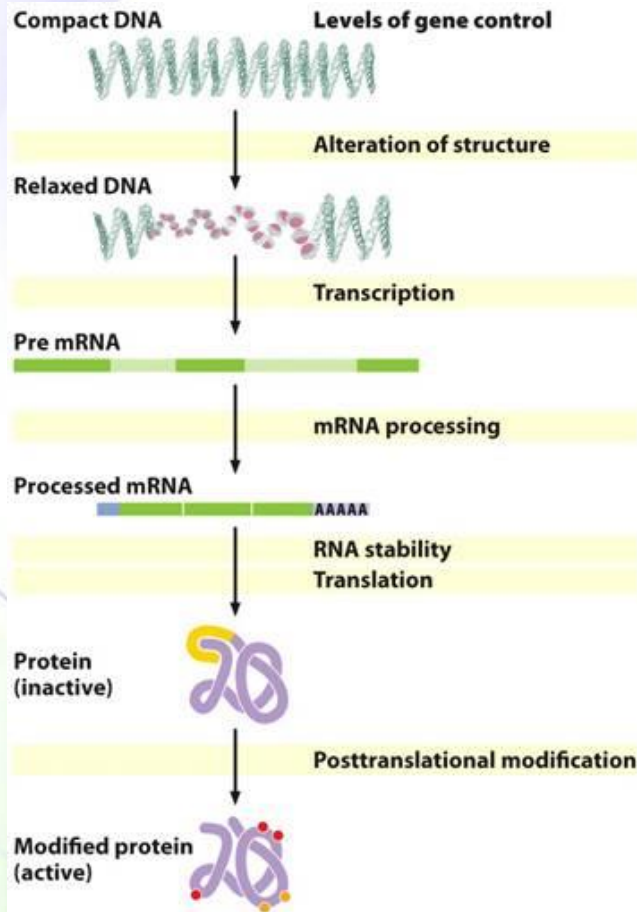


Figure 16.1
Genetics: A Conceptual Approach, Fifth Edition
© 2014 W. H. Freeman and Company

- Transcription *which change DNA structure or chemically by methylation...*
- RNA processing *Capping, poly-A tail, splicing ...*
- RNA transport
- mRNA stability *transferrin receptor mRNA (iron regulatory protein)*
- Translation
- Post-translational modification *splicing and microRNA*
- Protein activity *in Biochemistry*
- Protein degradation