Molecular Biology (11)
Translation

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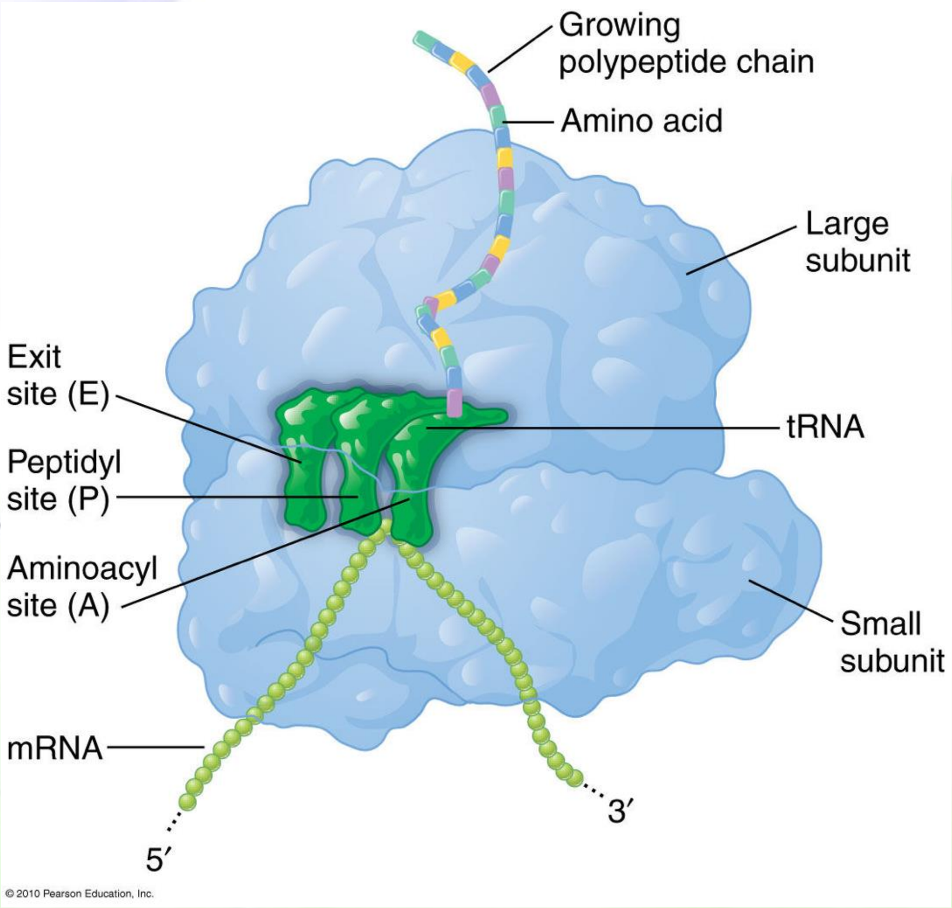
School of Medicine
Second year, First semester, 2024-2025

General information



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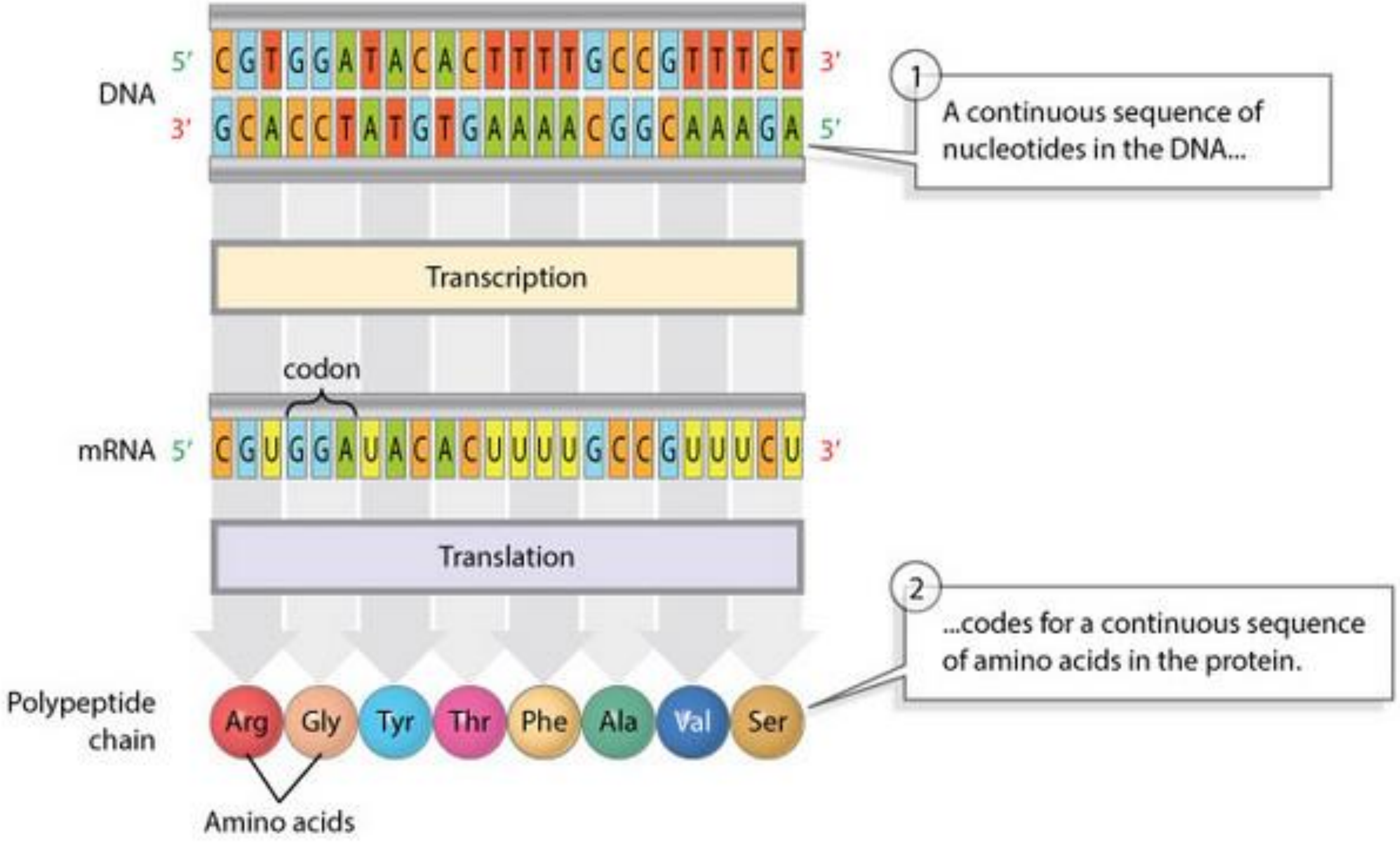
- Protein synthesis involves interactions between ribosomes with three types of RNA molecules:
  - tRNAs
  - rRNAs,
  - mRNA templates

Colinearity of genes, mRNAs, & proteins

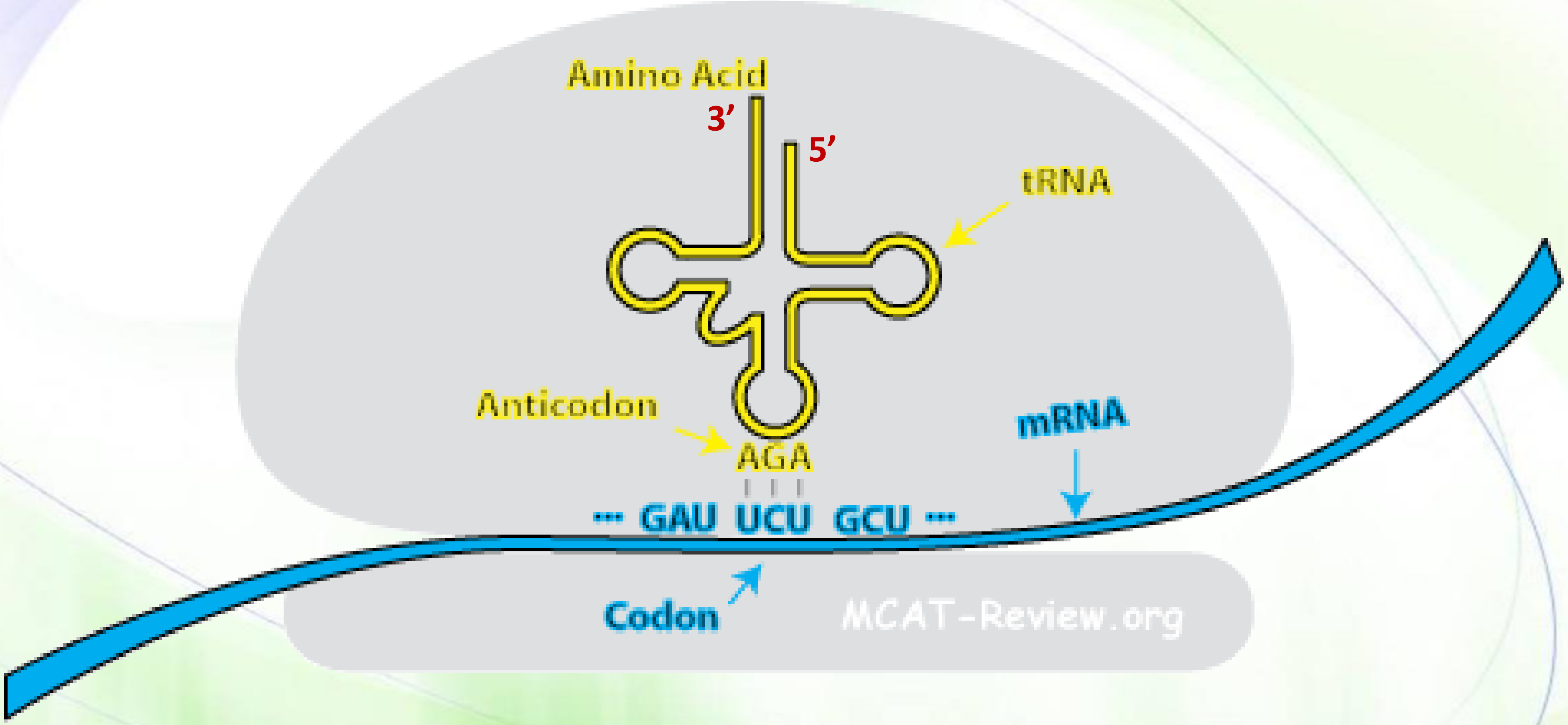


① A continuous sequence of nucleotides in the DNA...

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

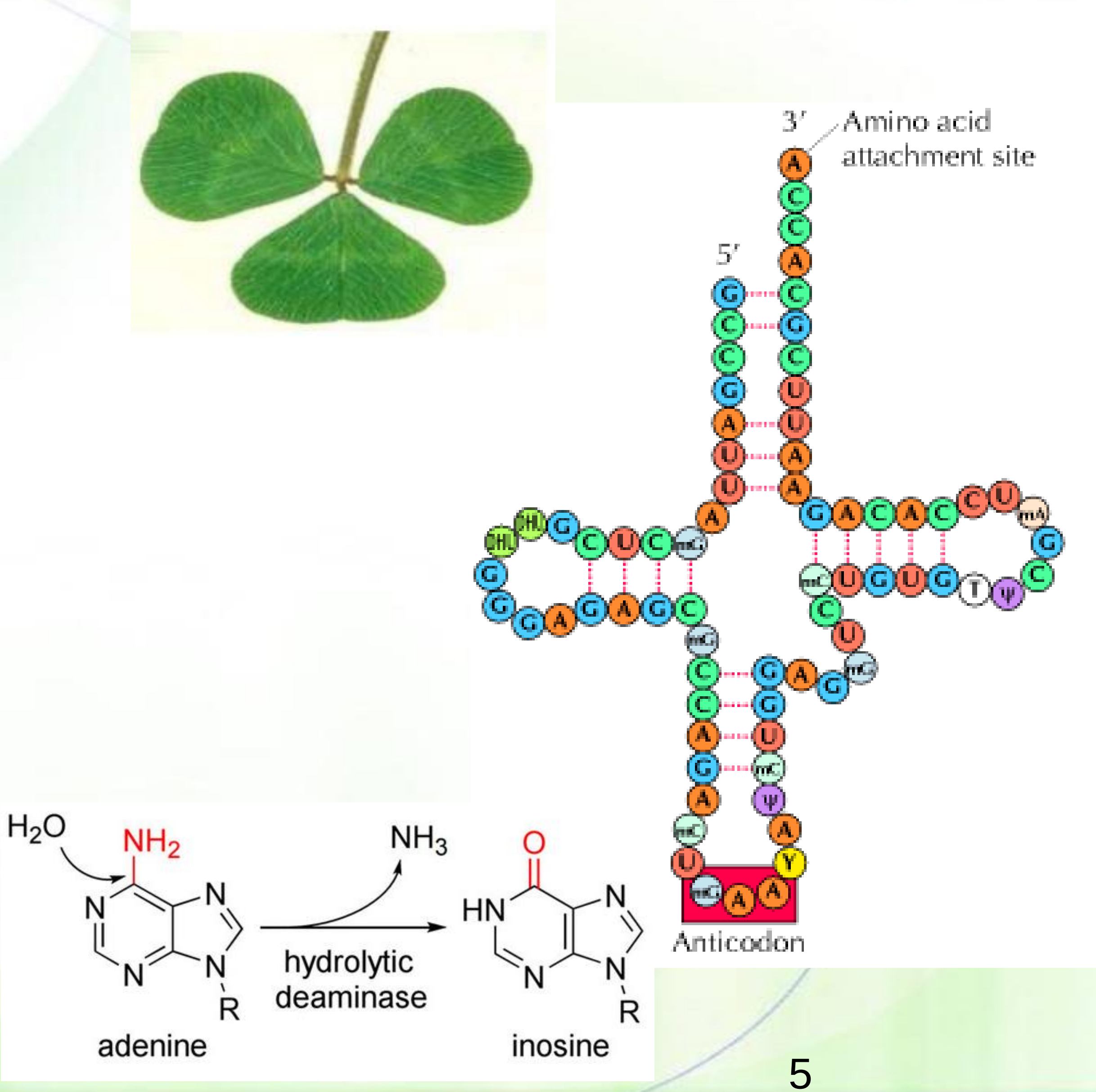
With colinearity, the number of nucleotides in the gene is proportional to the number of amino acids in the protein.

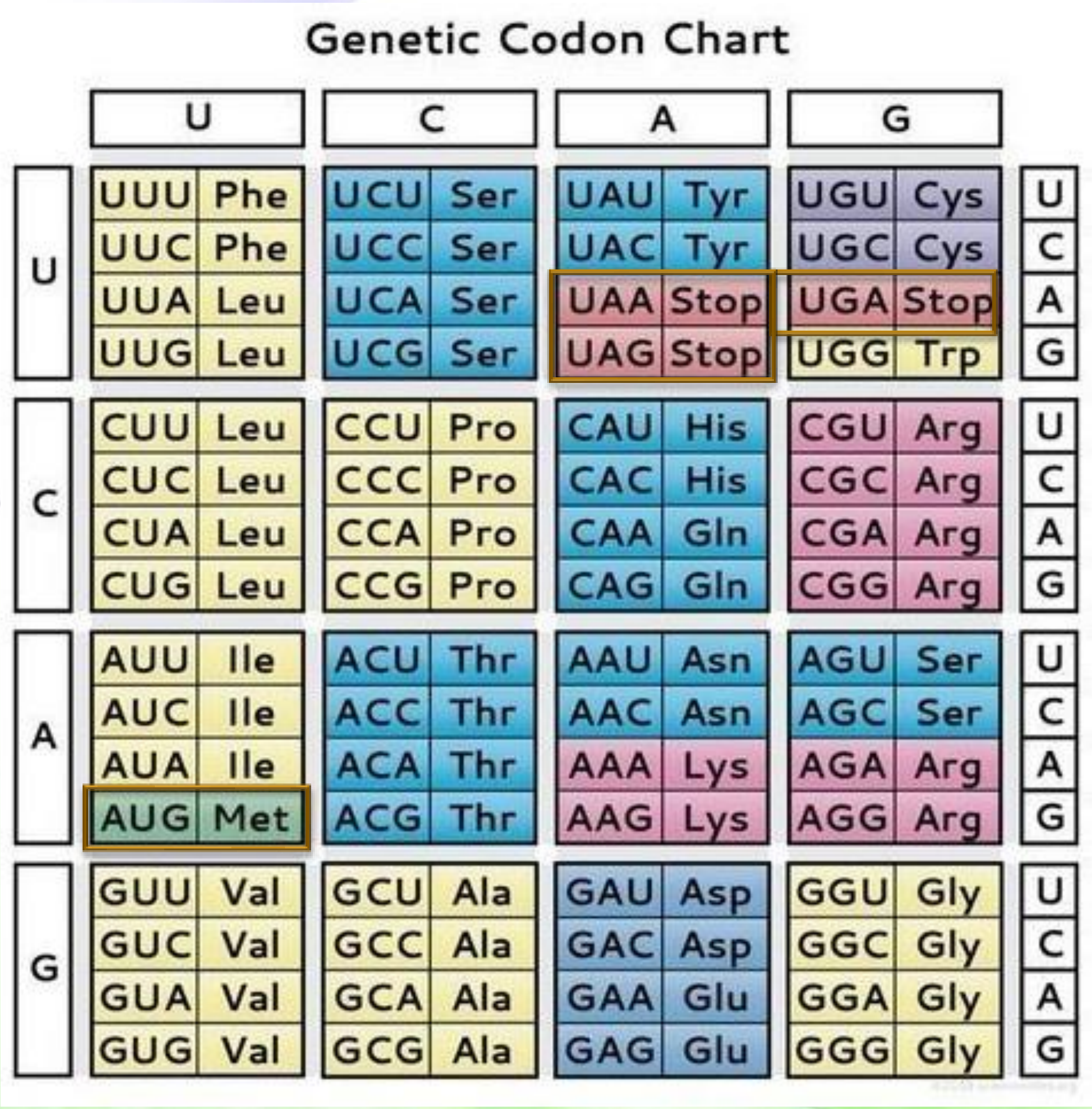
mRNA is read by tRNA in triplets



tRNA structure

- tRNAs are short single-stranded RNA molecules (80 bases long).
- “Charged” or “activated” tRNA carries one amino acid at the 3’-end.
- tRNAs contain stem loop structures, modified bases, and unusual bases (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

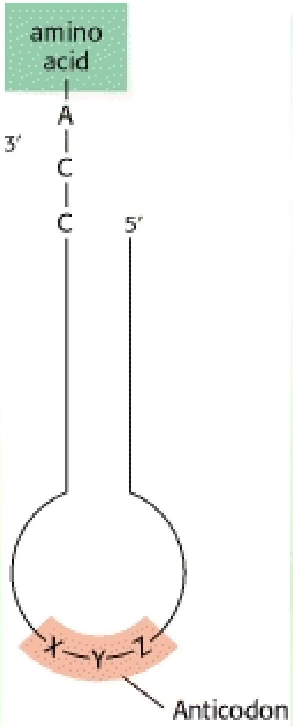
Cysteine

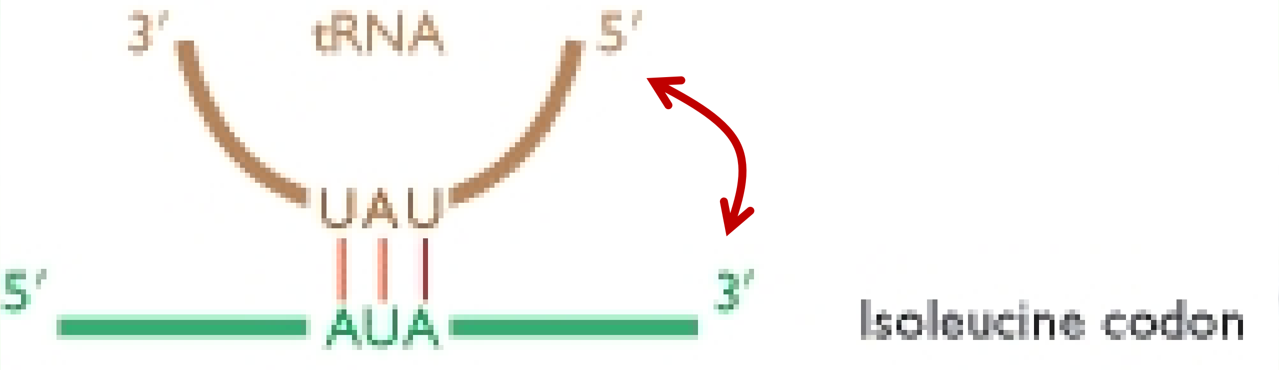
Features of the genetic codon

- 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.
- 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.

Codon vs. anticodon

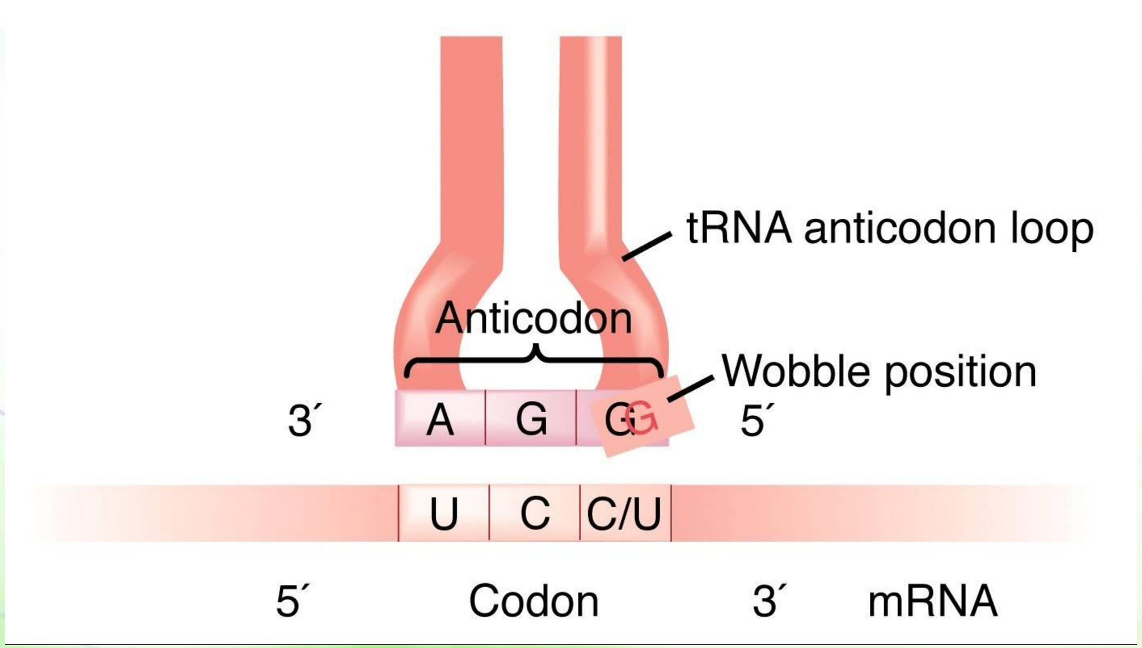
- tRNAs contain a three-nucleotide sequence known as “anticodon” that pairs in an anti-parallel manner with the “codon” of mRNA molecules.





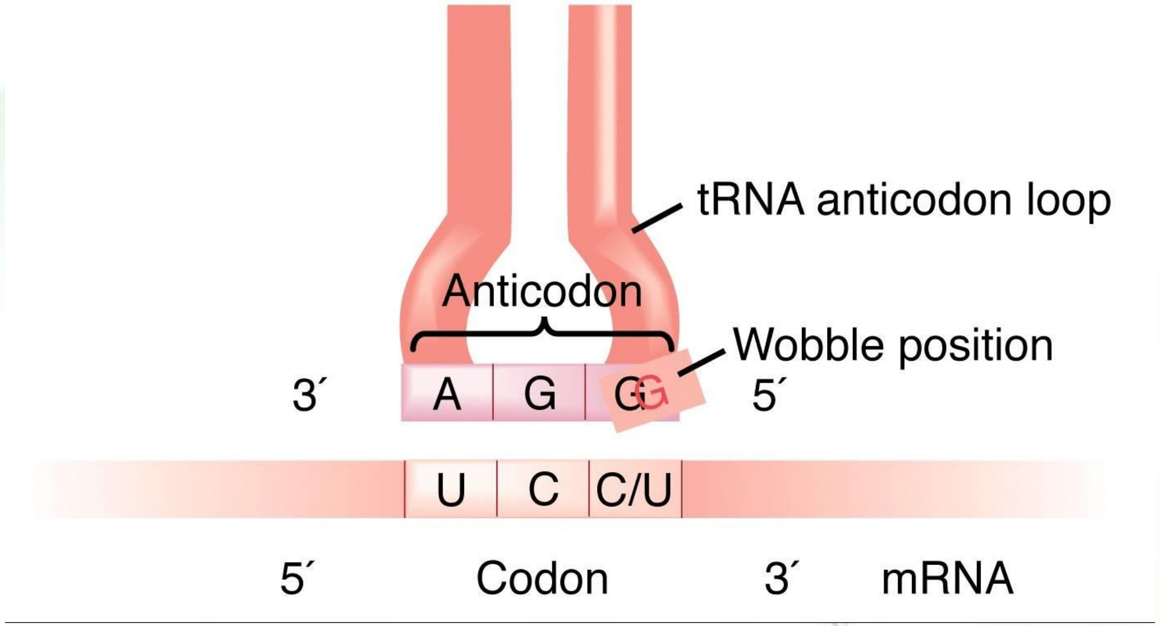
Fidelity of translation

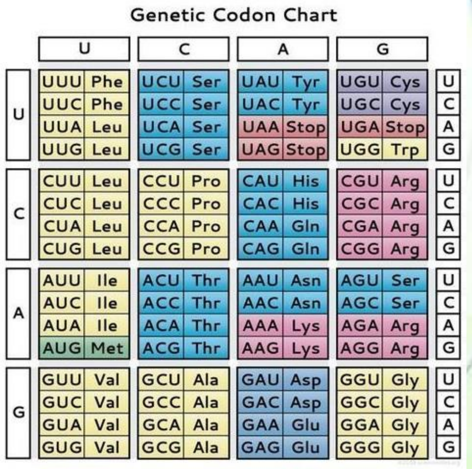
- Accurate translation requires two steps:
  - First: accurate association of amino acid to tRNA
  - Second: a correct match between the tRNA ‘s anticodon and the mRNA’s codon



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 genetic codon is degenerate.
  - It acts as a buffer against deleterious mutations.





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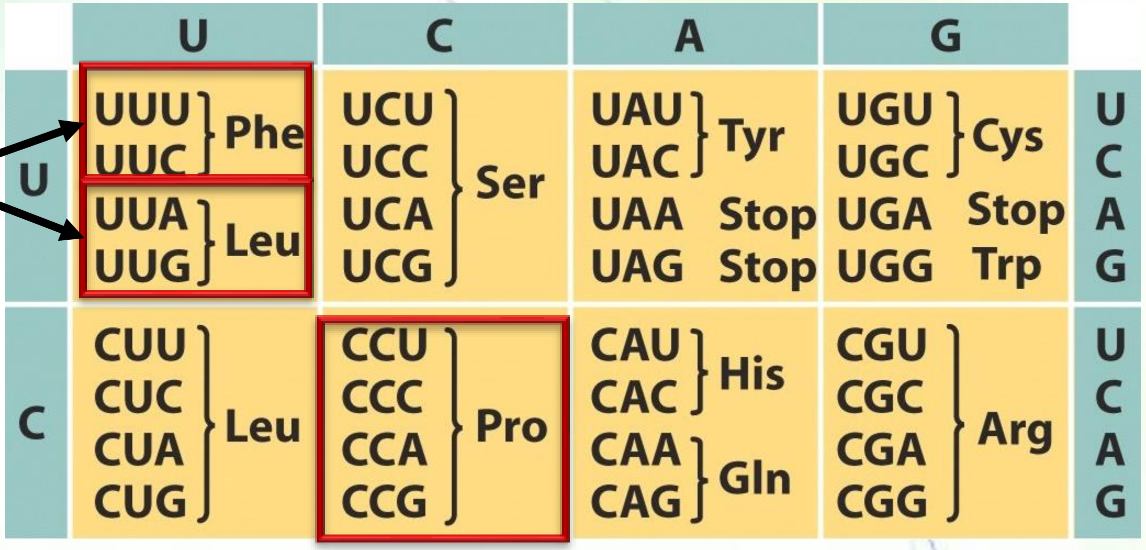
	UUA Leu	UCA Ser	UAA Stop	UGA Stop	A
	UUG Leu	UCG Ser	UAG Stop	UGG Trp	G
C	CUU Leu	CCU Pro	CAU His	CGU Arg	U
	CUC Leu	CCC Pro	CAC His	CGC Arg	C
	CUA Leu	CCA Pro	CAA Gln	CGA Arg	A
	CUG Leu	CCG Pro	CAG Gln	CGG Arg	G
A	AUU Ile	ACU Thr	AAU Asn	AGU Ser	U
	AUC Ile	ACC Thr	AAC Asn	AGC Ser	C
	AUA Ile	ACA Thr	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

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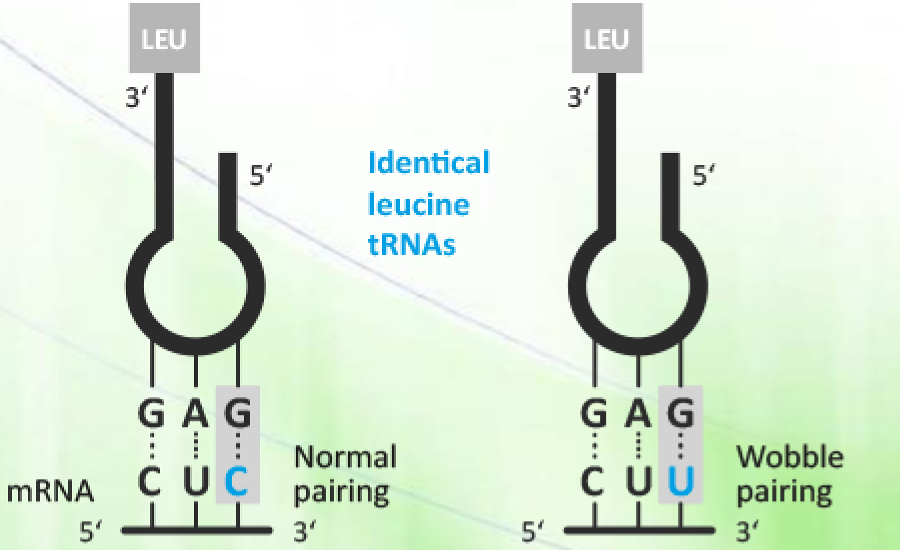
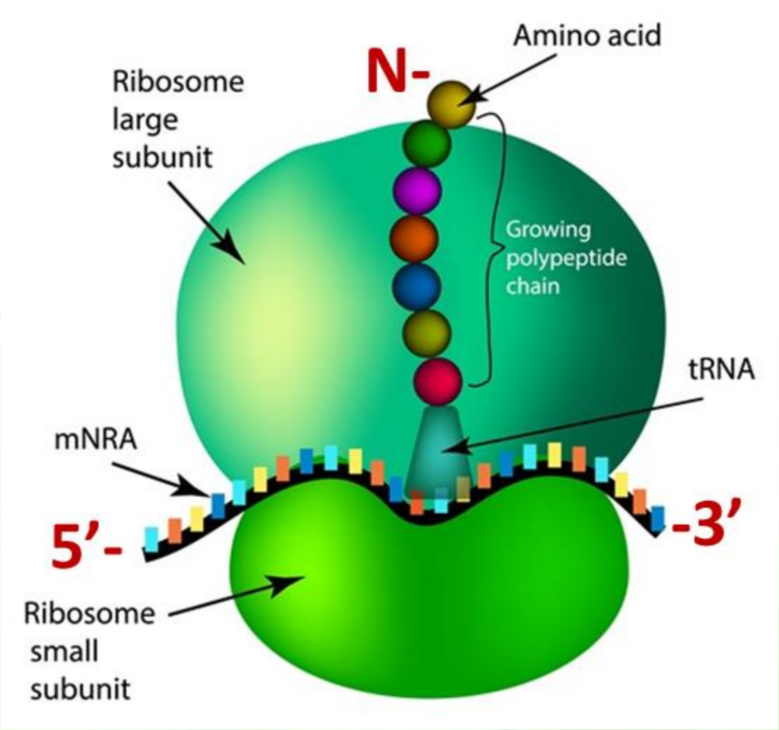
	UUC		UCC		UAC		UGC		C
	UUA	Leu	UCA		UAA	Stop	UGA	Stop	A
	UUG		UCG		UAG	Stop	UGG	Trp	G
C	CUU	Leu	CCU	Pro	CAU	His	CGU	Arg	U
	CUC		CCC		CAC		CGC		C
	CUA		CCA		CAA	Gln	CGA		A
	CUG		CCG		CAG		CGG		G



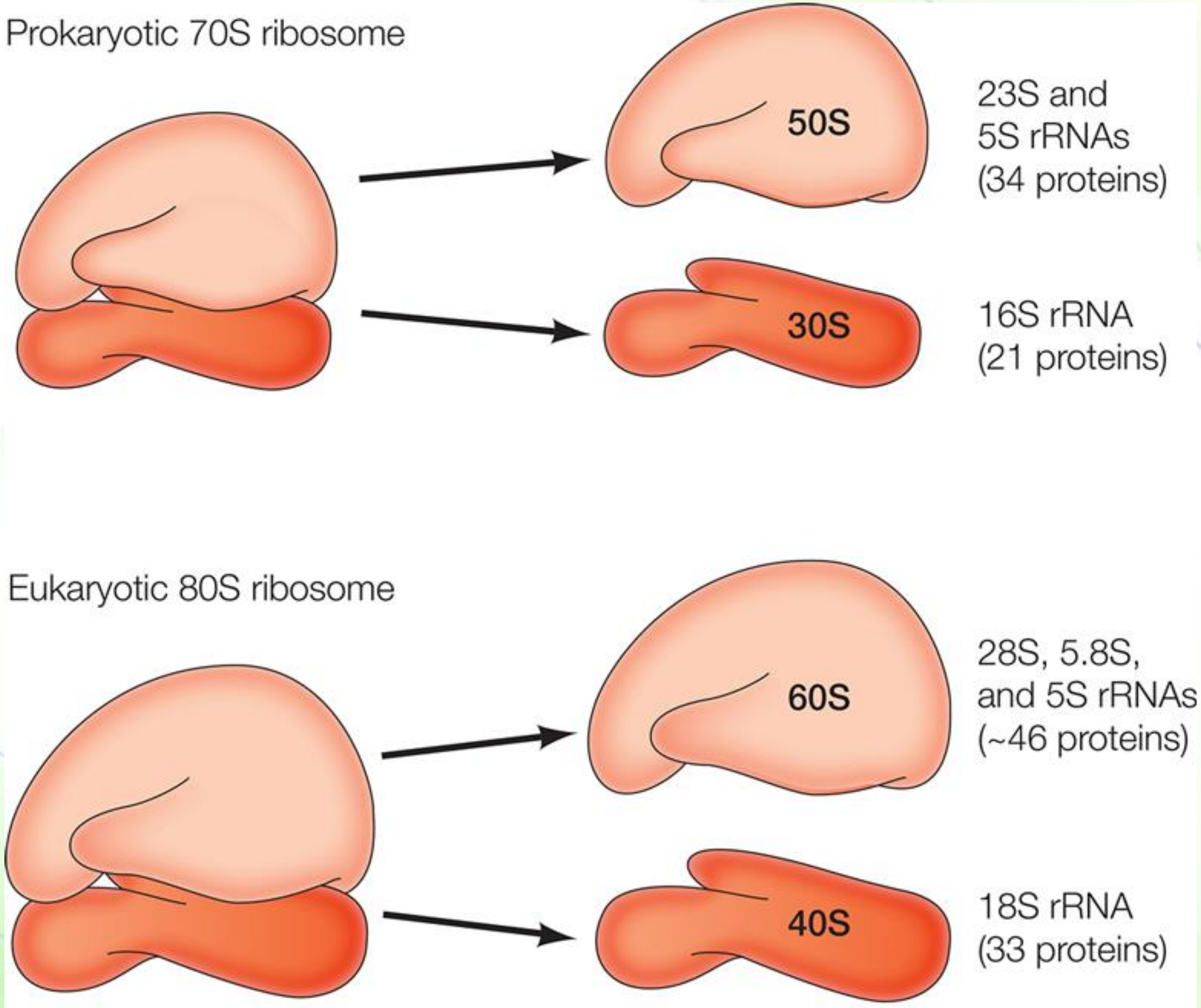
Ribosomes

- Ribosomes are the sites of protein synthesis.
- 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.

Ribosome structure



Prokaryotic 70S ribosome

23S and
5S rRNAs
(34 proteins)

16S rRNA
(21 proteins)

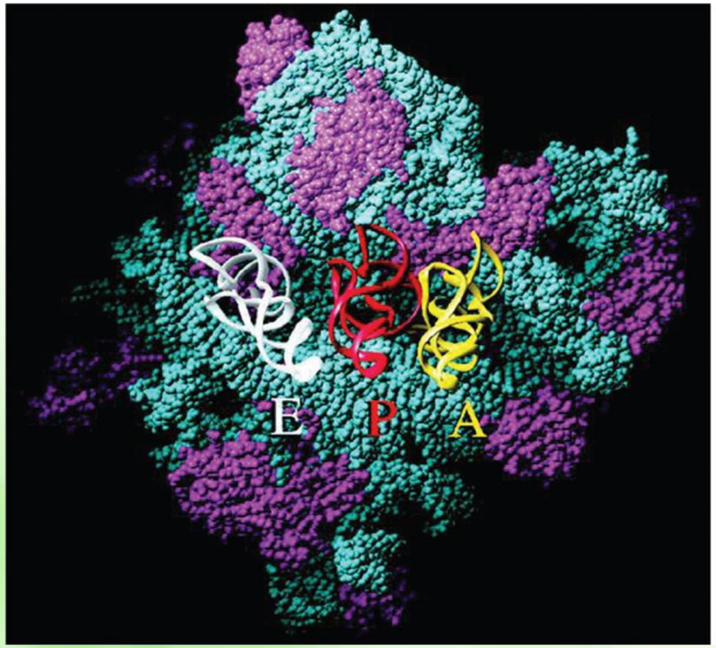
Eukaryotic 80S ribosome

28S, 5.8S,
and 5S rRNAs
(~46 proteins)

18S rRNA
(33 proteins)

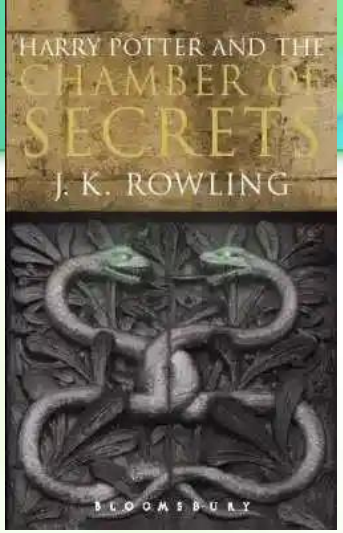
Functional and structural components of ribosomes

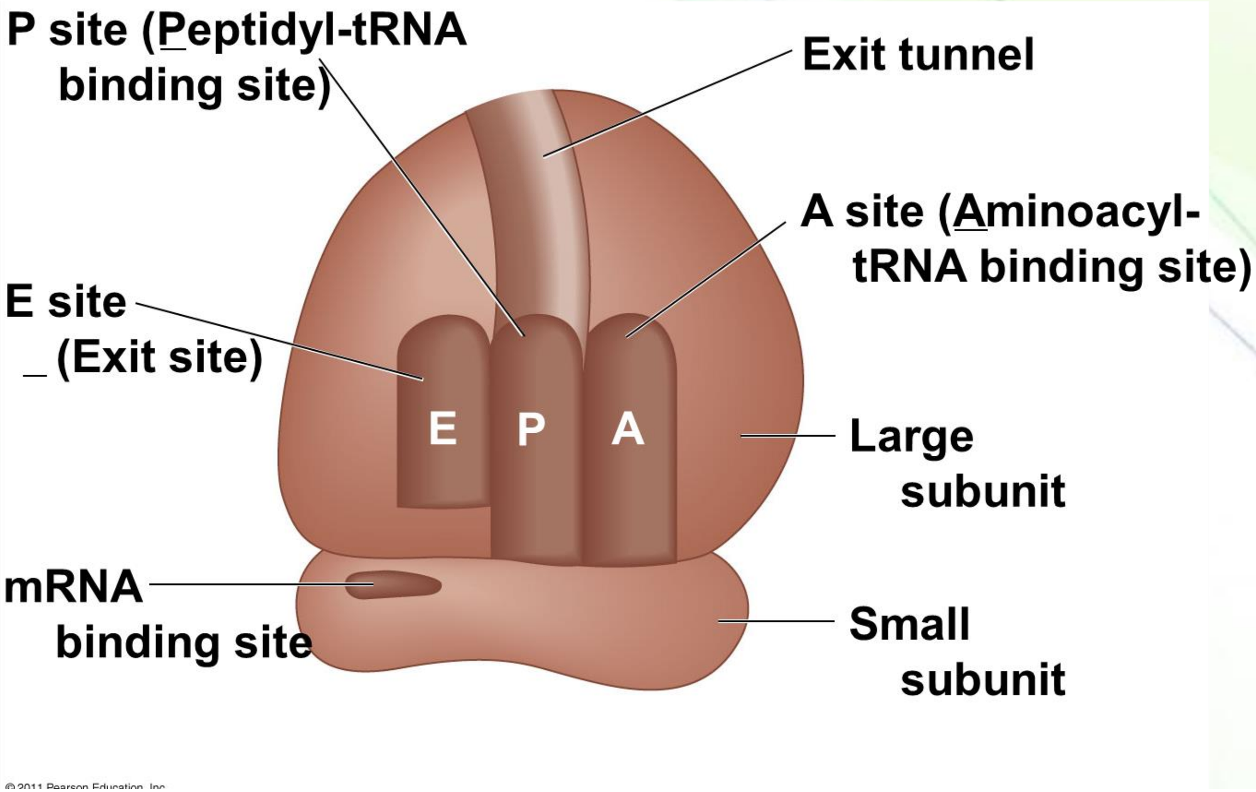
- Ribosomes facilitate specific coupling of tRNA anticodons with mRNA codons in protein synthesis.
- The RNA components are responsible for the catalytic function of the ribosome, and the protein components enhance the function of the rRNA molecules.



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

The chambers of secret





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tRNA binding sites on a ribosome:

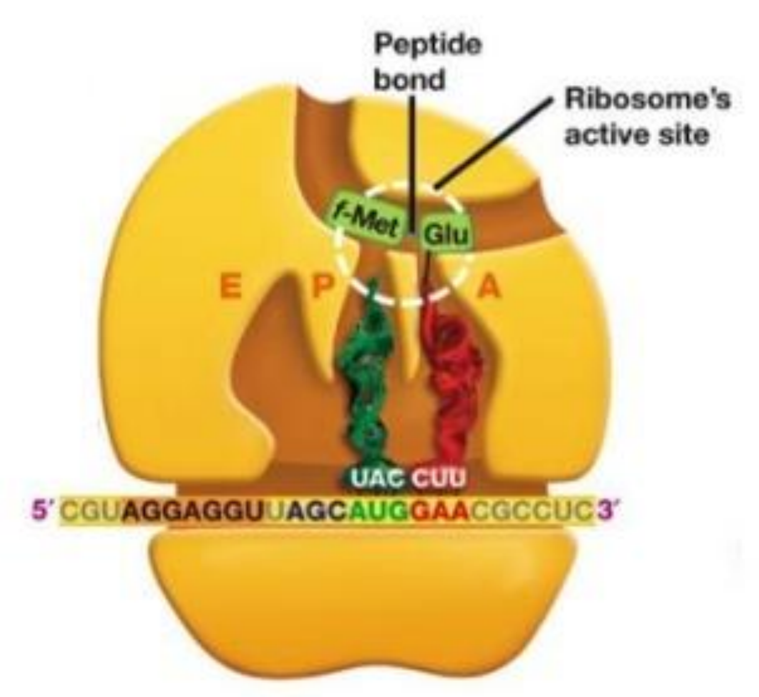
The **P site** holds the tRNA that carries the growing polypeptide chain

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

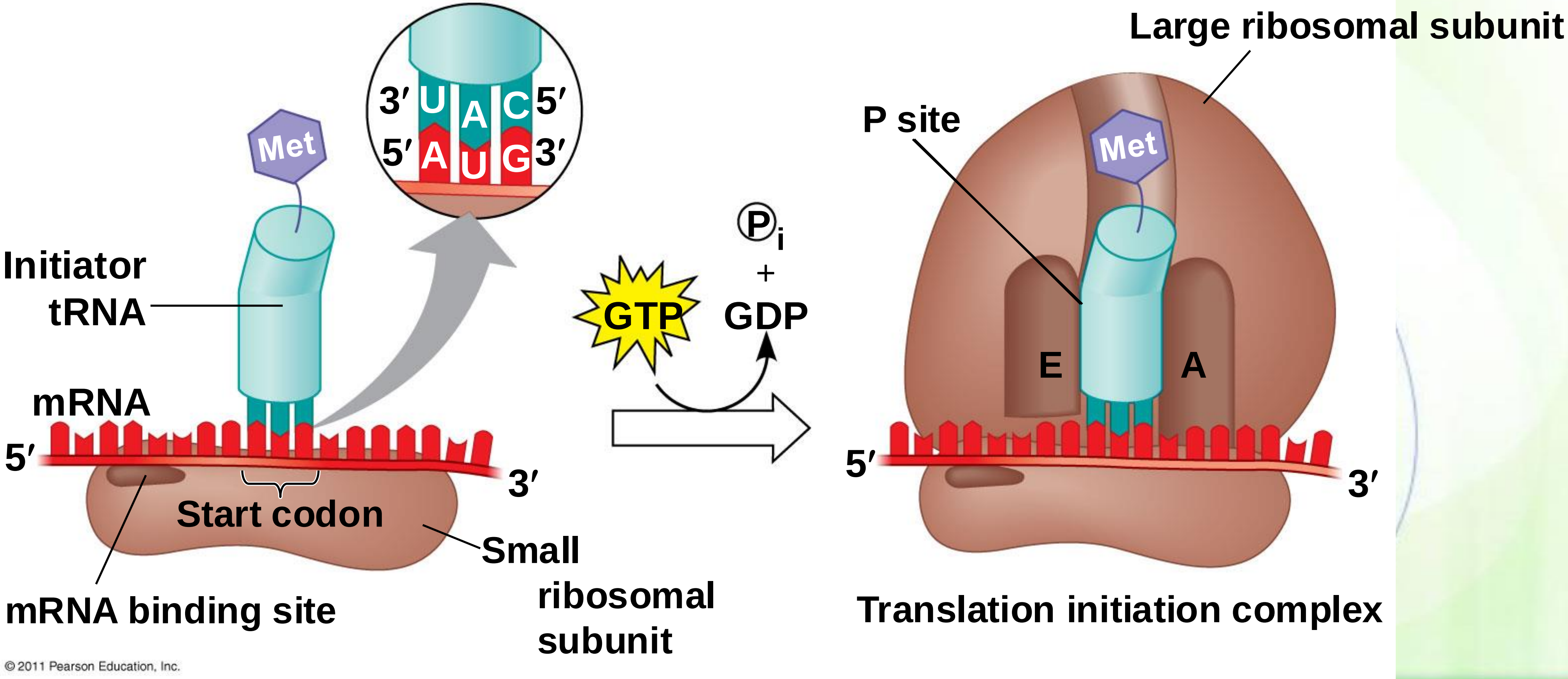
The general mechanism of translation

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



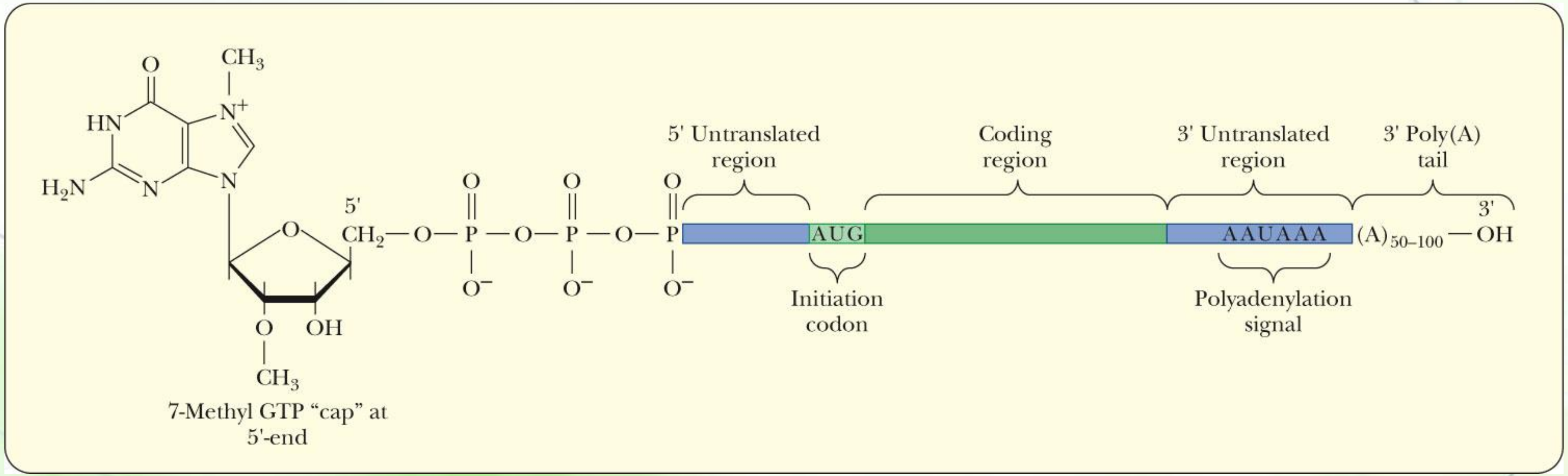
Start of translation

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



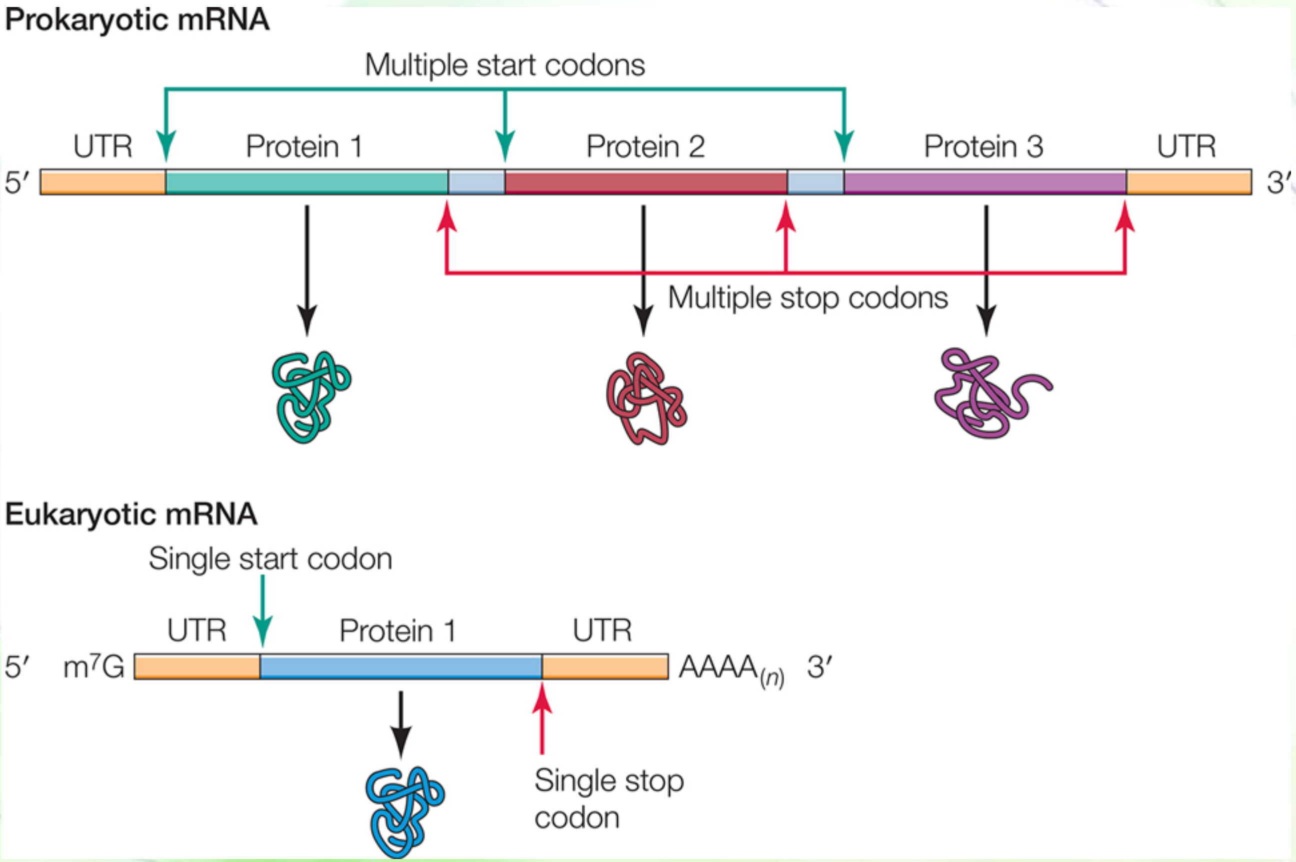
Untranslated regions

- 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.

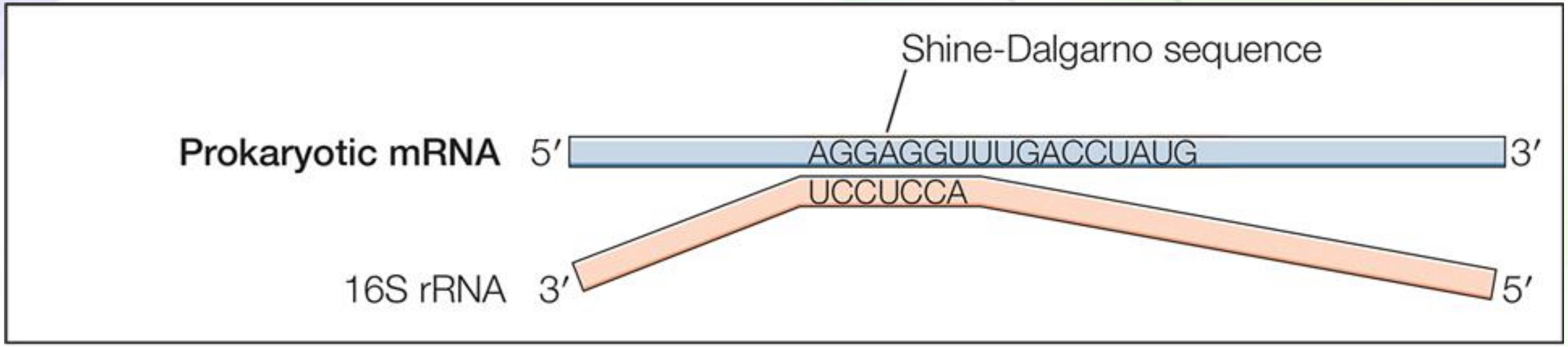


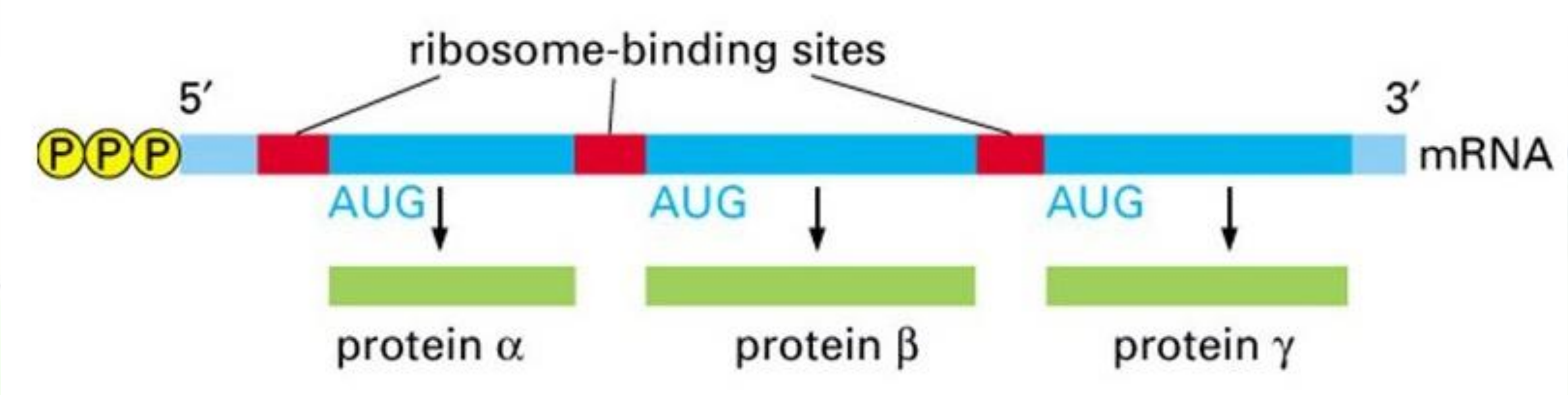
Remember…

- Bacterial mRNA is polycistronic
- Eukaryotic mRNA is monocistronic



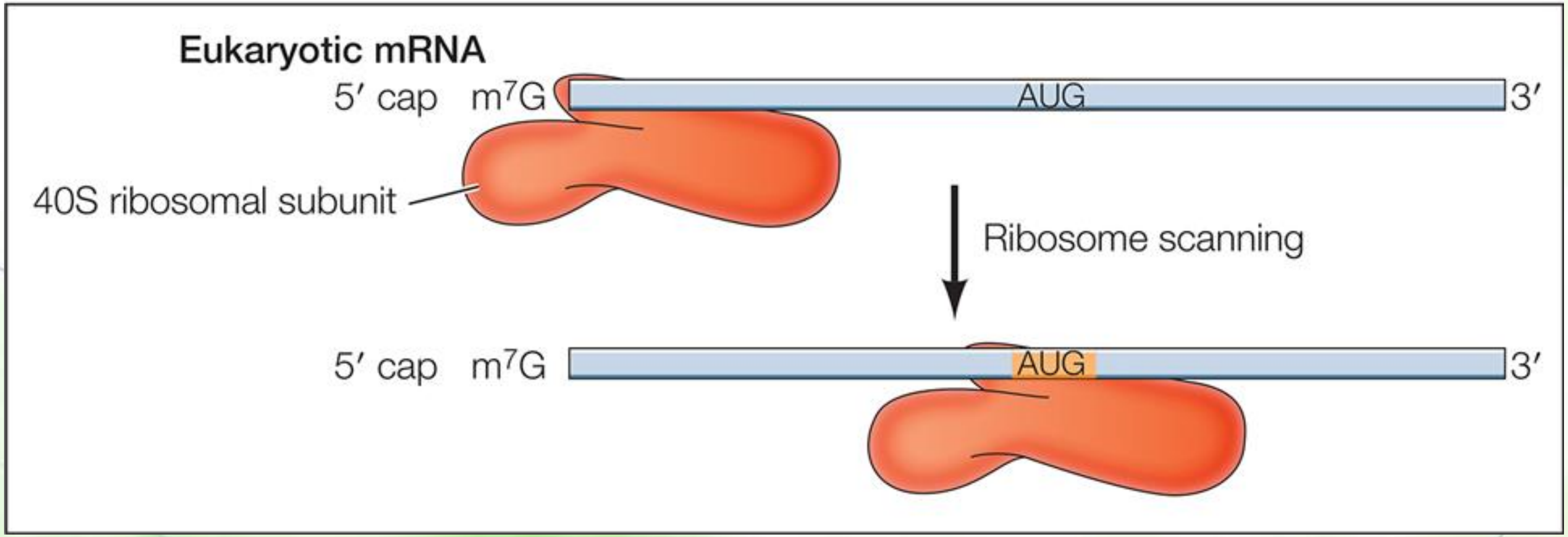
Shine-Dalgarno sequence





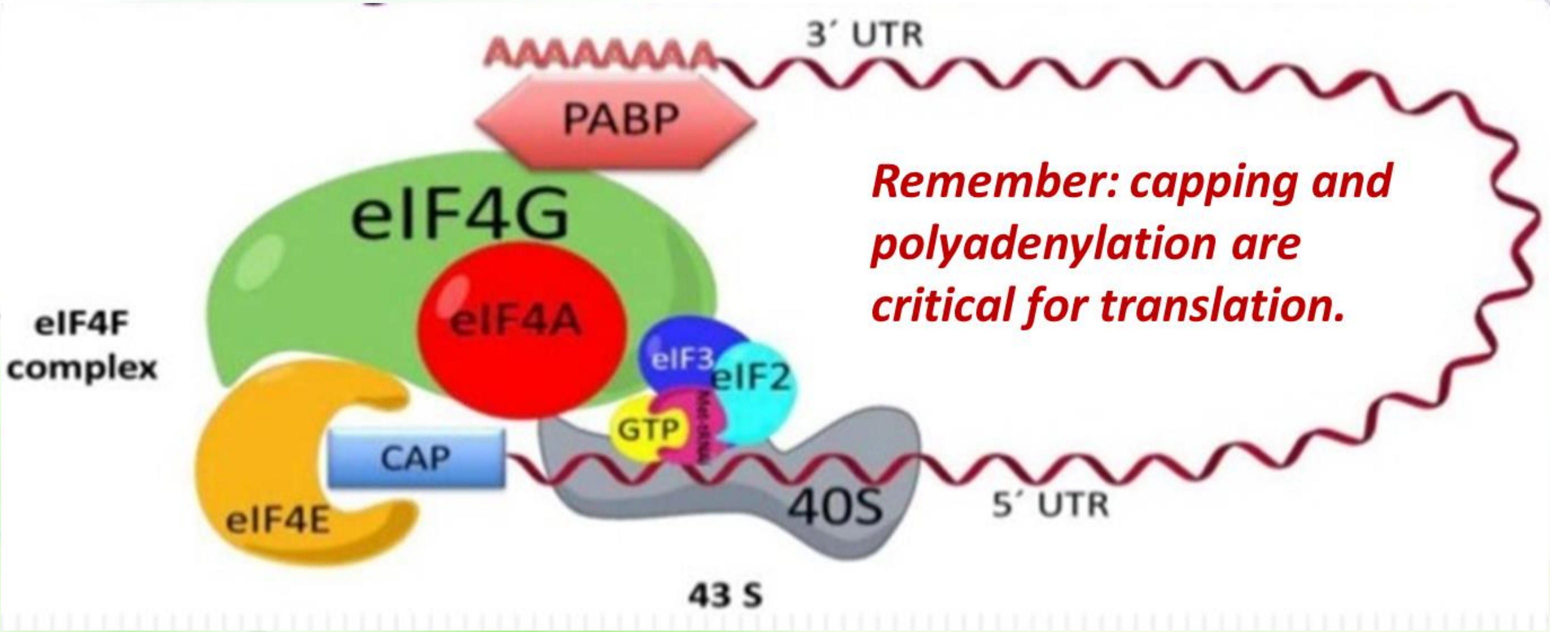
But in eukaryotes…

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



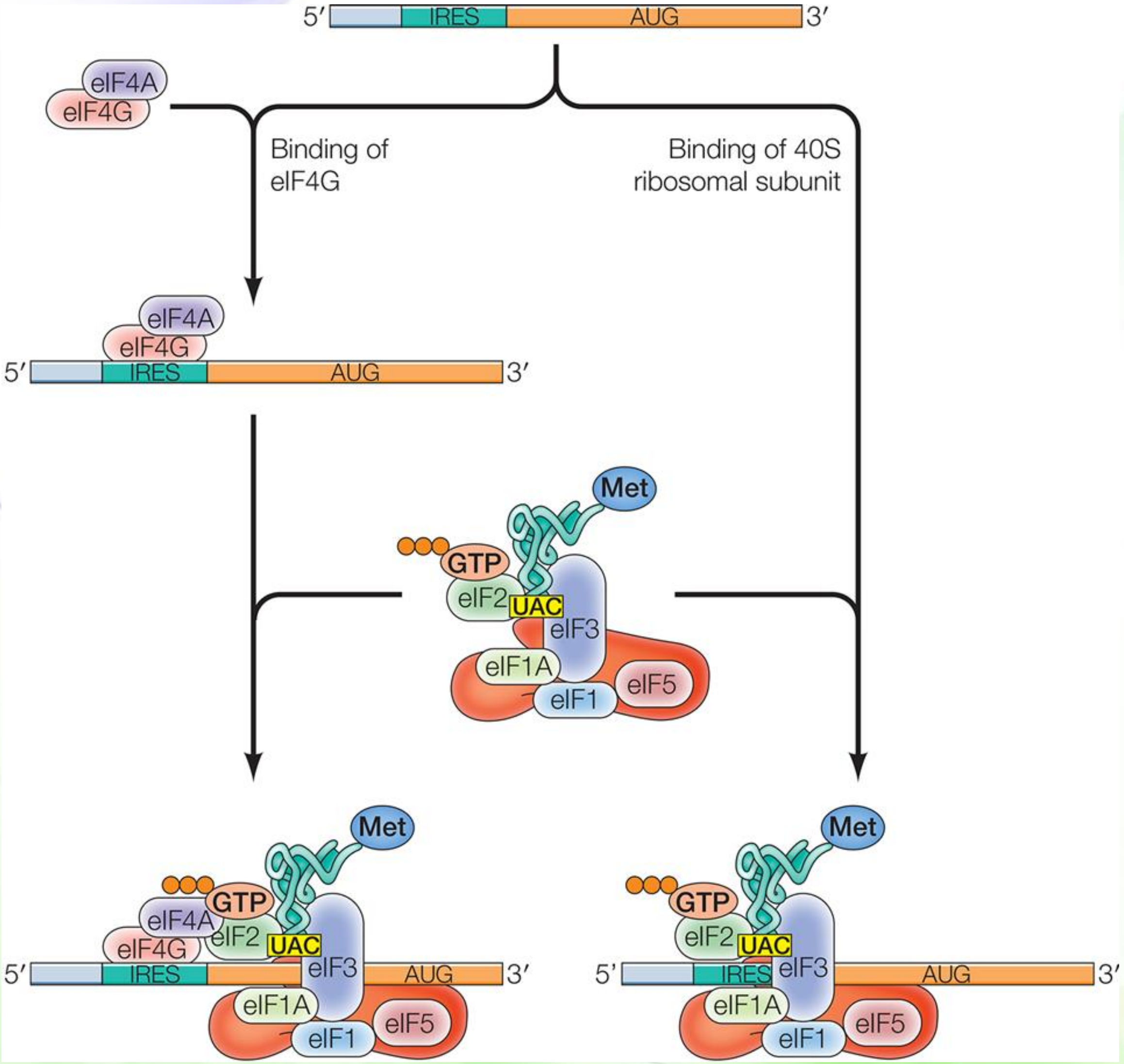
Translation initiation in eukaryotes

- 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.



Remember: capping and polyadenylation are critical for translation.

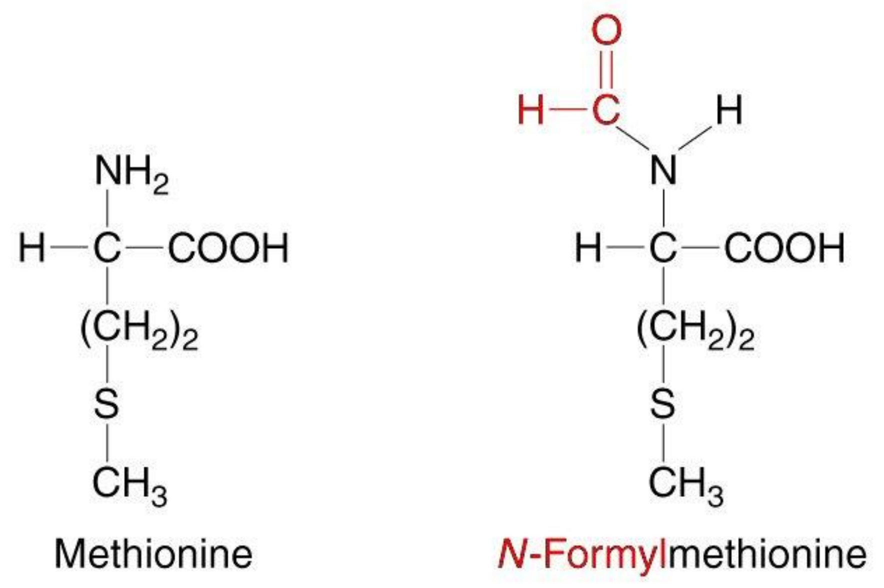
Internal ribosome entry site (IRES)



- 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.

The first amino acid

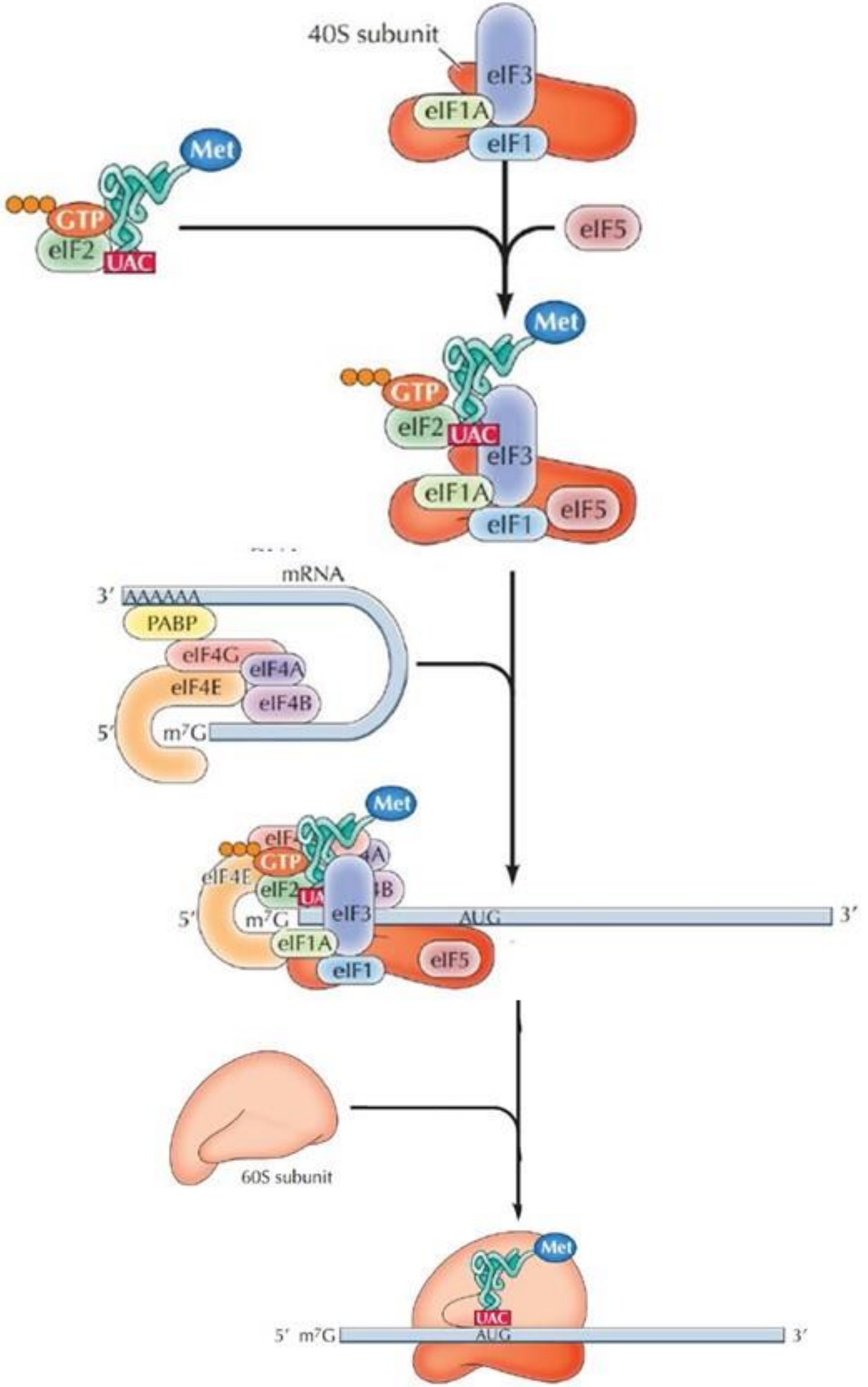
- Translation always initiates with the amino acid methionine, usually encoded by AUG.
- In bacteria, it is N-formylmethionine.



Building a polypeptide

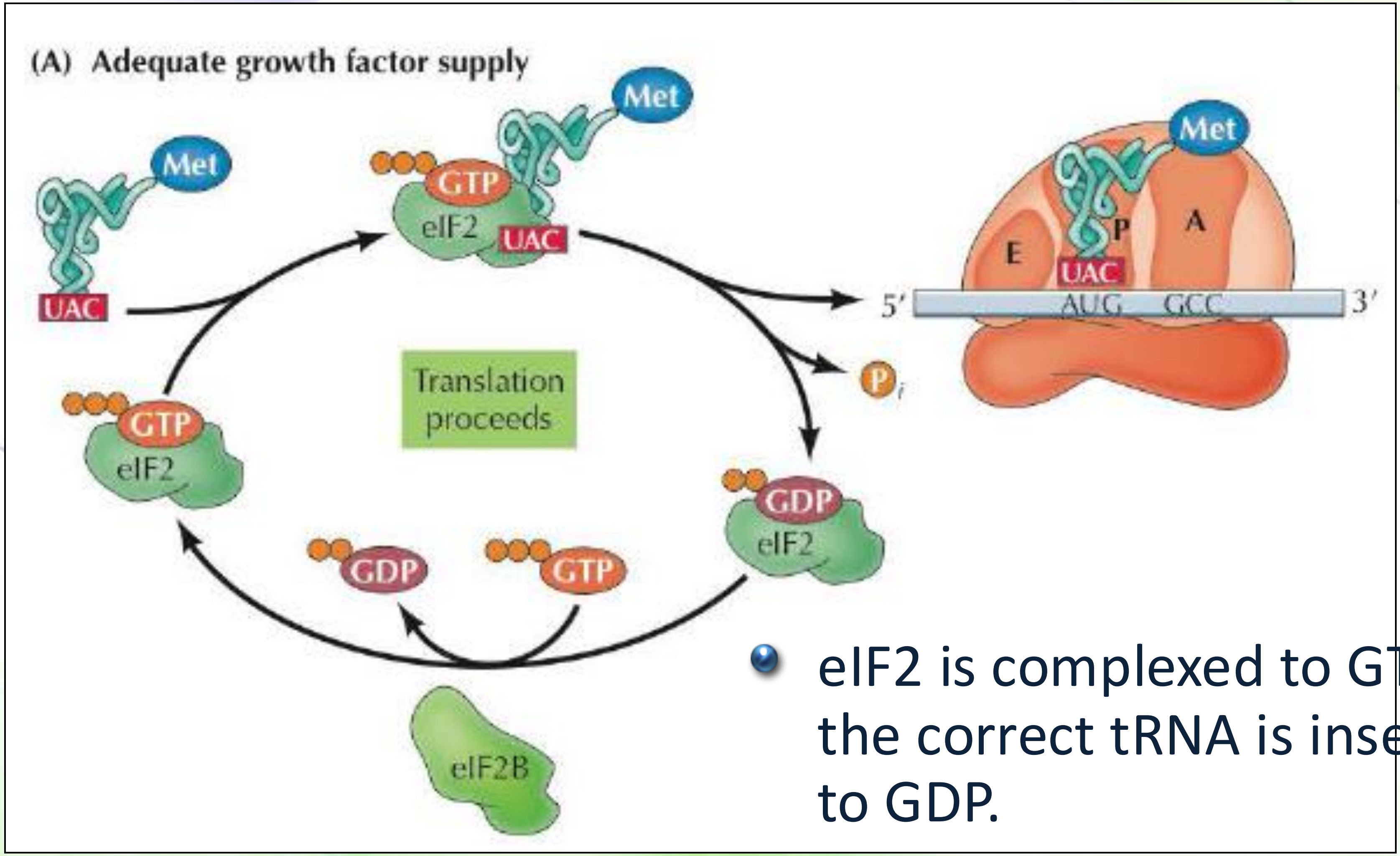
- The three stages of translation
  - Initiation
  - Elongation
  - Termination
- All three stages require protein “factors” that aid in the translation process.

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.

Regeneration of eIF2

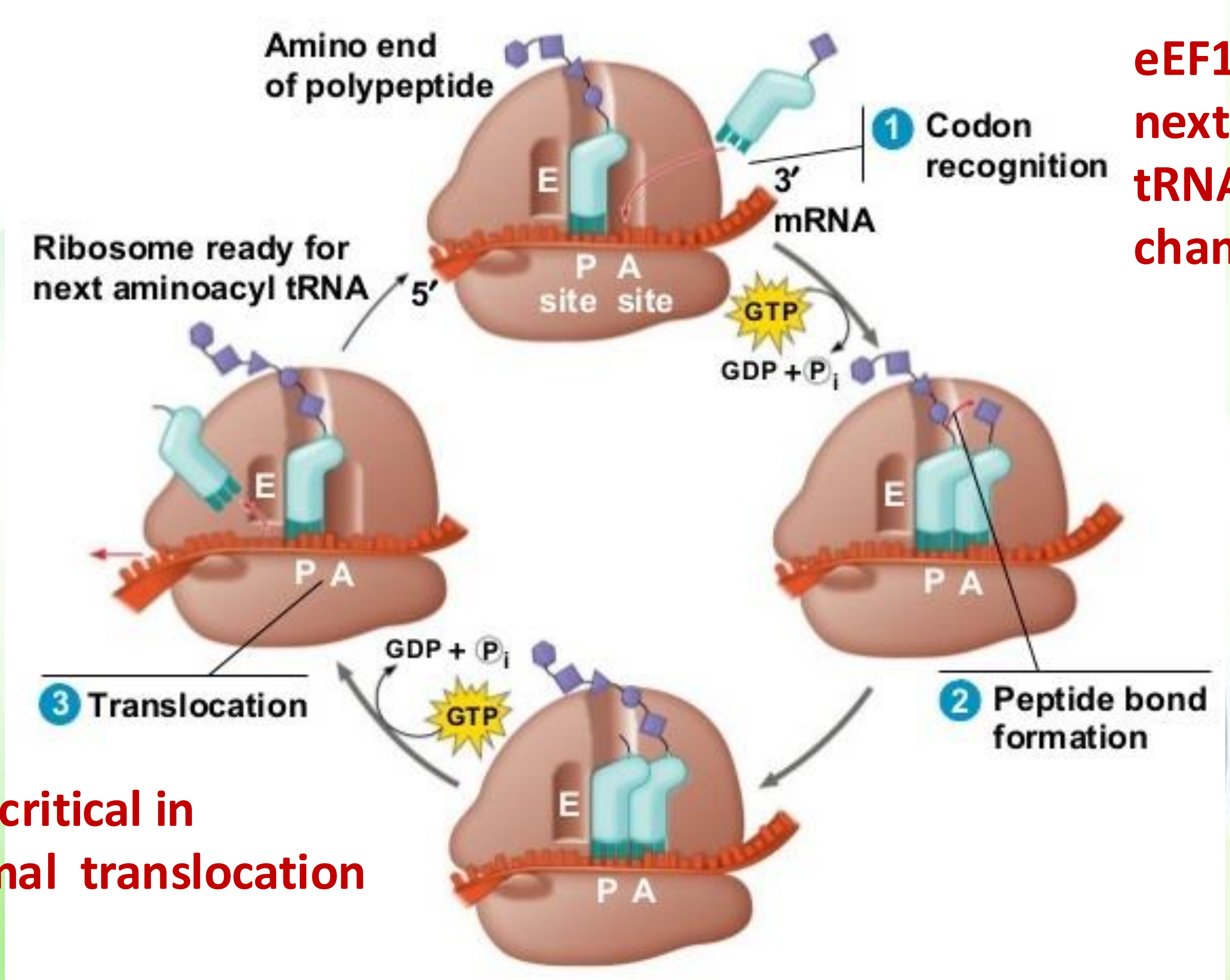


- eIF2 is complexed to GTP to be active. When the correct tRNA is inserted, GTP is hydrolyzed to GDP.
- The active eIF2/GTP complex must be regenerated by exchanging of the GDP for GTP.

Translation elongation

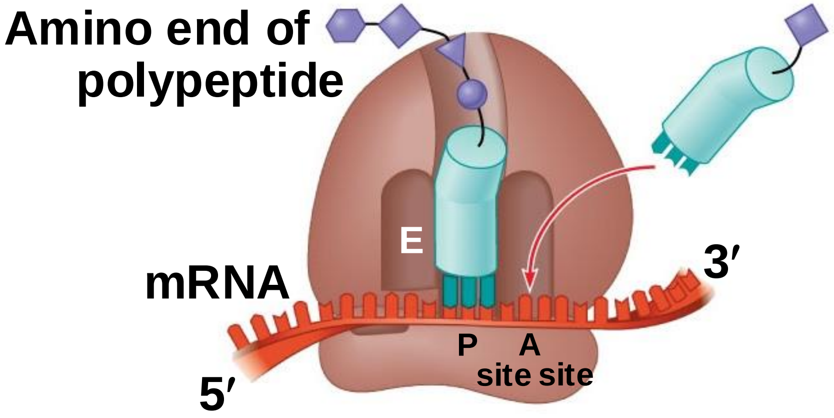
Three steps:

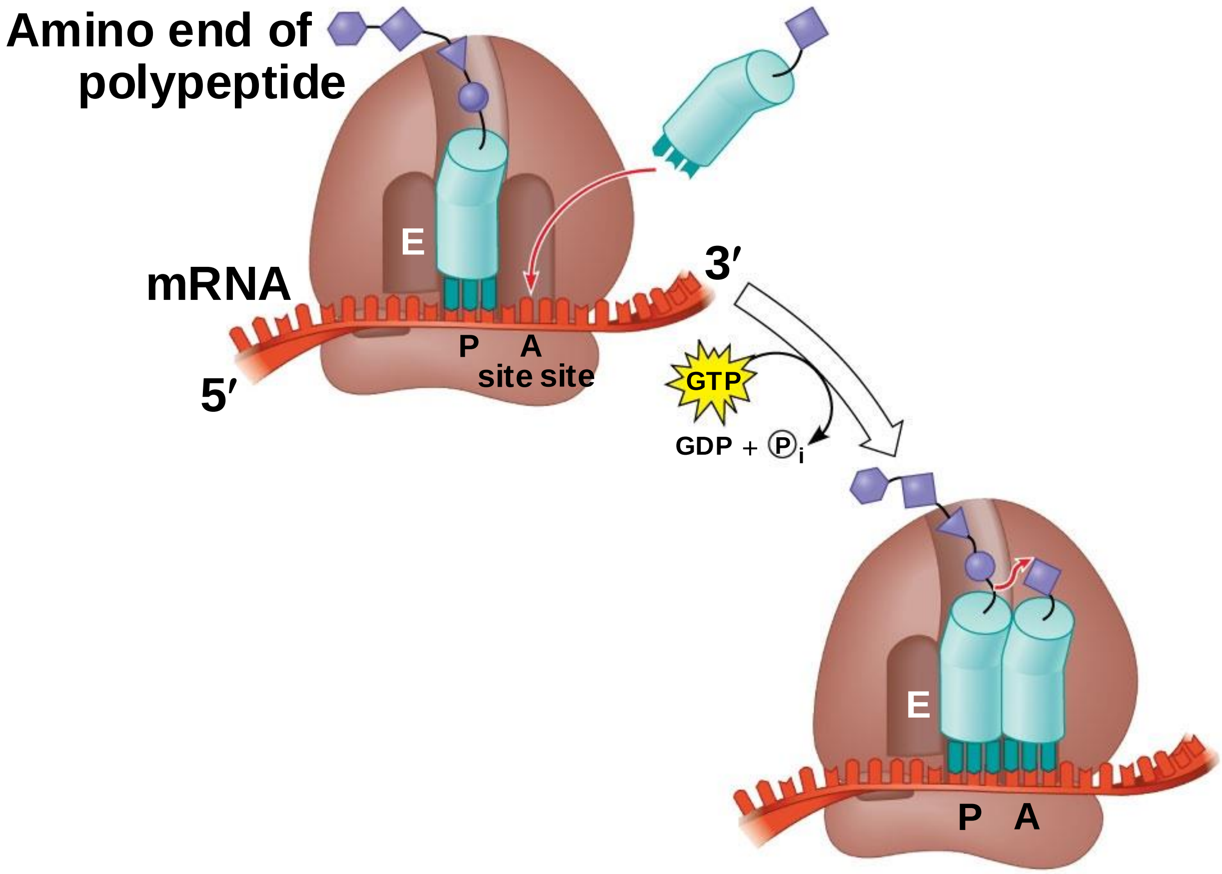
1. aminoacyl-tRNA binding
2. peptide bond formation
3. translocation with the help of elongation factors (eEF).

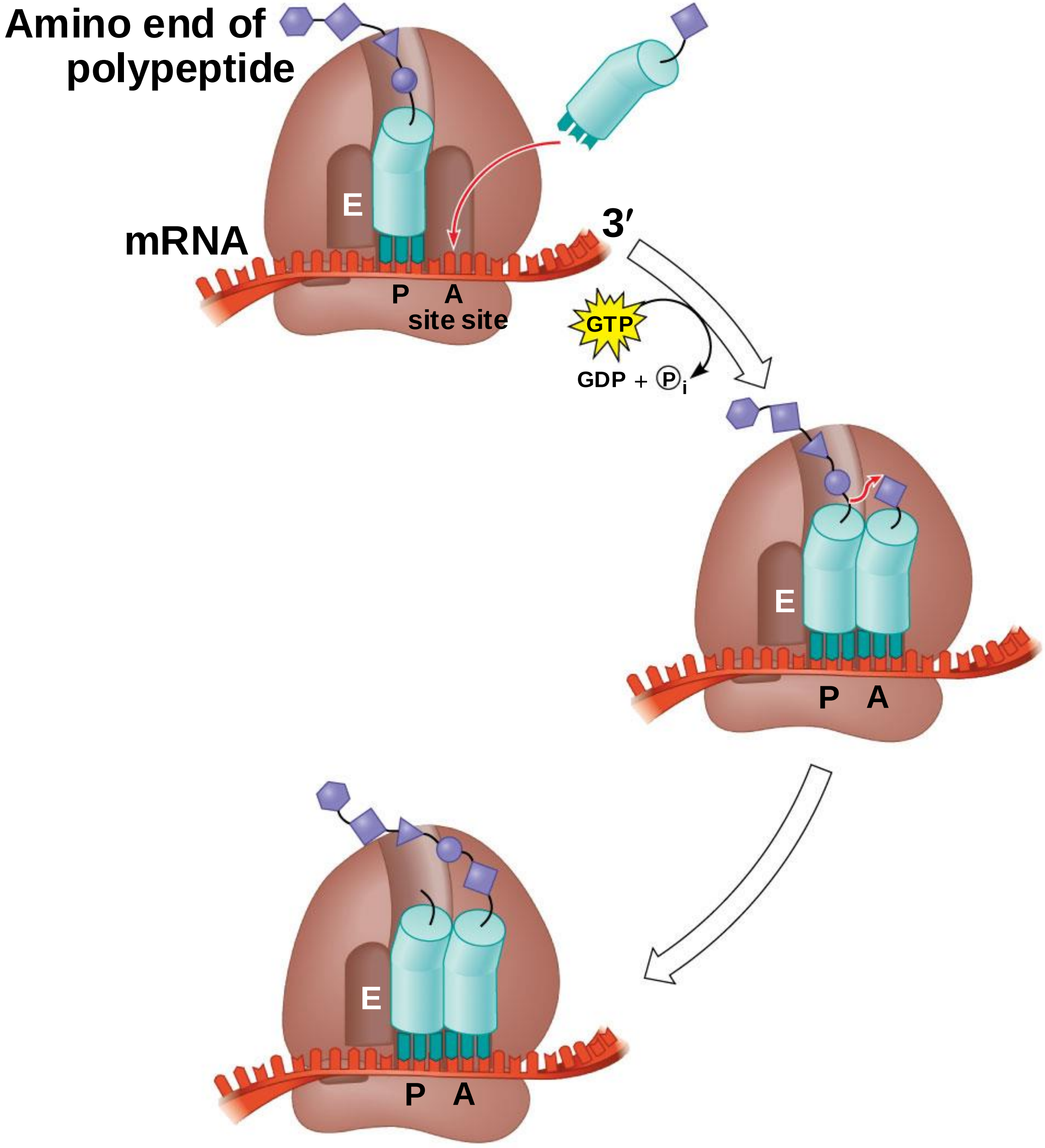


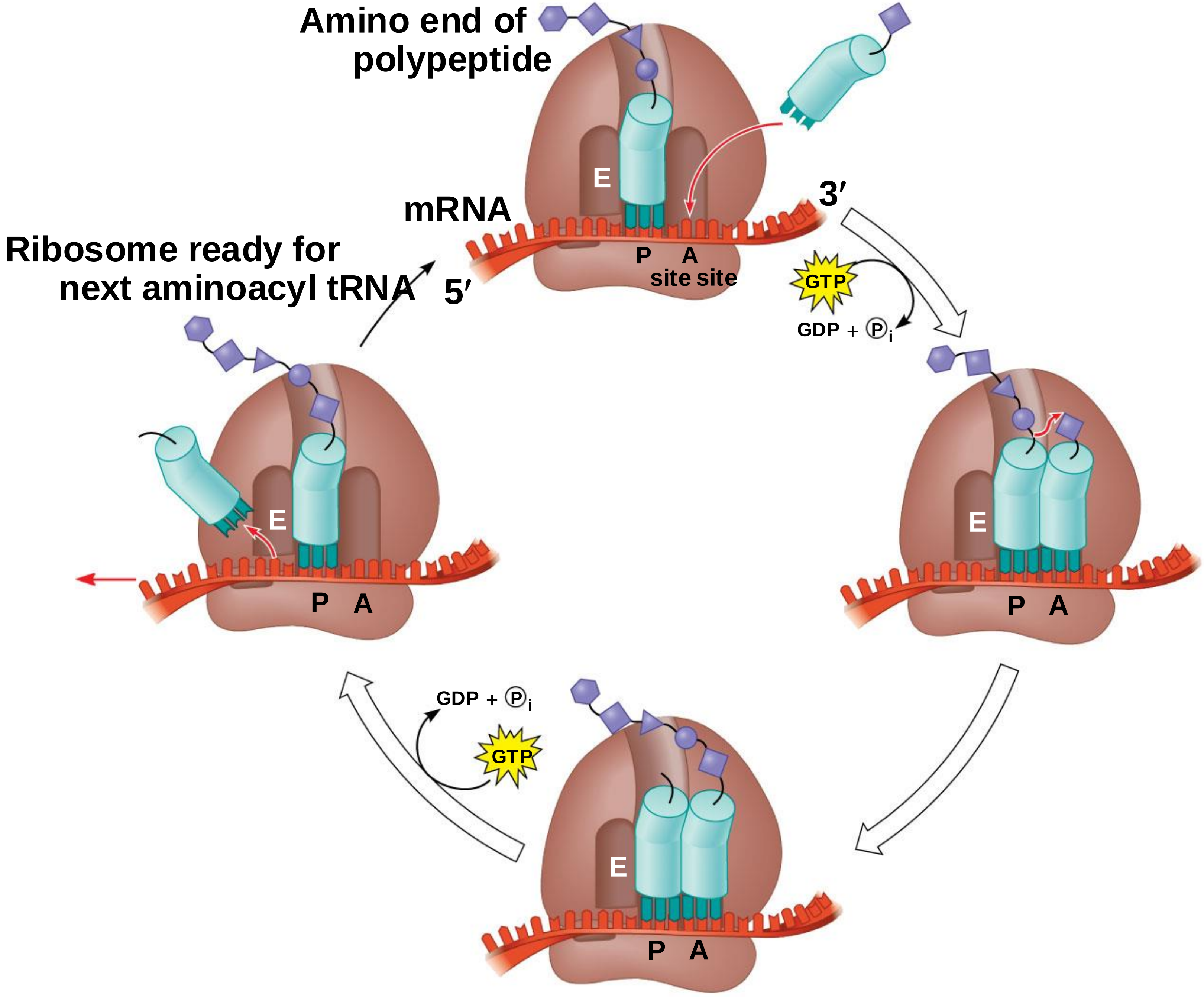
eEF1α brings next aminoacyl-tRNA to the A chamber

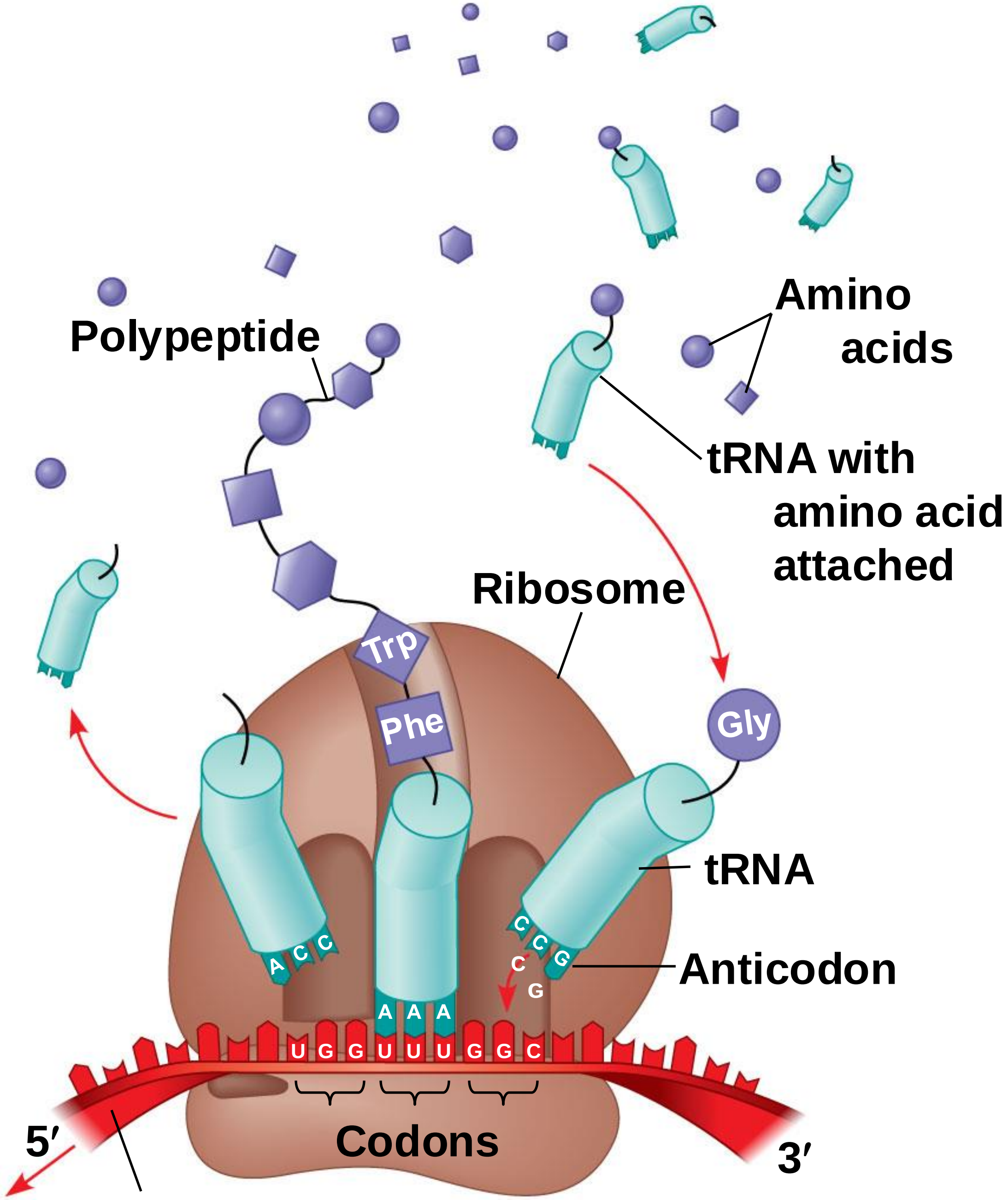
eEF2 is critical in ribosomal translocation

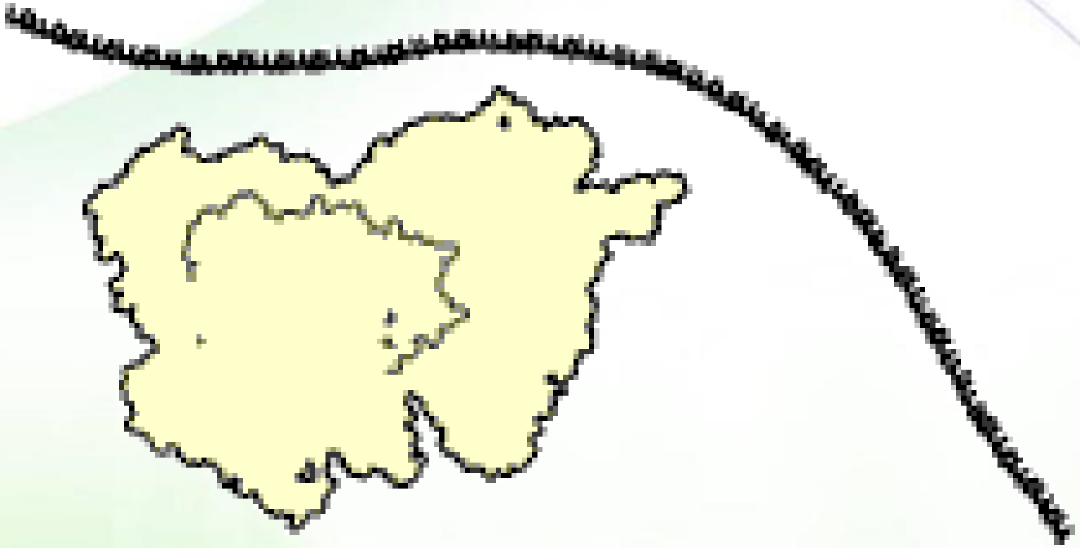

Amino end of polypeptide
E
mRNA
3′
P site
A site
5′


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


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

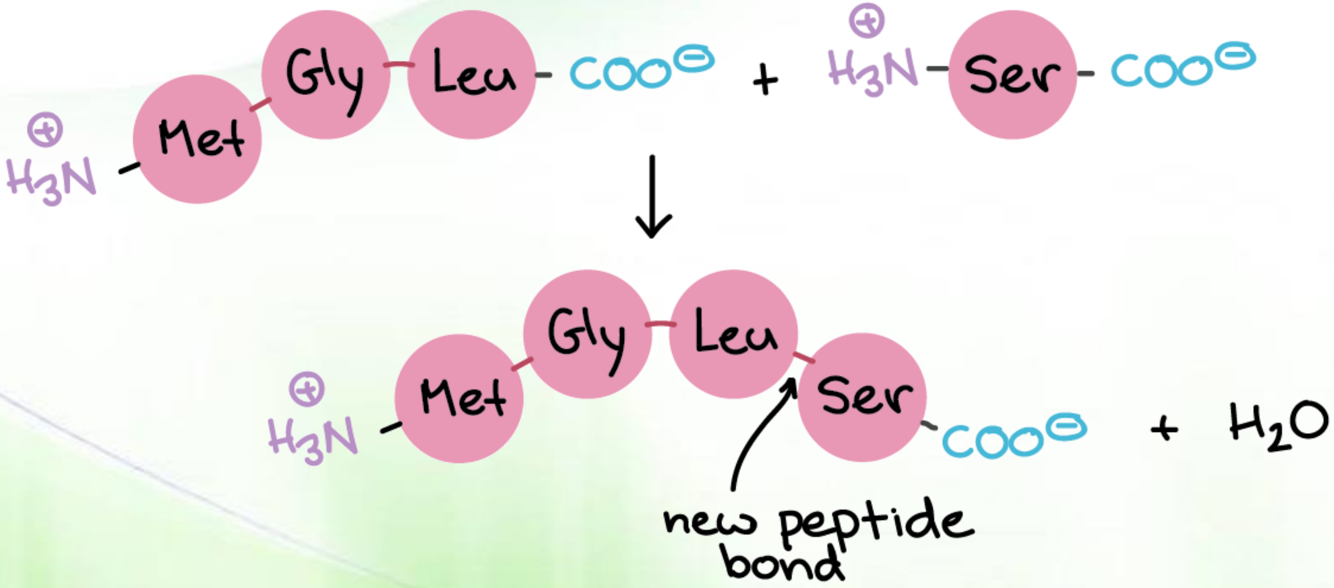

Amino end of polypeptide
E
mRNA
3′
5′
P site
A site
Ribosome ready for next aminoacyl tRNA
GTP
GDP + Ⓟi
E
P
A
E
P
A
GDP + Ⓟi
GTP
E
P
A


Polypeptide
Amino acids
tRNA with amino acid attached
Ribosome
Trp
Phe
Gly
tRNA
Anticodon
A C C
A A A
C C G
C
G
U G G U U U G G C
Codons
5′
3′
mRNA



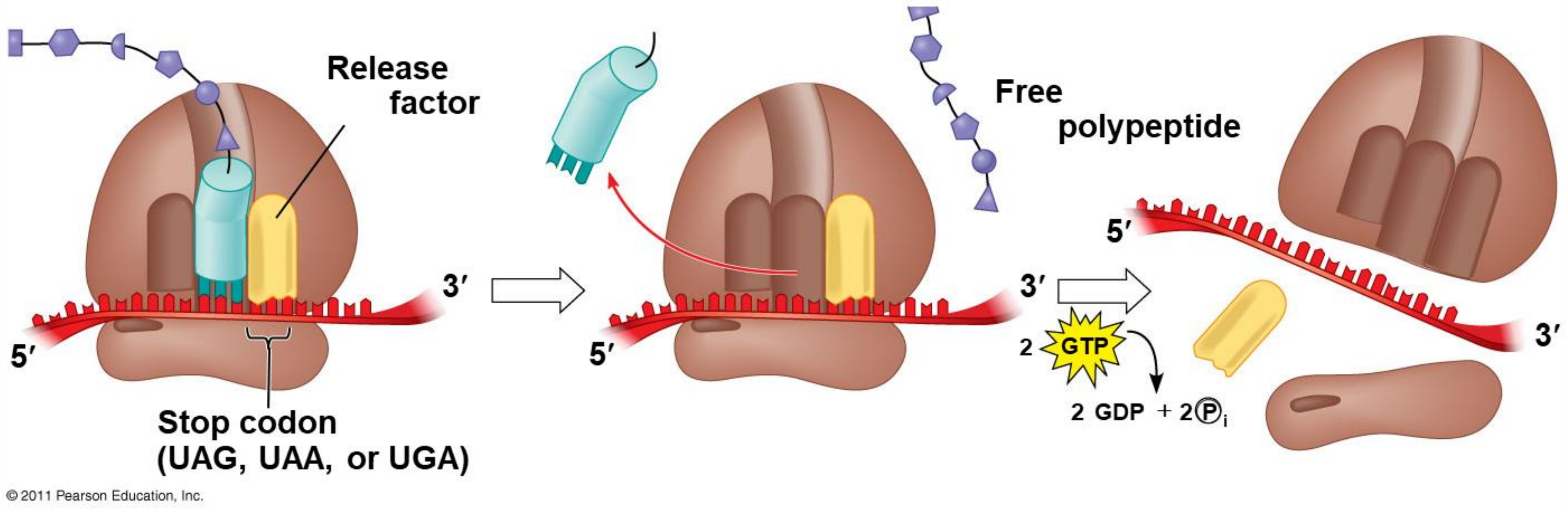
Elongation of the Polypeptide Chain

- 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.



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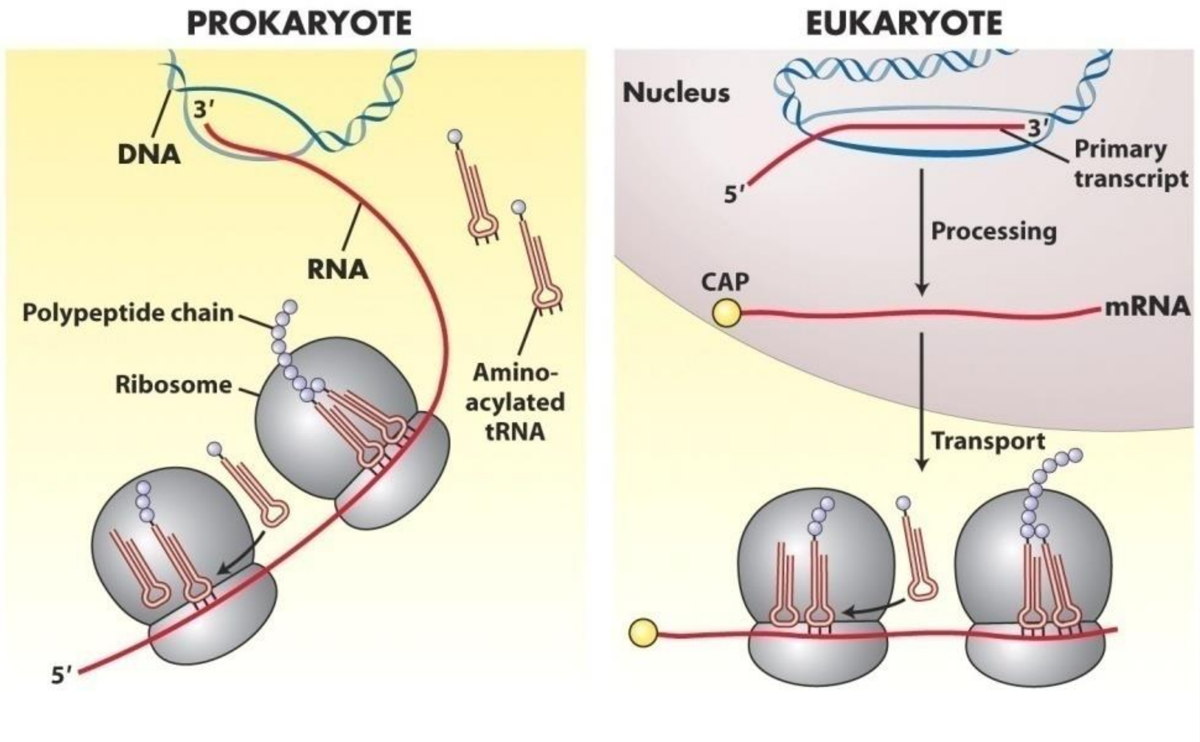
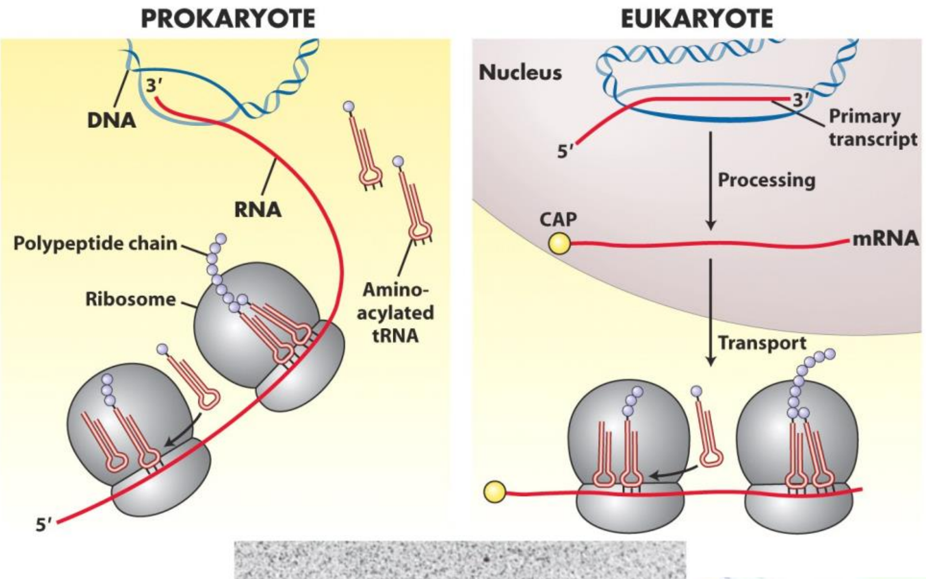
Transcription/translation Coupling

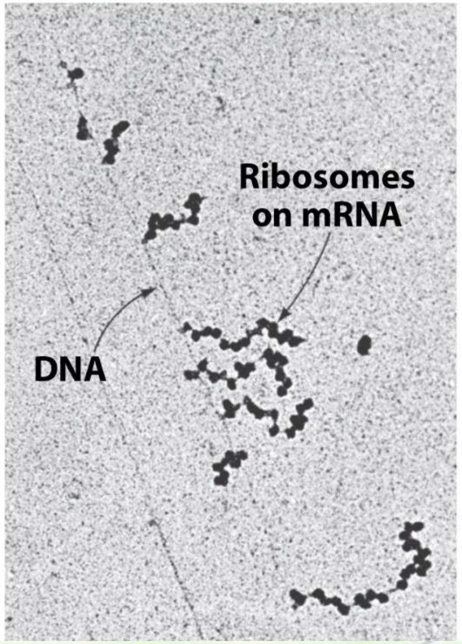
- Translation and transcription are coupled in space and time in prokaryotes.



Polyribosomes (polysomes)

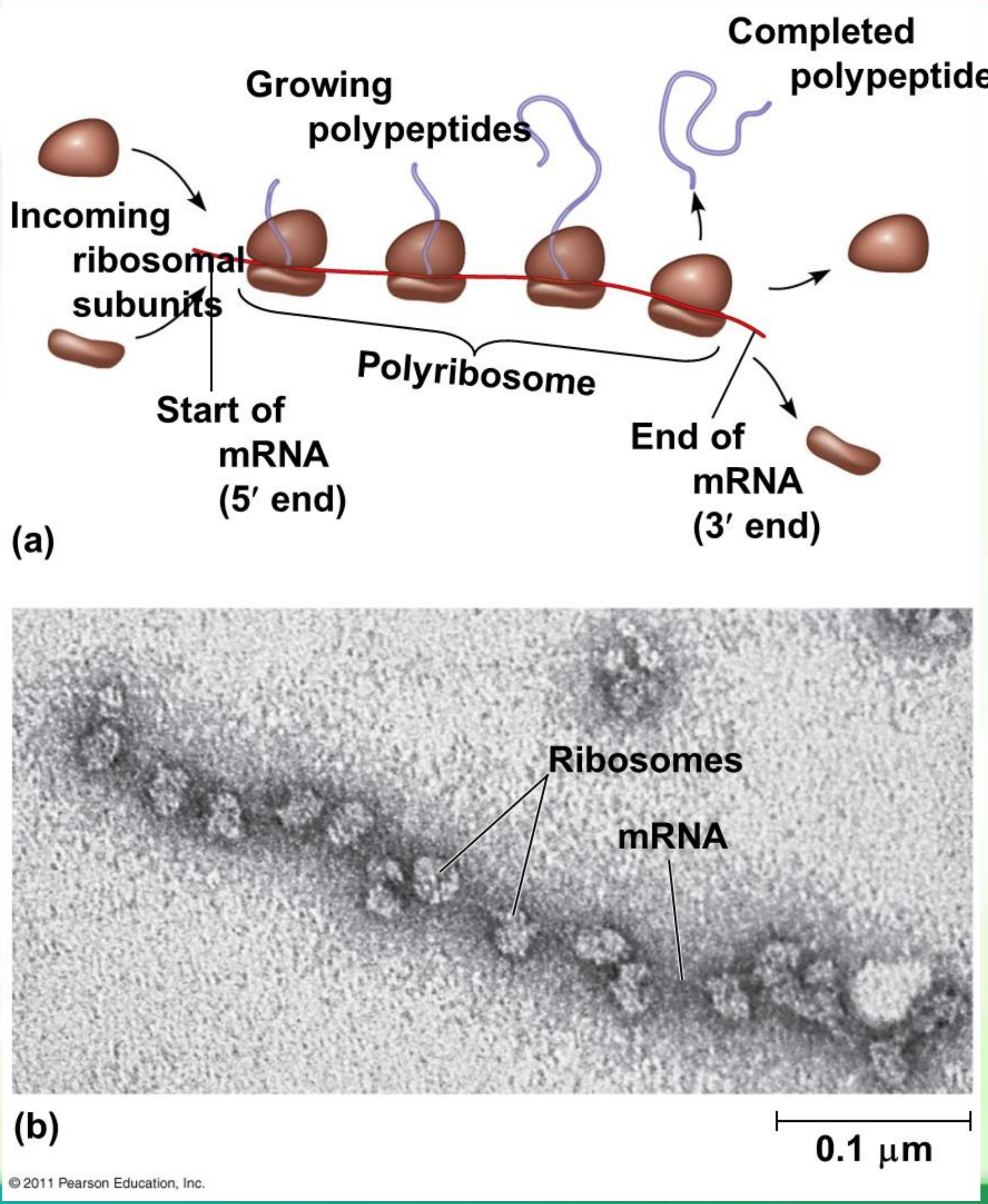
- A single mRNA molecule is translated by several ribosomes simultaneously. Each ribosome produces one copy of the polypeptide chain specified by the mRNA. When the protein has been completed, the ribosome dissociates into subunits that are used in further rounds of protein synthesis.





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.



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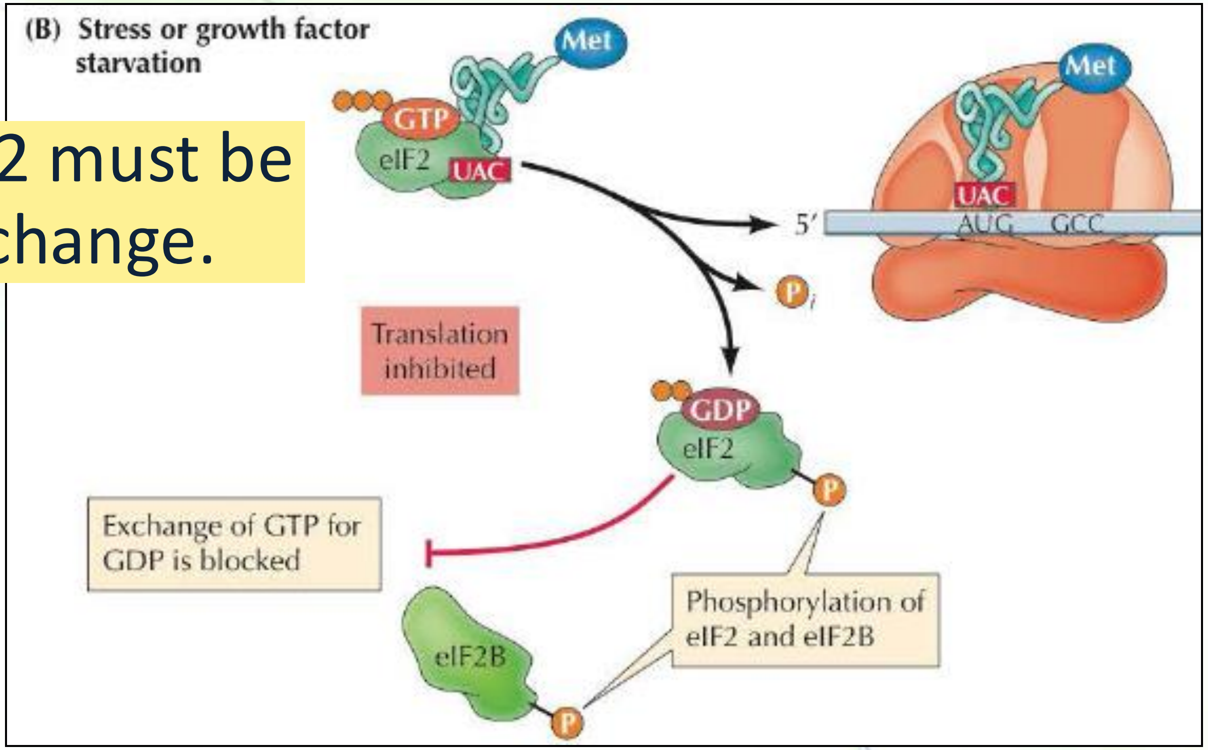
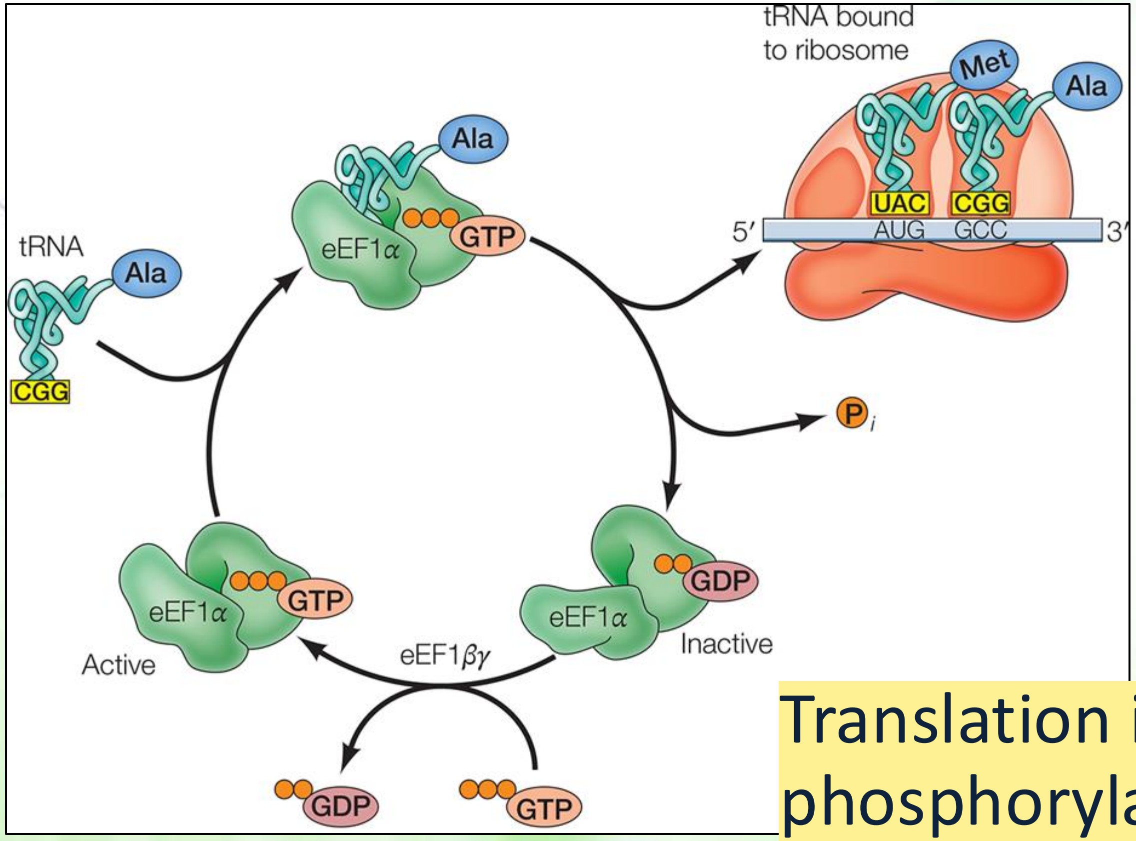
Regulation of translation

Global regulation



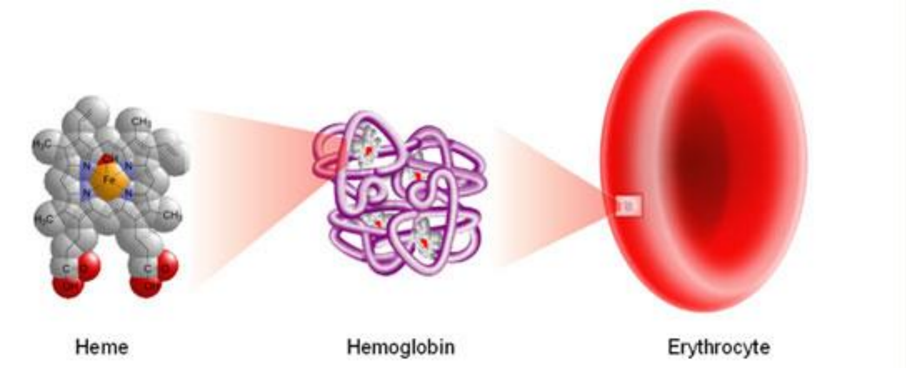
- To continue translation, eIF2 must be reactivated by GTP/GDP exchange.

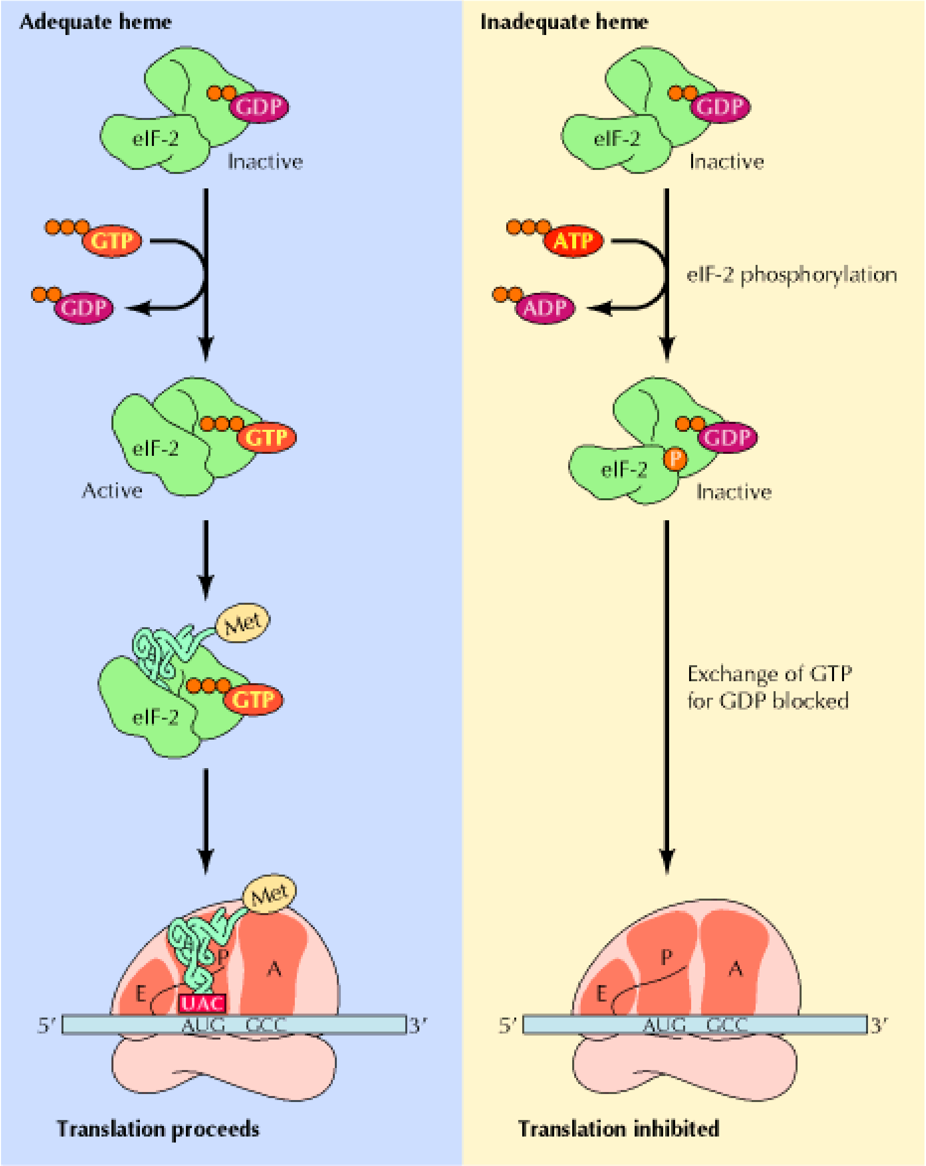


Translation is inhibited by regulatory kinases that phosphorylate eIF2 blocking the GTP/GDP exchange.

Heme and protein synthesis

- In reticulocytes (immature erythrocytes), if adequate heme is available, heme stimulates overall protein synthesis.
- If adequate heme is available, GDP-GTP exchange occurs and translation can proceed.
- If heme supplies are inadequate, a protein kinase phosphorylates eIF2.





Regulation of mRNA stability

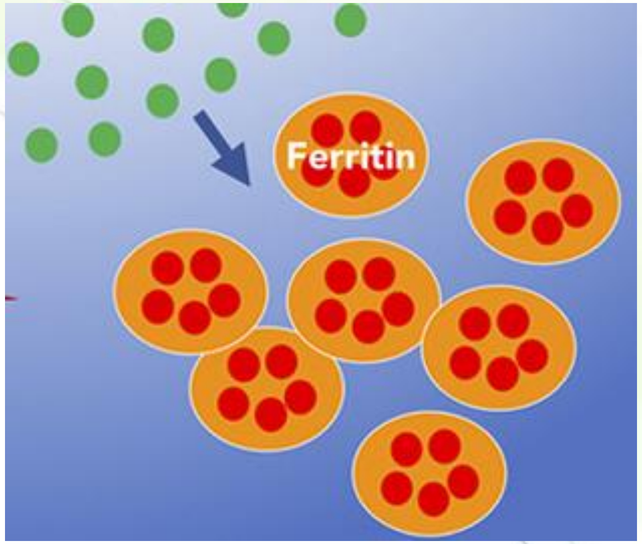
Physiology of iron

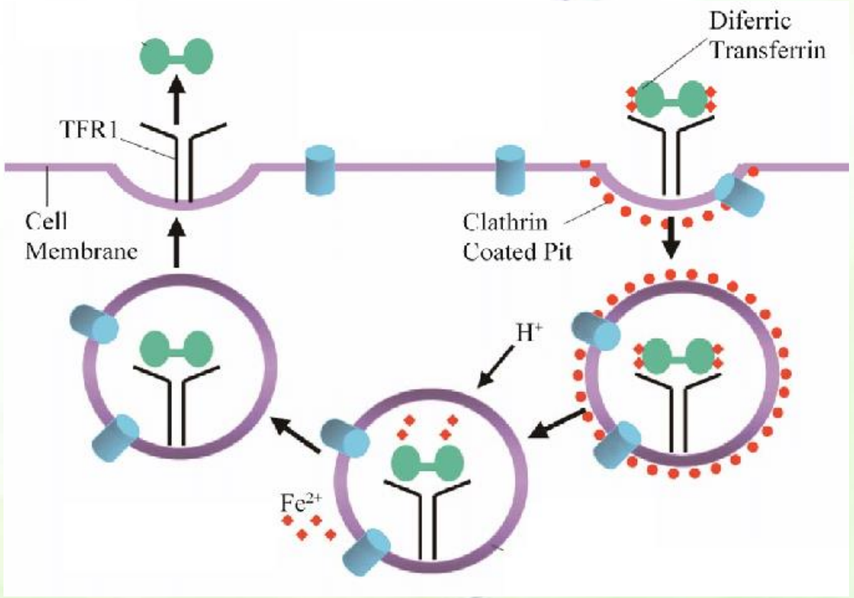
- Iron is an essential metal for the human body.
  - Oxygen transport
  - Enzyme function
- Too much iron can be toxic.
  - Organ failure
  - Bacterial infection
- The level of iron is intricately maintained.

The players

- Liver ferritin protein stores iron when abundant (in the liver).
- Transferrin receptor mediates iron entry via receptor-mediated endocytosis into peripheral cells when needed.

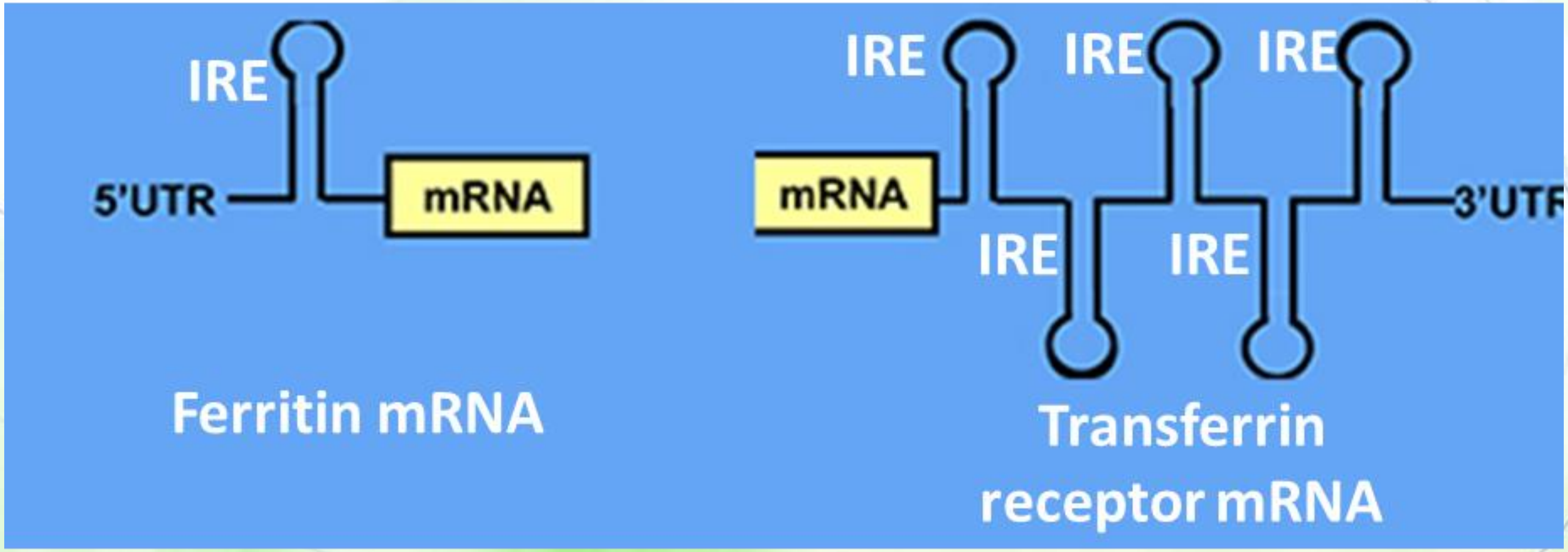
- When iron is high, expression of ferritin should be up-regulated and expression of transferrin receptor should be down-regulated, and vice versa.





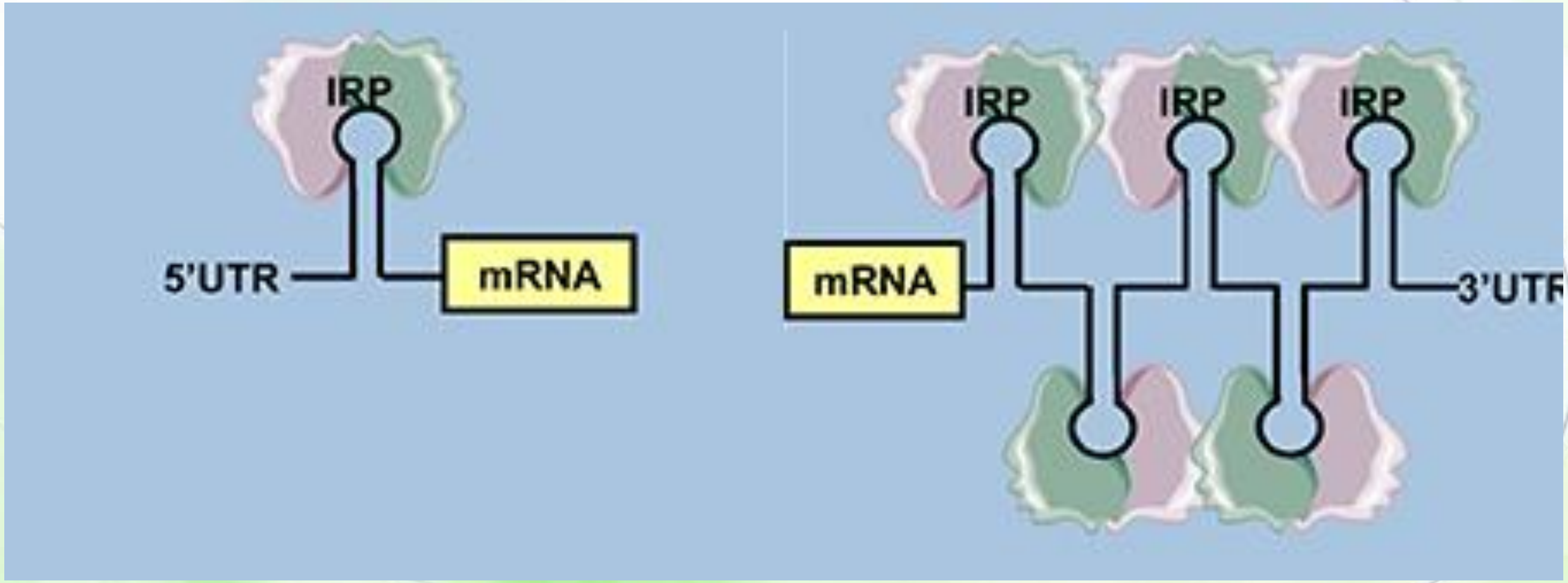
Iron-response elements

- 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.



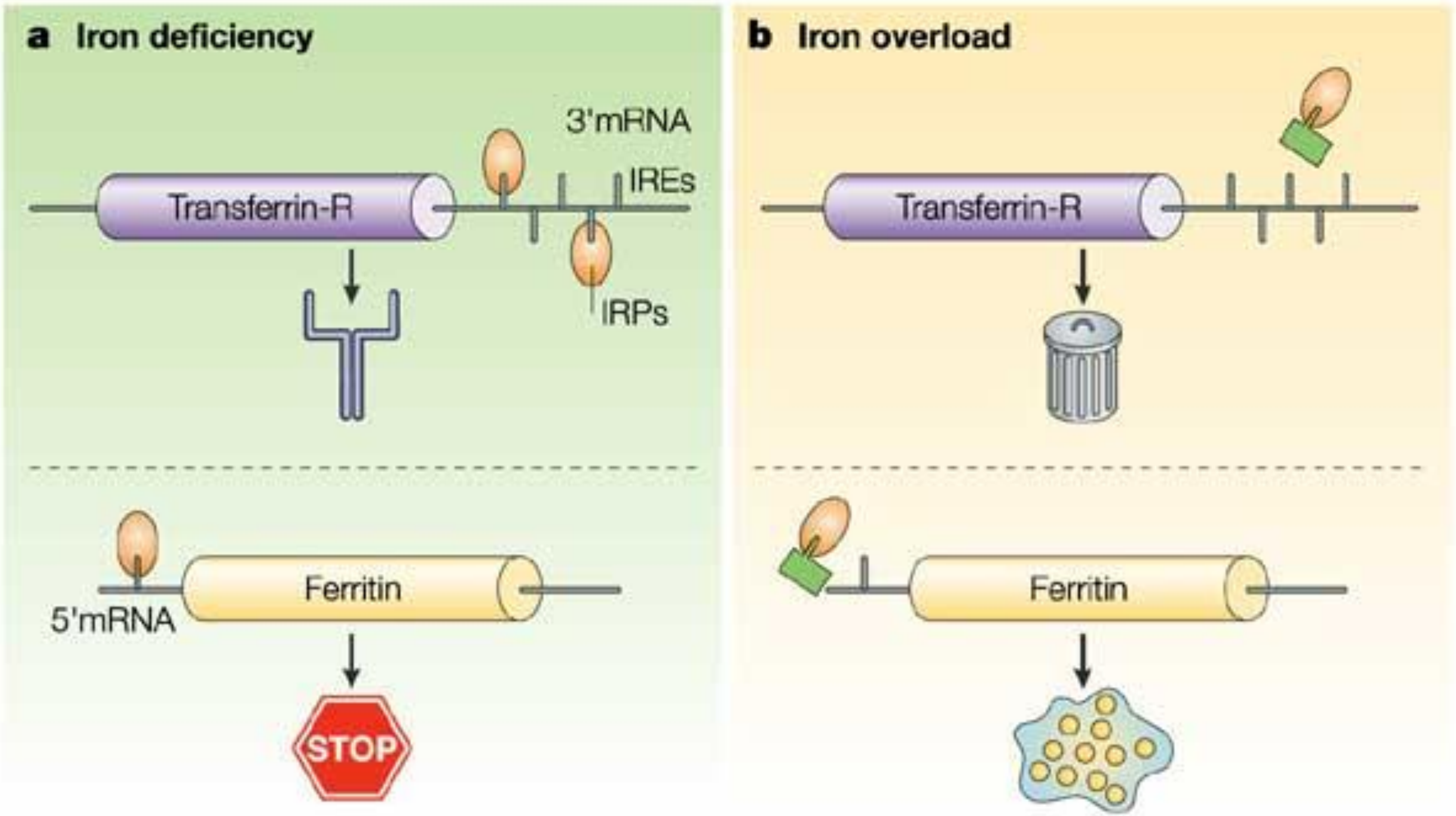
Iron regulatory protein

- 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.



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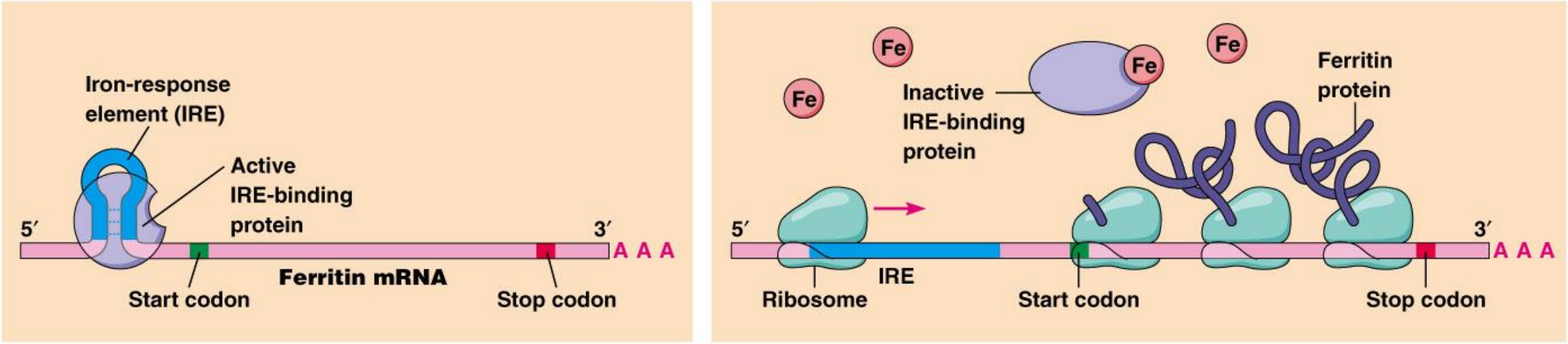
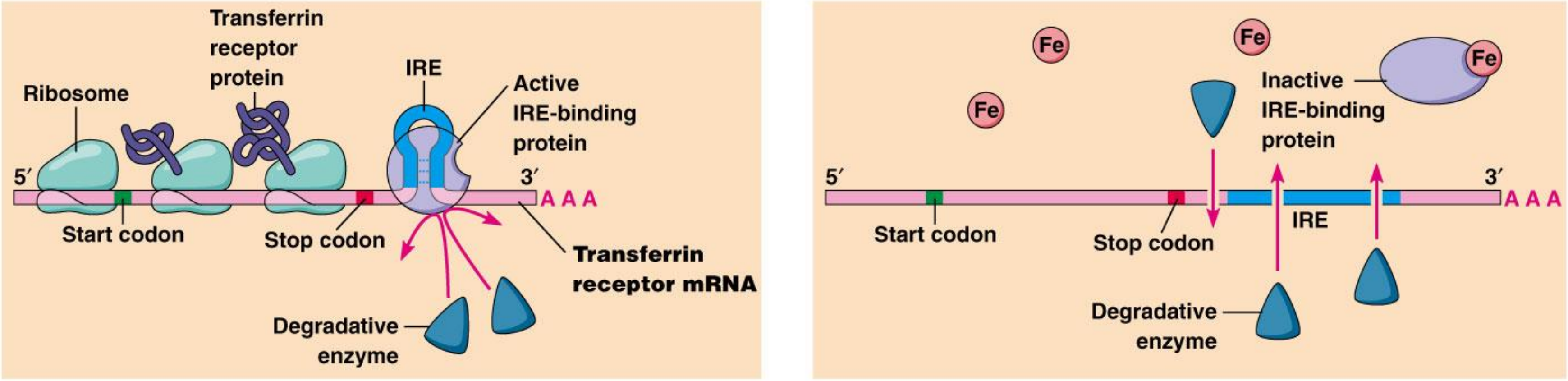


(a) Low iron concentration. IRE-binding protein binds to IRE, so translation of ferritin mRNA is inhibited.

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

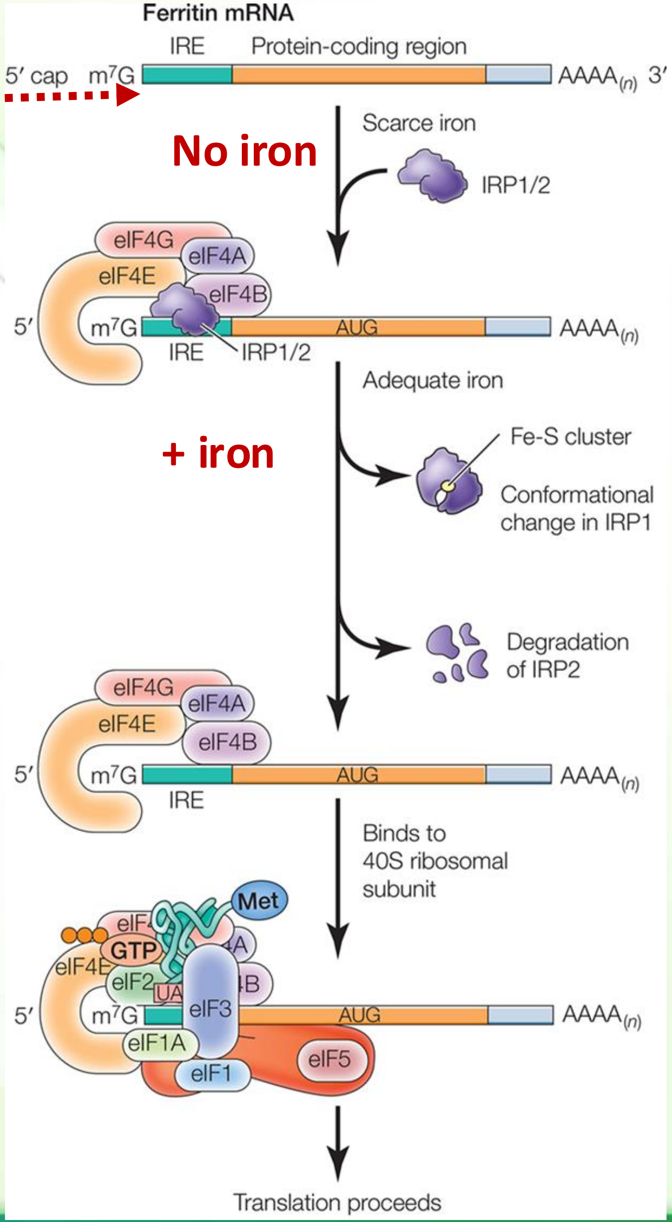


(a) Low iron concentration. IRE-binding protein binds to the IRE of transferrin receptor mRNA, thereby protecting the mRNA from degradation. Synthesis of transferrin receptor therefore proceeds.

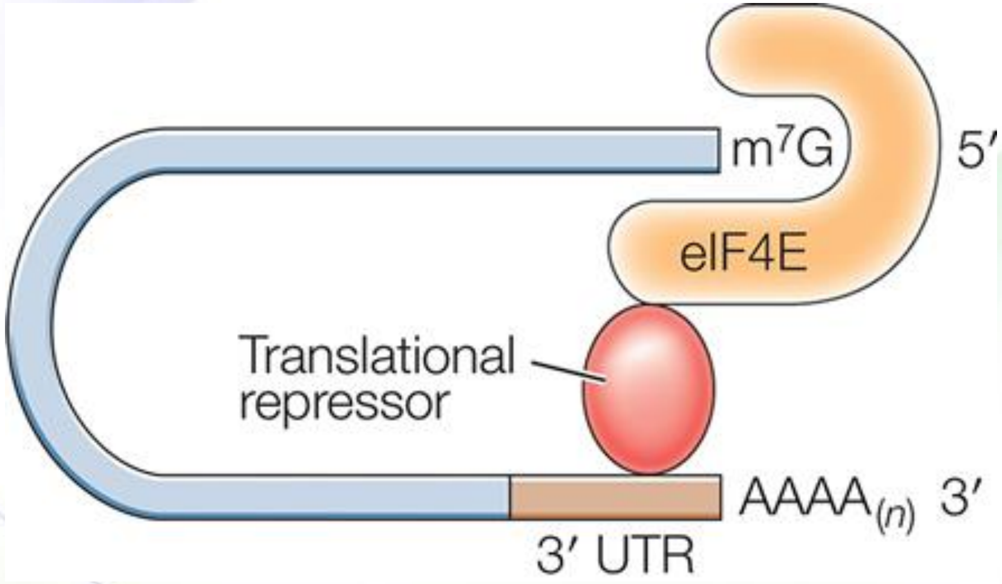
(b) High iron concentration. IRE-binding protein cannot bind to IRE, so mRNA is degraded and synthesis of transferrin receptor is thereby inhibited.

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.



Regulation of eIF4E



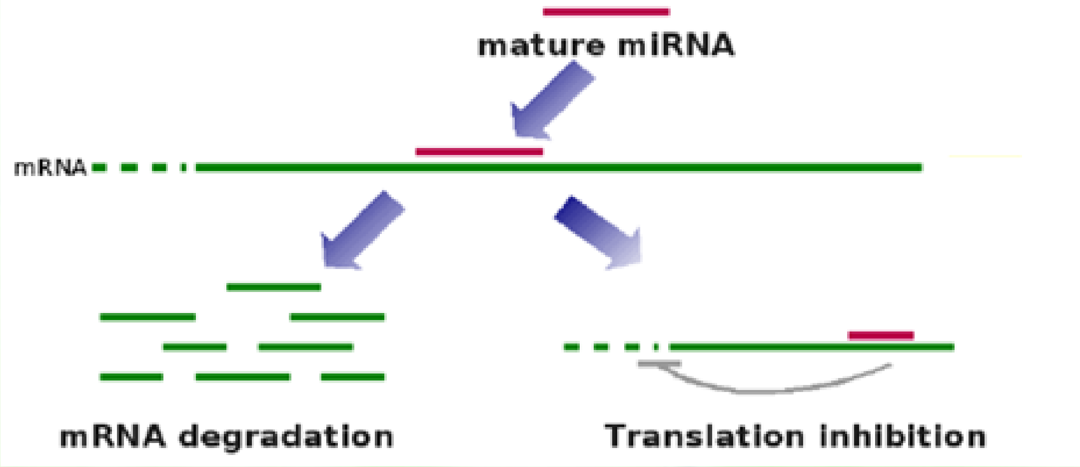
- Translational repressors can bind to regulatory sequences in the 3′ untranslated region (UTR) and inhibit translation by binding to the initiation factor eIF4E, bound to the 5′ cap.
- This interferes with translation by blocking the formation of a normal initiation complex

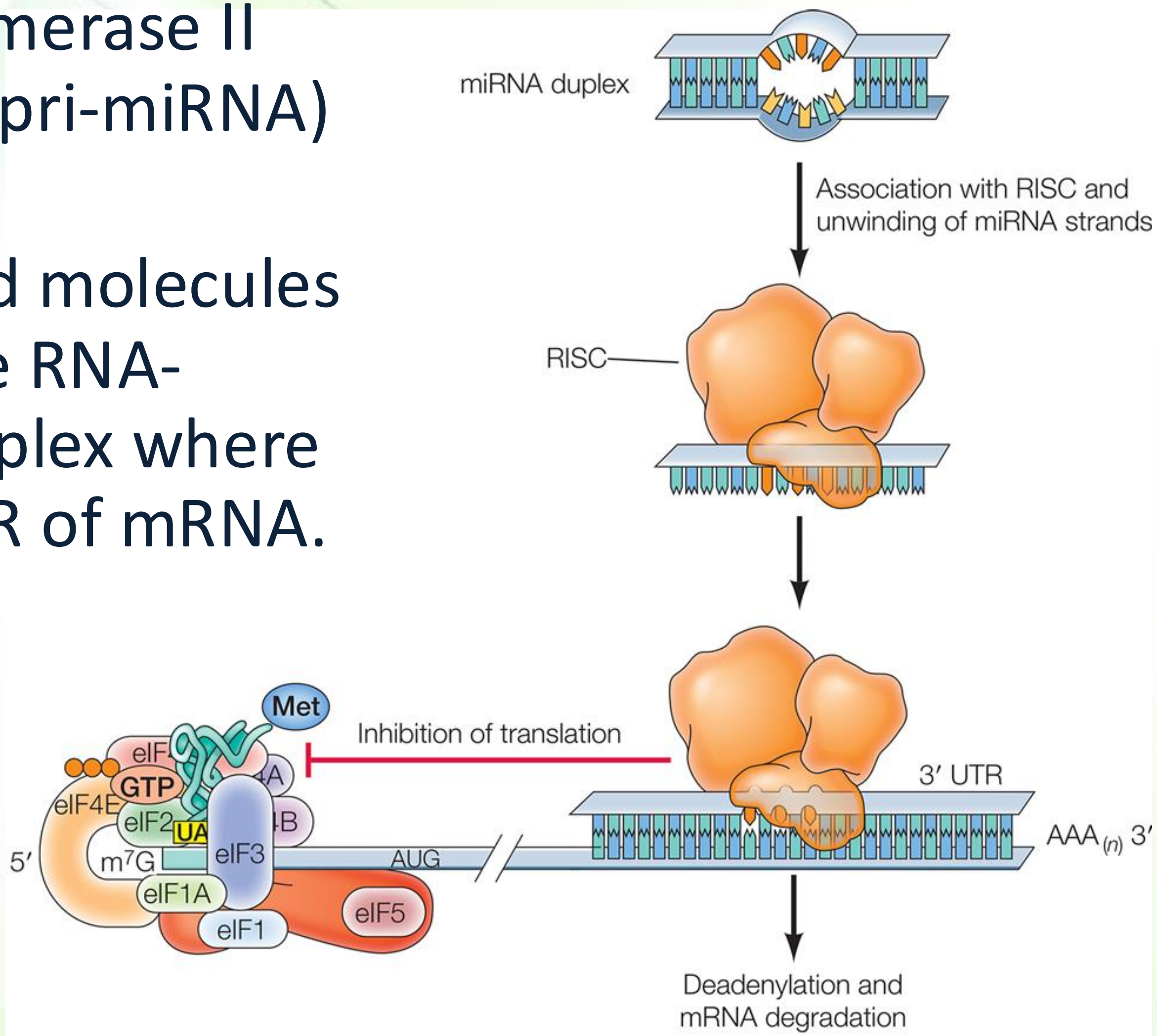
Regulation by microRNA (miRNA)

and siRNA

Regulation by microRNA (miRNA)

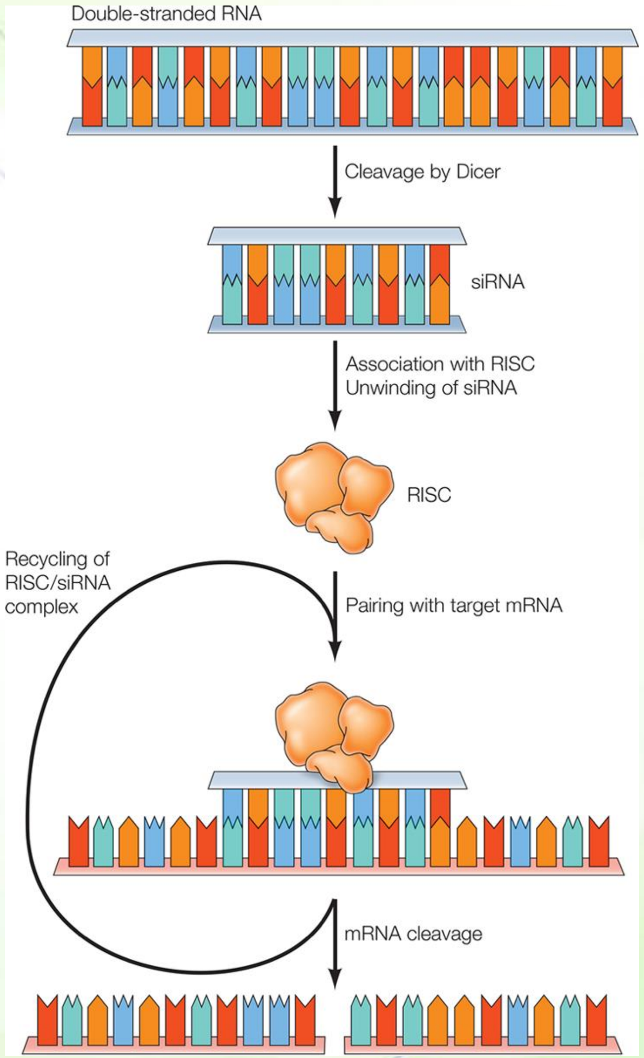
- MicroRNA is synthesized by RNA polymerase II into single-stranded, primary miRNA (pri-miRNA) transcript.
- It gets processed into double-stranded molecules but only one strand is loaded onto the RNA-induced silencing complex (RISC) complex where miRNA targets and binds to the 3’-UTR of mRNA.
- The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





RNA interference and short interfering RNA (siRNA)

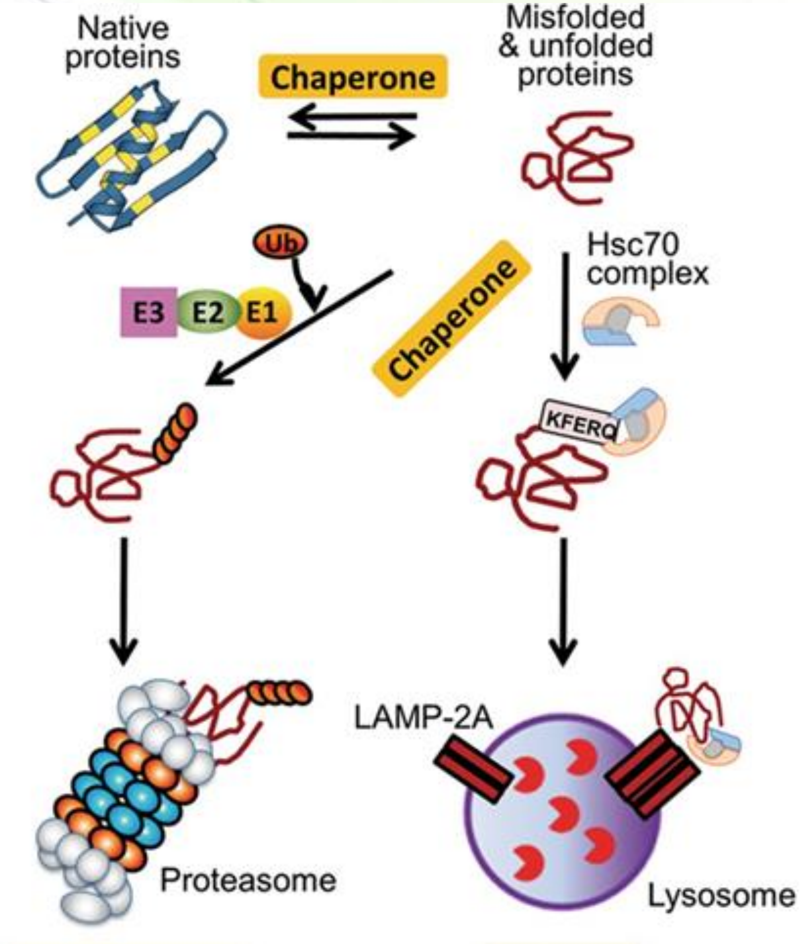
- Targeting mRNA by synthetic siRNA can be used for experimental and therapeutic purposes.
- The double-stranded siRNA associates with the RISC complex, which targets one strand onto a homologous mRNA.
- The mRNA is cleaved and, as a result, the protein level decreases or the protein is not expressed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



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.



The multi-levels of regulation

Levels of regulation

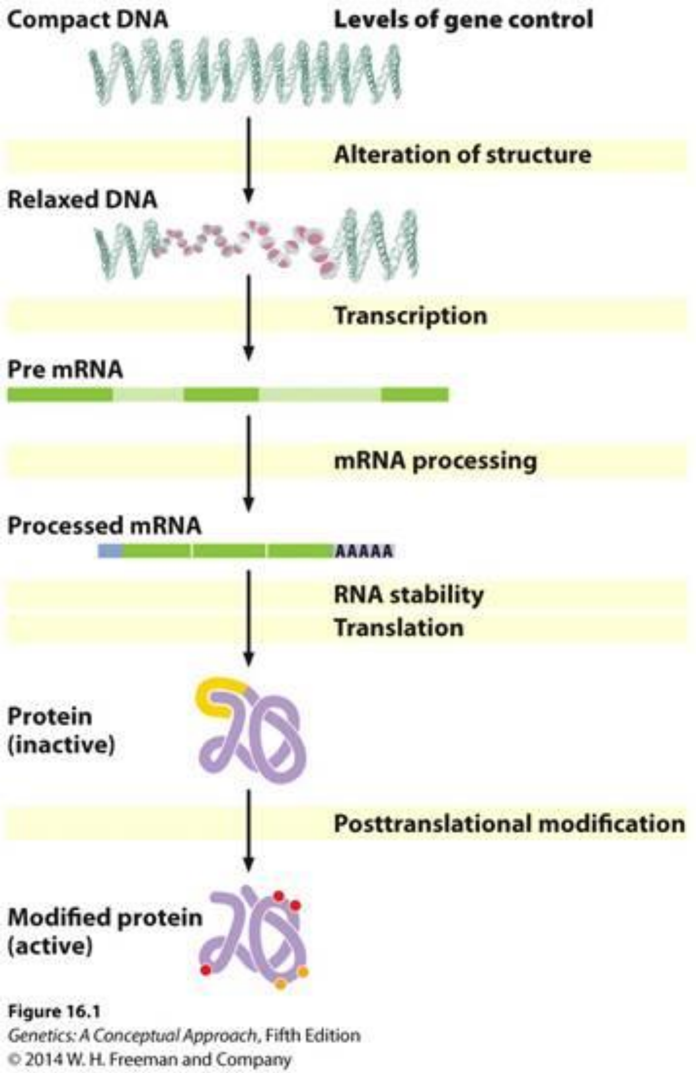


Figure 16.1
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- Transcription
- RNA processing
- RNA transport
- mRNA stability
- Translation
- Post-translational modification
- Protein activity
- Protein degradation