Synthesis of proteins. Posttranslational modifications of proteins.

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Translation – synthesis of proteins

Which cells: all cells having nuclear DNA

Where in the cell: ribosoms (free or attached to ER)
mitochondrias

Differences between prokaryotes and eukaryotes:

prokaryotes: transcription, processing of transcript and translation are not separated by space

eukaryotes: translation starts after the mRNA synthetized in nucleus is transported to cytoplasma

Molecules and other species necessary for synthesis of proteins

Species	Funkce
Amino acids	Substrates for proteosynthesis
Many enzymes	Catalysts
Protein factors	efectors
ATP, GTP	energy
Inorganické ions (Mg^{2+}, K^{+})	Cofactors of enzymes
tRNA	Transfer of AA to ribose
mRNA	Determines the order of AA in a protein
rRNA	Structural role, catalyzes the formation of peptide bond

Phases of translation

A. Inciation

B. Elongation

C. Termination

They occur in cytoplasma, in connection with ribosomes

Genetic code

Proteins – 20 different AA

RNA – 4 bases

Each amino acid is characterized by a triplet of bases in mRNA – **codon**

Codons consisting of three nucleotide can provide 64 variations → 61 of them code for 20 amino acids

3 codons are STOP codons (UAA,UAG, UGA)

Nierenberg (1961) – poly(U) sequence of mRNA produced polyphenylalanine by translation ⇒ sequence UUU is the codon for phenylalanine

Genetic codon

First base	Second base				Third base
5′	U	C	A	G	3′
	Phe	Ser	Tyr	Cys	U
	Phe	Ser	Tyr	Cys	C
U	Leu	Ser	Stop	Stop	A
	Leu	Ser	Stop	Trp	G
	Leu	Pro	His	Arg	U
	Leu	Pro	His	Arg	C
C	Leu	Pro	Gln	Arg	A
	Leu	Pro	Gln	Arg	G
	Ile	Thr	Asn	Ser	U
A	Ile	Thr	Asn	Ser	C
	Ile	Thr	Lys	Arg	A
	Met	Thr	Lys	Arg	G
G	Val	Ala	Asp	Gly	U
	Val	Ala	Asp	Gly	C
	Val	Ala	Glu	Gly	A
	Val	Ala	Glu	Gly	G

Prominent properties of genetic codon:

- it is degenerate (more than one codon can code for an amino acid)
- it is unambiguous (each codon specifies one and only one amino acid)
- it is almost universal (the code is identical in prokaryotes and eukaryotes with some exceptions : e.g. human mitochondrial mRNA the triplet UGA codes for trp instead of stop-codonu, AUA codes for methionine instead leucinu)
- it can wobble (base pairing between the last nucleotide of the triplet and corresponding nucleotide is not strictly given by Watson-Crick rule)
- it is nonoverlapping and „comaless“ (the codons are aligned without overlap and without empty spaces. Each base belongs to one codon and only one codon)

Degeneracy of the genetic code

GCA GCC GCG GCU	AGA AGG CGA CGC CGG CGU	GAC GAU	AAC AAU	UGC UGU	GAA GAG	CAA CAG	GGA GGC GGG GGU	CAC CAU	AUA AUC AUU	UUA UUG CUA CUC CUG CUU	AAA AAG	AUG	UUC UUU	CCA CCC CCG CCU	AGC AGU UCA UCC UCG UCU	ACA ACC ACG ACU	UGG	UAC UAU	GUA GUC GUG GUU	UAA UAG UGA
Ala	Arg	Asp	Asn	Cys	Glu	Gln	Gly	His	Ile	Leu	Lys	Met	Phe	Pro	Ser	Thr	Trp	Tyr	Val	stop

Relation between mRNA and the protein product

- Sequence of bases in mRNA is sorted into codons
- The start codon (AUG) sets the reading frame
- The order of codons in the mRNA determines the linear sequence of amino acids in the protein – the reading is given by reading frame

Only one of the three possible frames is the right one,
it begins at the start codon AUG recognized by the Met-tRNA^{Met}

Efect of mutations

Mutations are structural alterations that result from damage of DNA molecules or unrepaired errors during replication

They can be transcribed into mRNA

By translation of altered base an abnomal sequence of amino acids can appear in the protein

Types of mutation

1. point mutation → exchange of single base

a) No detectable effect – because of degeneration of the code – **silent mutation**

e.g. CGA→CGG (both sequences code for Arg)

b) Missence effect – different amino acid is incorporated at the corresponding site in the protein molecule

e.g. GCA→CCA results in replacement of arg by prolin

c) nonsense – they result in premature termination

e.g. CGA →UGA, the codon for Arg is replaced by stop-codon

Types of mutation (cont.)

2. insertion – one or more nucleotides are added to DNA

3. deletion – one or more nucleotides are removed from DNA

The damage of the protein depends on the number of deleted or inserted nucleotides

If three nucleotides (or more triplets) are inserted/deleted without a change of the reading frame, polypeptides with inserted/deleted amino acyl residues will be synthesized.

If one or two nucleotides are inserted/deleted, the result is a "frame-shift mutation,, that gives nonsense codons, distinct primary structure of proteins, etc.

Example of point mutation

Point mutations in the genes for hemoglobin:

There is known about 800 of structural variants of human hemoglobin

Most of them result from point mutations and are not harmfull.

Some of them cause diseases.

Methemoglobinemia – e.g. replacement of single histidine by tyrosine in α-chain ⇒ protein is unattackable for the treatment of methemoglobin reductase, the methemoglobin in blood rises

Sickle cell anemia – missense mutation, GTG replaces GAG ⇒ replacement of glutamate by valine in position 6 of β-chain ⇒ the chain become less soluble and precipitate when deoxygenated

Example of nonsens mutation

β^0-thalasemia

It is caused by mutation on codon 17 in the both allels. The synthesis of the β-chian is early terminated.

Significance of DNA molecules

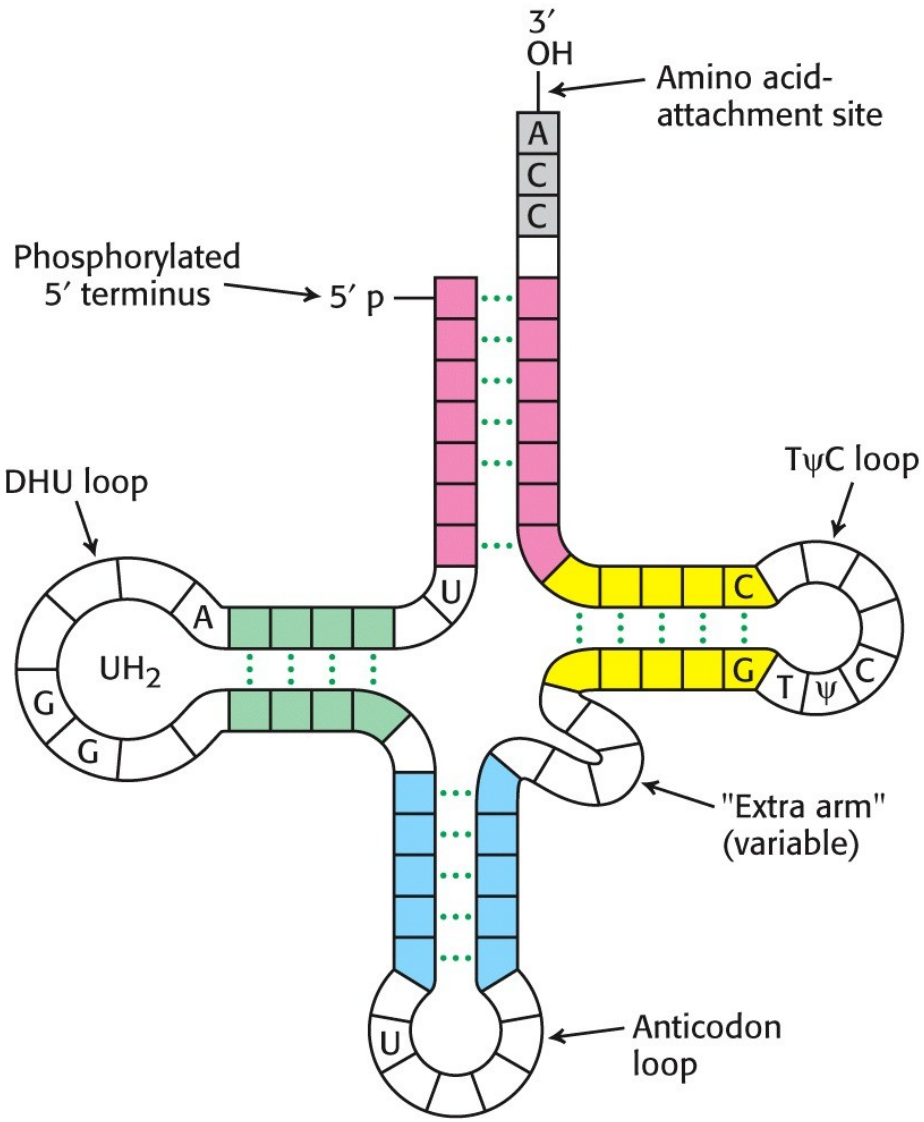
The genetic code defines the relationship between the base sequence of the messenger RNA and the amino acid sequence of the polypeptide:

Amino cids cannot react directly with bases

„the adapter molecules are tRNAs“

- each molecule of tRNA contains anticodon
- anticodon is a triplet of bases that are complementar with the codon in mRNA
- each tRNA can bind specific AA on its 3´-terminal

General structure of tRNA molecules

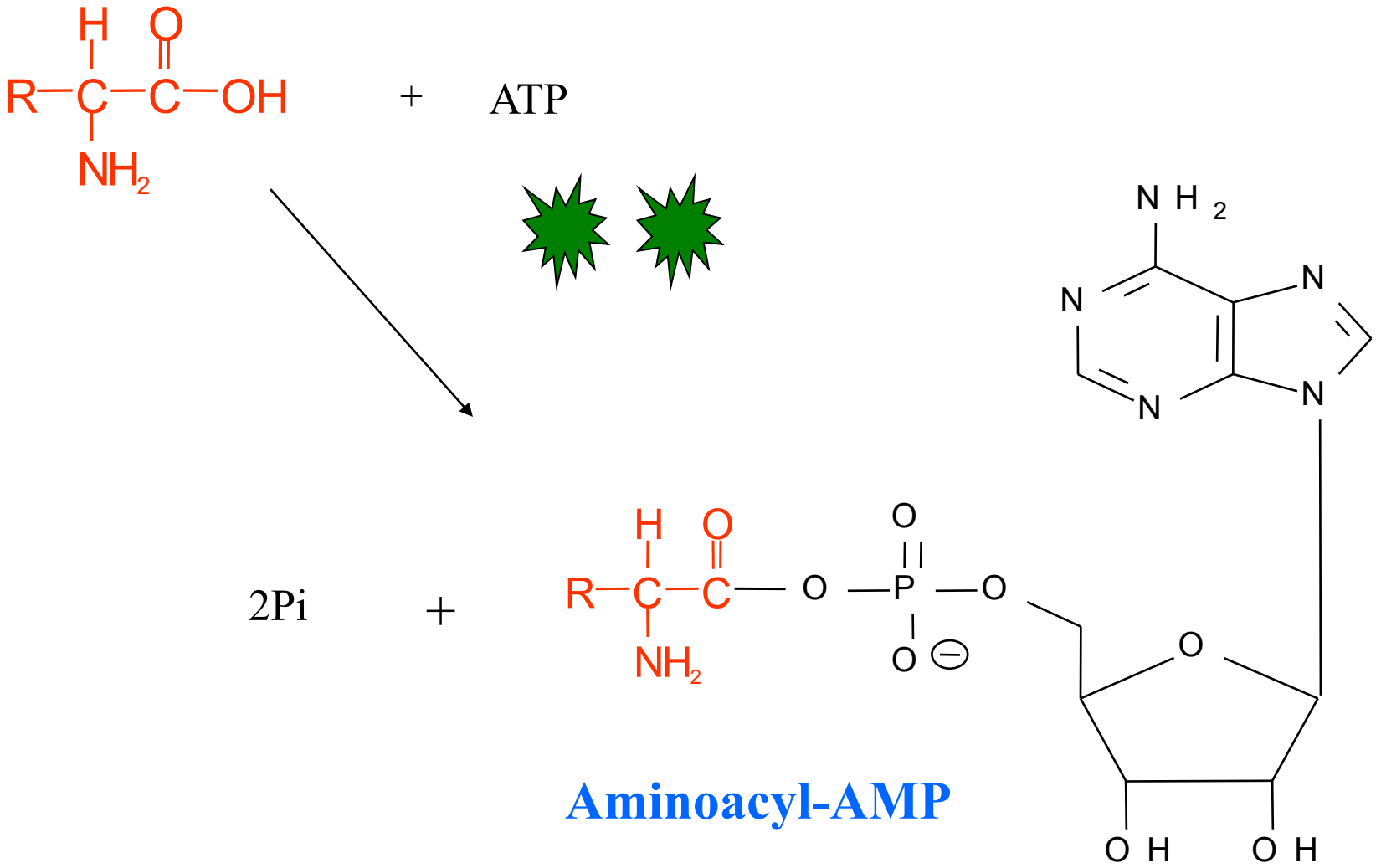


The cloverleaf structure

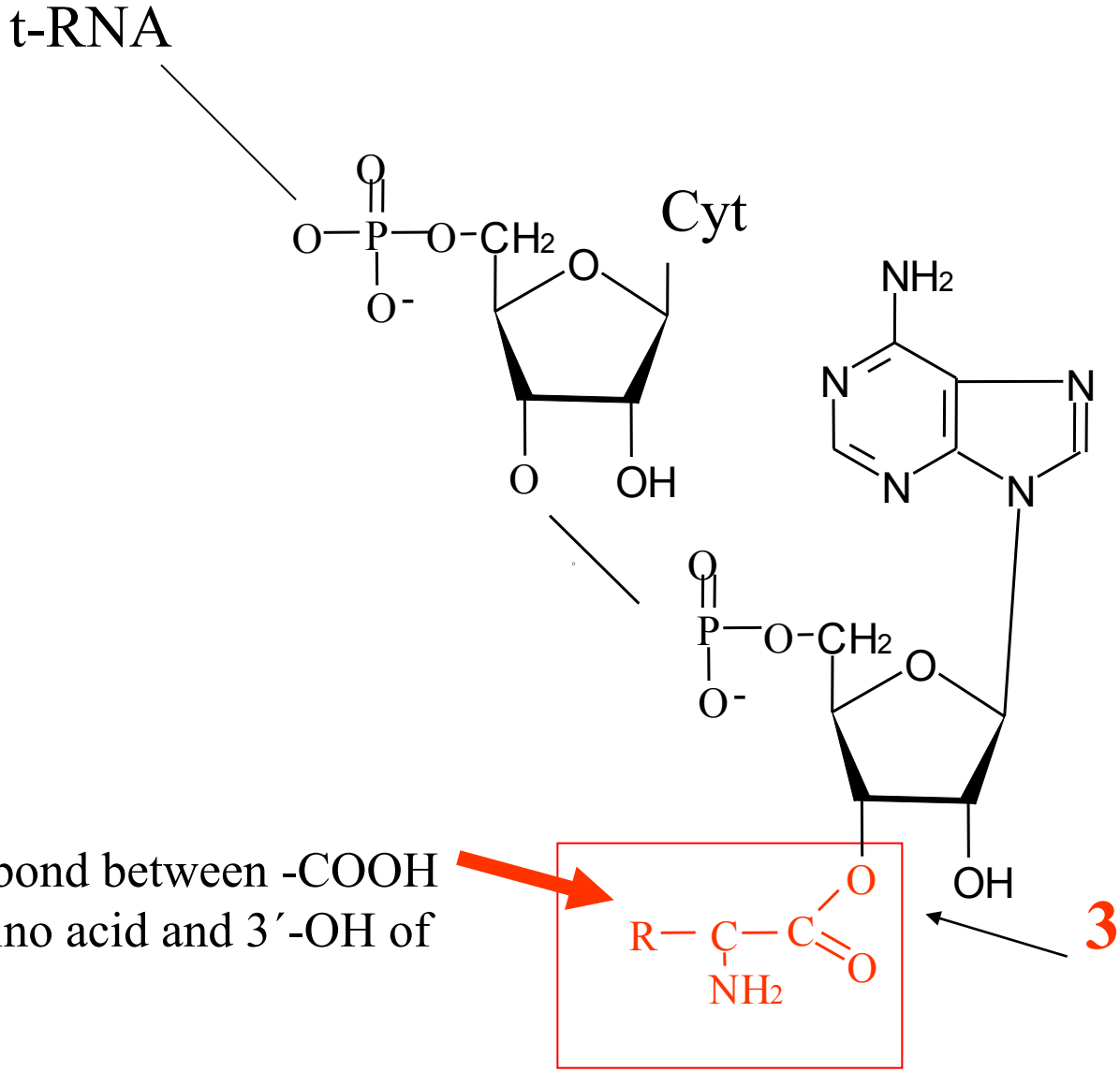
Formation of aminoacyl-tRNA

- aminoacid is firstly activated by a reaction of its carboxylic group with ATP to aminoacyl-AMP
- activated AA is transferred to 2´- or 3´- OH group of ribose on 3´terminal of tRNA
- reaction is catalyzed by specific enzymes (aminoacyl-tRNA synthetases)
- 20 different synthetases exist, one for each AA
- the cleavage of ATP in the first reaction provides energy

1. Activation of AA



2. Transfer of activated AA to 3´-end of tRNA



Ester bond between -COOH of amino acid and 3´-OH of ribose

3´- end of t-RNA

Amino acid

Aminoacyl-tRNA synthetases

(at least 20 distinct enzymes in cells) exhibit the **very high degree of specifity** for amino acids.

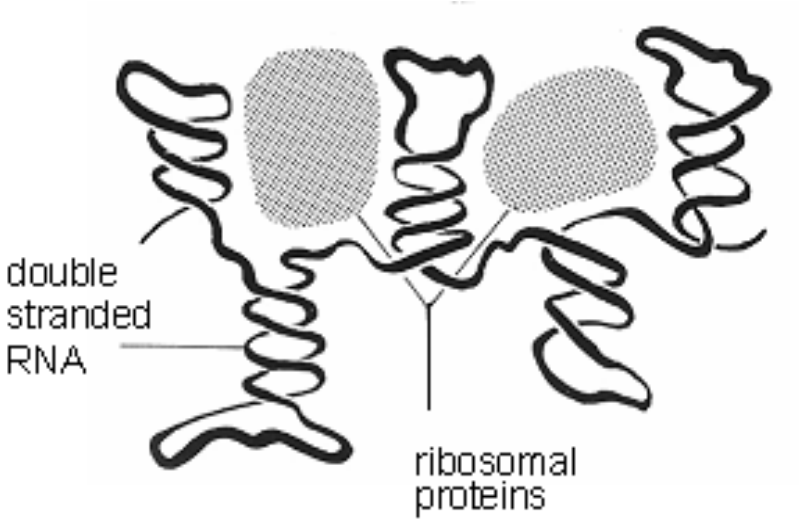
The enzyme molecule recognizes both a specific amino acid and a specific tRNA.

These enzymes discriminate accurately, the overall rate of occurence of errors in translating mRNA is less than 1 in 10 000.

This high specifity is oft called **the 2nd genetic code**.
It depends on specific location of some bases in tRNA molecules,
not on the sole anticodon.

Ribosomes

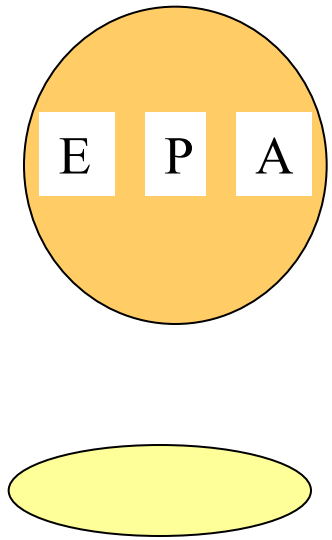
Ribosomes are workbenches for protein synthesis



Large ribonucleoprotein particles – composed of proteins and RNA

Free floating in the cytoplasma or attached to the membranes

Ribosomes



Ribosomes consist of a large and a small subunit

Inactive ribosomes exist as loose subunits that aggregate into complete particles when they get ready for protein synthesis.

Large ribosomal subunit has three binding sites for molecules of tRNA – P, A, E

P-peptidyl-tRNA

A-aminoacyl-tRNA

E-free tRNA (exit)

Prokaryotic x eukaryotic ribosomes

Property	Bacterial	Human
Sedimentation constants:		
complete ribosome	70S	80S
small subunit	30S	40S
large subunit	50S	60S
RNA content	65%	50%
RNA-small subunit	16S	18S
RNA-large subunit	5S 23S	5S 5,8S 28S
Placement in the cell	Free floating in cytoplazma or attached to plasmatic membrane	Free floating in cytoplazma or attached to ER membrane

Initiation

Ribosome has to associate with mRNA and the initiator tRNA

Formation of initiation complex.

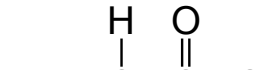
Soluble cytoplasmic factors help in initiation – **initiation factors**

Also GTP, ATP are involved

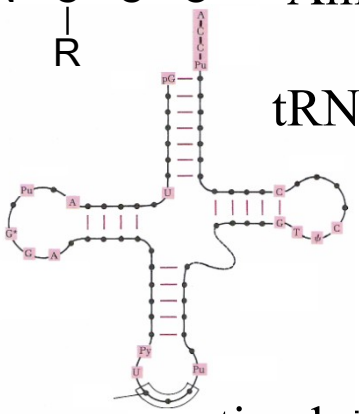
Differences between eukaryonic and prokaryonic cells

Initiation u eukaryotes

It involves formation of a complex composed of methionyl t-RNA^{met}, mRNA and a ribosome.

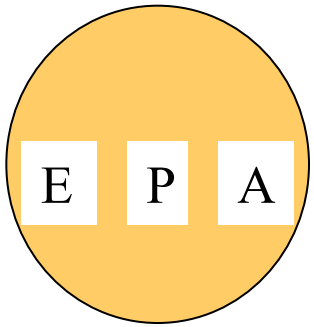


Aminocyl-tRNA

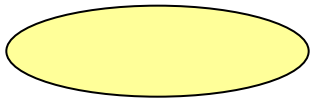


tRNA

anticodon



Large ribosomal subunit

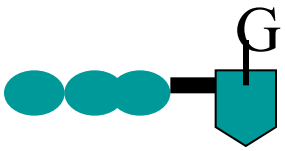


Small ribosomal subunit

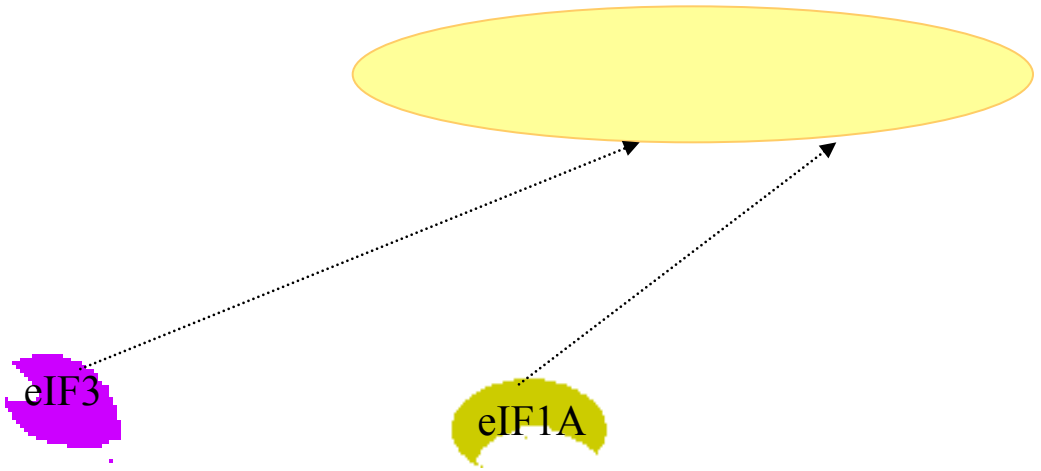
mRNA

Guanine cap

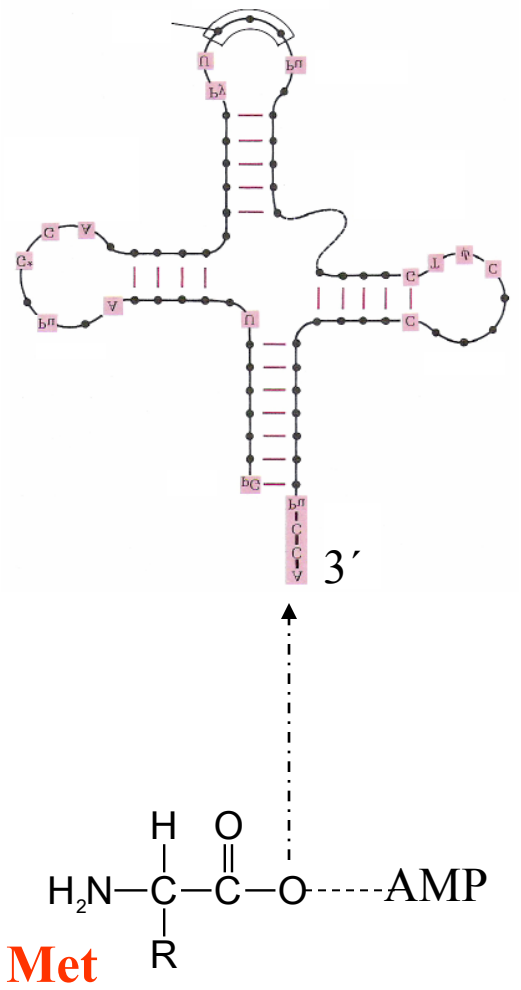
Iniciation factors



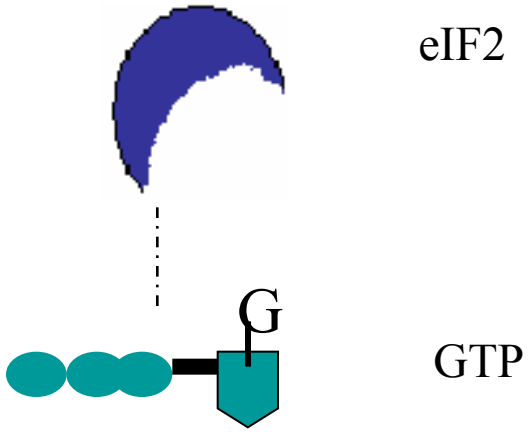
Binding of factors eIF3 and eIF1A to a small subunit



Binding of activated Met to tRNA^{Met}

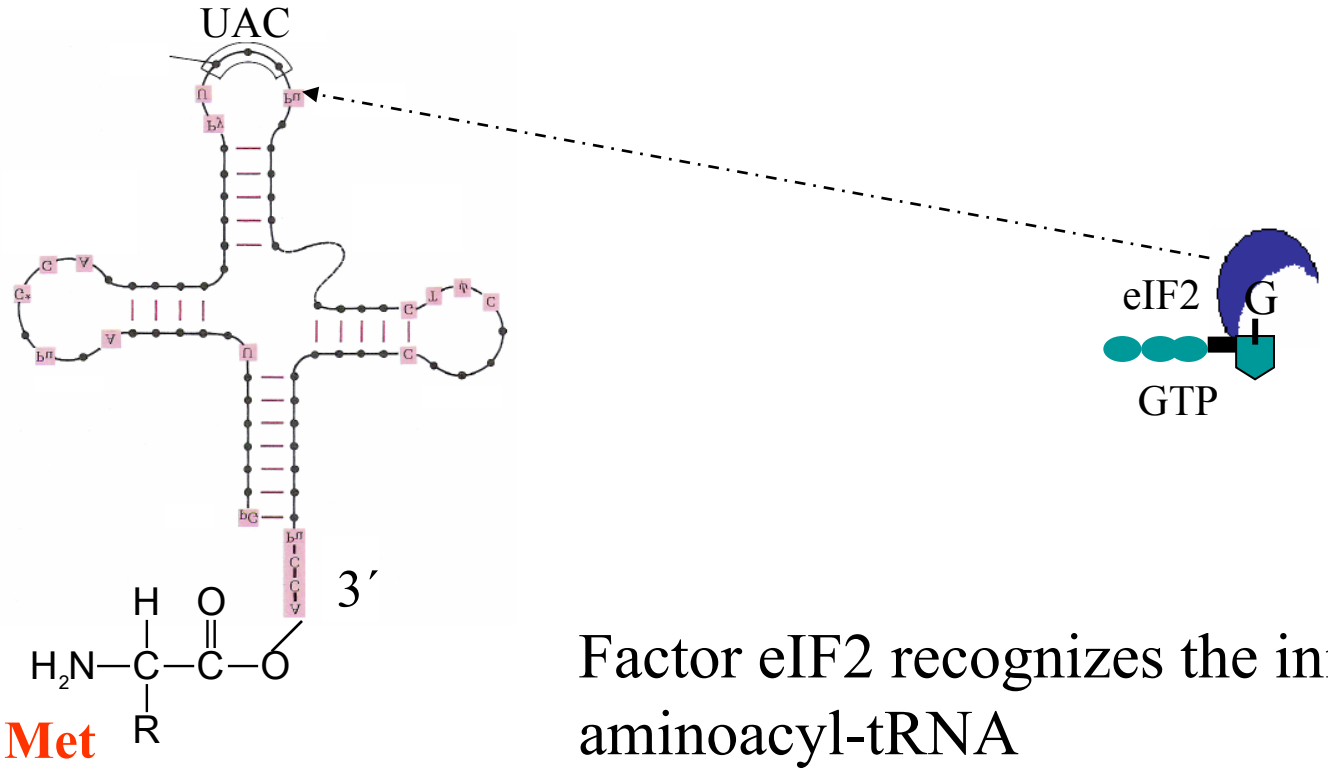


Binding of GTP to eIF2



eIF2 is heterotrimeric G-protein

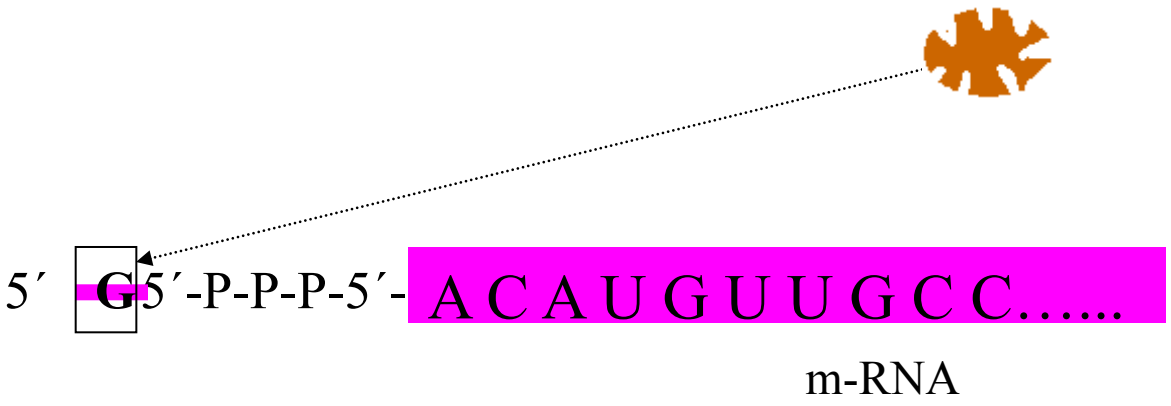
Binding of complex GTP-eIF2 to t-RNA^{Met}



Factor eIF2 recognizes the initiation aminoacyl-tRNA

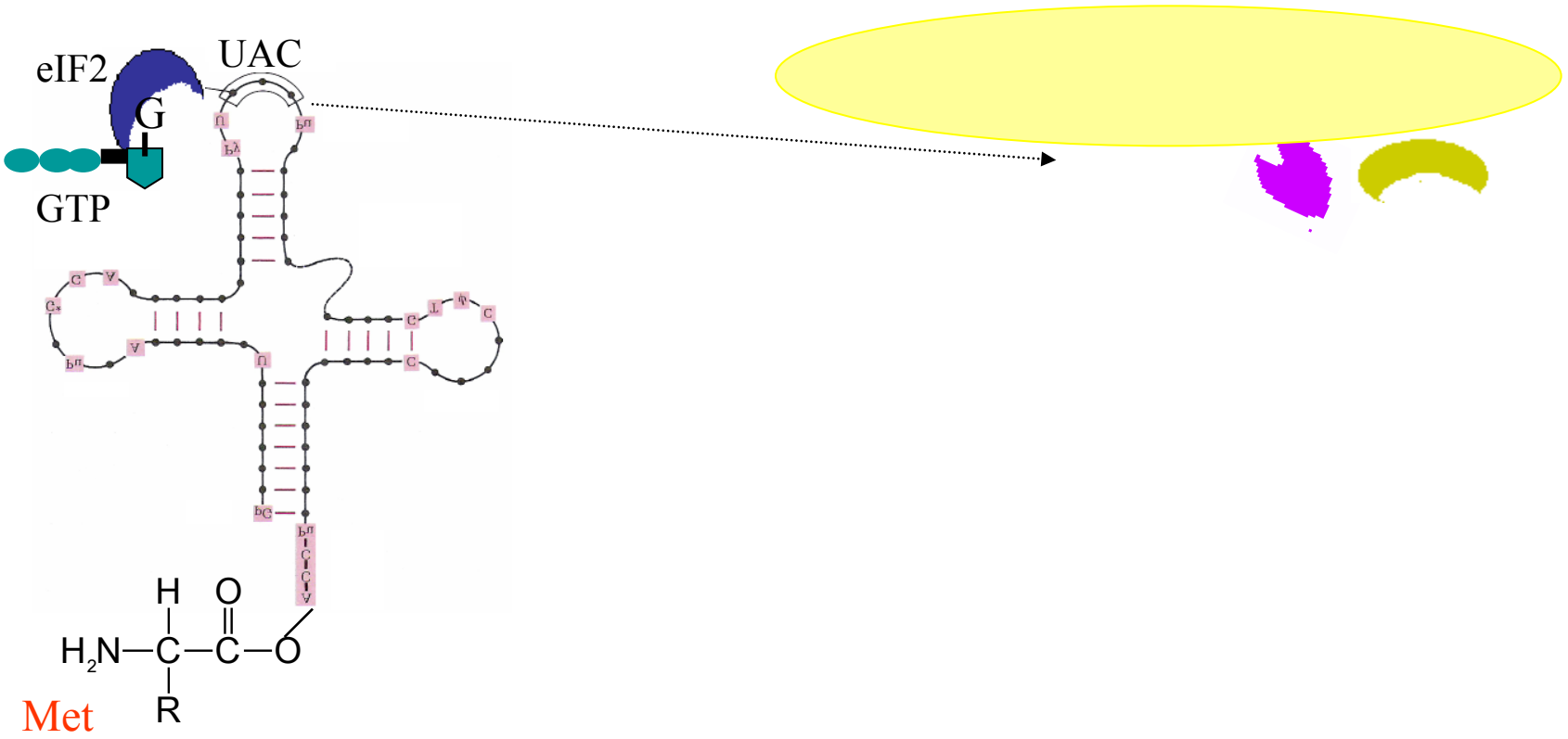
CBP (cap binding protein) binds to the cap on 5´end of mRNA

CPB (eIF-4F) is composed of a number of other initiation factors (eIFs)

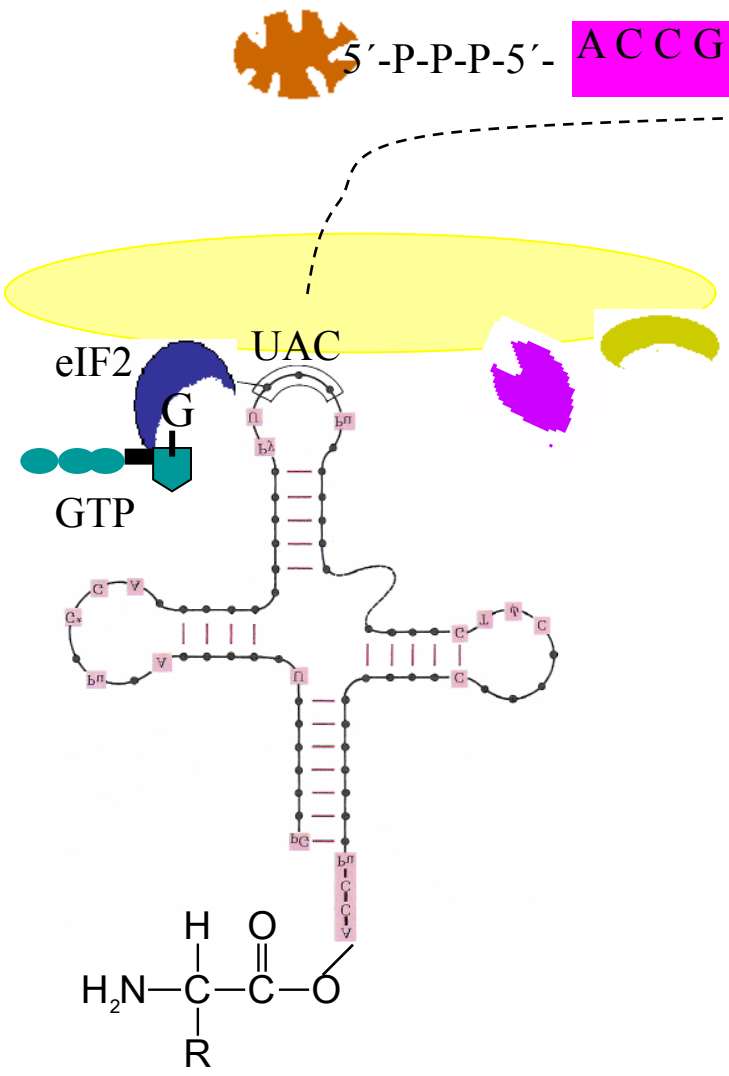


Complex Met-tRNA^{Met}, eIFs and GTP binds to the snaller ribosomal subunit

Preiniciation complex



Binding of m-RNA to preiniciation complex



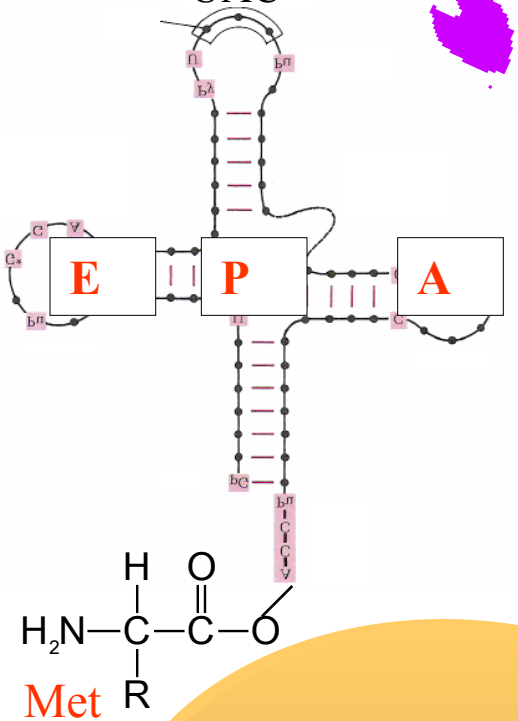
Reaction requires ATP for unwinding a hairpin loop in the mRNA (helicase activity of an eIF subunit)

The complex scans mRNA from 5´end until it locates the AUG start codon

Iniciation complex 80S

5´-P-P-P-5´- A C C G U A A C A U G U U G C C G

UAC



Pi + GDP

eIF

- GTP is hydrolyzed
- eIF separates

- The larger ribosomal unit is attached
- Met-tRNA bimds at the site P of larger ribosomal subunit

Differences in between prokaryotes and eukaryotes

	eukaryotes	prokaryotes
Binding of mRNA to smaller ribosomal subunit	Cap on the 5´end of mRNA binds IFs and 40S subunit containing t-RNA^{met}. mRNA is scanned until AUG	No cap, Shine-Dalgarno sequence in mRNA about 10 nucleotides upstream of the AUG start codon is attached to a complementary sequence in 16S RNA
First AA	methionine	formylmethionine
Iniciation factors	12 and more	3
ribosomes	80s (40s a 60s)	70s (30s a 50s)

eIF2 factor in eukaryotes

eIF2 has essential significance for initiation of translation – control point in proteosynthesis

It is heterotrimric G-protein, it binds GTP and GDP

Its phosphorylated form in inactive – regulation of its activity by action of proteinkionases

Conditions such starvation, heat shock, viral infection, glucose starvation result in phosphorylation of eIF2 by specific kinases (generally conditions when the energy expanditure required for synthesis of proteins would be deleterious)

Example of regulation:

Synthesis of globin in v reticulocytes

• In the absence of heme, the eIF2 is phosphorylated, the rate of initiation of globin synthesis decreases

• heme acts by inhibiting the phosphorylation of the eIF2 $\Rightarrow$ eIF2 is active in the presence of heme and globine synthesis is initiated

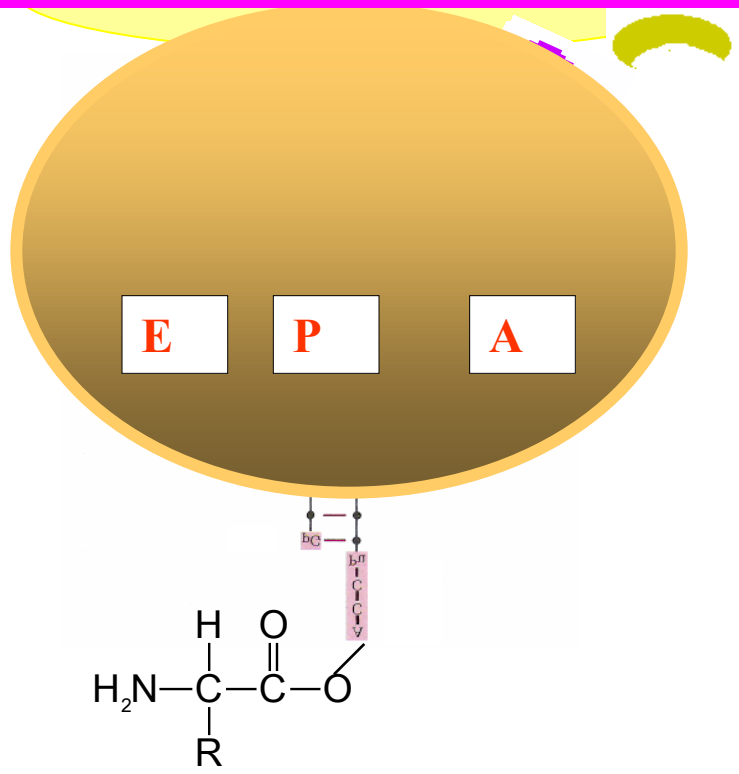
Elongation of peptide chain

- formation of the second aminoacyl-tRNA
- binding of an aminoacyl-tRNA to the A site on the ribosome
- formation of peptide bond
- translocation of peptidyl-tRNA to the site P

Which amino acid will be added?

The next codon is UUG

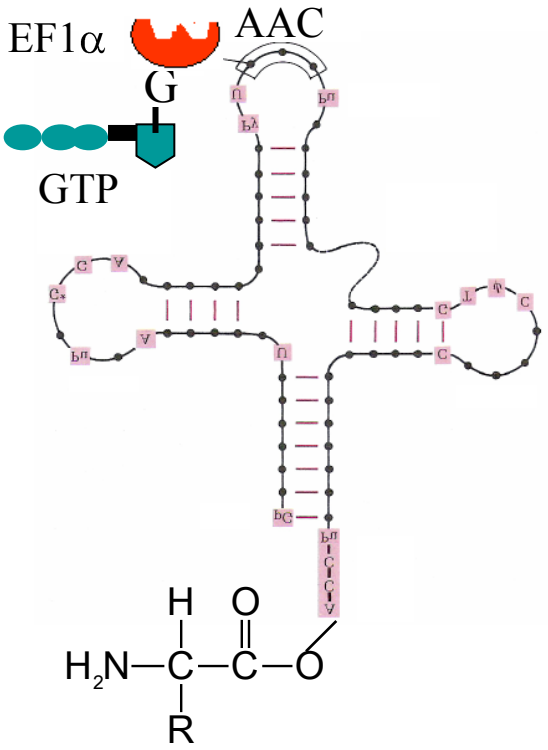
5´-P-P-P-5´- A C C G U A A C A U G U U G C C G



Antikodon is AAC

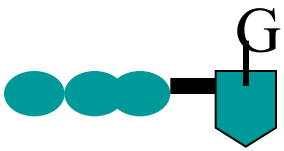
Amino acids is leucin

Formation of Leu-tRNA



Leu

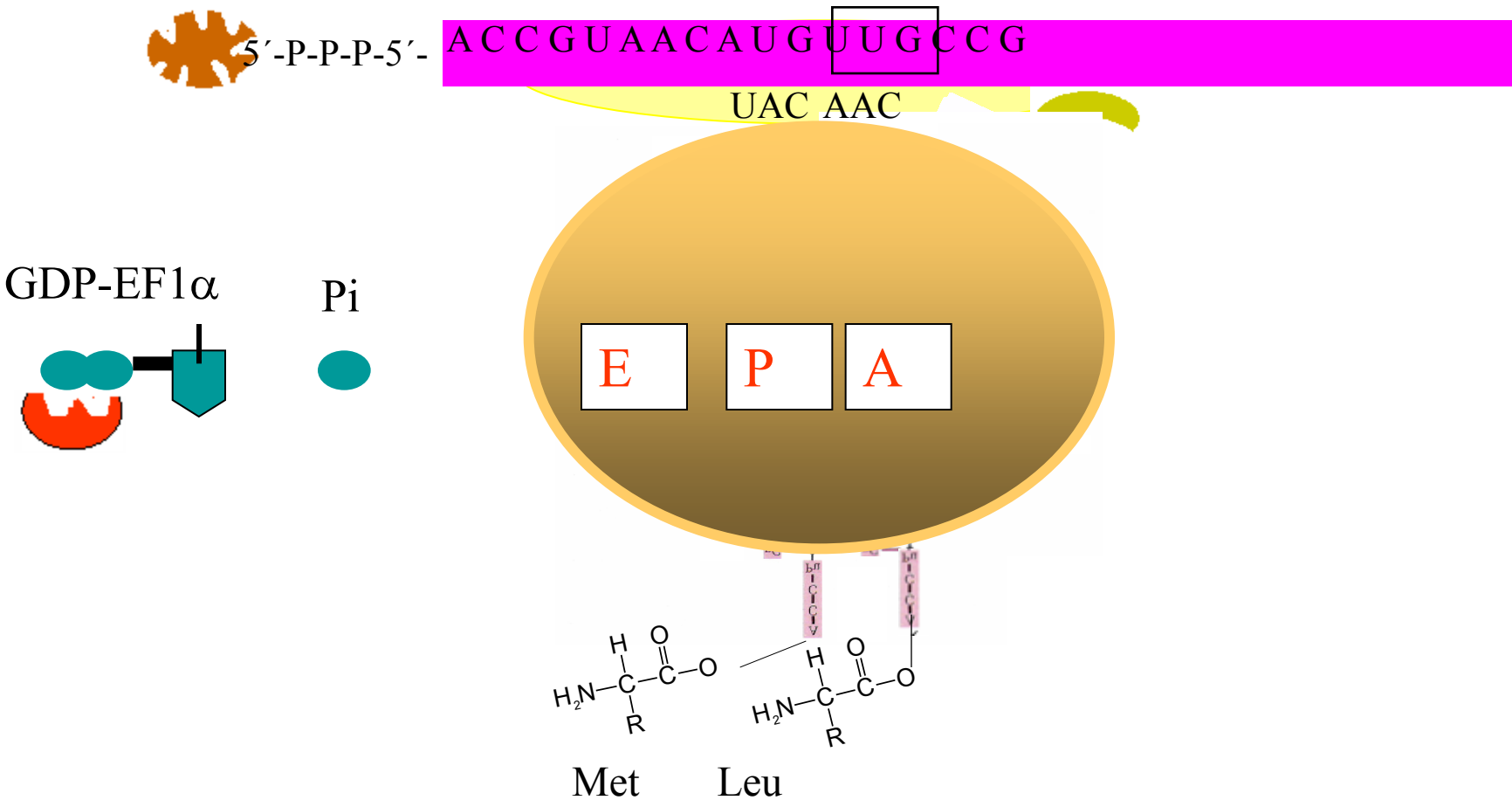
1. Activation of leucine by reaction with ATP → leucinyladenylate
2. formation of Leu-tRNA
3. + binding of GTP a EF1α



GTP

EF1α (elongation factor)

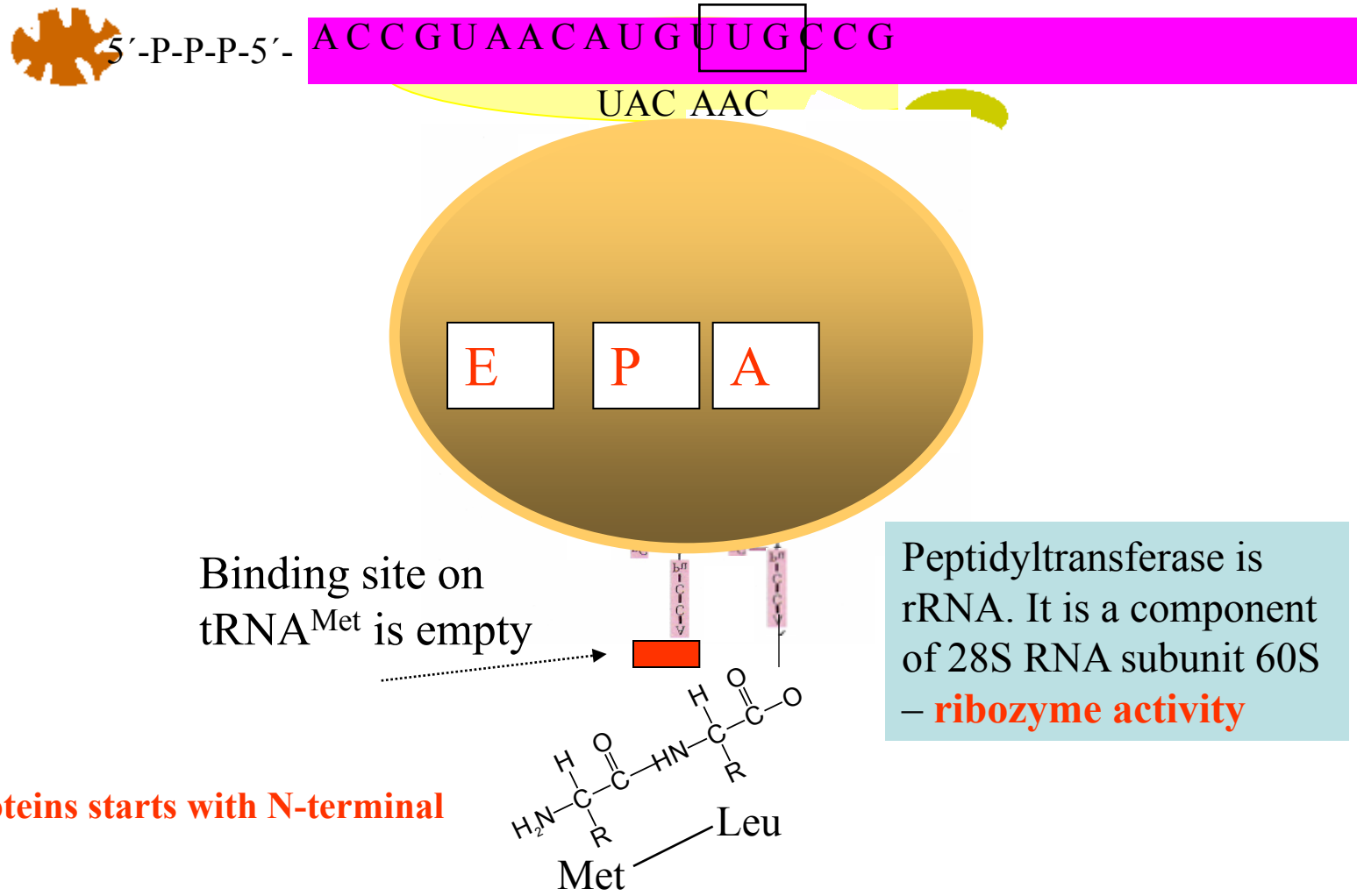
Leu-tRNA binds to the site A



GTP is hydrolyzed to GDP + Pi, complex GDP-EF1α is released

Process of elongation is very similar in eukaryotes and prokaryotes (different cofactors of elongation)

Formation of peptide bond (transpeptidation)

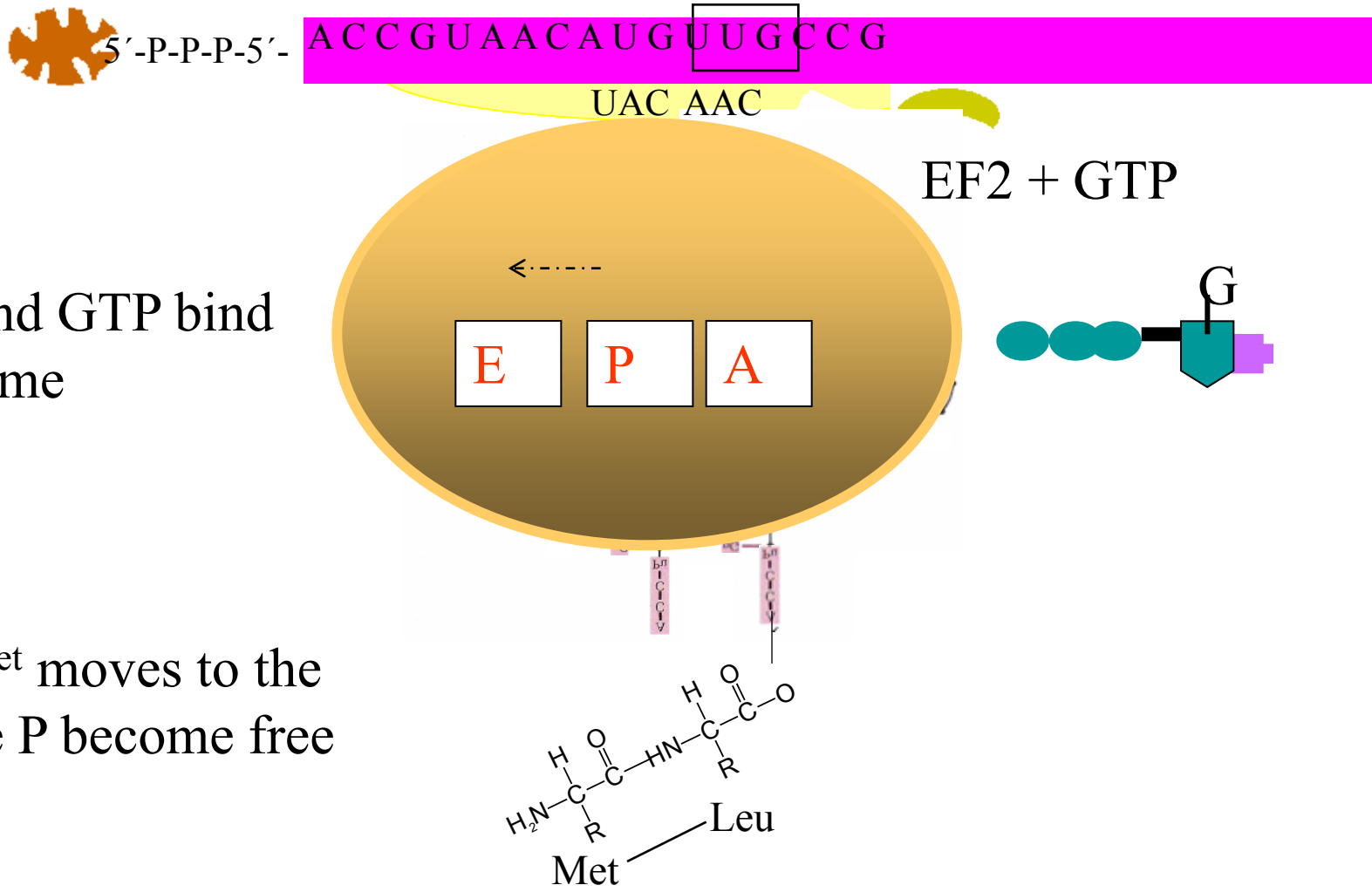


Peptidyltransferase is rRNA. It is a component of 28S RNA subunit 60S – **ribozyme activity**

Synthesis of proteins starts with N-terminal

Peptidyltransferase catalyzes the release of methionine from tRNA and its transfer to leucine. A peptide bond between carboxyl group of methionine and amino group of leucine is formed

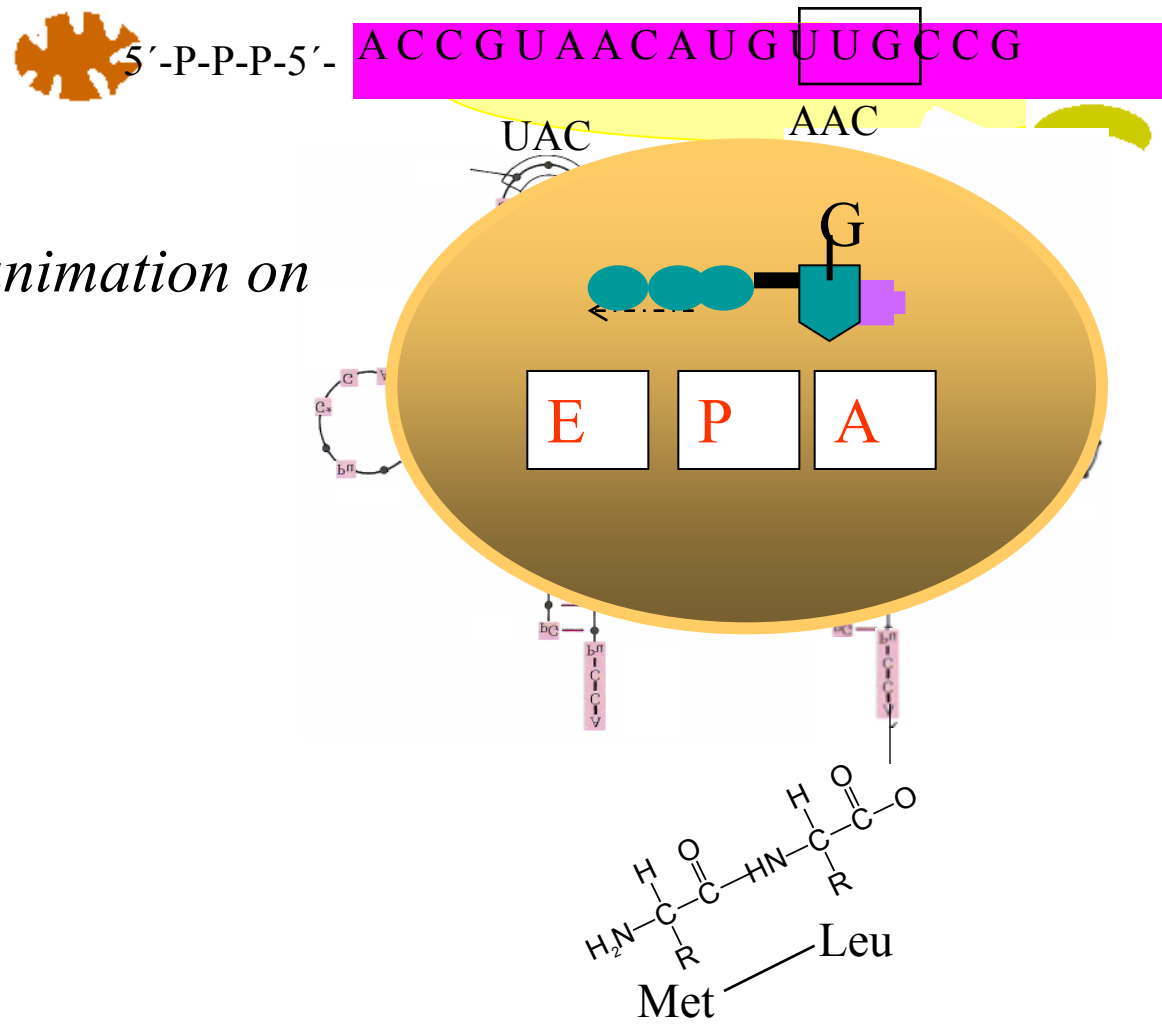
Movement of met-tRNA to the site E



1. EF2 and GTP bind to ribosome

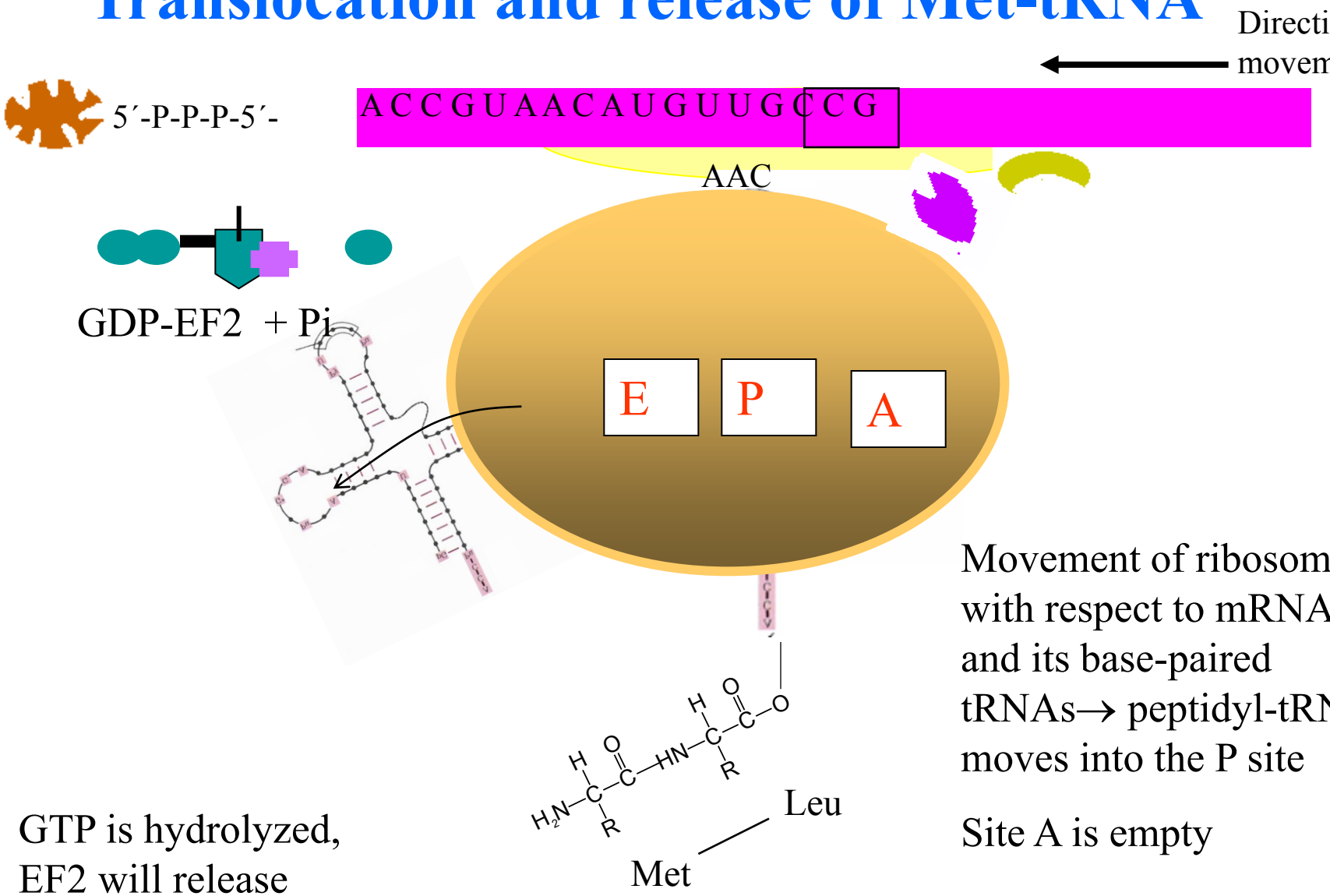
2. $tRNA^{Met}$ moves to the site E, site P become free

Movement of met-tRNA to the site E



Result of animation on page 42

Translocation and release of Met-tRNA



GTP is hydrolyzed, EF2 will release

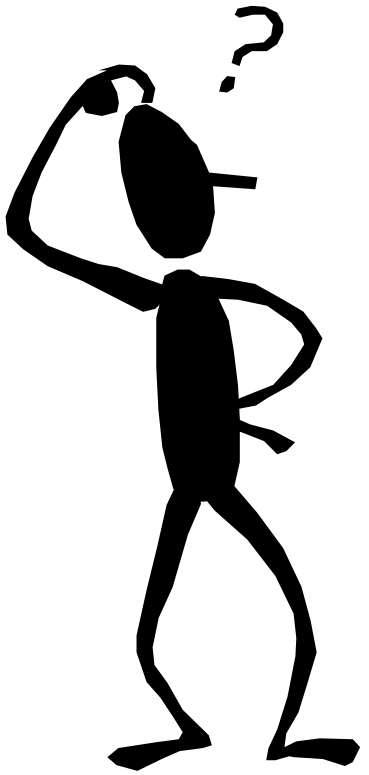
Movement of ribosome with respect to mRNA and its base-paired tRNAs→ peptidyl-tRNA moves into the P site

Site A is empty

Next step of elongation

Further tRNA charged with amino acid (proline) binds to the site A

Which further steps will follow?



Termination

- elongation steps are repeated until a termination (stop) codon moves into the A site of the ribosome
- no tRNA that can pair with stop codon is present in cytoplasma
- releasing factors bind to ribosome instead
- peptidyltransferase hydrolyzes the bond between peptide chain and tRNA
- newly synthesized peptide is released from ribosome
- ribosome dissociates into individual subunits, mRNA releases

Energy consumption

		Equivalent of ATP
Aaminoacyl-tRNA formation	ATP→ AMP + 2 Pi	2
Binding of aminacyl-tRNA to the site A	GTP → GDP + Pi	1
Translocation of peptidyl-tRNA to the site P	GTP → GDP + Pi	1
		4 ATP

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4 ATP are required for synthesis of one peptide bond.

Further energy is required for initiation and synthesis of nucleotides.

Proteosynthesis rate

Prokaryotes ~ 100 peptide bond s

Eukaryotes ~100 peptide bonds/min

Stimulation of proteosynthesis by insulin

Insulin is anabolic hormone, it stimulates proteosynthesis.

It affects the synthesis through the interaction with CBP.

CBP (cap binding protein complex) has subunit eIF4E

This subunit is blocked by protein 4E-BP

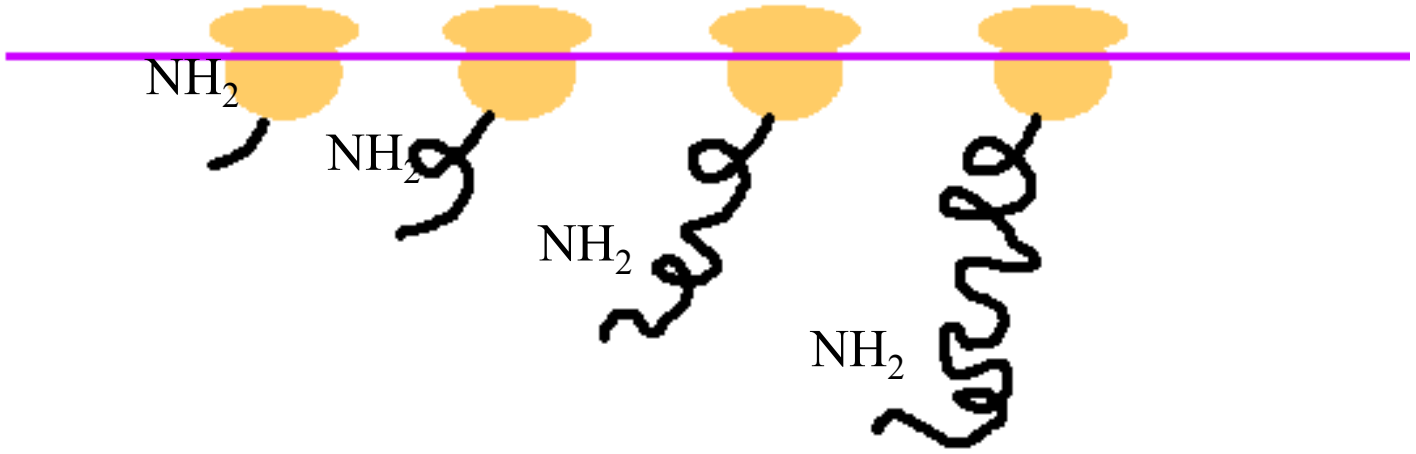
Insulin triggers phosphorylation of 4E-BP

Phosphorylated 4E-BP looses its afinity to eIF4E

$\rightarrow$ eIF4E become free for participation in protein synthesis

Polysomes

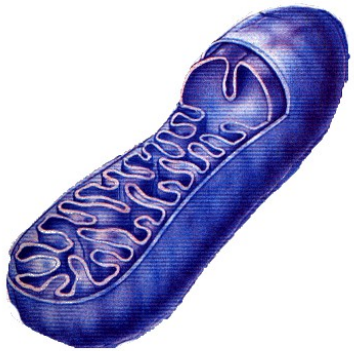
Simultaneous translation of mRNA on more ribosomes



As one ribosome moves along the mRNA produsing a polypeptide chain, a second ribosome can bind to the vacant 5´-end of mRNA. Many ribosomes can simultaneously translate a single mRNA, forming a complex known as polysome. A single ribosome covers approximately 80 nucleotides of mRNA. Therefore, ribosomes are positioned on mRNA at intervals of approximately 100 nucleotides.

Synthesis of proteins in mitochondria

- Mitochondria contain 2-10 copies of circular double stranded DNA
- The size varies depending on the species
- Animal mitochondrial DNA - $M_r \sim 10^7$
- Codes for rRNA, set of tRNAs and mRNA for several proteins
- Proteins synthesized in mitochondrial are a negligible proportion of total proteins of inner mitochondrial membrane but are essential for process of oxidative phosphorylatiob (a part of complexes I,III,IV and ATP-synthase)
- Synthesis of of proteins in mitochondria has many common features with synthesis in prokaryotes (e.g. initiation by formylmethionine, sensitivity to antibiotics)



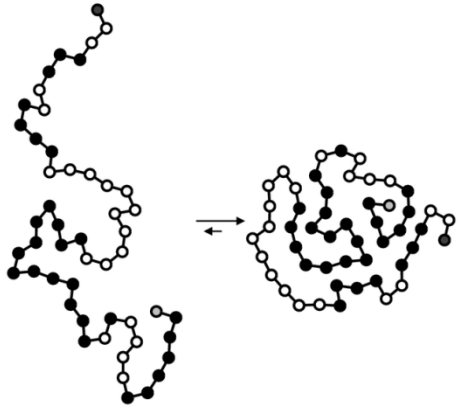
Effects of antibiotics on prokaryotic proteosynthesis

the differences in proteosynthetic procedure between eukaryotes and bacterias are exploited for clinical purposes

some antibiotics inhibits specifically proteins of bacterial ribosomes

Antibiotics	Effect
Streptomycine	Binds to 30S ribosomal subunit, inhibits formation of initiation complex.Triggers errors in reading frame of mRNA.
Tetracycline	Binds to 30S ribosomal subunit and inhibits binding of aminoacyl-tRNA to site A
Chloramfenikol	Binds to 50S ribosomal subunit and inhibits peptidyltransferase
Erytromycine	Binds to 50S ribosomal subunit and inhibits translocation
Puromycine	Binds to A-site on ribosome and trigers premature termination

Folding of proteins



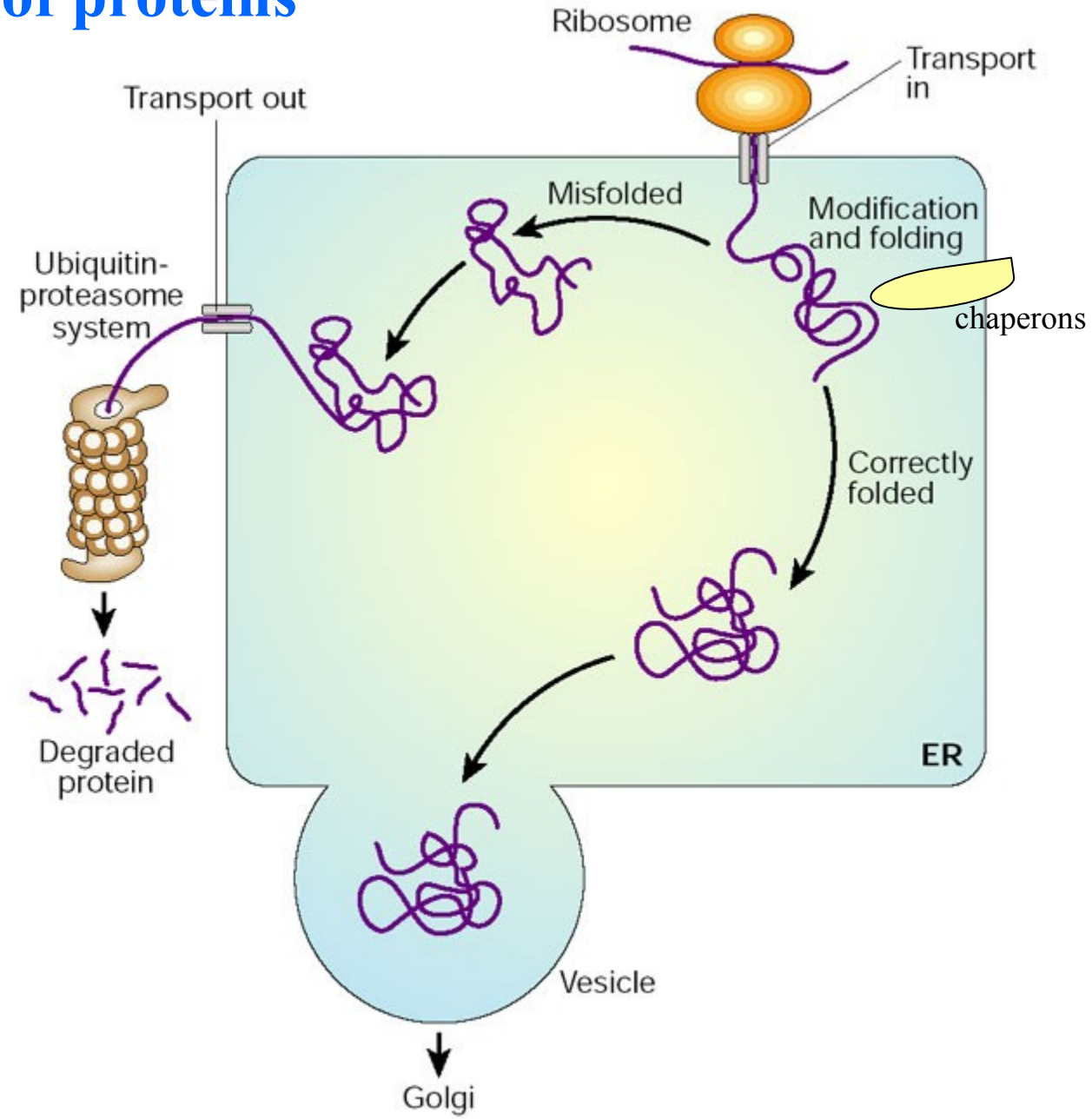
•Nascent polypeptide chain is transported across ribosomes

• Outside of the ribosome the N-terminus of the protein begins to fold (while the C-terminal portion of the protein is still being synthesized by the ribosome)

• Folding is the process by which a protein achieves its thermodynamically-stable three-dimensional shape to perform its biological function

•Specialized proteins called chaperones assist in the folding.

Folding of proteins



Folding

- **Protein folding** is the process by which a protein structure assumes its functional shape or conformation– only one of them is correct and is related to the native
- Misfolded proteins can be formed by means of high or low temperature, radiation, oxidative stress, chemicals etc. Folding is affected by chaperons.
- Incorrect folding can follow from mutation of a gene
- Misfolded proteins are ubiquitinated and degraded in proteasome.
- Accumulation of misfolded proteins in cell can occur due to overproduction of proteins, their damage or disfunction.
- Accumulation can cause disease, known as amyloid diseases.
- The most prevalent one is Alzheimer's disease, Parkinson's disease and Huntington's.
- Another diseases are caused by lack of a particular functioning protein, due to its degradation as a consequence of misfolding (e.g. cystic fibrosis (misfolded CFTR protein), Marfan syndrome (misfolded fibrillin), Fabry disease (misfolded alpha galactosidase),)

Chaperons

•Proteins stabilizing unfolded or partially folded structures, facilitate correct folding and assembly

• Many chaperones are heat shock proteins, that is, proteins expressed in response to elevated temperatures or other cellular stresses.

•They binnd mainly to hydrophobic areas of proteins

• they are present in mitochindria, cytoplasm, lumen ER

Targeting of proteins to subcelular and extracellular locations

Synthesis of proteins on polysomes in cytosol

↓

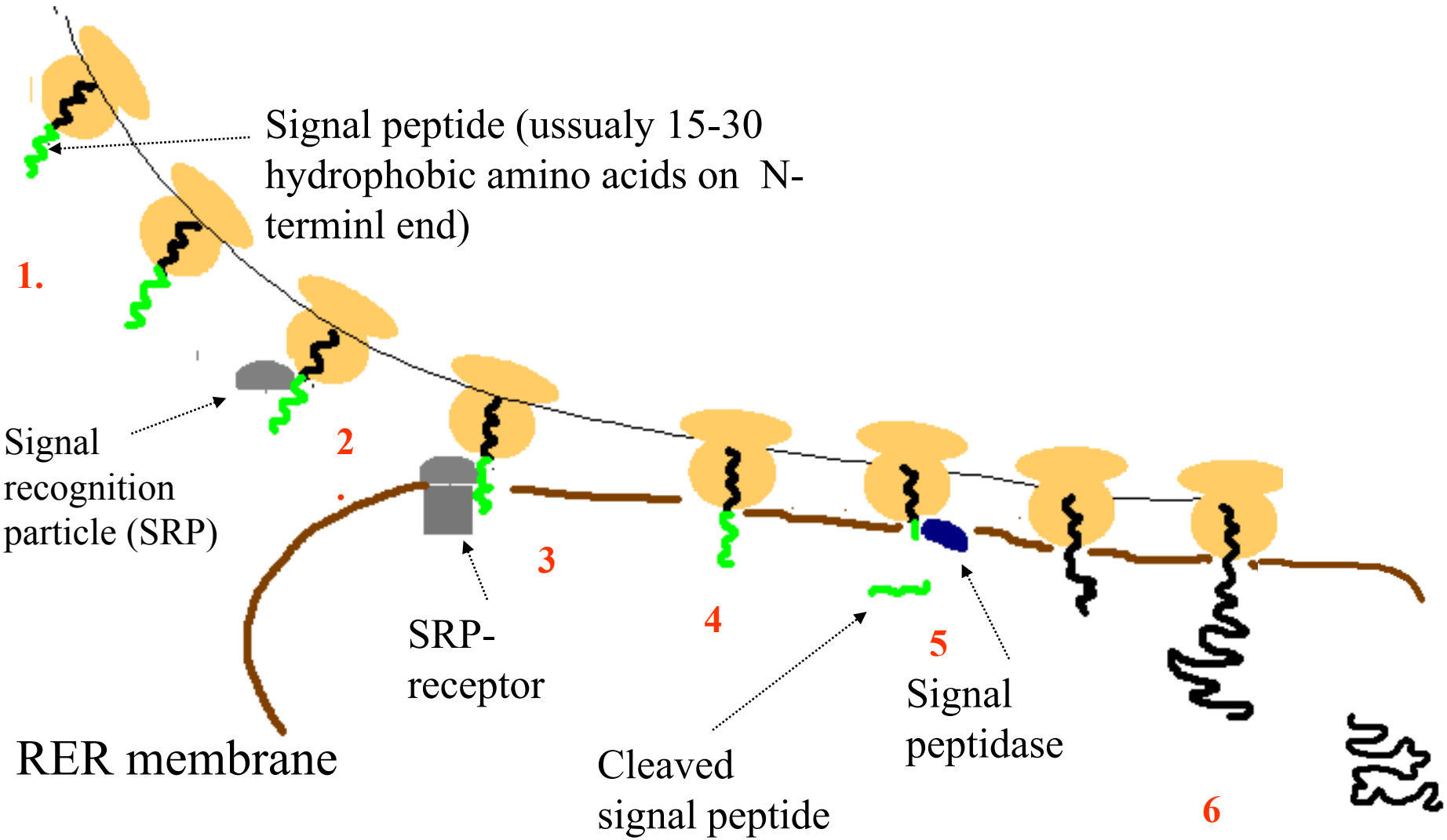
Proteins remain in the cytosol or enter the organeles (nucleus, mitochondria). They contain amino acid sequence (**targeting sequence)** that facilitate their transport into a certain organelle

Synthesis of proteins on ribosom bound to RER

↓

Transport to lysosomes, ER, Golgi complex, cellular membranes or secretion

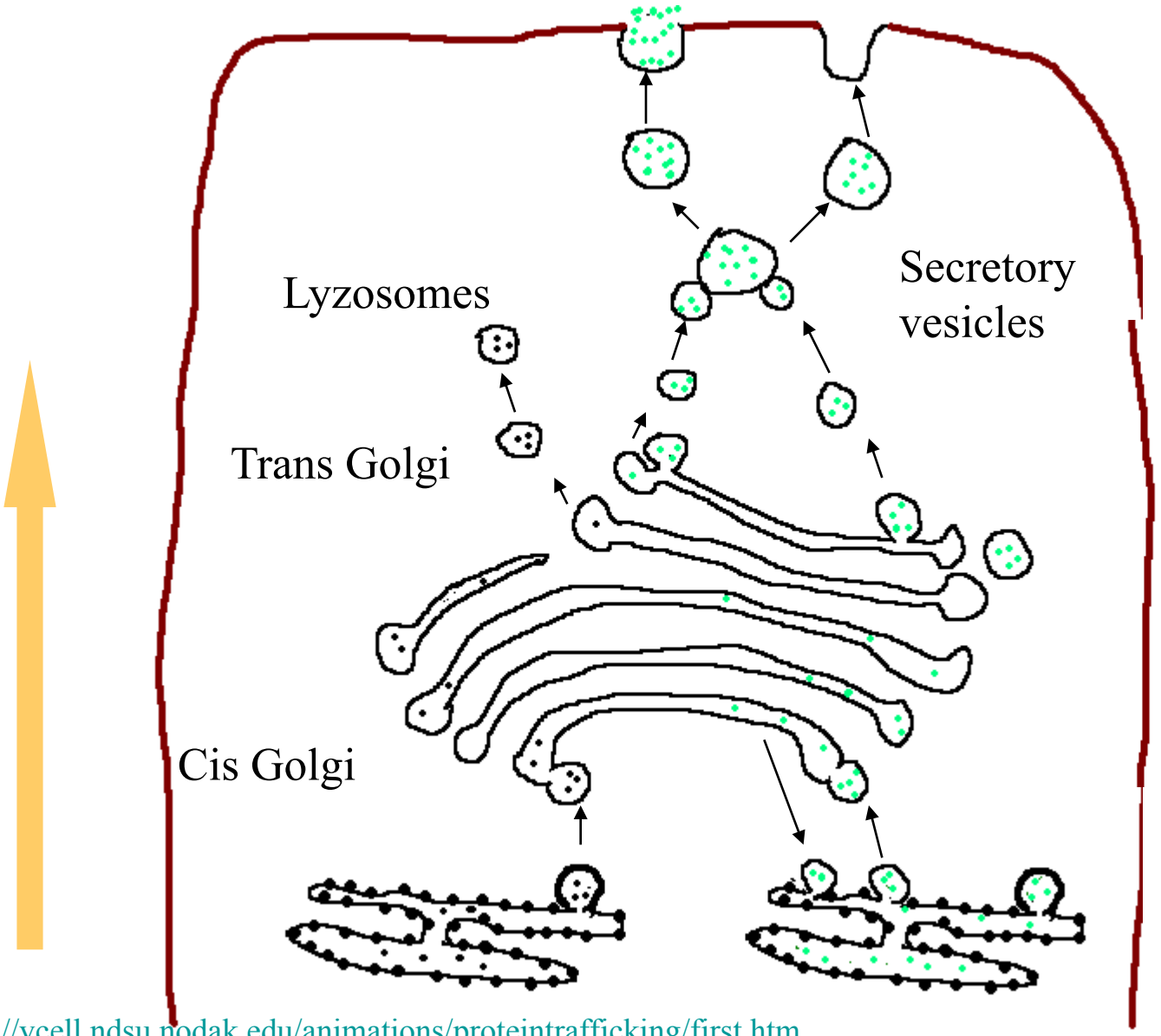
Transport of proteins syntesized on RER



Transport of proteins syntesized on RER

1. translation begins in the cytosol
2. As the signal protein emerges from the ribosome, a signal recognition particle (SRP) binds to it and to the ribosome snd inhibits further synthesis of the protein
3. the SRP binds to the SRP receptor in the RER membrane, docking the ribosome on the RER
4. The SRP is released and protein synthesis resumes
5. As the signal peptide moves through a pore into the RER, a signal peptidase removes the signal pepide
6. Synthesis of the nascent protein continues and the completed protein is released into the lumen RER

Transport of proteins syntesized on RER



http://vcell.ndsu.nodak.edu/animations/proteintrafficking/first.htm

Transport of proteins syntesized on RER

• Proteins syntesized ribosomes attached to RER travel in vesicles to the cis face of the Goolgi complex

• here is the sorting center – structural features of proteins determine their direction

• some remain in Golgi complex, some return to RER

• others bud from trans face of the Golgi complex in vesicles

• these vesicles become lysosomes or secretory vesicles depending on their contents

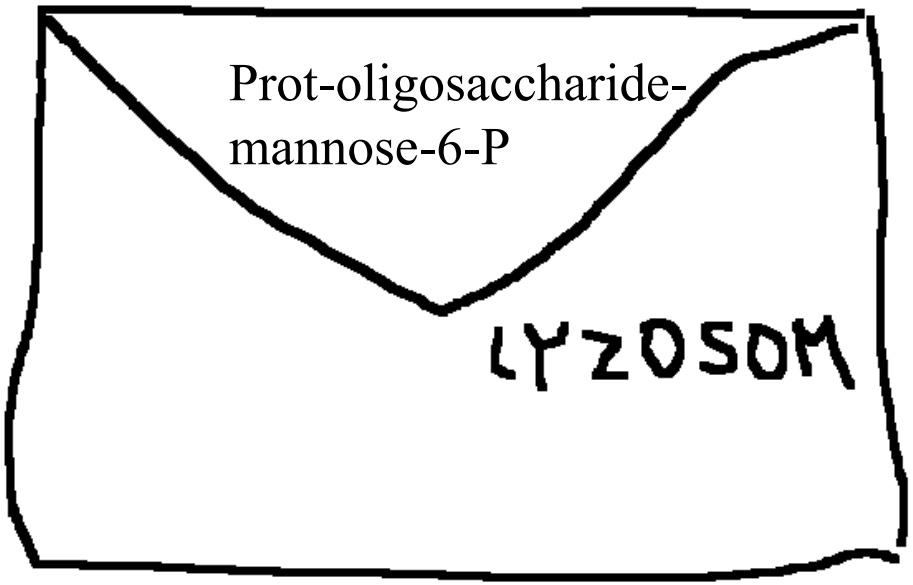
• secretory proteins are released from the cells when secretory vesicles fuse with the membranes

• proteins with hydrophobic regions embedded in the membrane of secretory vesicles become cell membrane proteins

Principles of intracellular sorting

Example 1:

Proteins determined for lysosomes are marked by N-bonded oligosacharides terminated by mannose-6-P

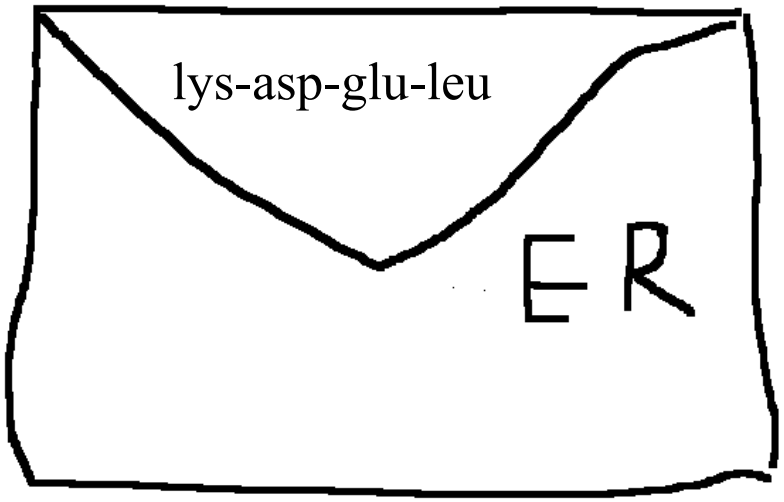


„address“ is recognised by specific membrane receptors in Golgi complex that embedds the protein into the clathrine coated vesicles

Principles of intracellular sorting

Example 2:

Proteins destinated for ER have sequence Lys-Asp-Glu-Leu on their carboxy terminal



Proteins are transported back from Golgi complex to ER

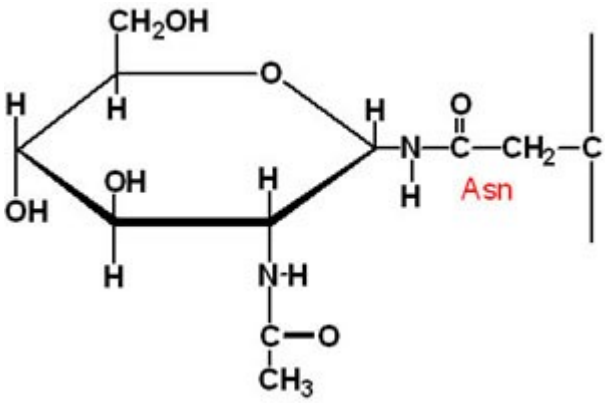
Posttranslational processing of proteins

After protein emerge the ribosome, they may undergo posttranslational modification

- Methionine removal
- ADP-ribosylation
- Glycosylation
- Fatty acylation
- Phosphorylation
- Acetylation
- Carboxylation
- Methylation
- Prenylation
- Hydroxylation
- Sulfatation ad.

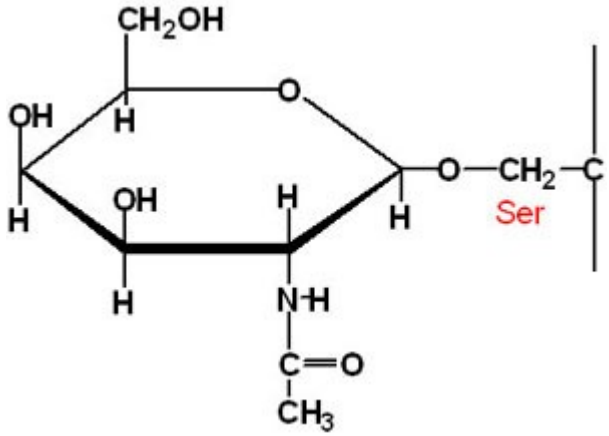
Glycosylation of proteins

Glycoproteins



N-linked carbohydrate chain

Involving the amide nitrogen of asparagine



O-linked carbohydrate chain

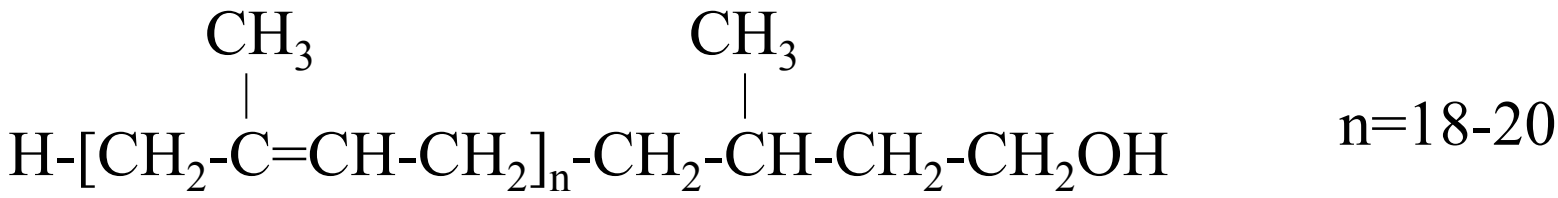
Involving hydroxyl side chain of serine or threonine

They differ in composition of saccharides and and the way of synthesis

Synthesis of N-glycoproteins

The oligosaccharide chain is first assembled on the dolichodiphosphate backbone

Dolichol is isoprene (see the synthesis of cholesterol)

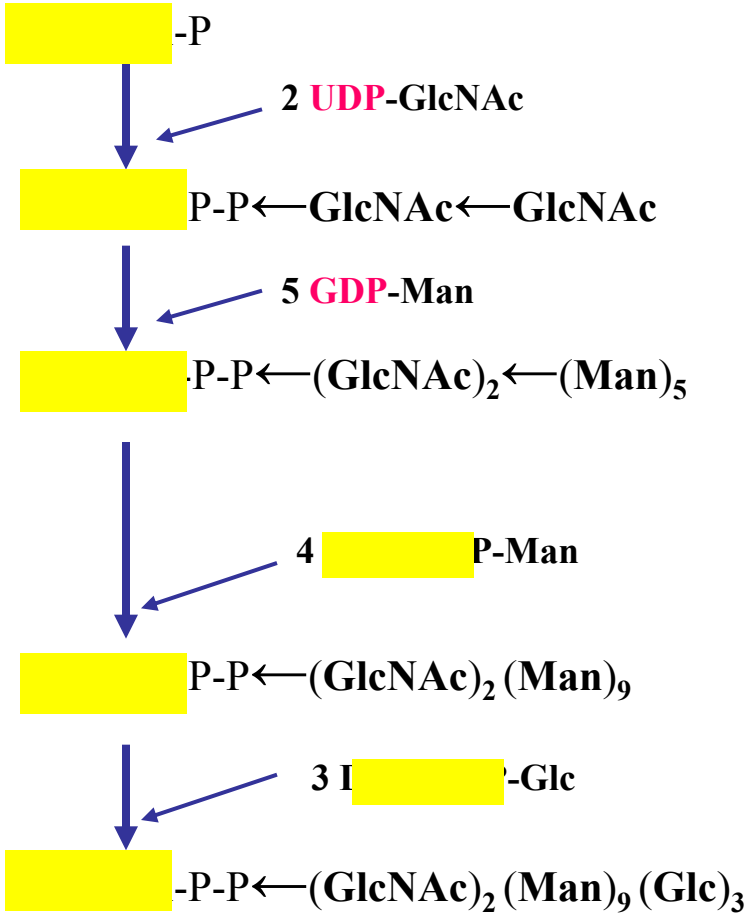


Dolicholdiphosphate is bonded to membrane of ER.

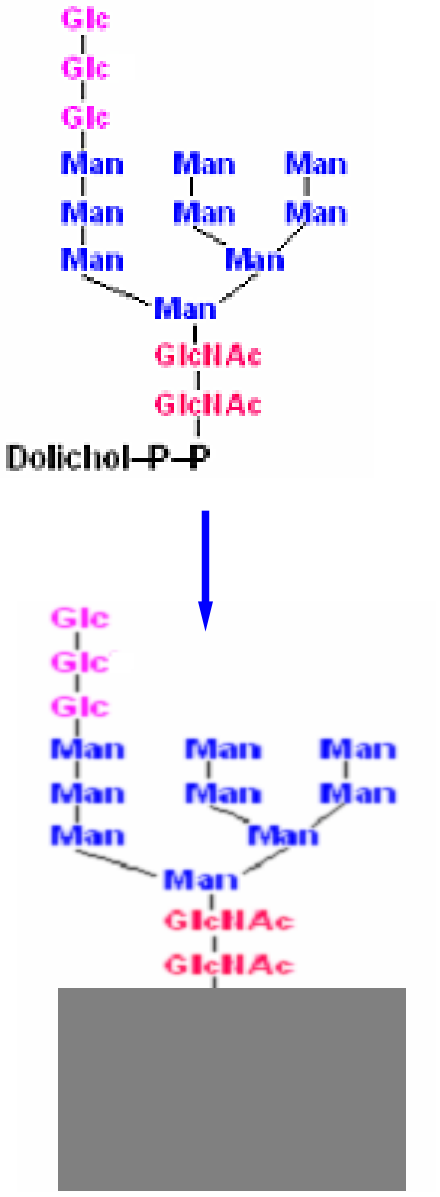
Activated monosaccharides are gradually attached to the terminal phosphate.

The oligosaccharide chain is then transferred en bloc to suitable Asn residues of apoglycoproteins during their synthesis on membrane-bound polyribosomes

Synthesis of oligosaccharide precursor



Transfer to protein



Trimming and final processing of *N*-linked glycoproteins:

$(Glc)_3\,(Man)_9\,(GlcNAc)_2$-protein

– **3** Glc

$(Man)_9\,(GlcNAc)_2$-protein

Trimming

up tp **4** Man removed

High-mannose *N*-linked glycoproteins

$(Man)_5\,(GlcNAc)_2$-protein

glycosylation

Hybride types

– **2** Man

$(Man)_3\,(GlcNAc)_2$-protein
pentasaccharide core region

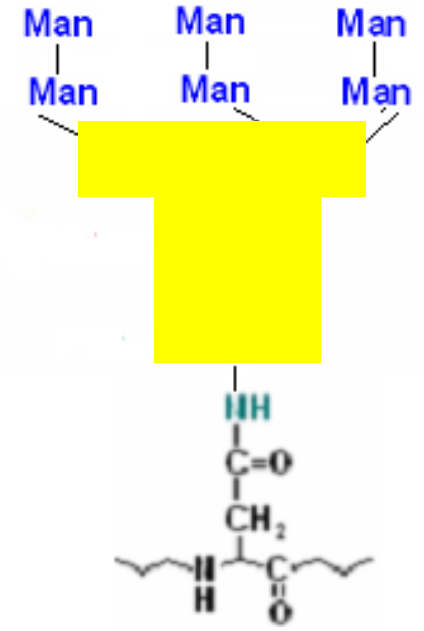
glycosylation

Late phase processing

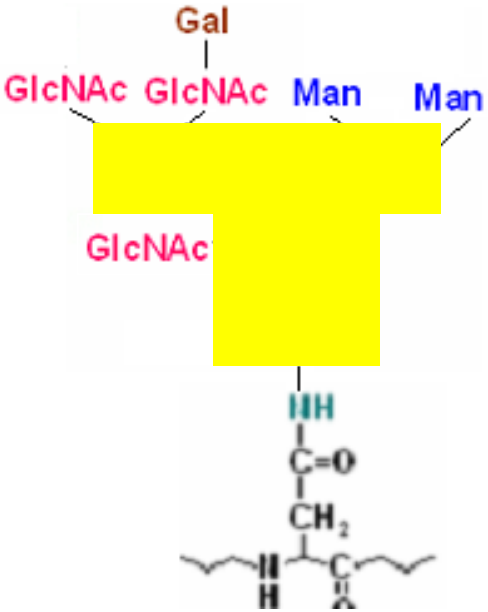
Complex type *N*-linked glycoproteins

Examples of plasma-type (*N*-linked) oligosaccharides

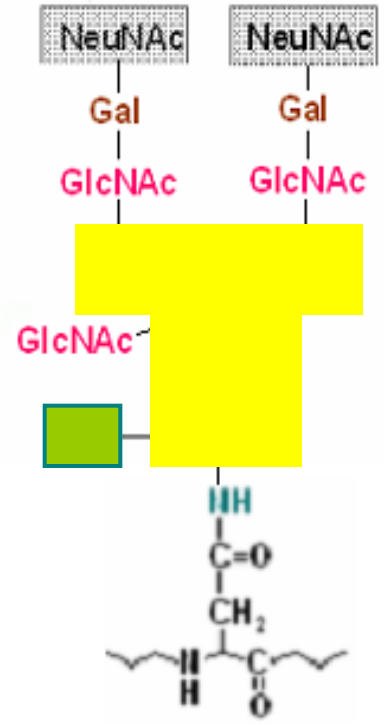
The boxed area encloses the pentasaccharide core region common to all *N*-linked glycoproteins.



High-mannose type
(before trimming to core region)



Hybride type
(one antenna)



Complex type
(triantennary chain)

Synthesis of O-glycoproteins

is **posttranslational** process that takes place exclusively in the Golgi complex and which is **direct** – glycosyls from nucleotide sugars (NuDP-glycoses) are transferred to side chains of Ser or Thr residues and elongated by other nucleotide sugars