MOLECULAR BIOLOGY

بسم الله الرحمن الرحيم

FINAL – Lecture 16

Translation

﴿ وَإِن تَتَوَلَّوْا يَسْتَبْدِلْ قَوْمًا غَيْرَكُمْ ثُمَّ لَا يَكُونُوا أَمْثَالَكُم ﴾

اللهم استعملنا ولا تستبدلنا

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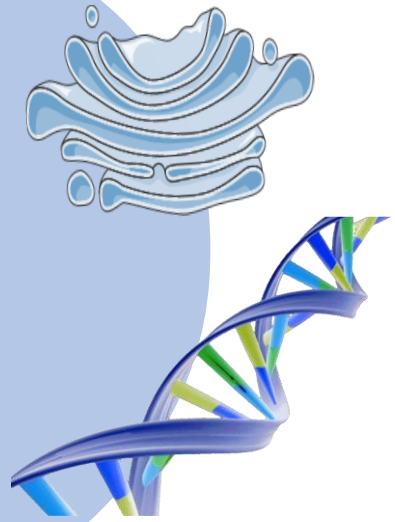
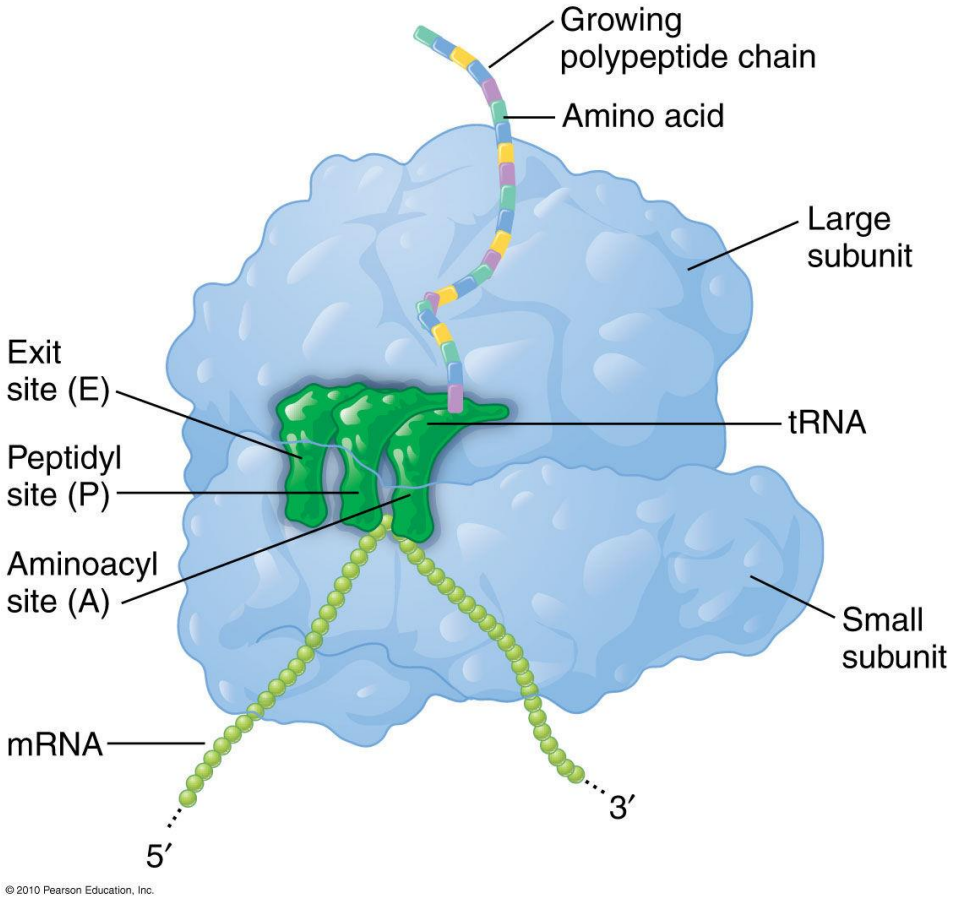


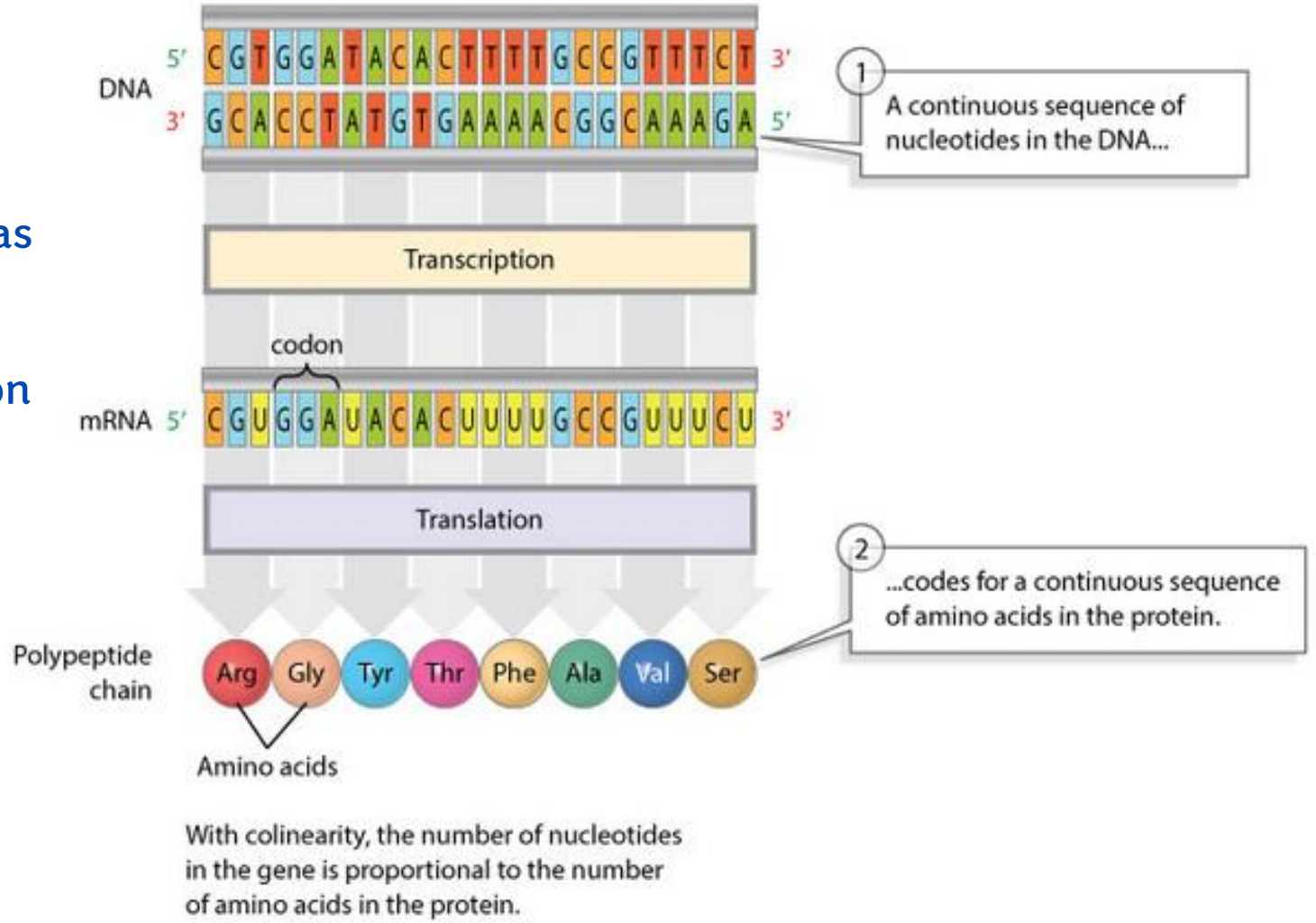
General information



- **Translation: The process of making proteins from mRNA.**
- Protein synthesis involves interactions between ribosomes with three types of RNA molecules:
  - tRNAs **(which carry the Amino acids)**
  - rRNAs,
  - mRNA templates

Colinearity of genes, mRNAs, & proteins

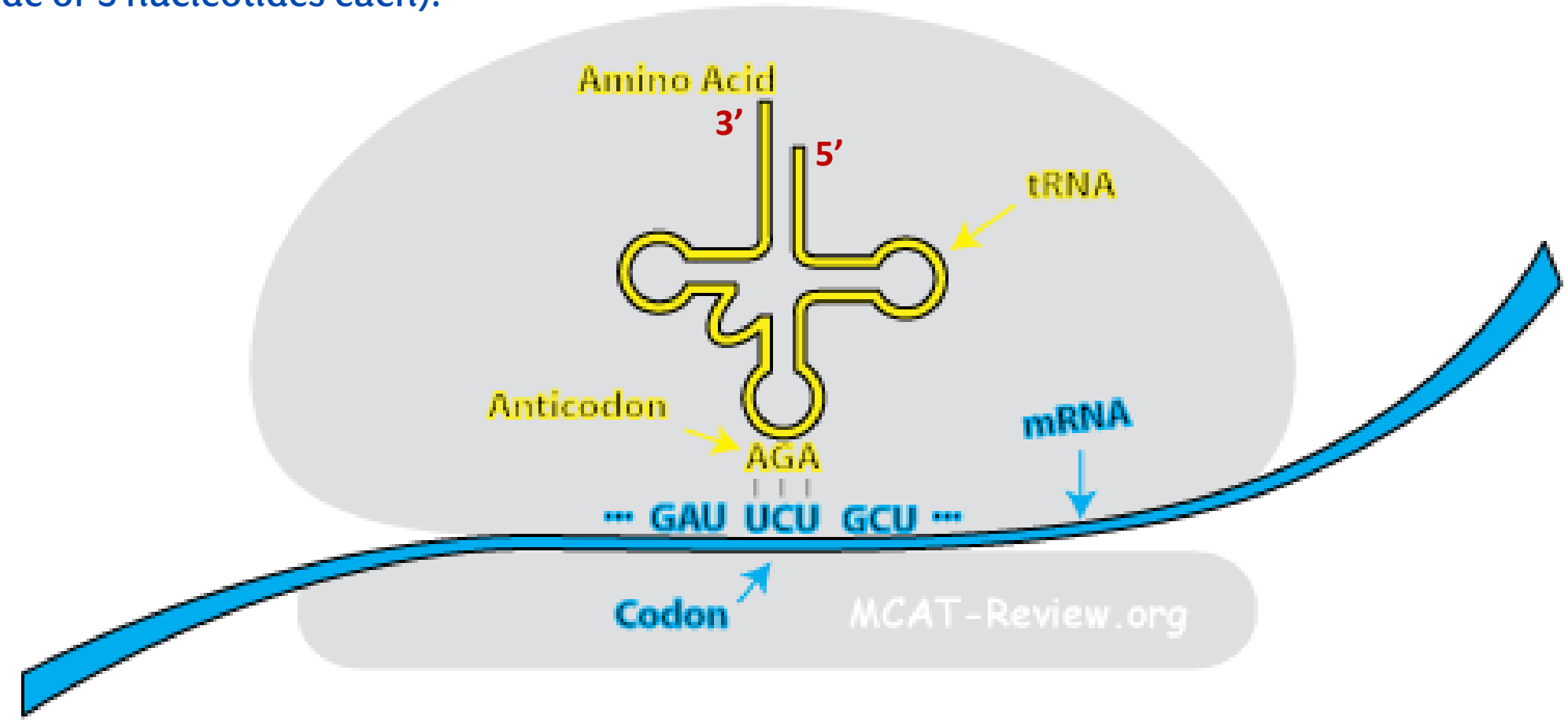
- Co-linearity: In linearity (they are parallel and accompany each other).
- mRNA has the same nucleotides sequence as the DNA , protein is co-linear with mRNA.
- So, if we have a mutation in a specific region in the DNA, it will reflect on the mRNA and the protein in the same specific region.



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

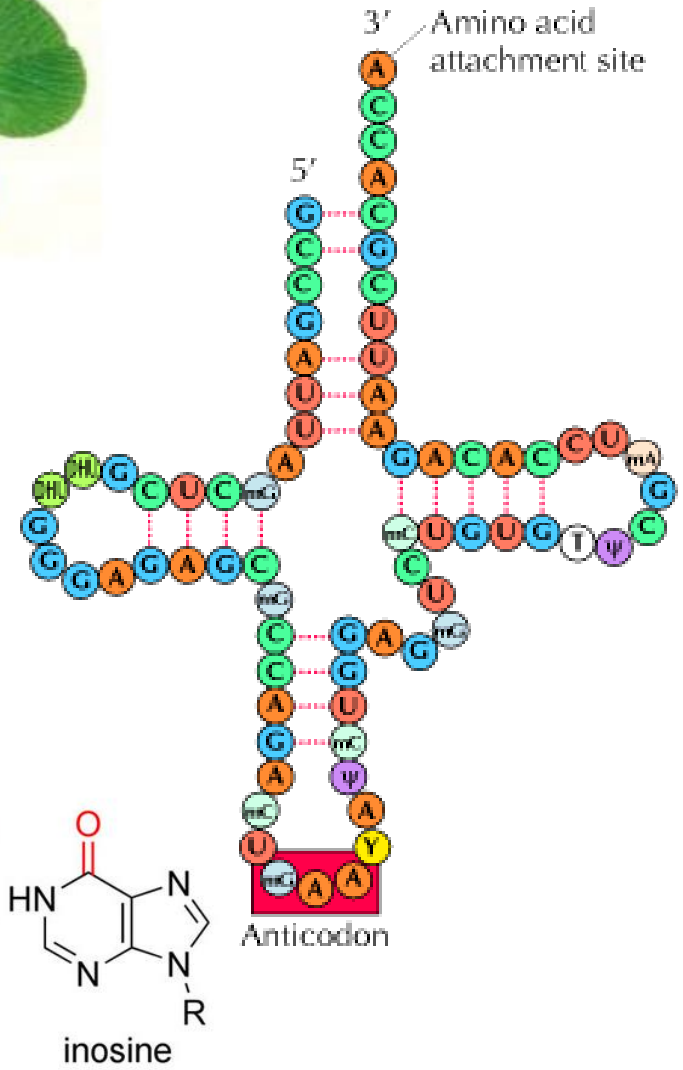
mRNA is read by tRNA in triplets

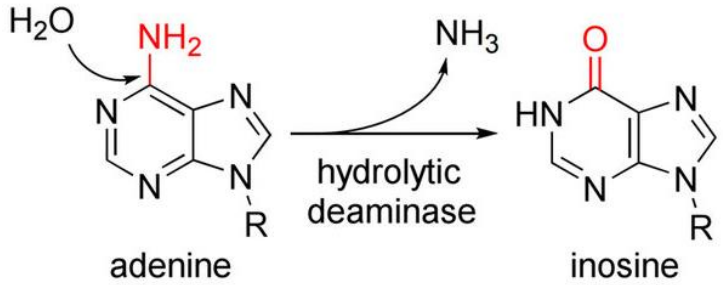
- tRNA reads the mRNA as triplets (codons are made of 3 nucleotides each).



tRNA structure

- tRNAs are short single-stranded RNA molecules (80 bases long).
- “Charged” or “activated” tRNA carries one amino acid at the 3’-end.
- **Amino Acid carried by the tRNA is determined by :**
  1. **Triplet of nucleotides (anticodon), which is complementary to the codon of mRNA.**
  2. **Internal sequence (special code inside the tRNA).**
- tRNAs contain stem loop structures, modified bases, and unusual bases (example: inosine).
- **In addition to the 4 nitrogenous bases we know, tRNA has additional modified base, for example (inosine).**





Genetic Codon Chart

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

Translation START codon

Translation STOP codon

Positively charged amino acids

Negatively charged amino acids

Hydrophobic amnio acids

Hydrophilic non-charged amino acids

Cysteine

- tRNA reads mRNA as triplets (codons).
- We have 64 probability of different triplets (codons) to encode 20 amino acids.
- These codons are <u>at the mRNA</u> not the tRNA.
- Amino acids cluster in groups (hydrophobic amino acids, hydrophilic amino acids, etc...), so its not random, which reduces the impact of mutations.

➢ AUG codes for methionine and it's usually the first codon in the translation.

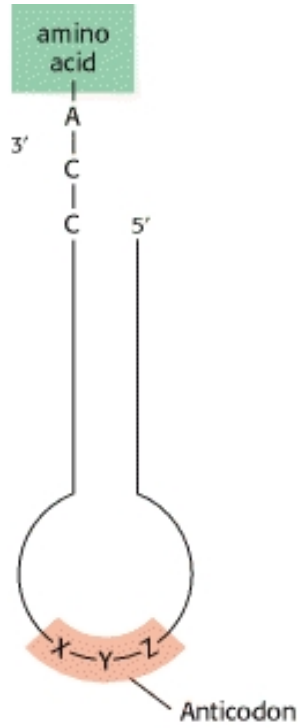
➢ UAA / UAG / UGA => stop codons.

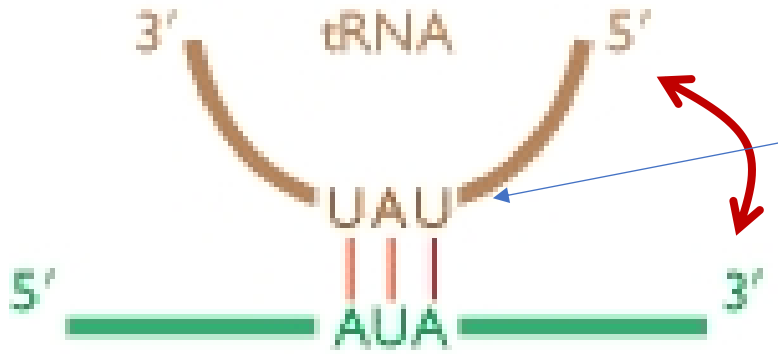
Features of the genetic codon

- 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.
- Protien synthesis begins at the amino terminus and extends toward the carboxyl terminus.
- 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) is typical of the universal code except for a few variants.
- mtDNA follow the same chart but there are some exceptions.

Codon vs. anticodon

- tRNAs contain a three-nucleotide sequence known as “anticodon” that pairs in an anti-parallel manner with the “codon” of mRNA molecules.





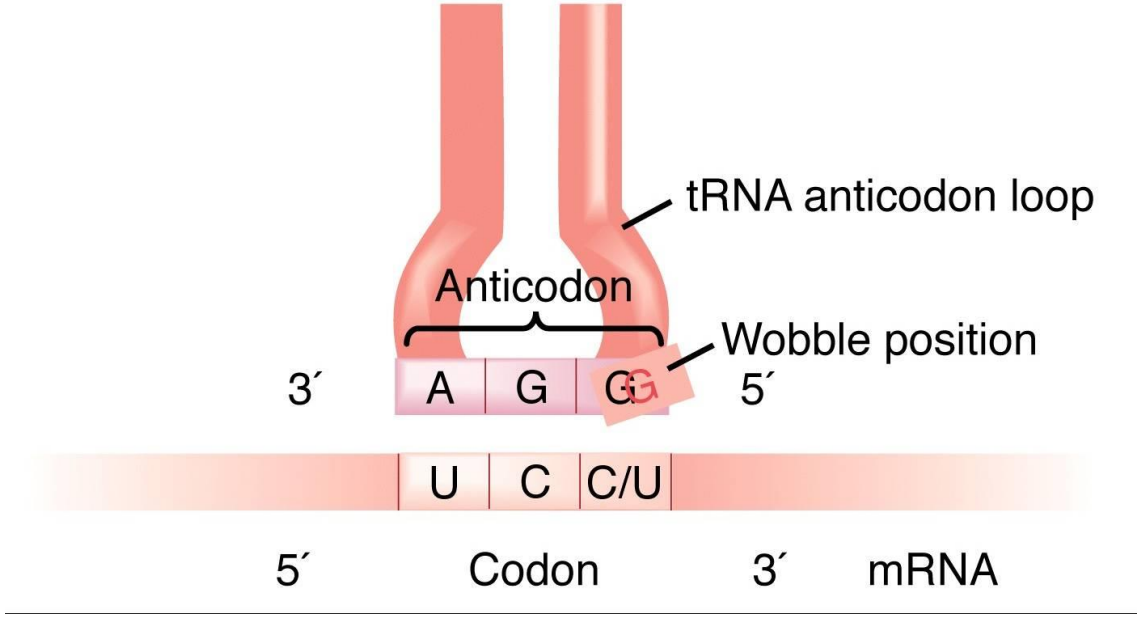
We read tRNA anticodon from here (5’ to 3’).

Isoleucine codon

Pairing between codon and the anticodon is anti-parallel.

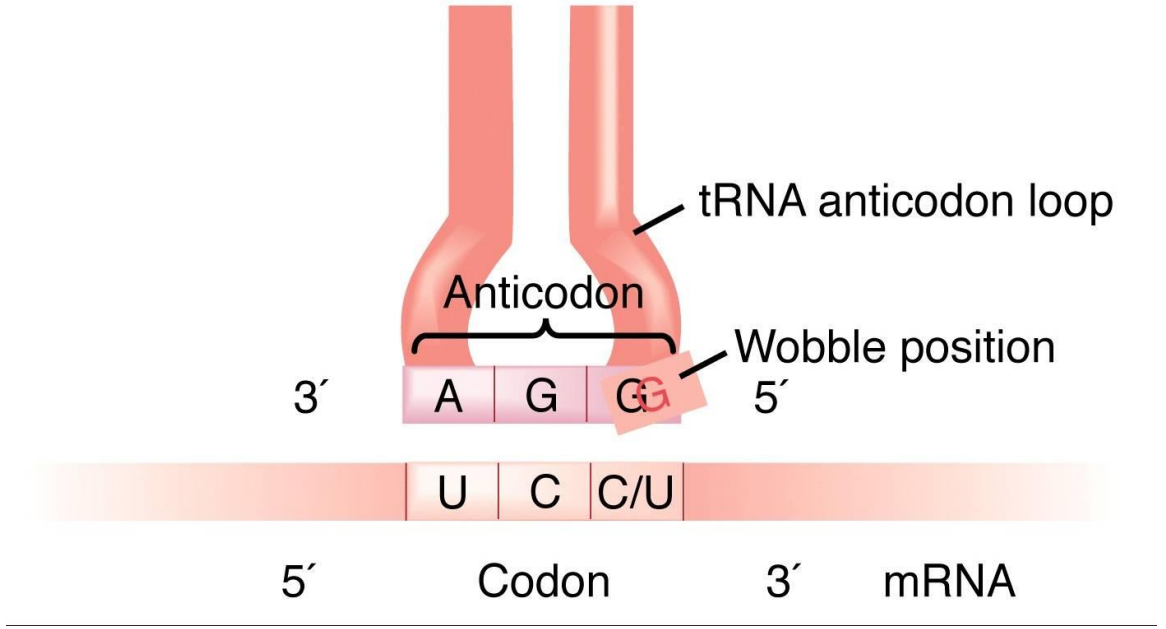
Fidelity of translation

- Accurate translation requires two steps:
  - First: accurate association of amino acid to tRNA (every amino acid attach with the right tRNA, so for every anticodon we have one amino acid that never change).
  - Second: a correct match between the tRNA 's anticodon and the mRNA's codon
  - The binding between the tRNA and the mRNA must be accurate (G binds with C / A binds with U), the third nucleotide pairing can be flexible.



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.
- Even if a mutation happened in the third nucleotide of a codon, most probably the amino acid will be the same.
  - 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 genetic codon is degenerate.
  - It acts as a buffer against deleterious mutations.



Genetic Codon Chart

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

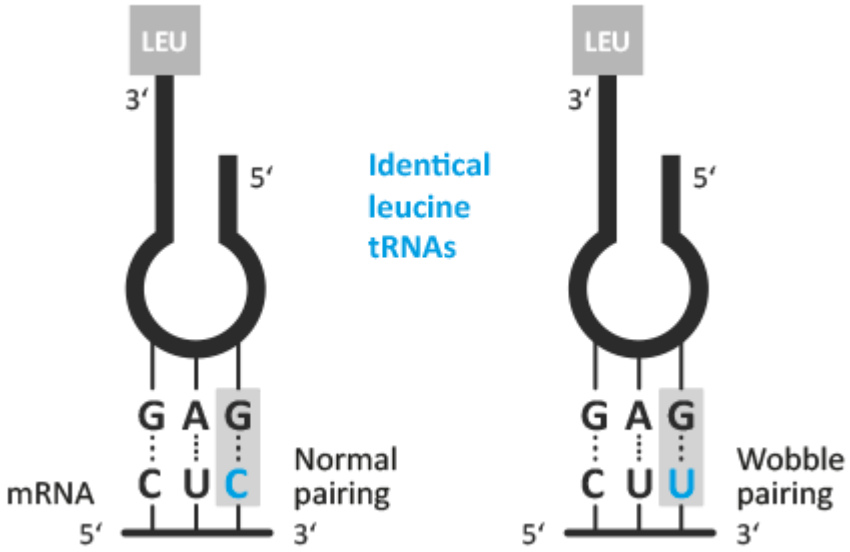
If we have a mutation in the second nucleotide, the amino acid will change but most probably the type of amino acids will be the same, so it gives some protection to the cell.

Examples of wobble base pairing

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

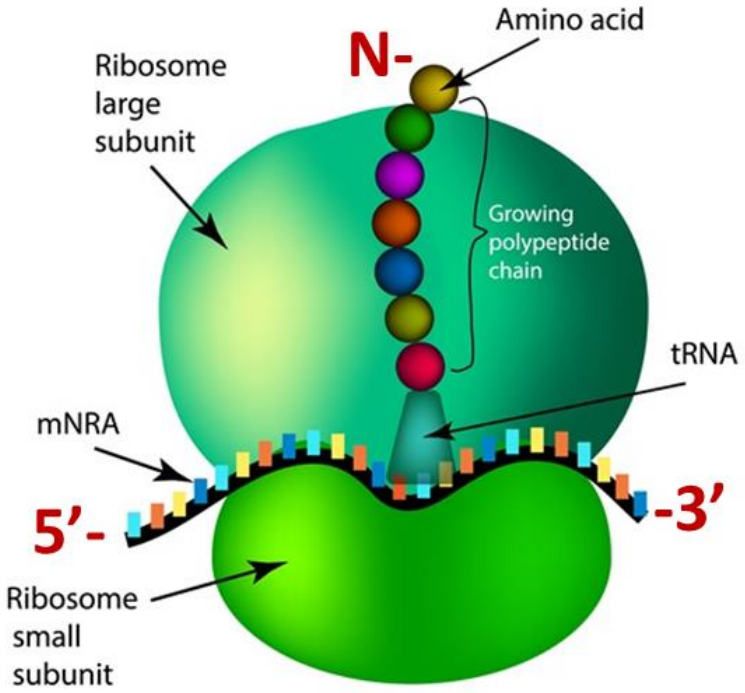
Both are hydrophobic amino acids

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



Ribosomes

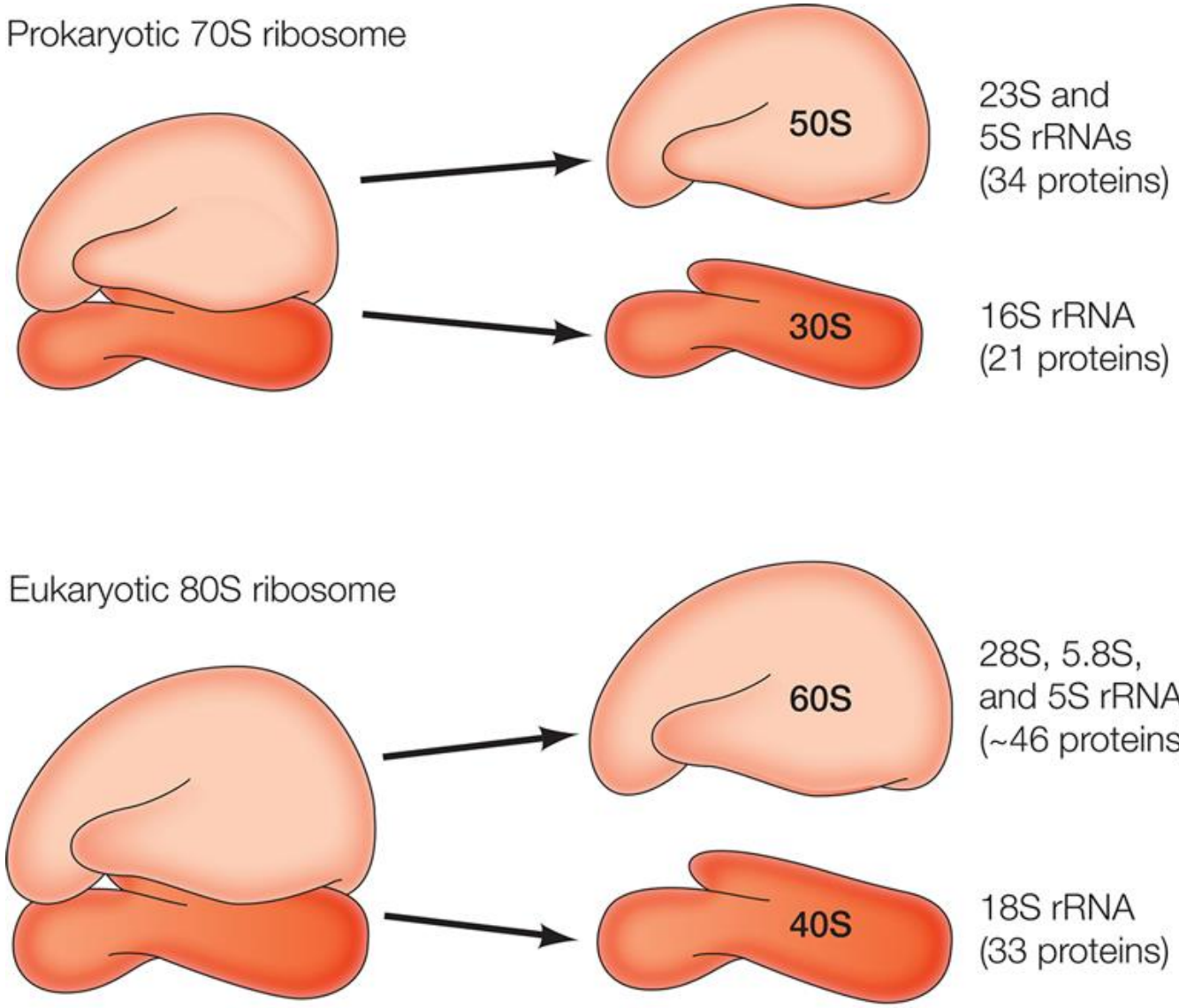
- Ribosomes are the sites of protein synthesis.
- Ribosomes are composed of proteins and rRNAs.
- E. coli contains about 20,000 ribosomes (~25% of the dry weight of the cell).
- Rapidly growing mammalian cells contain about 10 million ribosomes.



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

rRNA act as an enzyme, while protein provide structural support.

Ribosome structure

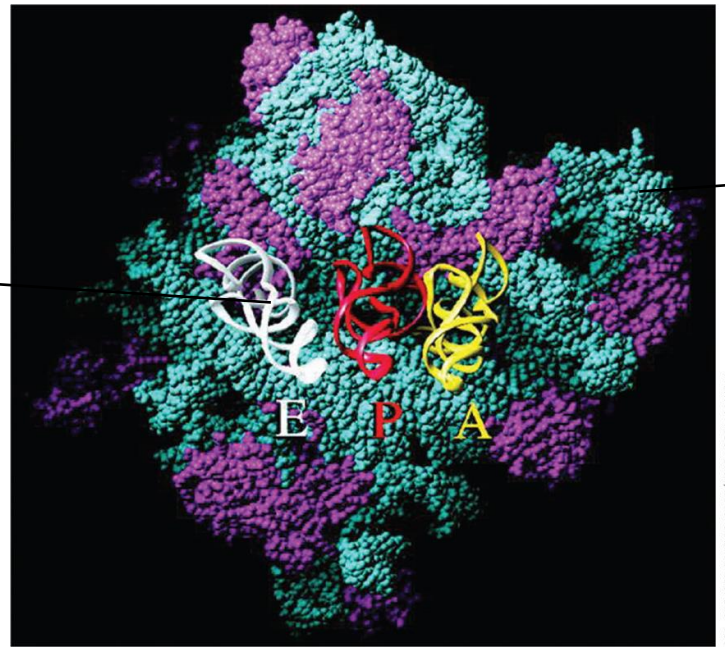


- Prokaryotes and eukaryotes share a similar ribosome structure, consisting of large and small subunits.
- The weight and the composition of both ribosomes in prokaryotes and eukaryotes are almost the same.

Functional and structural components of ribosomes

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

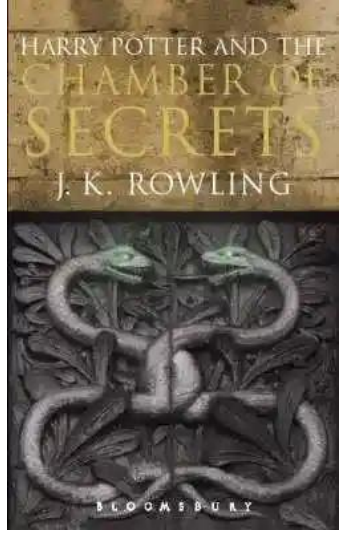
- rRNA (they are in the middle of the ribosome).
- Peptide synthesis take place around rRNA molecules.



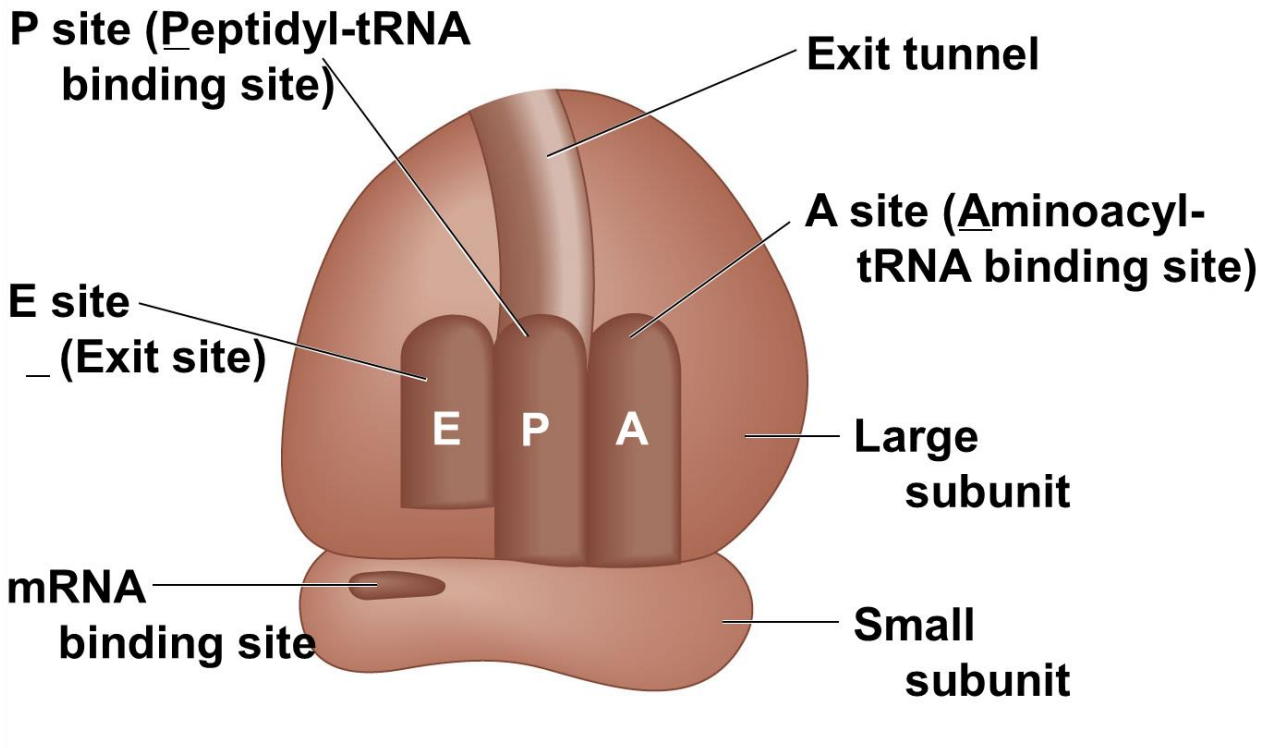
From P. Nissen et al., 2000. Science 289: 920–930

- Proteins (they provide structural support and enhance the function of the rRNA molecules).

The chambers of secret



- The large ribosomal subunit is composed of three chambers (E, P and A chamber).



tRNA binding sites on a ribosome:

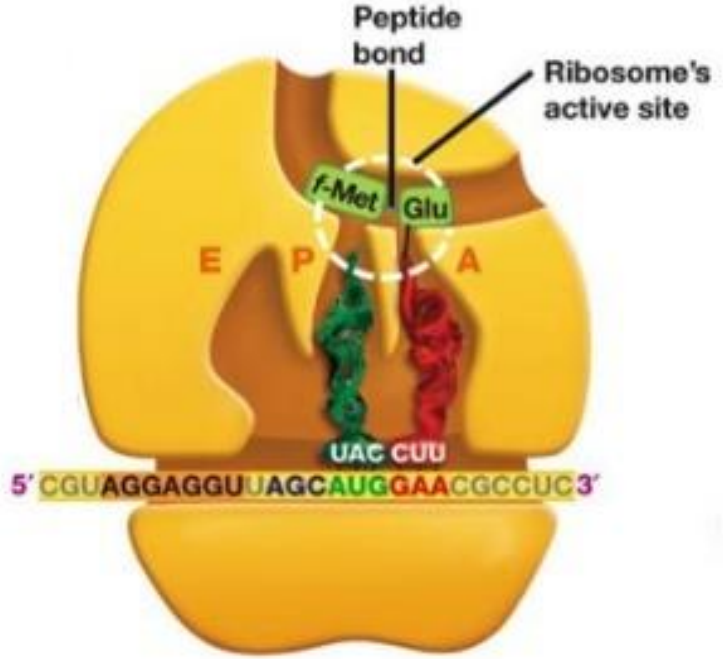
The **P site** holds the tRNA that carries the growing polypeptide chain

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 (when tRNA no longer carries an amino acid, it is referred to as discharged).

The general mechanism of translation

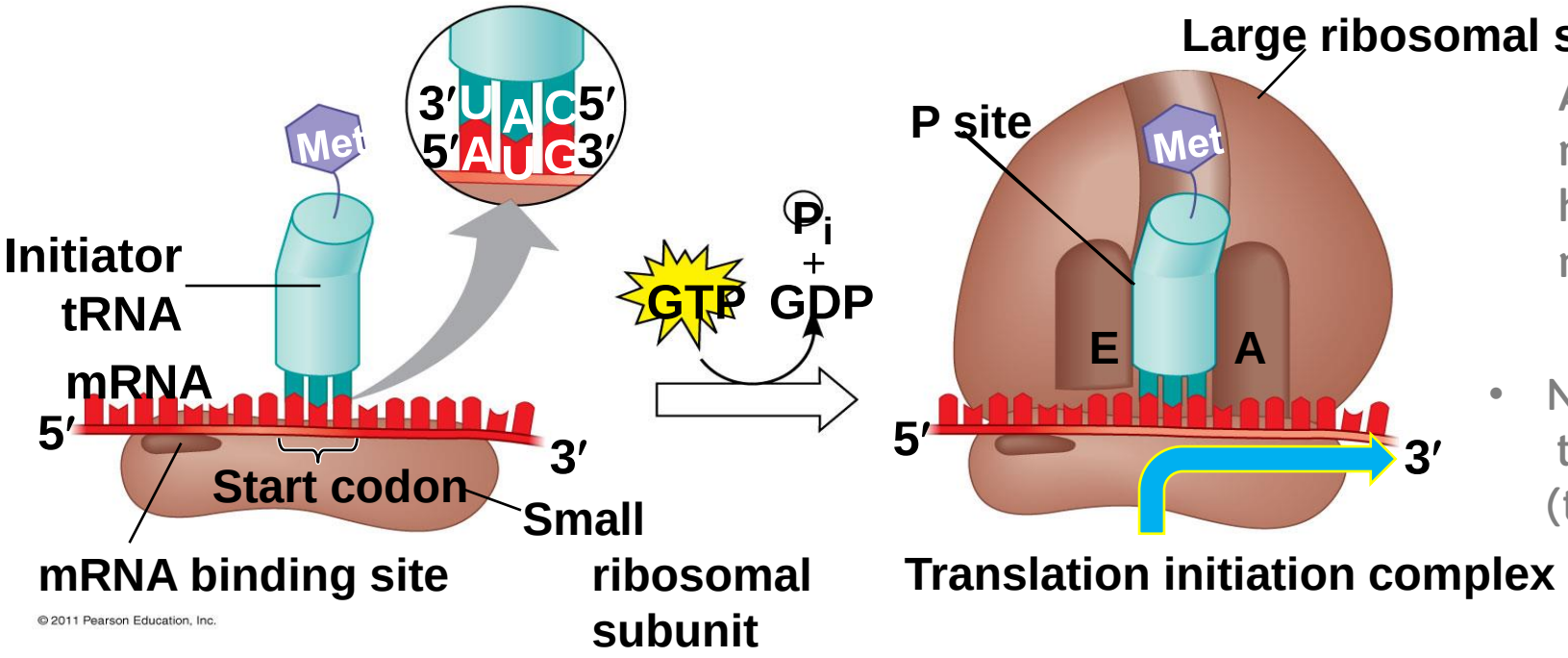
- Three stages: initiation, elongation, and termination.
- The direction is 5’ $\rightarrow$ 3’.
- Protein synthesis begins at the amino terminus and extends toward the carboxyl terminus.



Start of translation

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

- The large ribosomal unit joins after every other part of the translation initiation complex is already in place

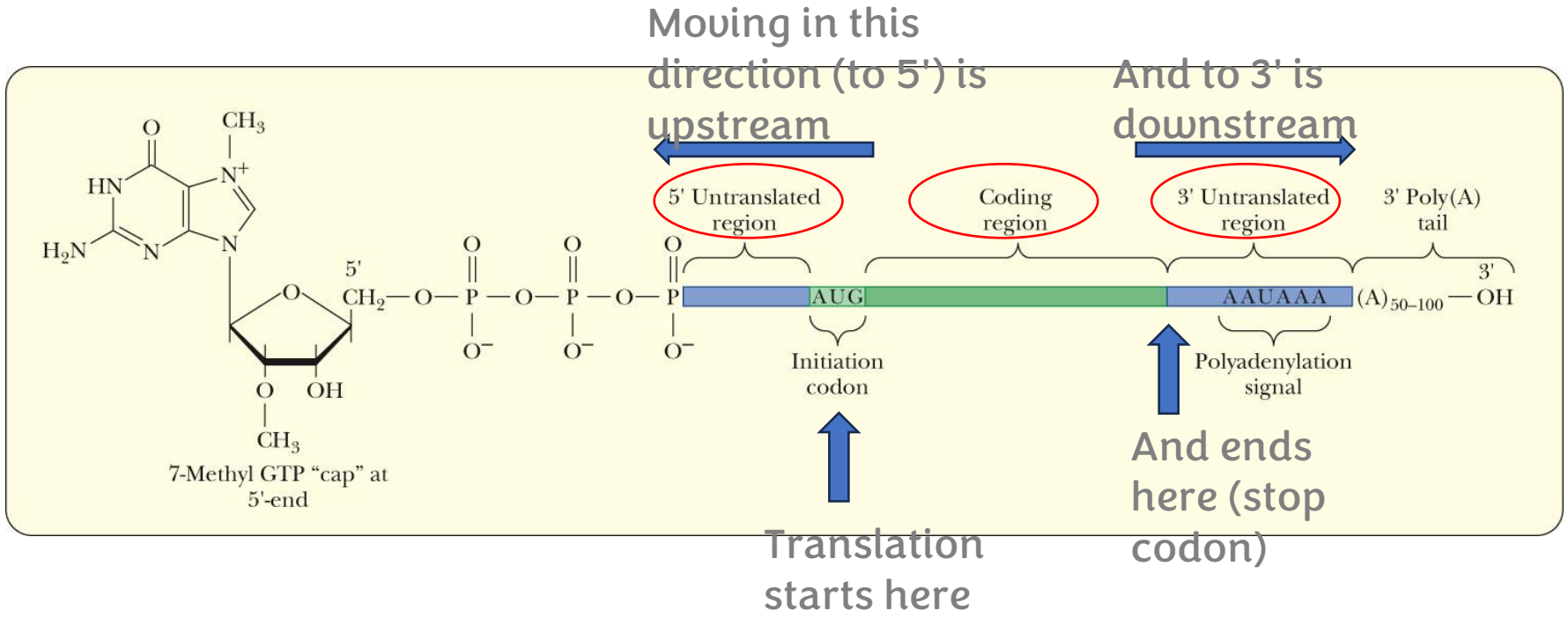


Also, for it to join it requires the hydrolysis of a GTP molecule.

- Notice where the translation begins (the blue arrow)

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.

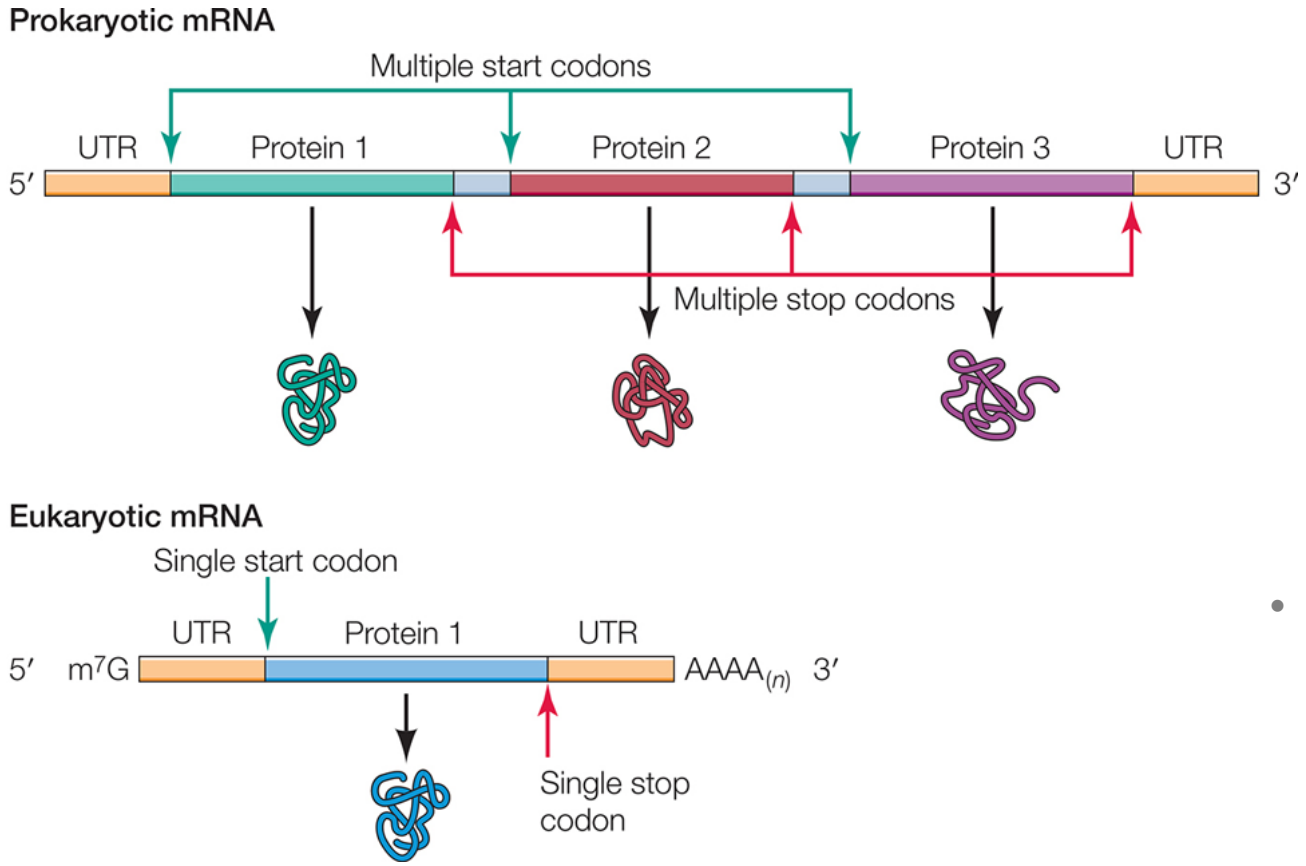


- This is the mRNA after "processing", which is done by:
  1) 5'Capping
  2) Splicing
  3) 3'Polyadenylation

Remember…

- Bacterial mRNA is polycistronic
- Eukaryotic mRNA is monocistronic

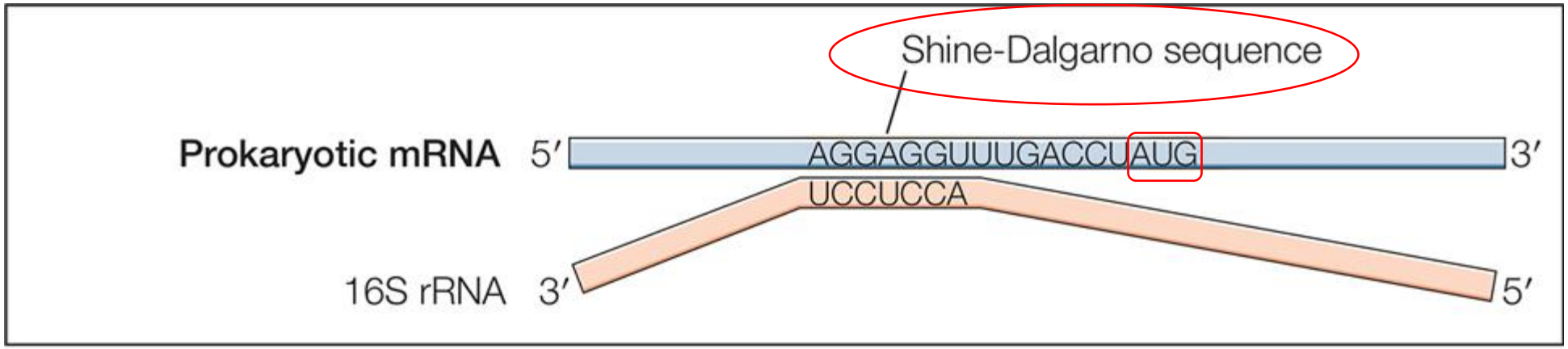
- One Bacterial mRNA can encode multiple proteins. In Eukaryotes, typically one mRNA encodes only one protein.



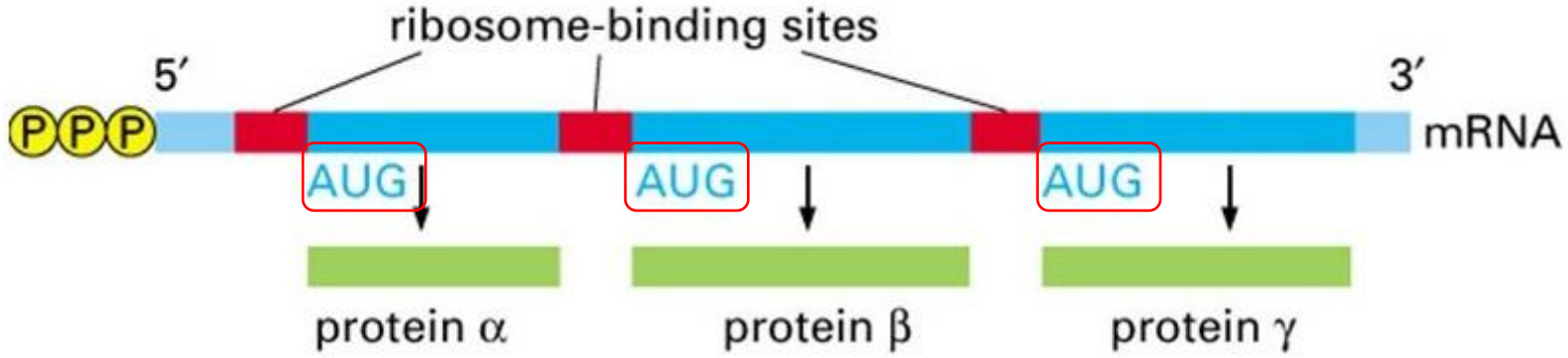
- But how can the ribosome find the start codon?? SEE UPCOMING SLIDES.

Shine-Dalgarno sequence

- In bacteria, we have Shine-Dalgarno sequence before the desired AUG start codon, SEE NEXT SLIDES.

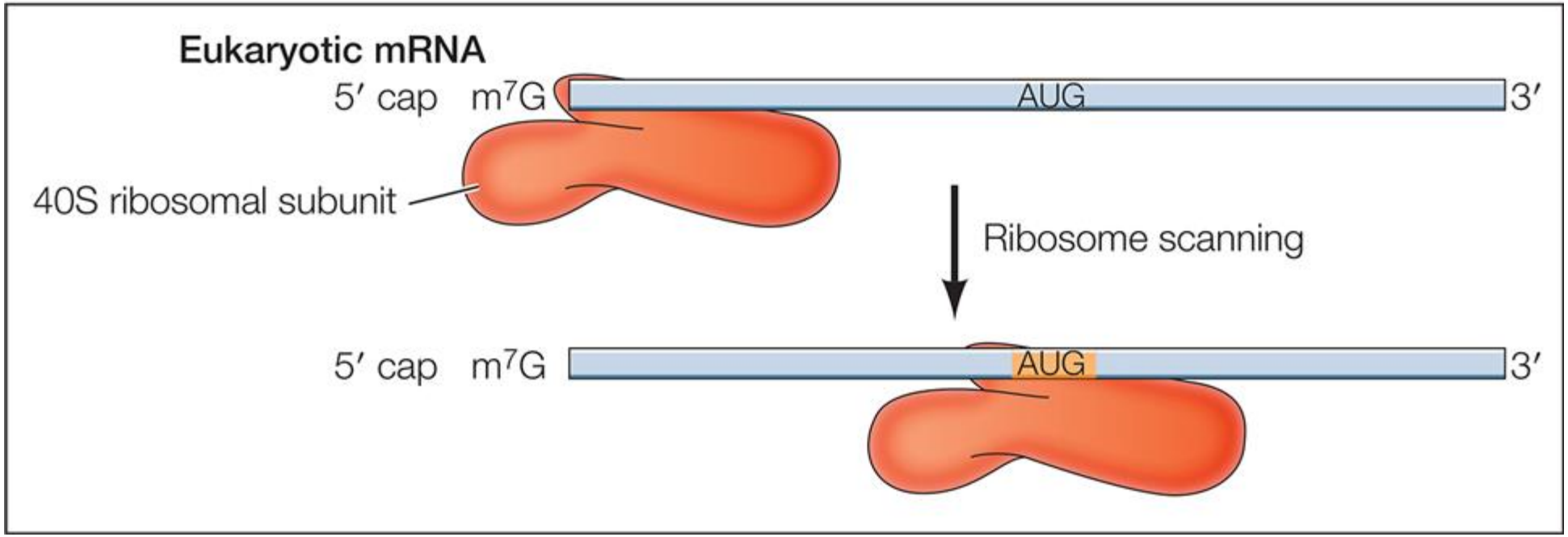


Contain (Shine-Dalgarno sequence)



But in eukaryotes…

- Eukaryotic ribosomes recognize mRNAs by binding to the 7-methylguanosine cap at their 5´ terminus. And once they reach the AUG start codon they start translating. FULL Explanation Next Slide…



Explanation of the previous slides:

We know that the translation starts with the start codon, but how does the ribosome 'find it'?

When the translation initiation complex starts working the mRNA passes through the ribosome. The ribosome is 'scanning' it in search of the start codon. In Eukaryotes, The small ribosomal unit binds to the 5' cap. Then mRNA passes through the ribosome and will reach the first AUG start codon downstream and start translating. The cap, as a result, helps the ribosome in locating the start codon easily. In bacteria, however, mRNA doesn't go through processing as in eukaryotes. Therefore, it doesn't have a 5' cap. Instead, bacteria have a specific sequence within their mRNA (can have multiple in one mRNA 'polycistronic') called the "Shine-Dalgarno Sequence".

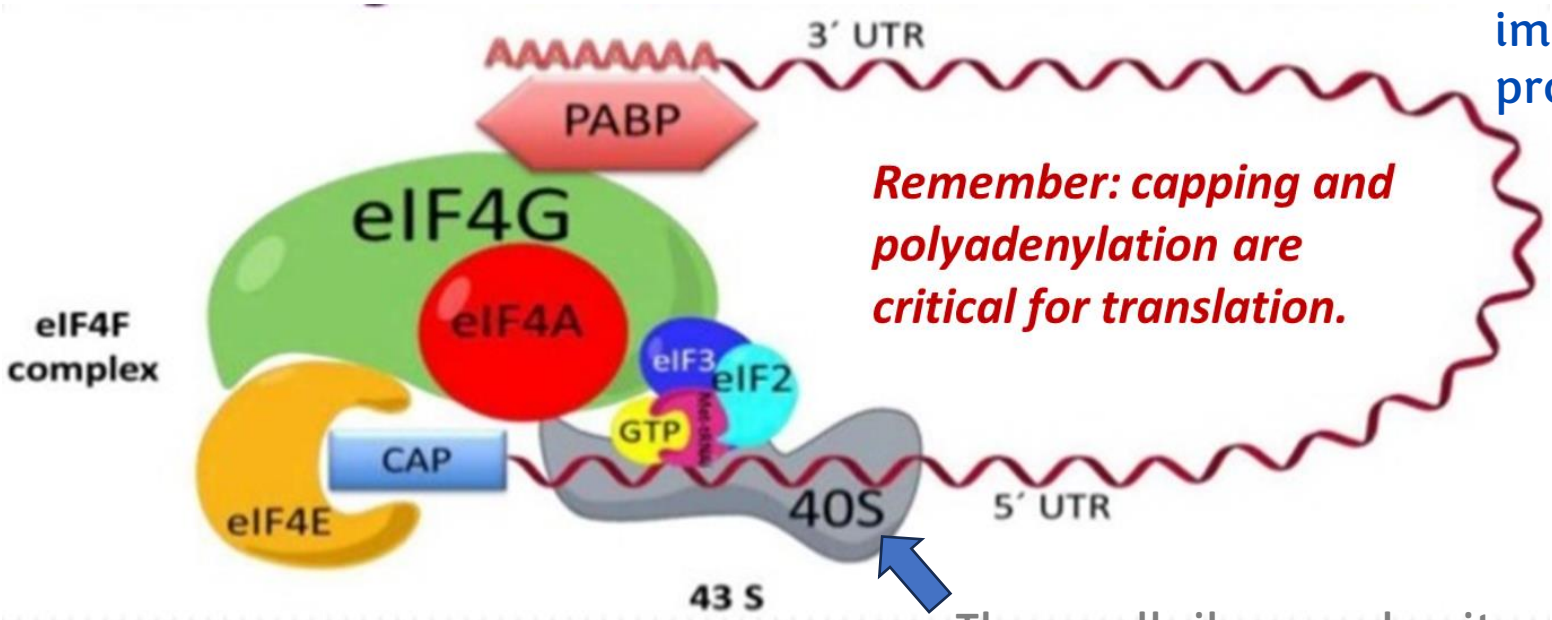
When bacterial ribosomes read the mRNA they will come upon this sequence, which precedes the start codon, and make hydrogen bonds between the bases in Shine-Dalgarno Sequence and the rRNA in the small ribosomal subunit, (see slide 19). This acts as a halt for the ribosomal reading, making it 'ready' to start translation from the start codon that follows the sequence.

Translation initiation in eukaryotes

(Eukaryotic initiation factor 4)

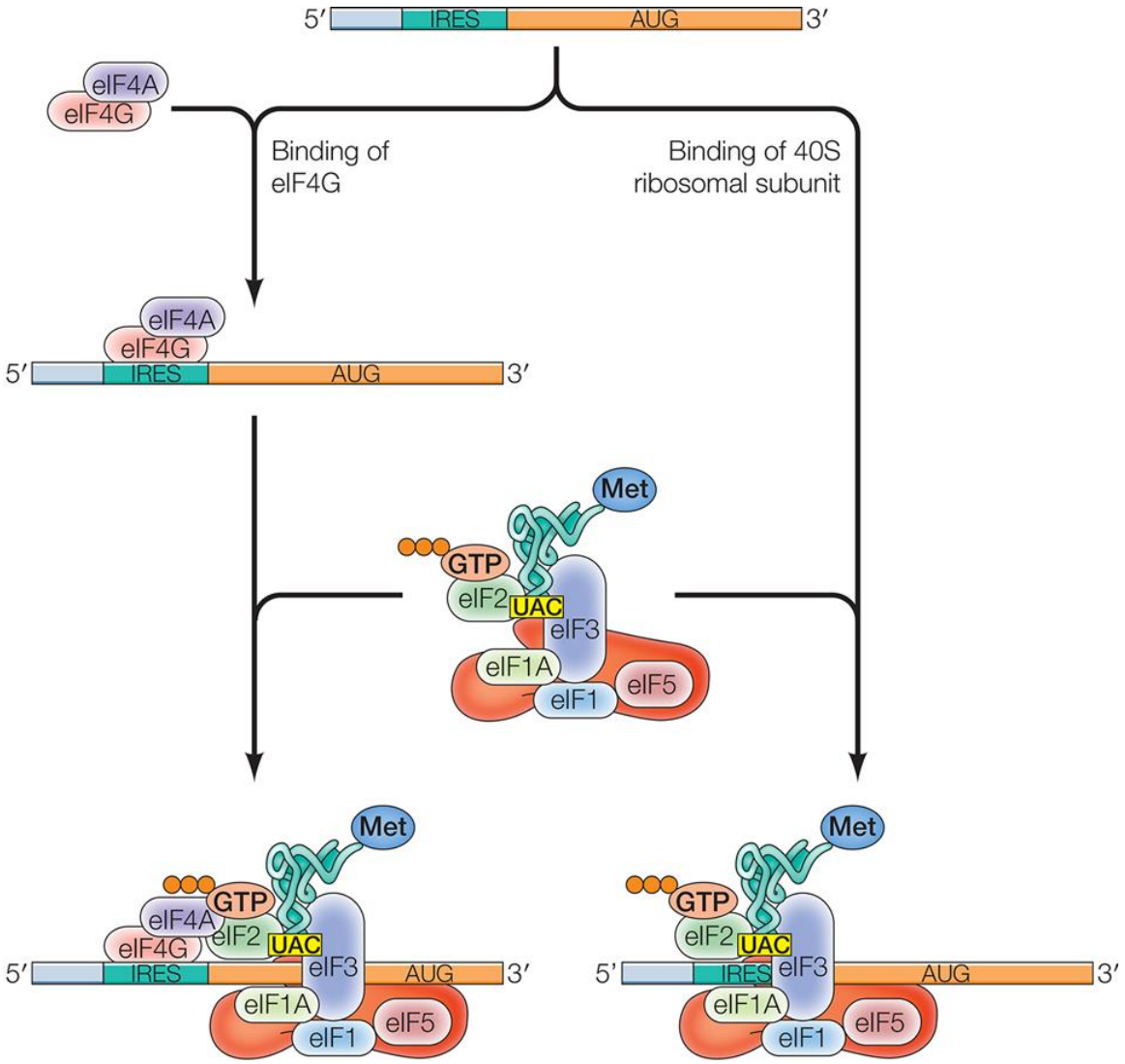
- The eIF4 initiation factors form a complex that links the poly-A tail to the CAP via poly-A binding protein (PABP).
- The eIF4 initiation factors then bring the mRNA to the small ribosomal subunit. Helping in getting the mRNA ready for translation.

Even though, the poly-A tail is not translated, it is important for the translation process.



The small ribosomal unit

Internal ribosome entry site (IRES)



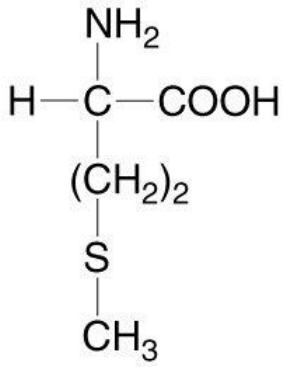
- Alternatively, internal ribosome entry site (IRES) exist in some other mRNAs and is recognized by the eIF4G protein followed by recruitment of the first tRNA and the small ribosome.

IRES helps in locating the AUG in cap-independent mRNAs (most mRNAs are cap-dependent). It is called cap-independent because it allows the ribosome to bind directly to IRES instead of to the 5' cap. **Know that it uses eIF4G. Also, the IRES is somewhat like the Shine-Dalgarno Sequence in bacteria in concept.**

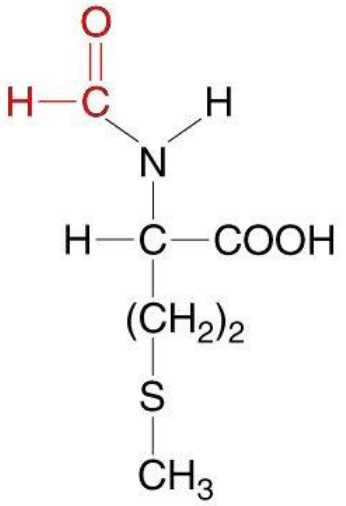
The first amino acid

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

Notice the differences between the eukaryotic and the prokaryotic systems in translation up until now



Methionine



N-Formylmethionine

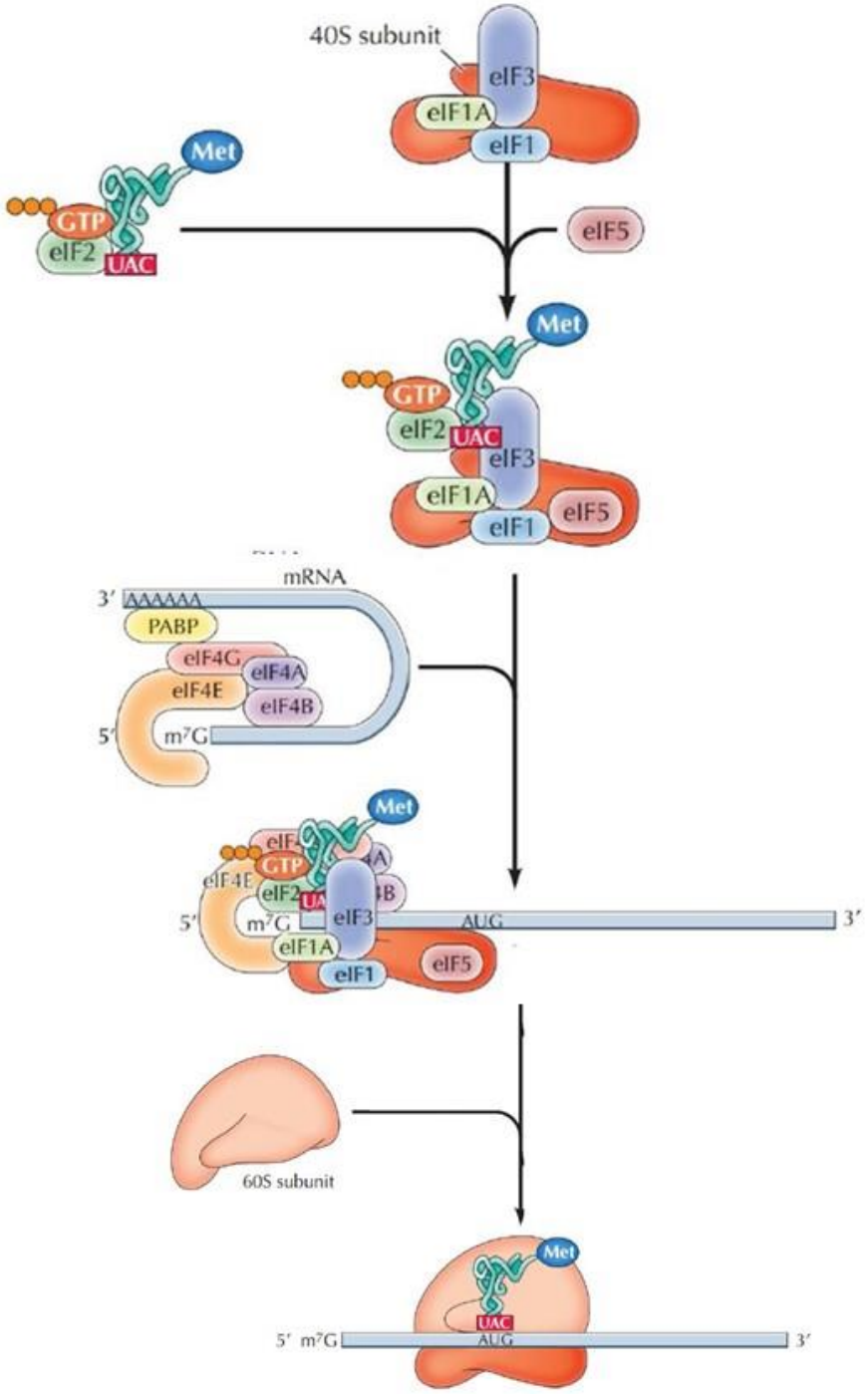
Building a polypeptide

- The three stages of translation
  - Initiation
  - Elongation
  - Termination
- All three stages require protein “factors” that aid in the translation process.

Specific factors to each stage:
(initiation factors for initiation.
elongation factors for elongation.
termination factors for termination.

Translation initiation

We will focus more on the eukaryotic system

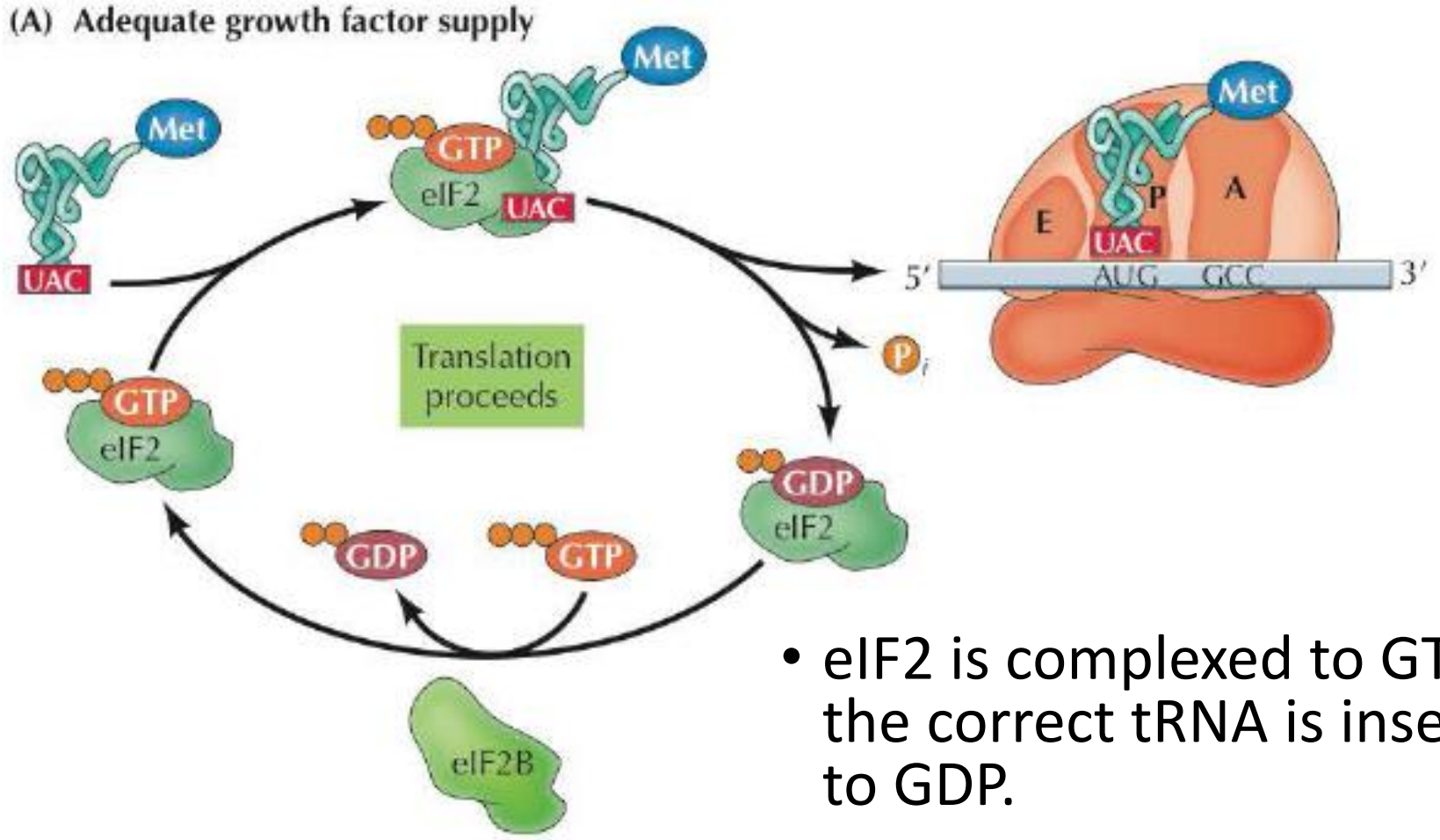


- tRNA forms a complex with the small ribosomal subunit with the help of eIF2.

Cap of mRNA linked with poly-A tail

- 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.

Regeneration of eIF2



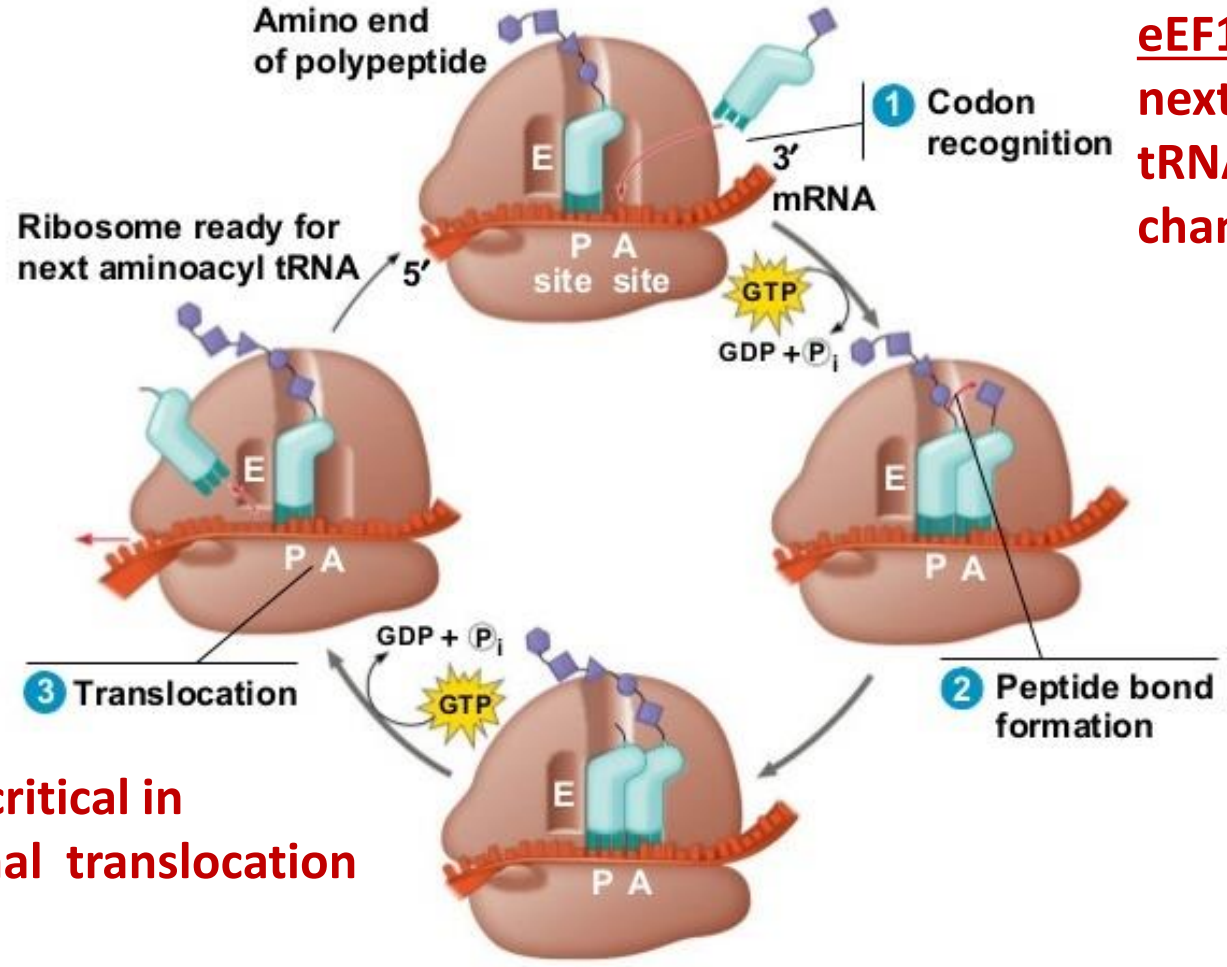
Regeneration of eIF2 is important to keep translation going in the cell

- 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

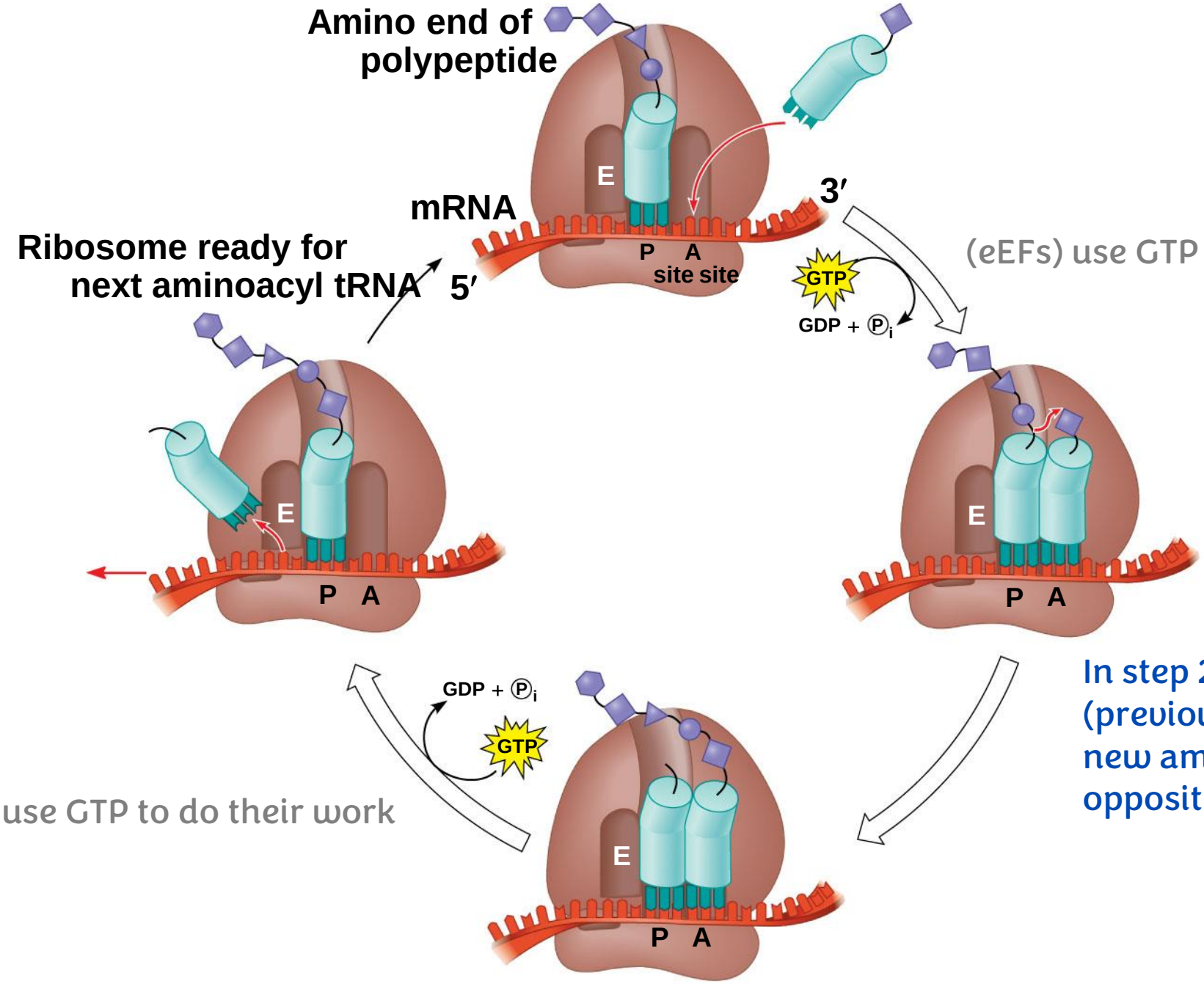
Three steps:

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

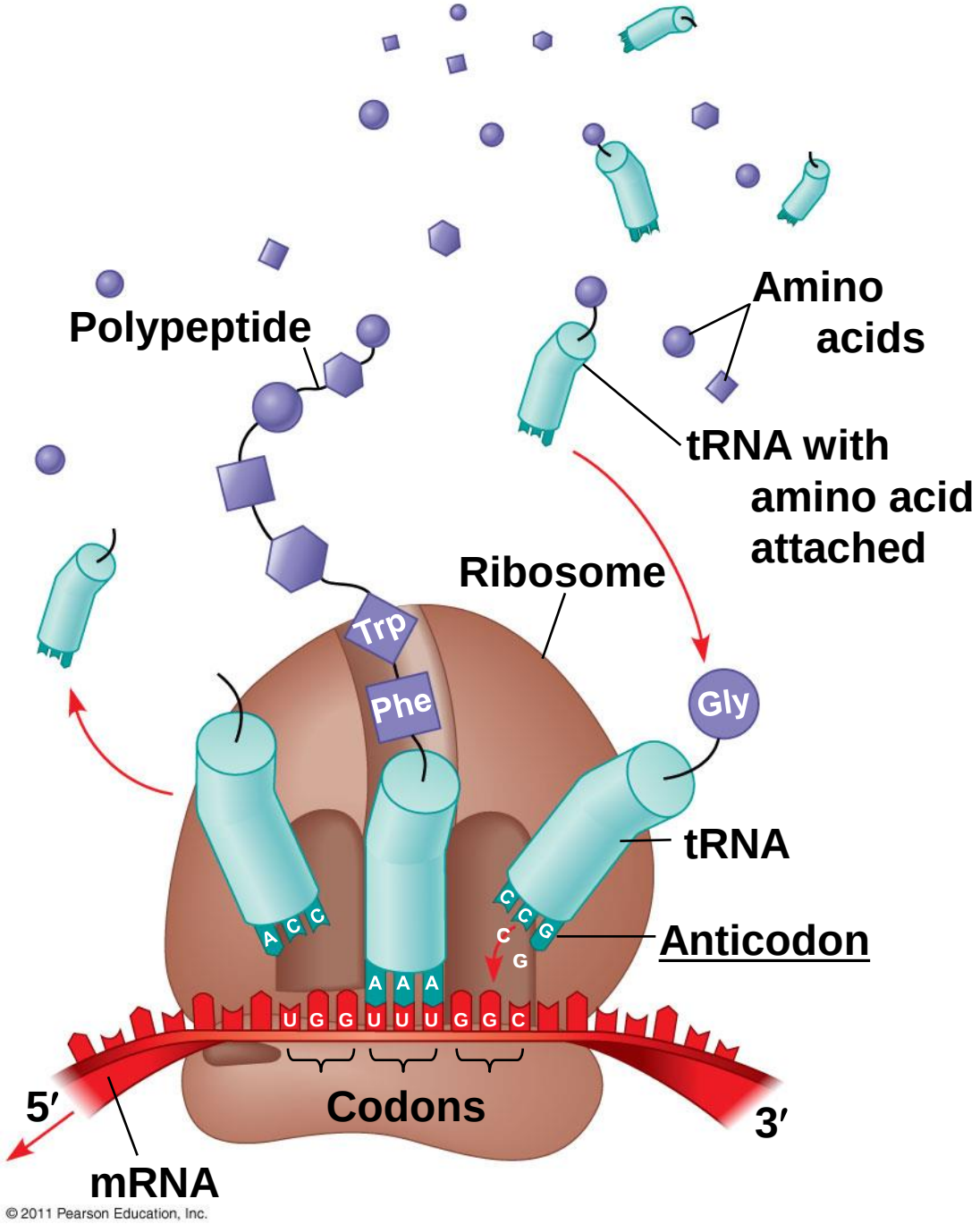


<u>eEF1α</u> brings next aminoacyl-tRNA to the A chamber

<u>eEF2</u> is critical in ribosomal translocation



In step 2, we add the big (previous chain) to the sole new amino acid, and not the opposite.


Polypeptide
Amino acids
tRNA with amino acid attached
Ribosome
Trp
Phe
Gly
tRNA
Anticodon
A C C
C C G
C G
A A A
U G G U U U G G C
Codons
5′
3′
mRNA
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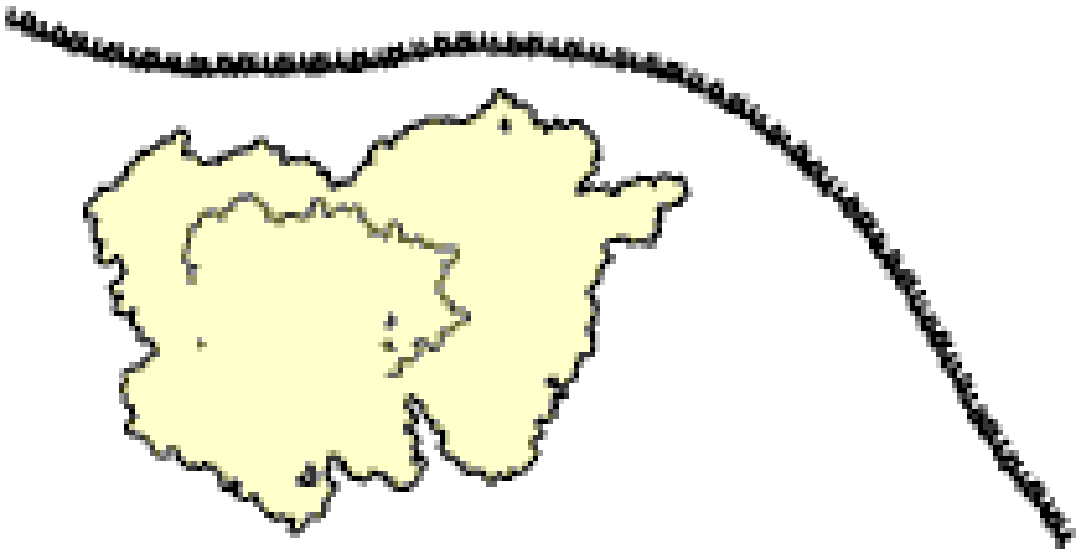
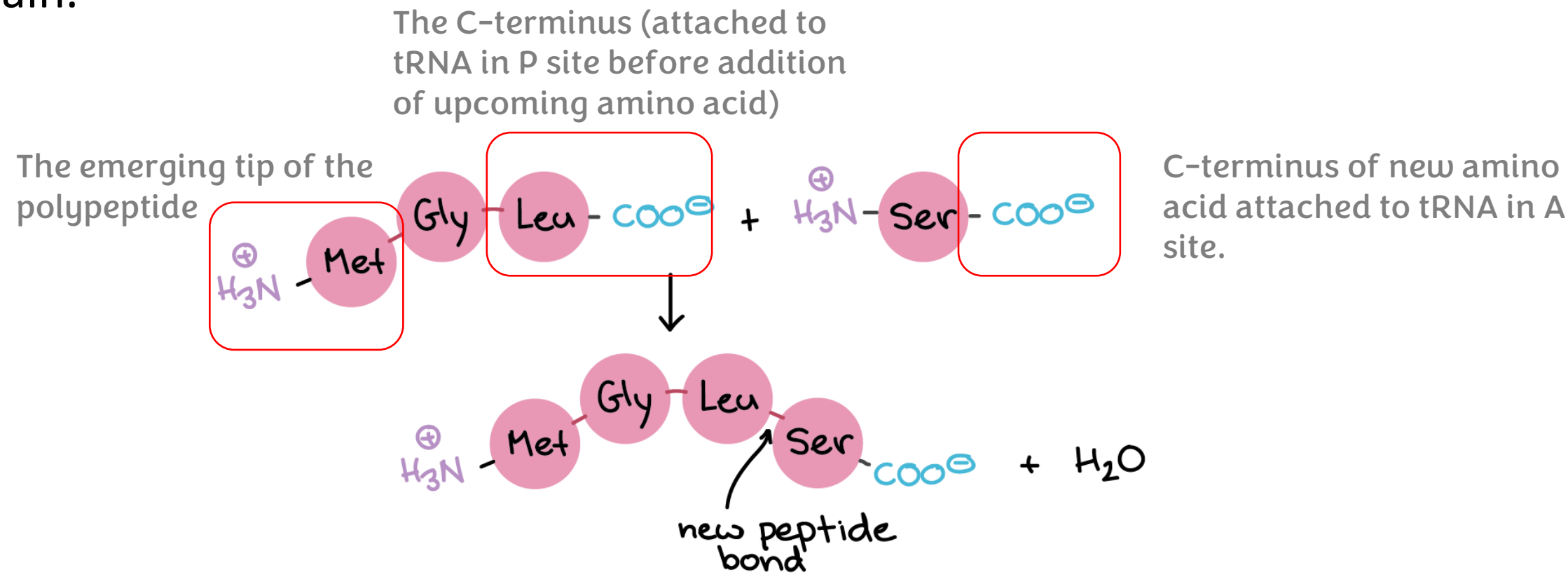
- There is an animation for those who want to see it; refer to the lecture at minute **42:41**



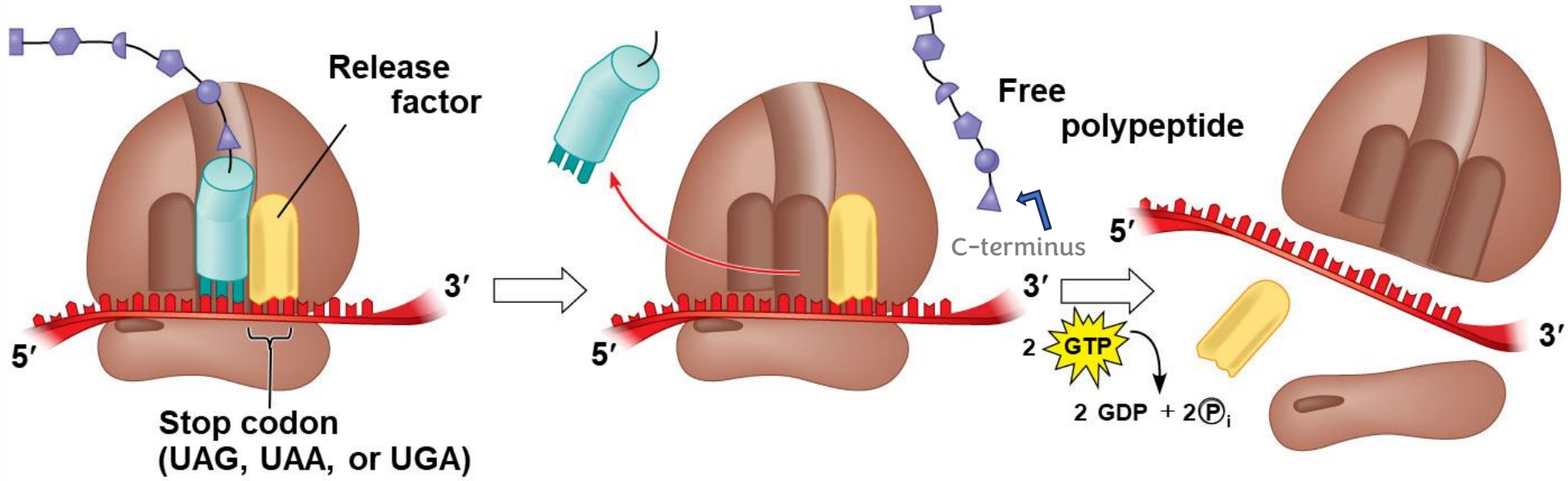
Elongation of the Polypeptide Chain

- 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 <u>not recognized by any tRNAs, but a release factor protein.</u>
- The empty A site accepts release factors, which cause the release of the polypeptide, and the translation assembly then <u>comes apart</u>.



For any feedback, scan the code or click on it.

Corrections from previous versions:

Versions	Slide # and Place of Error	Before Correction	After Correction
V0 → V1	22	(can have multiple in one mRNA ‘monocistronic')	(can have multiple in one mRNA ‘polycistronic')
V1 → V2			

Additional Resources:

رسالة من الفريق العلمي:

عن جابر بن عبد الله رضي الله عنهما قال: سمعت رسول الله صلى الله عليه وسلم يقول: أفضل الذكر لا إله إلا الله، وأفضل الدعاء الحمد لله.