Translation

Process of converting information stored in nucleic acid sequences into proteins

Charging of tRNA

- Linking amino acids to the correct t-RNAs catalyzed by **aminoacyl-tRNA synthetase** (aa-tRNA)

- Couples an amino acid to its cognate tRNA

- **Fidelity** of coupling – 20 different synthetases

Two steps

Activation of amino acid

Transfer of amino acid to tRNA

Translation

In prokaryotes, initiation of translation requires the formation of the **initiation complex** including

- an **initiator tRNA** charged with N-formylmethionine
- the small ribosomal subunit
- mRNA strand

The **ribosome binding sequence** of mRNA is complementary to part of rRNA

Translation

Elongation of translation involves the addition of amino acids

- a charged tRNA binds to the A site if its anticodon is complementary to the codon at the A site
- peptidyl transferase forms a peptide bond
- the ribosome moves down the mRNA in a 5’ to 3’ direction

Translation

Elongation continues until the ribosome encounters a stop codon.

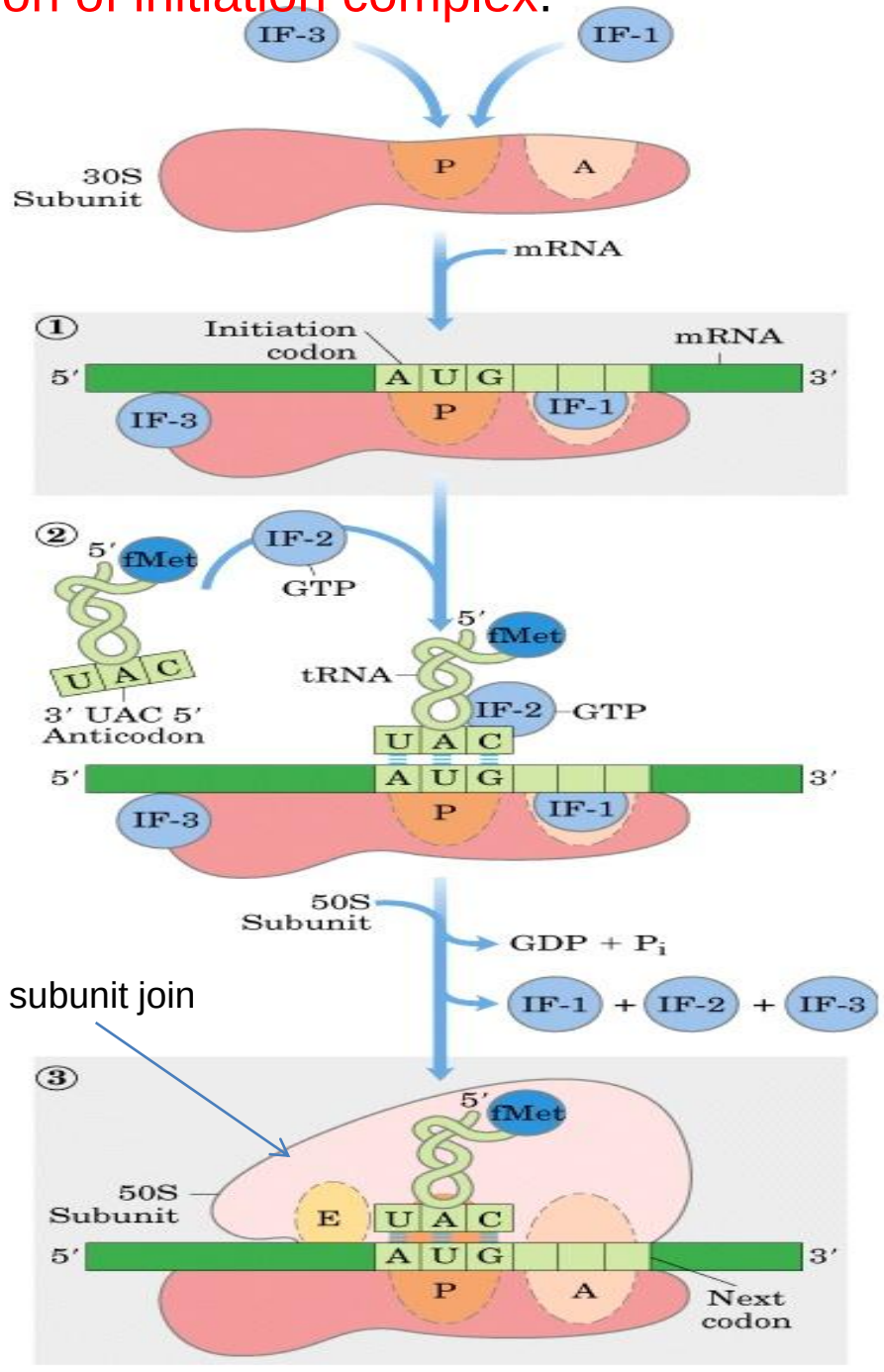
Stop codons are recognized by release factors which release the polypeptide from the ribosome.

tRNA and Ribosomes

The ribosome has multiple tRNA binding sites:

- **P site** – binds the tRNA attached to the growing peptide chain
- **A site** – binds the tRNA carrying the next amino acid
- **E site** – binds the tRNA that carried the last amino acid

Formation of initiation complex:



Large subunit join

The A and P sites bind to aminoacyl tRNAs

The initiating AUG is positioned at the P site, the only site to which fMet-tRNAfMet can bind

The fMet-tRNAfMet is the only aminoacyl-tRNA that binds to the P site;(the others on A site)

The E site is the site from which the "uncharged" tRNAs leave during elongation.

table 27–9

Protein Factors Required for Initiation of Translation in Bacterial and Eukaryotic Cells

Bacterial

Factor	Function
IF-1	Prevents premature binding of tRNAs to A site
IF-2	Facilitates binding of fMet-tRNA^{fMet} to 30S ribosomal subunit
IF-3	Binds to 30S subunit; prevents premature association of 50S subunit; enhances specificity of P site for fMet-tRNA^{fMet}

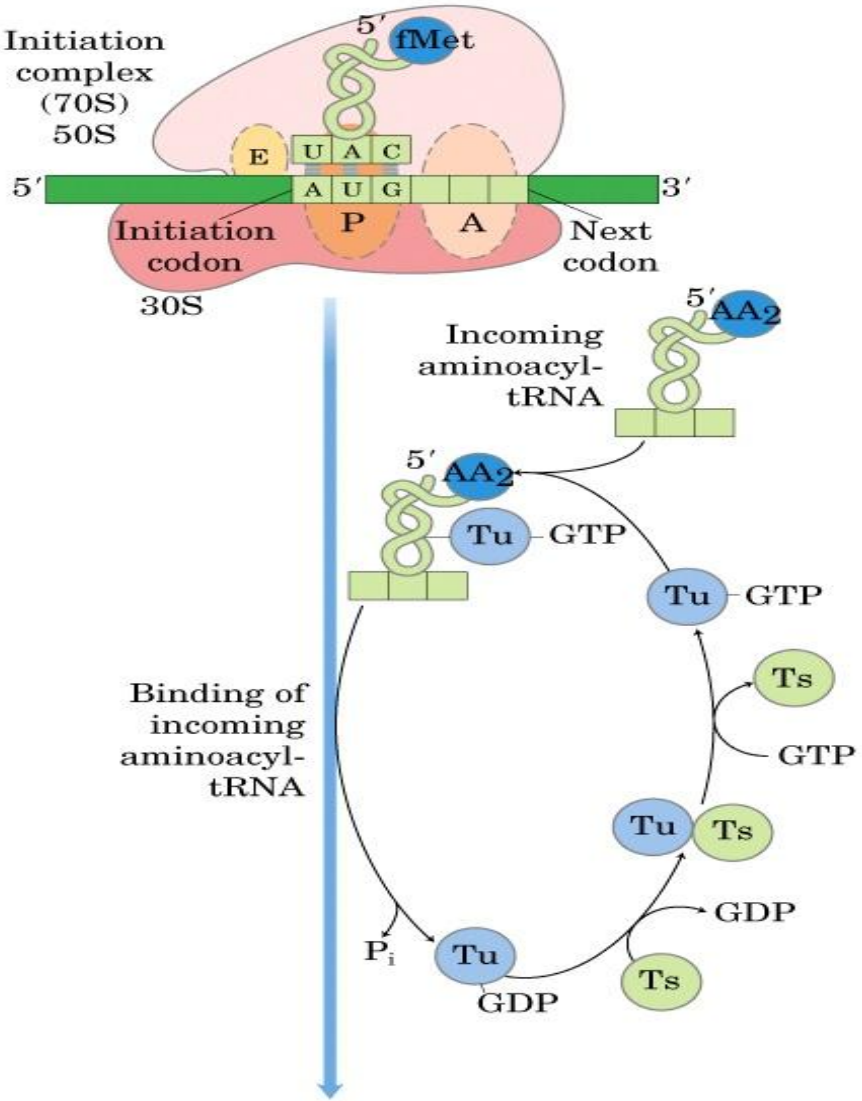
Eukaryotic

Factor*	Function
eIF2	Facilitates binding of initiating Met-tRNA^{Met} to 40S ribosomal subunit
eIF2B, eIF3	First factors to bind 40S subunit; facilitate subsequent steps
eIF4A	RNA helicase activity removes secondary structure in the mRNA to permit binding to 40S subunit; part of the eIF4F complex
eIF4B	Binds to mRNA; facilitates scanning of mRNA to locate the first AUG
eIF4E	Binds to the 5′ cap of mRNA; part of the eIF4F complex
eIF4G	Binds to eIF4E and to poly(A) binding protein (PAB); part of the eIF4F complex
eIF5	Promotes dissociation of several other initiation factors from 40S subunit as a prelude to association of 60S subunit to form 80S initiation complex
eIF6	Facilitates dissociation of inactive 80S ribosome into 40S and 60S subunits

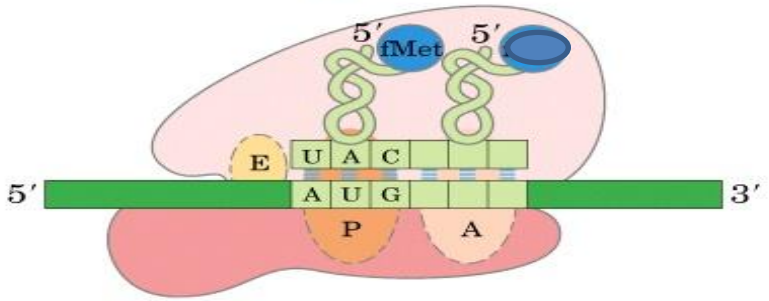
*The prefix "e" identifies these as eukaryotic factors.

Elongation:

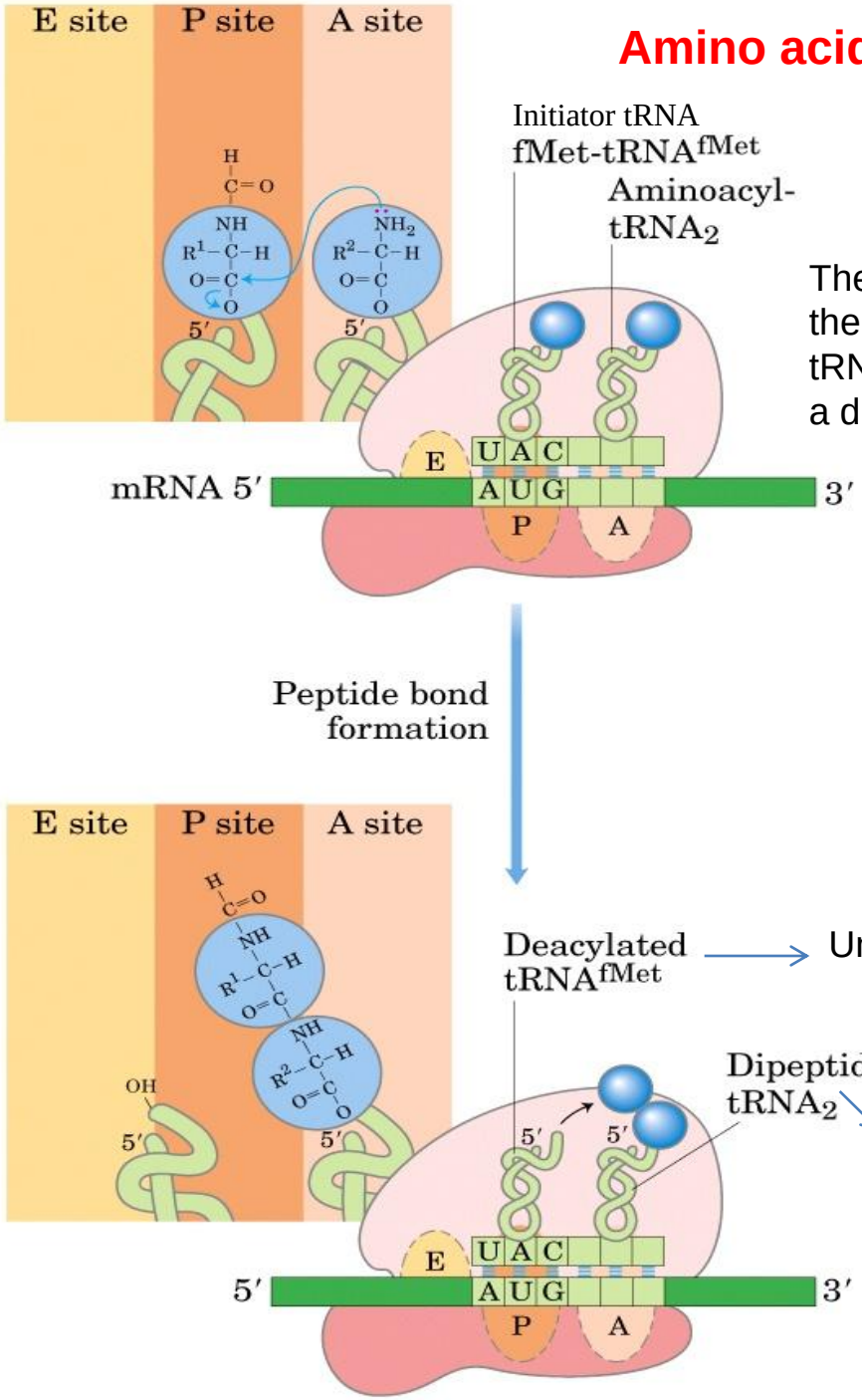
1-Codon recognition
2-peptide bond formation
3-translocation



2nd tRNA binds A site



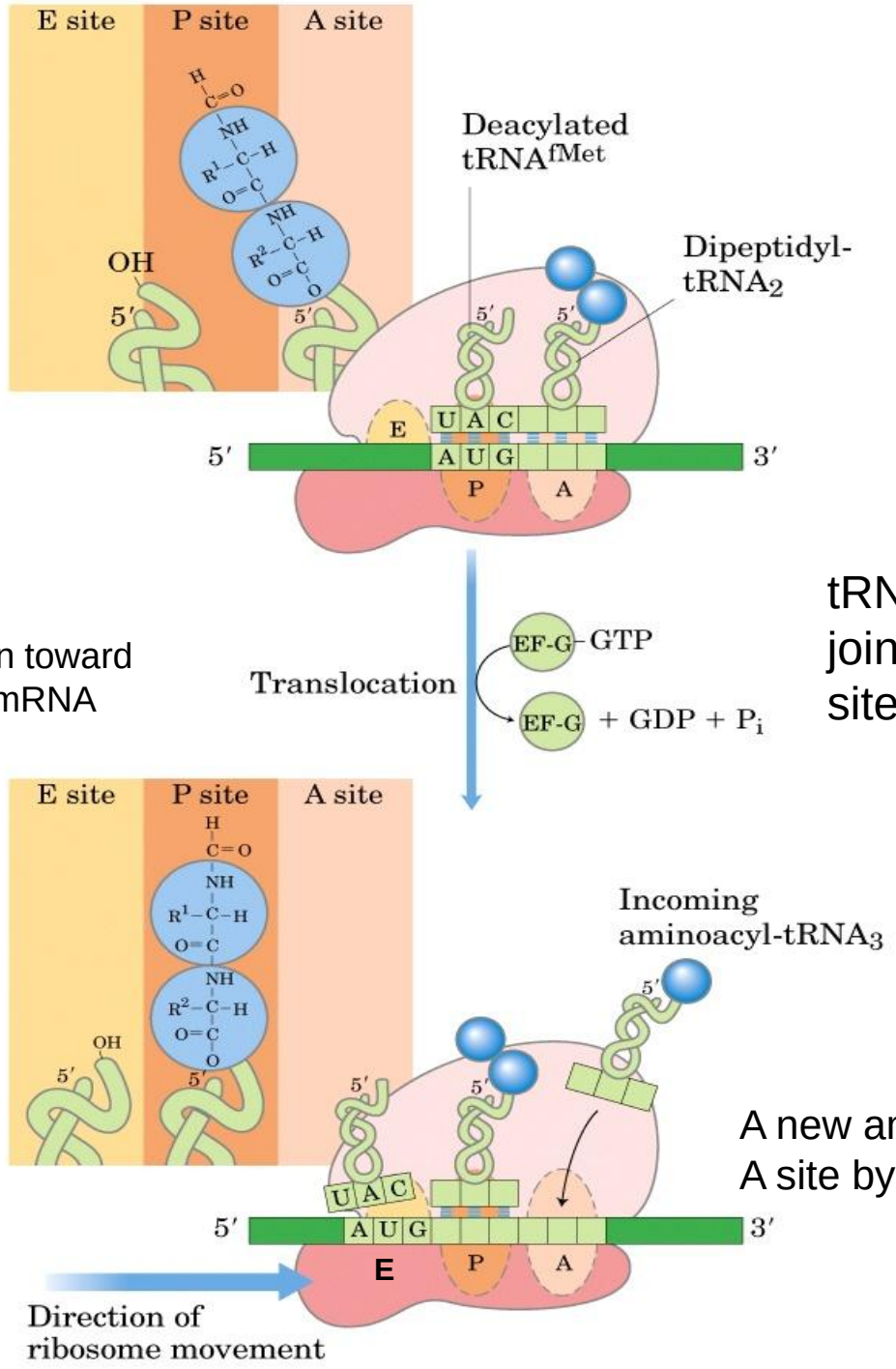
Amino acid connected: peptide bond formed



The -amino group of the amino acid in the A site acts as a nucleophile, displacing the tRNA in the P site to form the peptide bond.= a dipeptidyl-tRNA in the A site

Uncharged tRNA remains bound to p site

Peptide bond formed by **peptidyl transferase** ribozyme



the ribosome
moves one codon toward
the 3 end of the mRNA

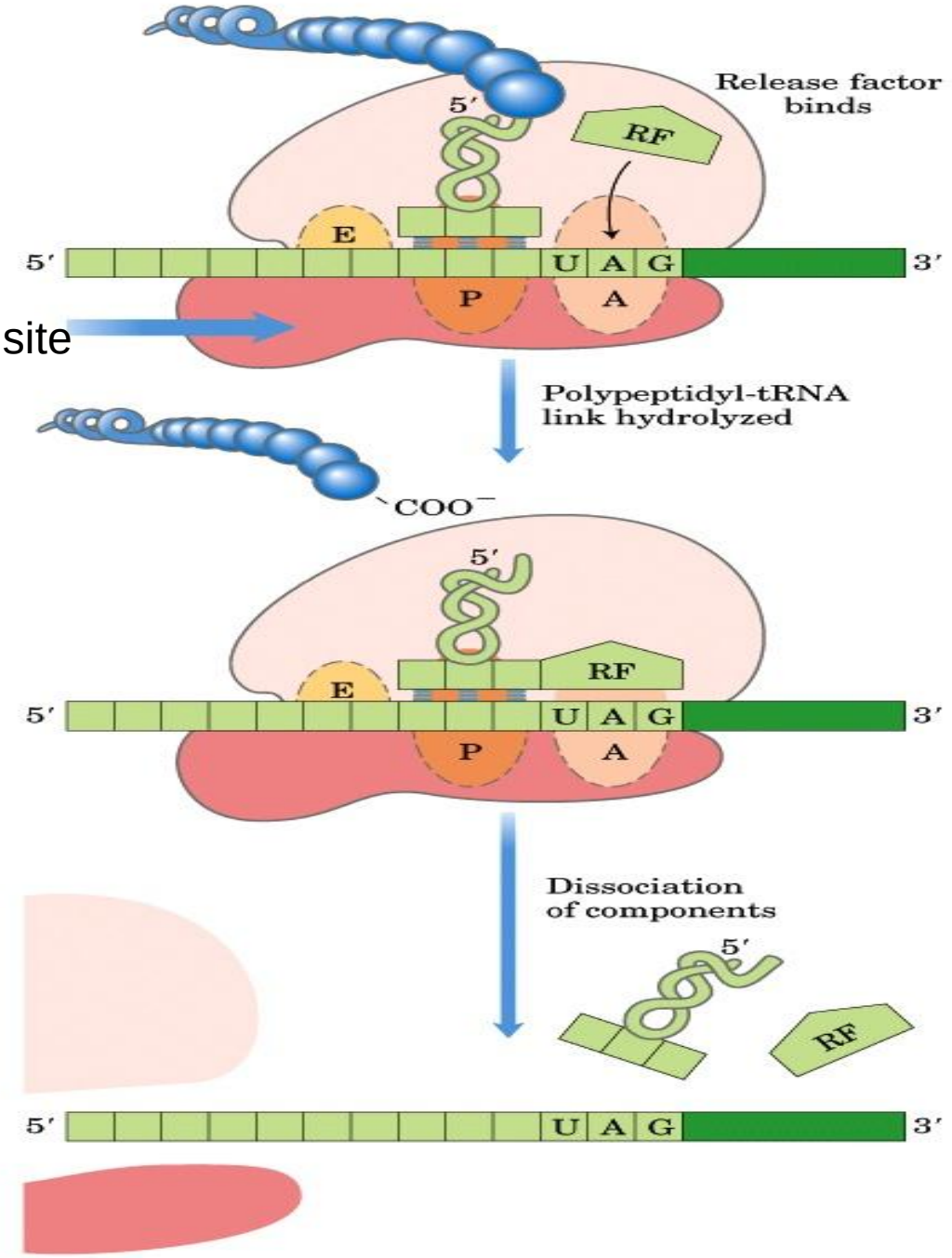
tRNA (with 2 amino acids joined) in A site moves to P site

A new amino –acylated tRNA moves in to A site by anti codon-codon pairing

Termination:

No tRNA for stop codon
Ribosomes stop

The release factor binds with A site
Ribosome disassembles
protein released



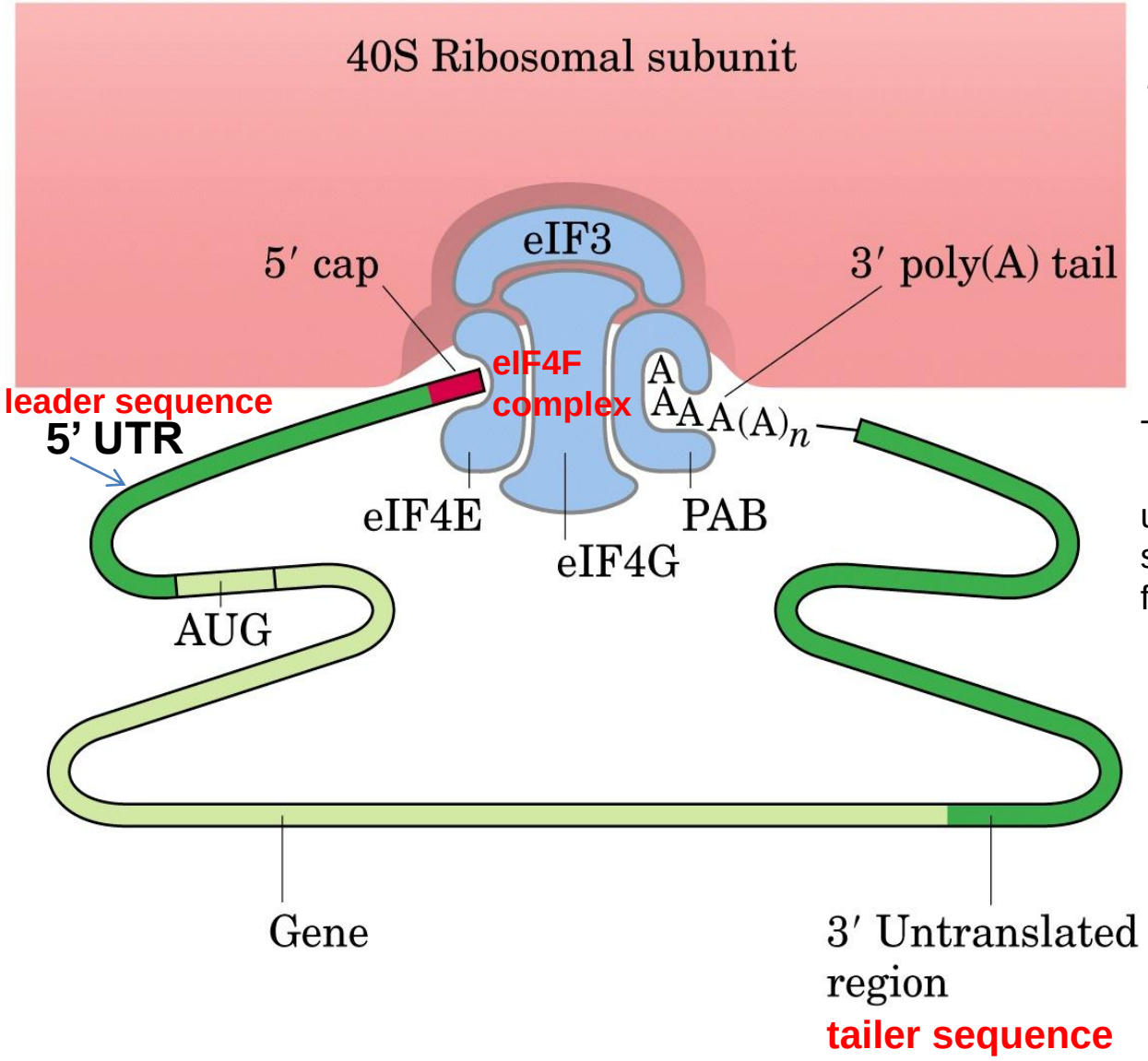
Eukaryotic elongation:

- 3 elongation factors (eEF1α, eEF1βγ, eEF2)

 analogous to bacterial (EF-Tu, EF-Ts, EF-G)

- No E site on ribosome, uncharged expelled directly from P site.

Eukaryotes



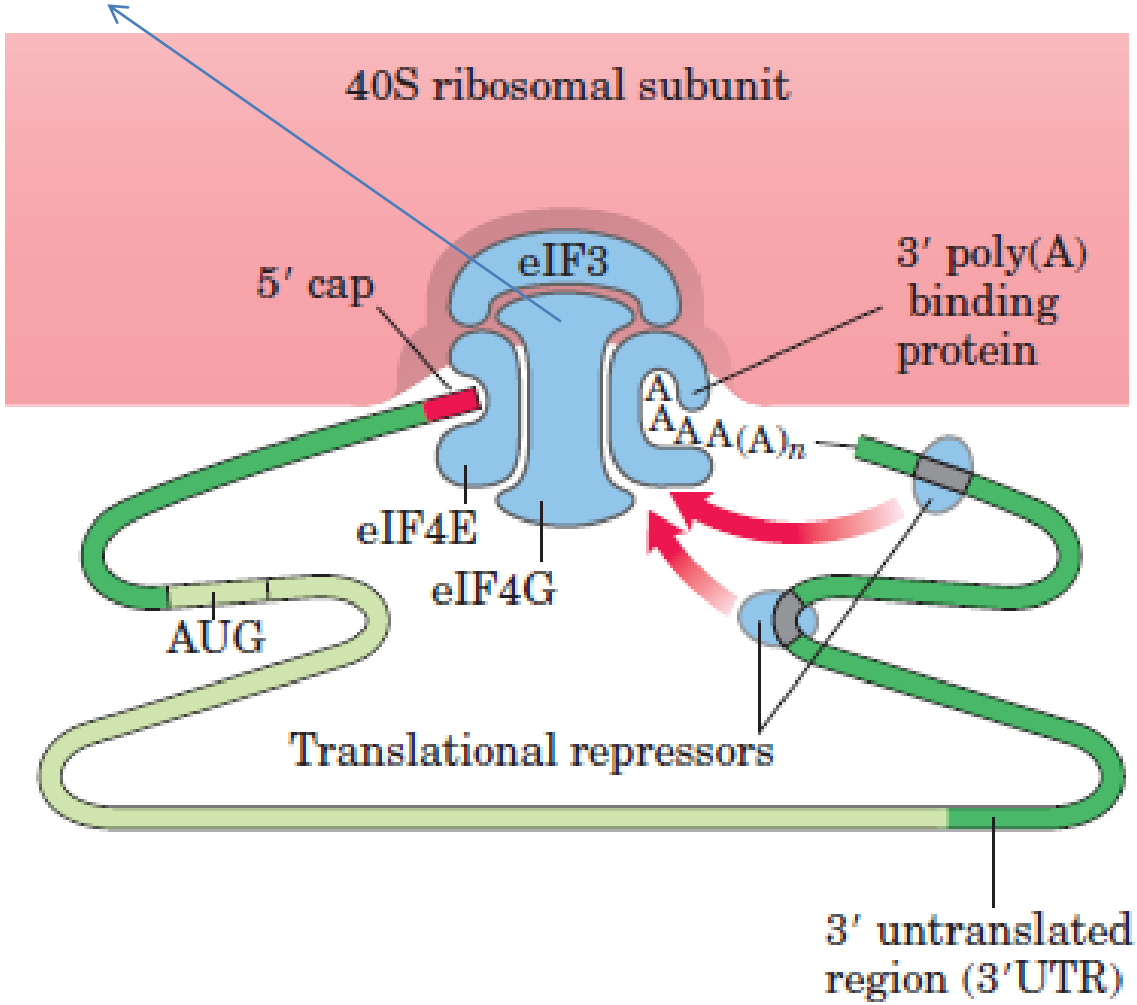
The 3 and 5 ends of eukaryotic mRNAs are linked by a complex of proteins that includes several initiation factors)

The initiating (5)AUG is detected by scanning the mRNA from the 5 end until the first AUG is encountered, signaling the beginning of the reading frame

The eIF4F complex is involved in scanning using the RNA helicase activity of eIF4A to eliminate secondary structure in the 5 untranslated portion of the mRNA.

translational regulation

phosphorylation



Some proteins bind directly to mRNA and act as translational repressors

these proteins interact with other translation initiation factors bound to the mRNA or with the 40S ribosomal subunit to prevent translation initiation

POLYSOME:

Many ribosomes on the same "string" of mRNA are called polysomes

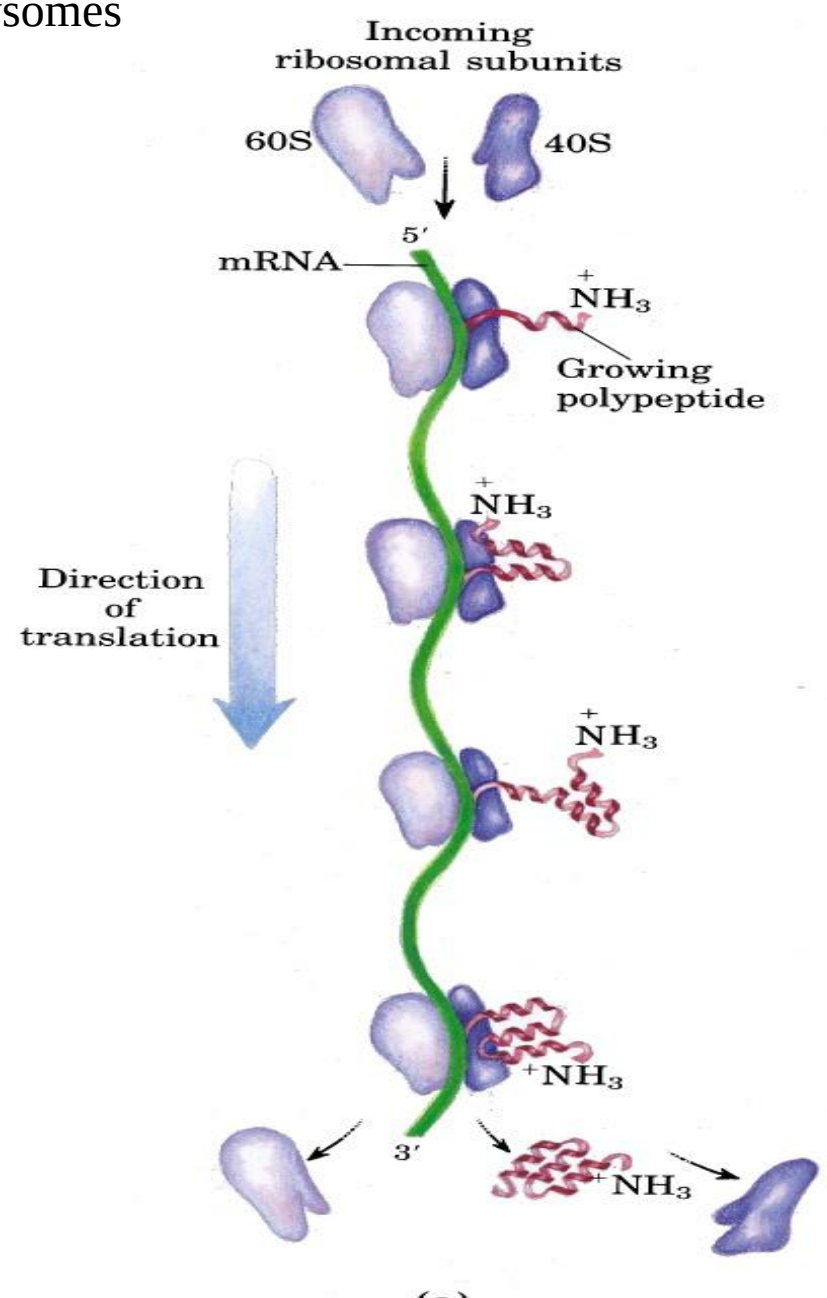
-

>several ribosomes translating a eukaryotic mRNA molecule simultaneously, moving from the 5 end to the 3 end and synthesizing a polypeptide from the amino terminus to the carboxyl terminus.

>Typically an m-RNA will have approximately one attached ribosome every 30 - 40 codons,

>Polysomes enable the cell to make several copies of polypeptide very quickly

>mRNA= 5>3'
Polypeptide =amino> carboxyl terminus



KEEP IN YOUR MIND

Accurate translation depends on:

1- correct match between tRNA and amino acid= amnioacyl tRNA synthetase
2- correct match between tRNA anti codon and mRNA codon

Newly Synthesized Polypeptide Chains Undergo Folding and Processing

Post translational modification

Post translational modification

Targeting to the appropriate cell compartment
polypeptides are folded
Formation of (s-s)

Proteolytic cleavage

Activation of inactive hormone (proinsulin)
Activation of enzyme (zymogens; Trypsinogen)
Removal of signal sequence (ER)

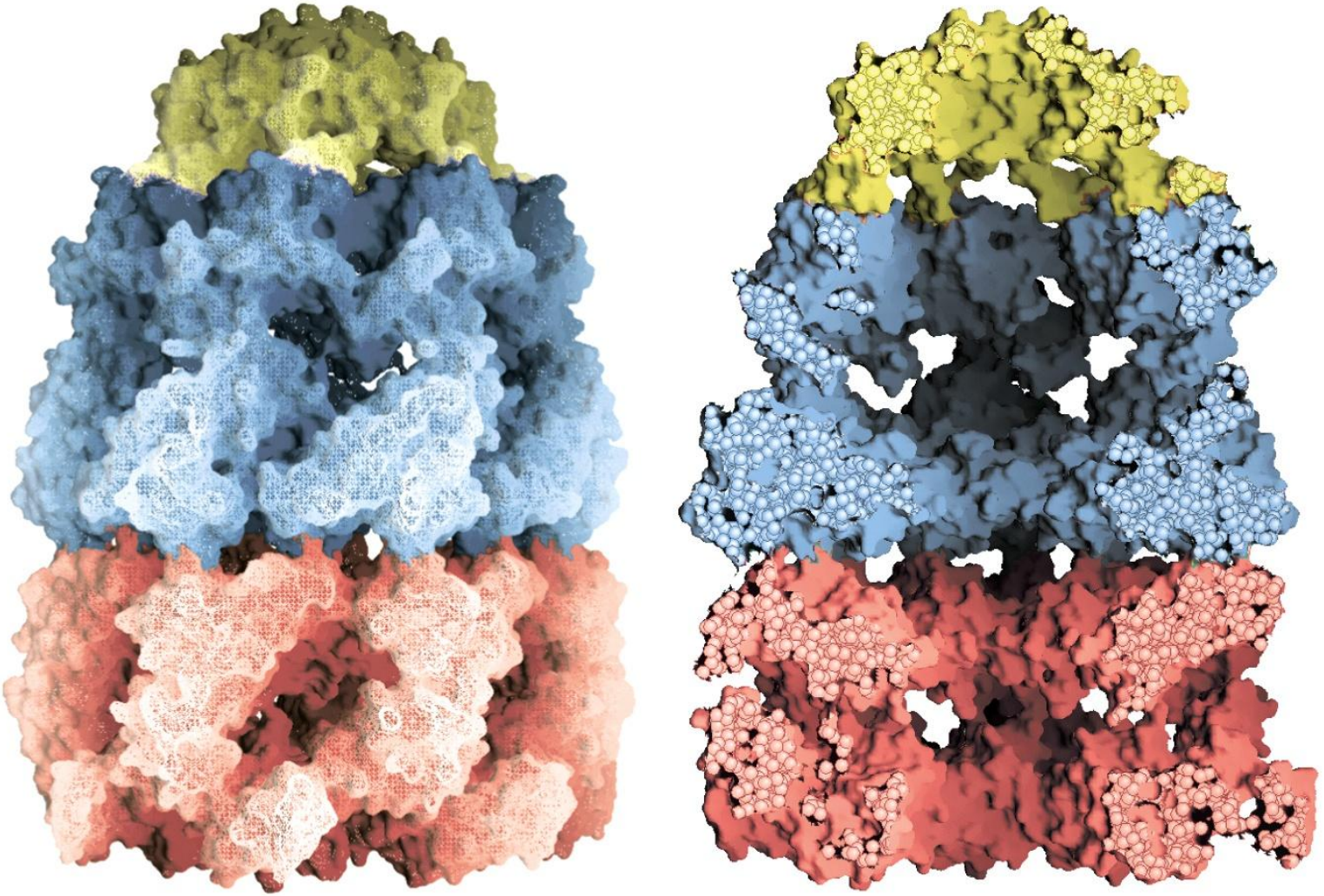
Amnio acid modification and group addition

Glucosylation (glycoproteins)
Acetylation
Phosphorylation (kinases)
Hydoxylation (Pro)
Methylation
Adition of prosethetic group (heme, biotin)
Adition of isoprenyl group

Chaperone in protein folding:

prevent newly synthesised polypeptide chains from aggregating into nonfunctional structures.

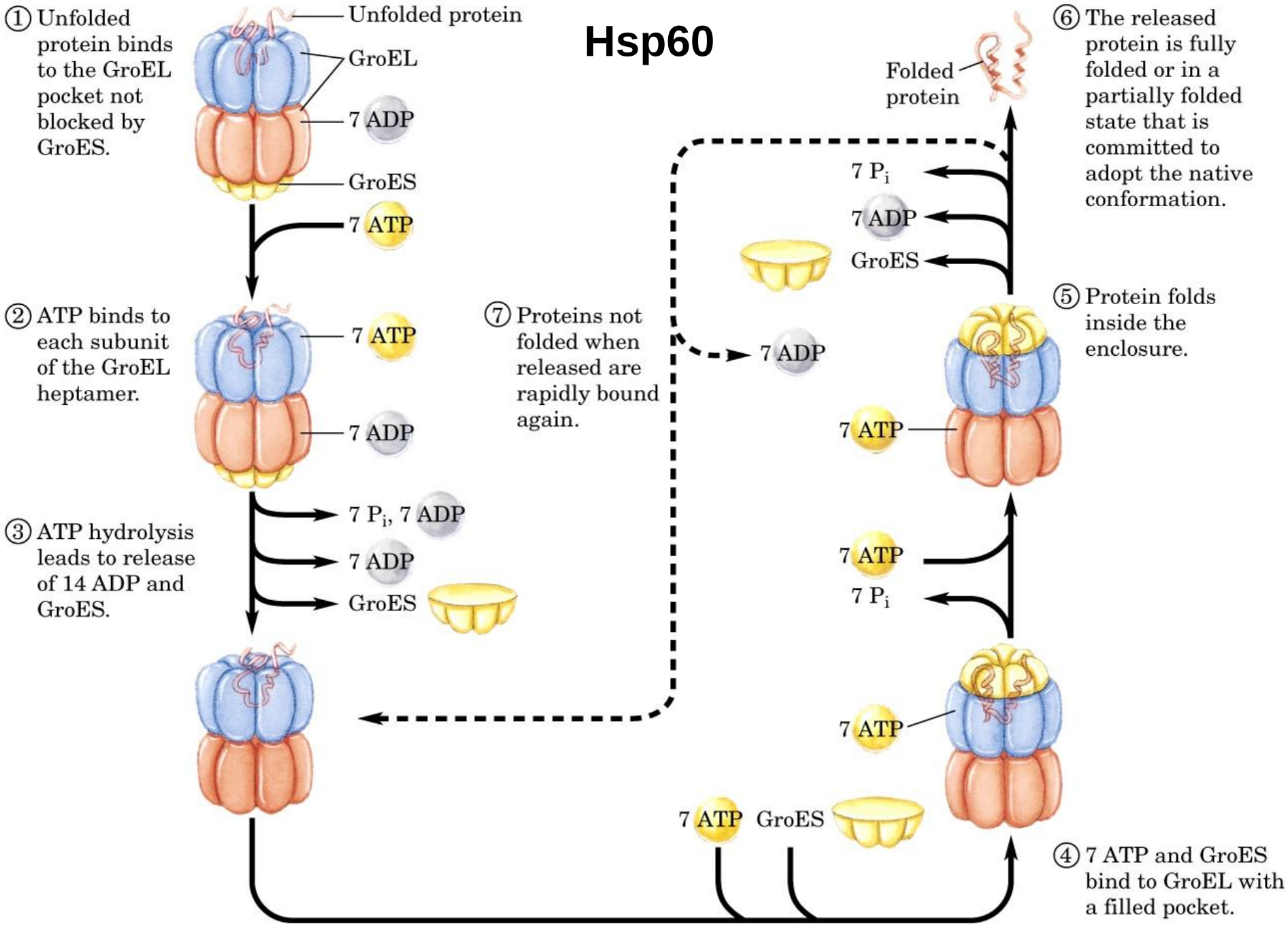
co-translationally



(b)

heat shock proteins: proteins expressed in response to elevated temperatures

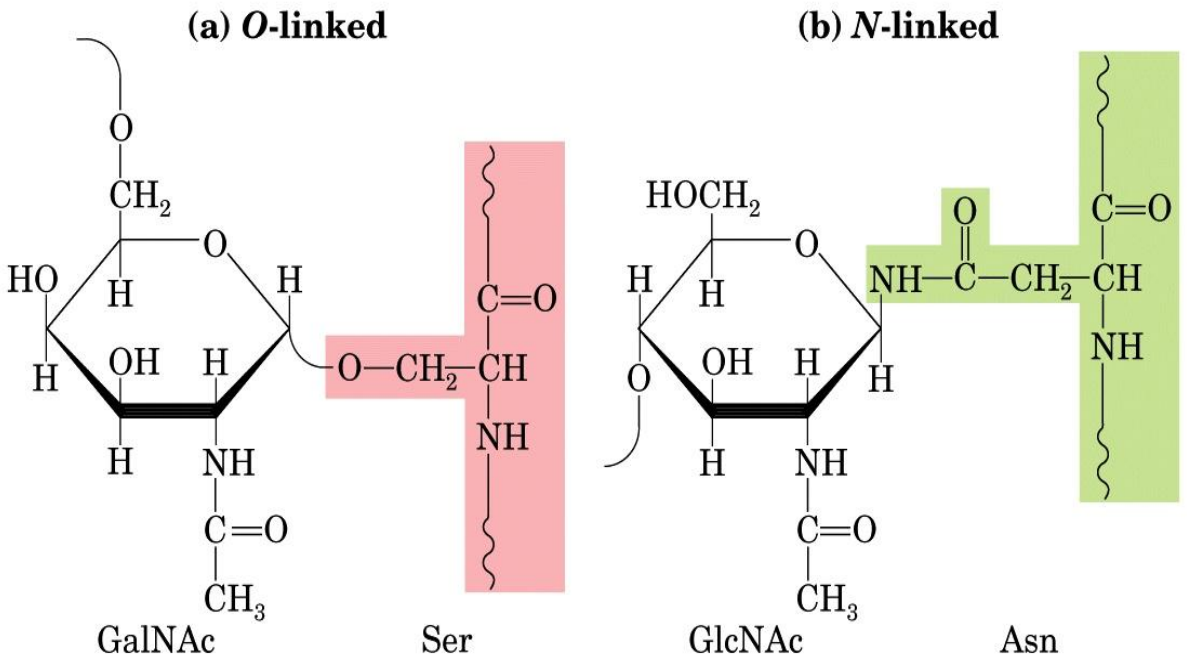
Hsp60


① Unfolded protein binds to the GroEL pocket not blocked by GroES.
Unfolded protein
GroEL
7 ADP
GroES
7 ATP
② ATP binds to each subunit of the GroEL heptamer.
7 ATP
7 ADP
③ ATP hydrolysis leads to release of 14 ADP and GroES.
7 P_i, 7 ADP
7 ADP
GroES
④ 7 ATP and GroES bind to GroEL with a filled pocket.
7 ATP
GroES
7 ATP
⑤ Protein folds inside the enclosure.
7 ATP
7 P_i
⑥ The released protein is fully folded or in a partially folded state that is committed to adopt the native conformation.
Folded protein
7 P_i
7 ADP
GroES
⑦ Proteins not folded when released are rapidly bound again.
7 ADP

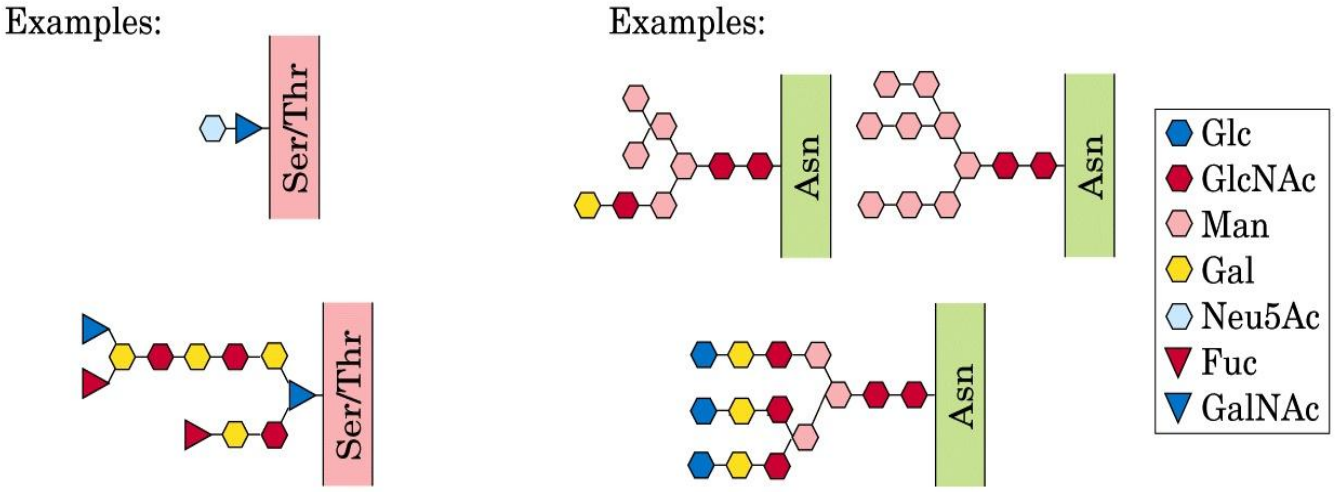
(a)

Glycosylation

.



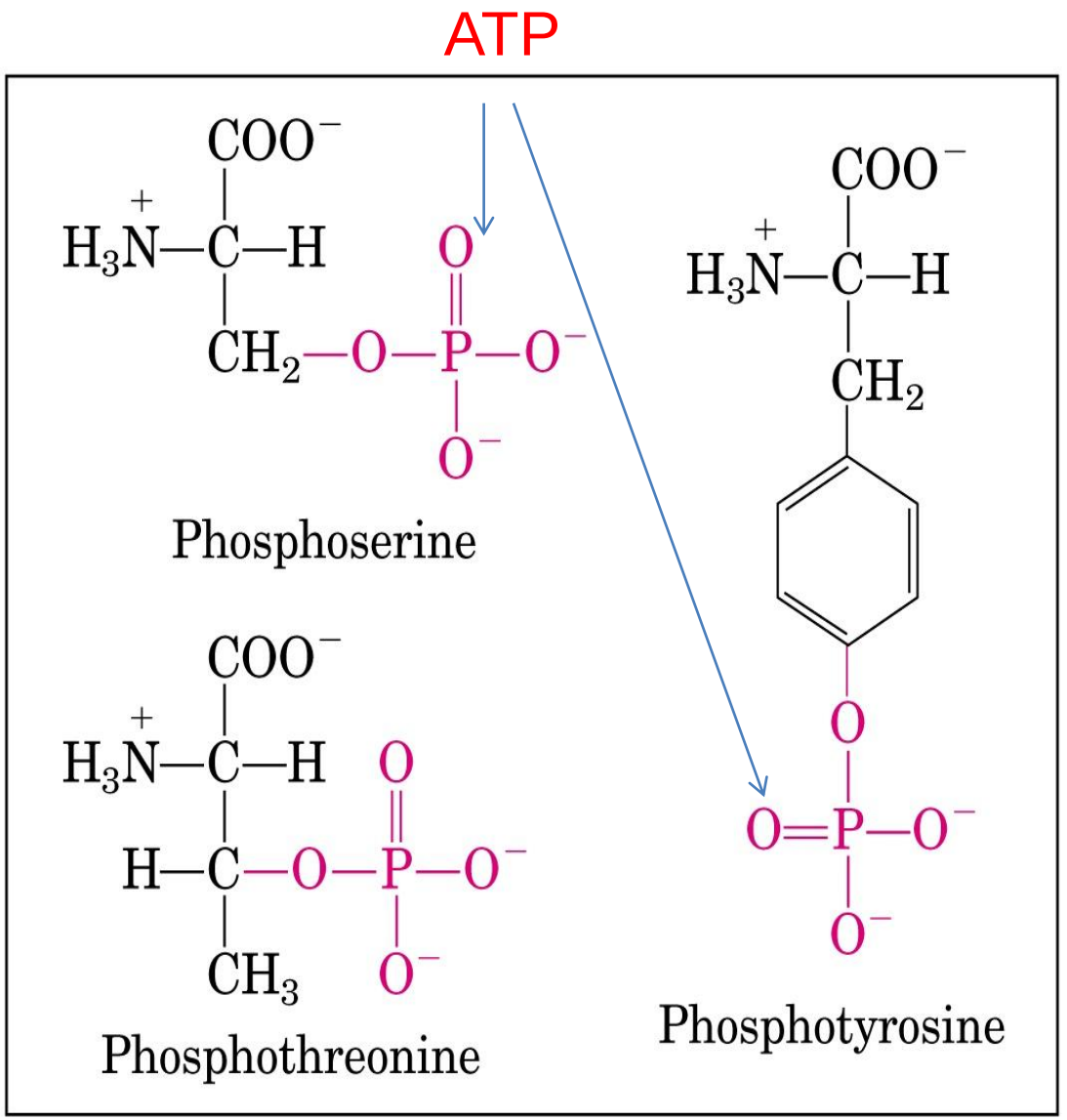
A few proteins are O glycosylated in the ER, but most O-glycosylation occurs in the Golgi complex or in the cytosol



1) Amino terminal and carboxyl-terminal modifications:

2) Loss of signal sequence

3) Modification of individual a.a :



(a)

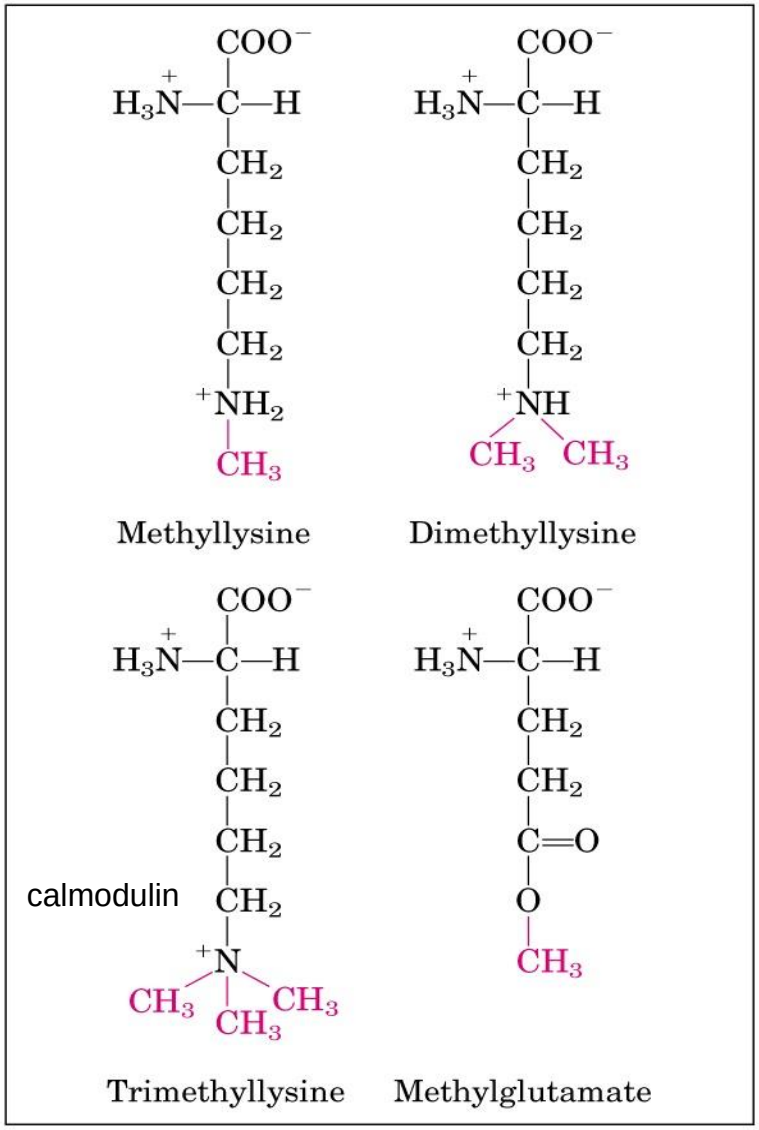
the phosphate groups add negative charges to these polypeptides

Examples:

1-Casein=P-serine= binds with Ca

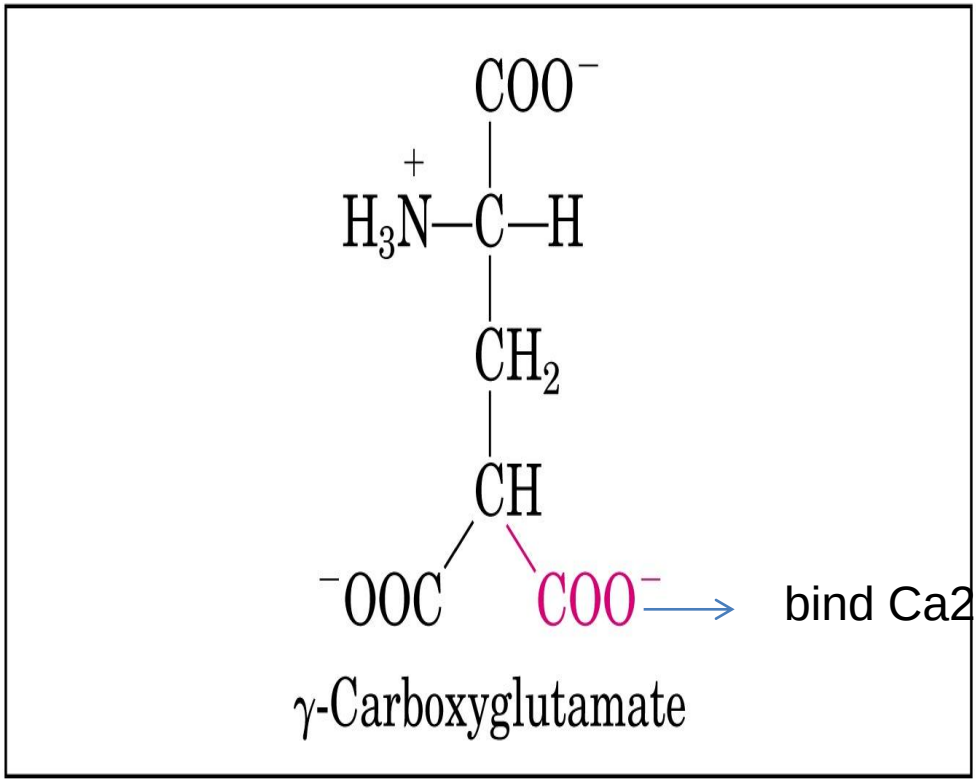
2- Phosphorylation=activation of regulatory Proteins

Carboxylation and Methylation:



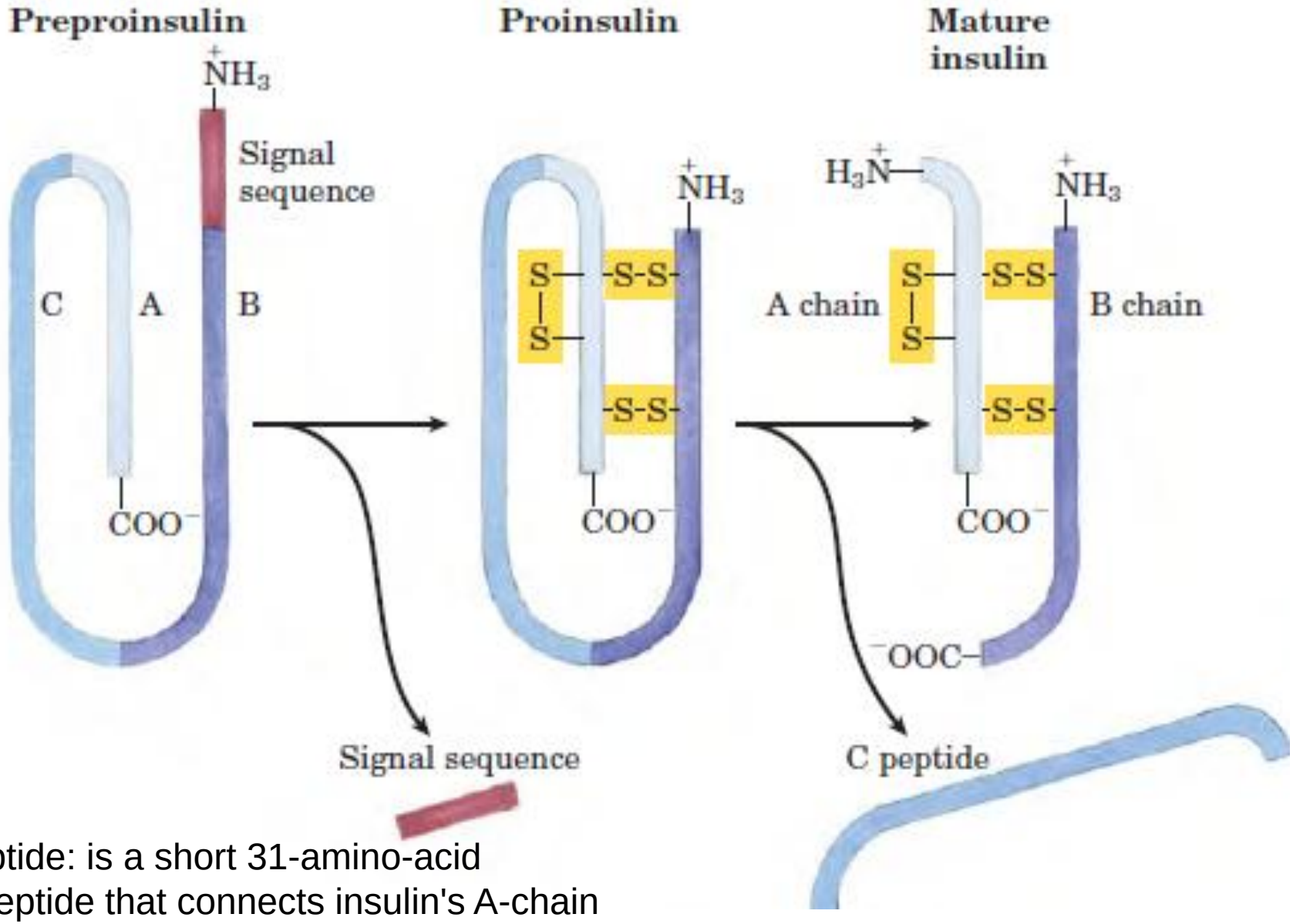
(c)

Extra carboxyl groups may be added to Glu residues

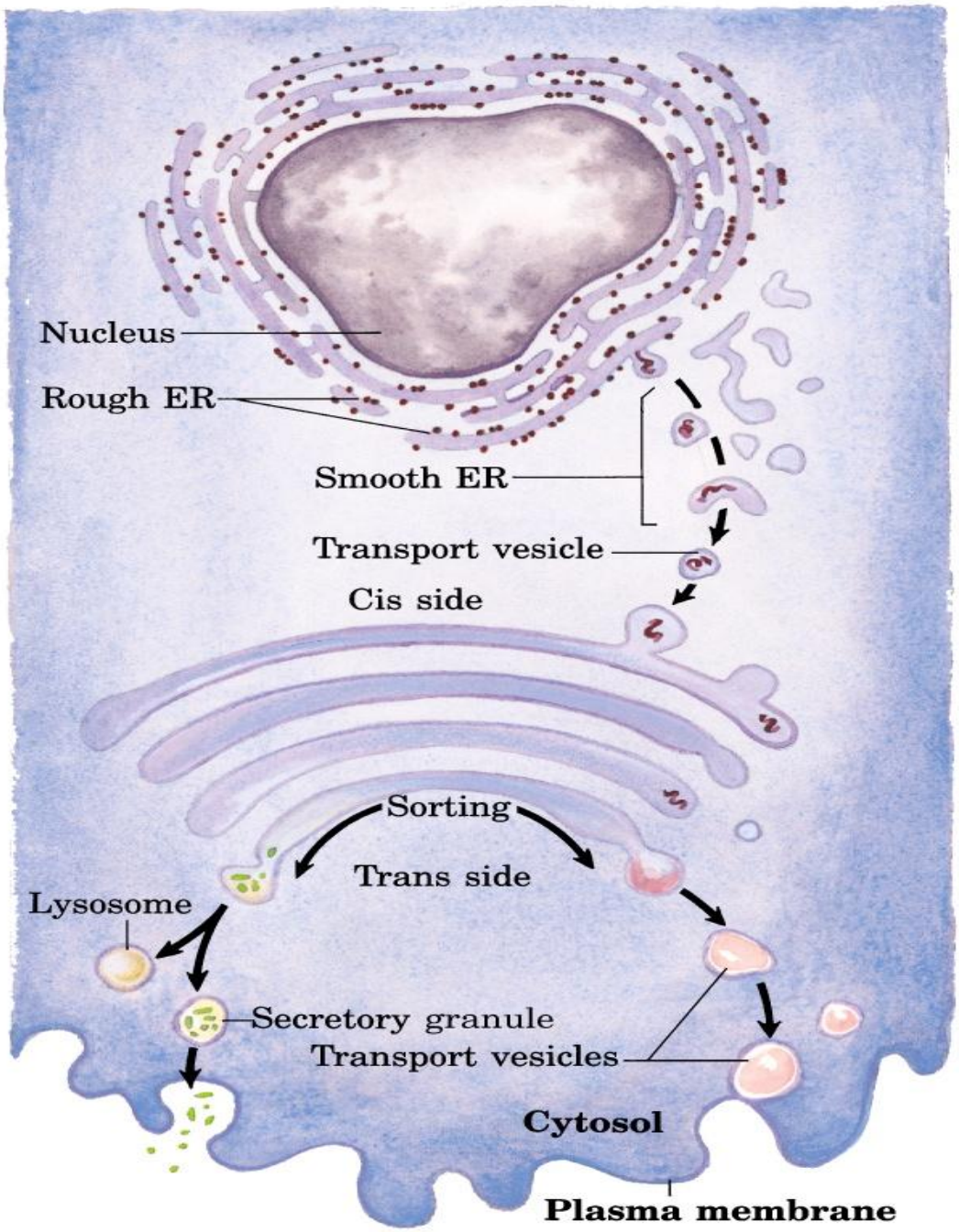


(b)

the blood-clotting protein prothrombin contains a number of –carboxyglutamate residues



C-peptide: is a short 31-amino-acid polypeptide that connects insulin's A-chain to its B-chain in the proinsulin molecule



Newly synthesized protein targeted to different locations:

-**Peptide signal** sequence (amino terminus)

-**SRP** (signal recognition particle) binds with peptide signal

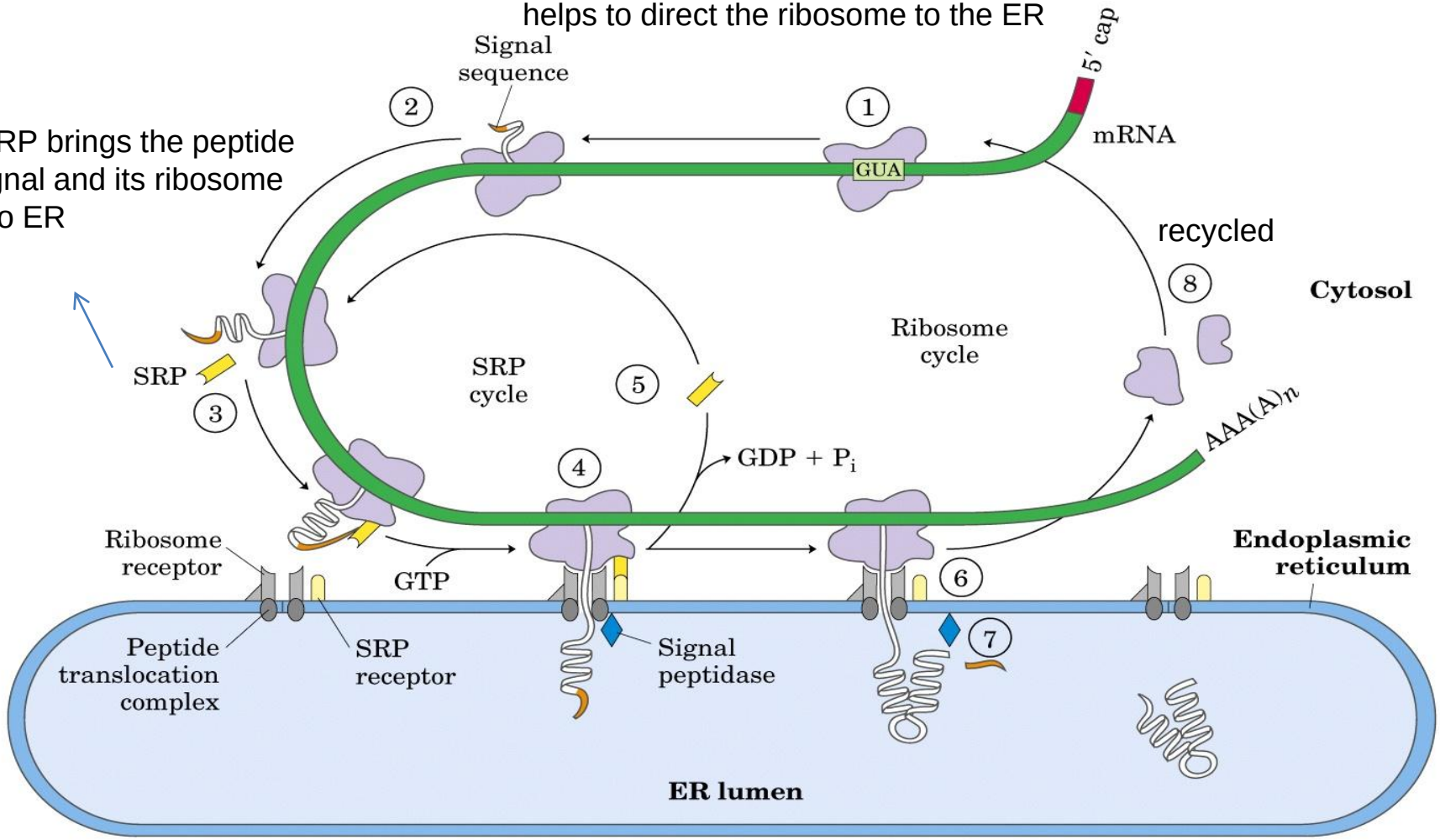
-Modification (ER)

- Golgi complex: Further modification and sorting

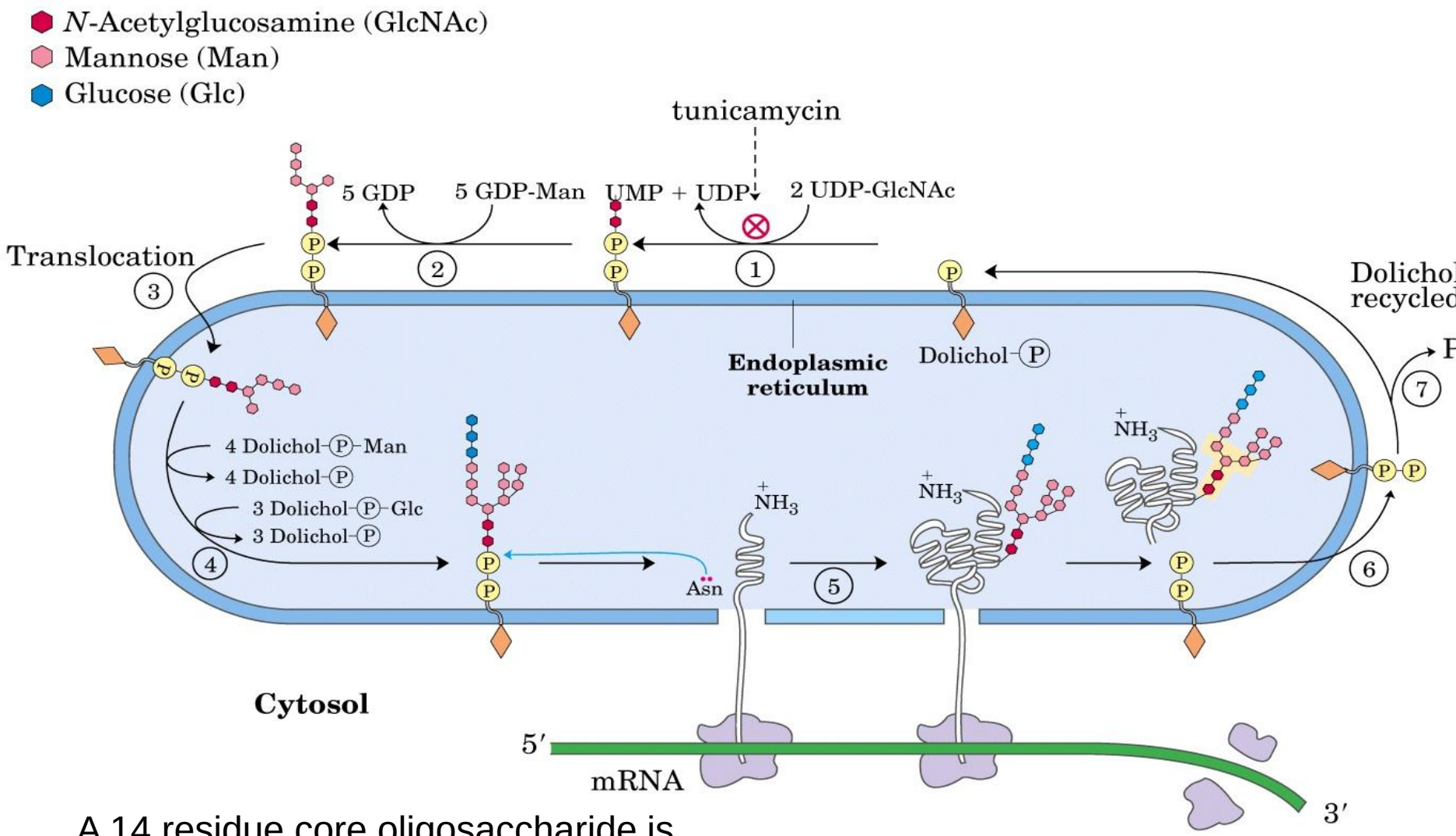
Directing eukaryotic proteins with the appropriate signals to the endoplasmic reticulum

helps to direct the ribosome to the ER

-SRP brings the peptide signal and its ribosome into ER



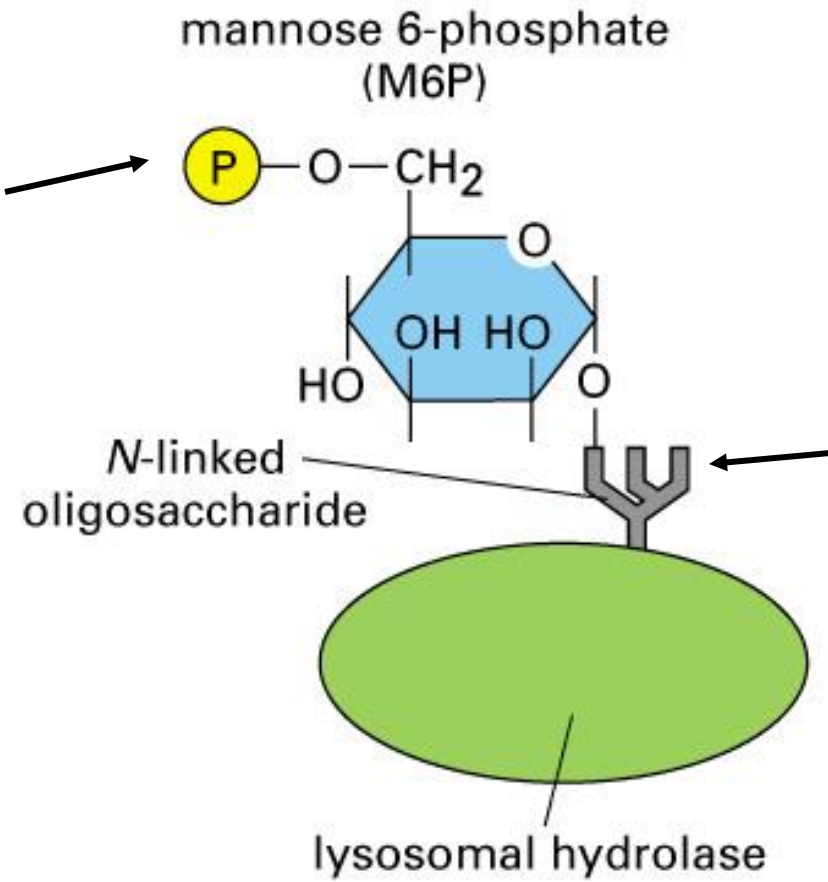
Glycosylation:



A 14 residue core oligosaccharide is built up in a stepwise fashion, then transferred from a dolichol phosphate donor molecule to certain Asn residues in the protein

The acid hydrolases in the lysosome are sorted in the Golgi complex based on the chemical marker mannose 6-phosphate.

The phosphate is added in the Golgi



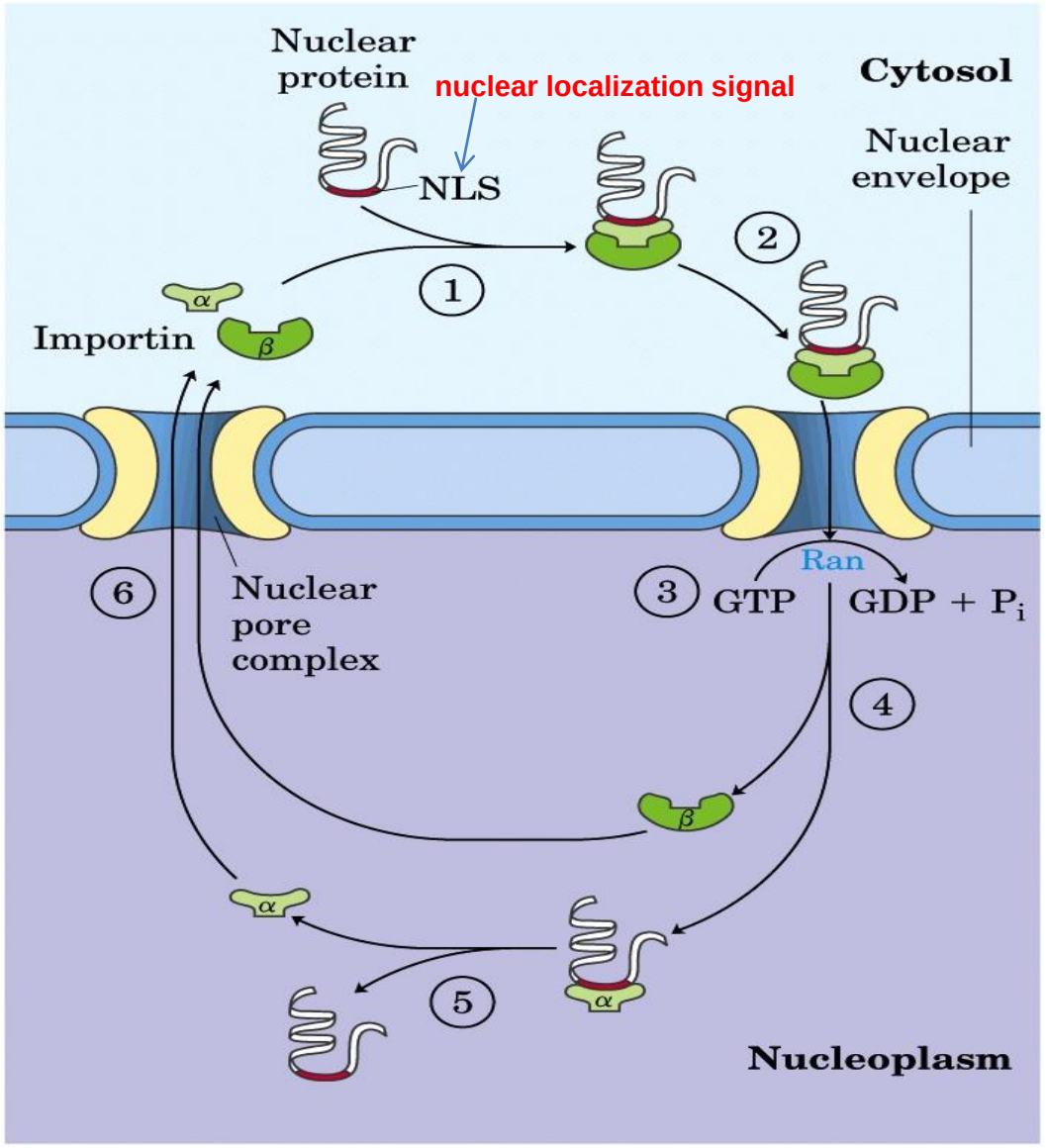
This was first attached in the ER.

Figure 13–36. Molecular Biology of the Cell, 4th Edition.

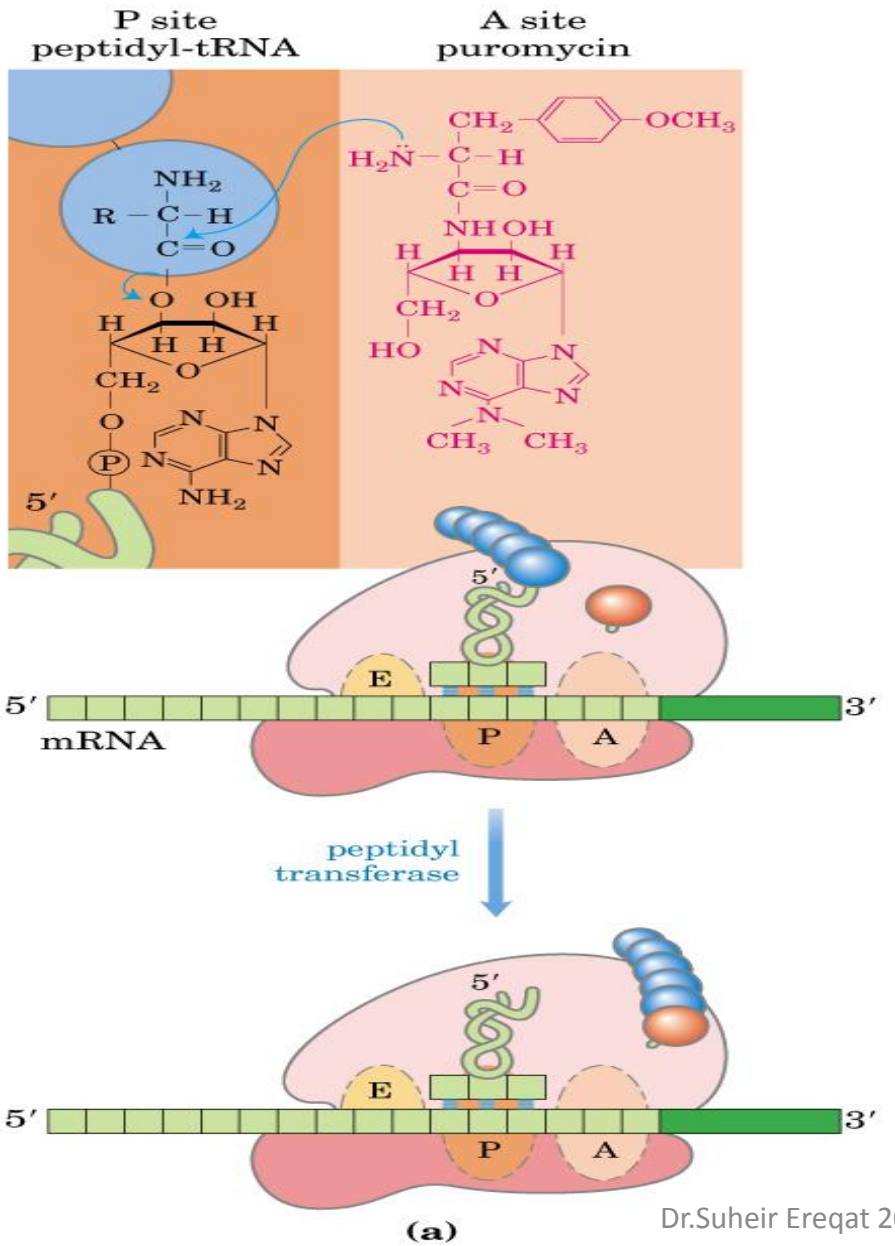
Targeting of nuclear proteins.

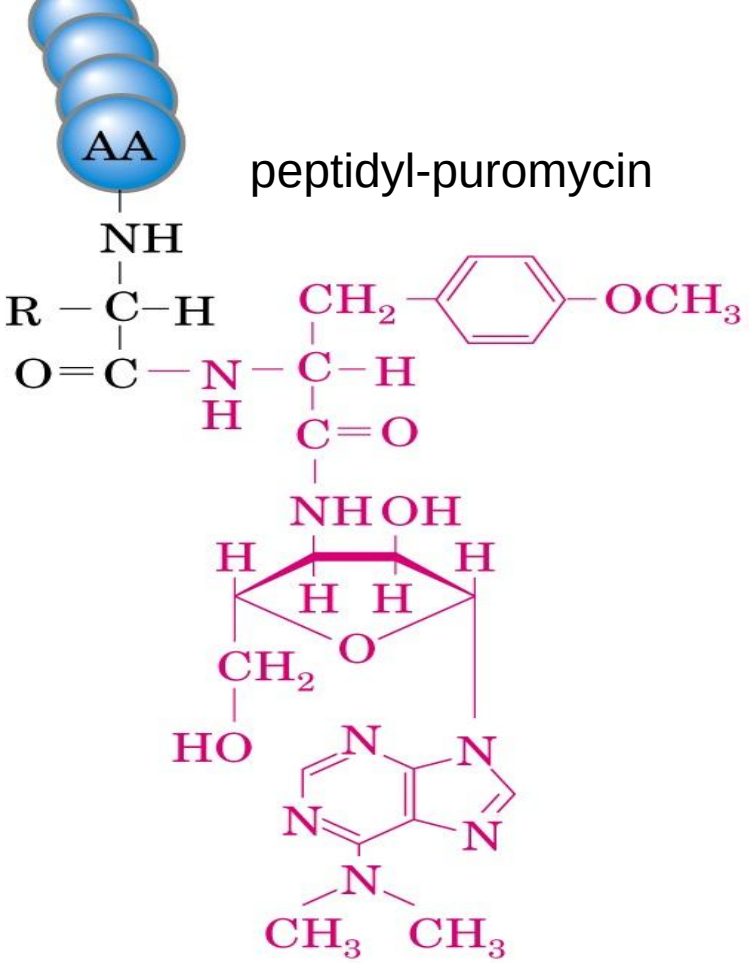
Nuclear importation (signal not cleaved):

-Ribosomal proteins



Puromycin





Its structure is very similar to the 3 end of an aminoacyl-tRNA, enabling it to bind to the ribosomal A site and participate in peptide bond formation

Table 6–4 Inhibitors of Protein or RNA Synthesis

INHIBITOR	SPECIFIC EFFECT
Acting only on bacteria	
Tetracycline	blocks binding of aminoacyl-tRNA to A-site of ribosome
Streptomycin	prevents the transition from translation initiation to chain elongation and also causes miscoding
Chloramphenicol	blocks the peptidyl transferase reaction on ribosomes (step 2 in Figure 6–66)
Erythromycin	binds in the exit channel of the ribosome and thereby inhibits elongation of the peptide chain
Rifamycin	blocks initiation of RNA chains by binding to RNA polymerase (prevents RNA synthesis)
Acting on bacteria and eucaryotes	
Puromycin	causes the premature release of nascent polypeptide chains by its addition to the growing chain end
Actinomycin D	binds to DNA and blocks the movement of RNA polymerase (prevents RNA synthesis)
Acting on eucaryotes but not bacteria	
Cycloheximide	blocks the translocation reaction on ribosomes (step 3 in Figure 6–66)
Anisomycin	blocks the peptidyl transferase reaction on ribosomes (step 2 in Figure 6–66)
α-Amanitin	blocks mRNA synthesis by binding preferentially to RNA polymerase II

The ribosomes of eucaryotic mitochondria (and chloroplasts) often resemble those of bacteria in their sensitivity to inhibitors. Therefore, some of these antibiotics can have a deleterious effect on human mitochondria.

Protein Degradation Is Mediated by Specialized Systems in All Cells

Protein degradation prevents the buildup of abnormal or unwanted proteins and permits the recycling of amino acids.

Regulated by:

N-end rule

Lysosome

upiqutination

N-end rule

the identity of the first residue that remains after removal of the amino-terminal Met residue, and any other posttranslational proteolytic processing of the amino-terminal end, has a profound influence on half-life

TABLE 27–9 Relationship between Protein Half-Life and Amino-Terminal Amino Acid Residue

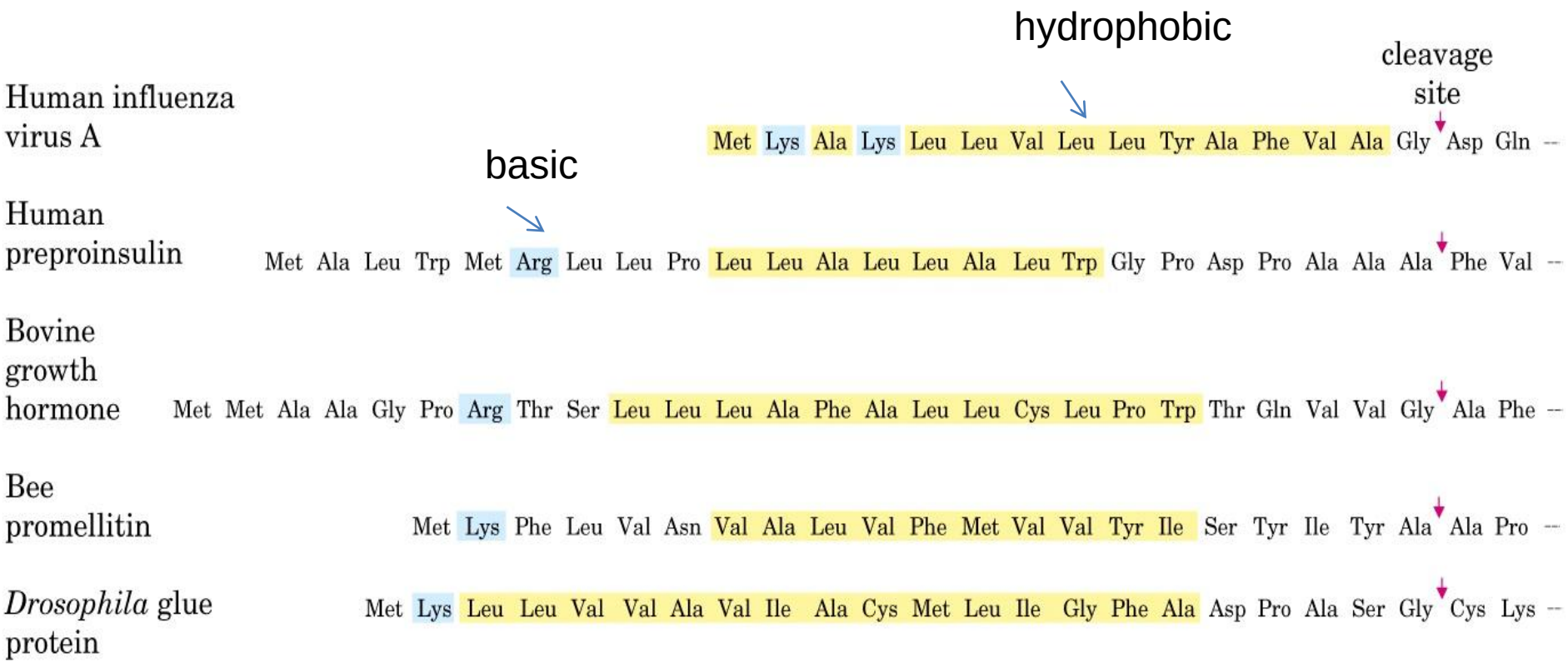
Amino-terminal residue	Half-life*
Stabilizing	
Met, Gly, Ala, Ser, Thr, Val	>20 h
Destabilizing	
Ile, Gln	~30 min
Tyr, Glu	~10 min
Pro	~7 min
Leu, Phe, Asp, Lys	~3 min
Arg	~2 min

Source: Modified from Bachmair, A., Finley, D., & Varshavsky, A. (1986) In vivo half-life of a protein is a function of its amino-terminal residue. *Science* **234**, 179–186.

*Half-lives were measured in yeast for the β-galactosidase protein modified so that in each experiment it had a different amino-terminal residue. Half-lives may vary for different proteins and in different organisms, but this general pattern appears to hold for all organisms.

Protein targeting:

Amino-terminal signal sequences of some eukaryotic proteins that direct their translocation into the ER.



Protein degradation

- For some proteins, more than 80% of peptides may not fold properly. These are removed from the ER and degraded.
- Retrotranslocation (or dislocation)
- N-glycanase removes the oligosaccharide.
- Ubiquitin chain added to protein which marks it for degradation in the proteasome.

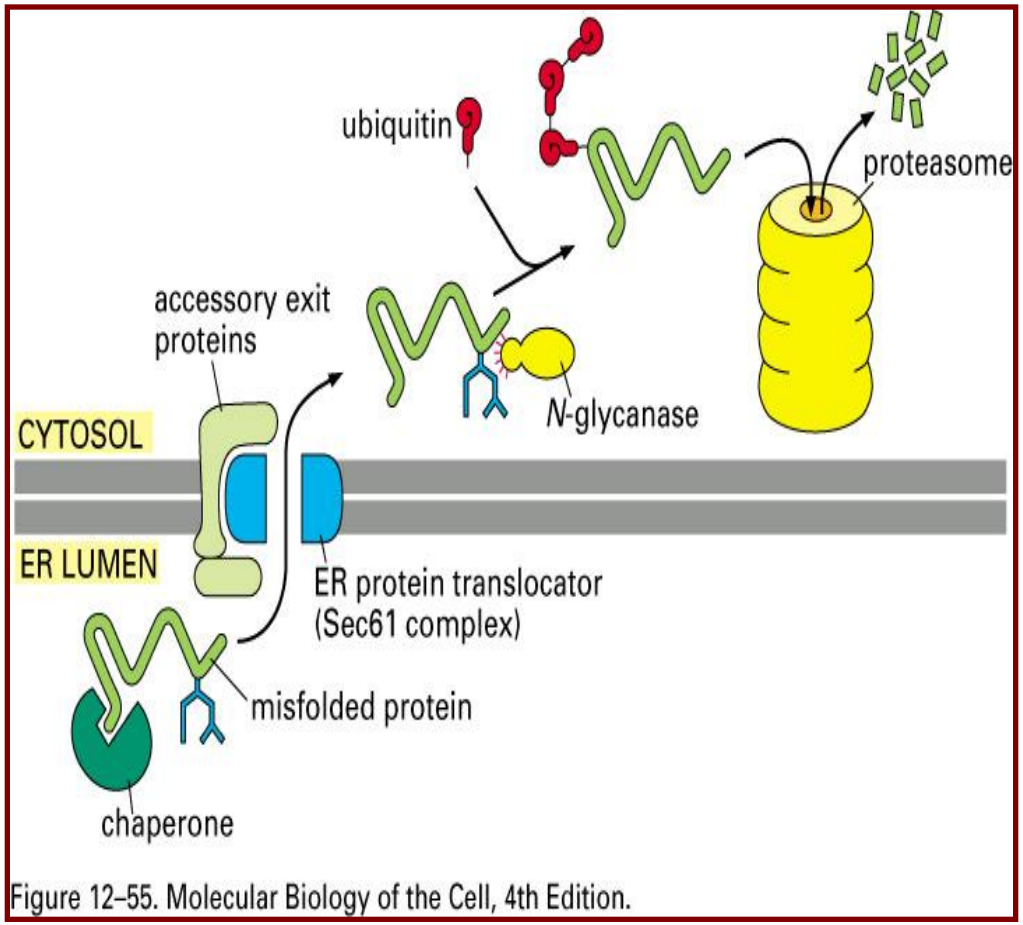
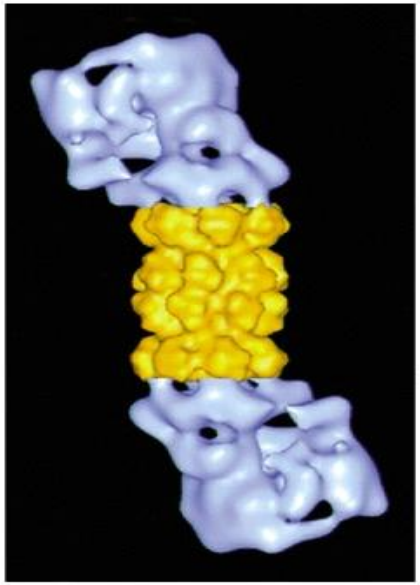
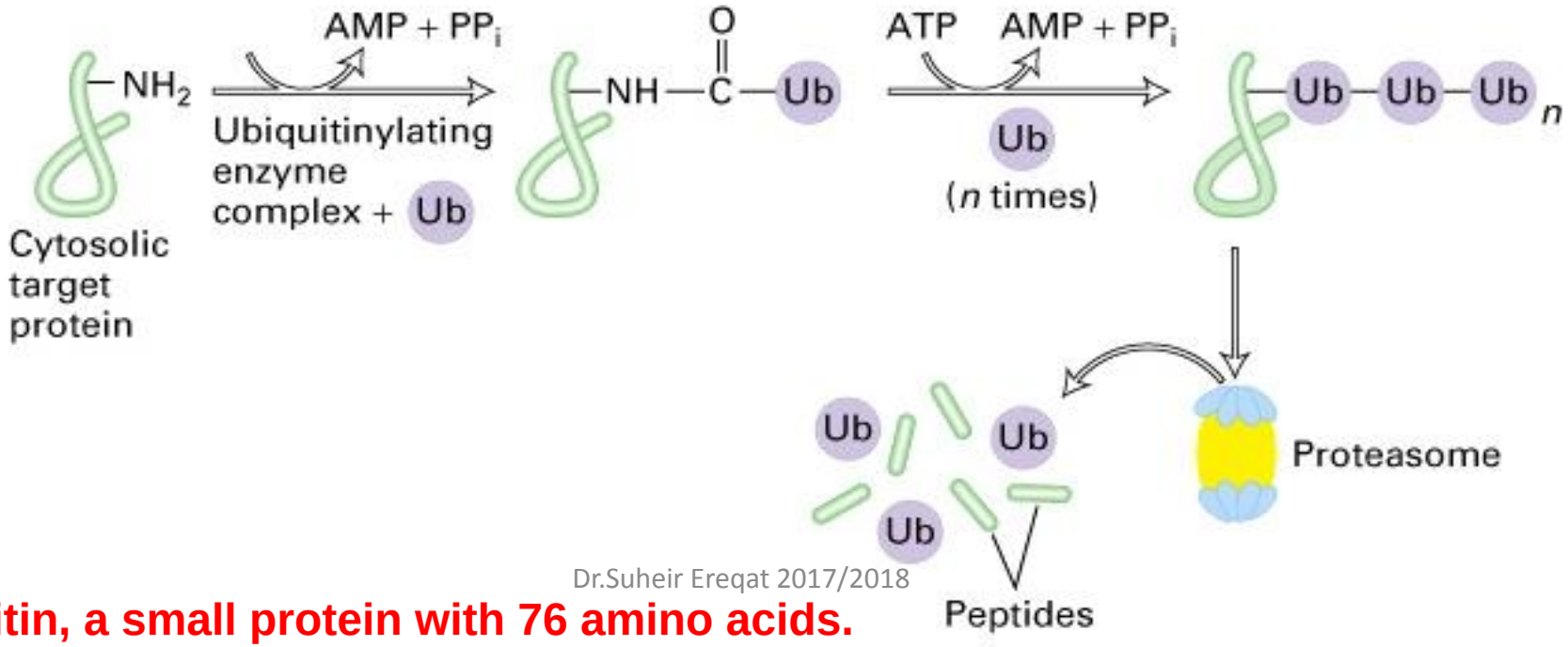


Figure 12–55. Molecular Biology of the Cell, 4th Edition.

Protein degradation by the ubiquitin- pathway





Ubiquitin, a small protein with 76 amino acids.