



Molecular Biology (9)

Translation

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Second semester, 2019-2020

Resources



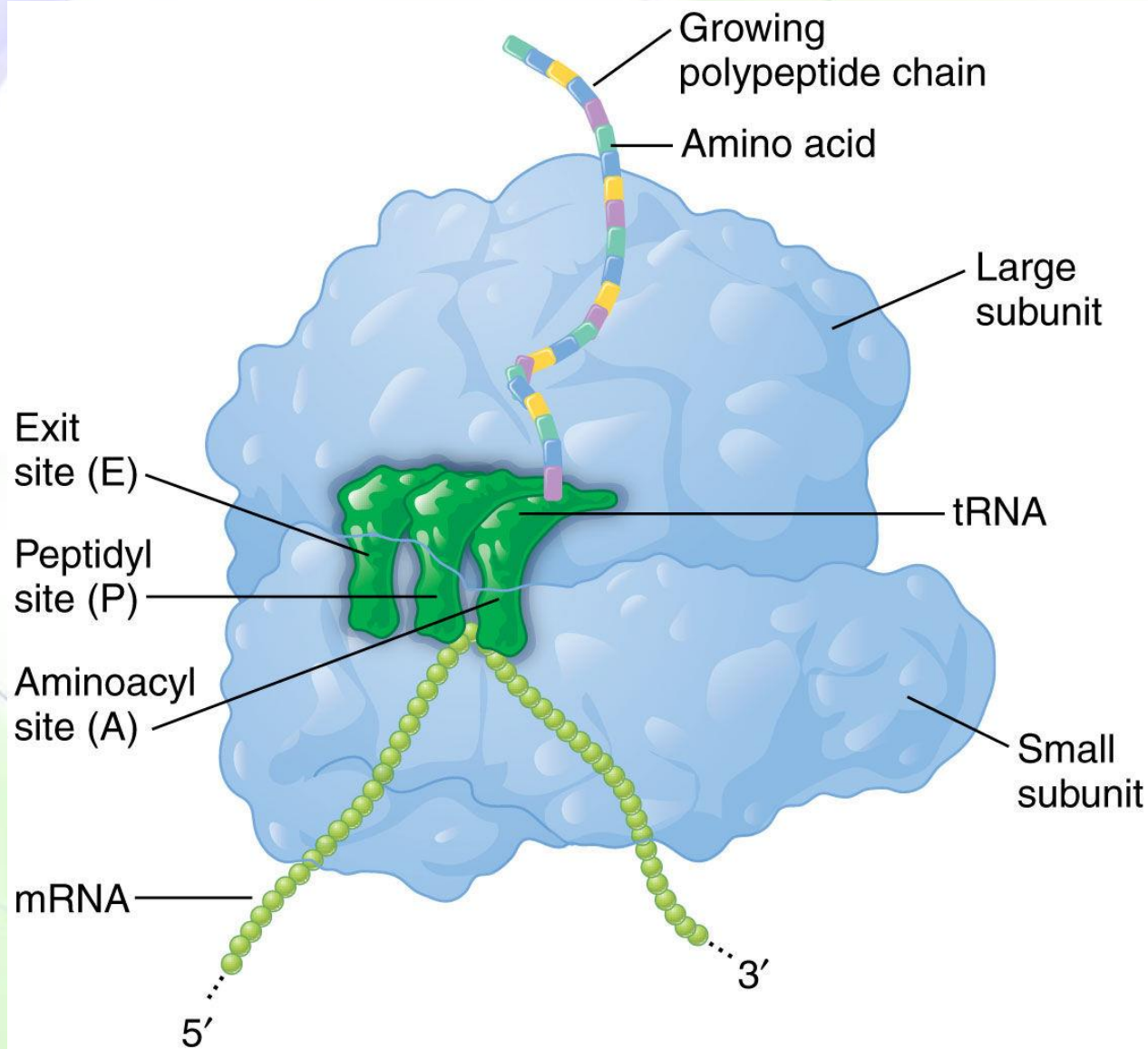
- This lecture
- Cooper, Ch. 9

General information

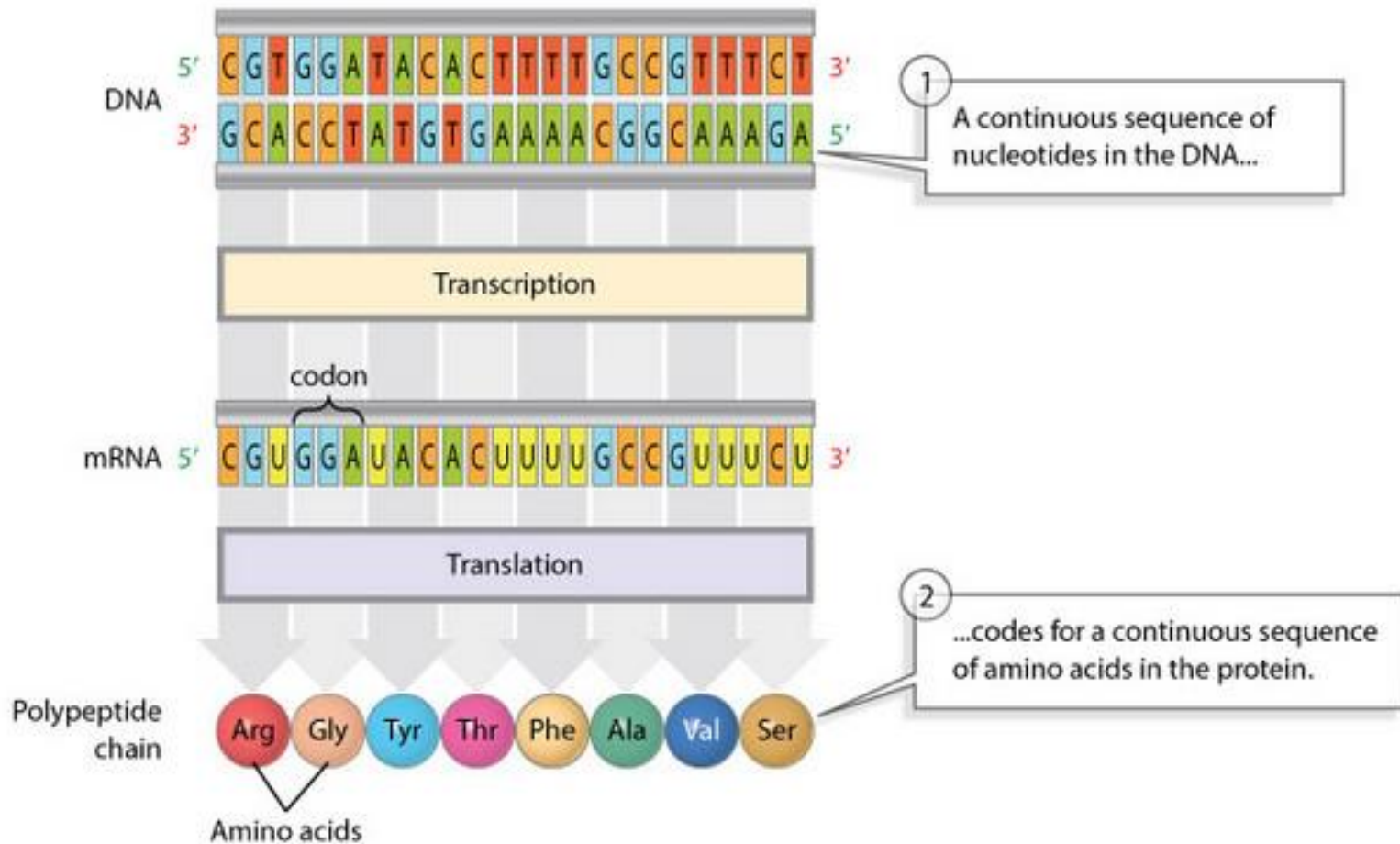


- Protein synthesis involves interactions between three types of RNA molecules:
 - tRNAs
 - rRNAs, which exist in ribosomes (the factories of protein synthesis)
 - mRNA templates

Components of the translation complex

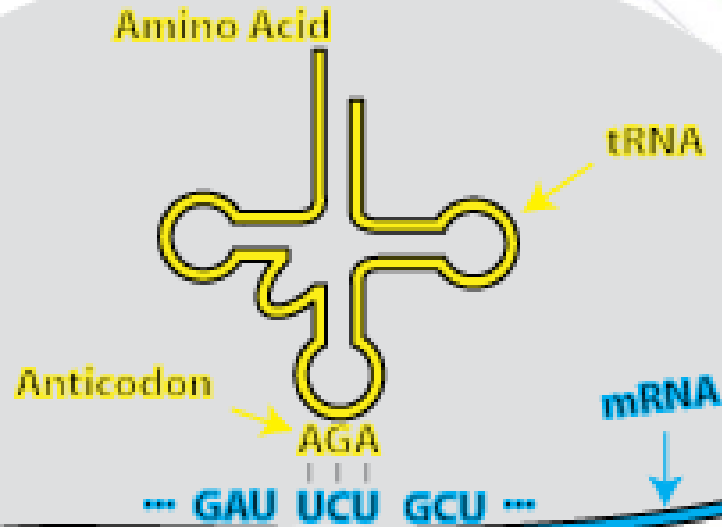


Colinearity of genes, mRNAs, & proteins



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



Codon

MCAT-Review.org

Second letter



First letter

Third letter

	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
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 } 7	U C A G

Features of the genetic codon



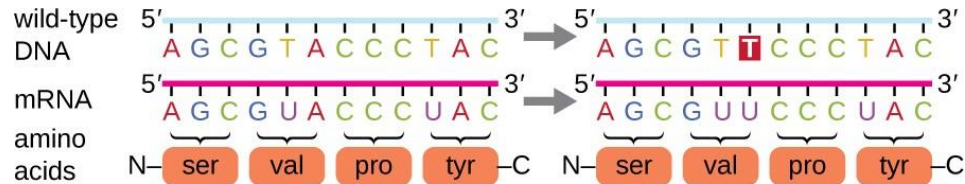
- All 64 possible codons of the genetic code and the amino acid specified by each, as read in the 5'→3' direction from the mRNA sequence.
- Sixty-one codons specify an amino acid.
 - Three **STOP** codons (UAA, UAG, and UGA) do not encode any amino acid.
- The genetic code for mitochondrial mRNA (mtDNA)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

Single point mutations

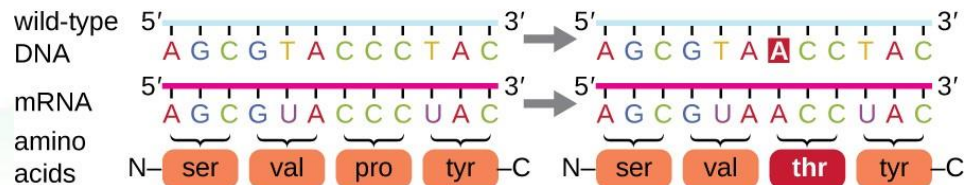


point mutation: substitution of a single base

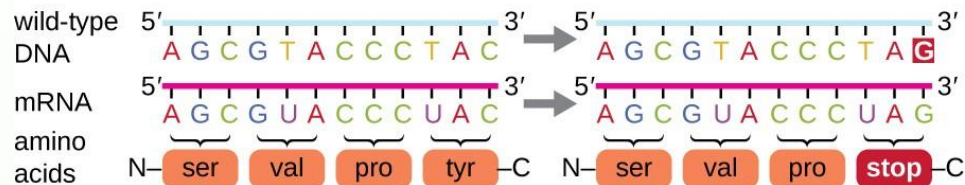
silent: has no effect on the protein sequence



missense: results in an amino acid substitution

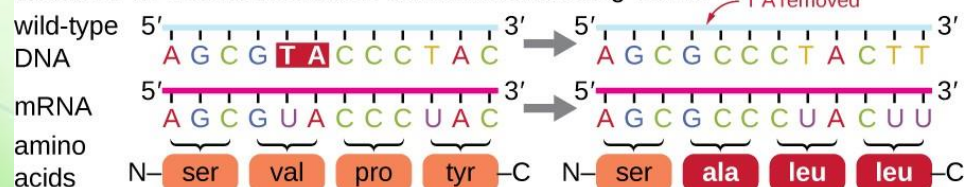


nonsense: substitutes a stop codon for an amino acid



frameshift mutation: insertion or deletion of one or more bases

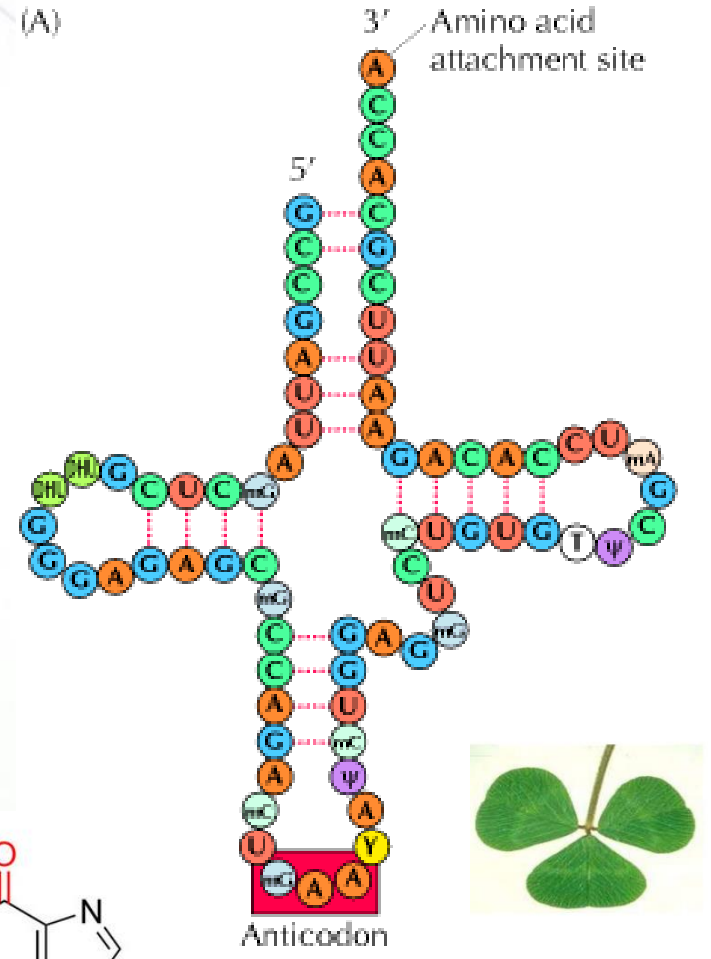
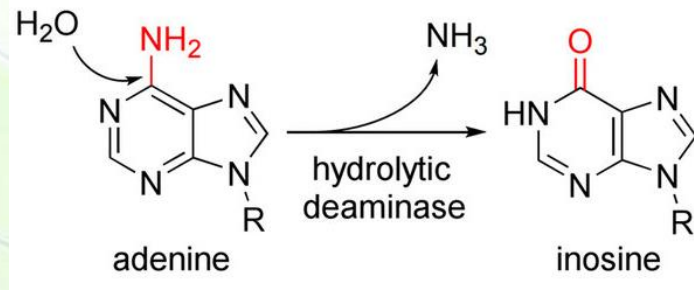
Insertion or deletion results in a shift in the reading frame.



tRNA structure



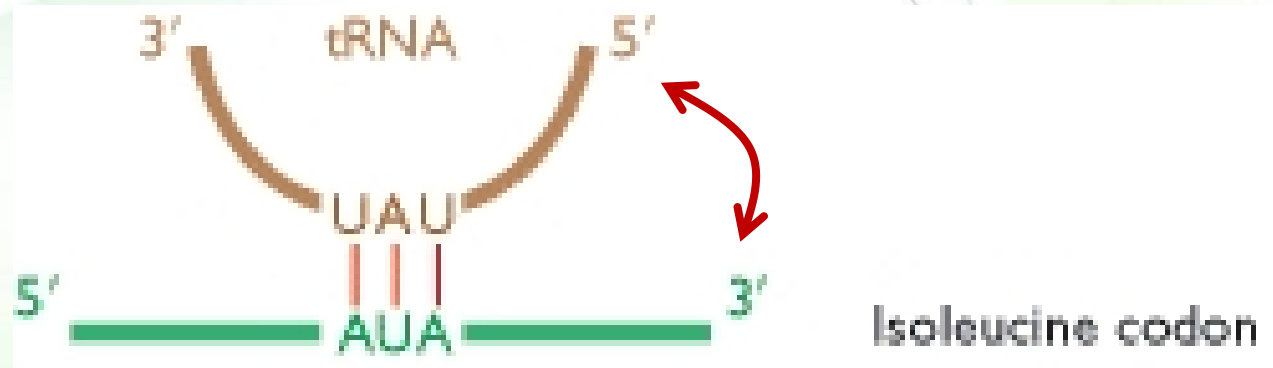
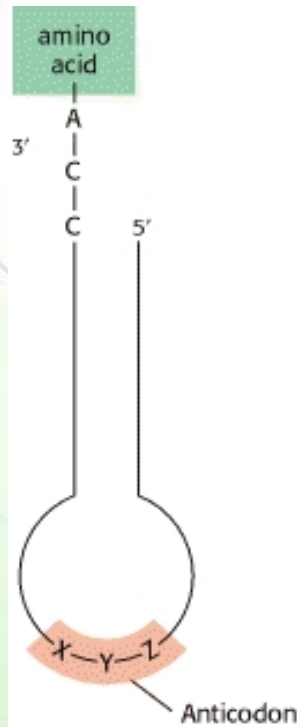
- ▶ tRNAs are short single-stranded RNA molecules (80 bases long).
- ▶ “Charged” or “activated” tRNA carries one amino acid, which is attached by aminoacyl-tRNA synthetases.
- ▶ An amino acid is covalently attached to the ribose of the terminal adenosine at CCA.
 - ▶ The amino acid attached to tRNA is 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).



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)

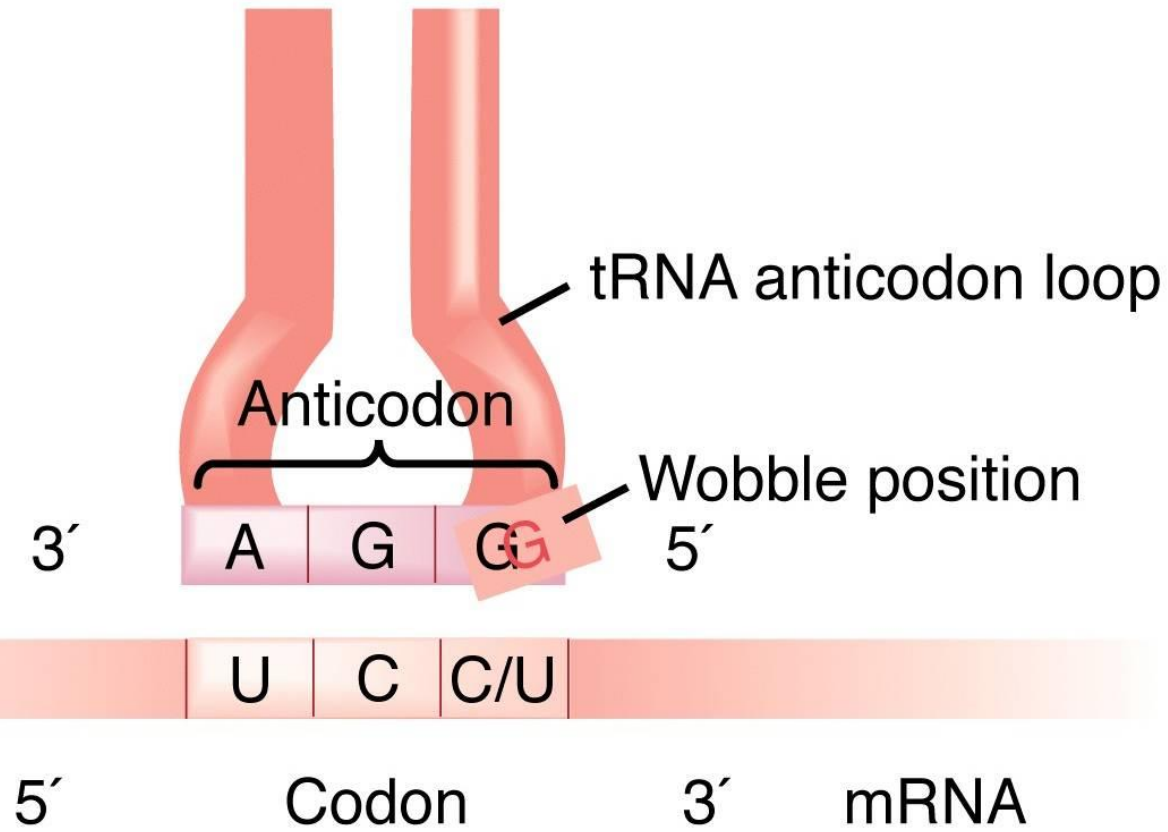


Fidelity of translation



- Accurate translation requires two steps
 - First: accurate association of amino acid to tRNA by **aminoacyl-tRNA synthetase**
 - Second: a correct match between the tRNA anticodon and an mRNA codon
- 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.
 - It acts as a buffer against deleterious mutations.

Wobble base pairing



Examples of wobble base pairing



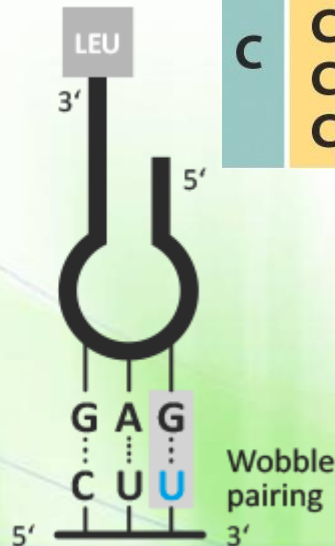
- Relaxed base pairing results from the formation of G-U base pairs.

	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

Both are hydrophobic amino acids



Identical leucine tRNAs

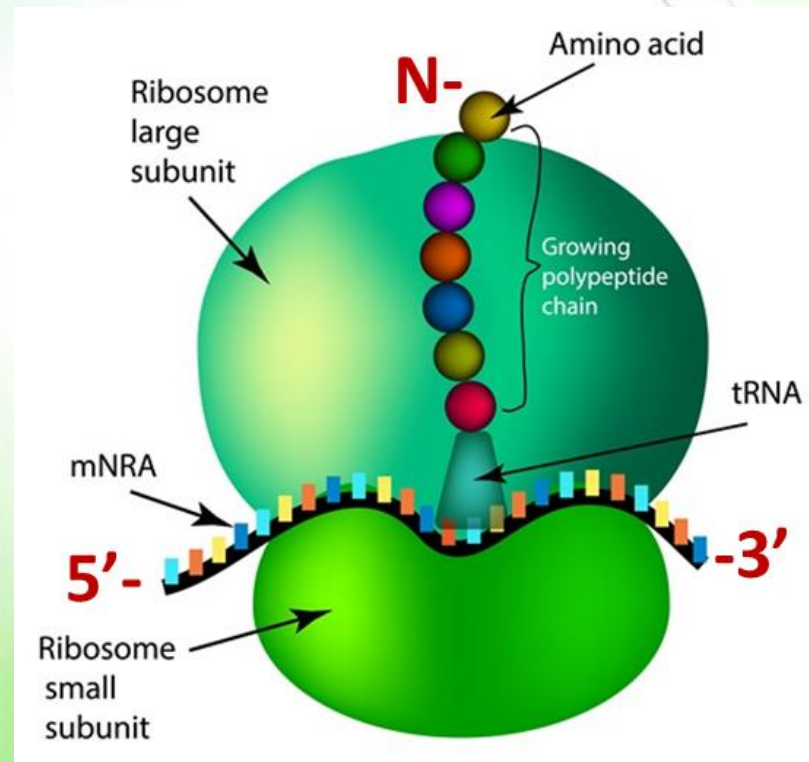


Ribosomes



- 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.

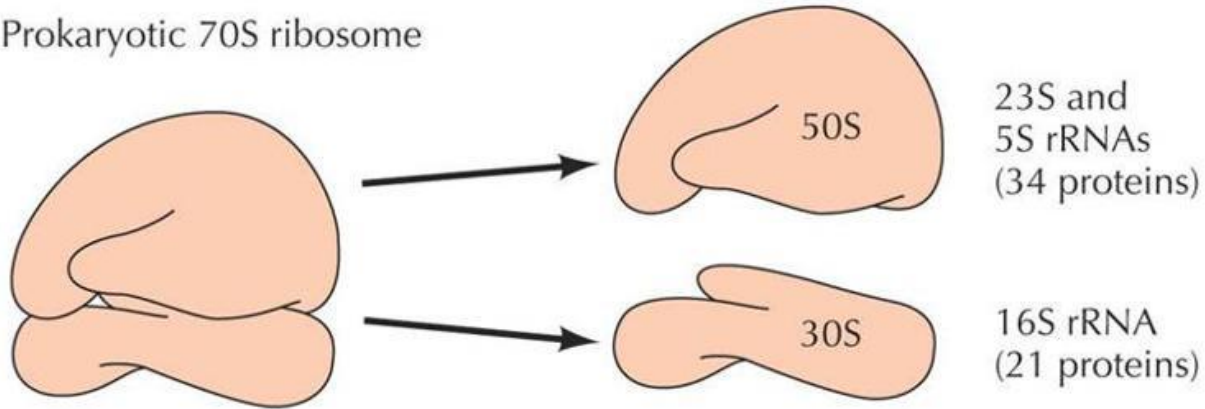


Ribosome structure

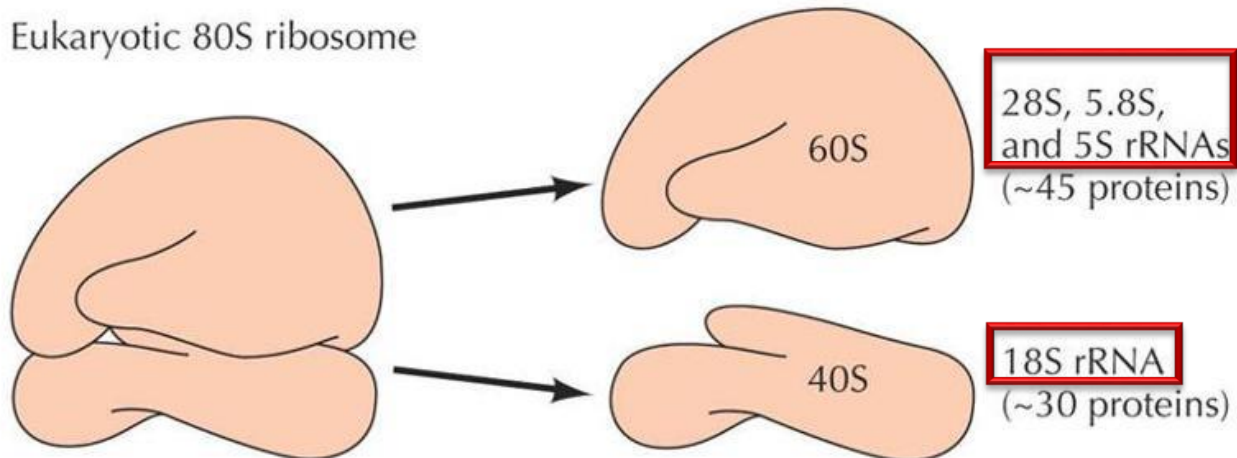


(A)

Prokaryotic 70S ribosome



Eukaryotic 80S ribosome



rRNA synthesis and processing



- The 18S, 5.8S, and 28S rRNAs are encoded by a single gene (13 kb long).
- 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 28S rRNAs associates with the large ribosomal subunit.
- The large ribosomal subunit also contains 5S rRNA, which is encoded by different genes transcribed by RNA polymerase III.

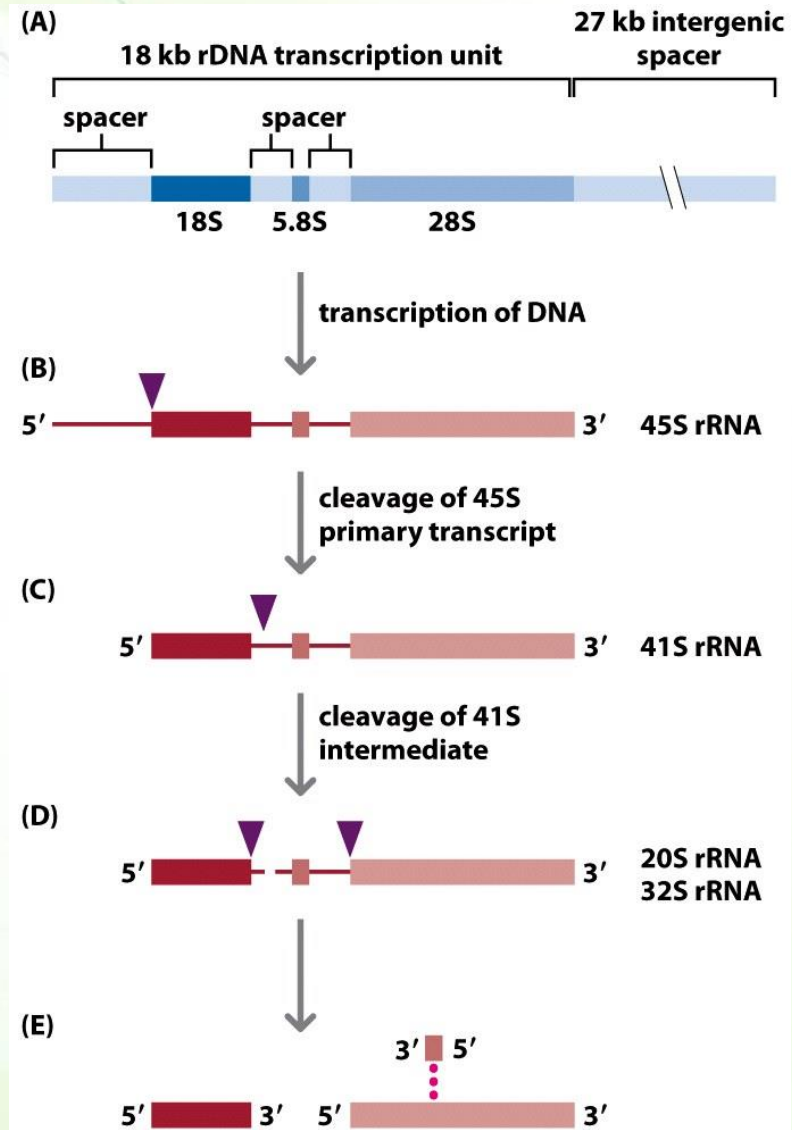


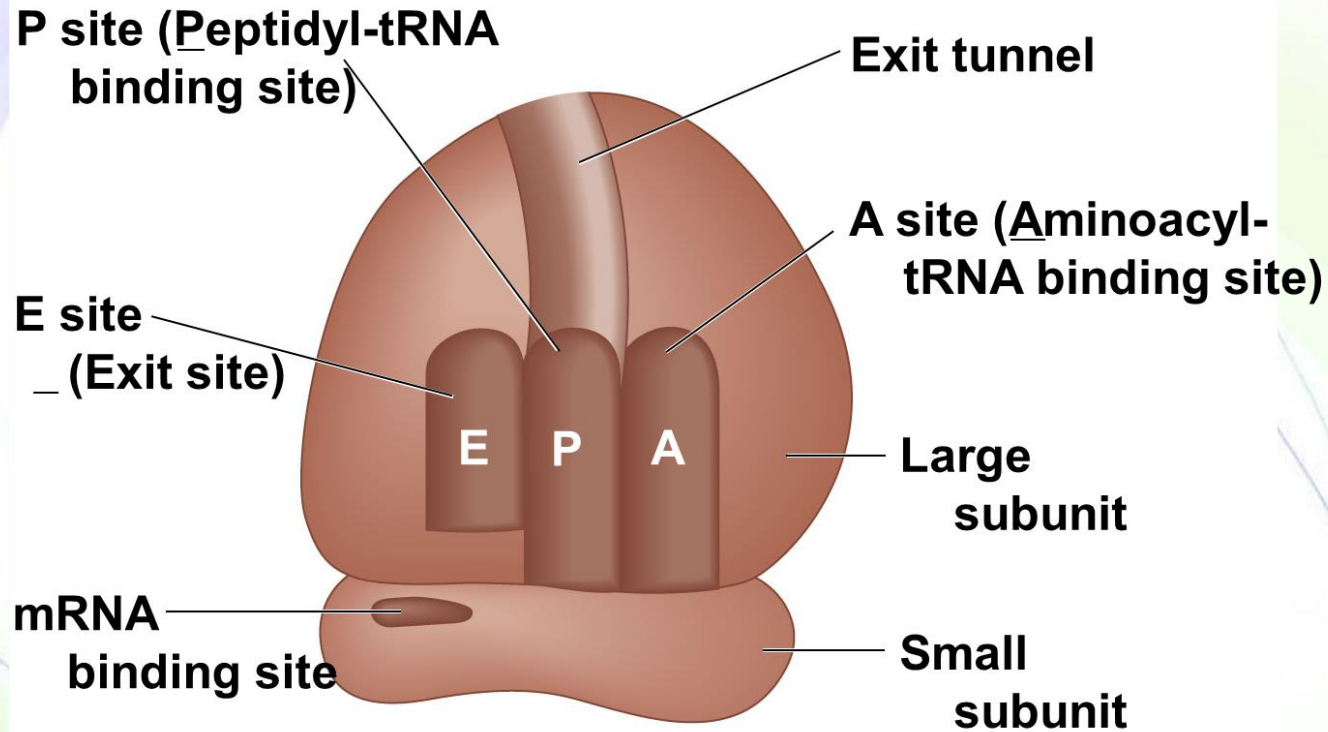
Figure 1.22 Human Molecular Genetics, 4ed. (© Garland Science)

Functional and structural components of ribosomes



- 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.

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

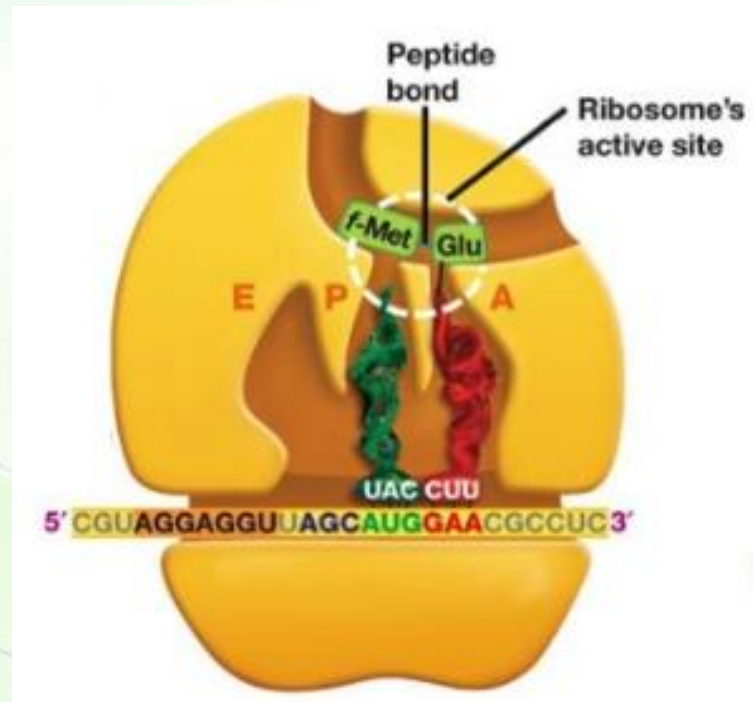
The **A site** holds the tRNA that carries the next amino acid to be added to the chain

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

The general mechanism of translation



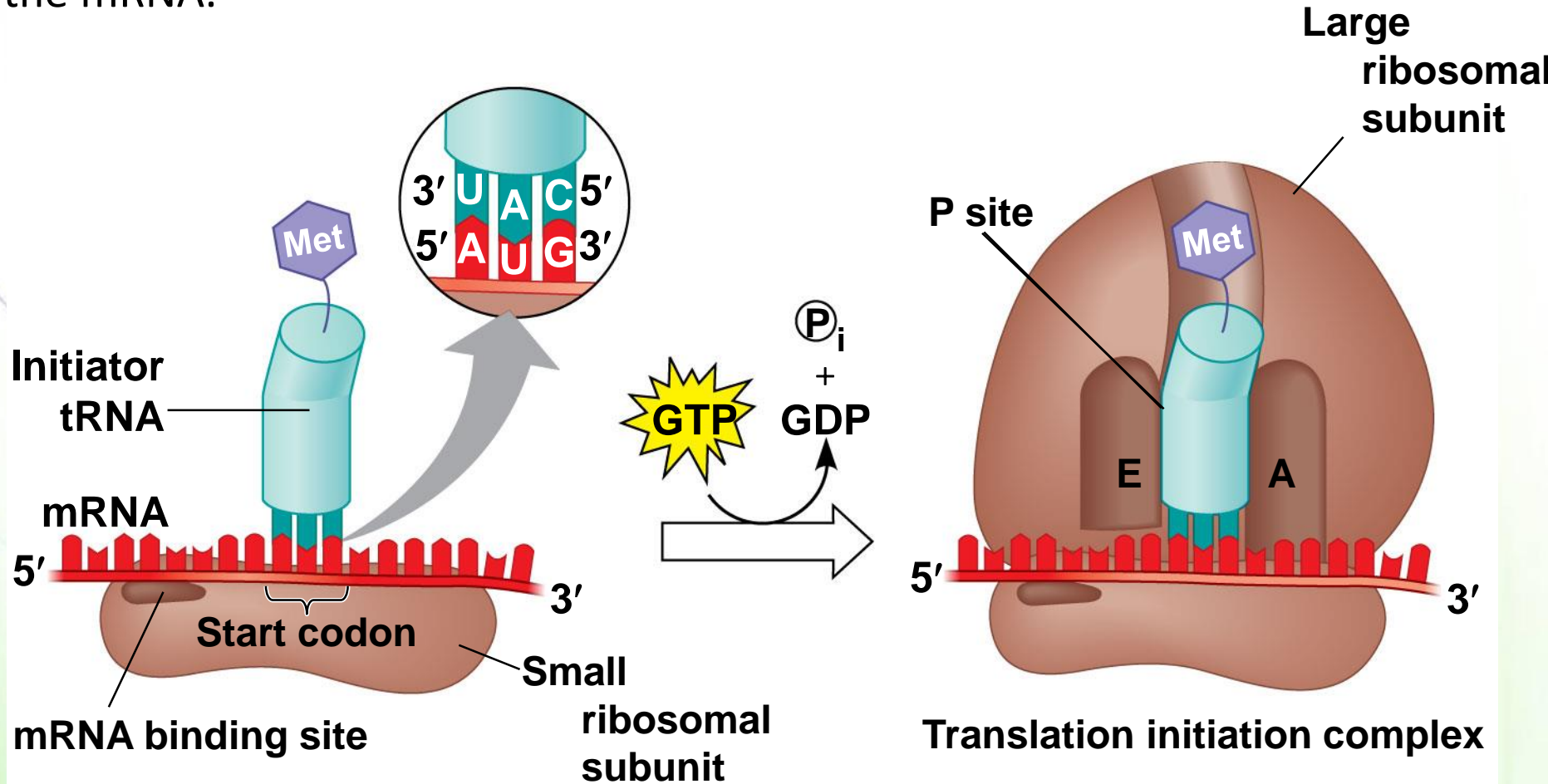
- Three stages: initiation, elongation, and termination.
- The direction is 5' → 3'.
- Protein synthesis begins at the amino terminus and extends toward the carboxyl terminus.



Start of translation



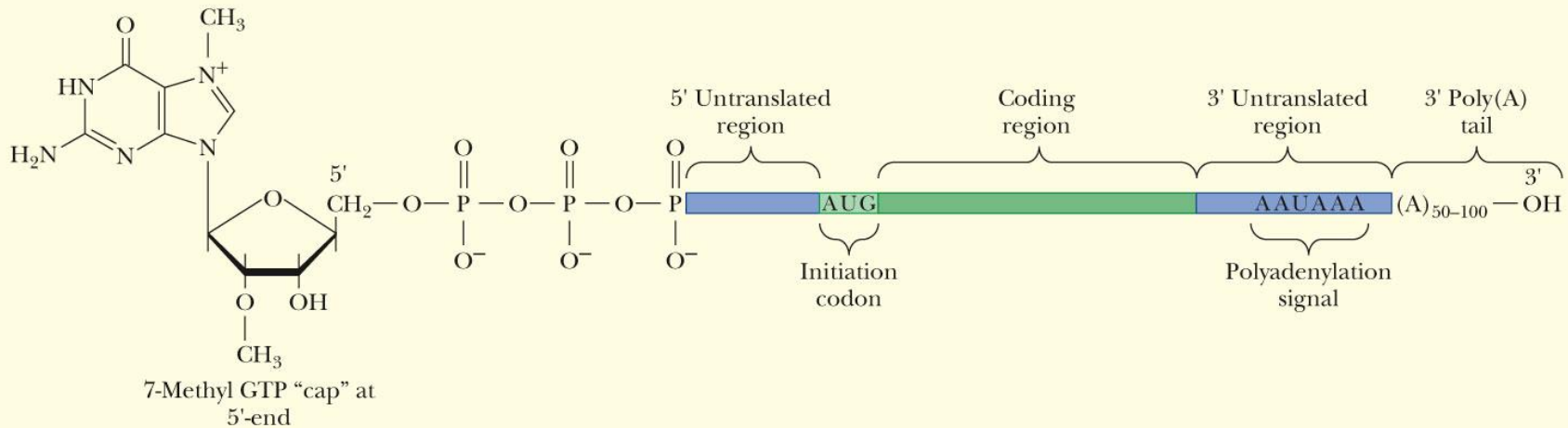
In both prokaryotes and eukaryotes, translation starts at specific initiation sites, which UAG (methionine), and not from the first codon of the mRNA.



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.

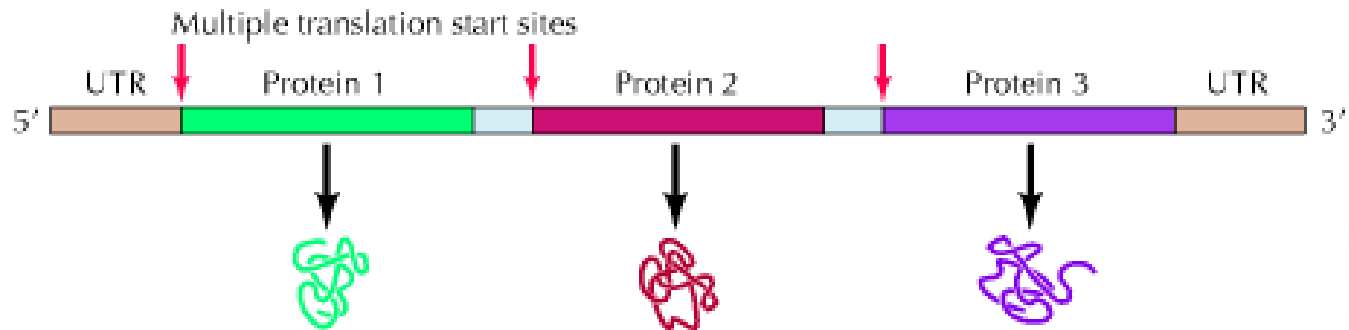


Remember...

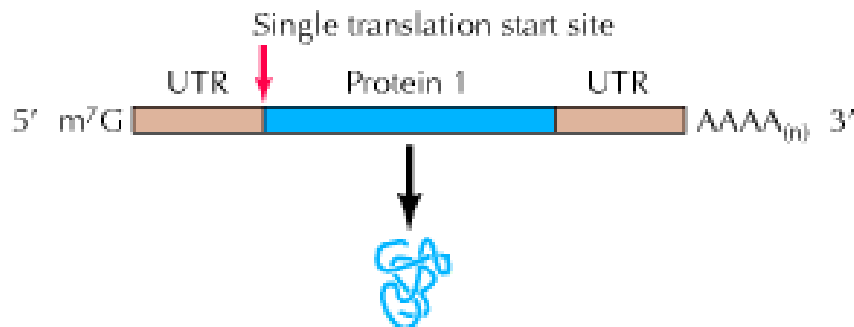


- Bacterial mRNA is polycistronic
- Eukaryotic mRNA is monocistronic

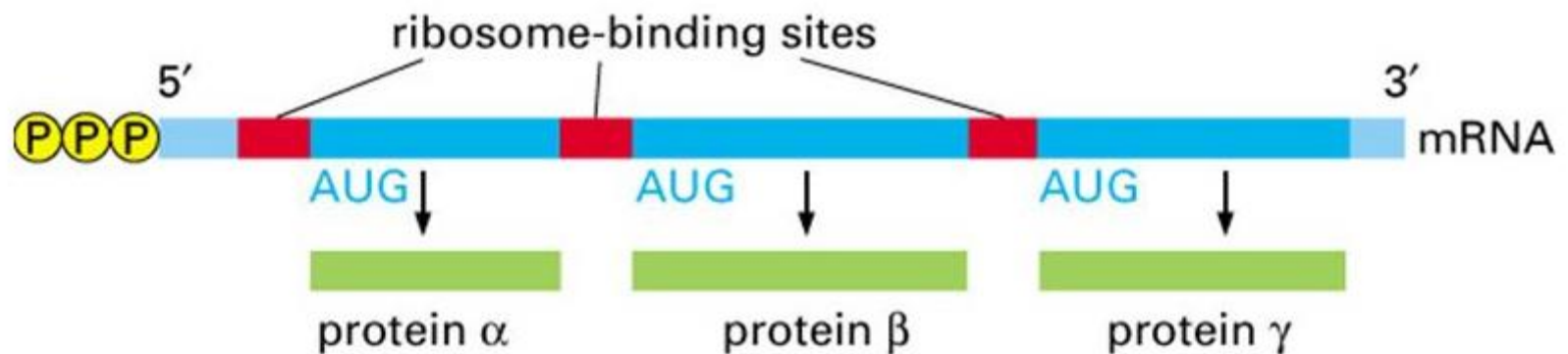
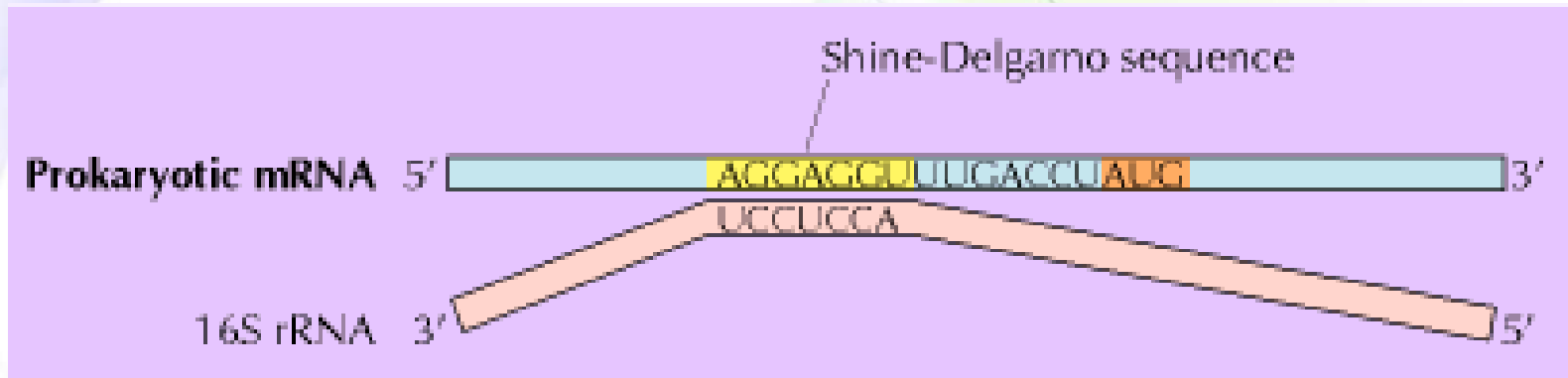
Prokaryotic mRNA



Eukaryotic mRNA



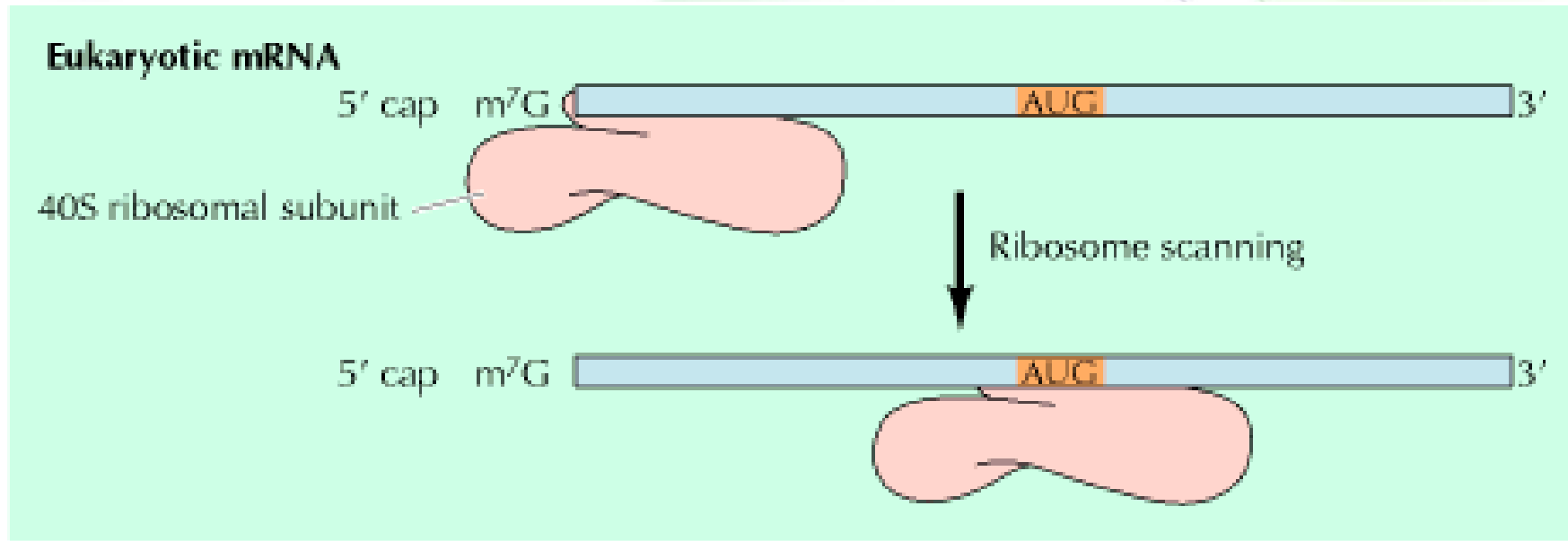
Shine-Dalgarno sequence



But in eukaryotes...



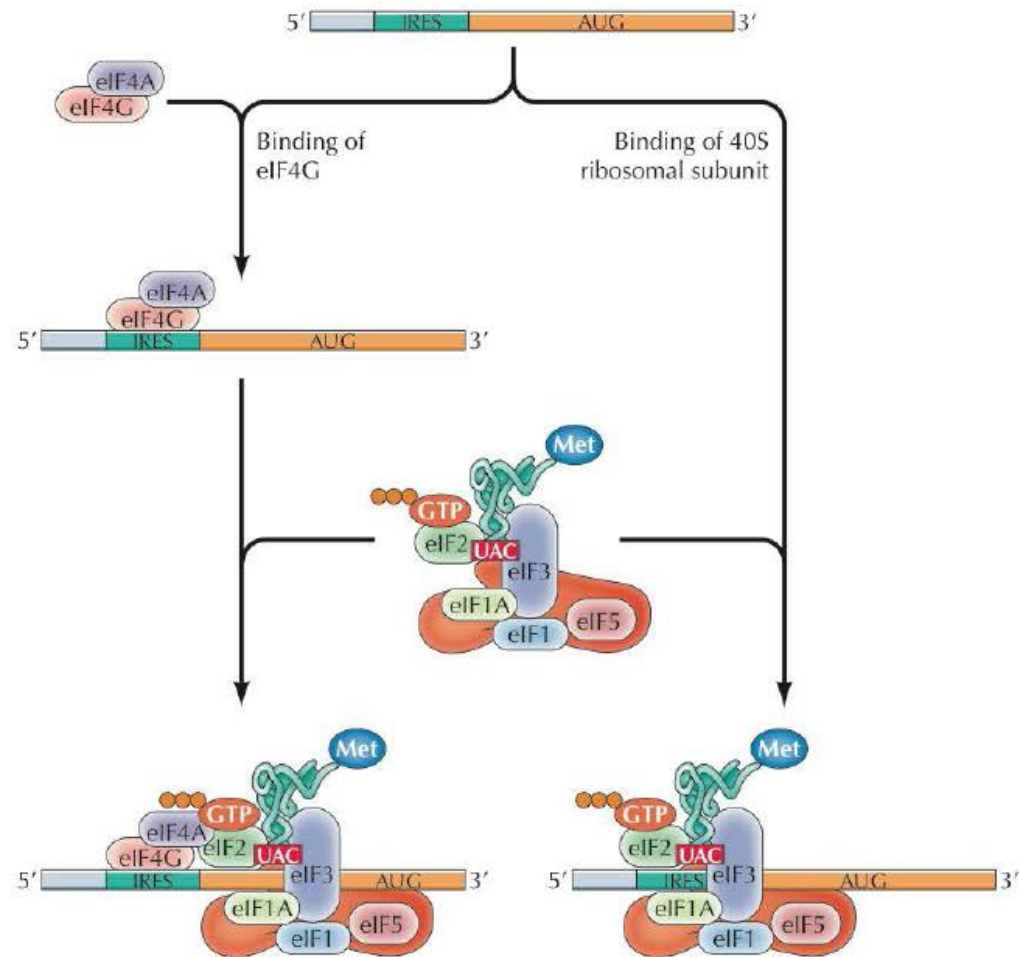
- Eukaryotic ribosomes recognize mRNAs by binding to the 7-methylguanosine cap at their 5' terminus



Internal ribosome entry site (IRES)



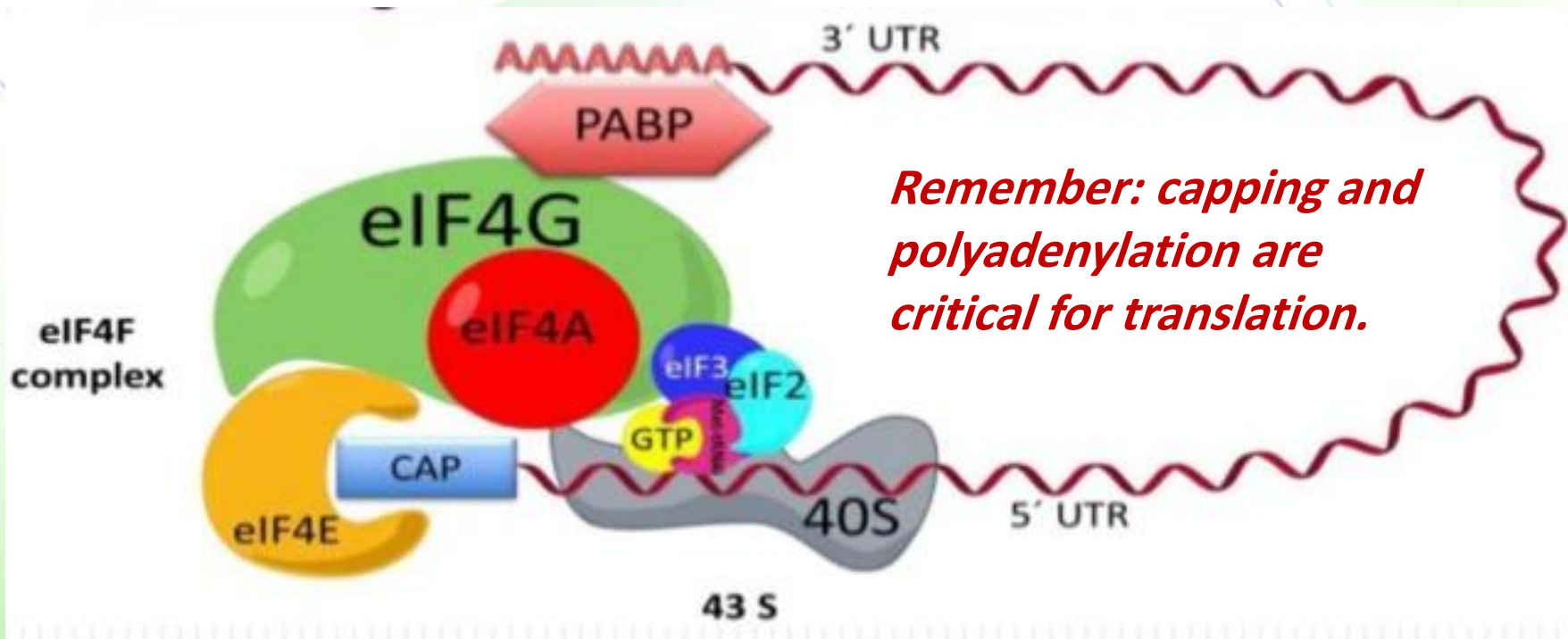
- Alternatively, internal ribosome entry site (IRES) exist in some other mRNAs and is recognized by the 40S ribosome or eIF4G protein followed by recruitment of the 40S ribosome.



Translation initiation in eukaryotes



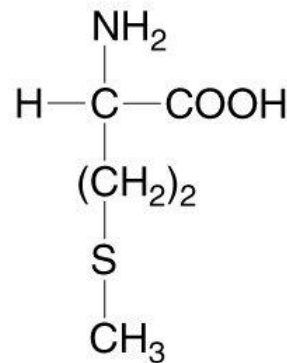
- The initiation factor, eIF4G, is member of a complex that links the poly-A tail to the CAP via poly-A binding protein (PABP) to the CAP-binding protein eIF4E.



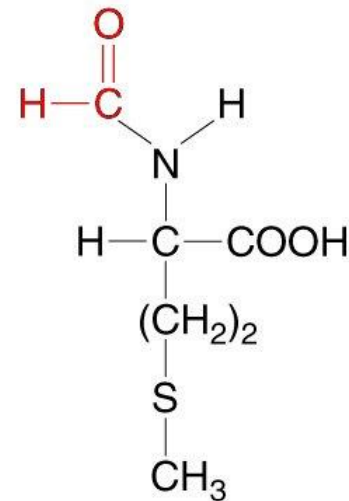
The first amino acid



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



Methionine



N-Formylmethionine

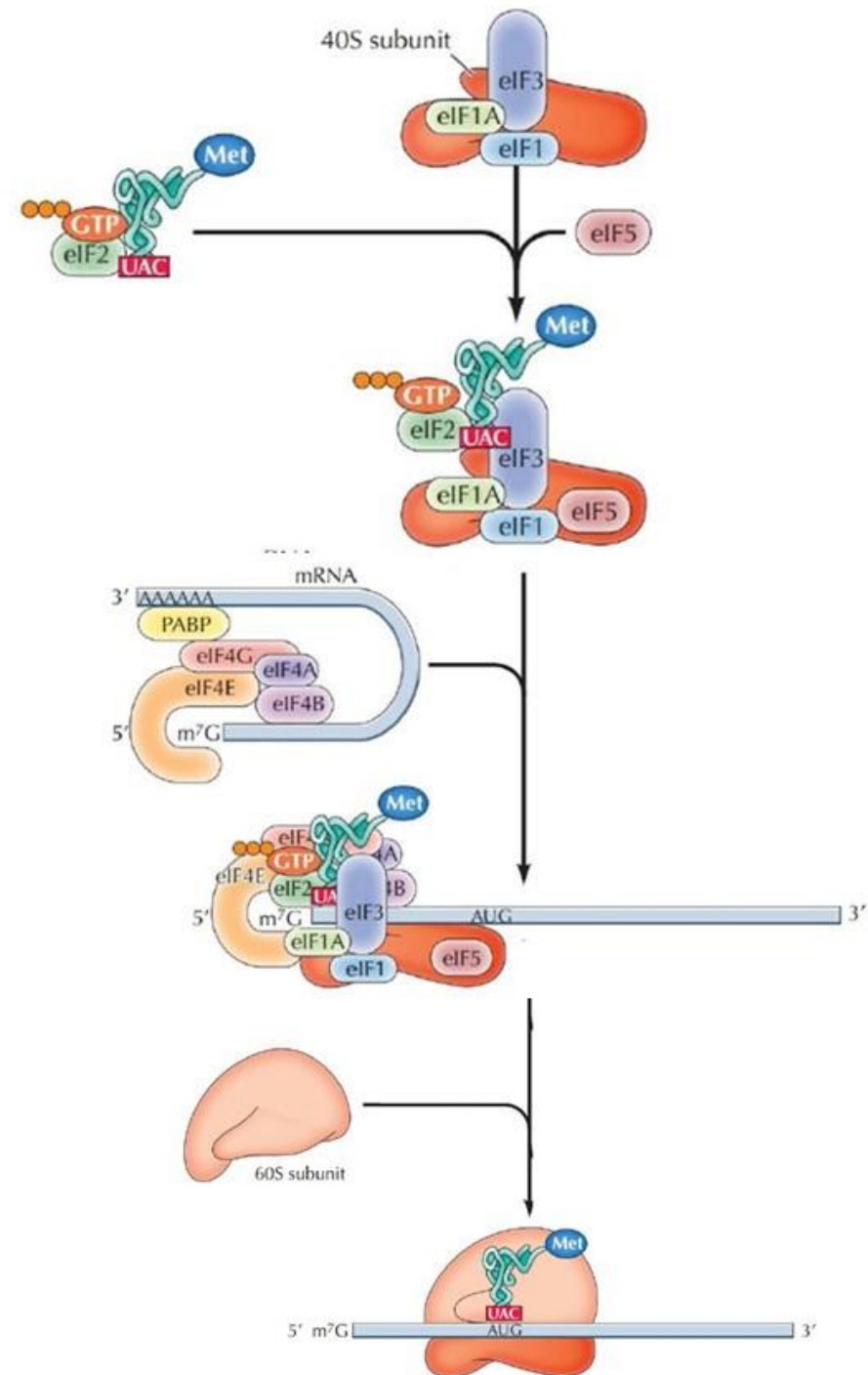
Building a Polypeptide



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

Translation initiation

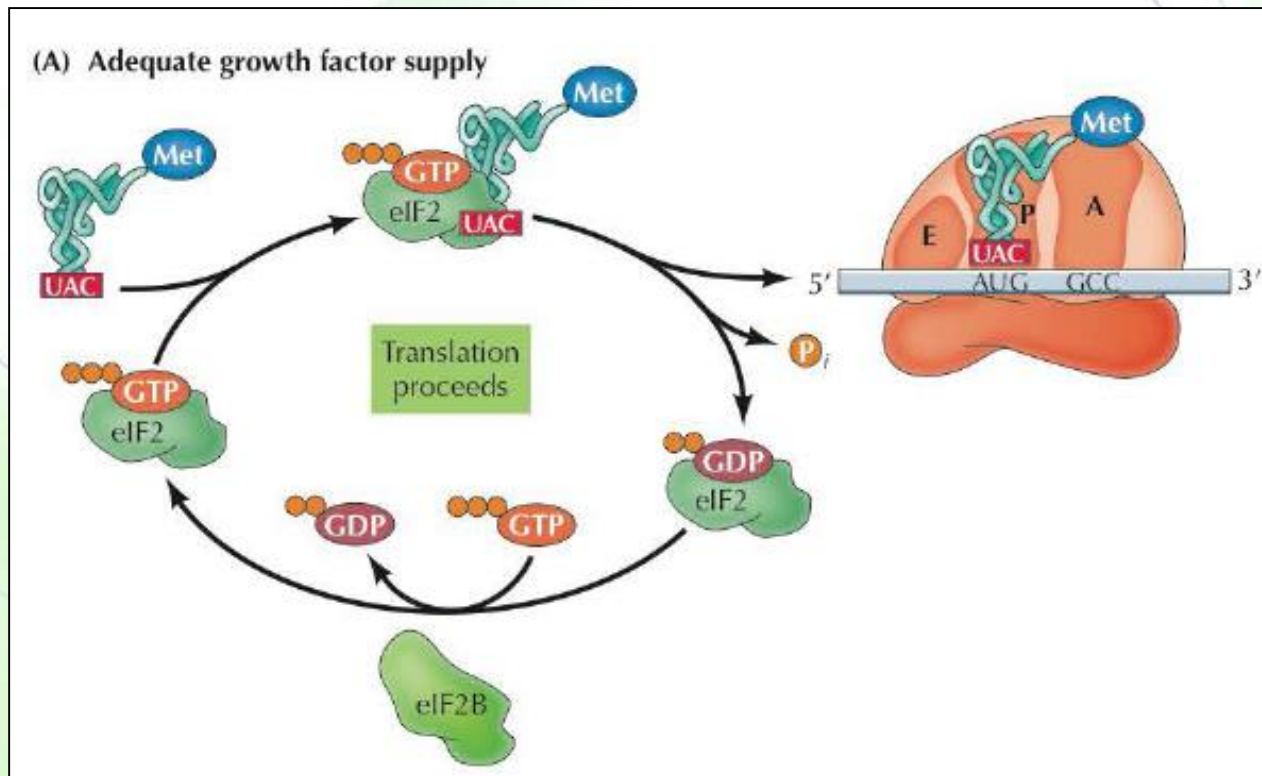
- tRNA forms a complex with 40S ribosomal subunit.
- mRNA joins the complex.
- The 40S ribosomal subunit scans for the first AUG.
- The large ribosomal subunit joins them all.
- A large group of initiation factors facilitate every step.
 - eIF2 brings tRNA to small ribosomal subunit.
 - eIF4F and eIF4G bring mRNA to tRNA/40S ribosomal subunit.



Regeneration of eEF2



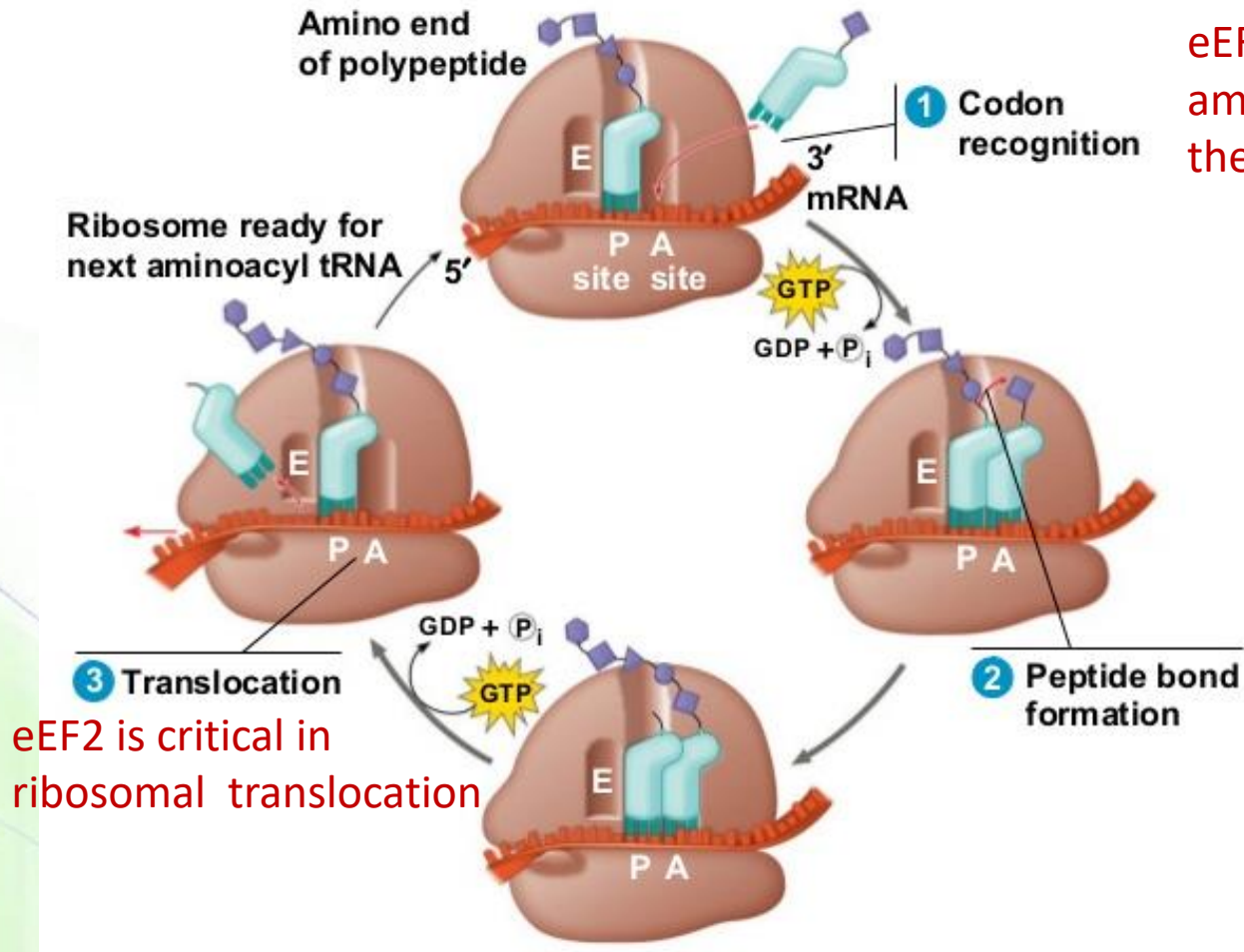
- eEF2 is complexed to GTP to be active. When the correct tRNA is inserted, GTP is hydrolyzed to GDP.
- The active eEF2/GTP complex must be regenerated by exchanging of the bound GDP for GTP.



Translation elongation I

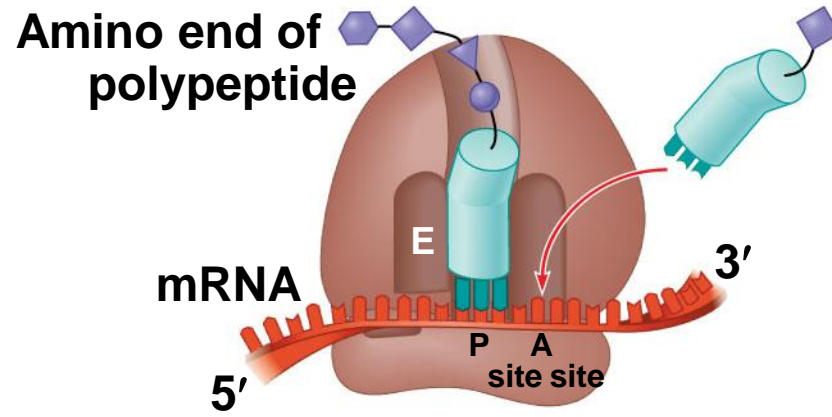


Three steps: aminoacyl-tRNA binding, peptide bond formation, and translocation with the help of elongation factors (eEF).



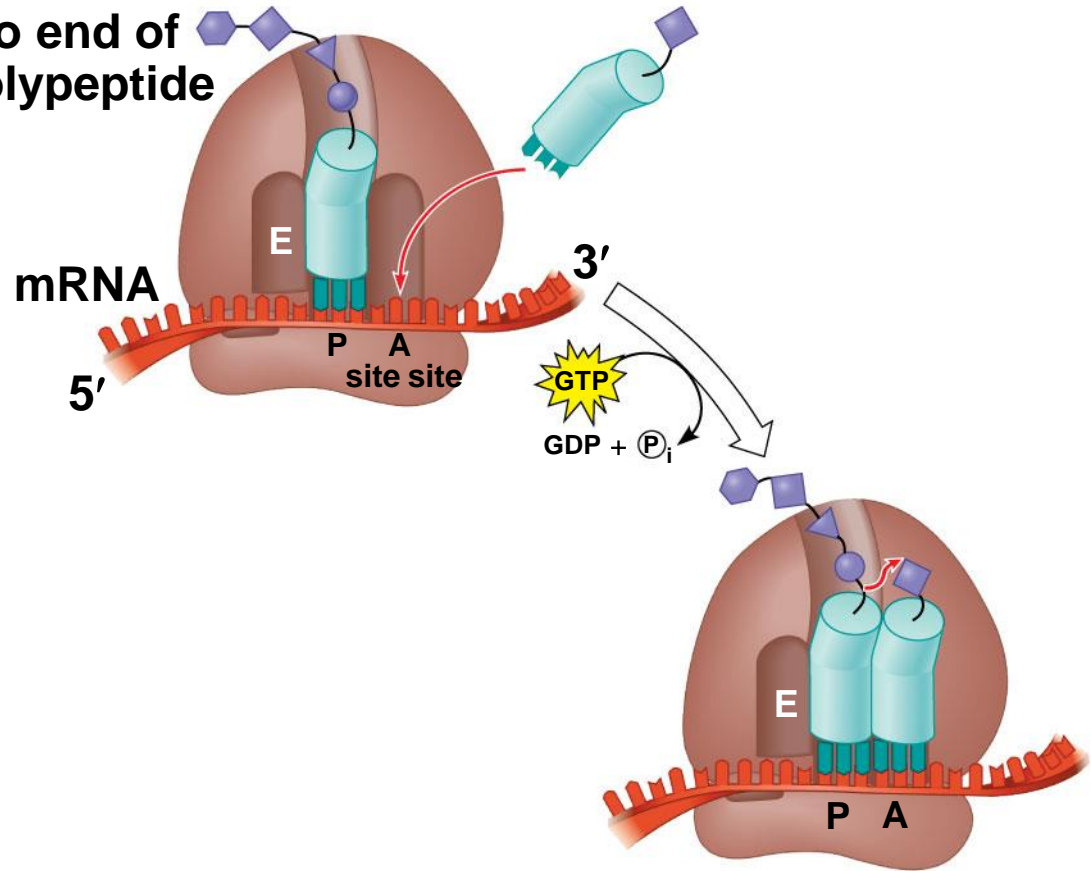
eEF1α brings next aminoacyl-tRNA to the A chamber

eEF2 is critical in ribosomal translocation



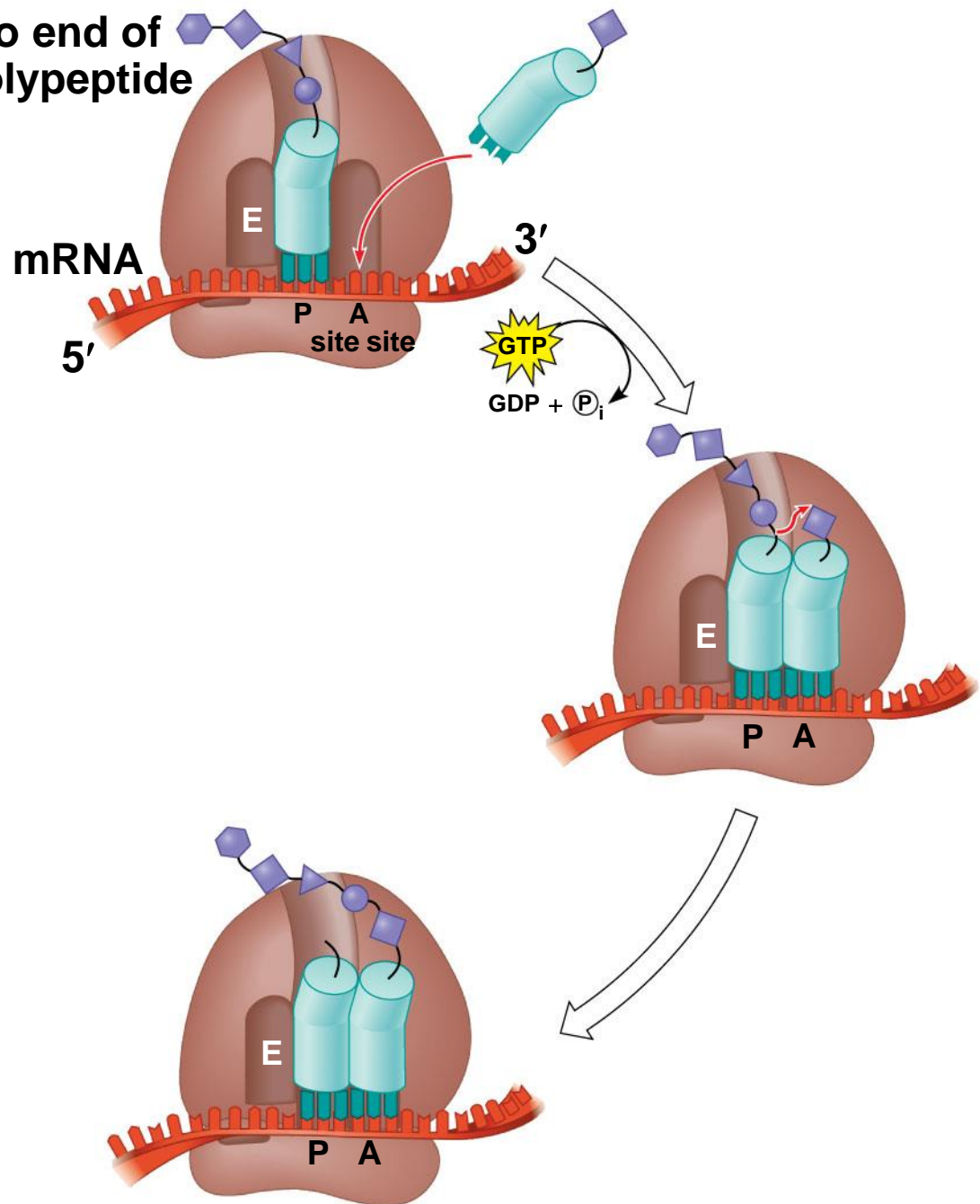


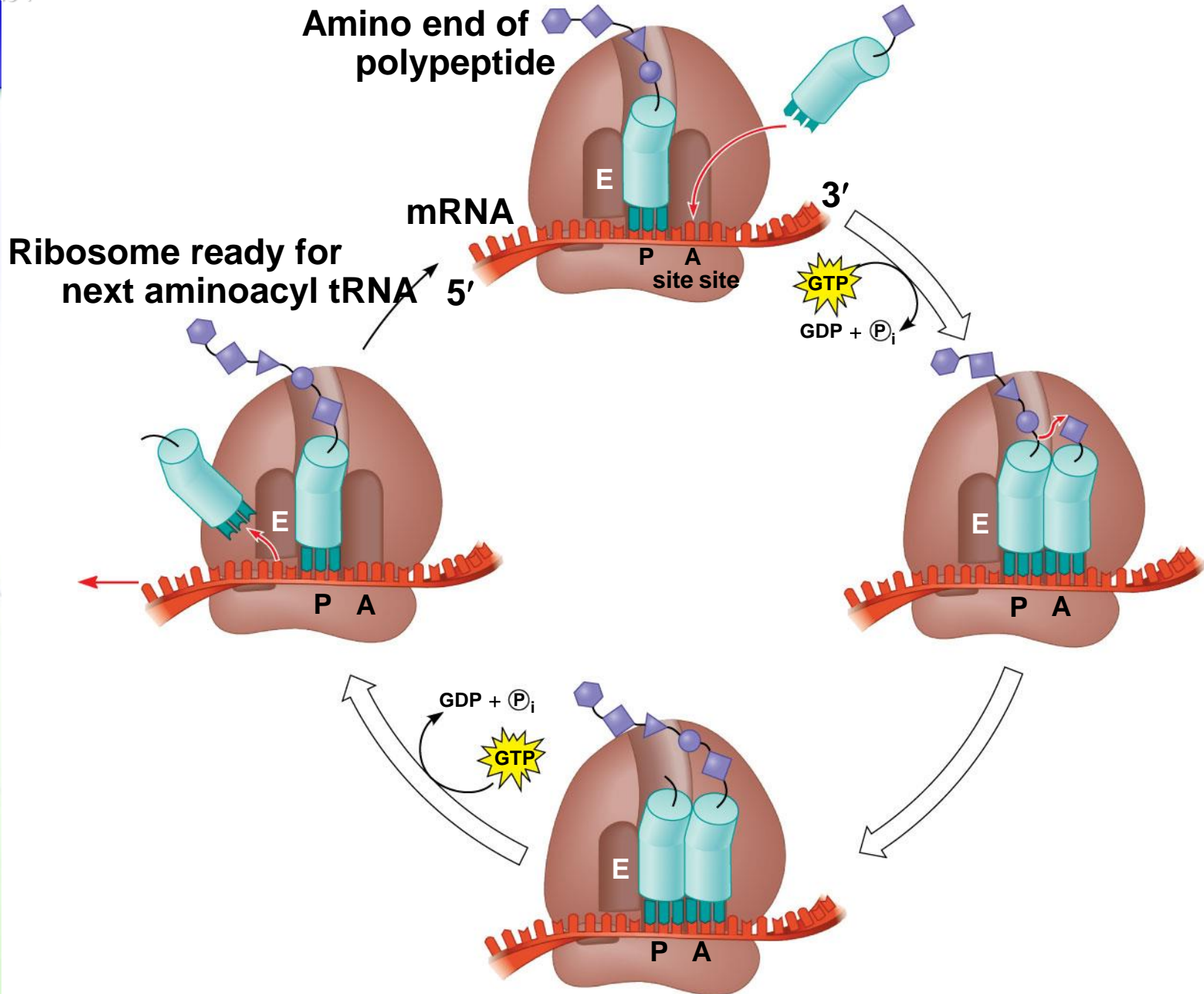
Amino end of polypeptide

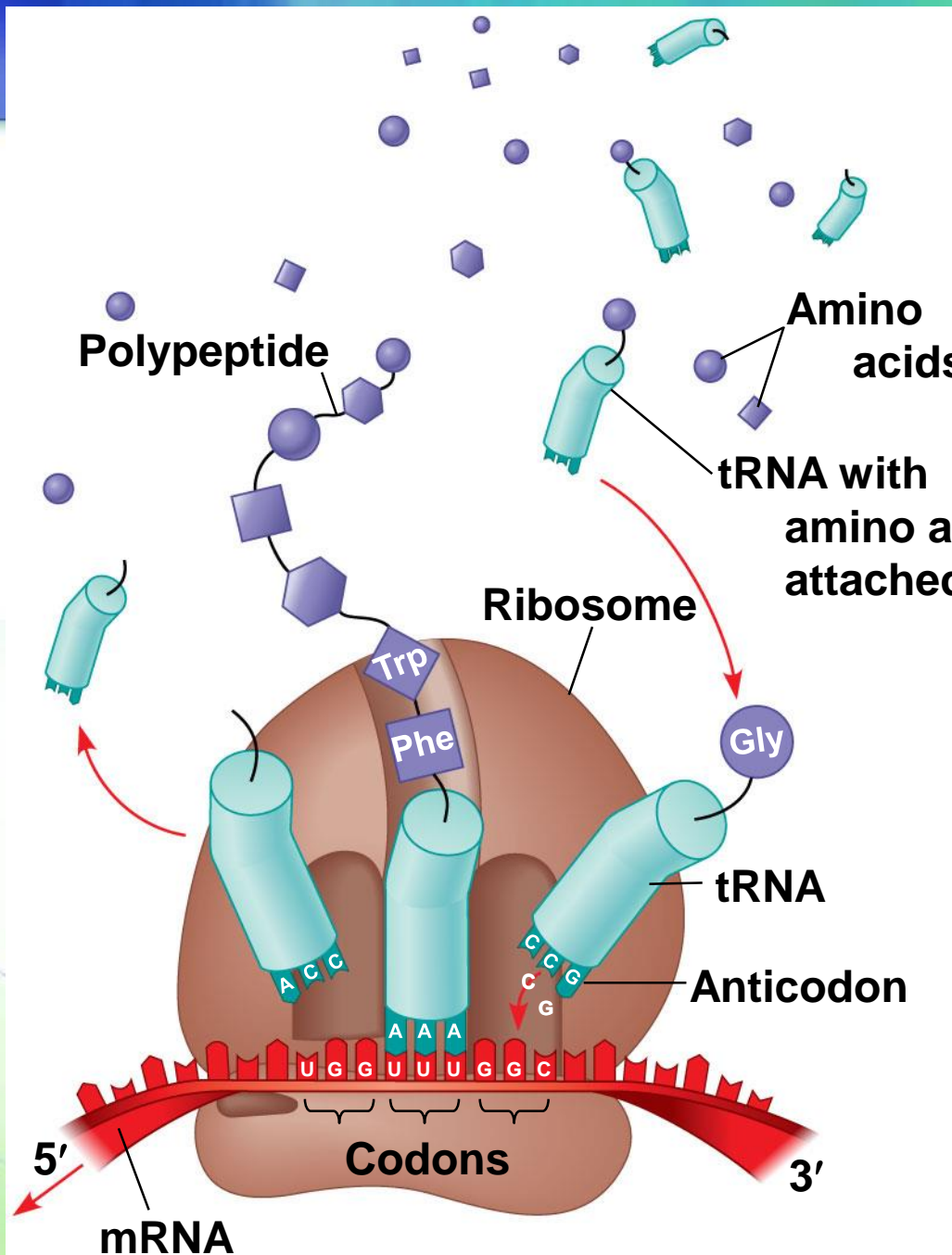




Amino end of polypeptide



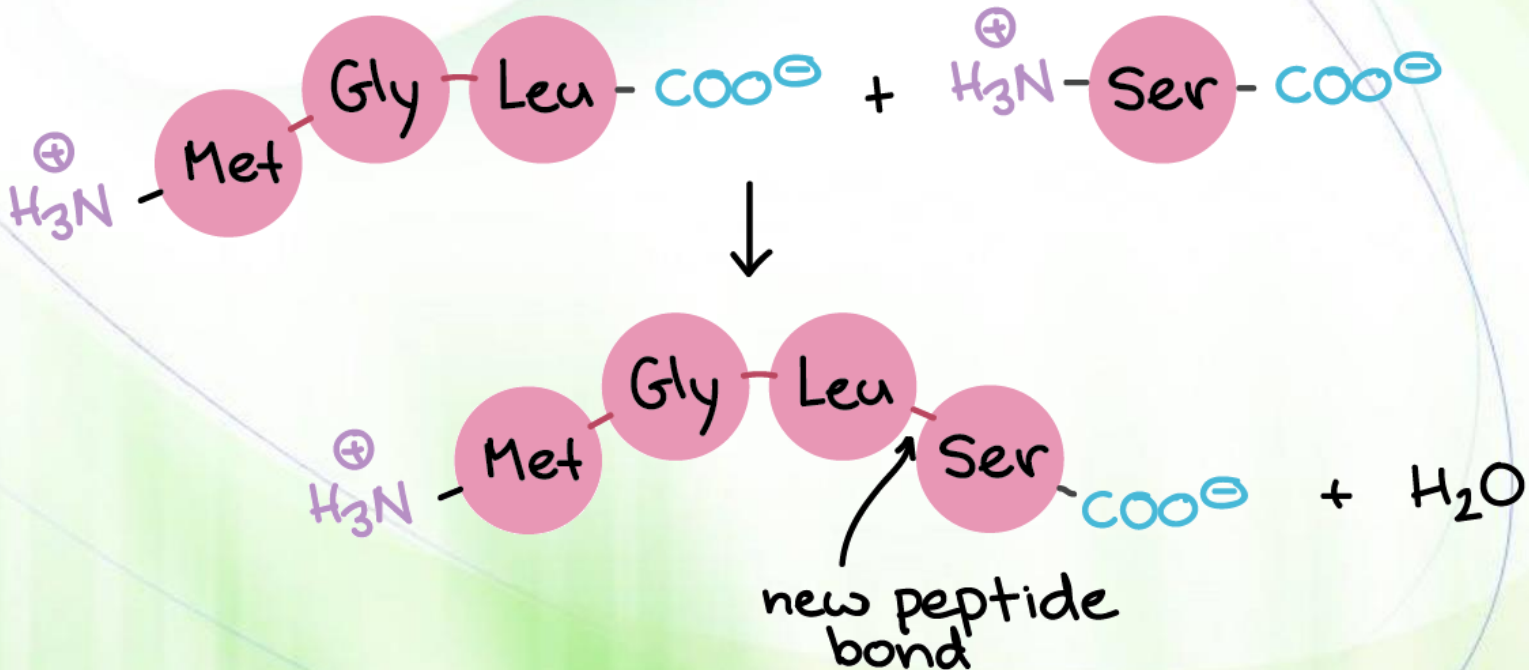




Elongation of the Polypeptide Chain



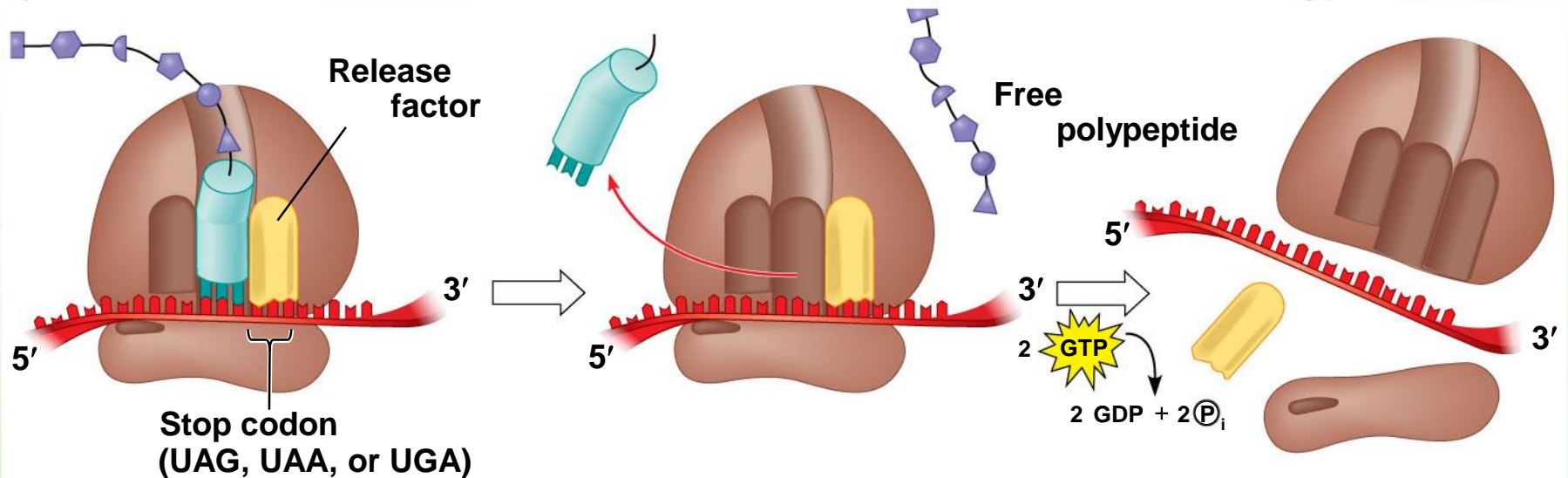
- During the elongation stage, amino acids are added one by one to the preceding amino (N)-terminus to the carboxy (C)-terminus of the growing chain.



Termination of Translation



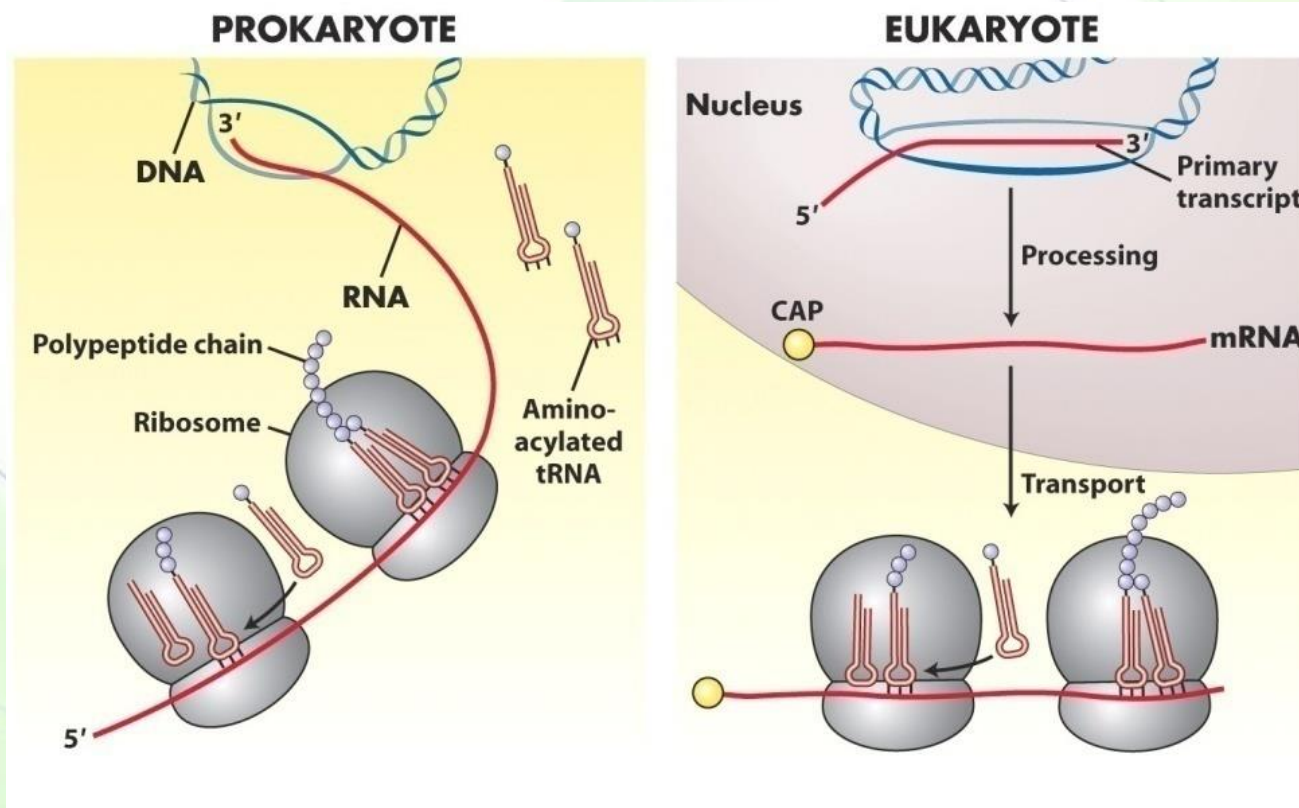
- The codons UAA, UAG, and UGA are the stop signals. They are not recognized by any tRNAs, but a release factor protein.
- The A site accepts **the release factor**, which causes the release of the polypeptide, and the translation assembly then comes apart.



Transcription/translation Coupling



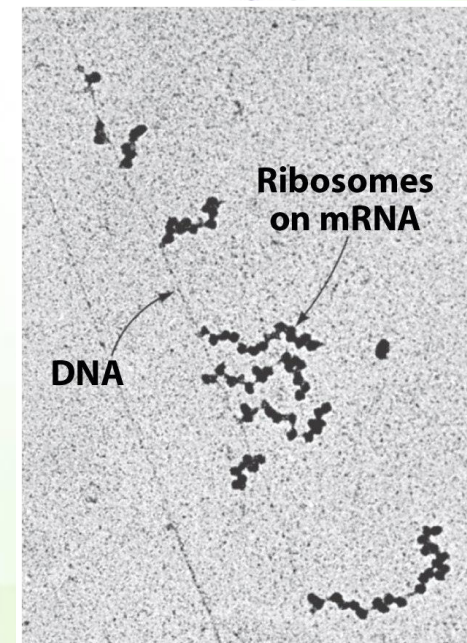
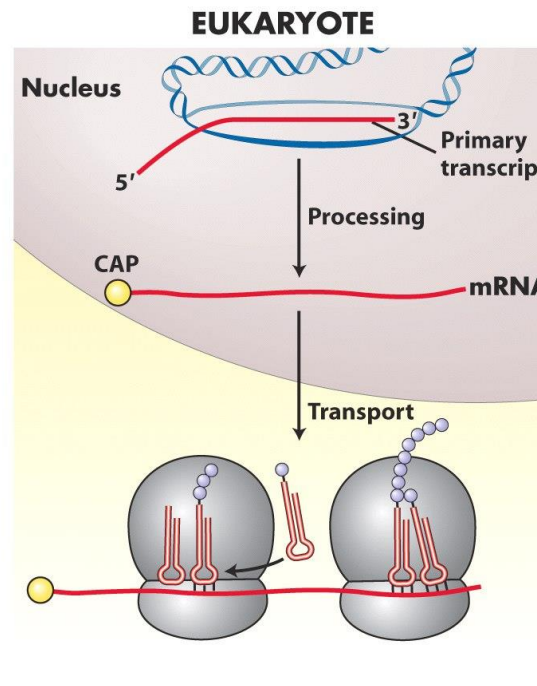
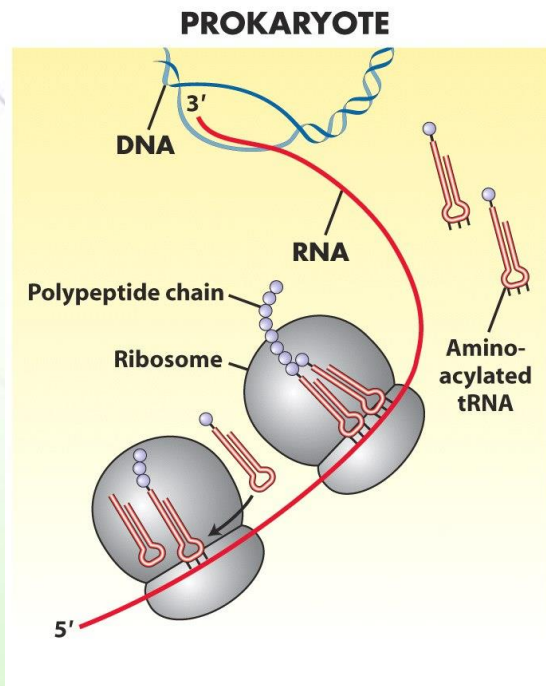
- Translation and transcription are coupled in space and time in prokaryotes.



Polyribosomes (polysomes)



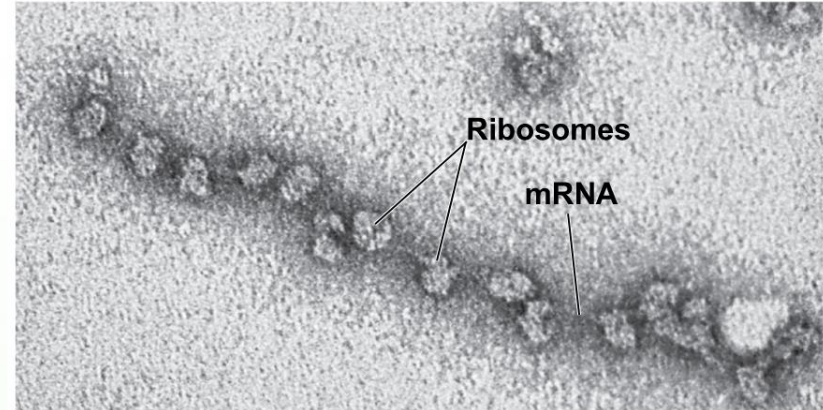
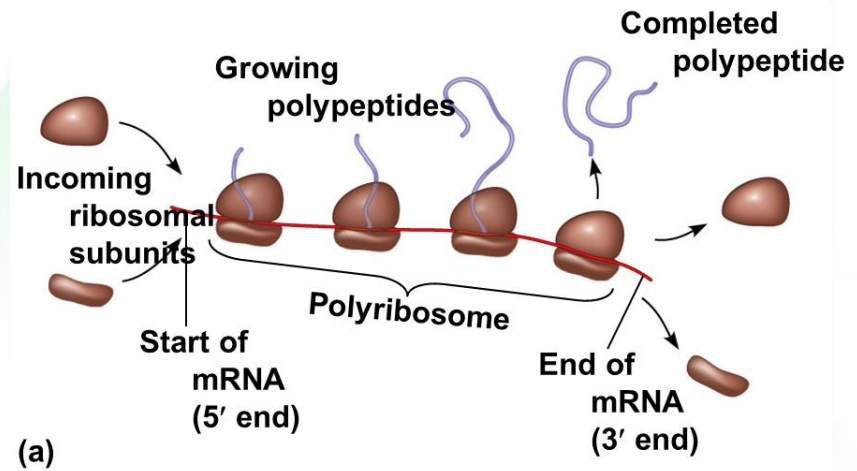
- A single mRNA molecule is translated by several ribosomes simultaneously. Each ribosome produces one copy of the polypeptide chain specified by the mRNA. When the protein has been completed, the ribosome dissociates into subunits that are used in further rounds of protein synthesis.



Polysomes (in eukaryotes)



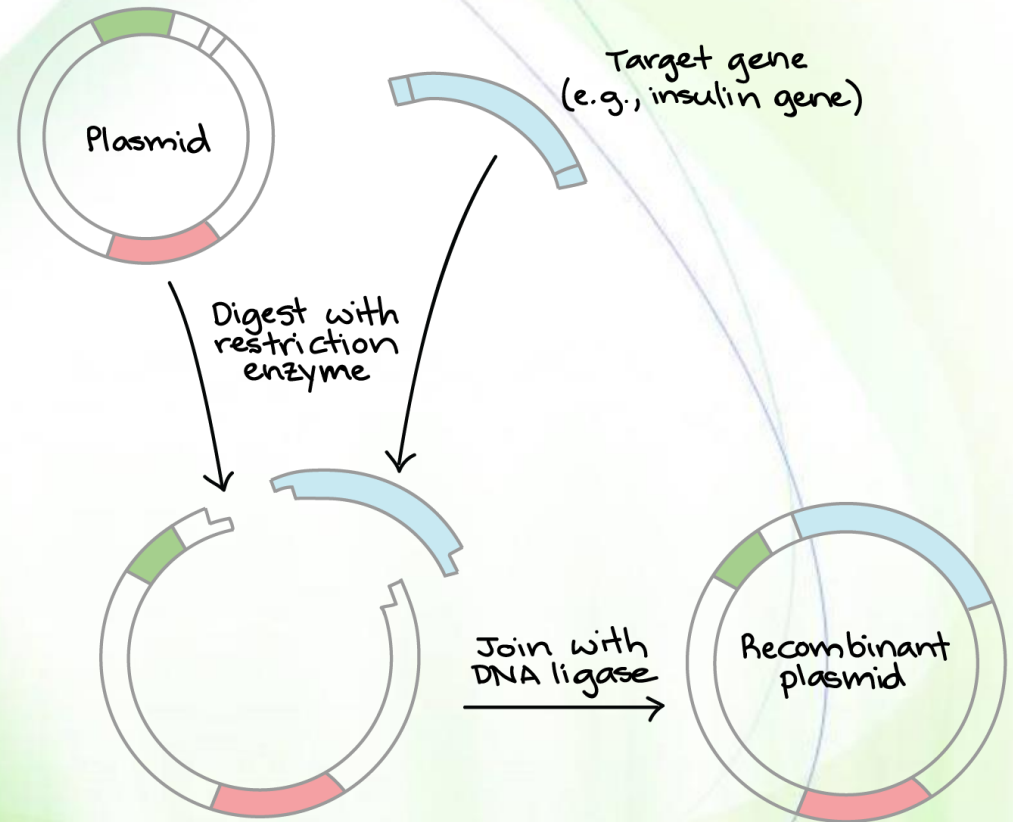
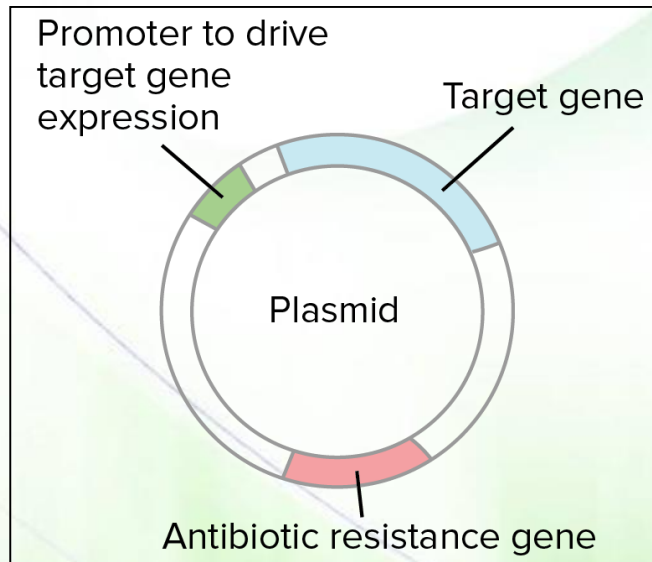
- A number of ribosomes can translate a single mRNA simultaneously, forming a **polyribosome** (or **polysome**).
- Polyribosomes enable a cell to make many copies of a polypeptide very quickly.



(b)

0.1 μm

A benefit of restriction endonucleases (cloning to produce of hormones)



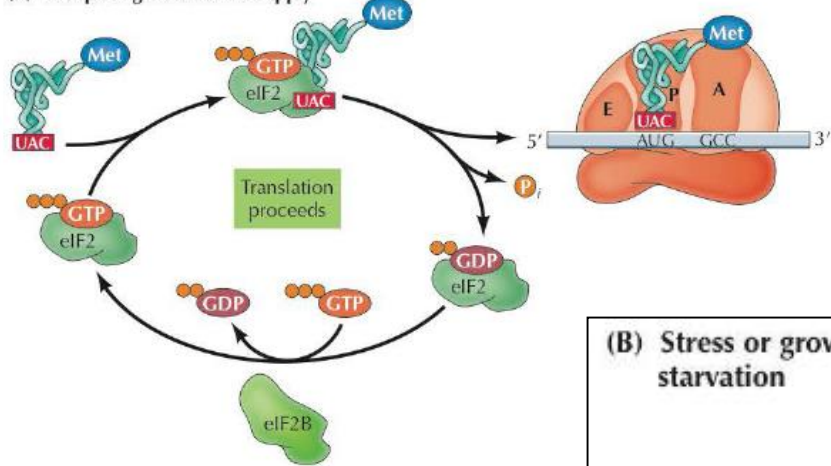


Regulation of translation

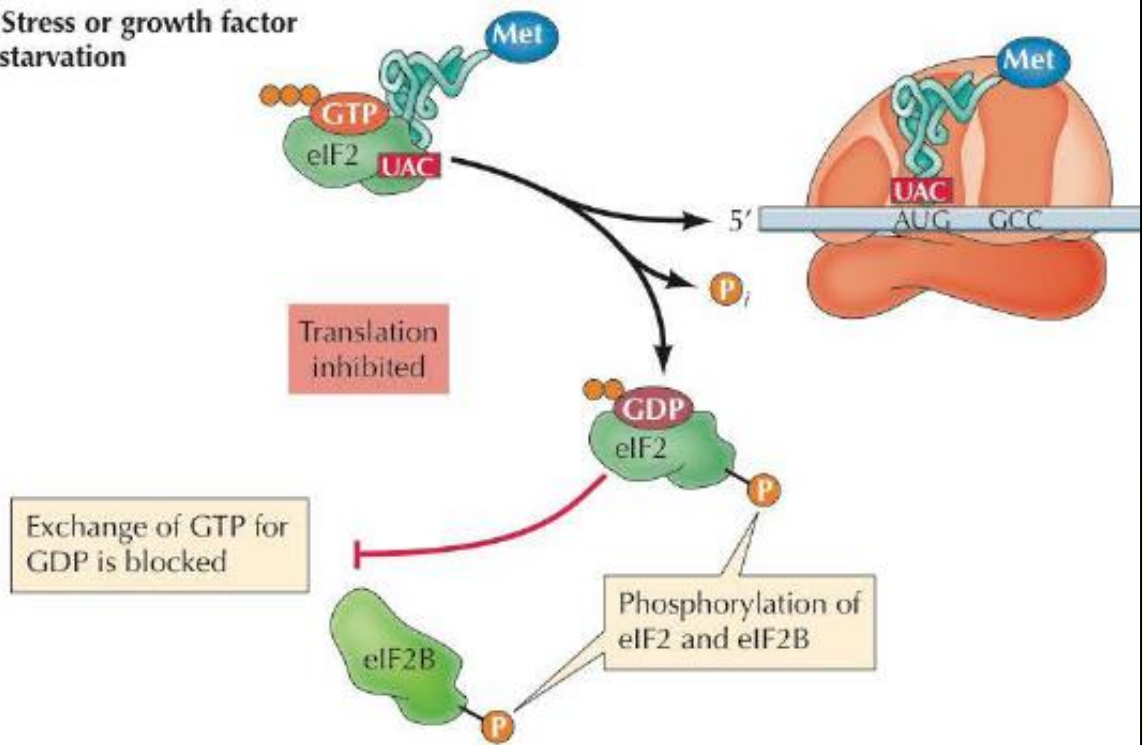
Global regulation



(A) Adequate growth factor supply



(B) Stress or growth factor starvation



Heme and protein synthesis

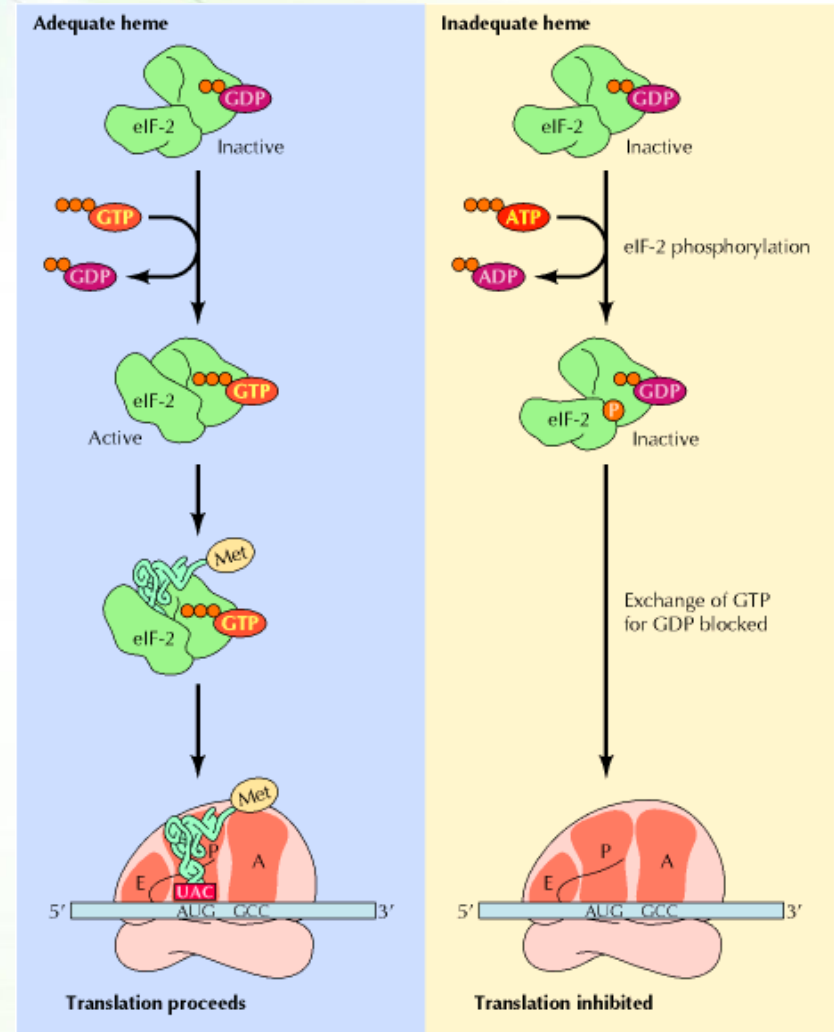


- In reticulocytes (immature erythrocytes), heme stimulates protein synthesis.
- The mRNA is translated only if adequate heme is available to form functional hemoglobin molecules.
- This is done via regulating the activity of eIF-2, which is responsible for escorting initiator methionyl tRNA to the ribosome.
- eIF-2 must be bound to GTP to be active. When it is released from the ribosome, GTP is hydrolyzed to GDP, which must be exchanged with GTP for eIF-2 to be active again.

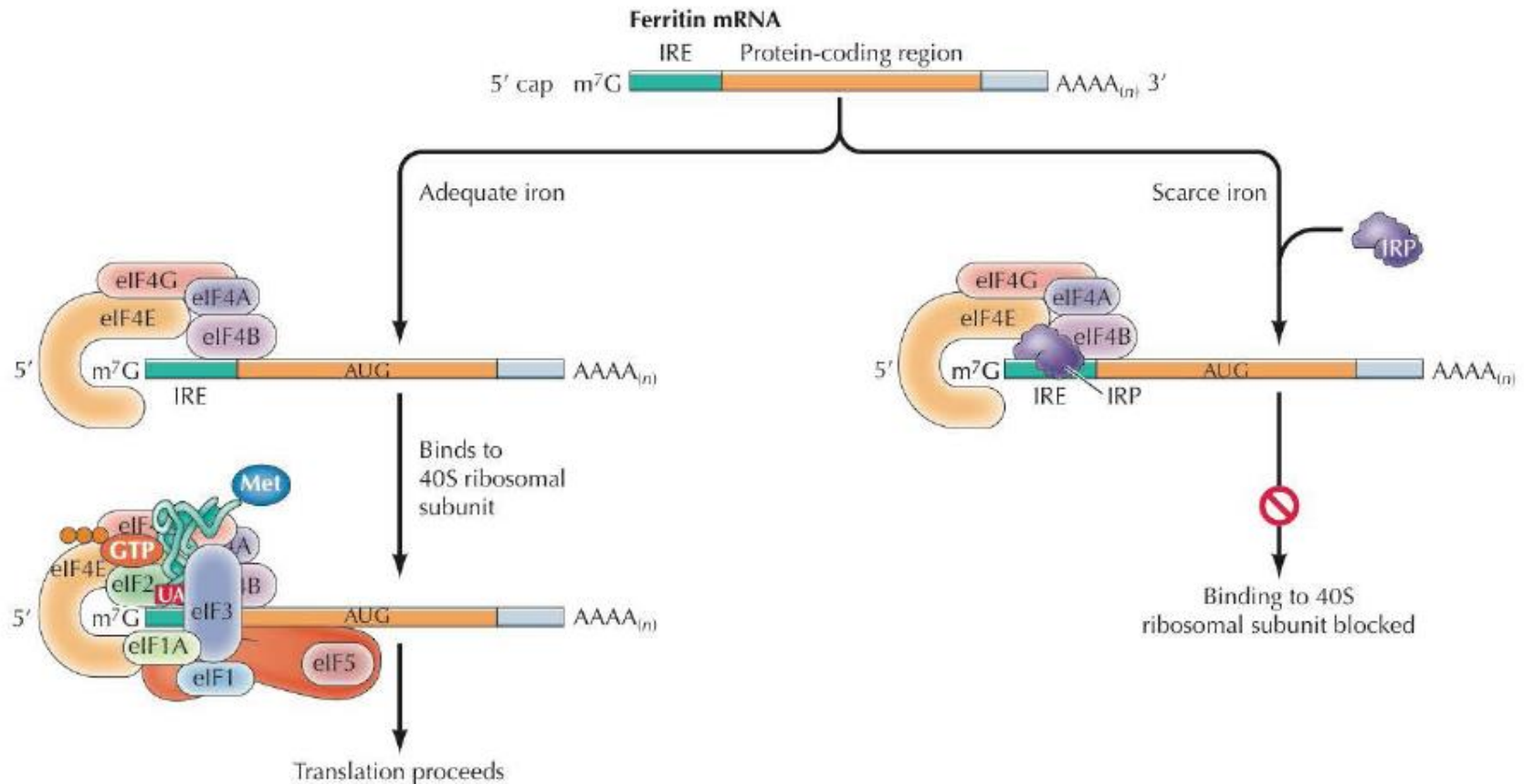
Regulation



- If adequate heme is available, GDP-GTP exchange occurs and translation is able to proceed.
- If heme supplies are inadequate, a protein kinase that phosphorylates eIF-2 is activated. Phosphorylation of eIF-2 blocks the exchange of GTP for GDP, so eIF-2/GTP cannot be regenerated and translation is inhibited.



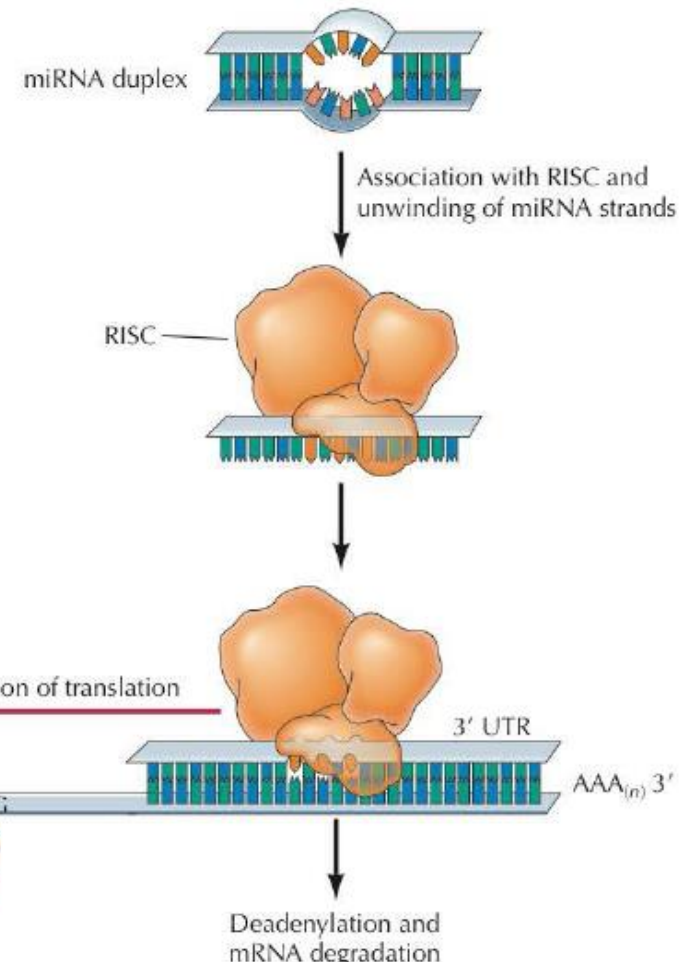
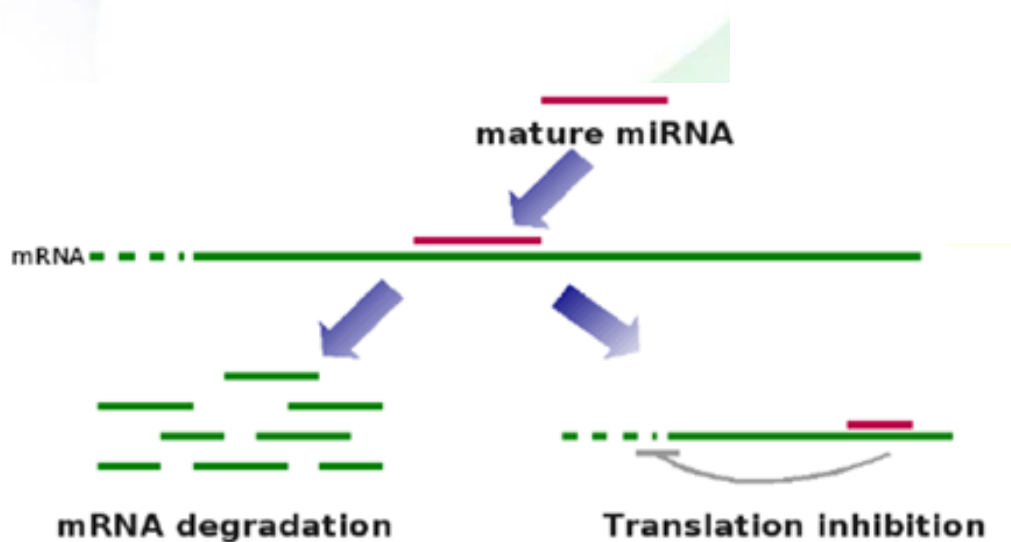
Also, remember...ferritin



Regulation by microRNA (miRNA)



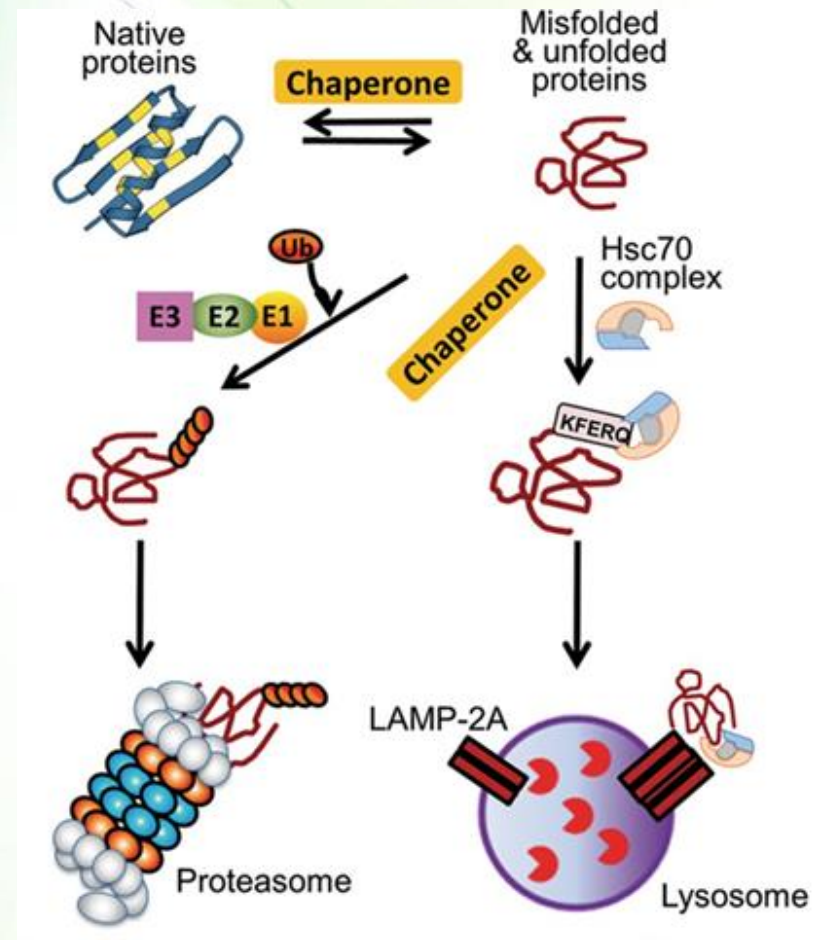
- MicroRNA is synthesized by RNA Pol II into single-stranded, primary miRNA (pri-miRNA) transcript.
- It gets processed and one strand and is loaded onto a RISC complex where miRNA is targeted to the 3'-UTR of mRNA.



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



- Proteins are degraded either in degradative subcellular organelles like lysosomes or by the macromolecular **proteasomes**.
- Proteins are targeted for destruction in a proteasome by **ubiquitylation** which involves labeling by small polypeptides known as ubiquitin.





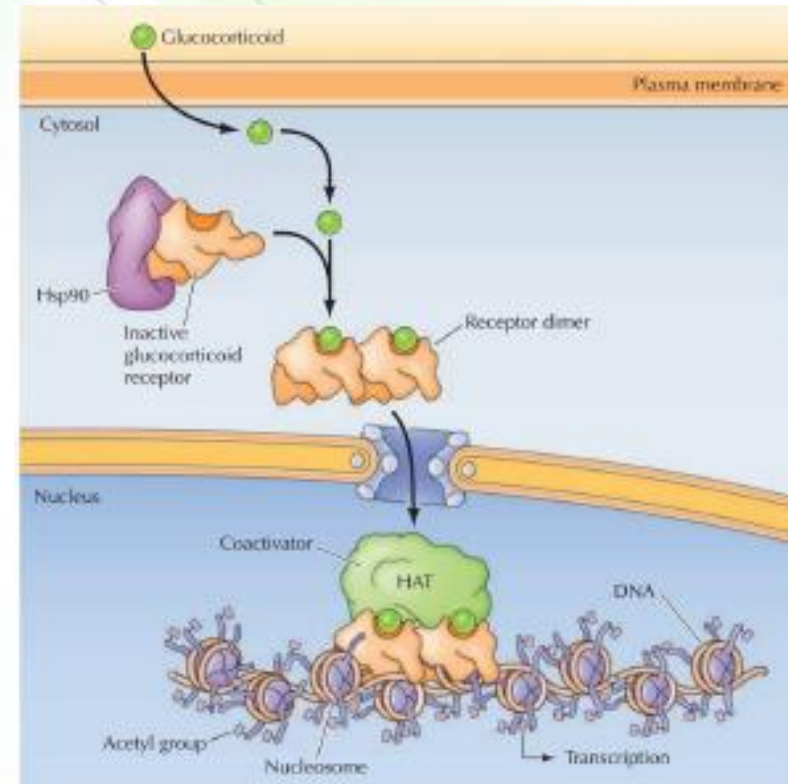
Connecting outside to inside: from cell signaling to protein synthesis

Different modes of regulating protein synthesis

Glucocorticoids



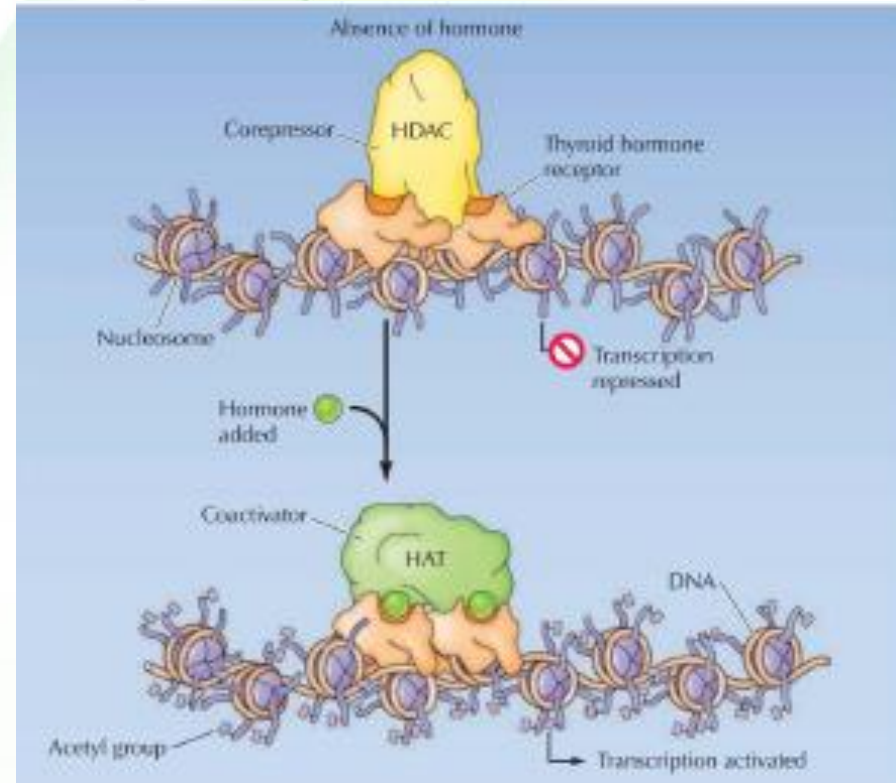
- Glucocorticoids, like androgens and other steroid hormones, diffuse across the plasma membrane and bind to the glucocorticoid receptor.
- Glucocorticoid binding allows the formation of receptor dimers.
- The activated receptors translocate to the nucleus, bind DNA, and associate with coactivators (example: histone acetyltransferase (HAT)) to stimulate transcription of their target genes.



Thyroid hormone



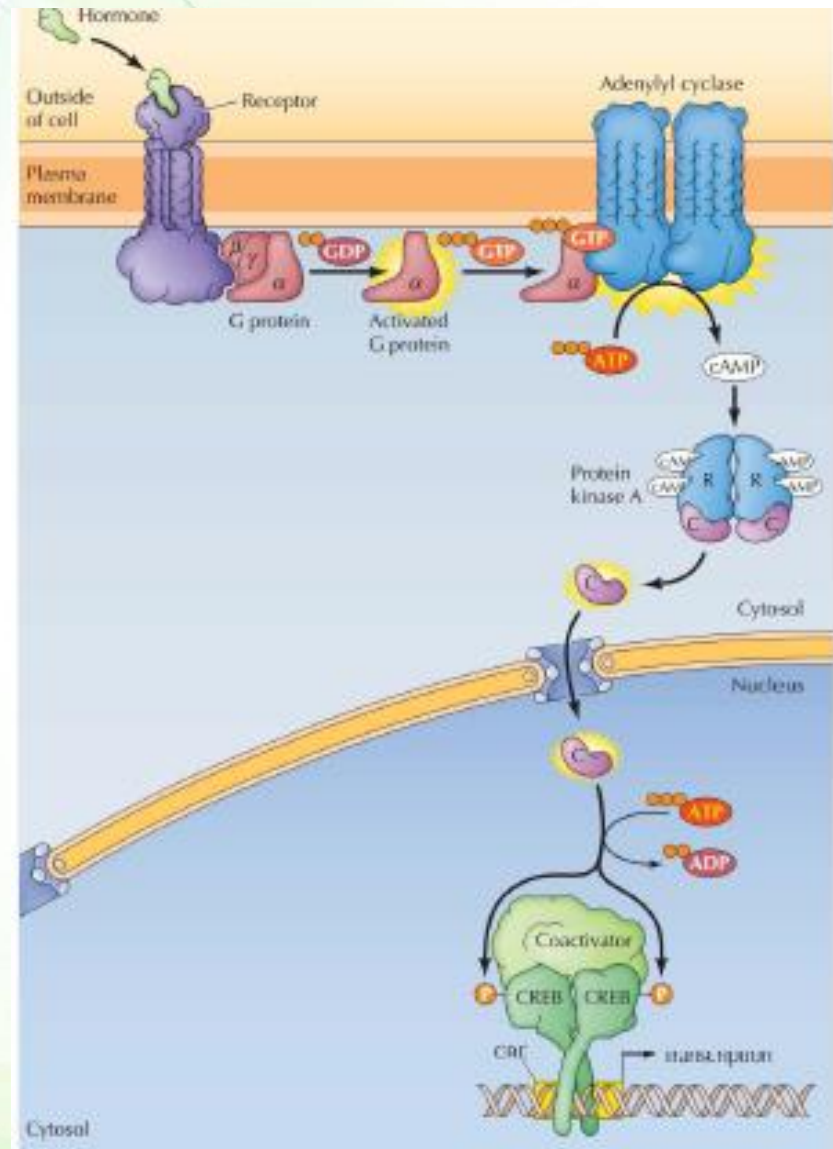
- Thyroid hormone receptor binds DNA in either the presence or absence of hormone.
- In the absence of hormone, the receptor associates with corepressors with histone deacetylase (HDAC) activity.
- In the presence of hormone, the receptor associates with coactivators with histone acetyltransferase (HAT) activity.



cAMP-inducible gene expression



- Receptor stimulation leads to activation of adenylyl cyclase, synthesis of cAMP, and activation of protein kinase A.
- The catalytic subunit of protein kinase A is freed, translocates into the nucleus, and phosphorylates the transcription factor CREB (CRE-binding protein), leading to the recruitment of coactivators and expression of cAMP-inducible genes.

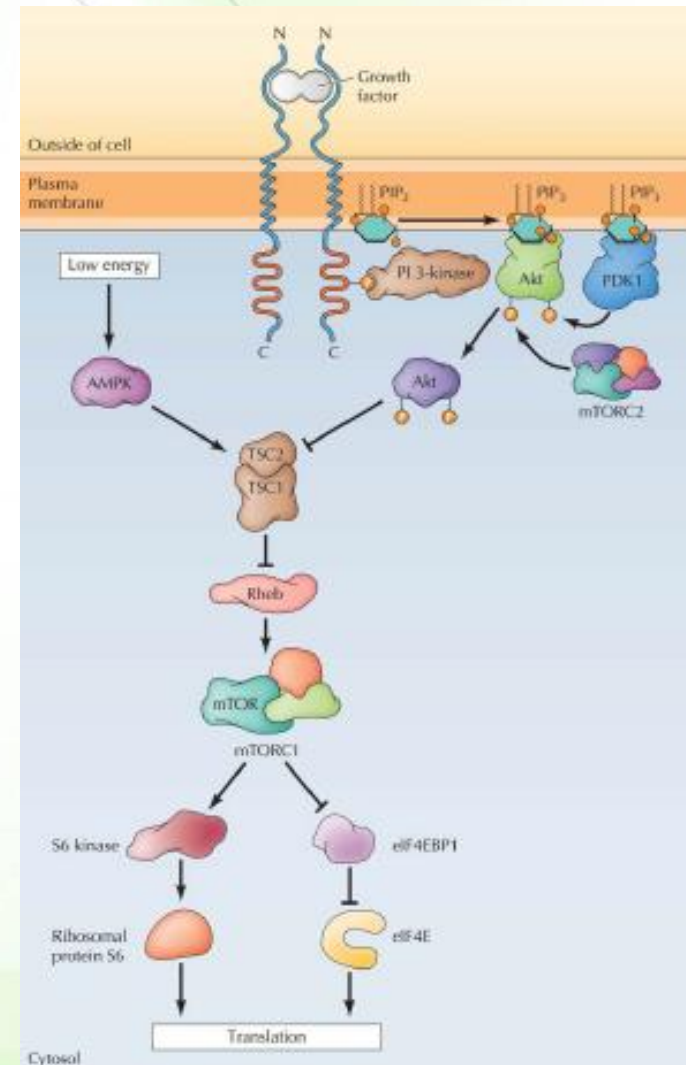


The mTOR pathway



Coupling growth to energy stores

- mTORC1 stimulates translation by phosphorylating 56 kinase (which phosphorylates ribosomal protein 56) and by phosphorylating eIF4E binding protein-1 (4E-BP1), relieving inhibition of translation initiation factor eIF4E.
- At high energy, Akt is activated leading to activation of mTORC1 and, hence, translation.
- At low energy stores (AMP>ATP), AMP kinase (AMPK) inhibits translation by inhibiting mTORC1.

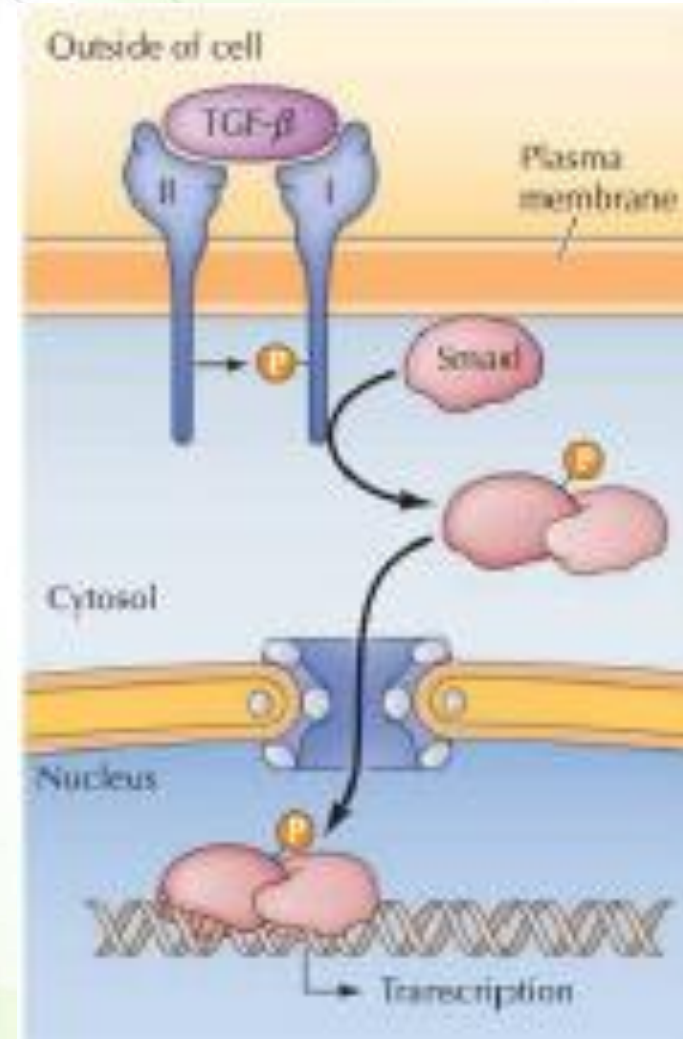


Direct activation of transcription factors



(TGF- β receptors \rightarrow Smad)

- When bound to their ligand, transforming growth factor receptors (TGF- β receptors) phosphorylate a Smad protein.
- Phosphorylated Smads form complexes and translocate into the nucleus to activate transcription of target genes.

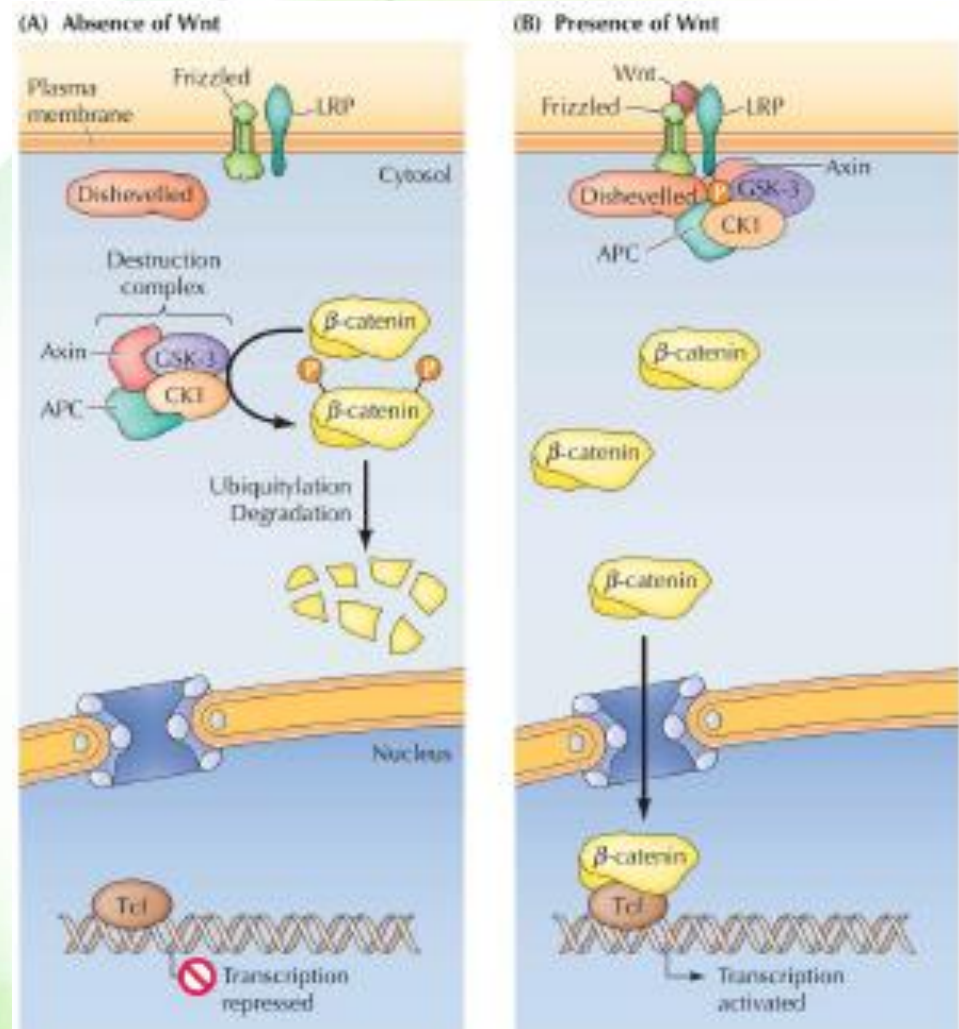


Trapping of transcription factors

(The Wnt pathway)



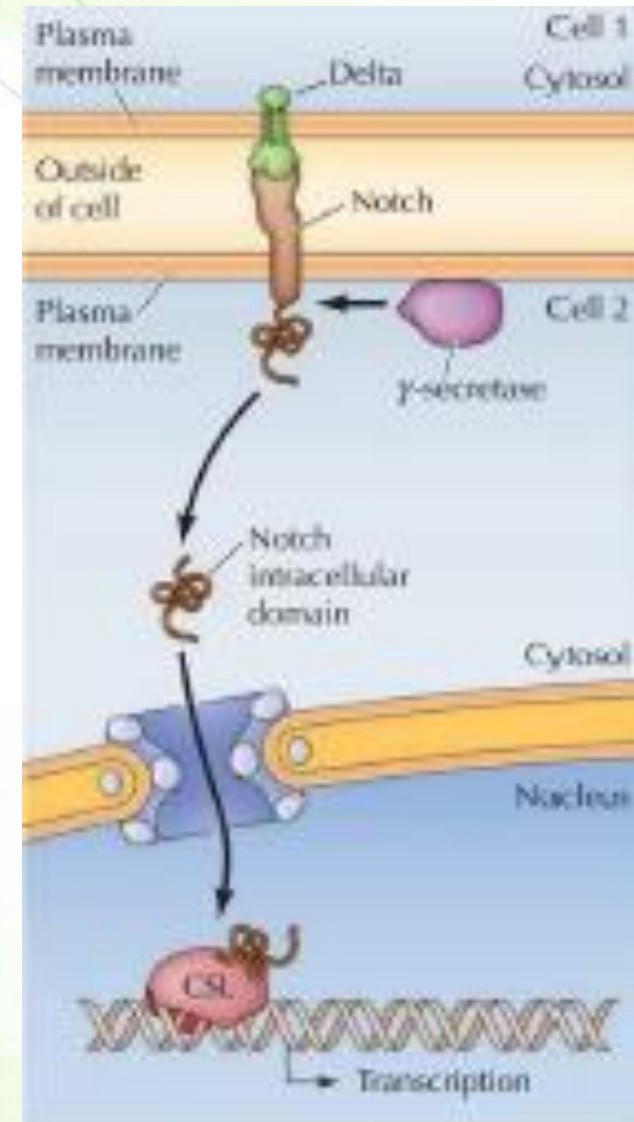
- (A) In the absence of the ligand Wnt, β -catenin is ubiquitylated and degraded.
- (B) When Wnt is present, the destruction complex is inactivated and β -catenin is stabilized, translocates into the nucleus and forms a complex with other transcription factors activating transcription.



The TF is within the membrane receptor (The Notch pathway)



- Notch is a receptor.
- The binding of its ligand leads to proteolytic cleavage of Notch by γ -secretase.
- This releases the Notch intracellular domain, which translocates to the nucleus and interacts with a transcription factor to induce gene expression.



Levels of regulation



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

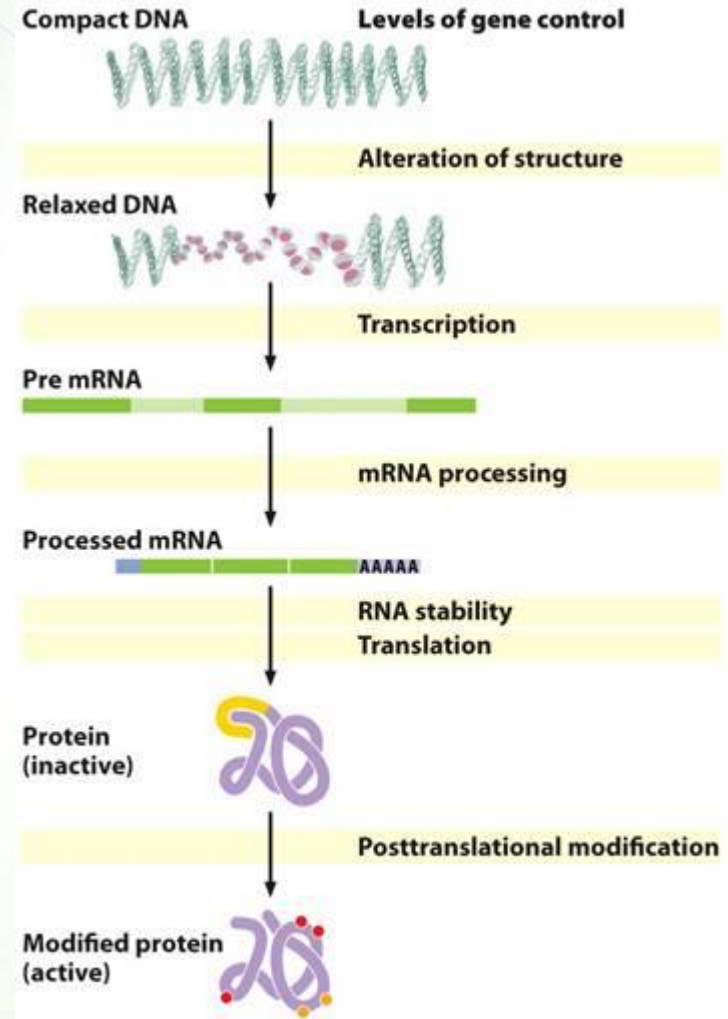


Figure 16.1
Genetics: A Conceptual Approach, Fifth Edition
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This is molecular biology in a nutshell

