

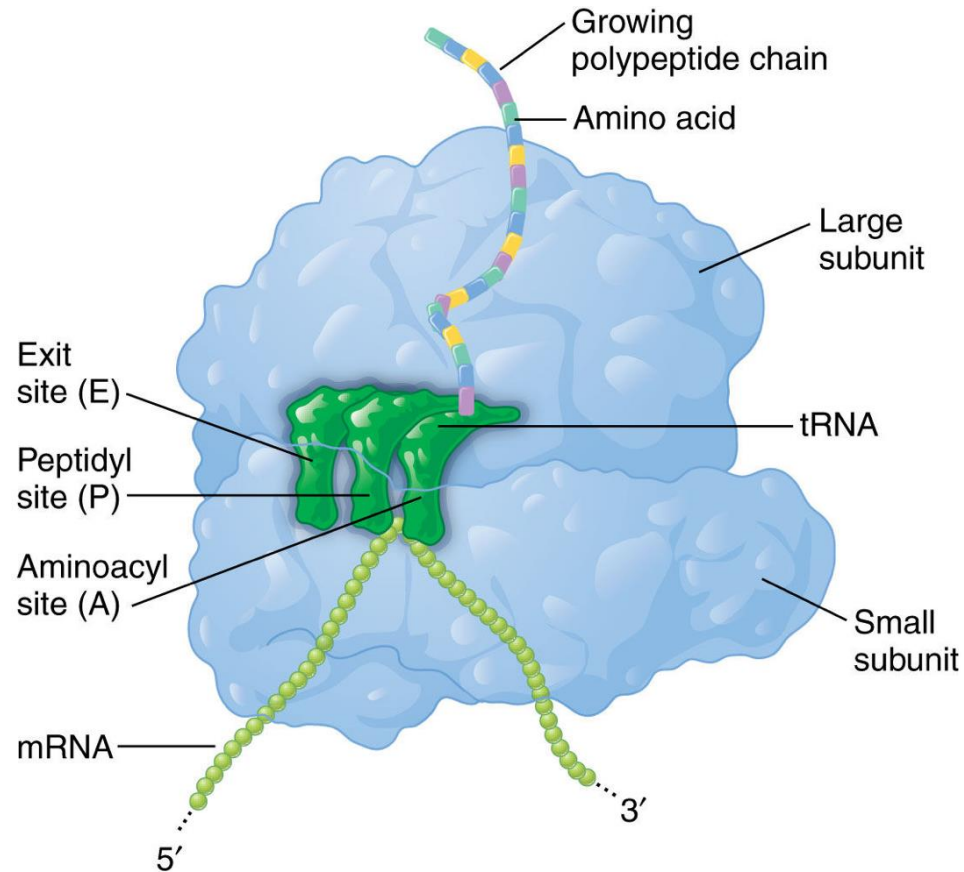


Molecular Biology (12)

Translation and its regulation

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School of Medicine
Second year, First semester, 2025-2026

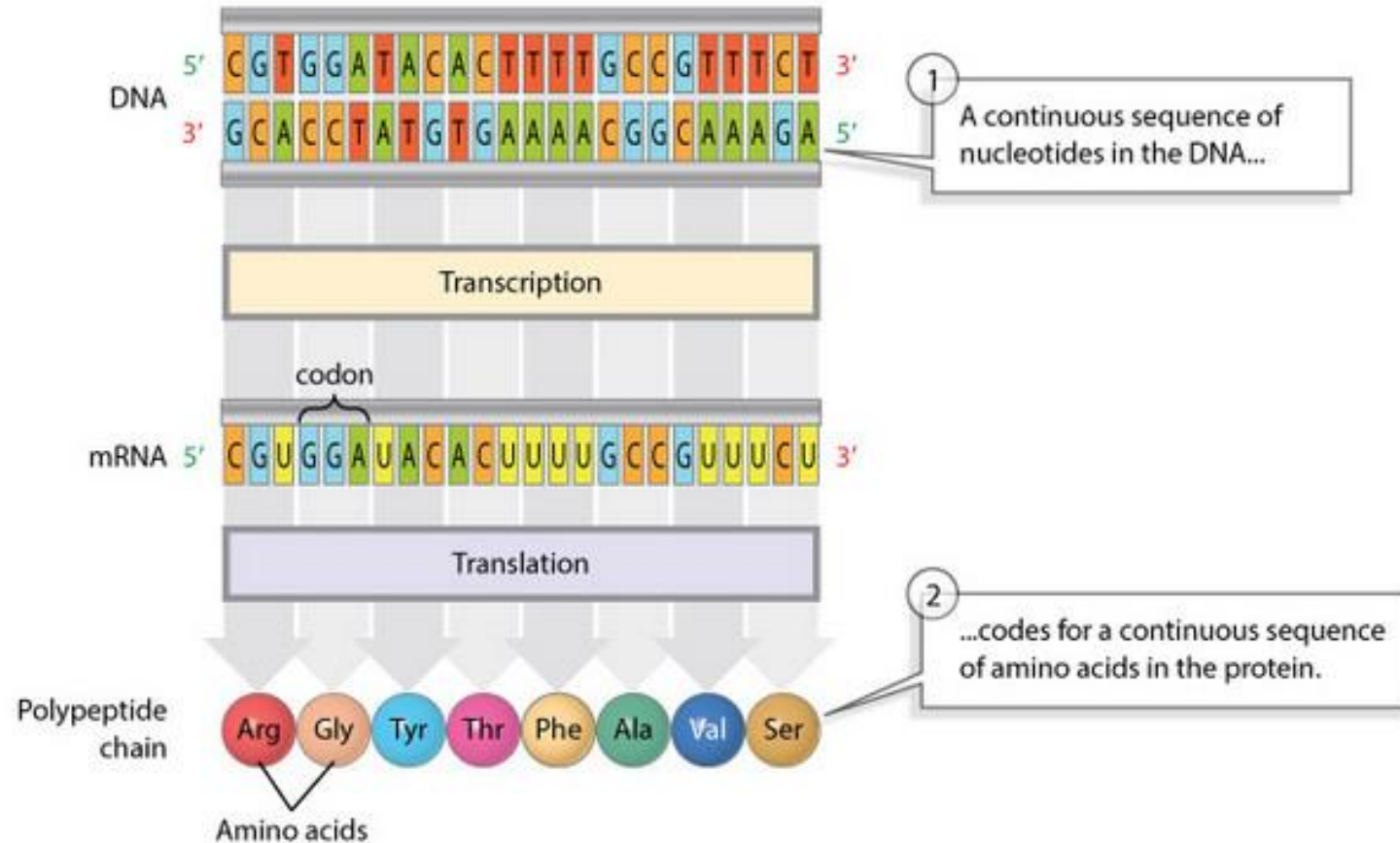
General information



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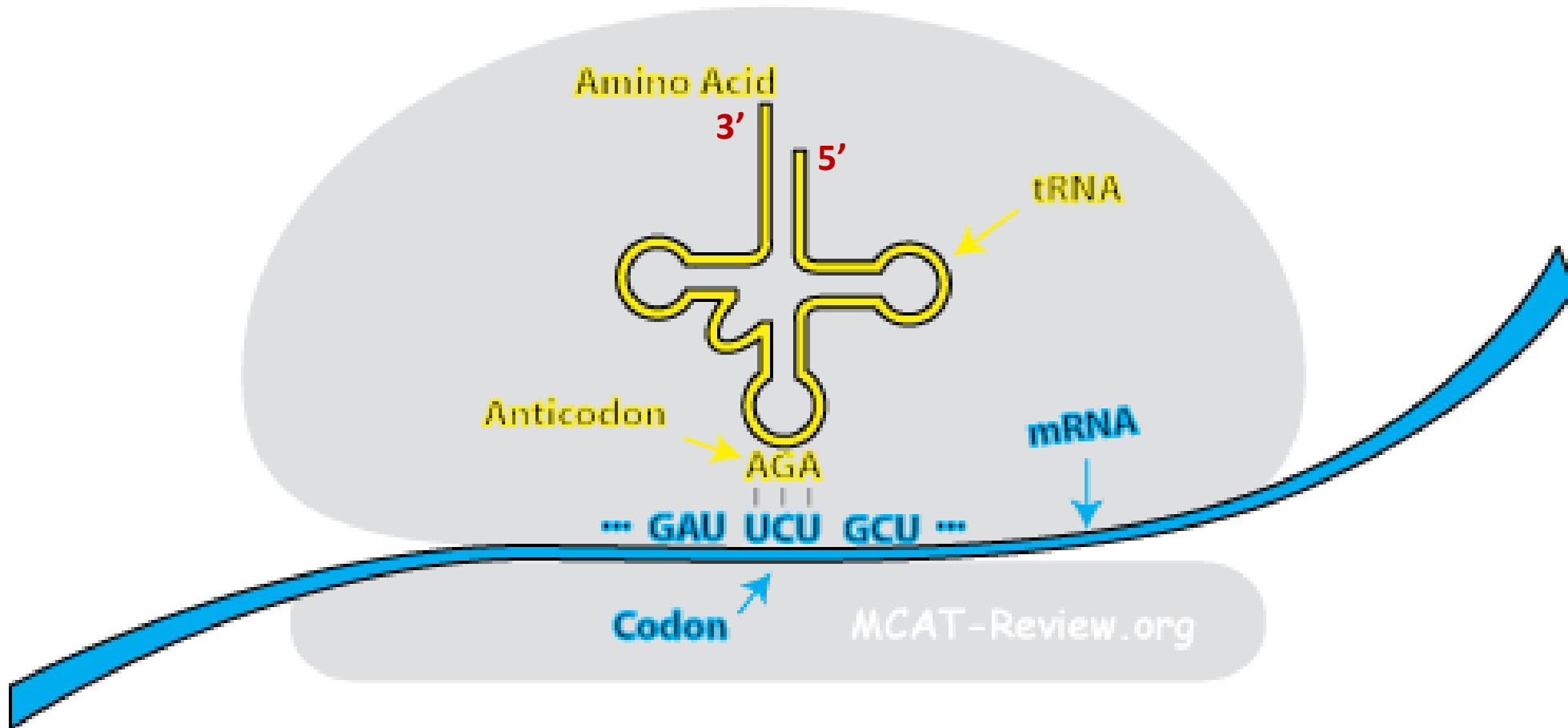
- Protein synthesis involves interactions between ribosomes with three types of RNA molecules:
 - Transfer RNA (tRNAs)
 - Ribosomal RNA (rRNAs)
 - Messenger RNA (mRNA) templates

Colinearity of genes, mRNAs, & proteins



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

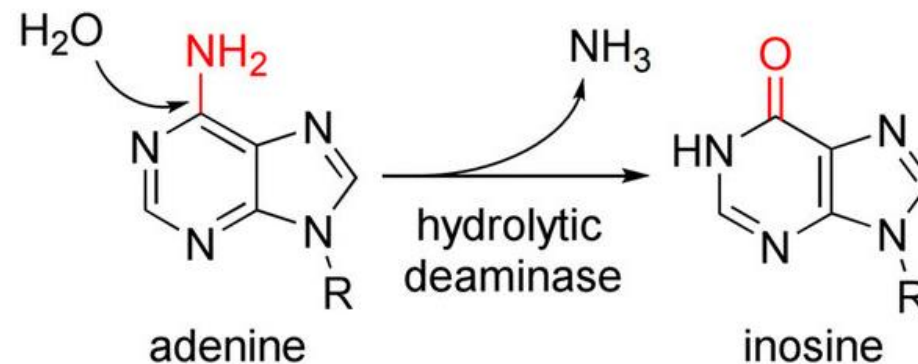
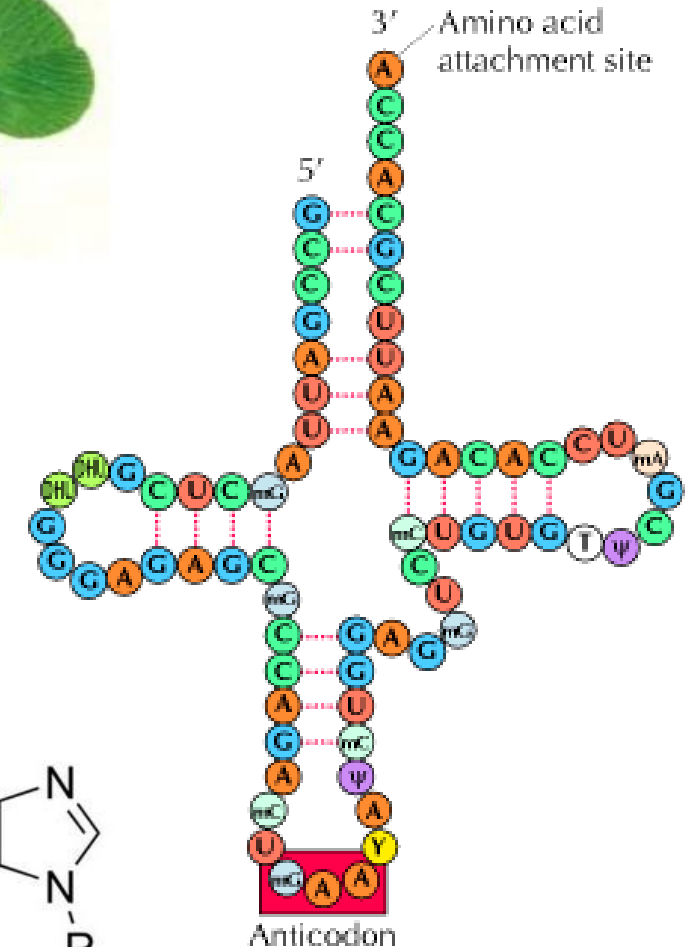
mRNA is read by tRNA in triplets



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 and modified 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 amino acids

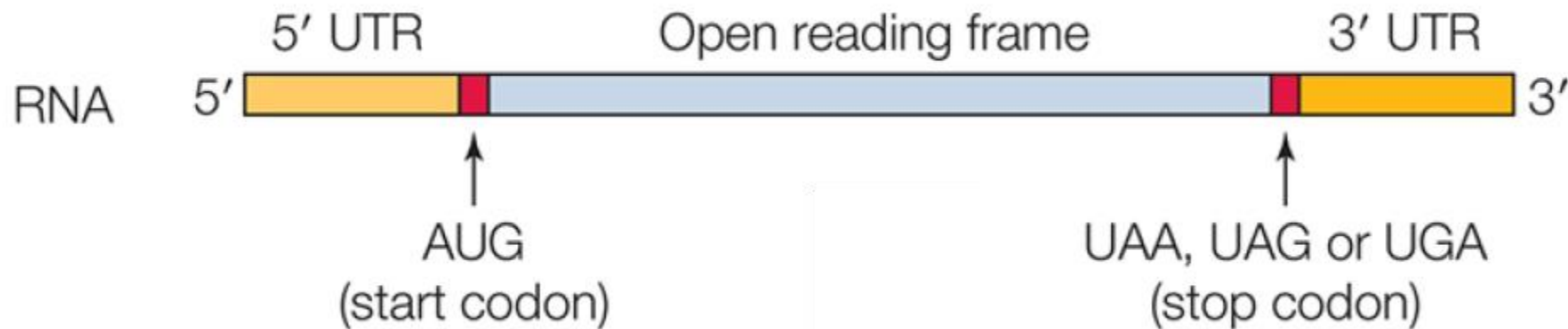
Hydrophilic non-charged amino acids

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.

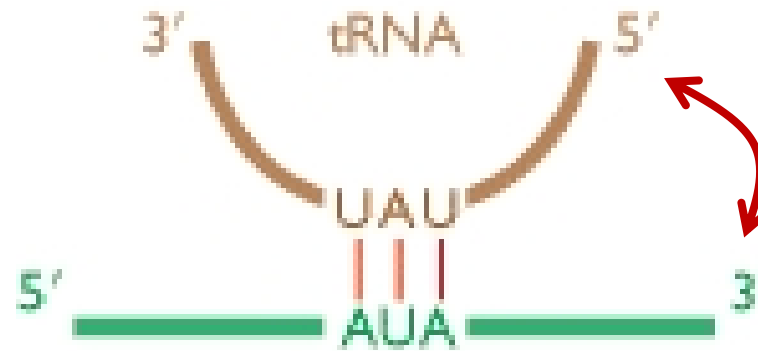
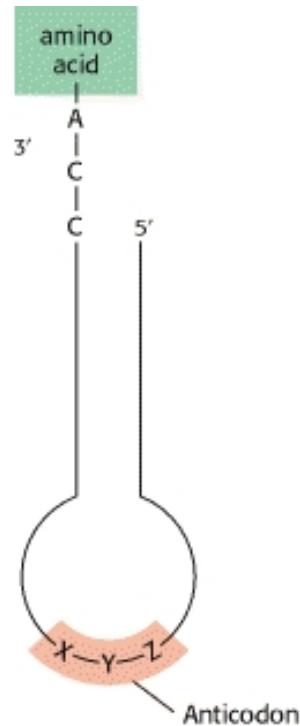


open-reading frame: A stretch of nucleotide sequence that encodes a polypeptide excluding the stop codons.

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.

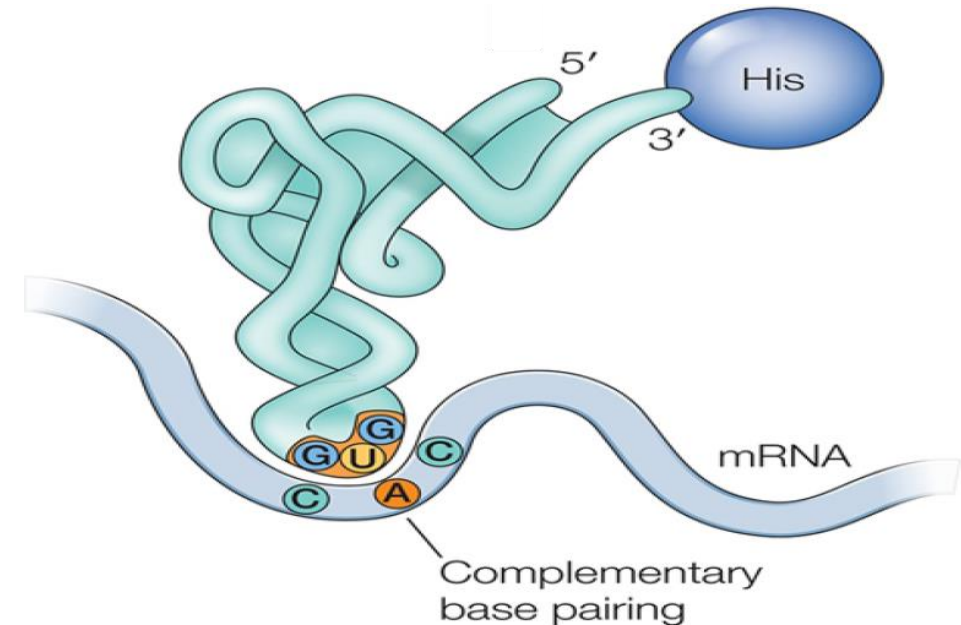
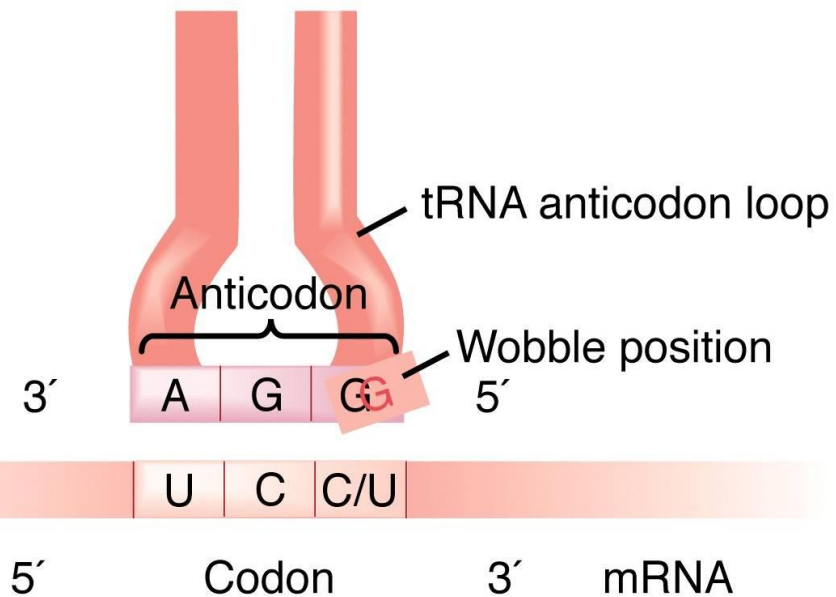


Isoleucine codon

Fidelity of translation



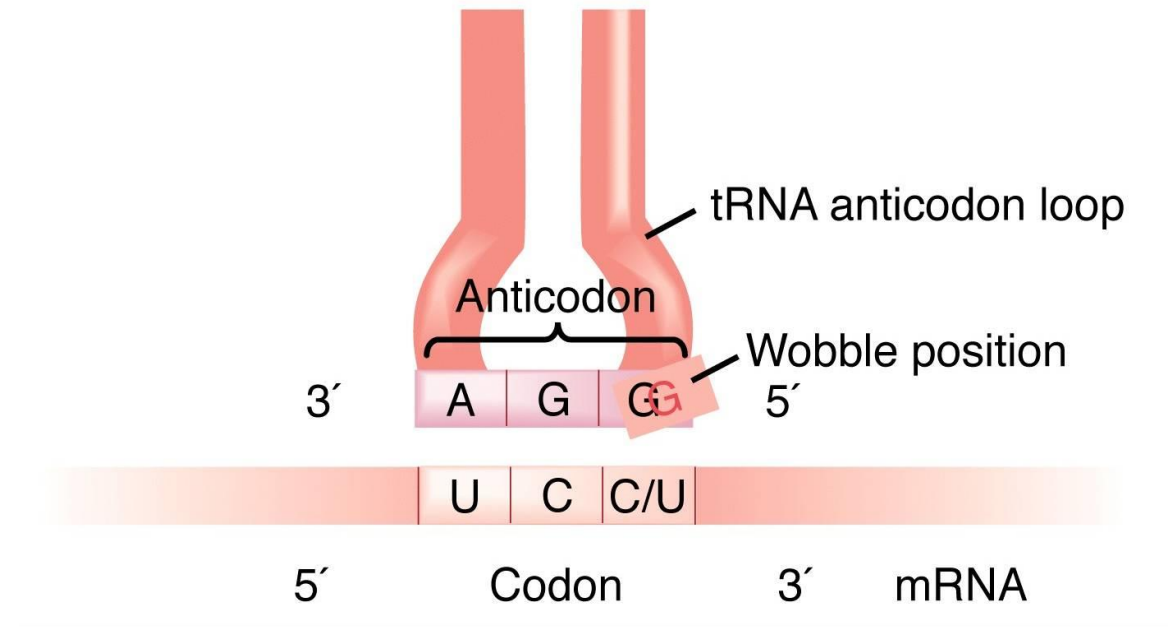
- Accurate translation requires two steps:
 - First: accurate association of amino acid to tRNA
 - Second: a correct match between the tRNA 's anticodon and the mRNA's codon



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.



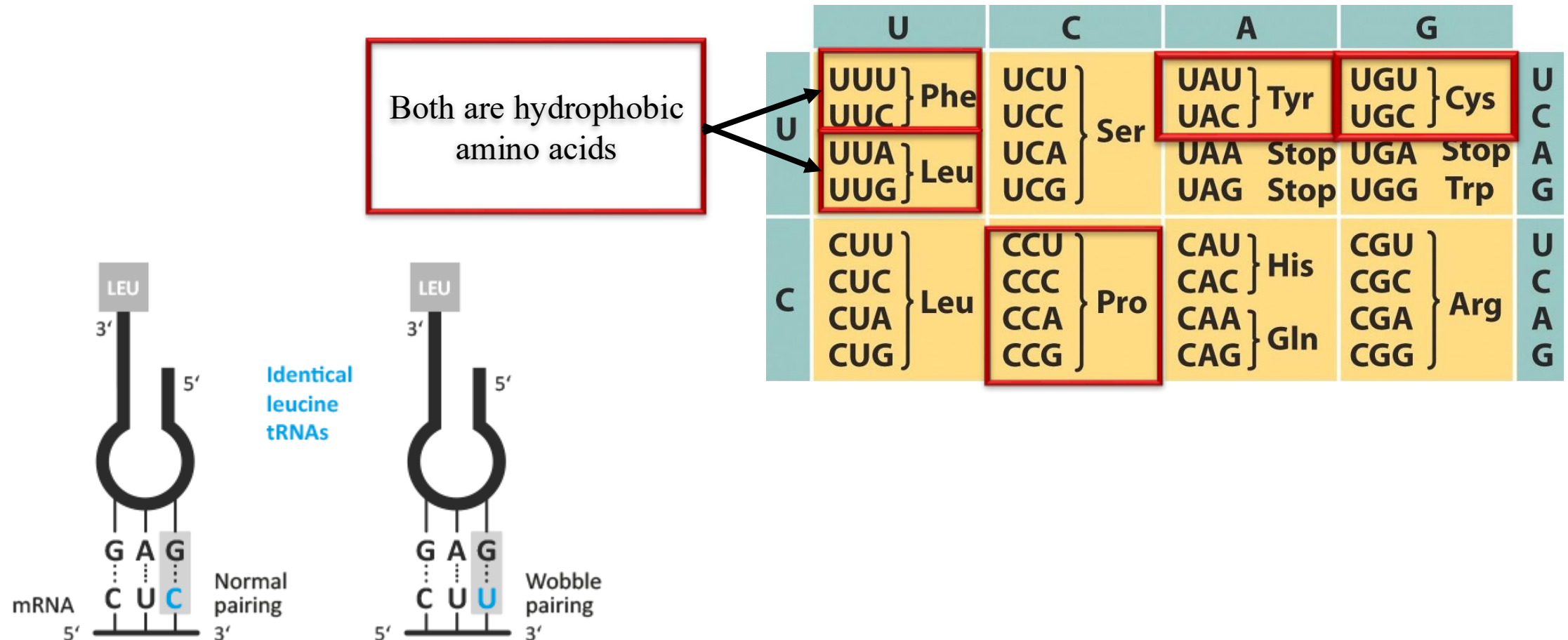
Genetic Codon Chart

	U	C	A	G	
U	UUU Phe UUC Phe UUA Leu UUG Leu	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
A	AUU Ile AUC Ile AUA Ile AUG Met	ACU Thr ACC Thr ACA Thr ACG Thr	AAU Asn AAC Asn AAA Lys AAG Lys	AGU Ser AGC Ser AGA Arg AGG Arg	U C A G
G	GUU Val GUC Val GUA Val GUG Val	GCU Ala GCC Ala GCA Ala GCG Ala	GAU Asp GAC Asp GAA Glu GAG Glu	GGU Gly GGC Gly GGA Gly GGG Gly	U C A G

Examples of wobble base pairing



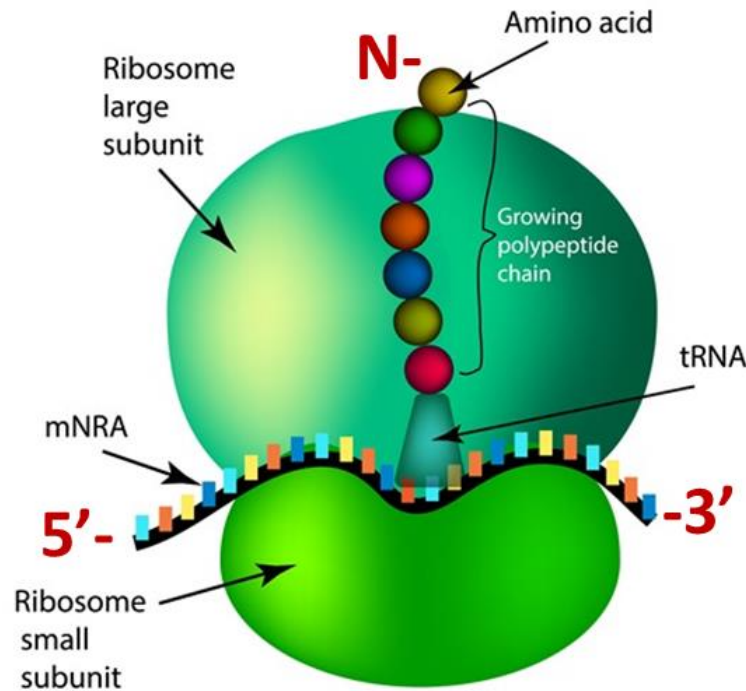
- Relaxed base pairing results from the formation of G-U base pairs.



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.

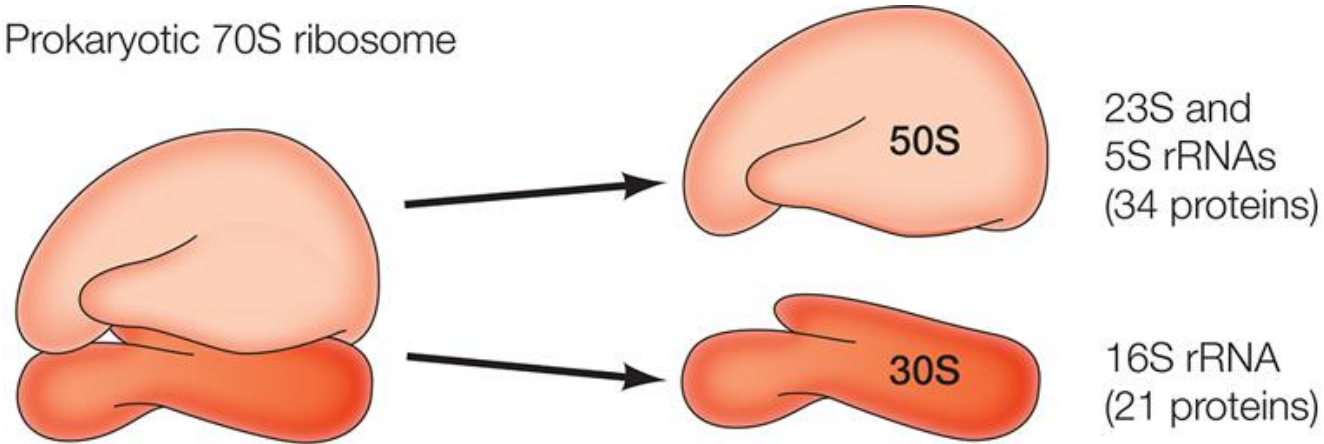


The peptidyl transferase reaction of a peptide bond is catalyzed by the rRNA of the large ribosomal subunit.

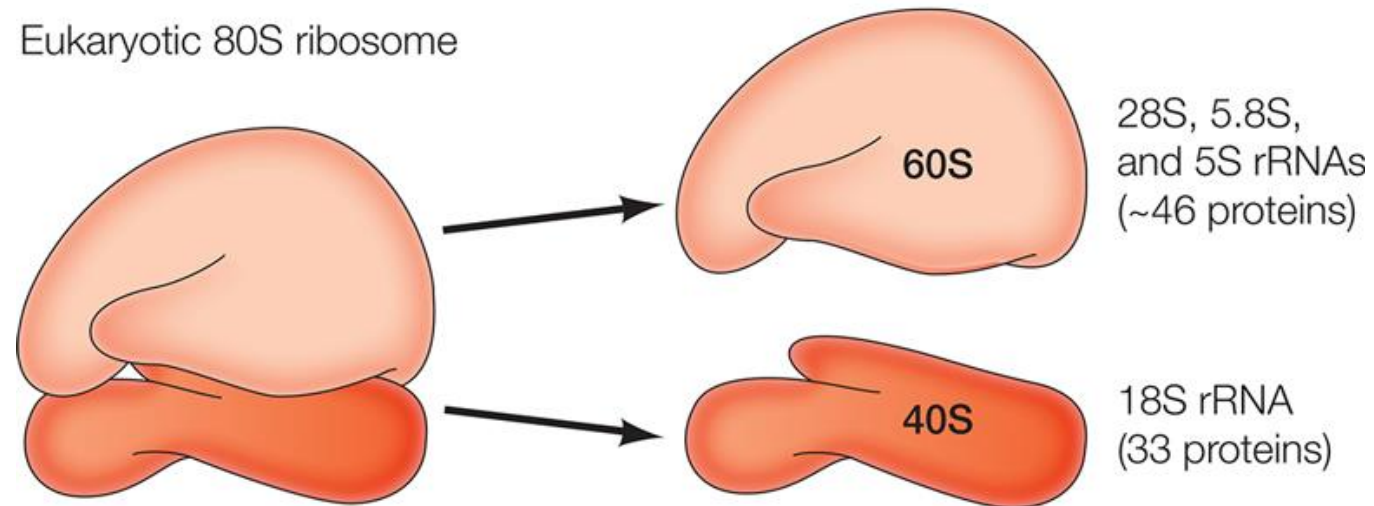
Ribosome structure



Prokaryotic 70S ribosome



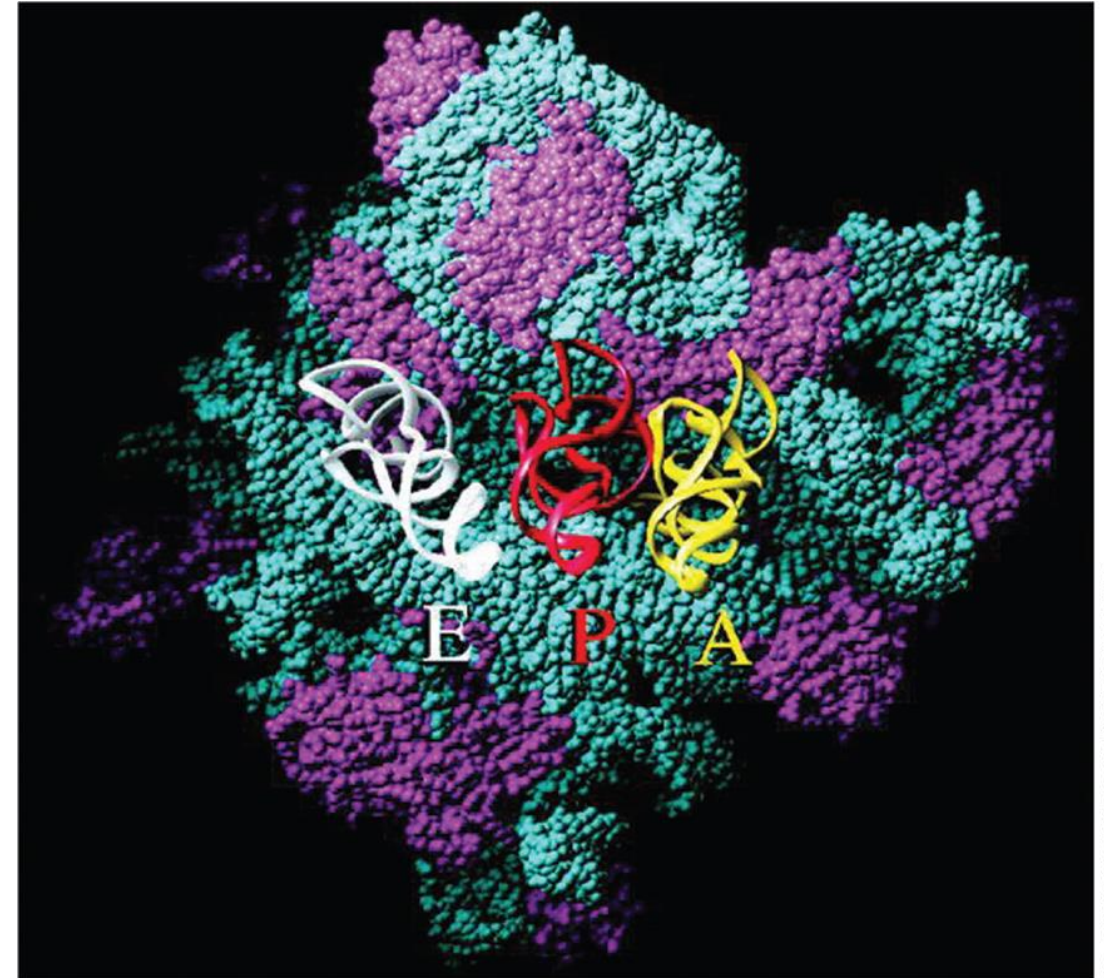
Eukaryotic 80S ribosome



Functional and structural components of ribosomes

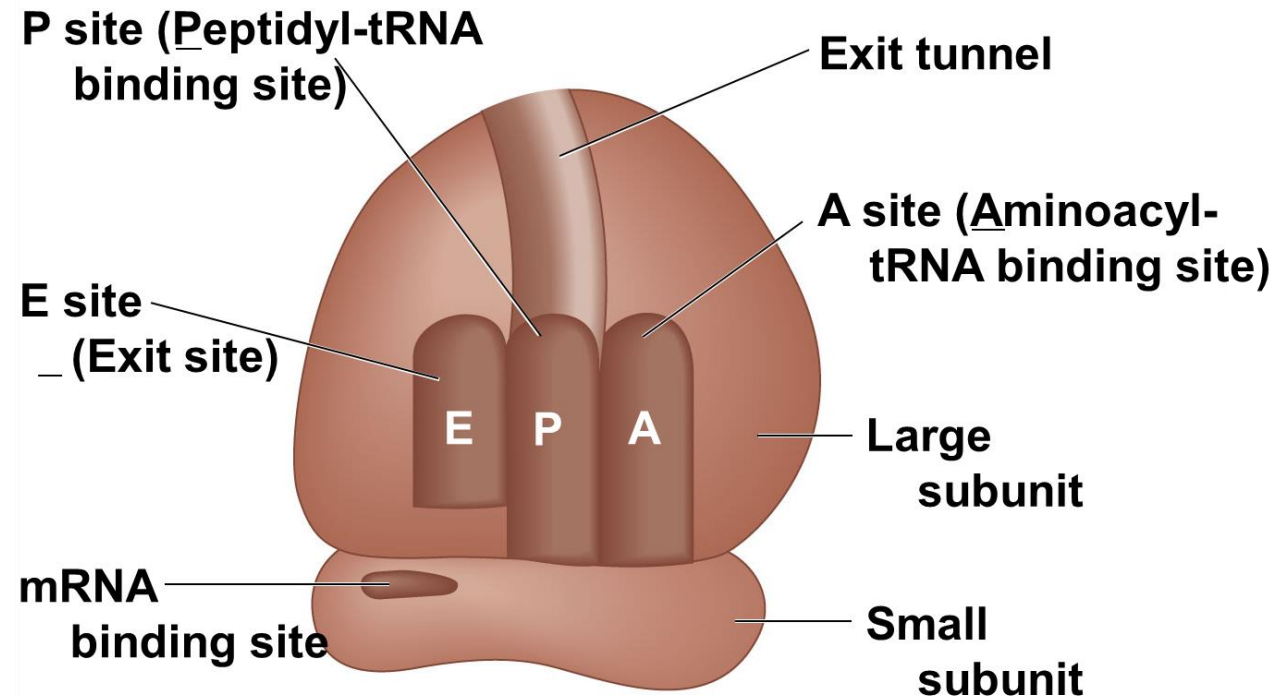
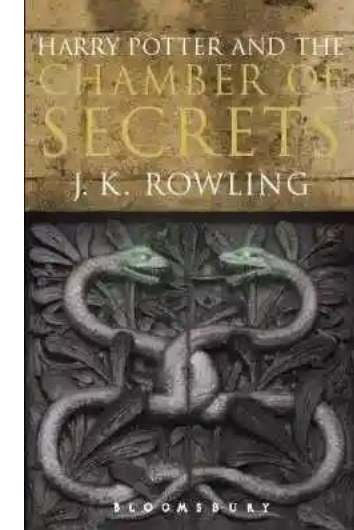


- Ribosomes facilitate specific coupling of tRNA anticodons with mRNA codons in protein synthesis.
- The RNA components are responsible for the catalytic function of the ribosome, and the protein components enhance the function of the rRNA molecules.



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

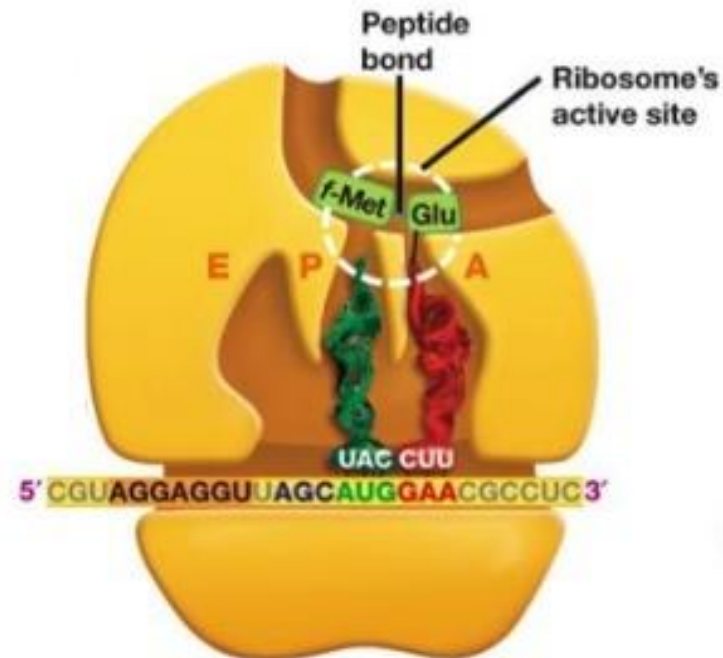
The **A site** holds the tRNA that carries the next amino acid to be added to the chain

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

The general mechanism of translation



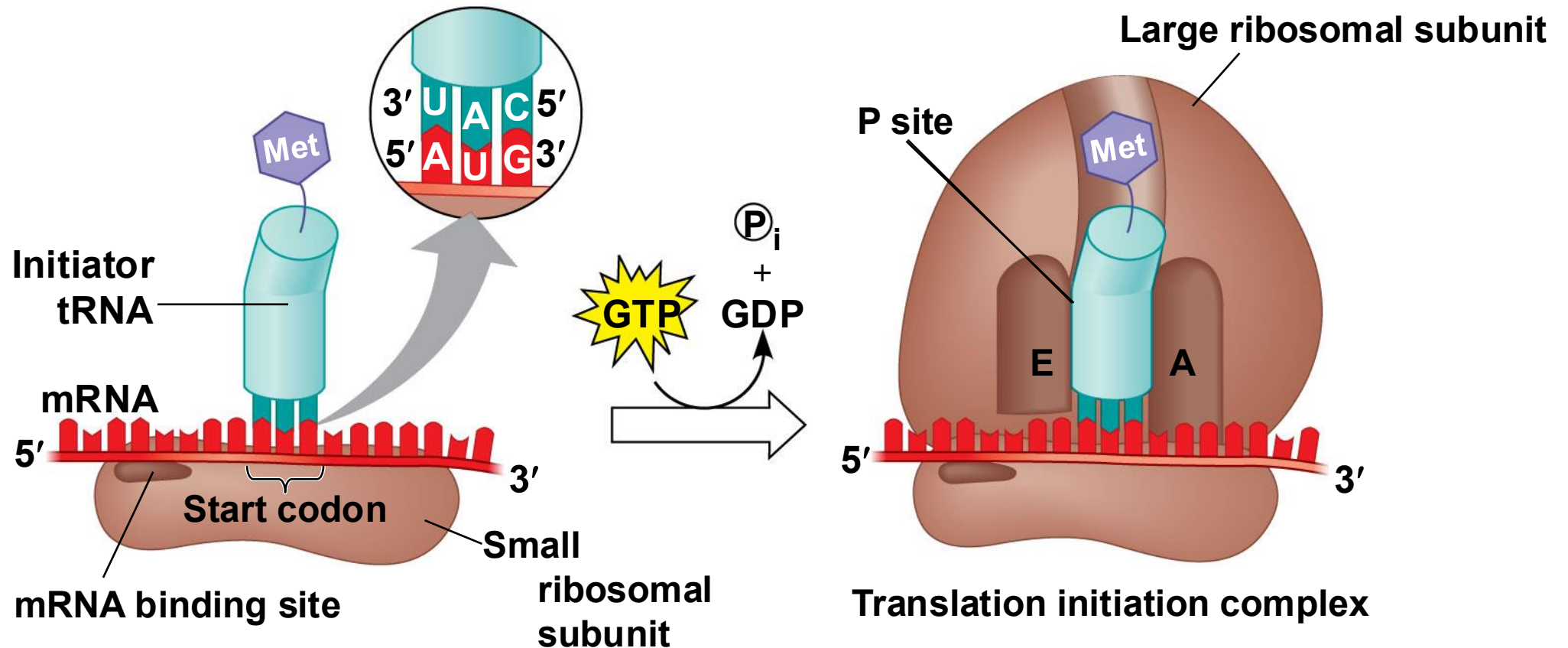
- Three stages: initiation, elongation, and termination.
- The direction is 5' → 3'.
- Protein synthesis begins at the amino terminus and extends toward the carboxyl terminus.



Start of translation



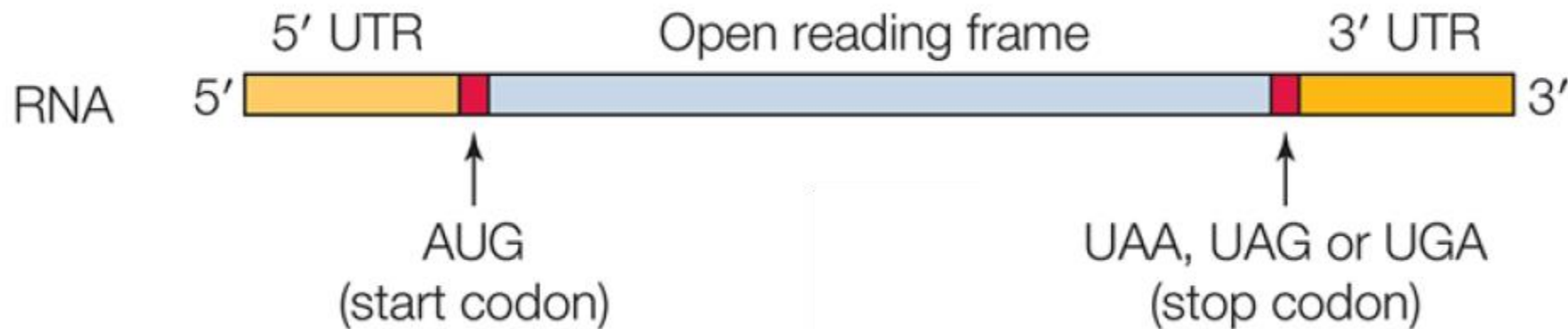
- In both prokaryotes and eukaryotes, translation starts at specific initiation sites, which is AUG (methionine), and not from the first codon of the mRNA.



Untranslated regions



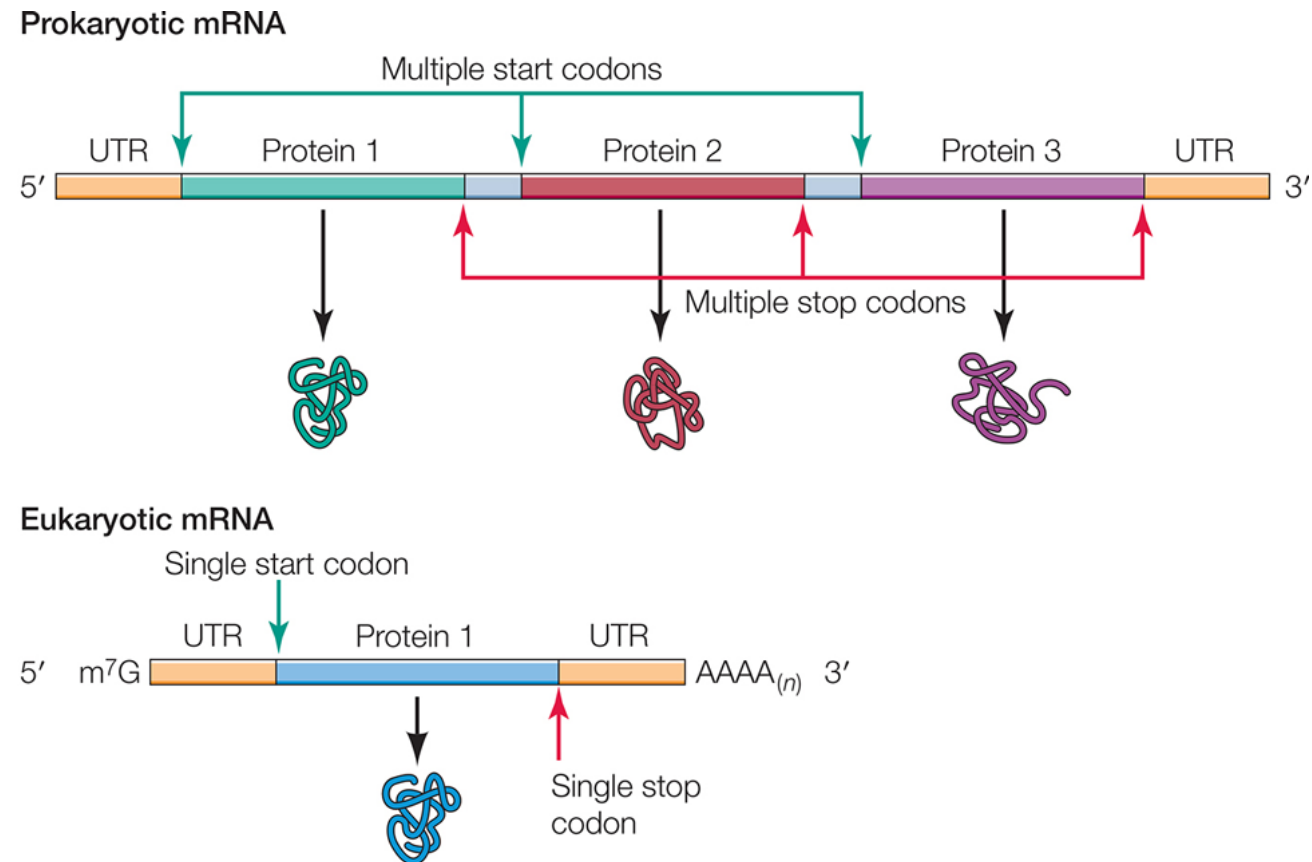
- The 5' terminal portions upstream of the initiation sites of both prokaryotic and eukaryotic mRNAs contain noncoding sequences, referred to as 5' untranslated regions (UTRs).
- There is also a 3'-untranslated region, which follows any of the three stop codons.



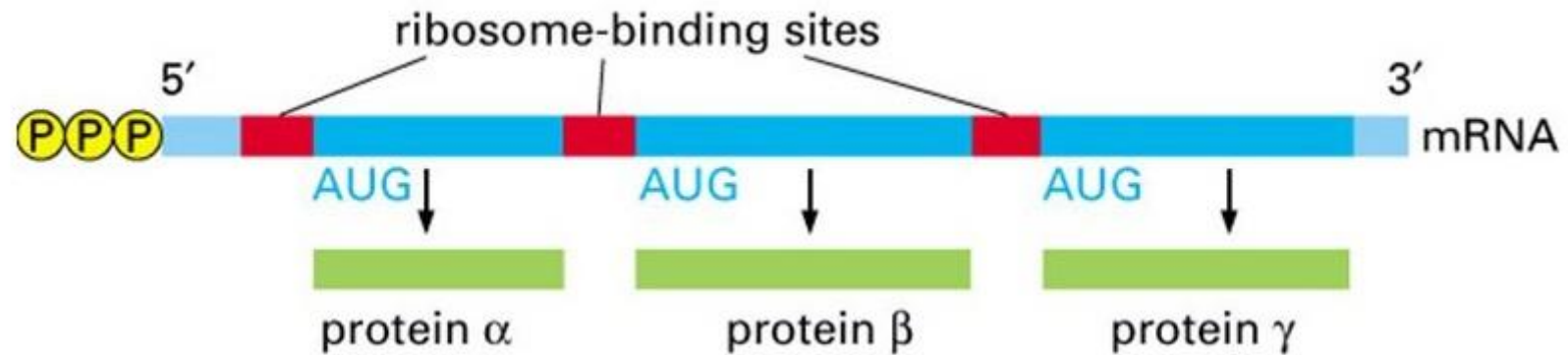
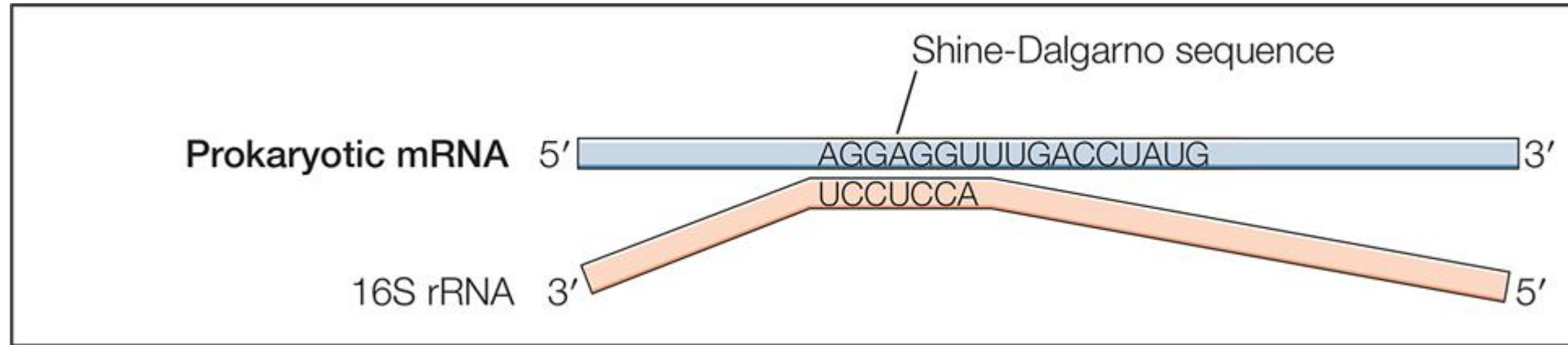
Remember...



- Bacterial mRNA is polycistronic
- Eukaryotic mRNA is monocistronic



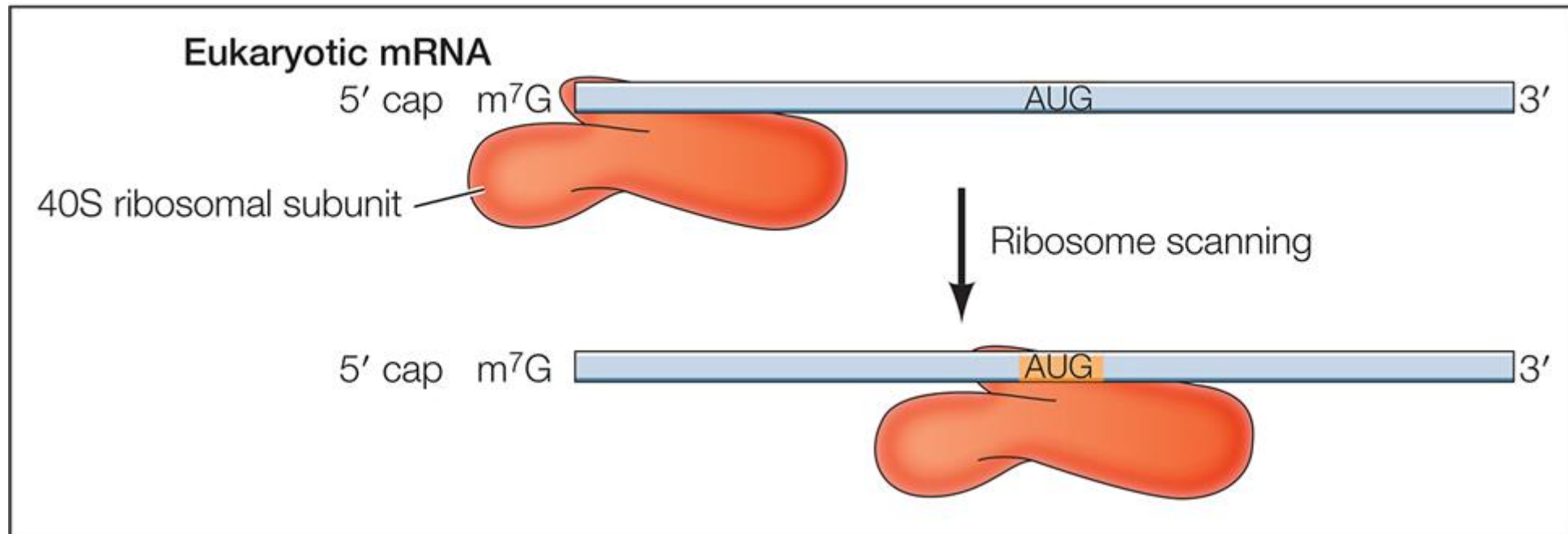
Shine-Dalgarno sequence



But in eukaryotes...



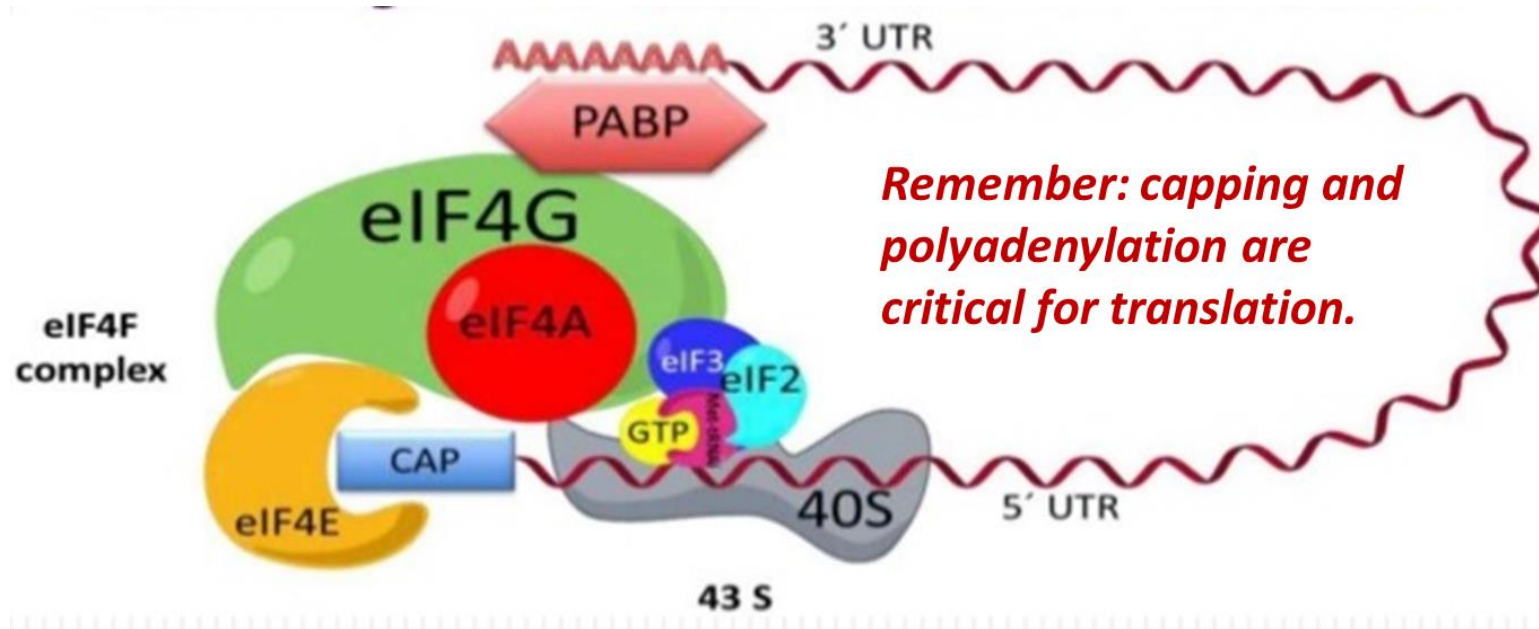
- Eukaryotic ribosomes recognize mRNAs by binding to the 7-methylguanosine cap at their 5' terminus.



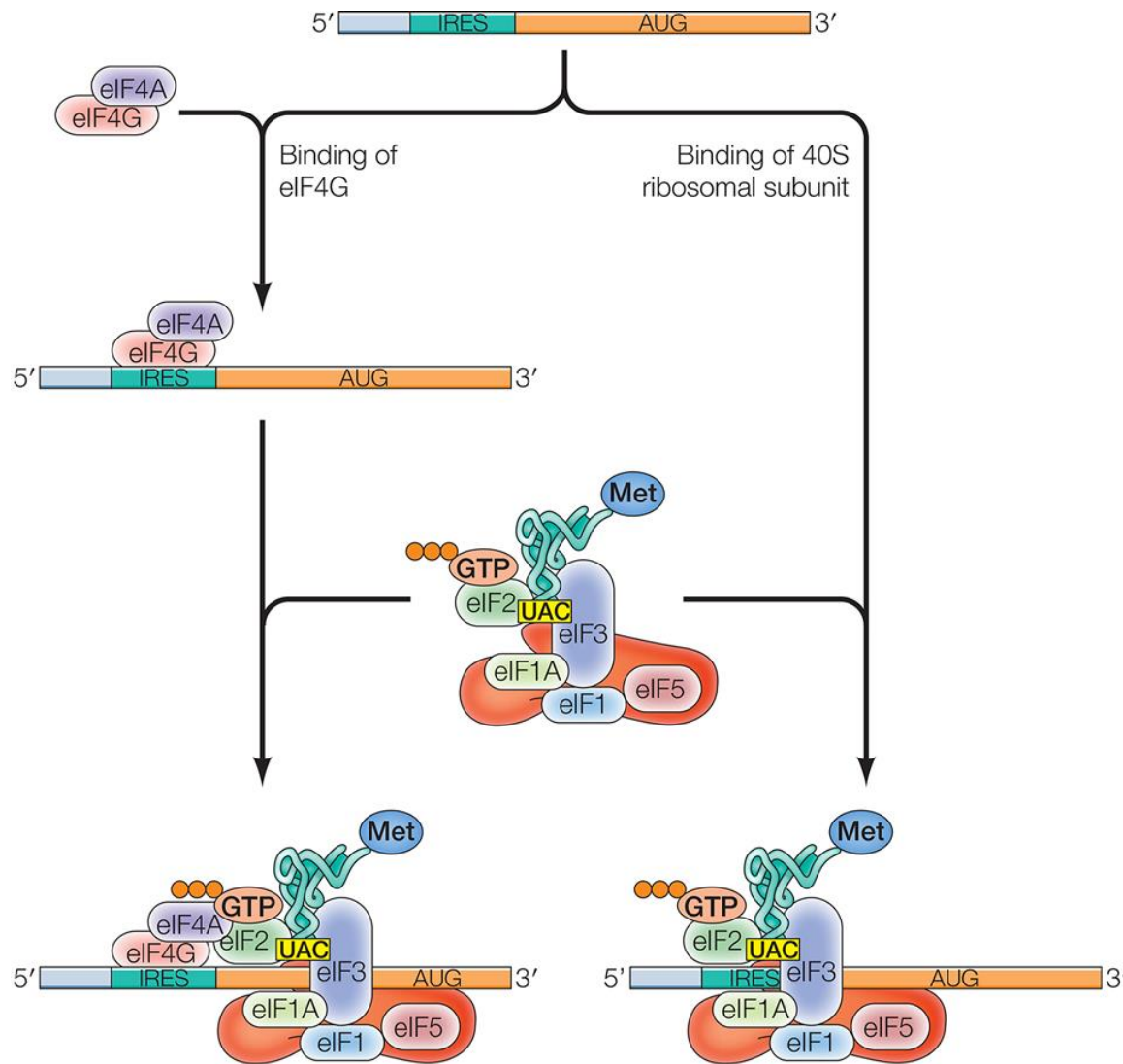
Translation initiation in eukaryotes



- 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.



Internal ribosome entry site (IRES)

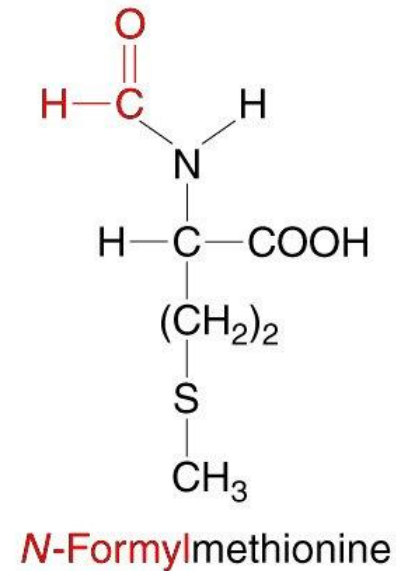
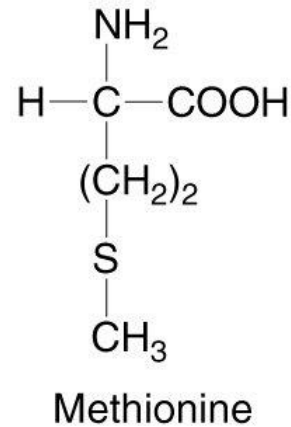


- Alternatively, internal ribosome entry site (IRES) exist in some other mRNAs and is recognized by the eIF4G protein followed by recruitment of the first tRNA and the small ribosome.
- IRESs in other mRNAs may also be recognized directly by the 40S ribosomal subunit.

The first amino acid



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



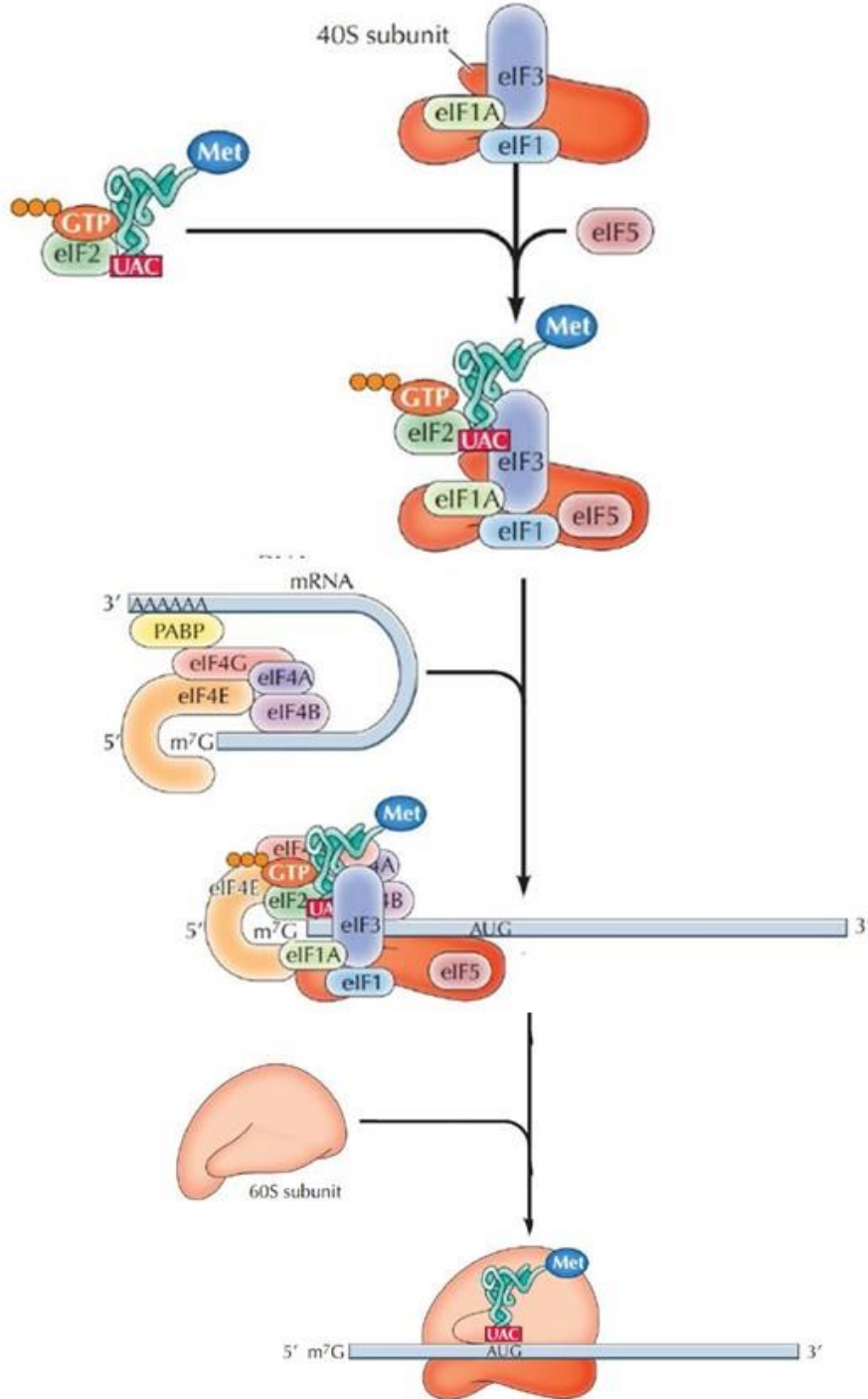
Building a polypeptide



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

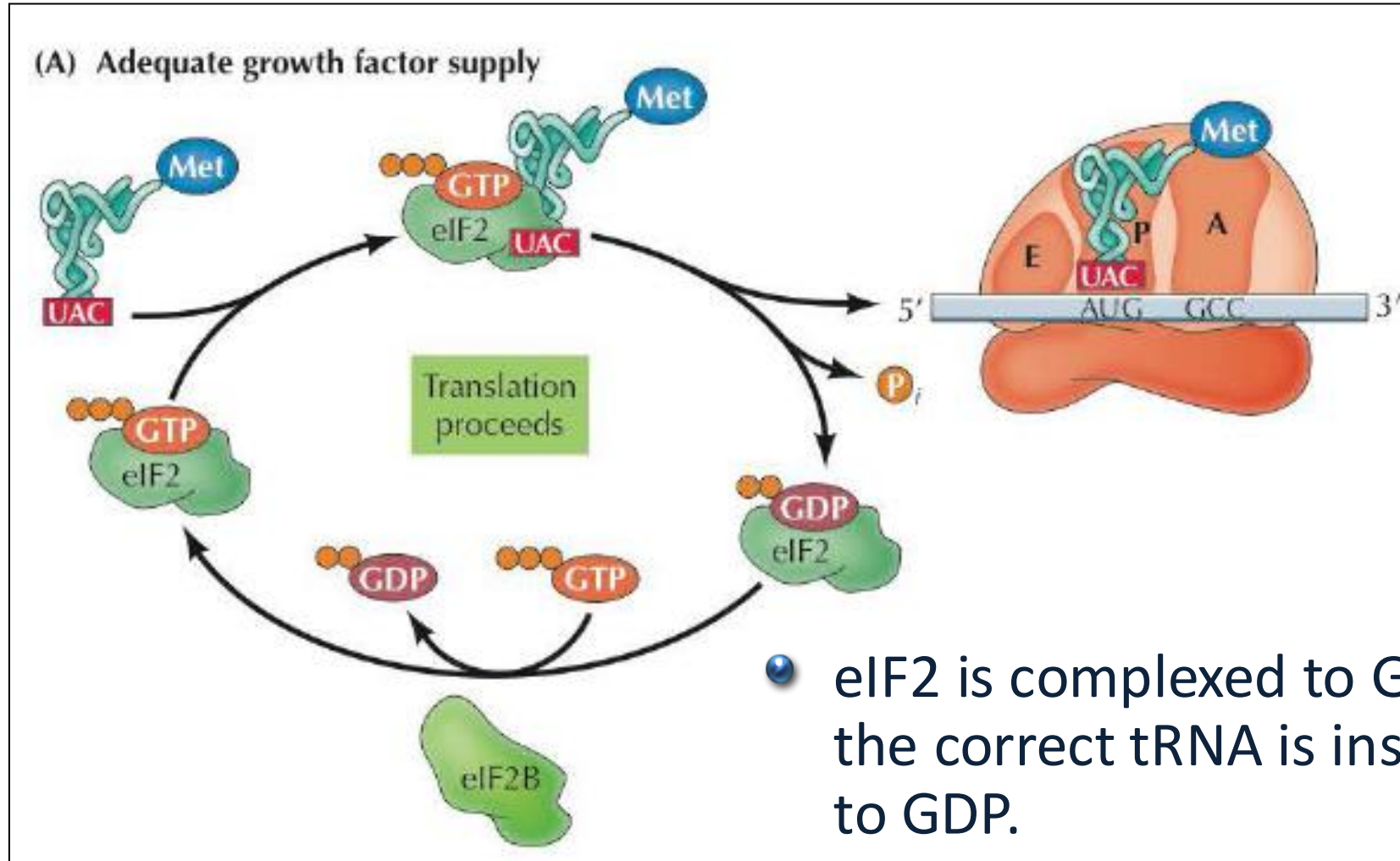


Translation initiation



- 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



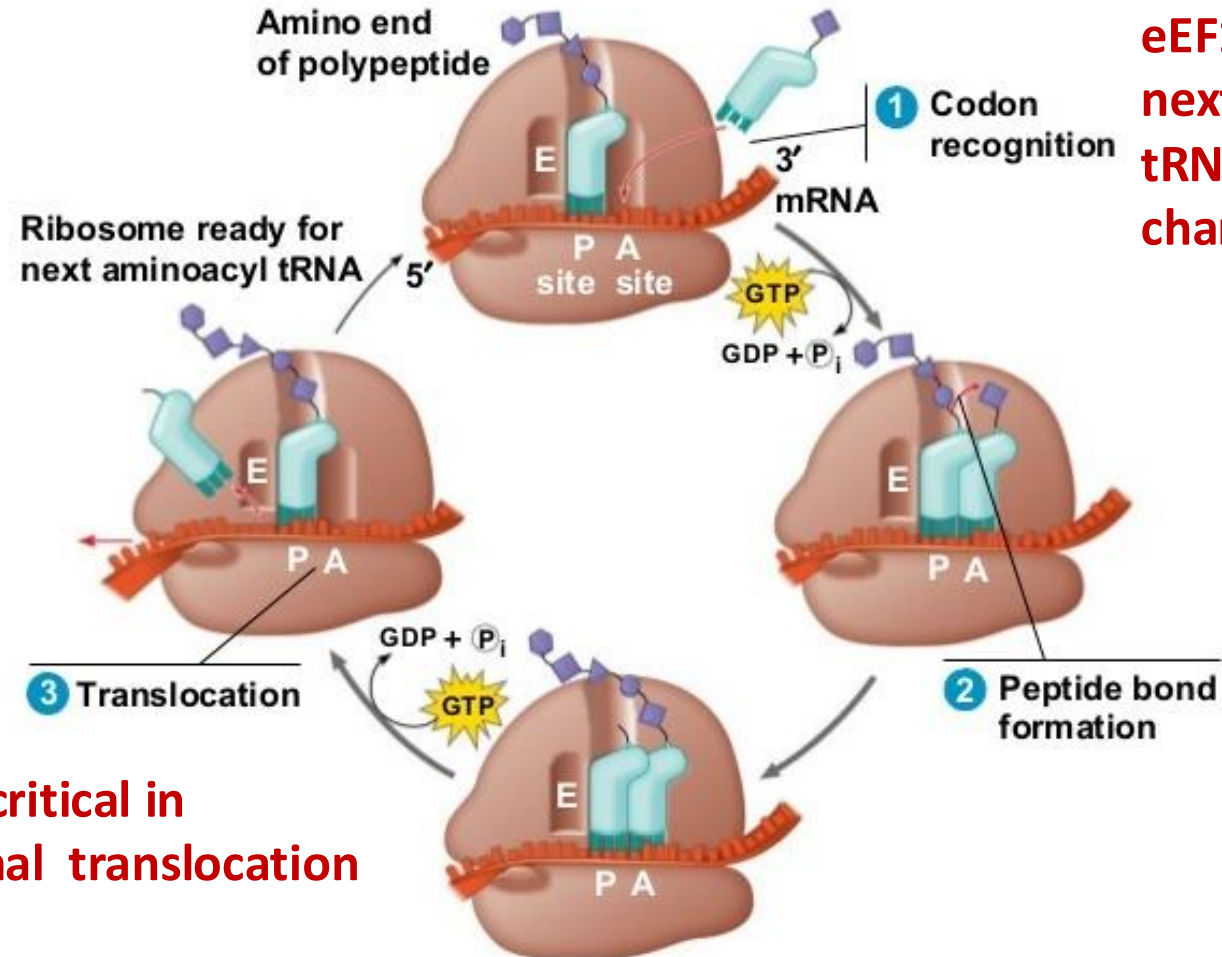
- eIF2 is complexed to GTP to be active. When the correct tRNA is inserted, GTP is hydrolyzed to GDP.
- The active eIF2/GTP complex must be regenerated by exchanging of the GDP for GTP.

Translation elongation



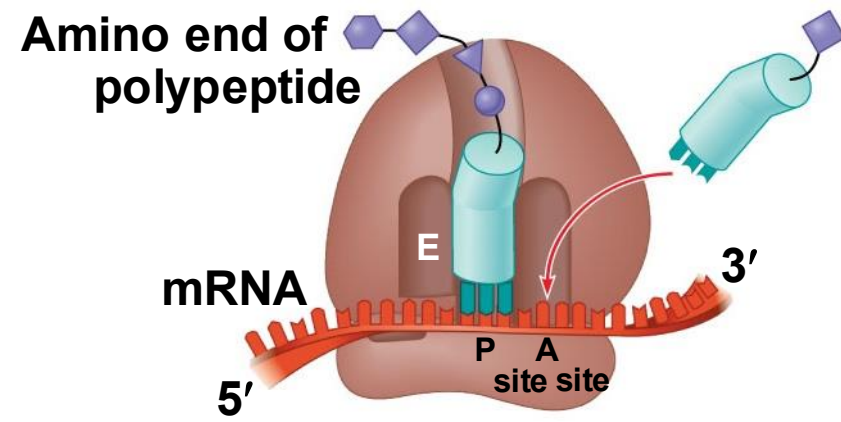
Three steps:

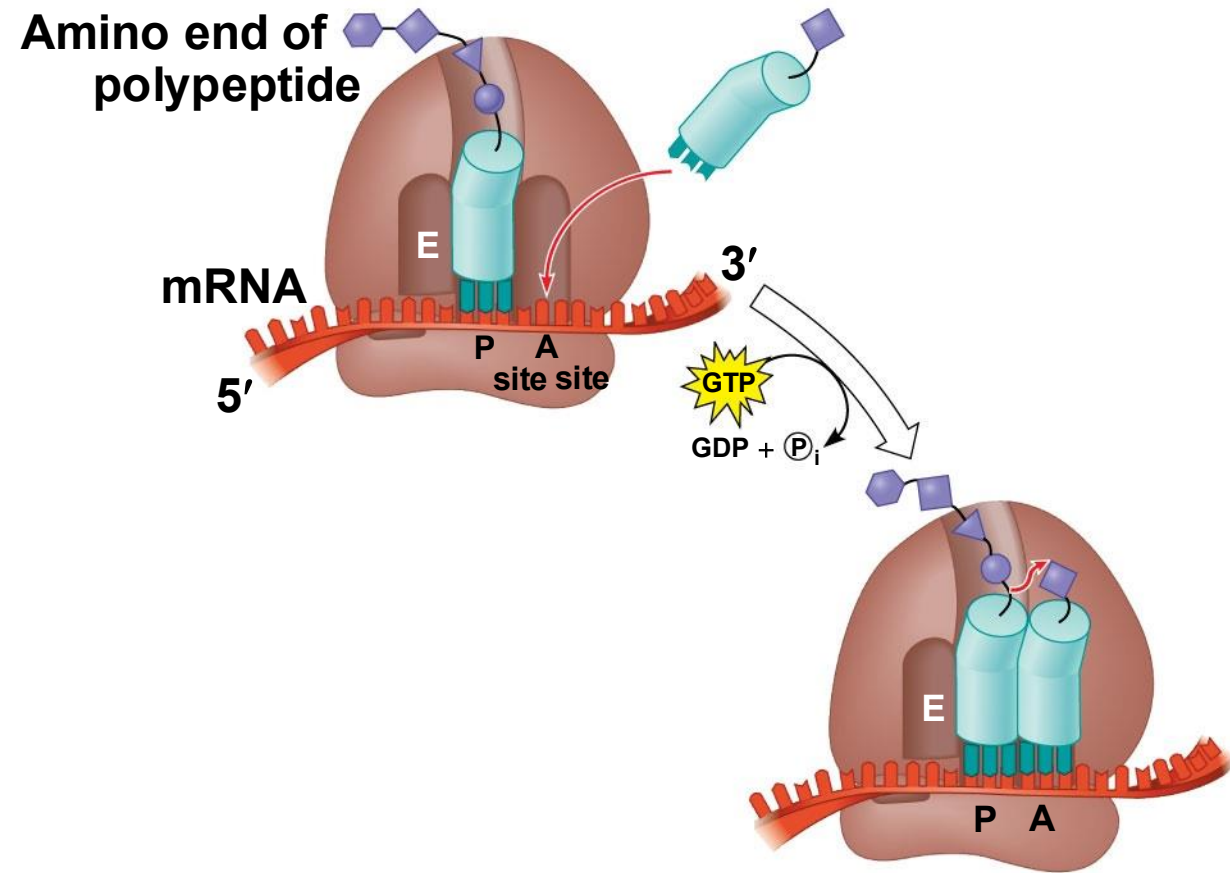
1. aminoacyl-tRNA binding
2. peptide bond formation
3. translocation with the help of elongation factors (eEF).

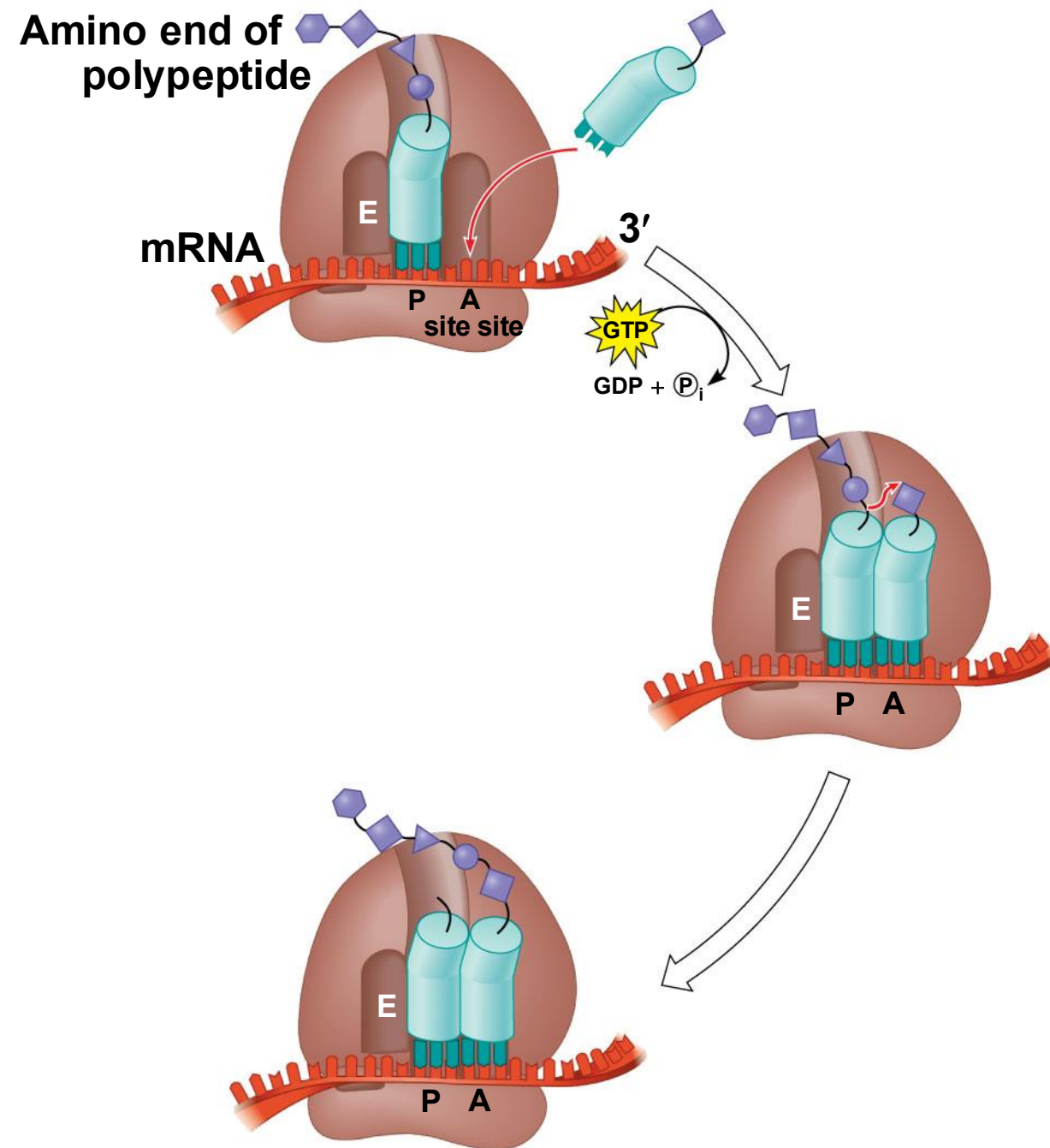


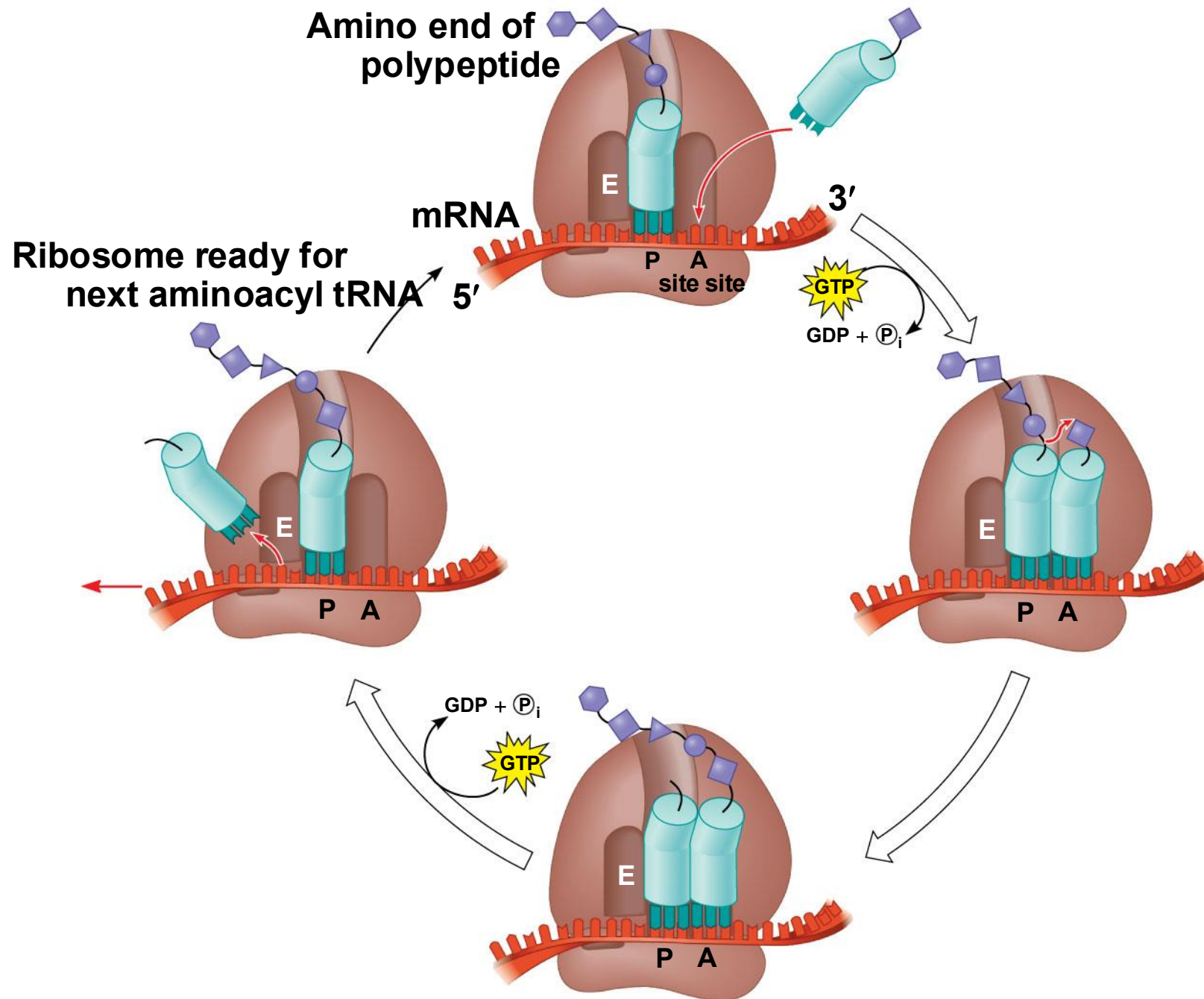
eEF1 α brings next aminoacyl-tRNA to the A chamber

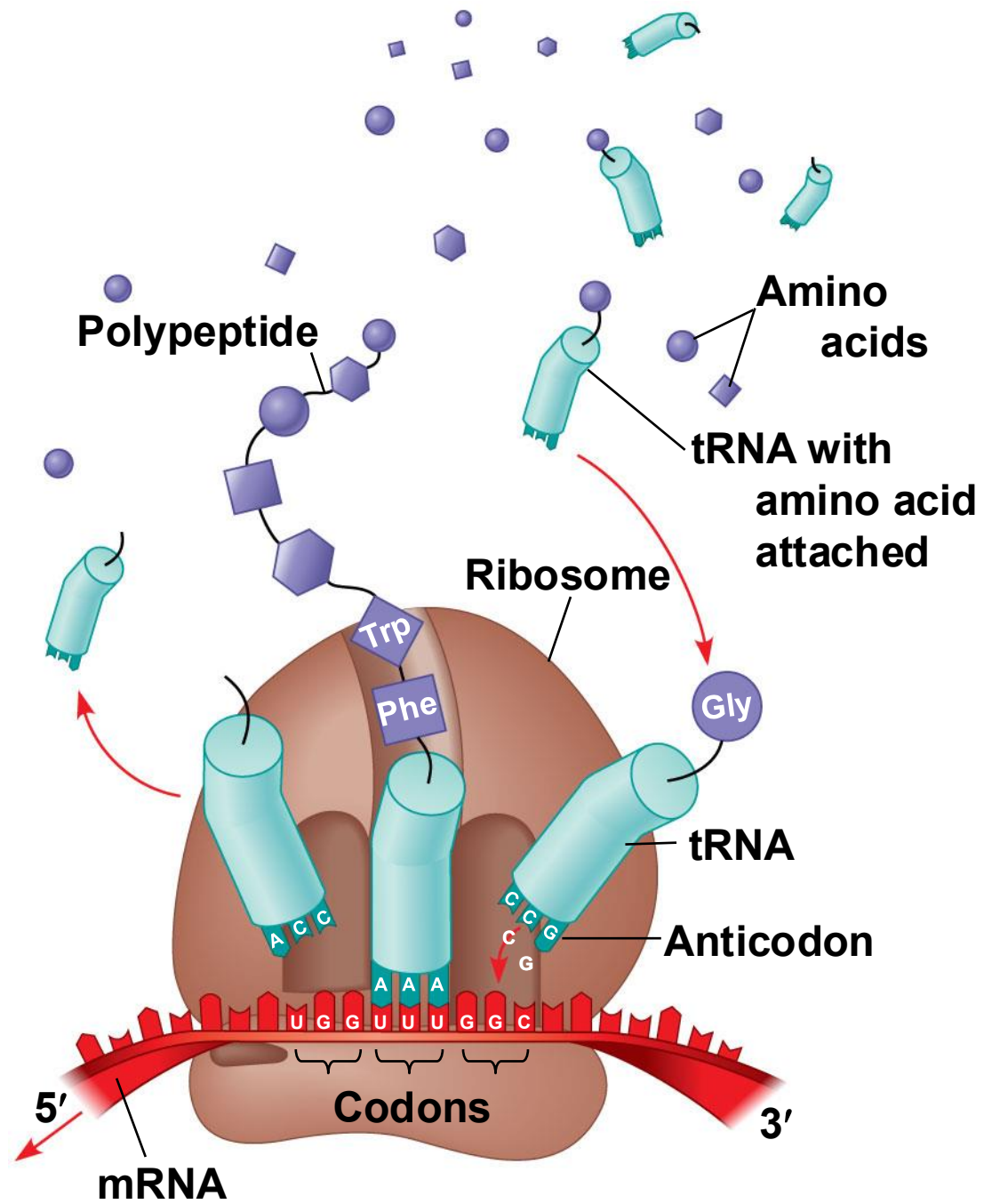
eEF2 is critical in ribosomal translocation

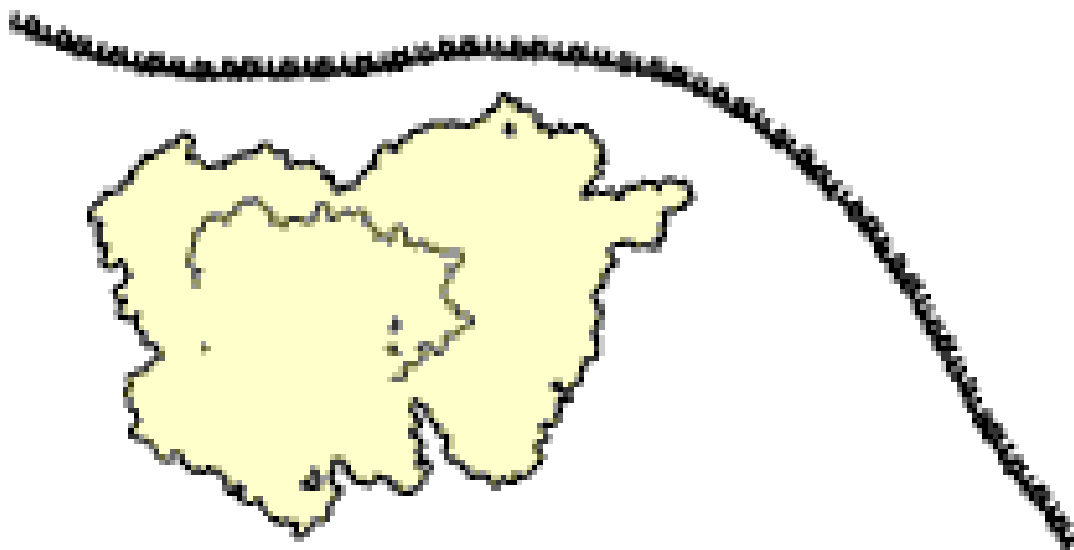








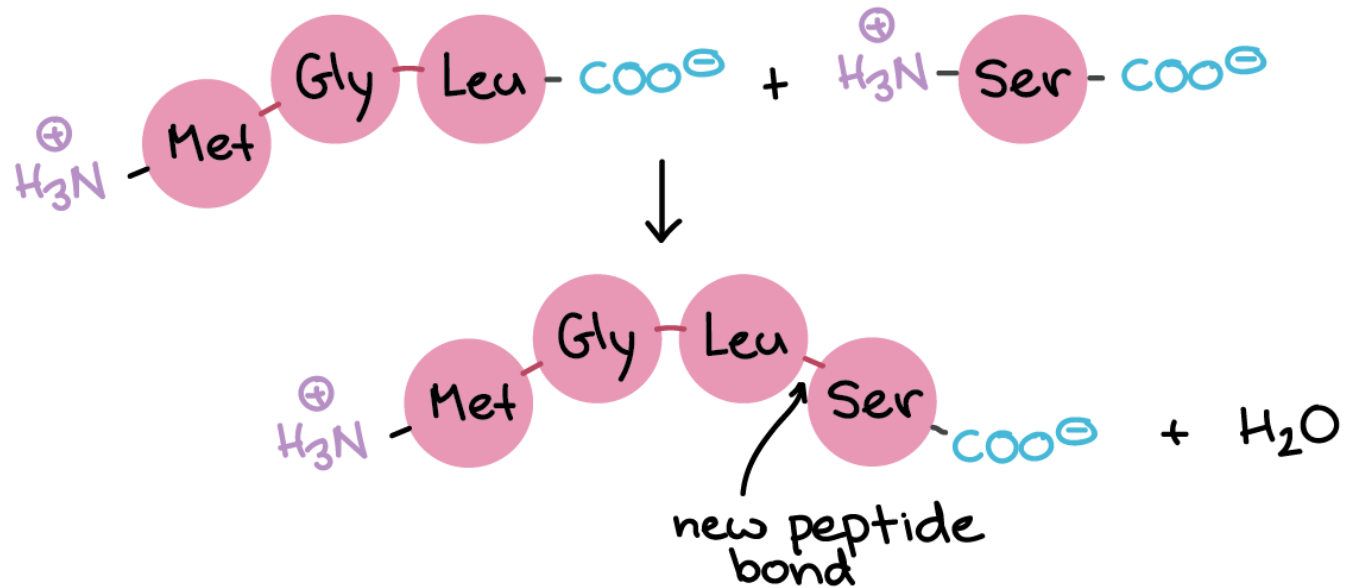




Elongation of the Polypeptide Chain



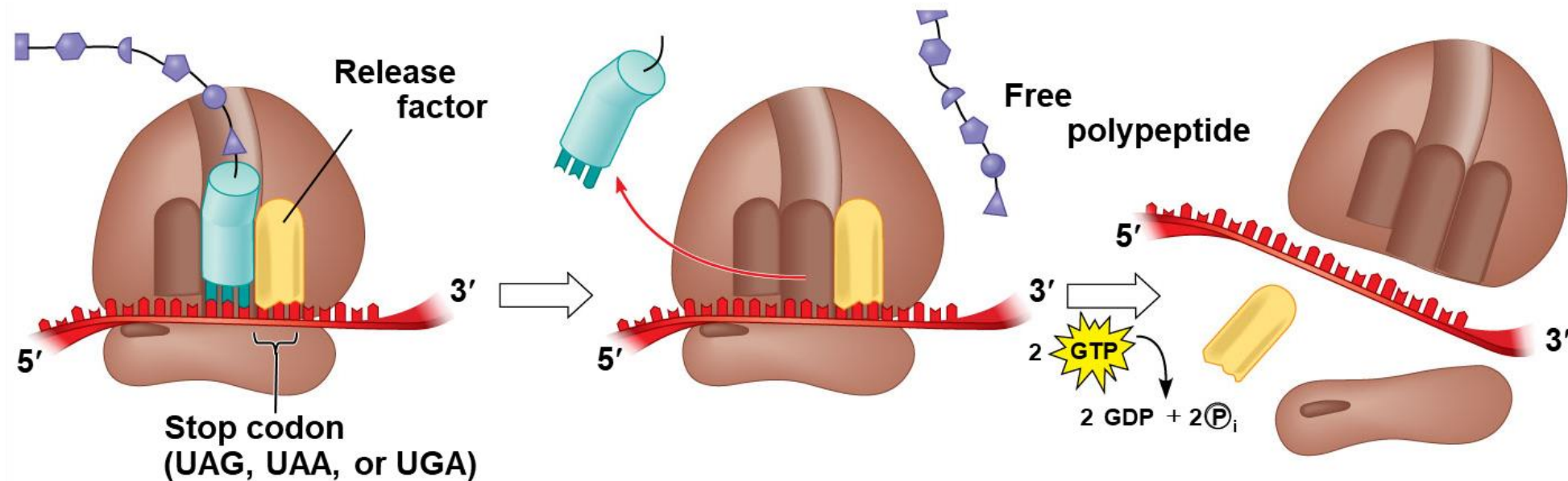
- During the elongation stage, amino acids are added one by one to the preceding amino (N)-terminus to the carboxy (C)-terminus of the growing chain.



Termination of Translation



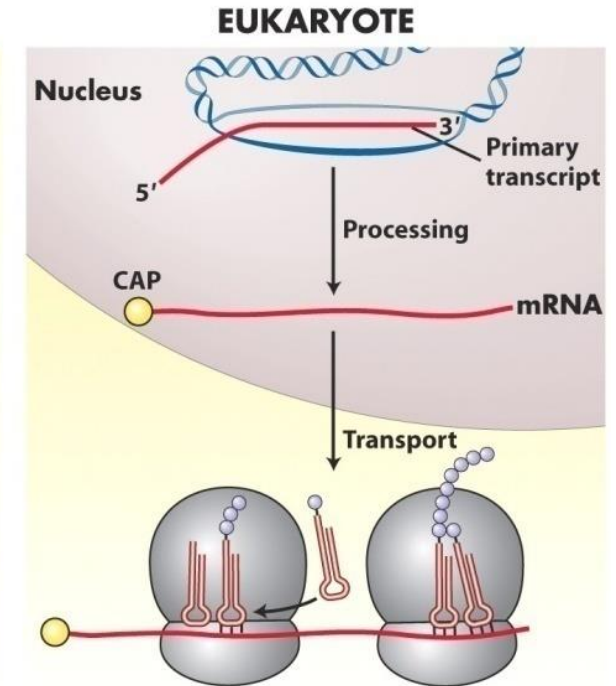
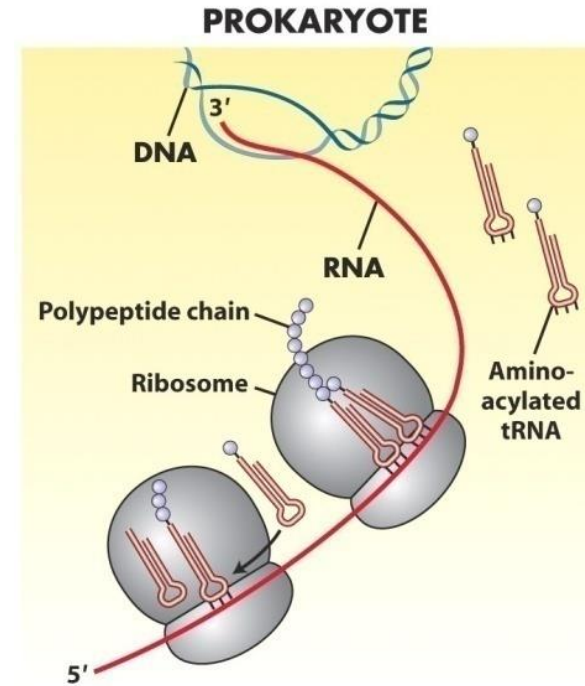
- The codons UAA, UAG, and UGA are the stop signals. They are not recognized by any tRNAs, but a release factor protein.
- The empty A site accepts release factors, which cause the release of the polypeptide, and the translation assembly then comes apart.



Transcription/translation Coupling



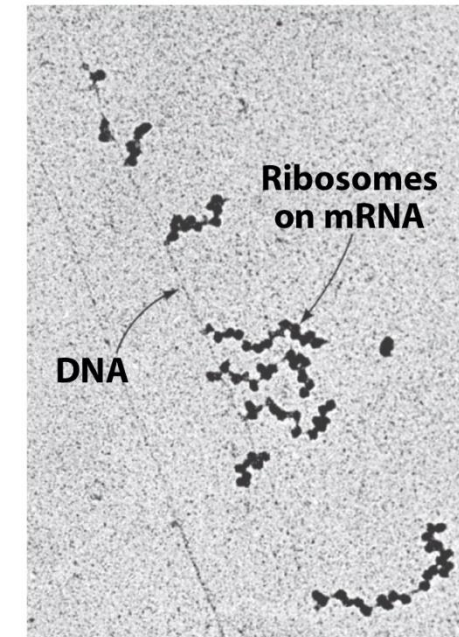
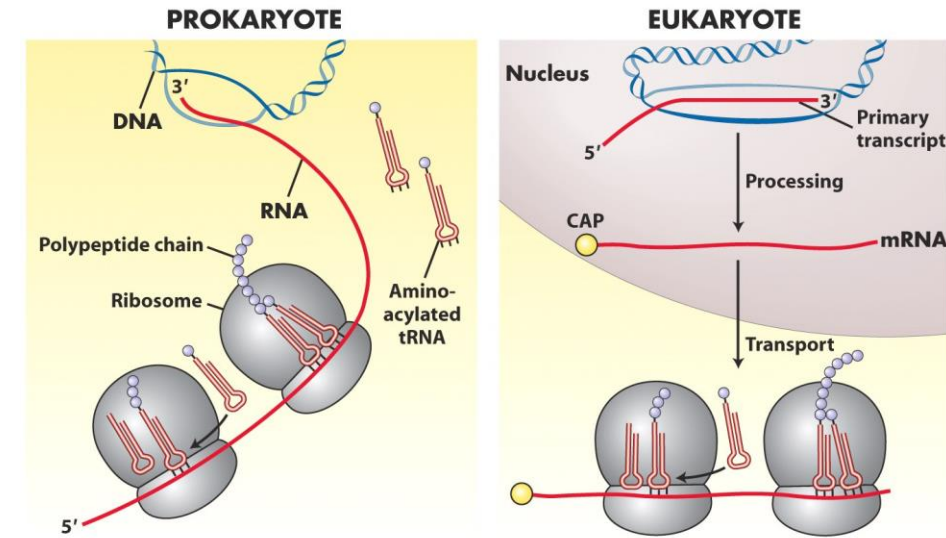
- Translation and transcription are coupled in space and time in prokaryotes.



Polyribosomes (polysomes)



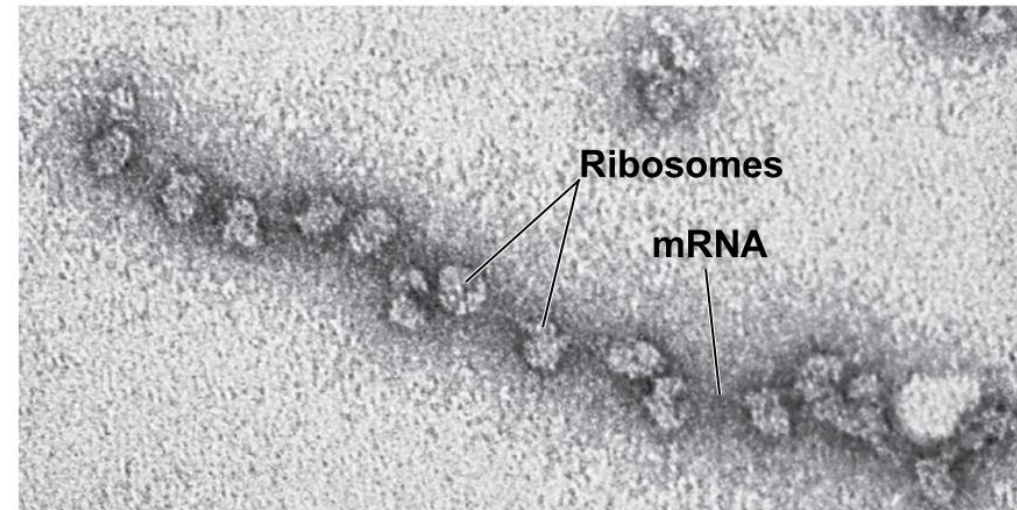
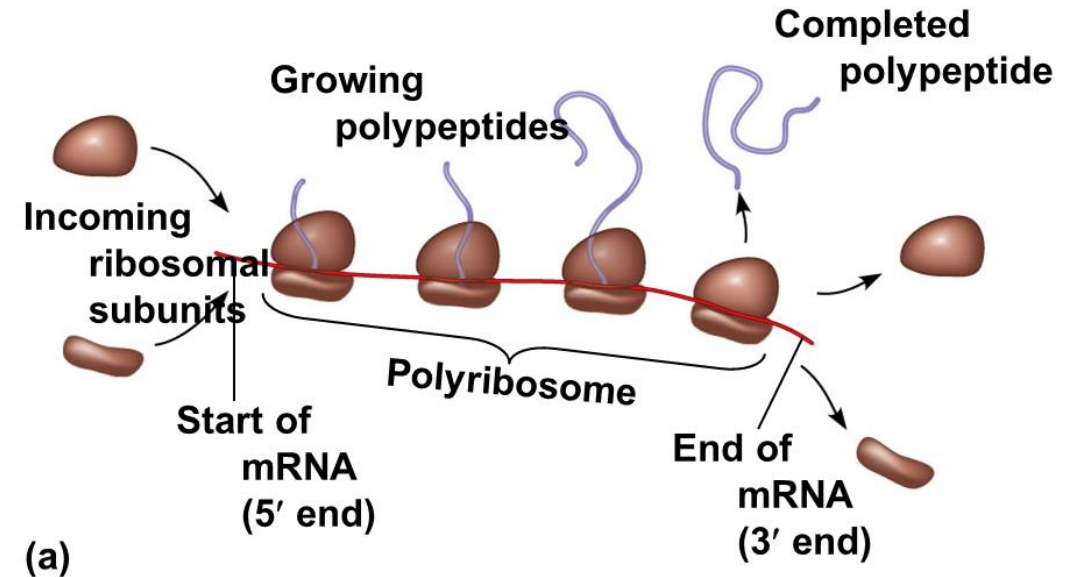
- A single mRNA molecule is translated by several ribosomes simultaneously. Each ribosome produces one copy of the polypeptide chain specified by the mRNA. When the protein has been completed, the ribosome dissociates into subunits that are used in further rounds of protein synthesis.



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.



(b)

0.1 μm

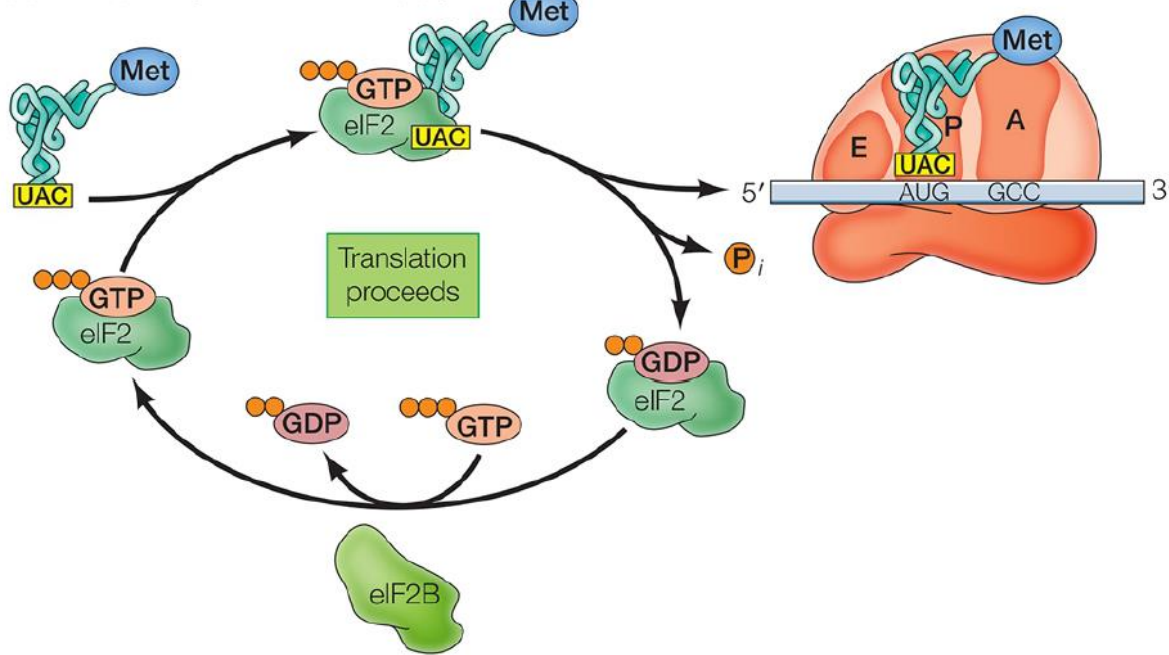


Regulation of translation

Global regulation

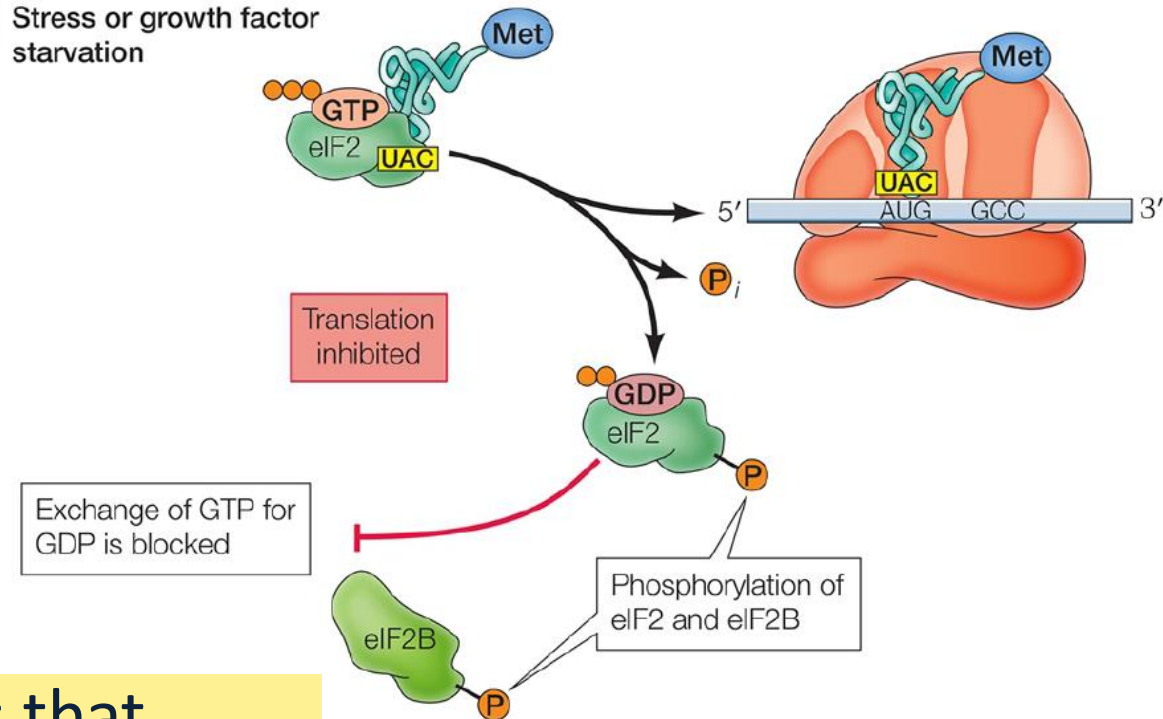


(A) Adequate growth factor supply



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

(B) Stress or growth factor starvation

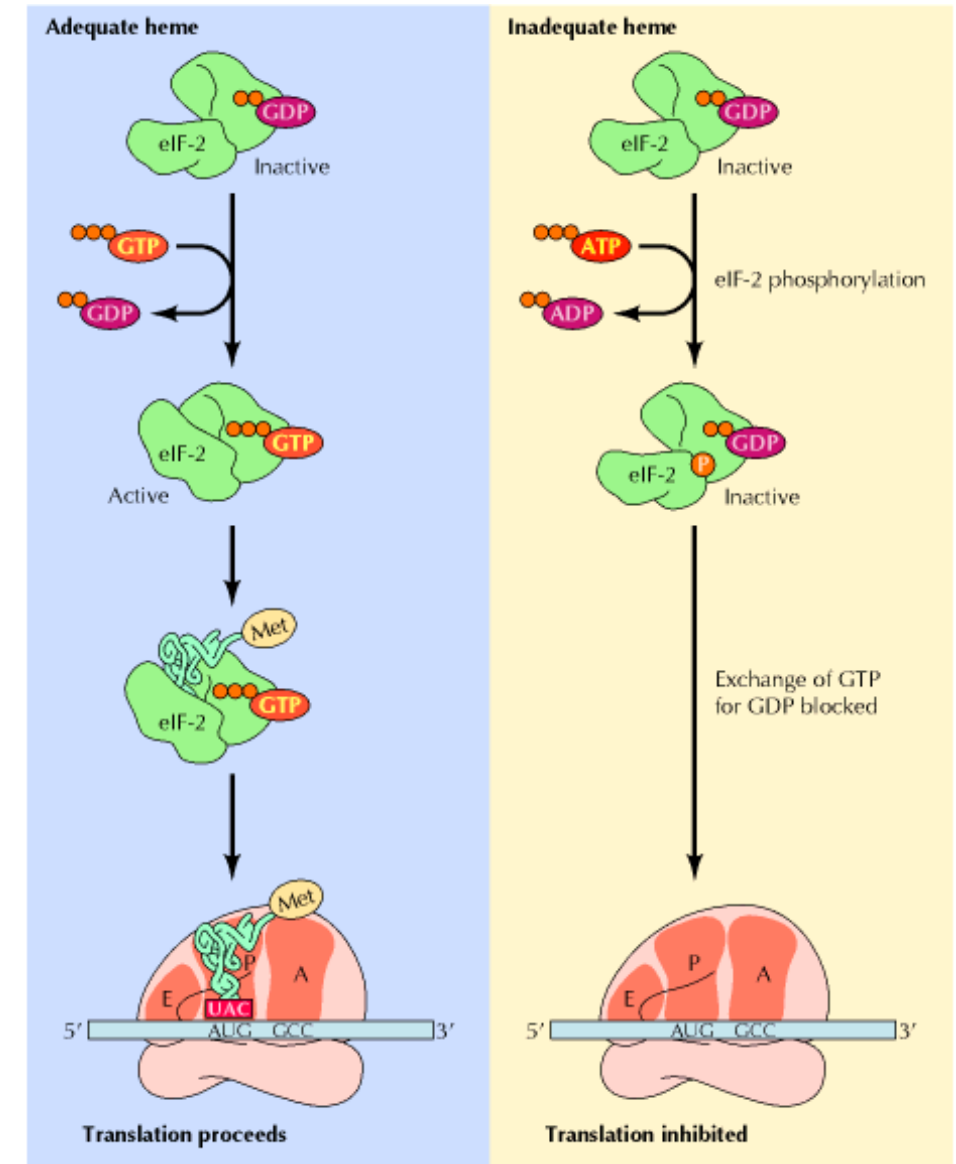
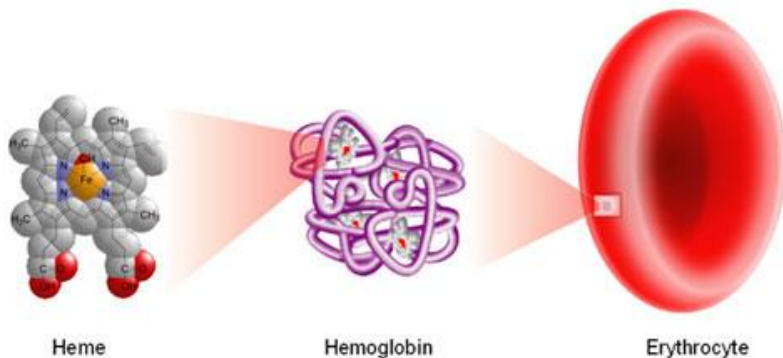


Translation is inhibited by regulatory kinases that phosphorylate eIF2 blocking the GTP/GDP exchange.

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.





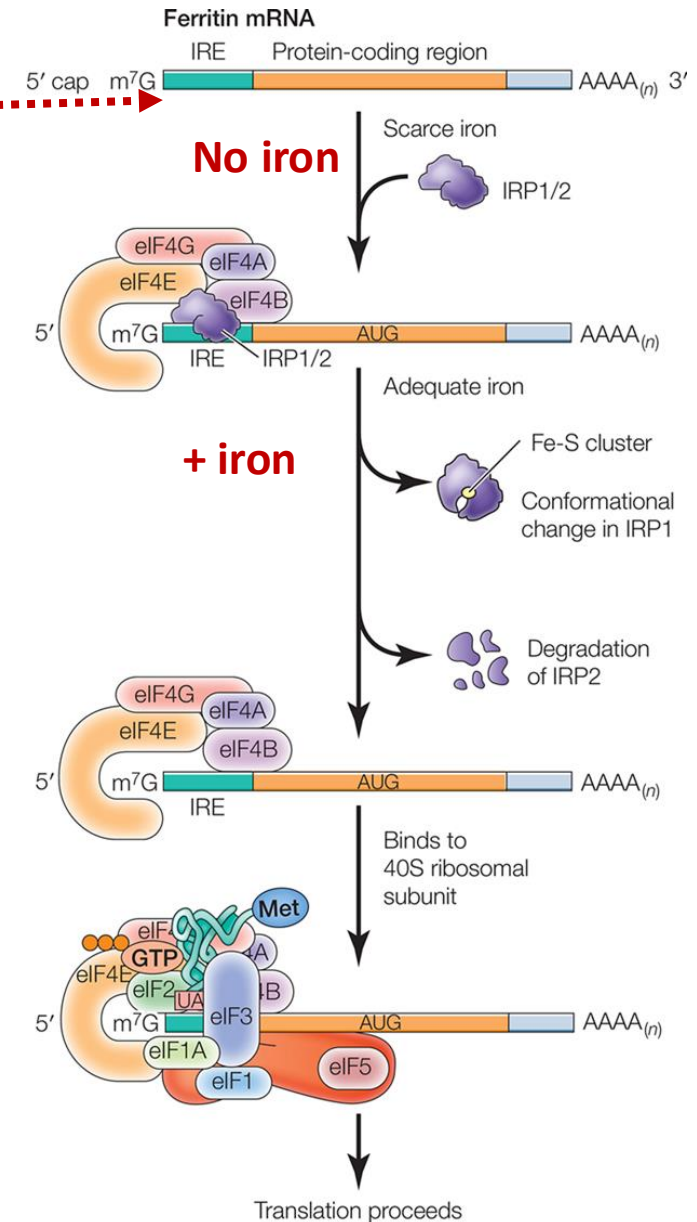
Regulation of mRNA stability

Remember the iron-regulatory mechanism

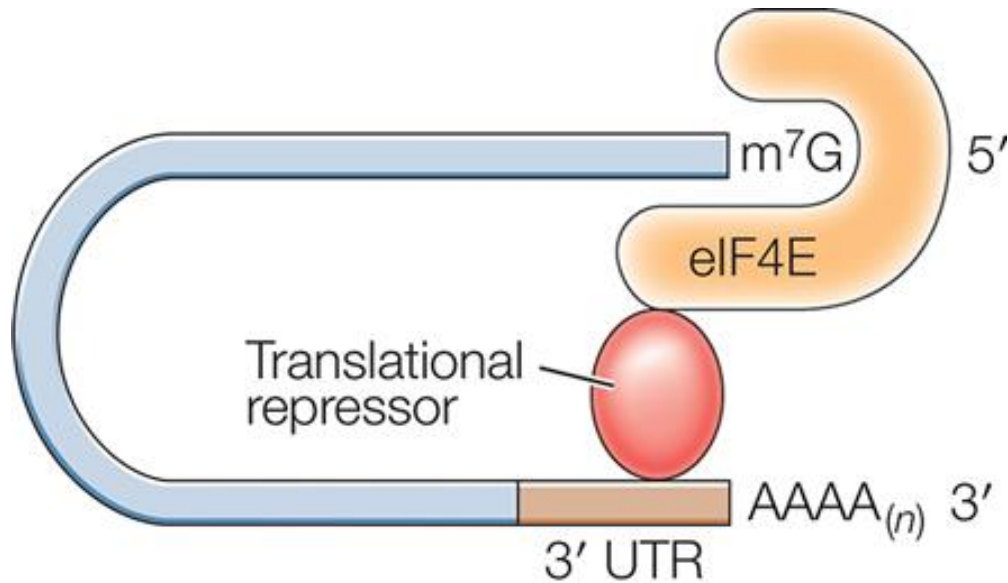
Regulation of ferritin synthesis



- The ferritin mRNA contains an iron response element (IRE) near its 5' cap.
- If iron is scarce, a protein called iron regulatory protein (IRP2) binds to the IRE, blocking translation by interfering with the binding of the mRNA to the 40S ribosomal subunit.
- In the presence of iron, IRP is neutralized 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

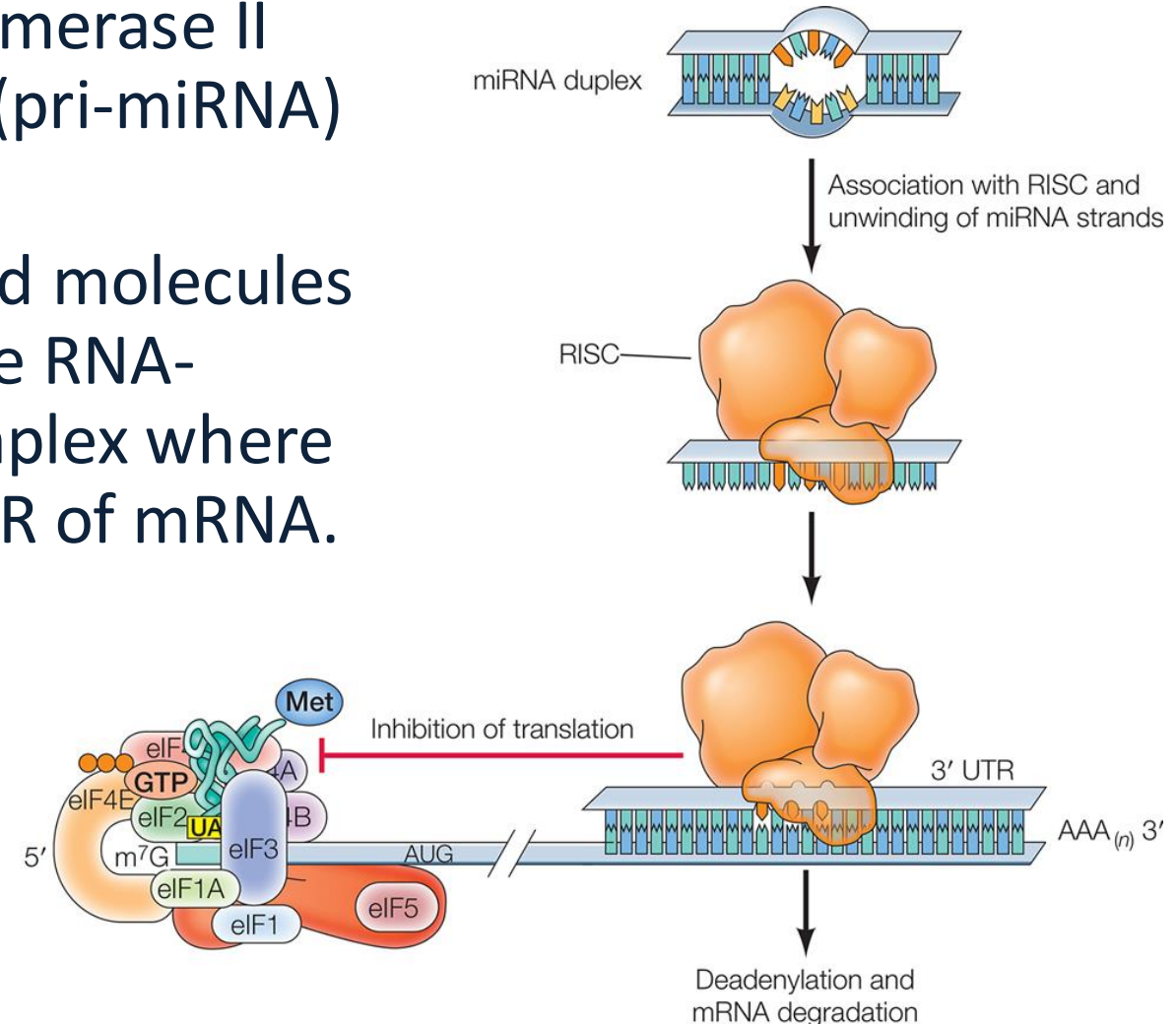
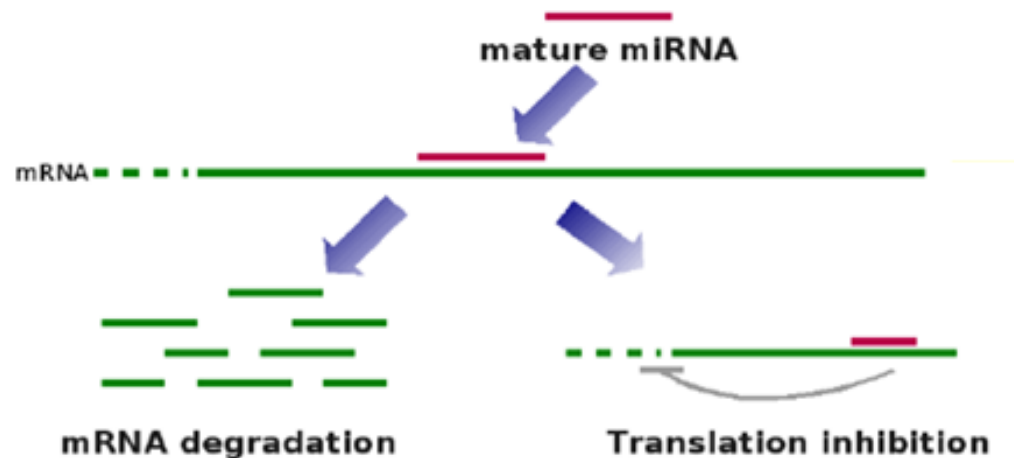


Regulation by microRNA (miRNA) *and siRNA*

Regulation by microRNA (miRNA)



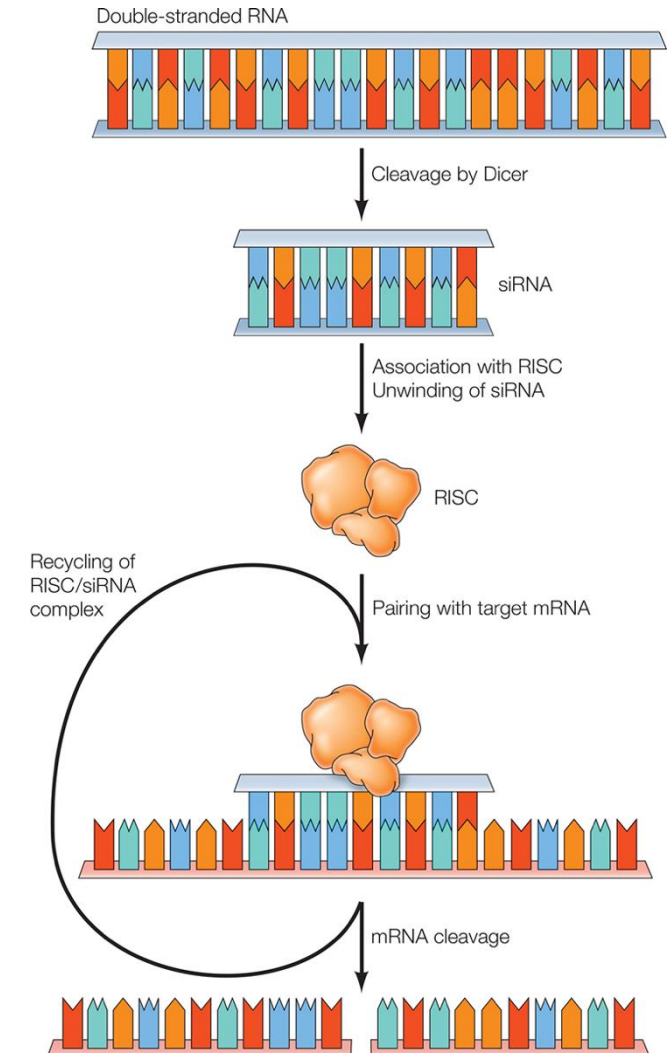
- MicroRNA is synthesized by RNA polymerase II into single-stranded, primary miRNA (pri-miRNA) transcript.
- It gets processed into double-stranded molecules but only one strand is loaded onto the RNA-induced silencing complex (RISC) complex where miRNA targets and binds to the 3'-UTR of mRNA.
- The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



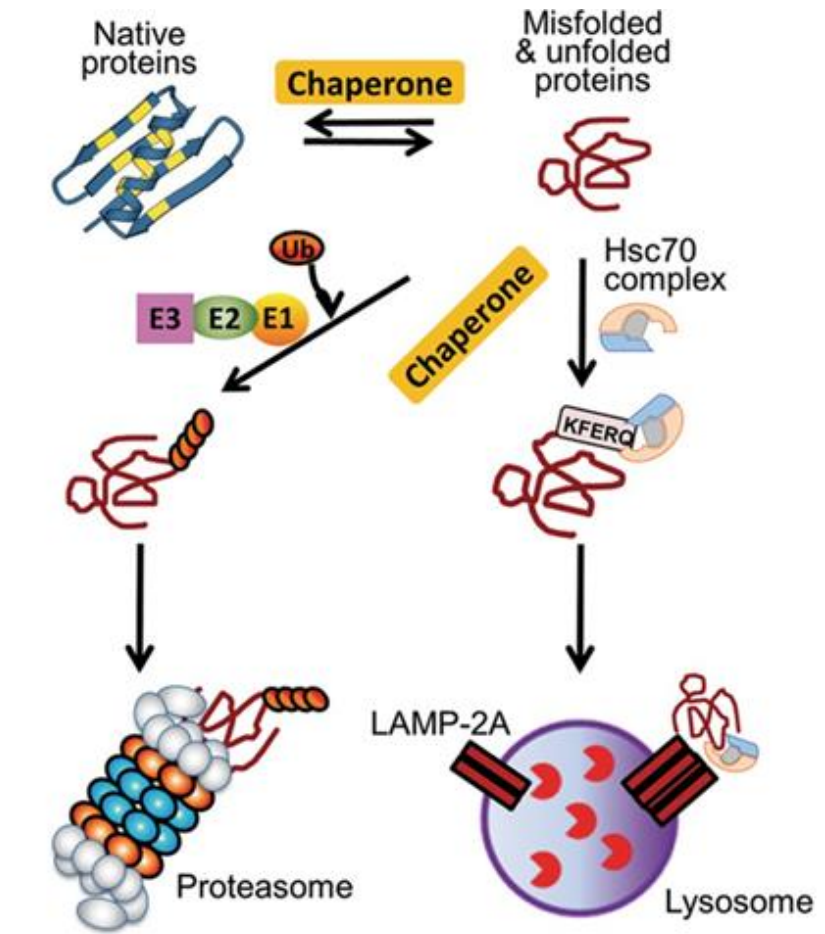


What else?

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 ubiquitinated.





The multi-levels of regulation

Levels of regulation

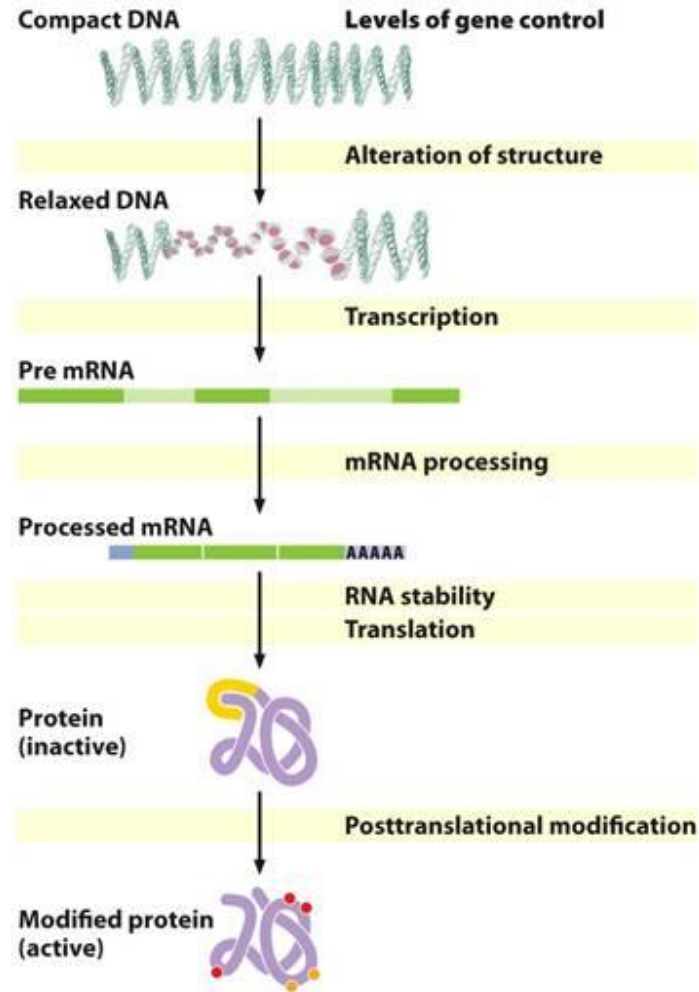


Figure 16.1
Genetics: A Conceptual Approach, Fifth Edition
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- Transcription
- RNA processing
- RNA transport
- mRNA stability
- Translation
- Post-translational modification
- Protein activity
- Protein degradation