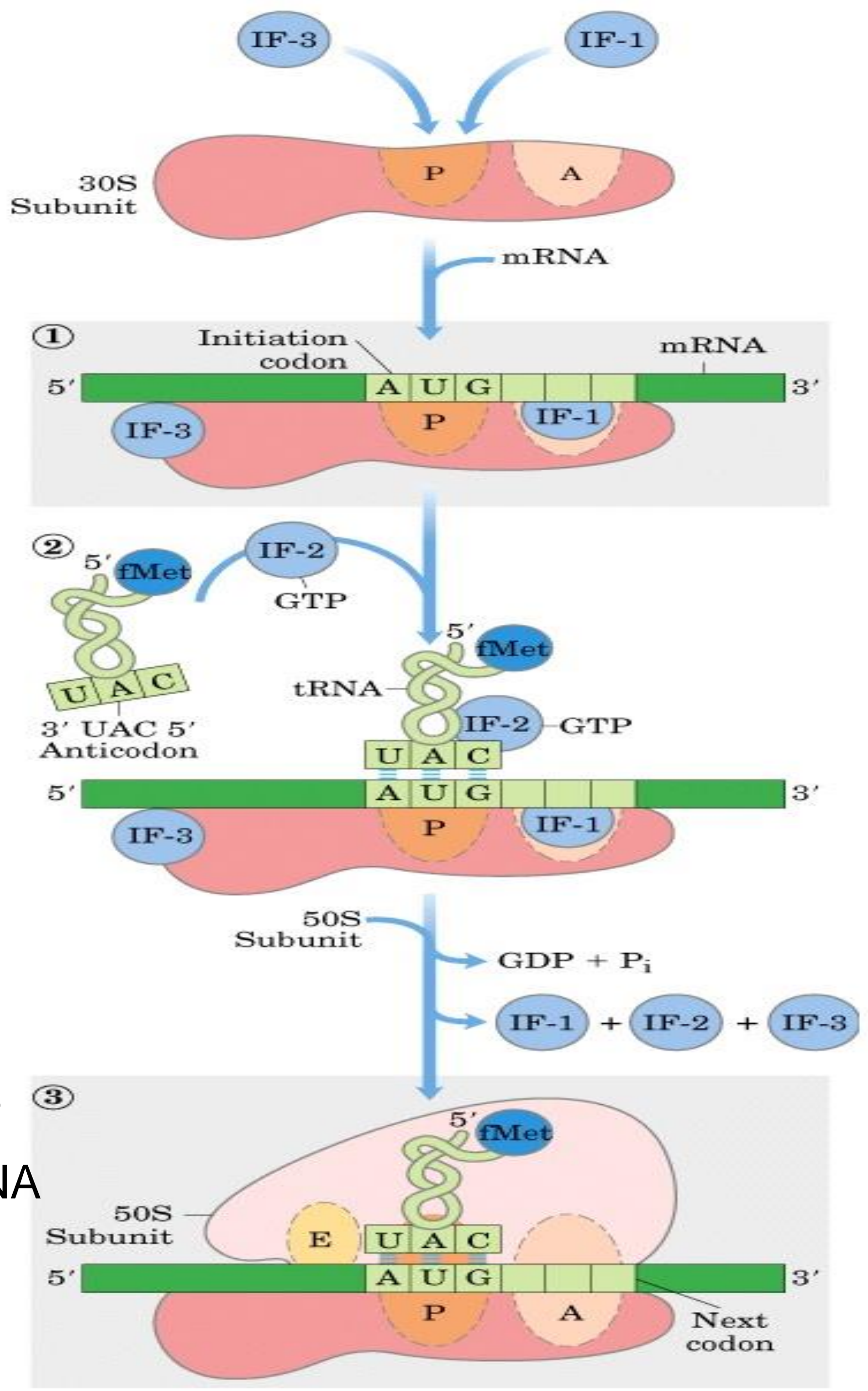
Formation of initiation complex:

Requirements:

1) 30S ribosomal subunit
2) mRNA
3) GTP
4) Mg2+
5) IF-1, IF-2, IF-3
6) 50S ribosomal subunit
7) Initiating fMet-tRNA ^{fMet}

-At shine dalgarno sequence mRNA –rRNA interaction positions the AUG in precise position to base pair with fMet-tRNA ^{fMet}

- IF-1 binds at A site to prevent tRNA binding
- IF-3 prevent premature binding of 30 and 50S
- GTP-bound IF2 guides tRNA to pair with mRNA
- 70S initiation complex formed



Eukaryotic initiation complex:

5`cap and 3`poly A linked by poly A binding protein (PAB) elongation factors (at least 9).

Complex eIF4F:

eIF4E, eIF4G, bind the cap

eIF4A helicase activity

→solve secondary structures in mRNA

eIF4B: scan 5` mRNA for initiation codon 5`-AUG signaling start of reading frame.

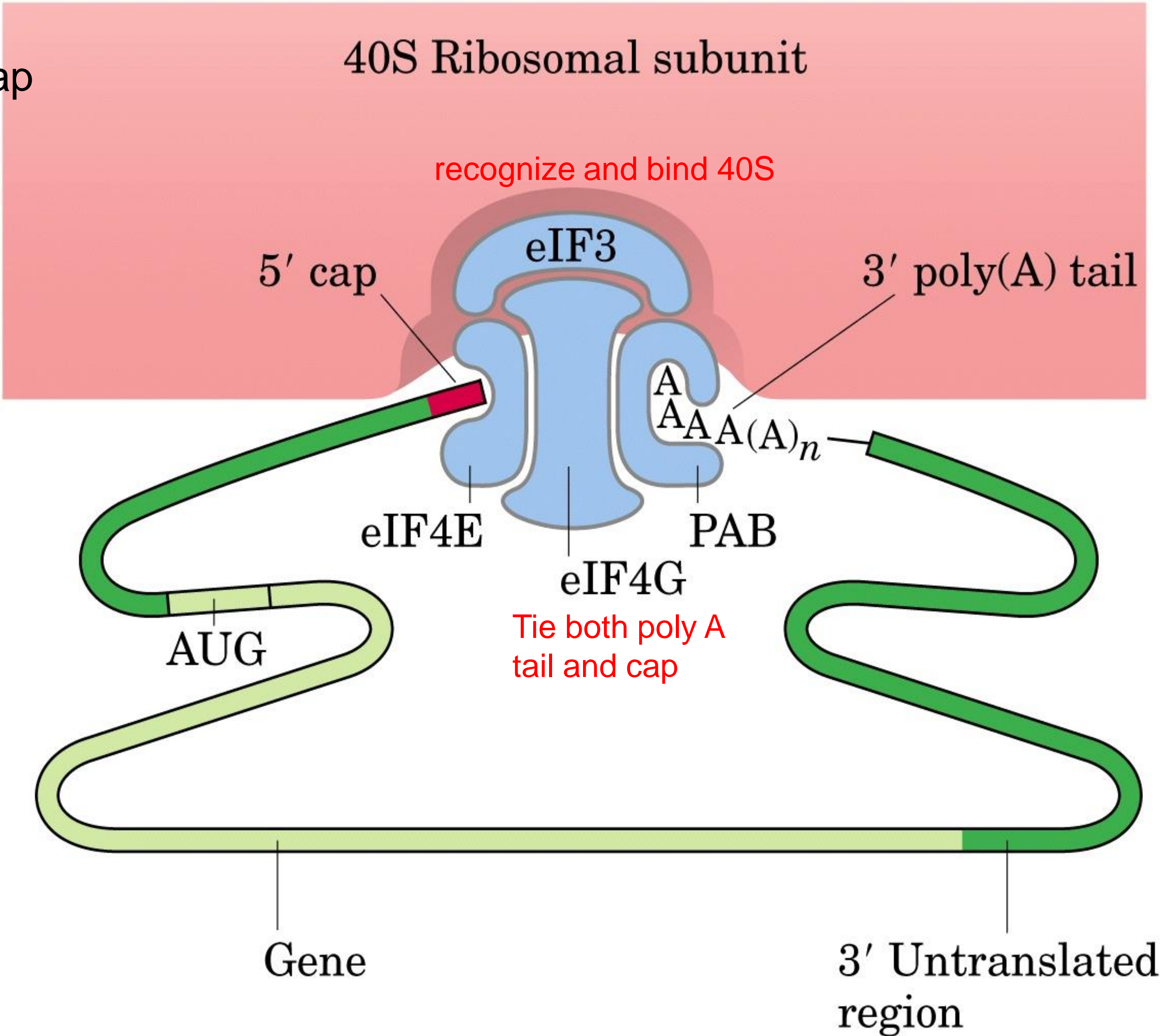


table 27–9

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.

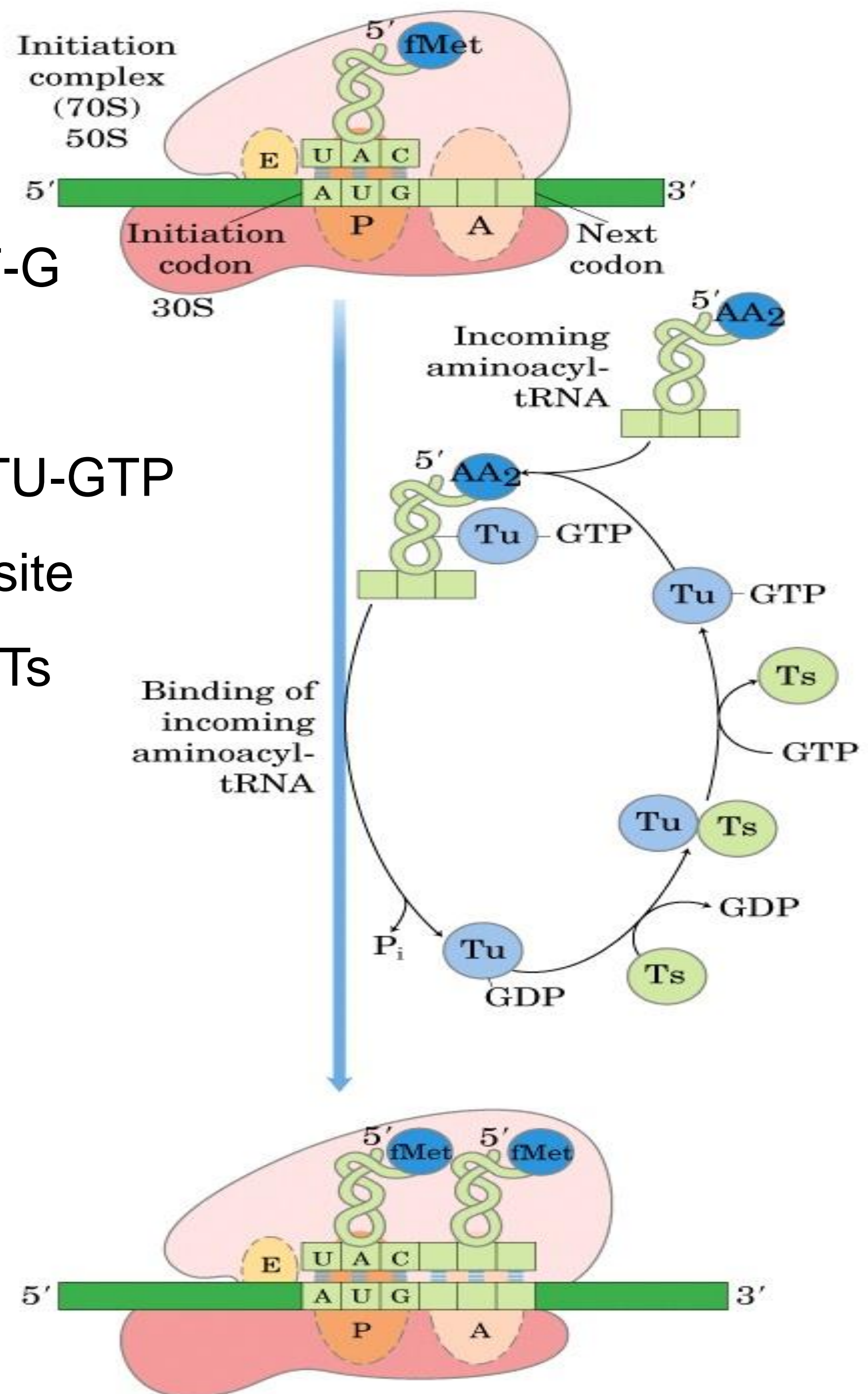
Elongation: Requirements

1) Initiation complex
2) Aminoacyl-tRNA
3) Elongation factors EF-Tu, EF-Ts , EF-G
4) GTP

Incoming aminoacyl-tRNA binds to EF-TU-GTP

→aminoacyl –tRNA-EF-Tu-GTP bind A site

GTP hydrolyzed and EF recycled using Ts



Peptide bond formation:

α- amino group of a.a (A site) acts

as a nucleophile forming :

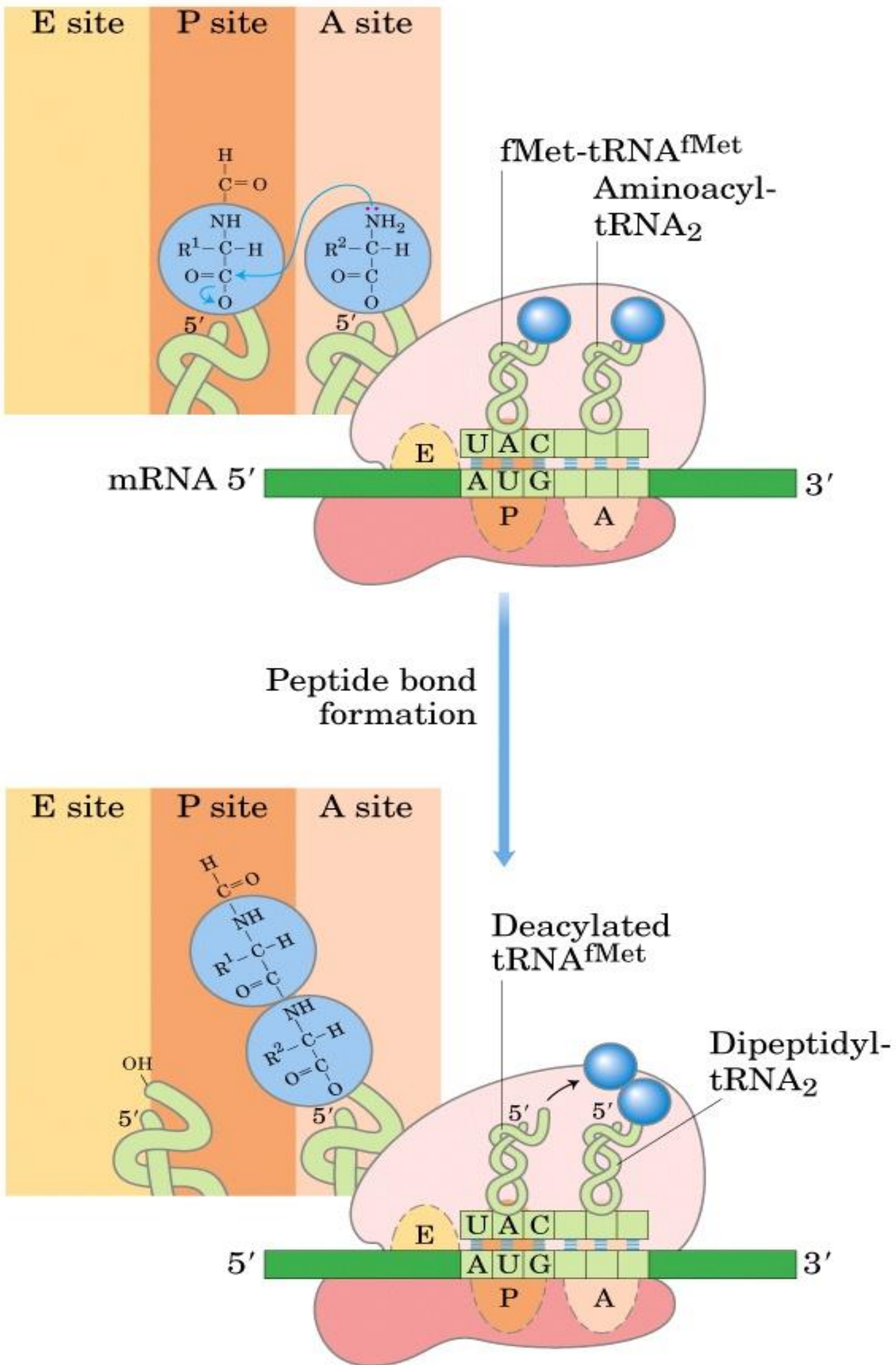
1) a peptide bond = dipeptidyl on A site

2) Uncharged tRNA on P site

→ tRNAs shift to hybrid binding state

→ Spanning 2 different sites on ribosome.

but anticodons remain in A and P position.



Peptidyl transferase = not a protein/s

at the large ribosomal subunit but

23S rRNA (catalytic RNA)

Translocation:

Movement of ribosome one codon towards 3` site .

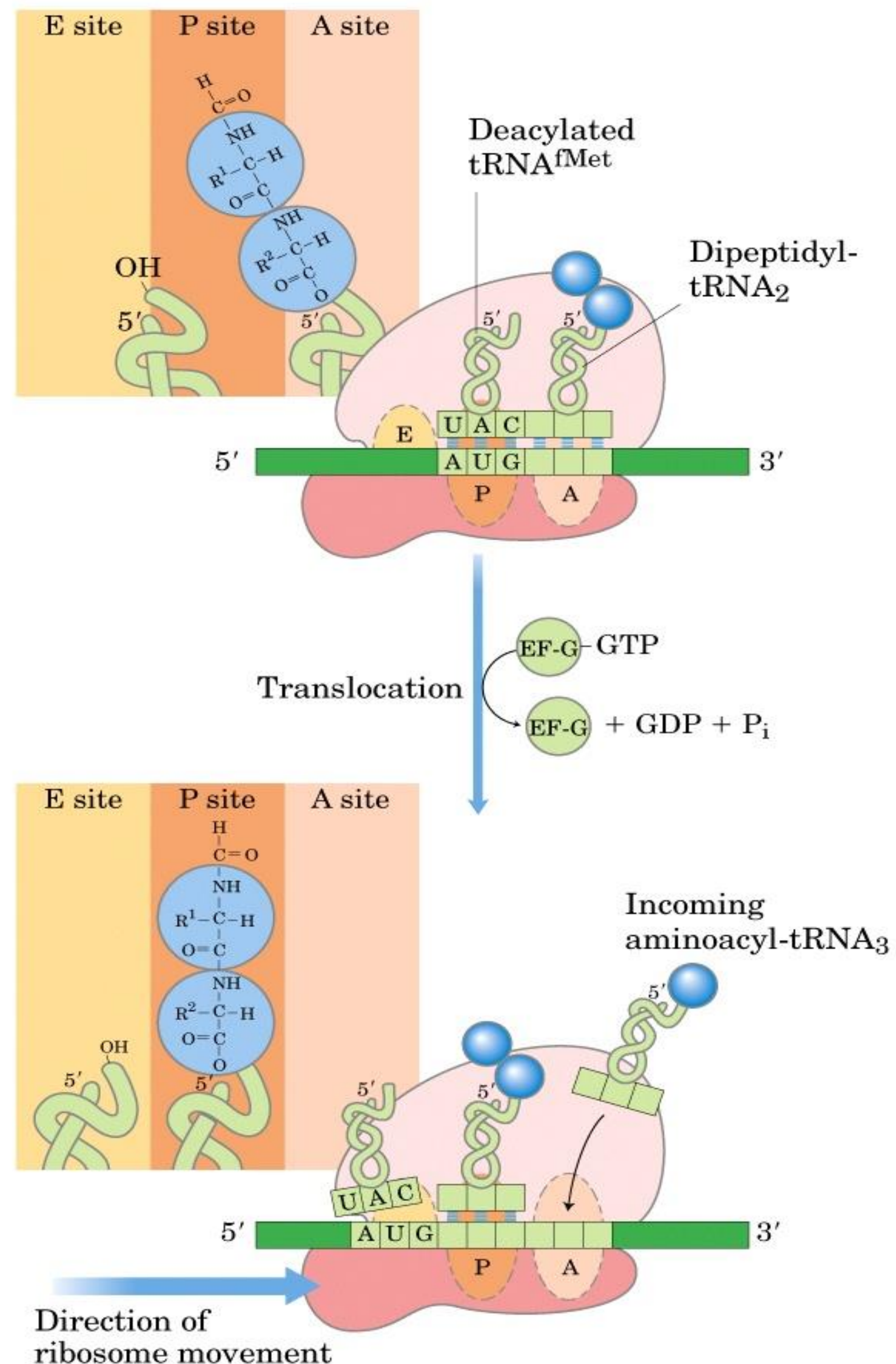
Uncharged shift P→E site

Dipeptidyl-tRNA shift A→P site

Ribosome movement requires:

translocase = EF-G + energy from GTP

Ester linkage bw tRNA and carboxyl terminus of the growing polypeptide activate terminal carboxyl group for nucleophilic attack by incoming a.a → form a new peptide bond.



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.

- Accurate translation requires two steps:

– First step:

a correct match between a tRNA and an amino acid, done by enzyme aminoacyl-tRNA synthetase.

– Second step:

a correct match between the tRNA anti-codon and an mRNA codon.

Proofreading on the ribosome:

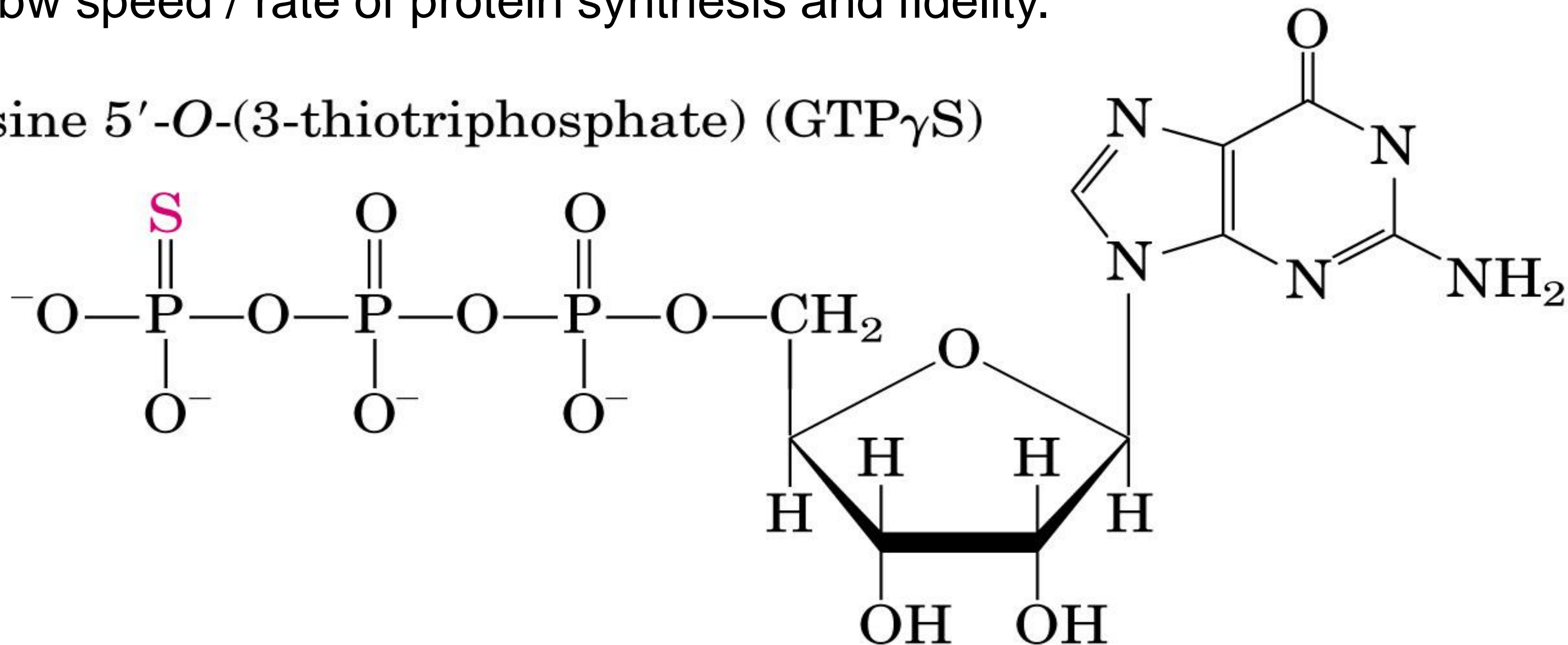
EF-Tu has GTPase activity. EF-Tu –GTP and EF-Tu-GDP exist for milliseconds before they dissociate.

This interval allow codon-anticodon interaction to be proofread.

Incorrect aminoacyl-tRNA dissociate from A site during this time.

- If GTP analog used →slower hydrolysis →higher fidelity (increasing proofreading interval).

Balance bw speed / rate of protein synthesis and fidelity.



Termination:

Termination codon (UAA, UAG, UGA)

-A site occupied by a termination codon
→ 3 release / termination factors:
Hydrolysis of terminal peptidyl tRNA bond

1) Release of free polypeptide chain
2) Release of last tRNA (uncharged)
3) 70S ribosome dissociation into 30S, 50S

RF1:recognize codon (UAG, UAA)

RF2:recognize codon (UGA, UAA

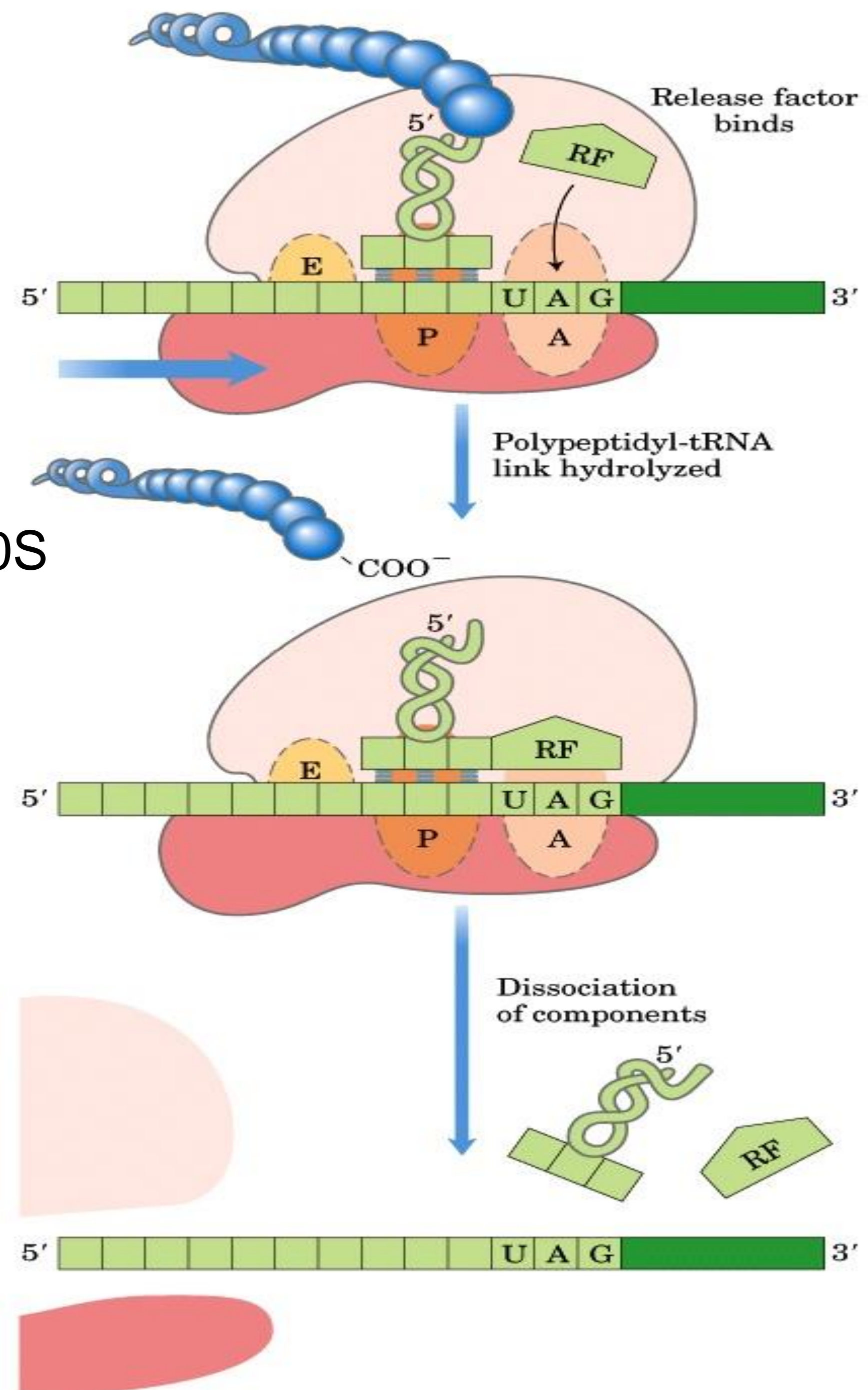
RF1/RF2 (depending on present codon)

transfer of polypeptide chain not to a

new a.a but to water(hydrolysis)

RF3: release of ribosome subunits.

Eukaryotes one release factor (eRF)



Mutation in termination codon → deleterious to cell.

Thalassemia

The α chain of human haelogmobin is normally 141 a.a residues long.

A mutation (U→C) converts the termination codon UAA to CAA = glutamine.

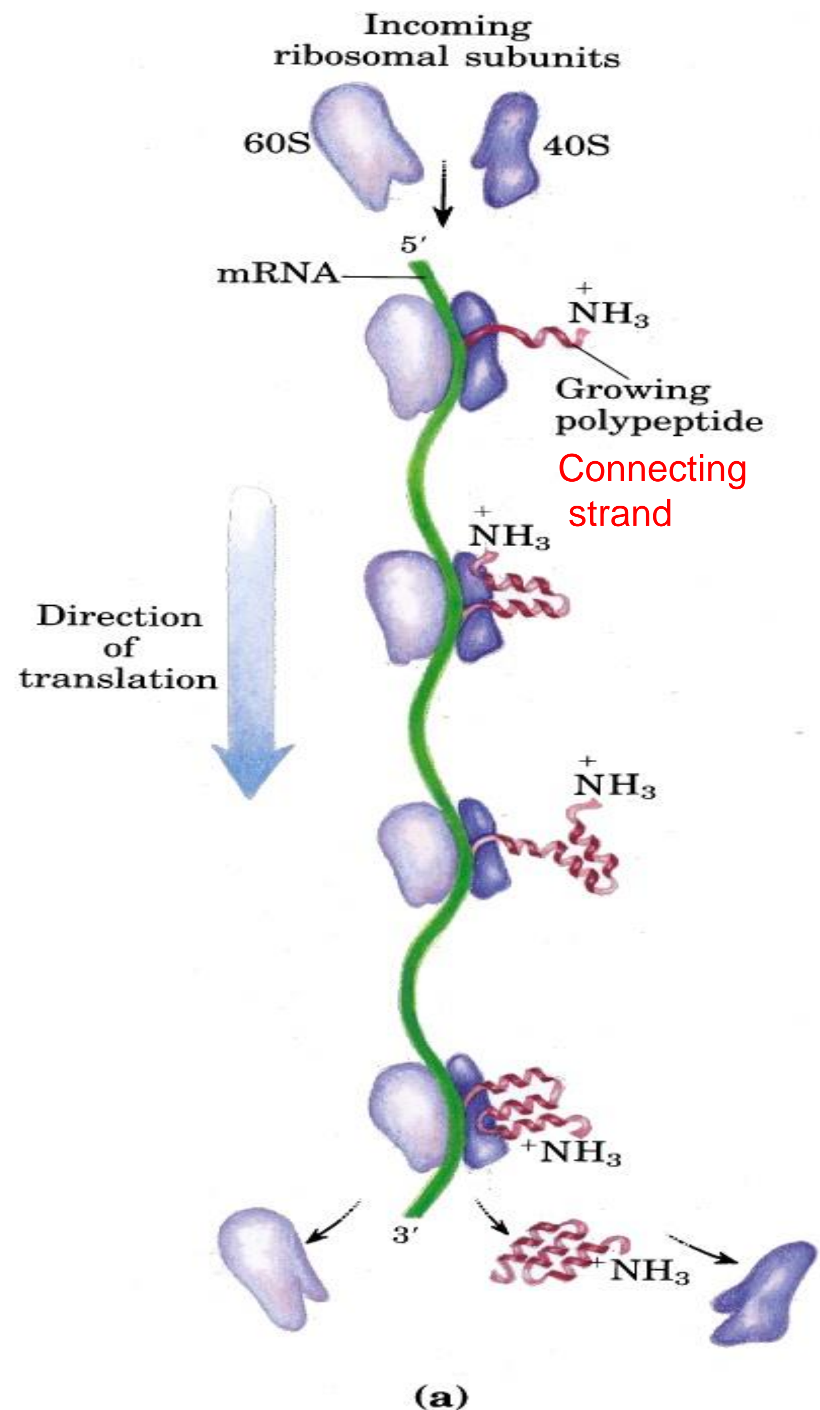
→producing a polypeptide chain containing 172 a.a

A **nonsense mutation** is a point mutation results in a premature stop codon .

A **missense mutation** is a point mutation where a single nucleotide is changed to cause substitution of a different amino acid.

POLYSOME:

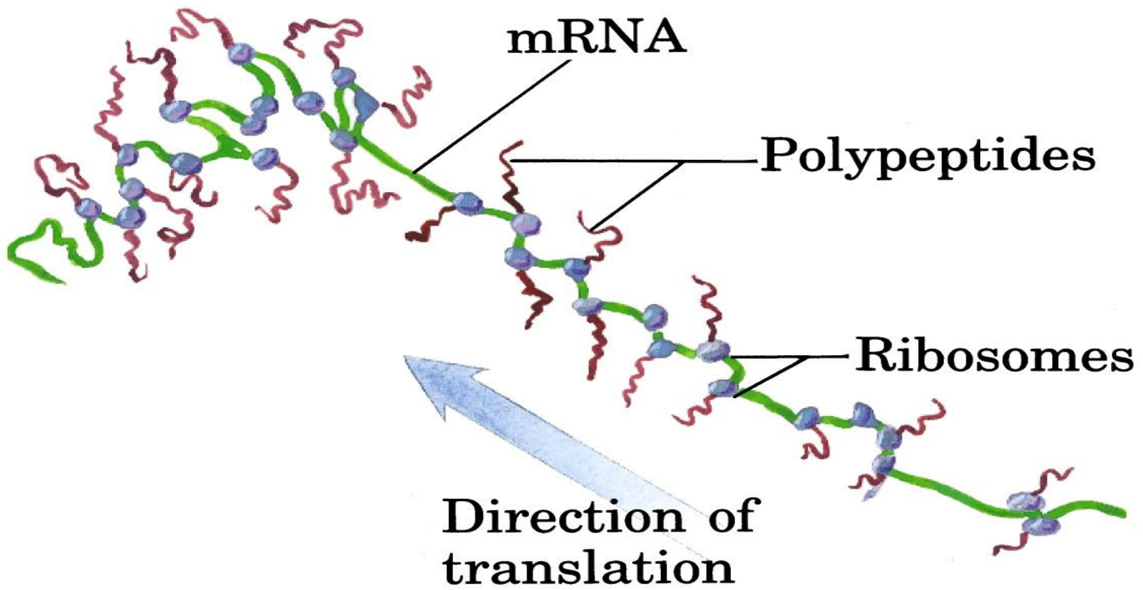
- Several ribosomes cluster of 10-100) can translate a single mRNA simultaneously, forming a polyribosome.
- Polyribosomes enable a cell to make many copies of a polypeptide very quickly.
- In both prokaryotes and Eukaryotes.
- mRNA from 5’→ 3’
  Polypeptide from amino → carboxyl terminus
- Half life of mRNA minutes,
  Translation with high efficiency by polysomes.



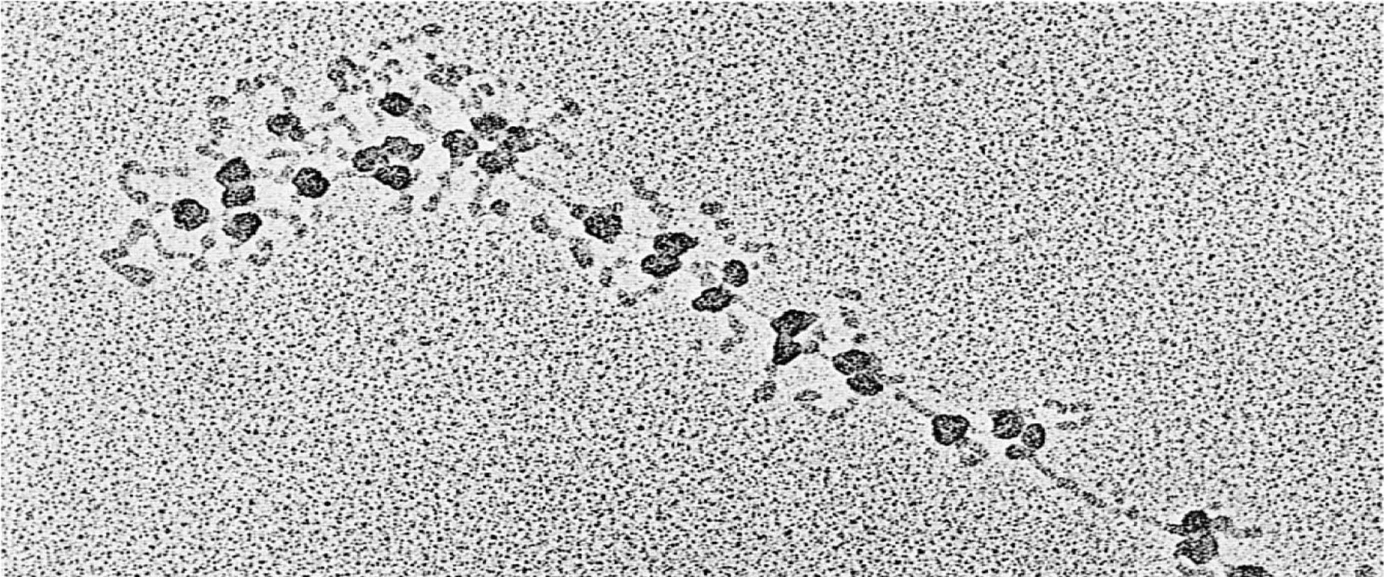
(a)

Polysome:

polypeptide chain gets longer as ribosomes move toward 3`



(b)



Protein Folding:

Often translation is not sufficient to make a functional protein.

During and after synthesis, a polypeptide chain spontaneously coils and folds into its three dimensional shape.

Chaperons has a role in post translational folding.

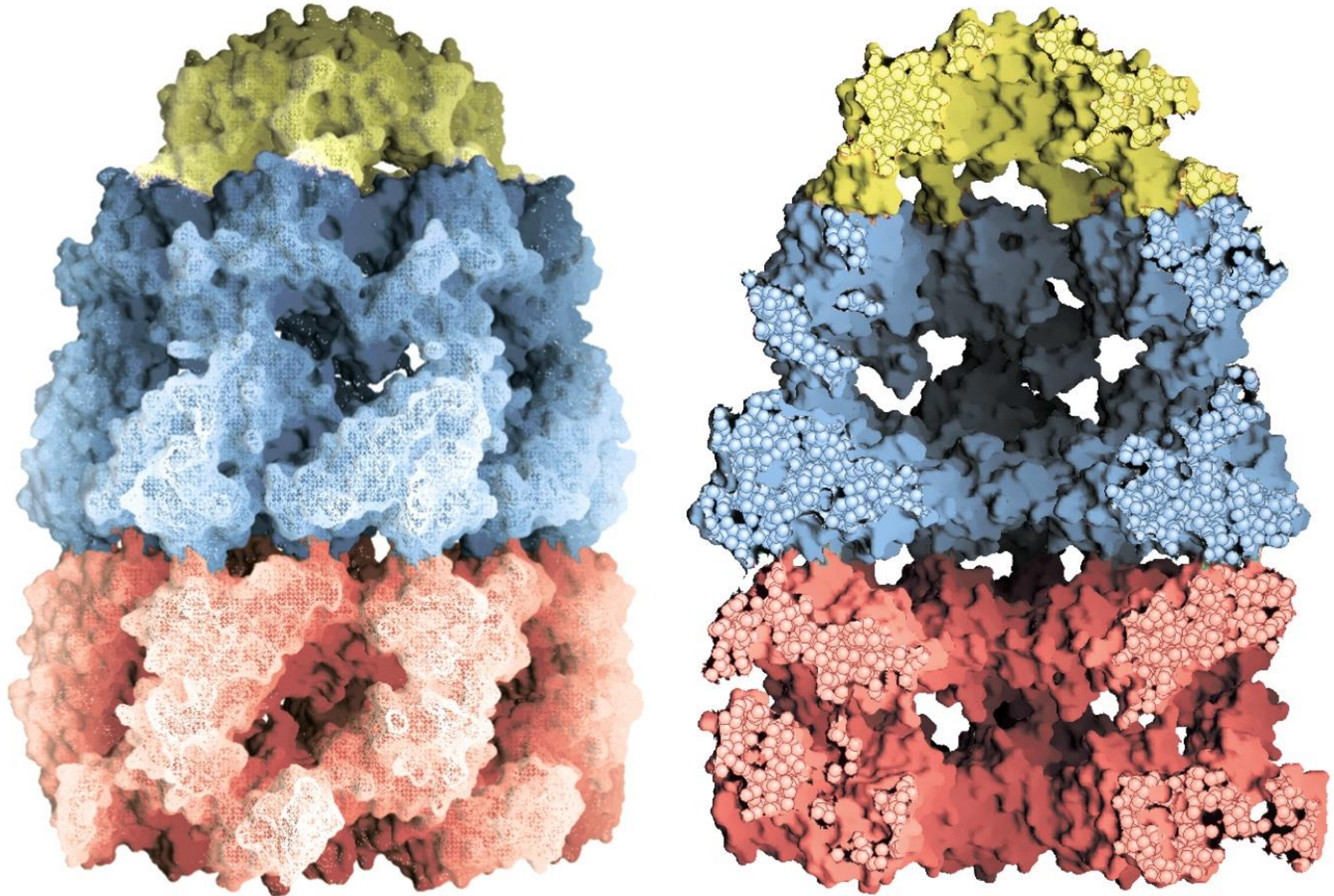
Polypeptide chain assumes its conformation by appropriate interactions:

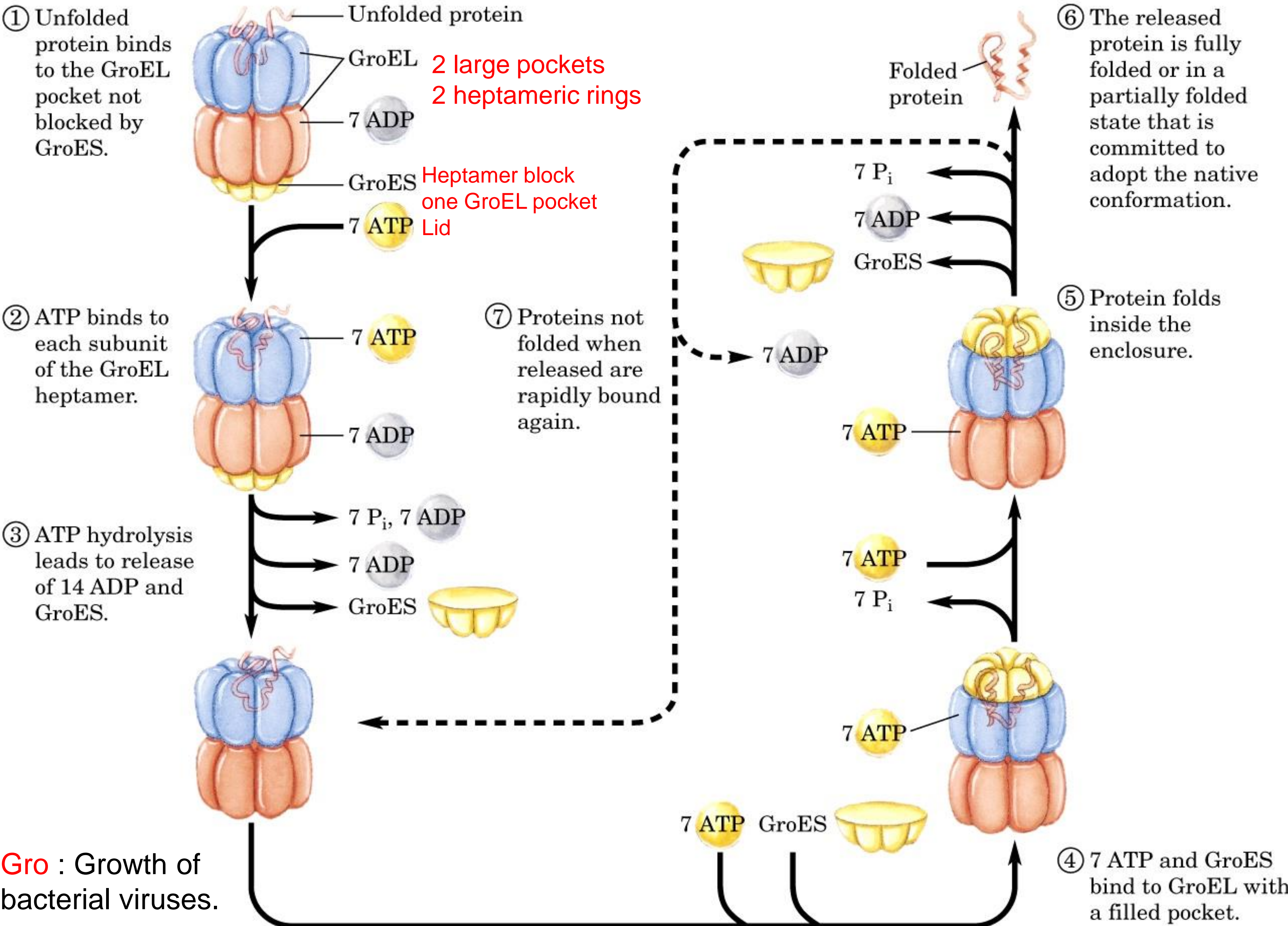
H-bonds, Van der Waals, ionic, hydrophobic interactions.

Linear genetic information in DNA → mRNA→ three dimensional structure of protein.

Chaperonins in protein folding:

Surface and cut away images.





(a)

1) Amino terminal and carboxyl-terminal modifications:

In prokaryotes (N-formyl Methionine).

Eukaryotes (Methionine).

Removed / cleaved enzymatically.

50% eukaryotic proteins amino group of amino terminal end N-acetylated.

Caboxyl-terminal end modified.

2) Loss of signal sequence:

15-30 residue at amino terminal end directs the protein to its destination,

Ultimately removed by peptidases.

3) Modification of individual a.a :

Hydroxyl group Ser, Tyr, Thr.

Phosphorylated by ATP

Phosphate adds –ve charge

Casein many phosphoserine

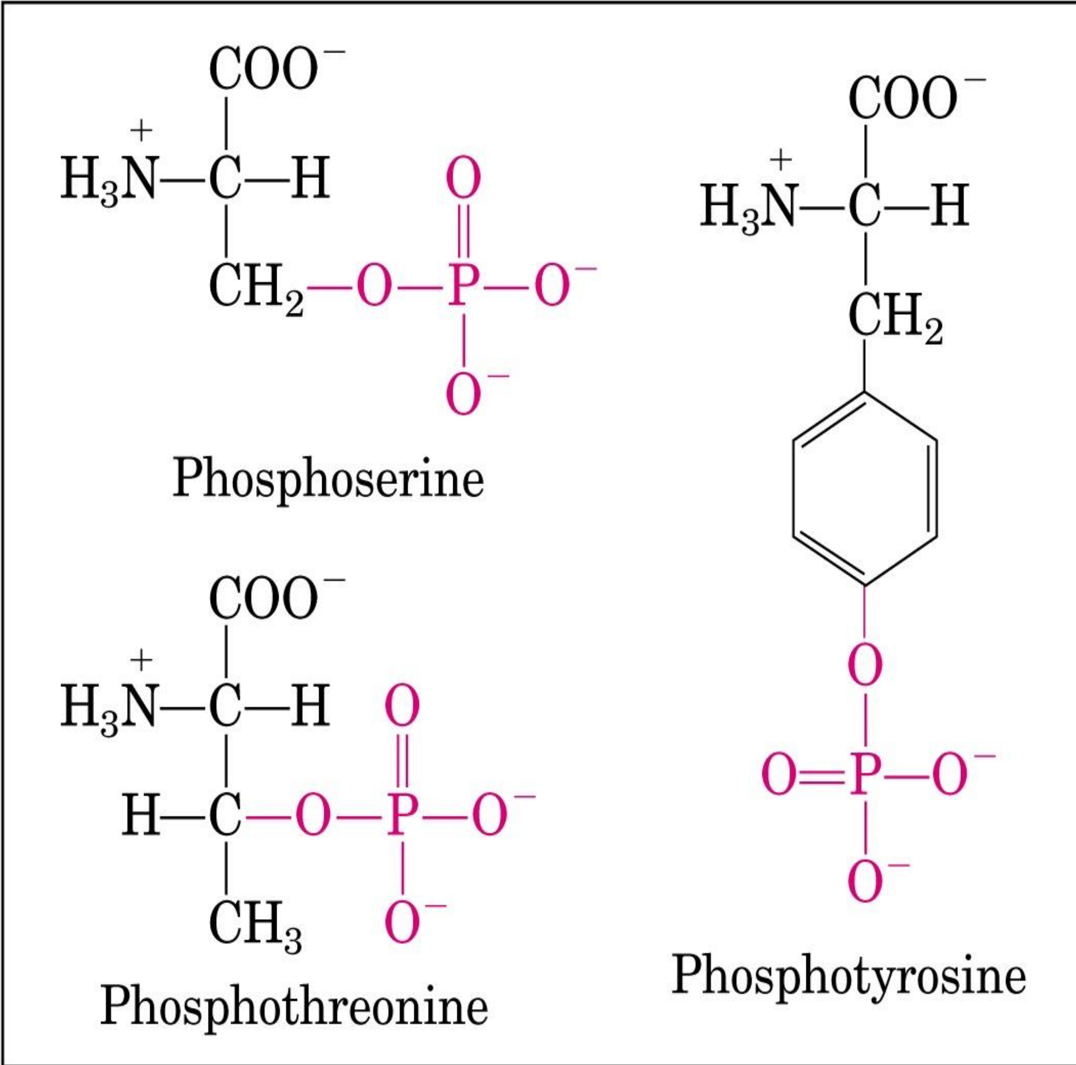
Groups to bind Ca2+

Phosphorylation, dephosphorylation

enzyme regulation

Other modifications:

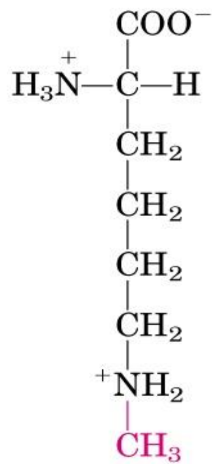
- Acetylation
- Hydroxylation of Pro

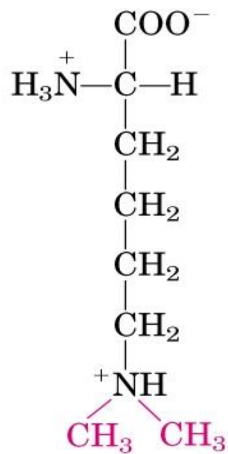


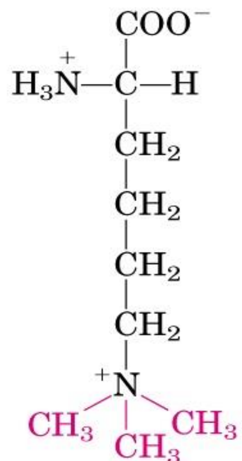
(a)

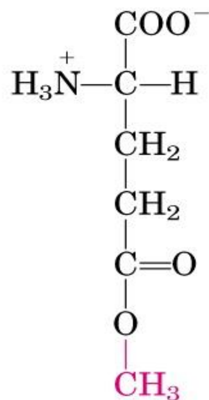
Carboxylation : e.g prothrombin contain several carboxyglutamate for Ca^{2+} binding required for blood clotting.

Methylation: mono/ di / tri , methylation of Glu removes its –ve charge

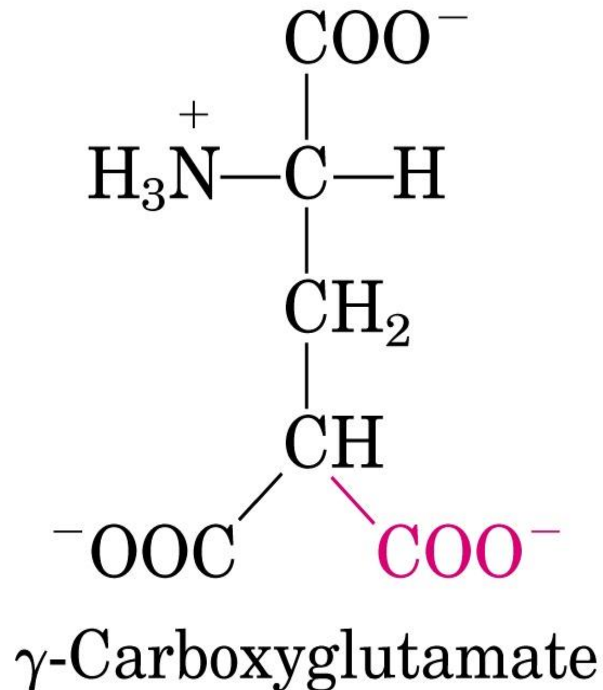








(c)



(b)

5) Addition of prosthetic group:

Many proteins covalently link a prosthetic group.

e.g. biotin for acetyl-CoA carboxylase. Heme in Myoglobin and Hemoglobin.

6) Proteolytic processing: (most common)

Initially synthesized as large inactive precursors → proteotically trimmed to

to smaller active forms

- activation of large inactive hormone e.g. proinsulin
- removal of signal sequence (ER secretion)
- activation of enzymes (zymogen e. g. Trypsinogen)

7) Formation of disulfide cross-links:

Interchain or intrachain disulfide bonds bw Cys residues.

disulfide bonds common in proteins to be exported:

Protect the native conformation of the protein from denaturation in extracellular

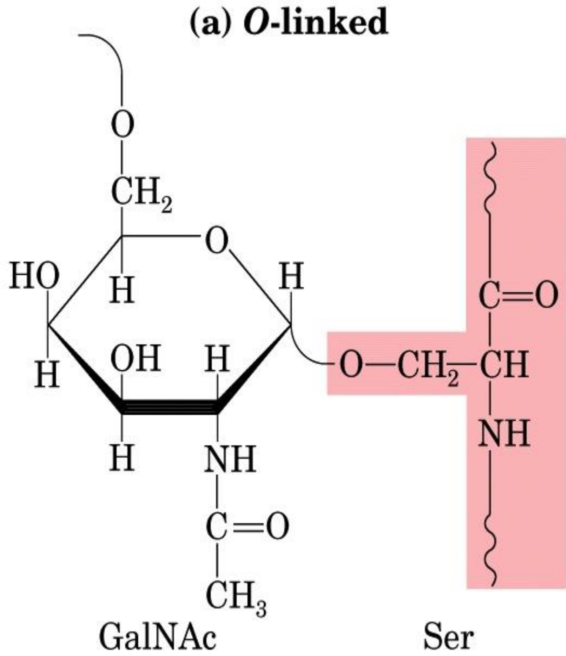
environment which is oxidizing.

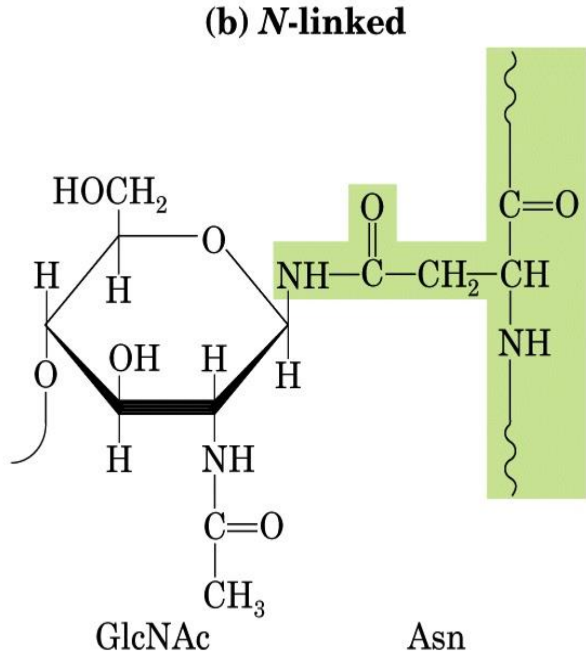
8) Attachment of CHO side chains to proteins = glycoproteins:

Occurs during/ after protein synthesis.

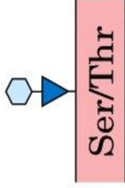
Ser/Thr → O-linked oligosaccharide

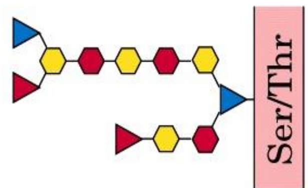
Asn→ N-linked oligosaccharide



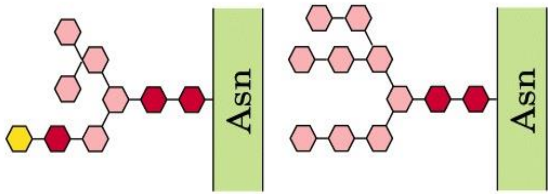


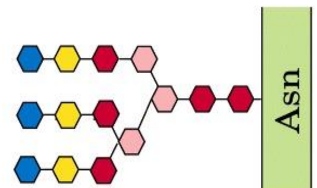
Examples:





Examples:





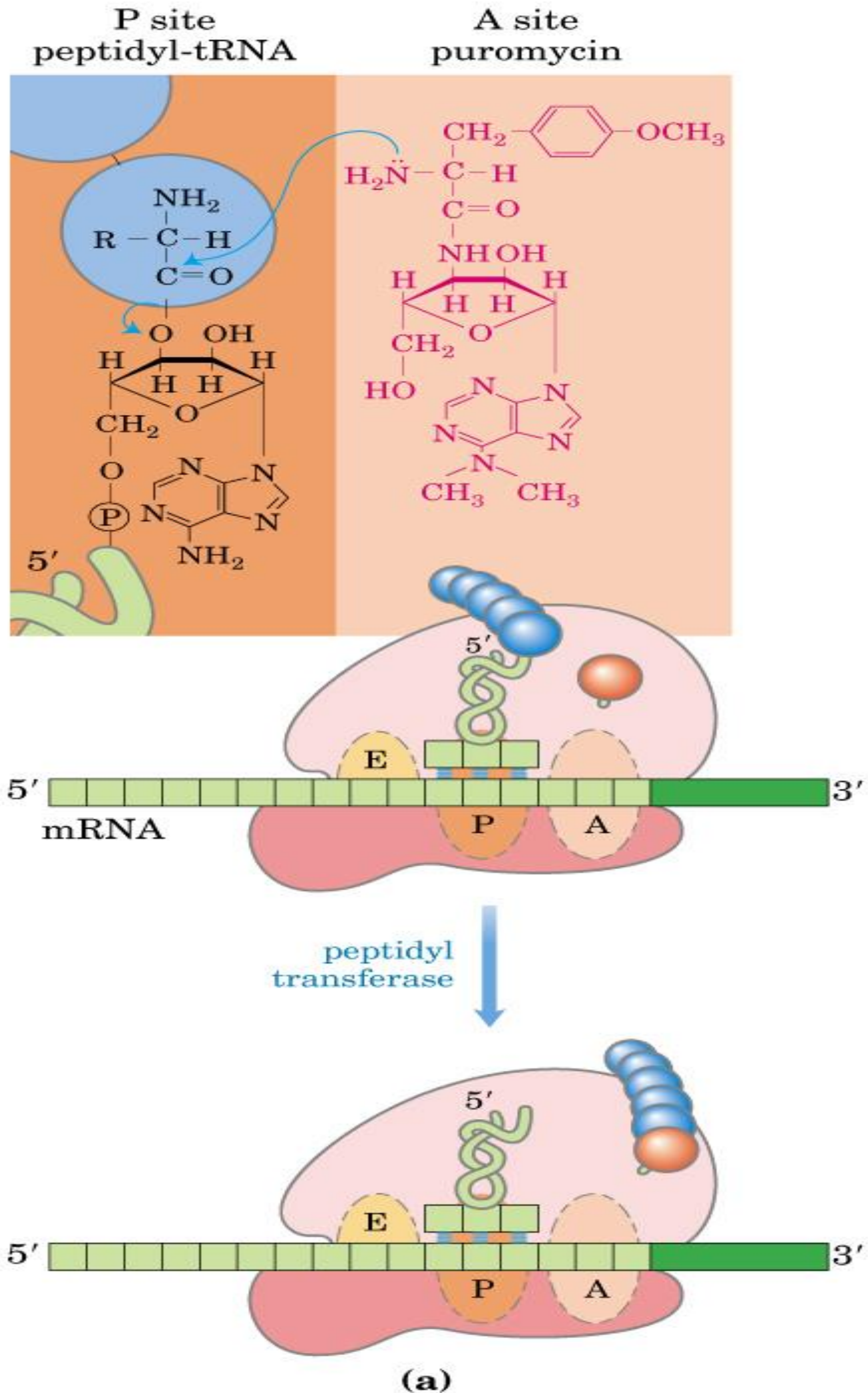
Glycoproteins and Proteoglycans

Glycoproteins	**Proteins conjugated to saccharides lacking a serial repeat unit**	**Protein >> carbohydrate**
Proteoglycans	**Proteins conjugated to polysaccharides with serial repeat units**	**Carbohydrate >> protein**

Protein synthesis a target of antibiotics

Puromycin antibiotic :

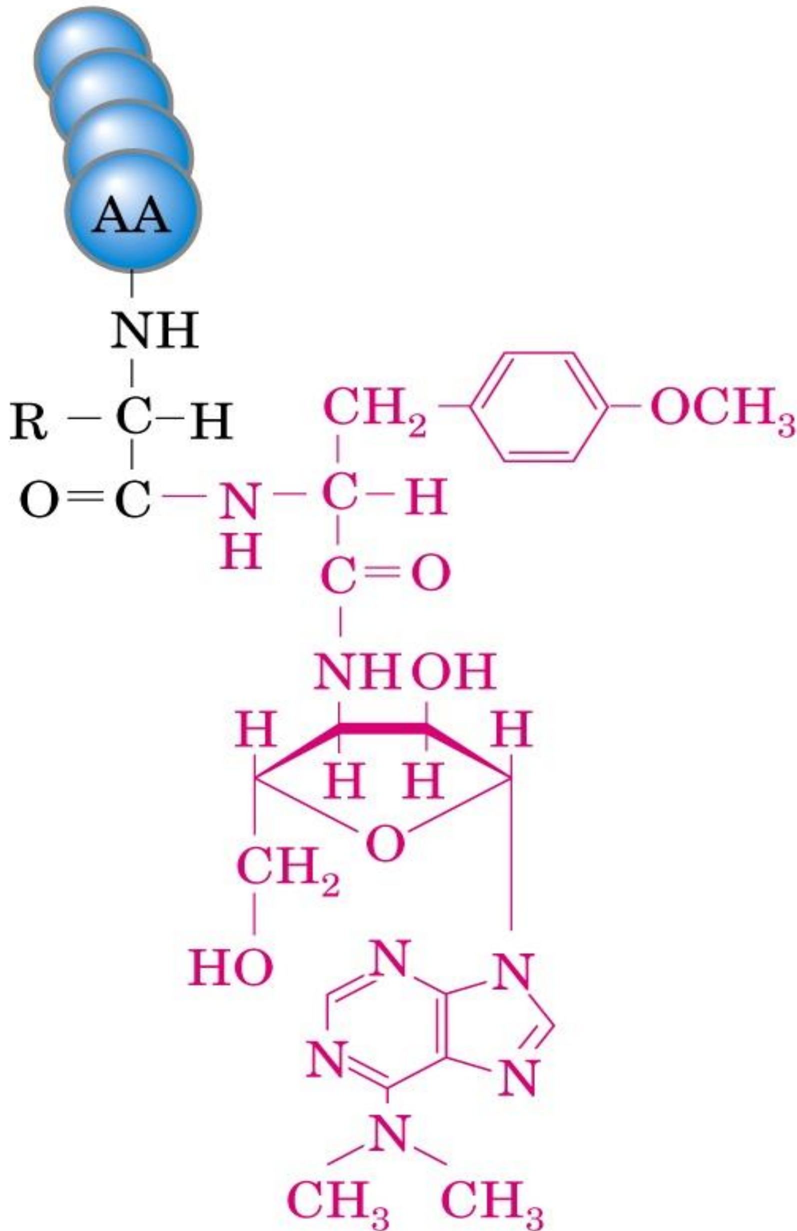
similar structure to 3` end of an amioacyl- tRNA → bind ribosomal A site → form a peptide bond→ peptidyl-puromycin



(a)

Peptidyl-puromycin:

Not engaged in translocation and dissociate from ribosome with a premature polypeptide chain termination.



Protein Targeting:

Signal Sequence :

a short sequence of a.a directs a protein to its location, removed during transport or when destination reached.

Targeting capacity of these signals confirmed by fusing the signal sequence from one protein to a 2nd . Signal of 1st directs the 2nd to the 1st location.

Amino-terminal signal sequence marks proteins for translocation into ER lumen.

Carboxyl-terminus defined by a cleavage site.

Protease removes the signal after protein imported into ER lumen.

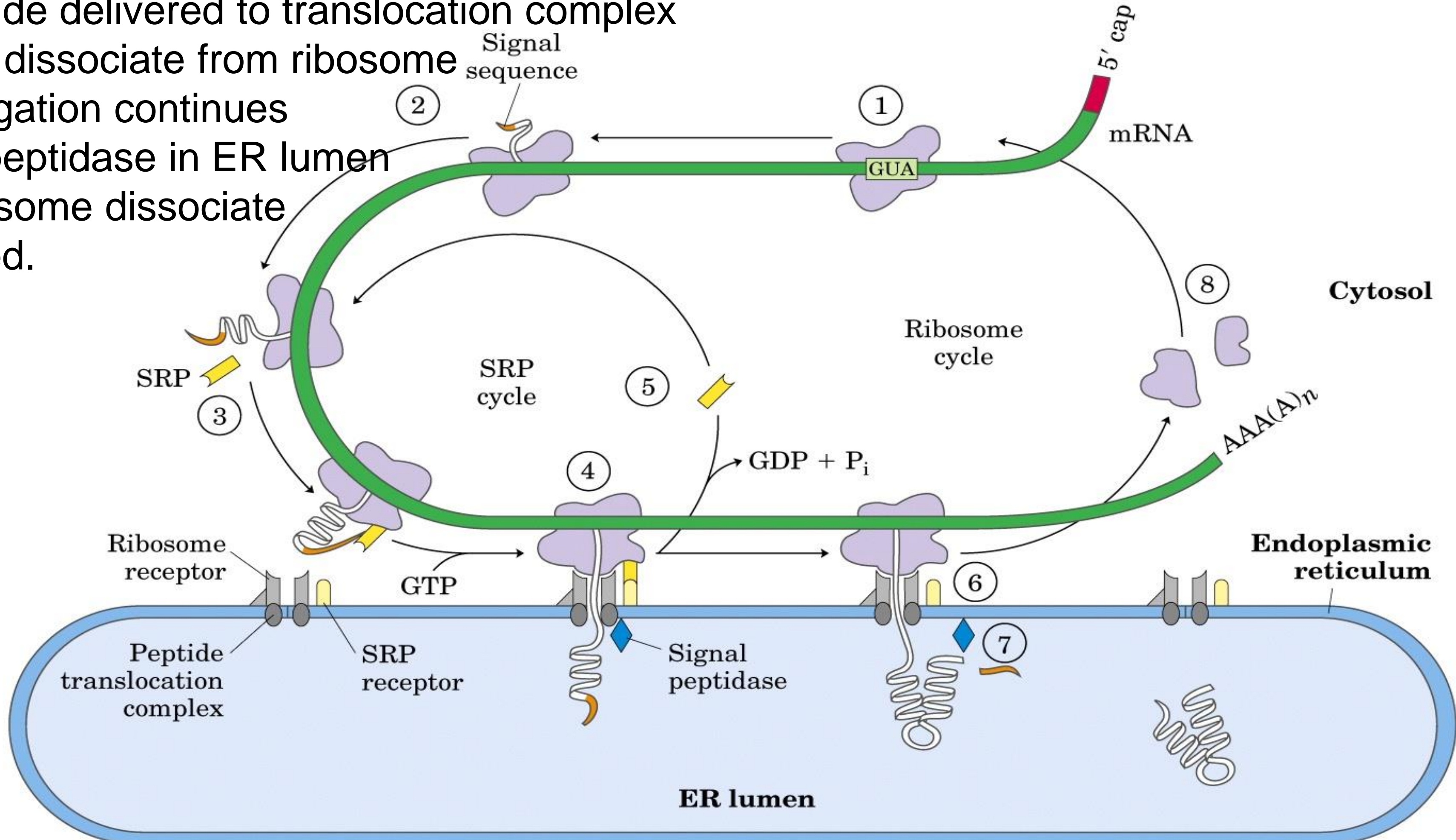
Signal sequence 13-36 a.a

1) 10-15 Hydrophobic a.a core.
2) One/ more +ve charged basic a.a near N-terminus.
3) Short sequence at carboxyl terminus near cleavage site with a.a (short R e.g. Ala).

	cleavage site
Human influenza virus A	Met Lys Ala Lys Leu Leu Val Leu Leu Tyr Ala Phe Val Ala Gly ↓ Asp Gln --
Human preproinsulin	Met Ala Leu Trp Met Arg Leu Leu Pro Leu Leu Ala Leu Leu Ala Leu Trp Gly Pro Asp Pro Ala Ala Ala ↓ Phe Val --
Bovine growth hormone	Met Met Ala Ala Gly Pro Arg Thr Ser Leu Leu Leu Ala Phe Ala Leu Leu Cys Leu Pro Trp Thr Gln Val Val Gly ↓ Ala Phe --
Bee promellitin	Met Lys Phe Leu Val Asn Val Ala Leu Val Phe Met Val Val Tyr Ile Ser Tyr Ile Tyr Ala ↓ Ala Pro --
Drosophila glue protein	Met Lys Leu Leu Val Val Ala Val Ile Ala Cys Met Leu Ile Gly Phe Ala Asp Pro Ala Ser Gly ↓ Cys Lys --

Proteins with such signals synthesized on ribosomes attached to ER or signal direct ribosome to the ER.

1) Protein synthesis initiation on free ribosome
2) Signal sequence appears early at its amino terminus
3) Attached to SRP +bound to GTP when 70a.a & signal sequence completely emerge from ribosome →stops elongation
4) GTP bound SRP directs ribosome + mRNA to receptor to ER cytosolic face
5) Peptide delivered to translocation complex
6) SRP dissociate from ribosome
7) Elongation continues signal peptidase in ER lumen
8) Ribosome dissociate recycled.

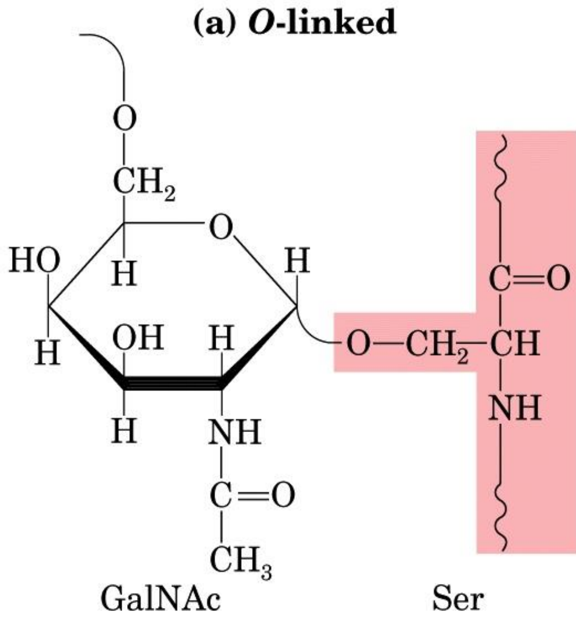


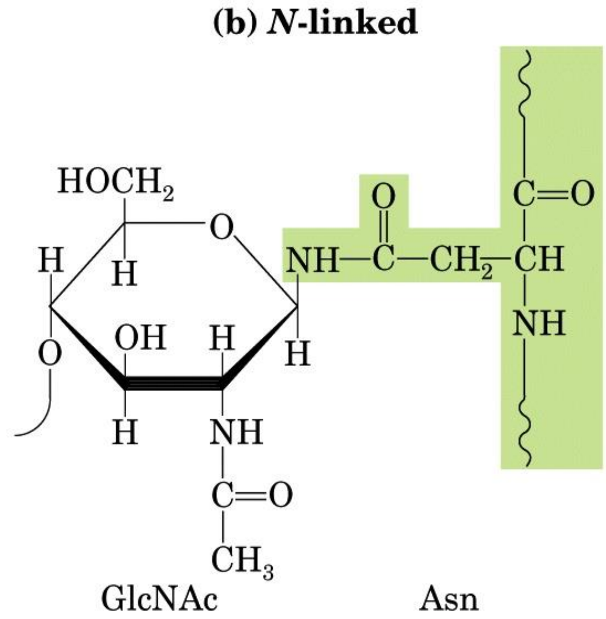
ER lumen disulfide bonds formed.

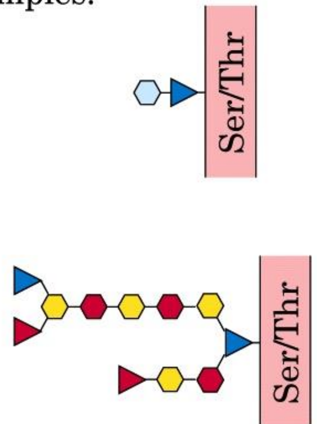
Proteins glycosylated to form glycoproteins.

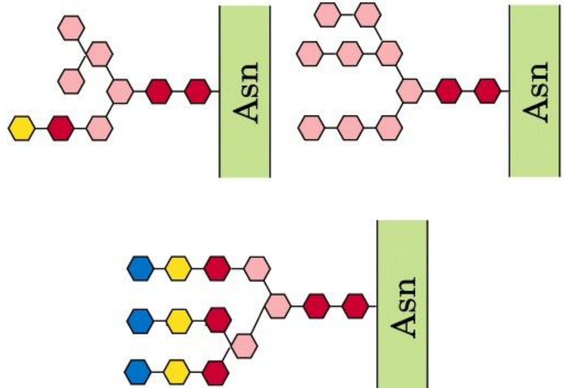
Glycosylation may be Asn → N-linked oligosaccharides.

- If glycosylation on Ser/Thr→O linked occurs in Golgi complex /cytosol.





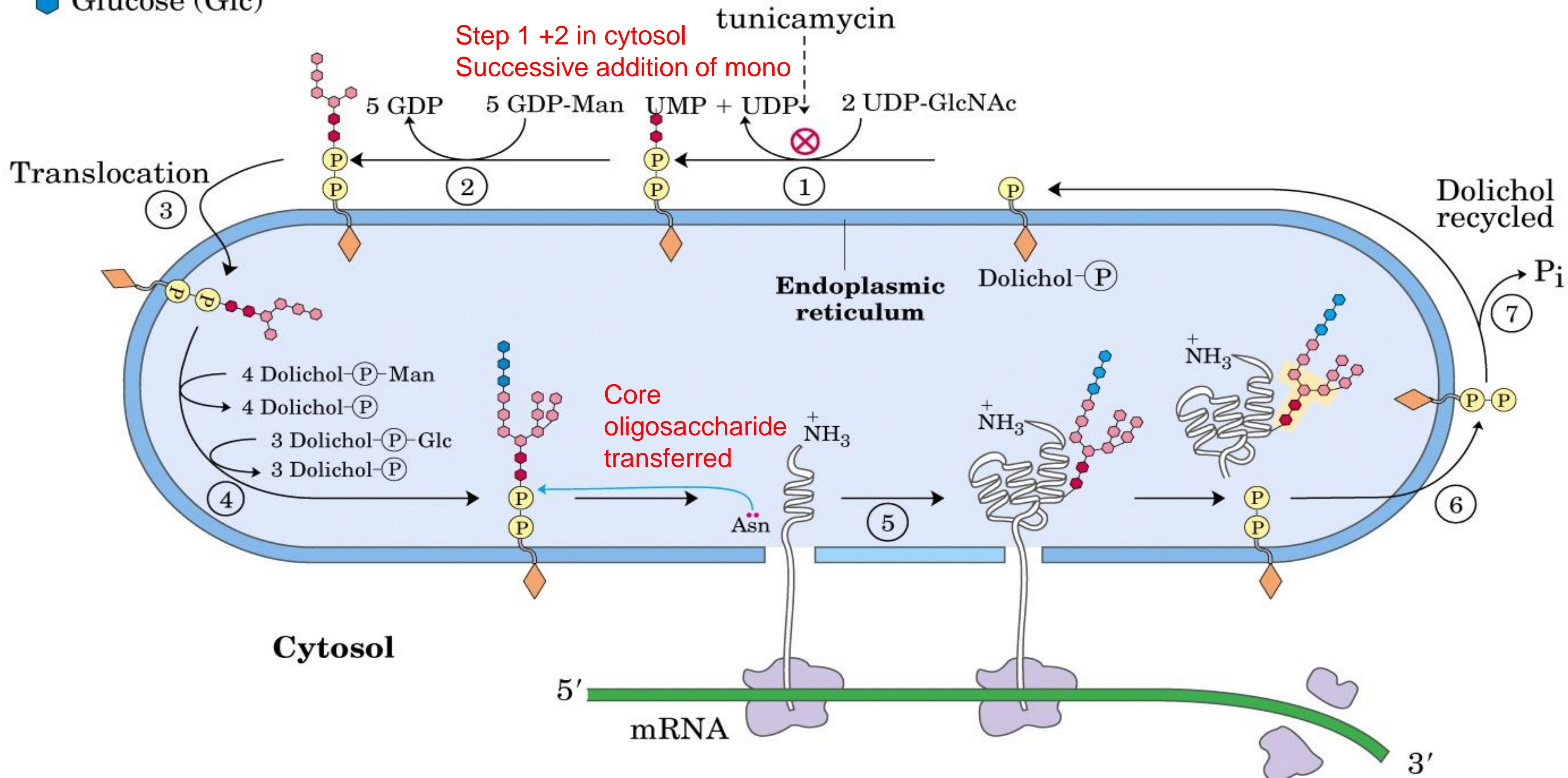




Glycosylation:

A core oligosaccharide of ~12 residue transferred from dolichol phosphate donor to Asn.

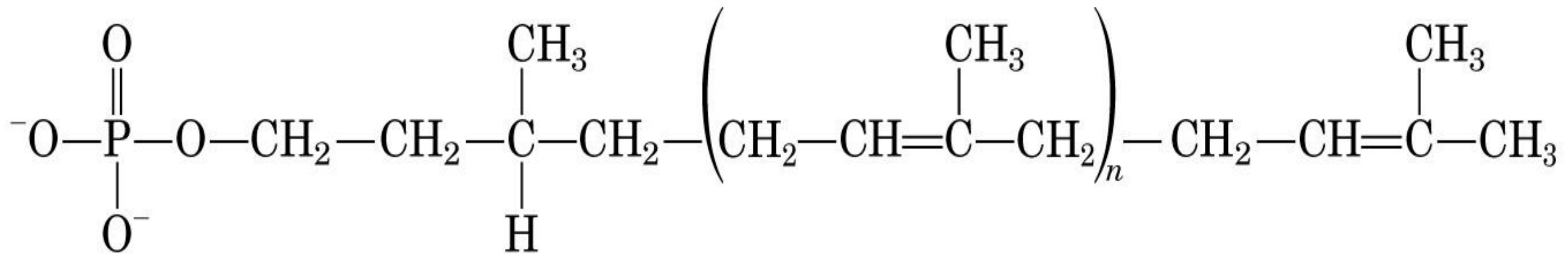
Transferase is on the lumenal face of ER→ cant catalyze glycosylation of cytosolic proteins. (oligosaccharide core modified/ trimmed except for the 5).



Dolichol phosphate derivates =

donors of Glc units in ER lumen.

An intermediate in the glycosylation of proteins & lipids.

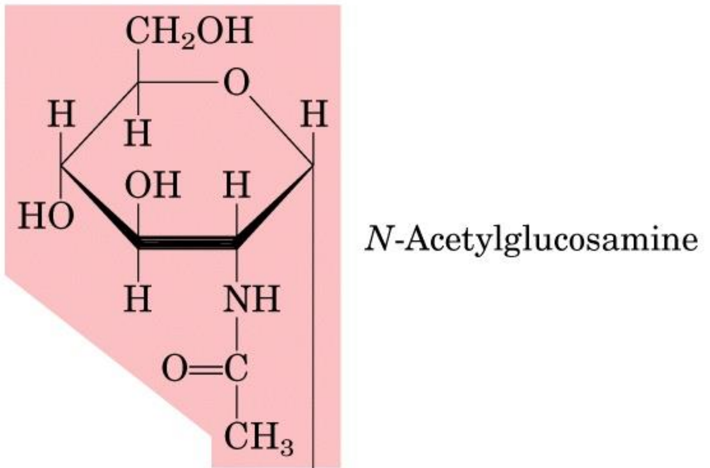


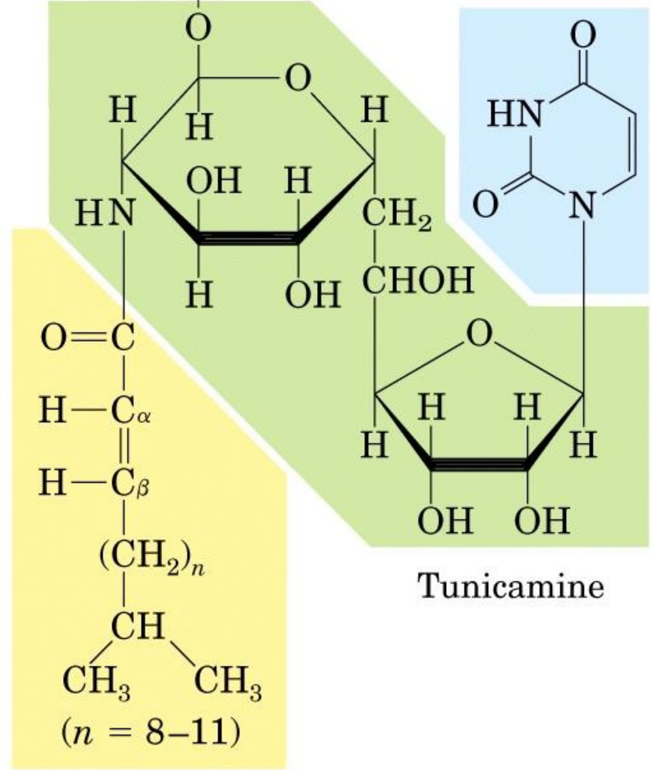
Dolichol phosphate
(n = 9–22)

Antibiotics can interfere with glycosylation step:

Tunicamycin resembles (UDP-GlcNAc)

Blocks 1st step



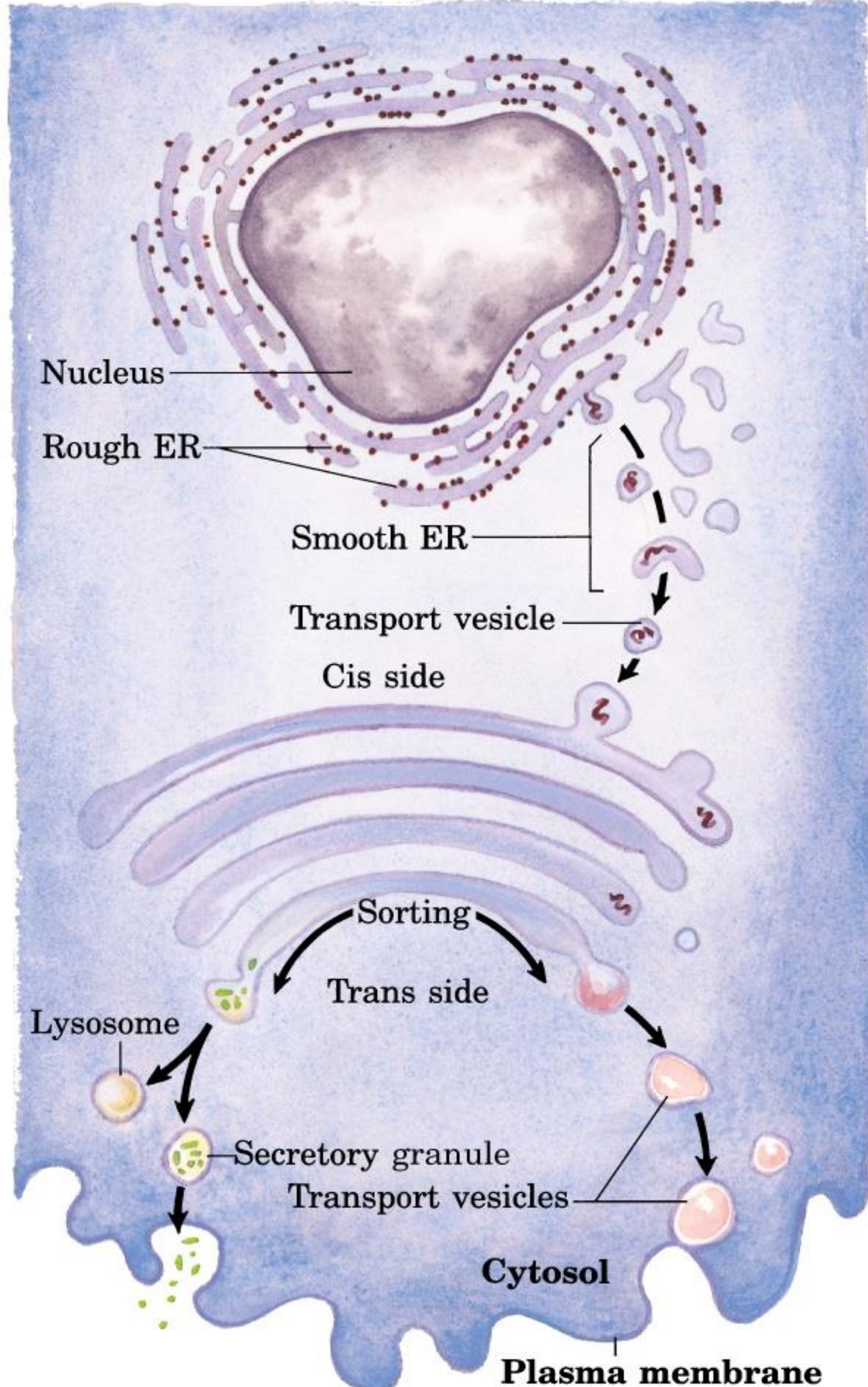


Proteins travel from ER → Golgi complex

- In Golgi oligosaccharides O-linked to some proteins. N-linked (from ER) further modified.

- In Golgi sorting of proteins transport vesicles, endocytosis / lysosomes / plasma mb.

- Relying not on signal sequence (removed) but on <u>structural features.</u>



Sorting in Golgi complex: ER →Golgi →lysosomes.

In Golgi phosphotransferase recognize hydrolase's 3 dimensional structure

phosphorylates certain Man residue in oligosaccharide.

Structural signal = one/more Man

N-linked oligo

target protein to lysosome

A receptor in Golgi mb recognizes

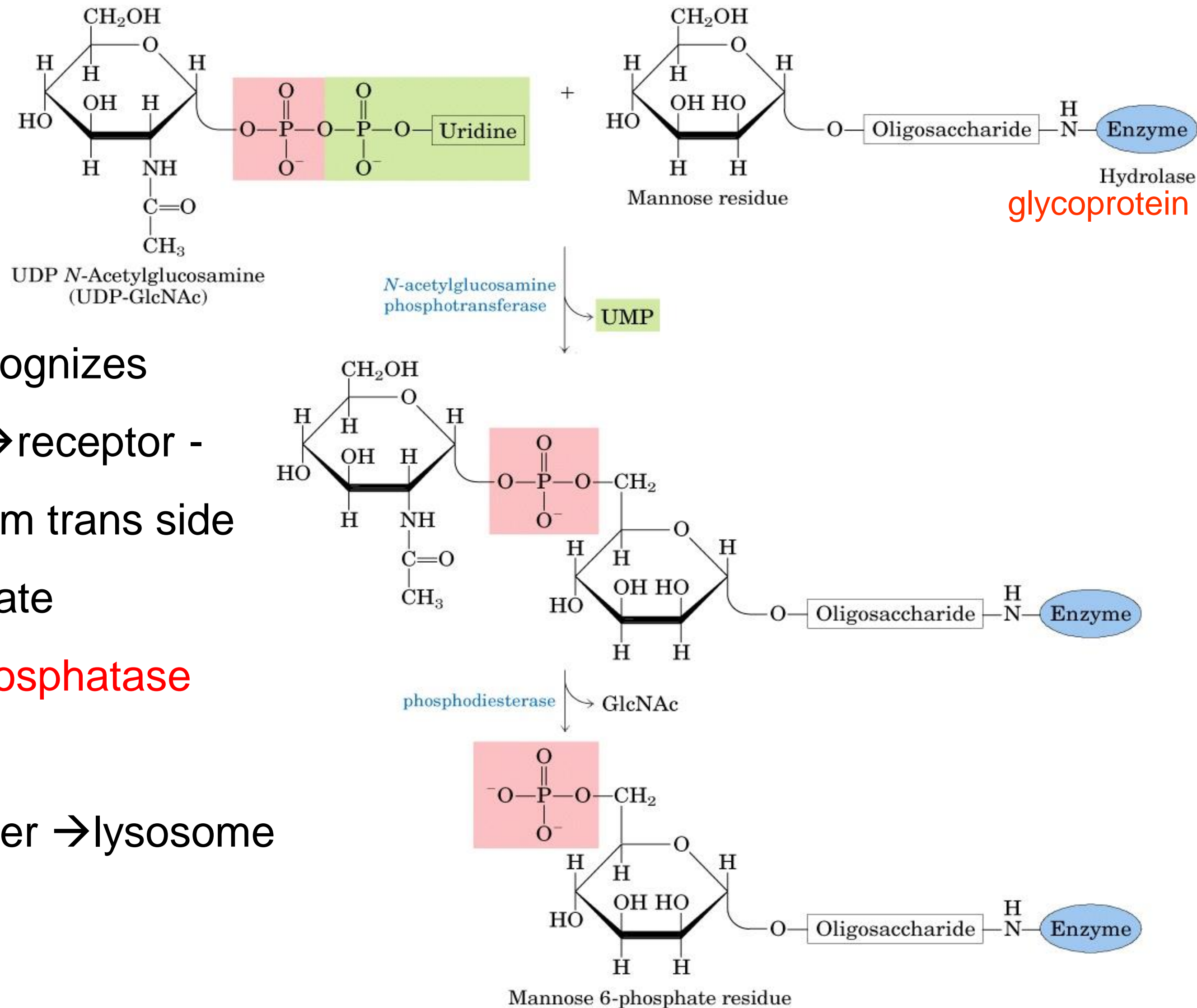
Man-6-p binds hydrolase→receptor -

hydrolase vesicles bud from trans side

In vesicle receptor dissociate

- recycled-by low pH & phosphatase

-Tunicamycin inhibit transfer →lysosome instead secreted.



Nuclear importation (signal not cleaved):

-Ribosomal proteins synthesized in cytosolic ribosomes imported→nucleus →assembled to 40s and 60S in nucleolus.

Completed subunits→exported to cytosol.

-Nuclear proteins (pol, topo, histones) synthesized in cytosol →nucleous

-In cell division nuclear mb cleaves & reestablished.

To allow repeated nuclear importation → Nuclear Localization Signal NLS not cleaved + localized anywhere in protein

- Many 4-8 a.a with several basic (Arg, Lys)

Nuclear Importation:

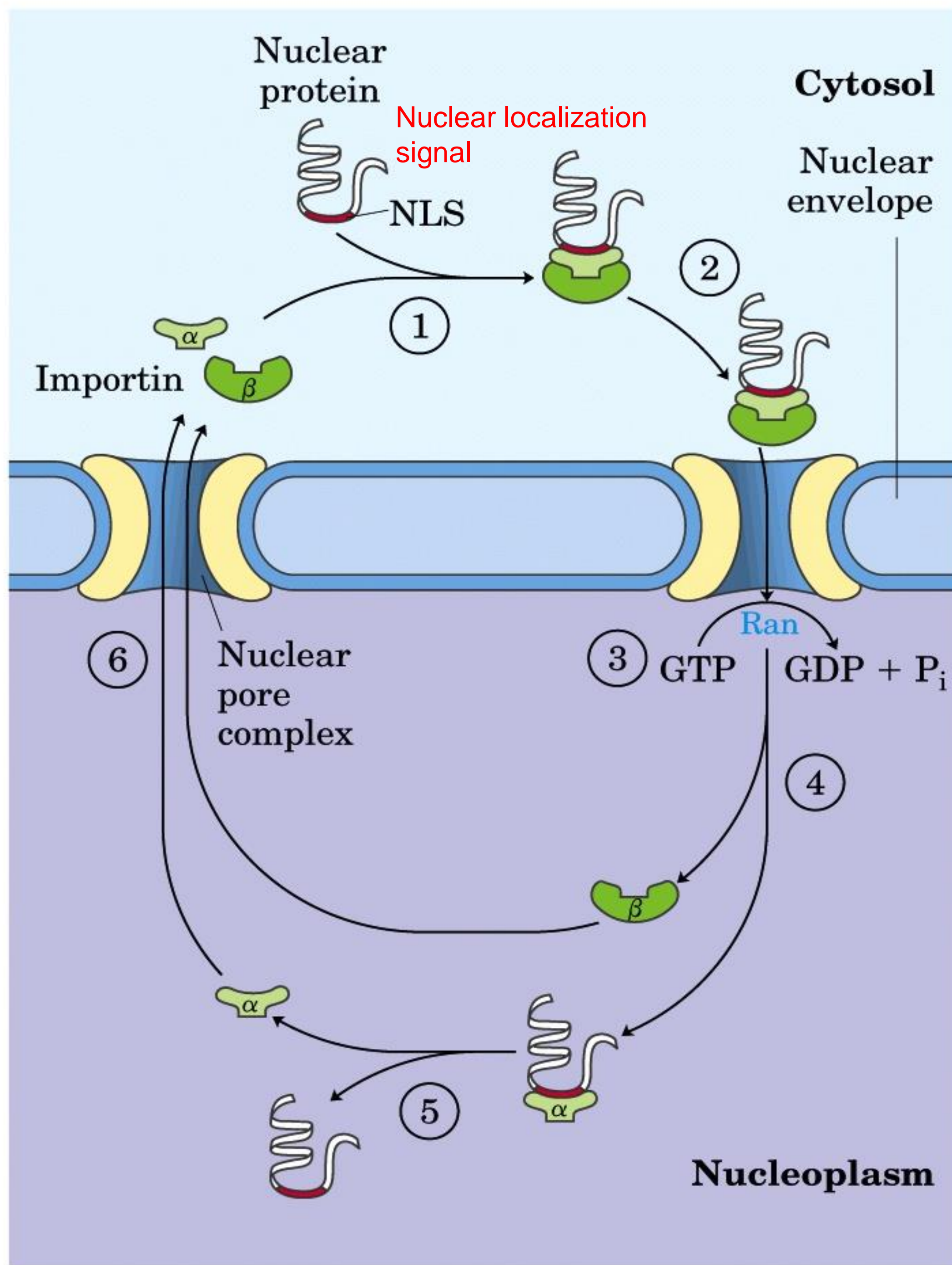
Mediated by Proteins cycle bw cytosol & nucleous.
Importin (α , β) GTPase (Ran).

1) Importin heterodimer of α & β = soluble receptor for protein targeted to nucleous.

2) α binds NLS bearing protein in cytosol .

3) Complex docks at nuclear pore translocated through pore by Ran GTPase.

4) α & β subunits dissociate & exported to cytosol.



Protein degradation:

1) Allows recycling of a.a

2) Prevent the buildup of ubnormal /defective/ unwanted protein

Half lives of proteins 30 sec - several days.

Hemoglobin last for 110 days = lifetime of RBC

Two systems for protein degradation:

1) In eukaryotes, prokaryotes: ATP dependent cytosolic systems
2) In eukaryotes, mainly vertebrates: lysosomes (endocytosis)

E. coli ATP-dependent protease called Lon “Long form” of protein.

protease activated only when protein is degraded /defective/ turn over time

2ATP hydrolyzed / one peptide bond cleaved.

Eukaryotes ATP-dependent ubiquitin system.

Signals that trigger ubiquitination:

One simple signal:

N-end rule :

signal that trigger ubiquitination & affect half life:

1st residue after removal of N-terminal Met & any posttranslational proteolytic cleavage.

table 27–10

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 a single protein modified so that in each experiment it had a different amino-terminal residue. (See Chapter 29 for a discussion of techniques used to engineer proteins with altered amino acid sequences.) Half-lives may vary for different proteins and in different organisms, but this general pattern appears to hold for all organisms: amino acids listed here as stabilizing when present at the amino terminus have a stabilizing effect on proteins in all cells.

UBIQUITIN

- 76 a.a. polypeptide
- Highly conserved in evolution
- 3 a.a. differences between yeast & human homologues

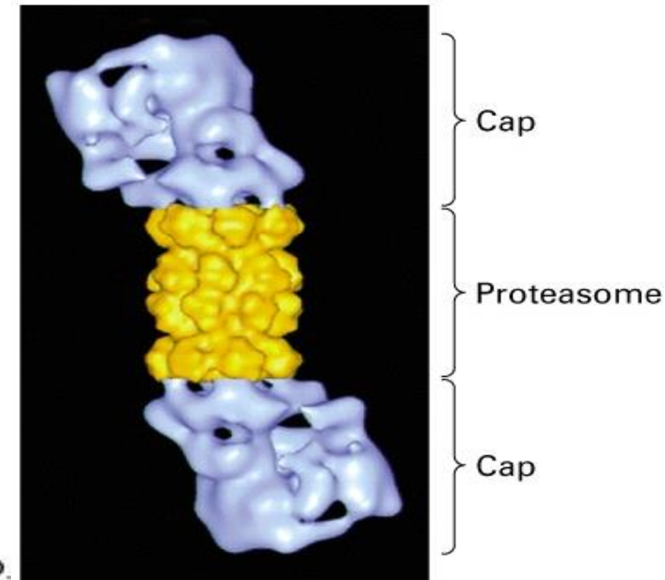
Protein degradation by the ubiquitin- pathway

Ubiquitin common throughout the animal kingdom

76 aa (8.5 kDa) i.e. small

- E1 (ubiquitin activating enzyme)
- E2 (ubiquitin carrier protein)
- E3 (ubiquitin protein ligase) selects proteins

to tag by N-term



NH_2
$AMP + PP_i$
Ubiquitinylating enzyme complex + Ub
Cytosolic target protein
$NH - C(=O) - Ub$
ATP
$AMP + PP_i$
Ub
(n times)
$Ub - Ub - Ub_n$
Proteasome
Ub
Ub
Ub
Peptides