Charging of tRNAs Wobble position

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Charging of tRNAs

For protein synthesis, the amino acids have to be carried from cytosol to ribosomes

The adaptor molecule (tRNA) is the carrier of amino acids

First, the amino acids have to be attached to tRNAs

Each tRNA has an anticodon loop

The anticodon loop contains an anti-codon

The anticodon is complementary to a particular codon

A given tRNA can combine with only one amino acid

The amino is selected according to the anticodon present on the tRNA

Binding of amino acid to tRNA is known as charging of tRNA

The binding is catalysed by amino acyl tRNA synthetase

The amino acid, ATP and the enzyme react to form amino acyl-AMP-enzyme complex

Amino acyl-AMP-enzyme complex reacts with tRNA to form amino acyl tRNA

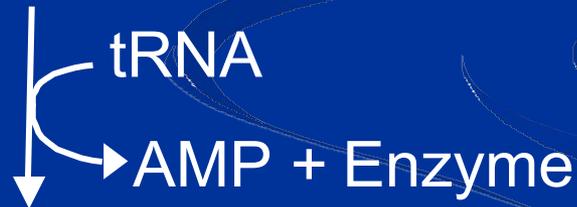Enzyme, AMP and PPi are released; PPi is hydrolysed

Amino acyl tRNA is known as charged tRNA

R−CH−COOH + ATP + Enzyme

   |
NH_2

Amino acid

H_2O

PPi $\longrightarrow$ 2 Pi

R−CH−C−AMP−Enzyme

  | ||
NH_2 O

Amino acyl-AMP-Enzyme complex

tRNA

AMP + Enzyme

R−CH−C−tRNA

  | ||
NH_2 O

Amino acyl tRNA

The tRNA carries the amino acid to ribosome

The anticodon on tRNA finds the complementary codon on mRNA

Amino acids are added in a sequence directed by the codons on mRNA

Twenty amino acids are required for protein synthesis

Therefore, there must be at least 20 species of tRNA

Amino acid is bound to 3'-end of tRNA; the anticodon is present far away

Therefore, the anticodon does not play any role in recognition of amino acid

This function is performed by the enzyme, amino acyl tRNA synthetase

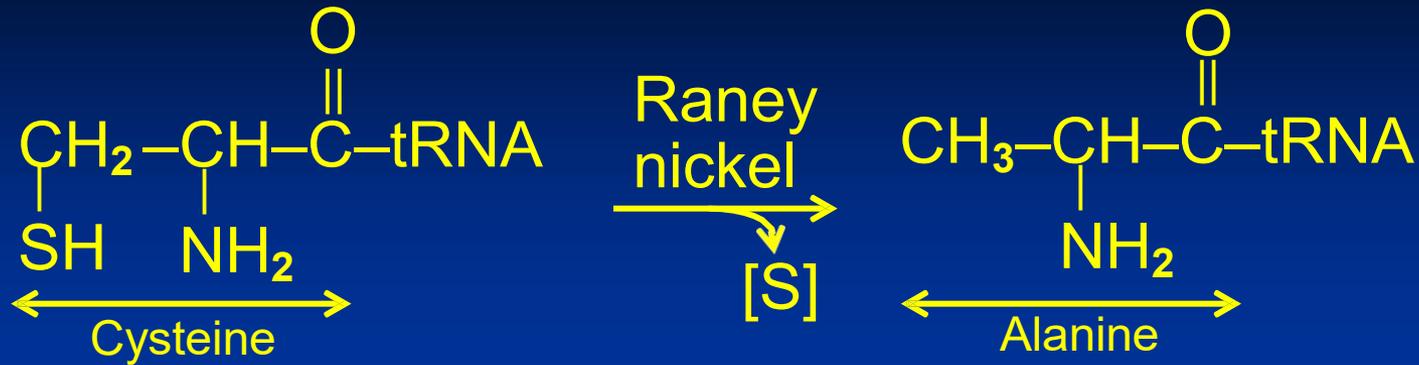There are at least 20 different species of amino acyl tRNA synthetase

Each amino acyl tRNA synthetase charges one tRNA with a specific amino acid

Once a tRNA is charged, its anticodon will find the complementary codon on mRNA

One experiment showed that amino acid has no role in recognition of codon

In this experiment, the tRNA for cysteine (tRNA$_{cys}$) was charged with cysteine

The cysteine residue was then chemically converted into an alanine residue

$$CH_2-CH-C-tRNA \xrightarrow[\ [S]\ ]{\text{Raney nickel}} CH_3-CH-C-tRNA$$

Cysteine (with SH and NH_2 groups, and C=O)

Alanine (with NH_2 group, and C=O)

Anticodon of this tRNA recognized the codon for cysteine

Therefore, this $tRNA_{cys}$ added alanine in place of cysteine during translation

Wobble

When an anticodon pairs with a codon, the first two bases of the codon are recognized precisely

Recognition of the third base is not precise; the third base may be mis-paired

This is known as wobble in base pairing

tRNA

mRNA

GAG
CUC

Normal
pairing

GAG
CUU

Wobble
in pairing

RCG
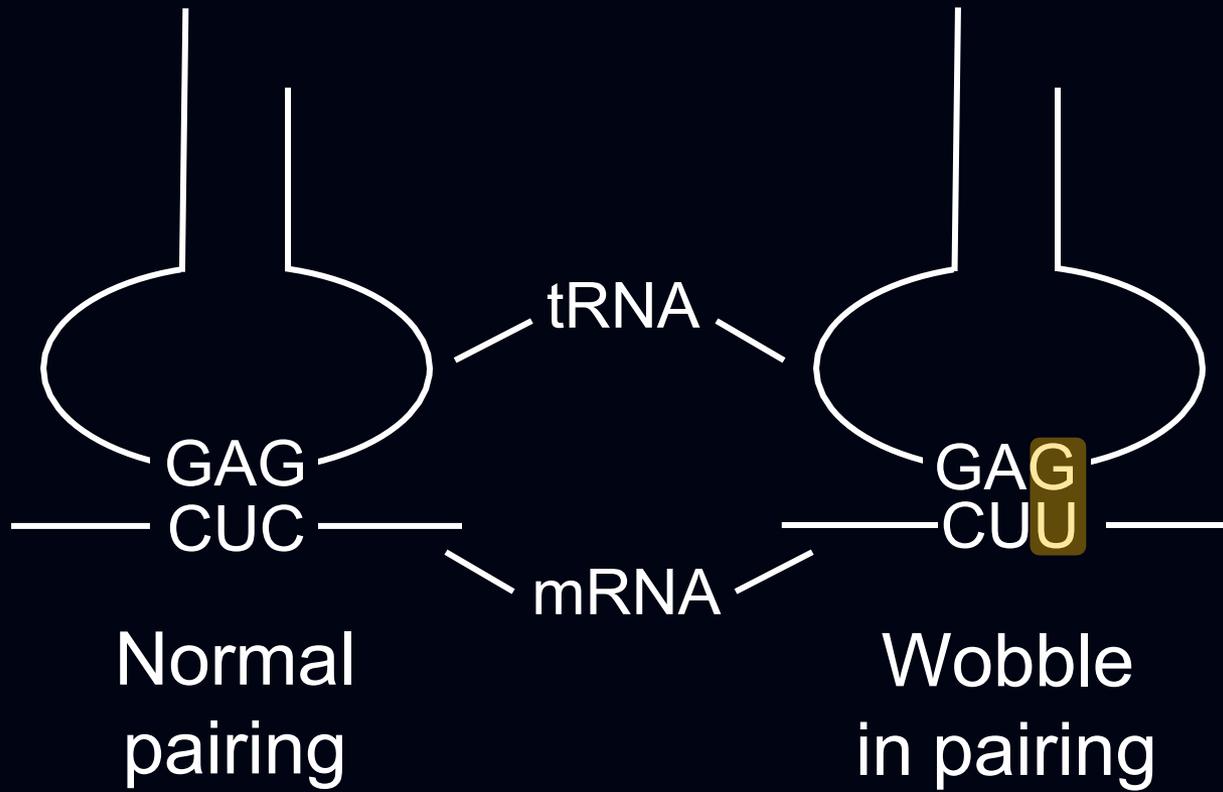
Due to degeneracy, codons usually differ in the third base

They will be read as code words for the same amino acid because of wobble

Therefore, correct amino acid will be incorporated in the protein

Translation

The actual process of protein synthesis is known as translation

Translation occurs on ribosomes

It can be divided into: (i) initiation, (ii) elongation and (iii) termination

Initiation is binding of mRNA and the first amino acyl tRNA to the ribosome

Elongation is addition of subsequent amino acids to the first one

Termination is conclusion of elongation and release of the polypeptide

Initiation

Initiation of protein synthesis requires the interaction of:

- Ribosome
- mRNA
- First amino acyl tRNA
- GTP
- ATP
- Eukaryotic initiation factors (eIFs)
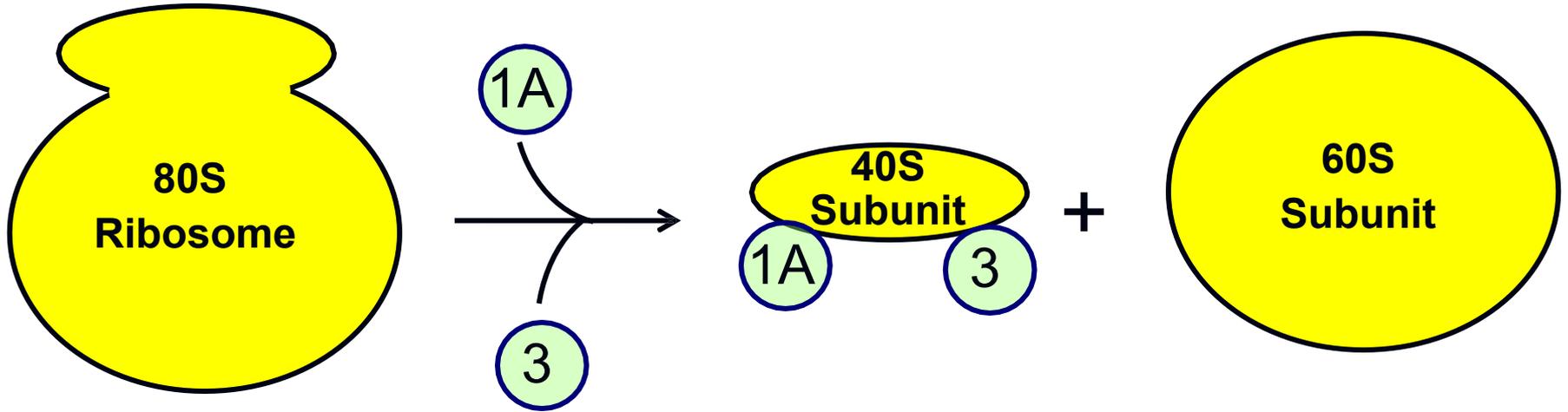
Initiation occurs in four steps:

- Dissociation of ribosomal subunits
- Formation of 43S pre-initiation complex
- Formation of 48S initiation complex
- Formation of 80S initiation complex

Dissociation of ribosomal subunits

The 80S ribosome dissociates into its 40S and 60S subunits

Dissociation occurs in the presence of eIF-1A and eIF-3

eIF-1A and eIF-3 bind to 40S subunit, and prevent its re-association with 60S subunit
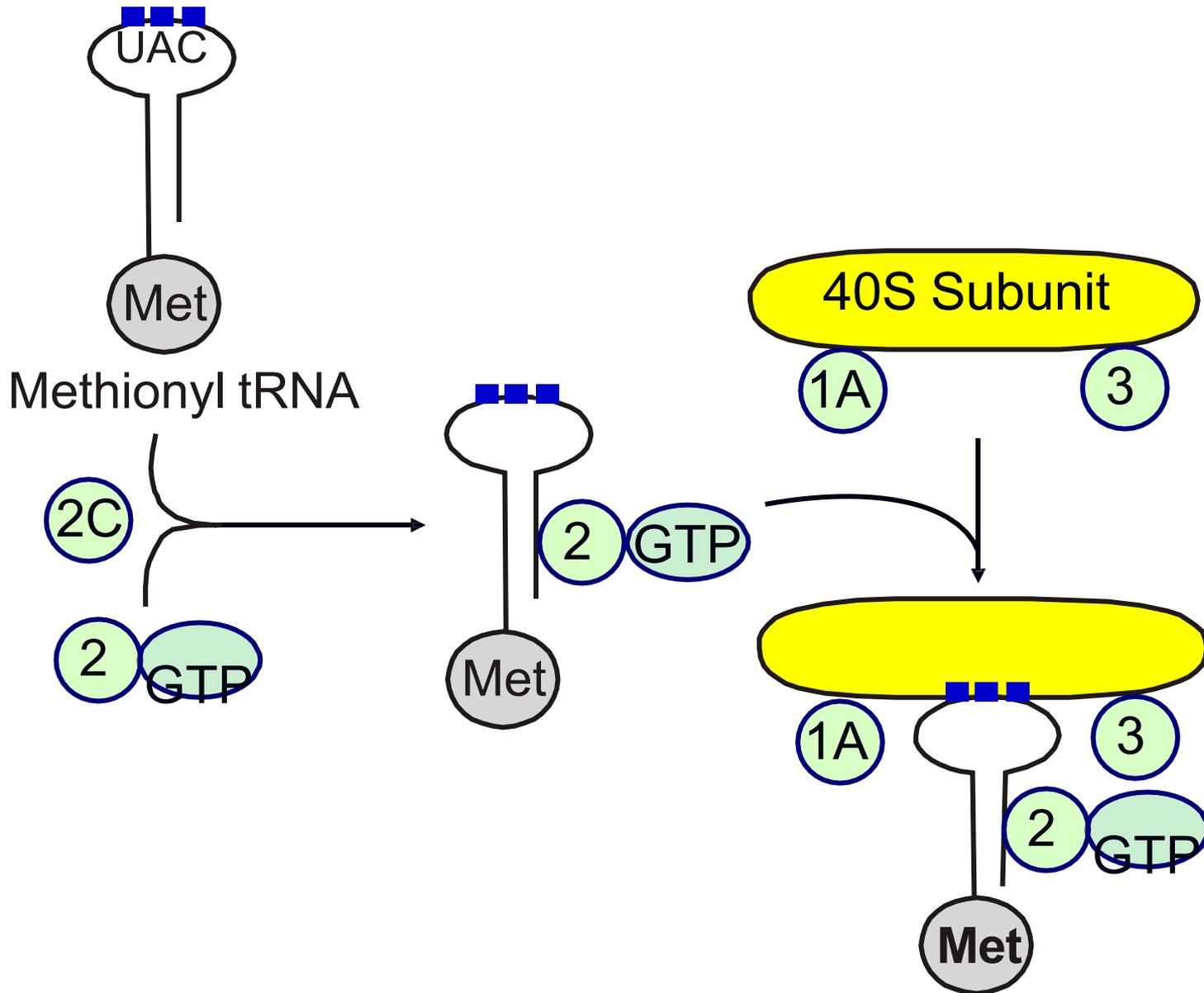
Formation of 43S pre-initiation complex

In the presence of eIF-2C, eIF-2 and GTP bind to the first amino acyl tRNA

In eukaryotes, the first amino acyl tRNA is always methionyl tRNA

This complex binds to 40S subunit to form the 43S pre-initiation complex

UAC

Met

Methionyl tRNA
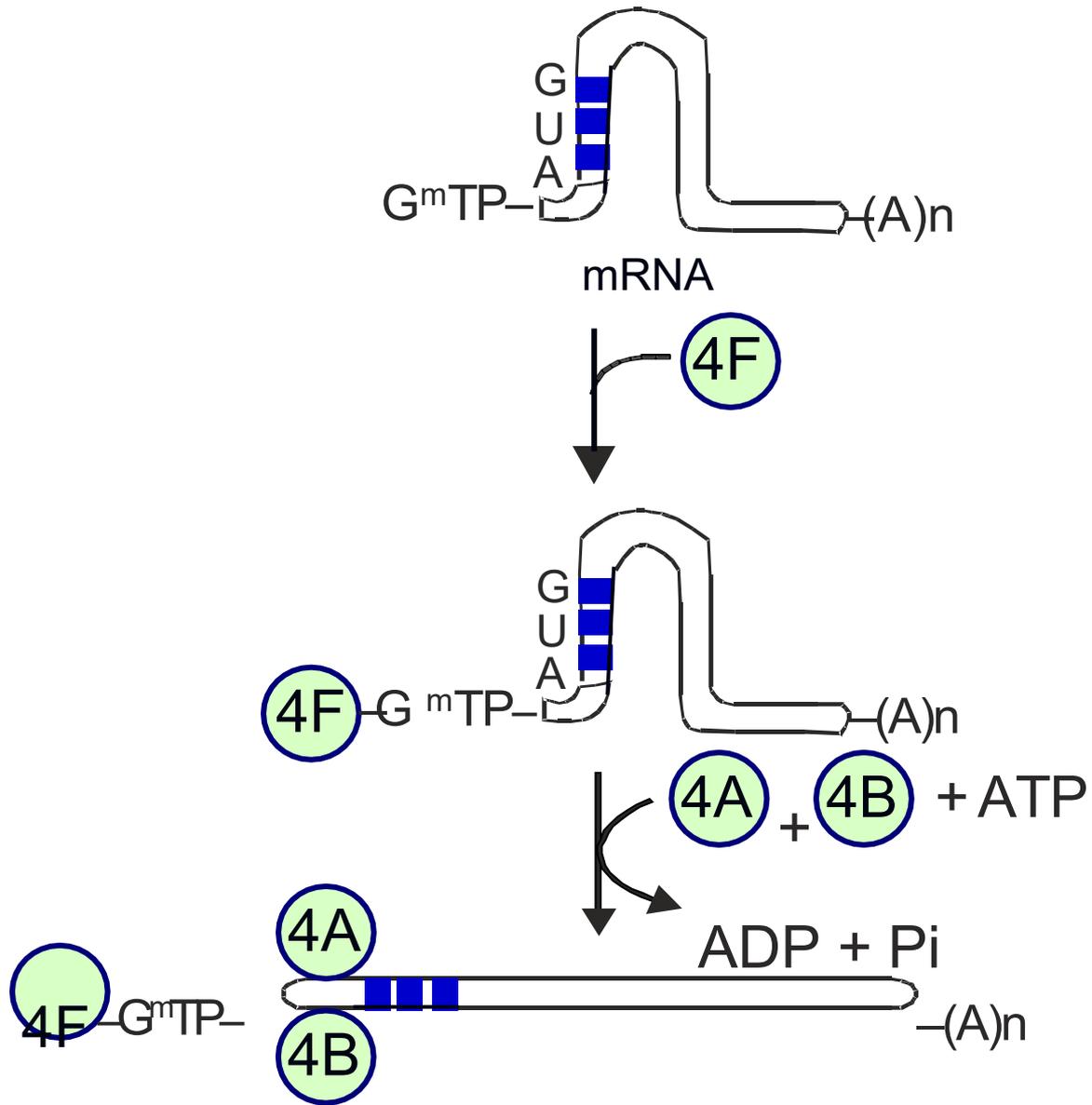
2C

2 GTP

40S Subunit

1A   3

2 GTP

Met

1A   3

2 GTP

Met

43S Pre-initiation complex

RCG

Formation of 48S initiation complex

eIF-4F binds to the 5' cap of mRNA
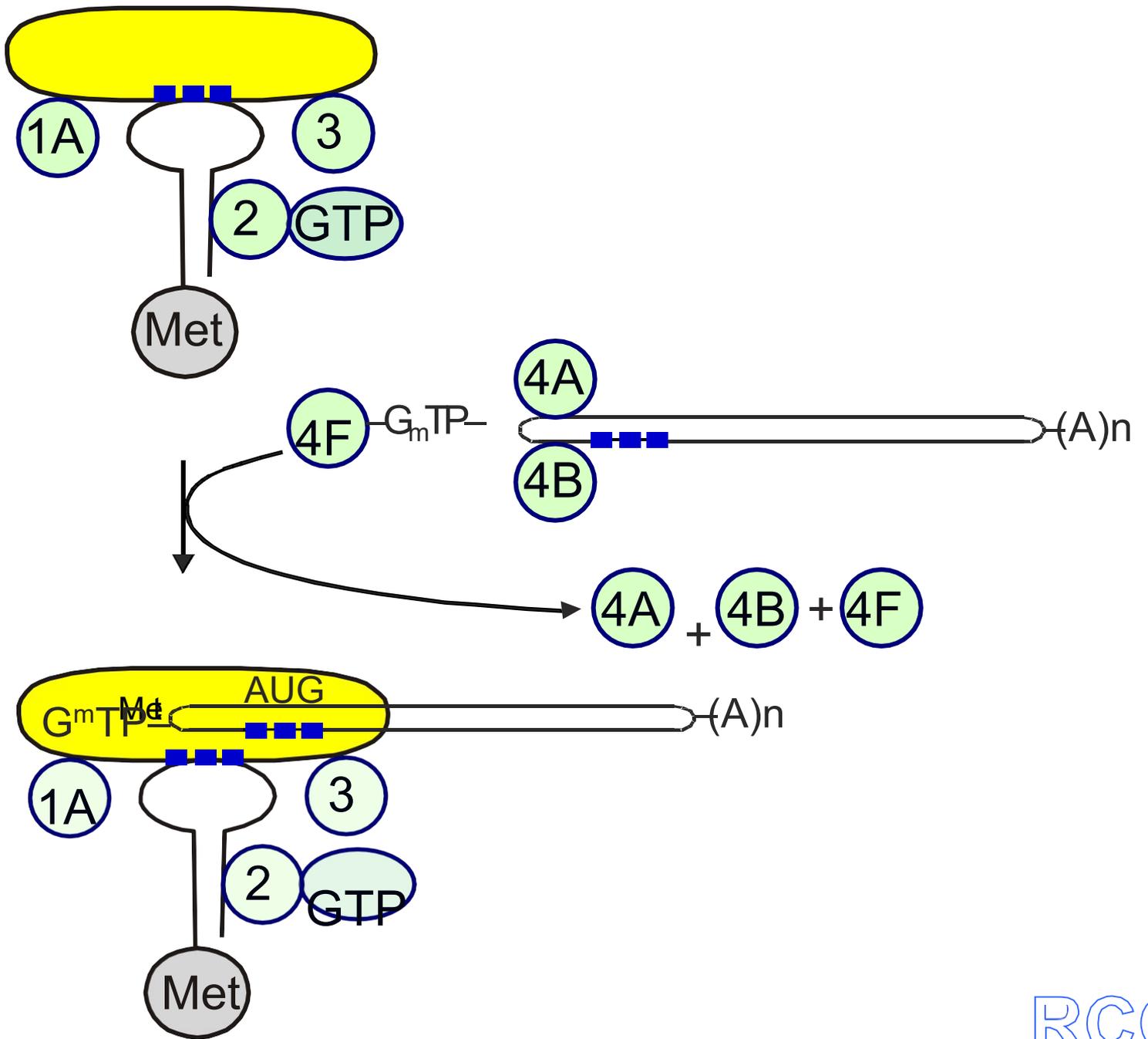
eIF-4A and eIF-4B bind to mRNA in the presence of ATP

IF-4A hydrolyses ATP intoADP and Pi

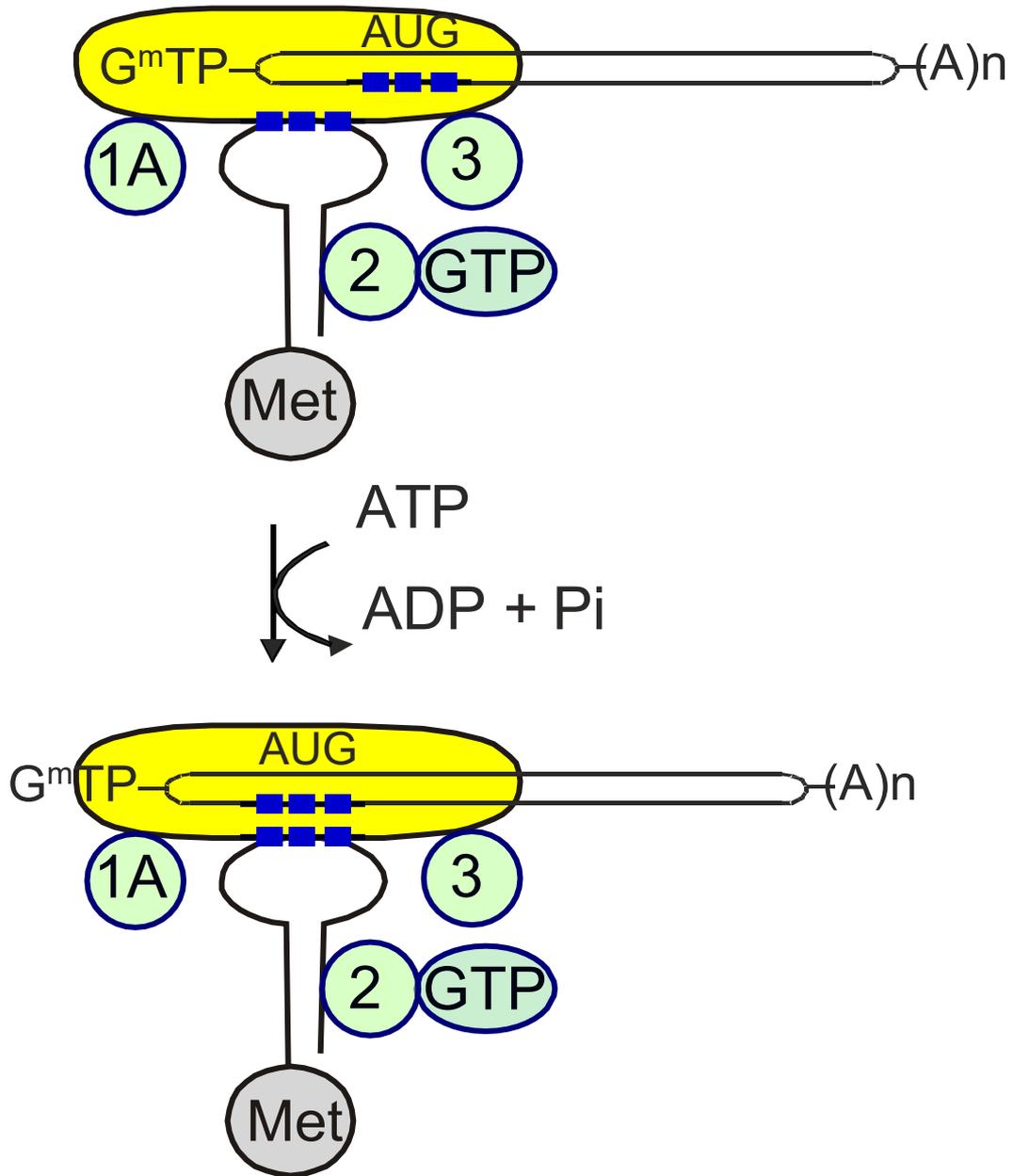Using this energy, eIF-4B uncoils the mRNA near its 5'-end

The mRNA binds to the 40S ribosomal subunit

eIF-4A, eIF-4B and eIF-4F are released

40S subunit moves along the mRNA until AUG is opposite the anticodon of methionyl tRNA

This complex is known as the 48S initiation complex

ATP

ADP + Pi

48S Initiation complex

Formation of 80S initiation complex

In the presence of eIF-5, 60S ribosomal subunit binds to 40S subunit to form the 80S initiation complex

80S Initiation complex consists of 80S ribosome, mRNA and methionyl tRNA

After the formation of 80S initiation complex, all the eIFs are released

The GTP attached to eIF-2 is hydro-lysed into GDP and Pi
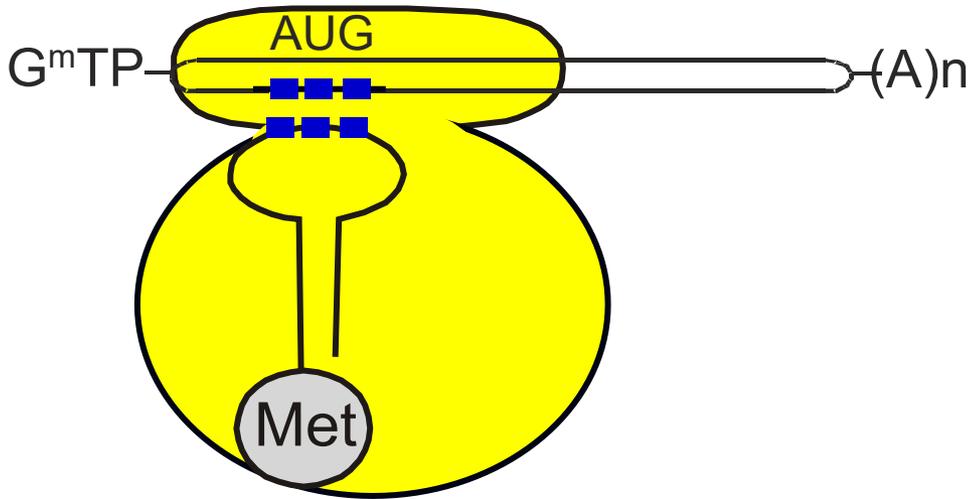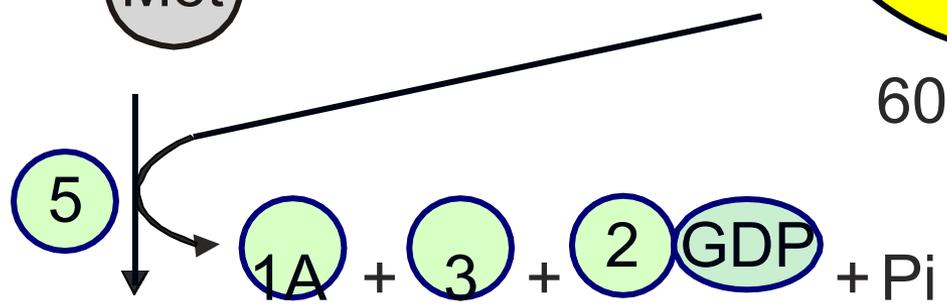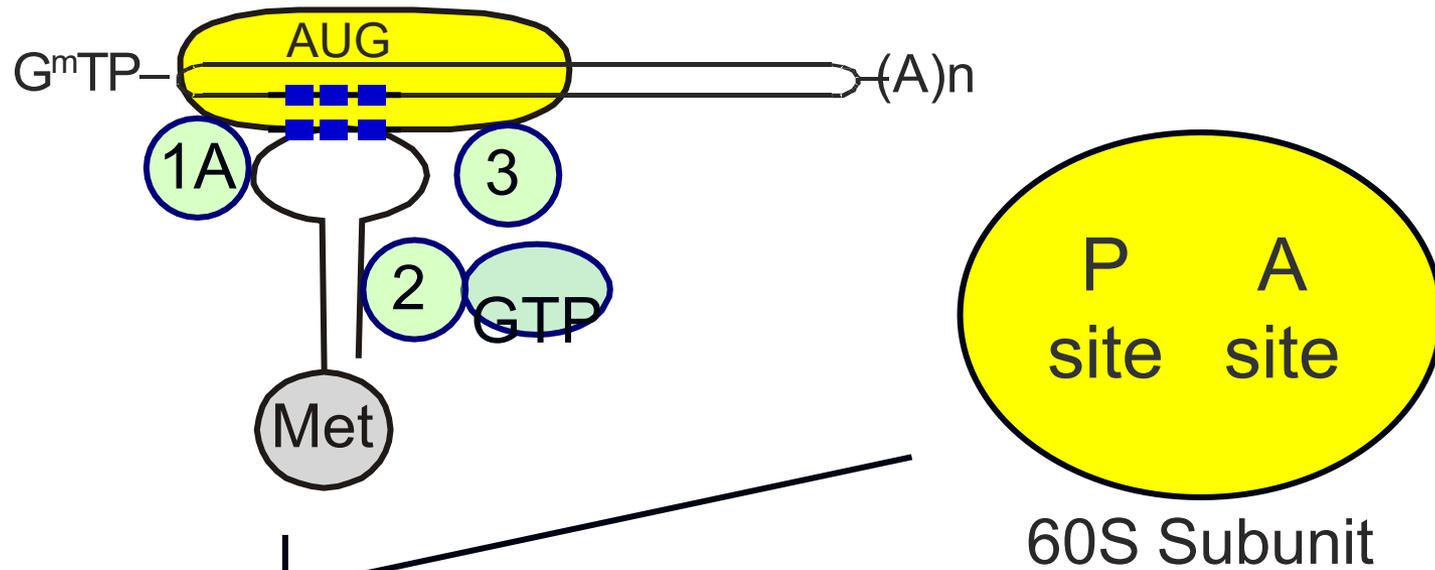
GᵐTP AUG (A)n
1A 3 2 GTP Met
60S Subunit P site A site
5 1A + 3 + 2 GDP + Pi
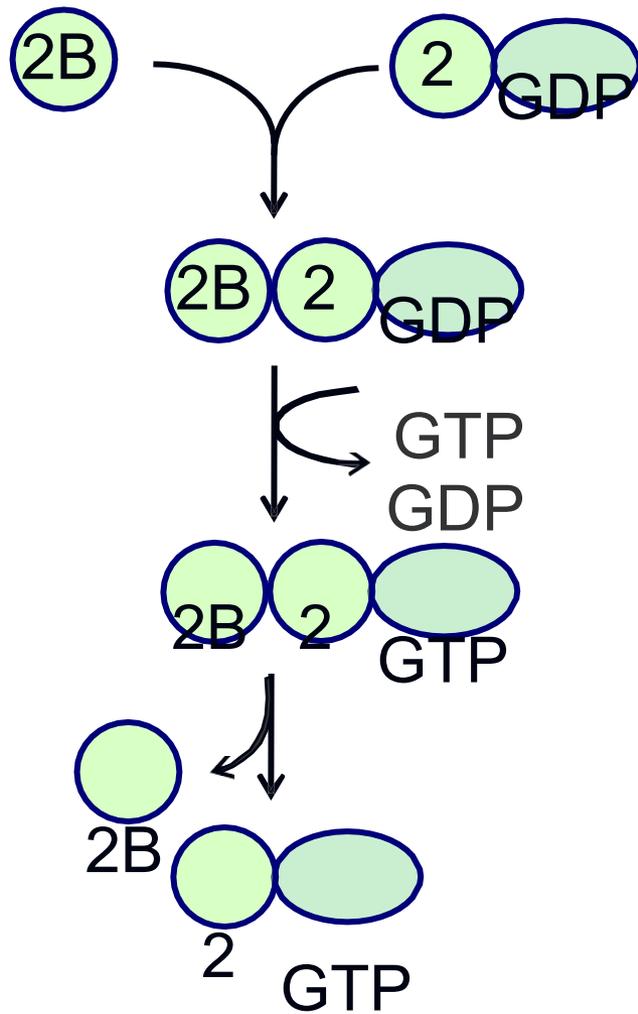GᵐTP AUG (A)n Met
80S Initiation complex

GDP attached to eIF-2 is released

It is replaced by GTP
This occurs in the presence of eIF-2B

A new cycle of initiation can start now

Elongation

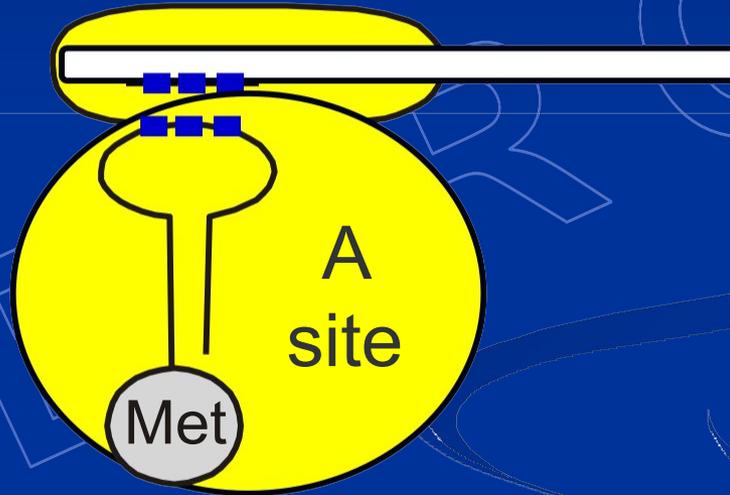**Elongation requires:**

Amino acyl tRNAs

GTP

Eukaryotic elongation factors (eEFs)

Eukaryotic elongation factors are:

eEF-1A

eEF-2

The 60S ribosomal subunit has got P (peptidyl) site and A (amino acyl) site

A site

Met

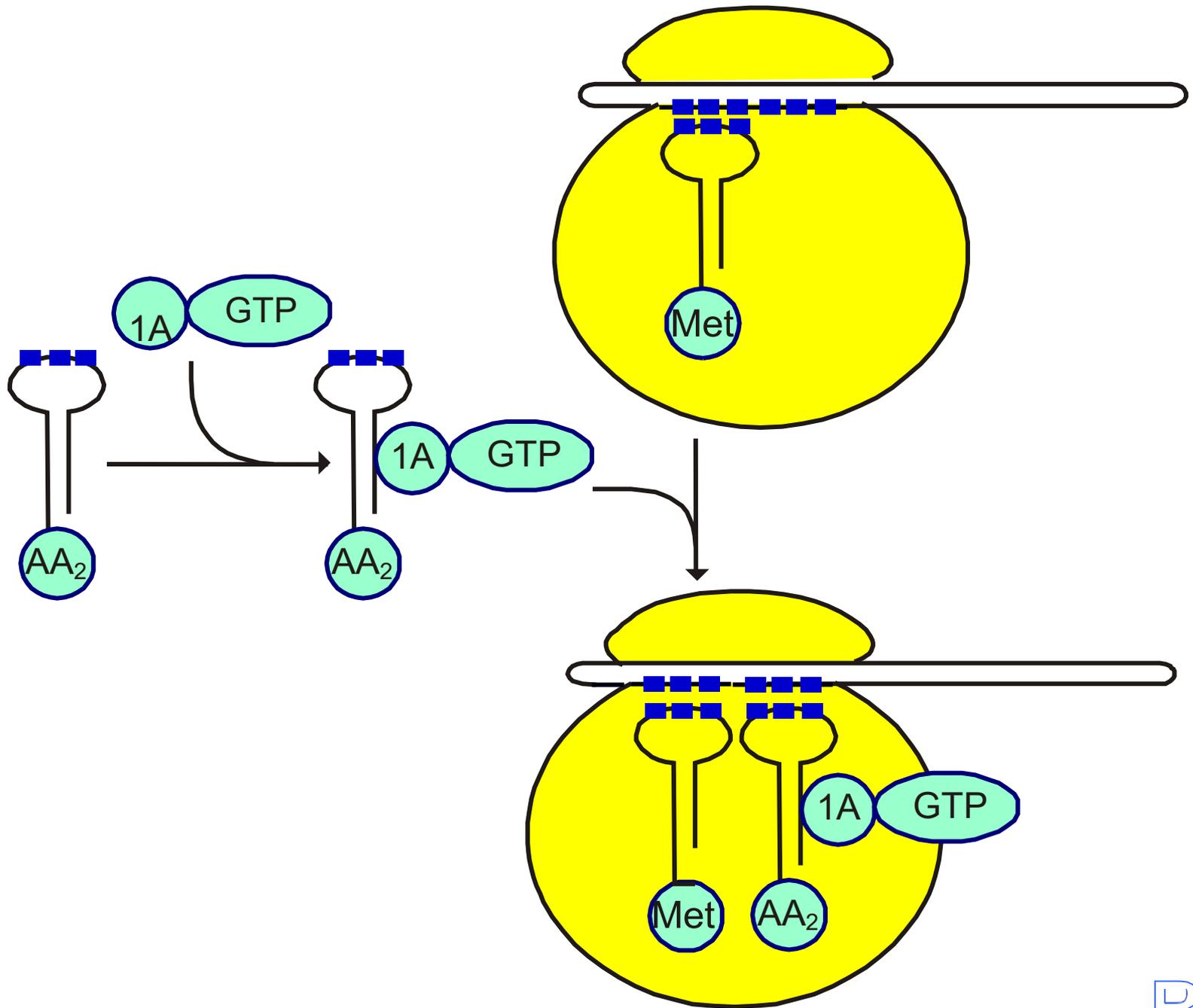After initiation, P site is occupied by methionyl amino acyl tRNA; A site is vacant

Amino acyl tRNA having anticodon complementary to the second codon comes

eEF-1A and GTP are attached to the amino acyl tRNA

This complex binds to the ribosome; the second amino acid is at the A site

After binding of the second amino acyl tRNA to ribosome:

GTP is hydrolysed

eEF-1A:GDP complex and Pi are released

The 60S ribosomal subunit possesses peptidyl transferase activity

This activity is present in the 28S rRNA which is a ribozyme

This enzyme forms a peptide bond between carboxyl group of first amino acid and amino group of second amino acid
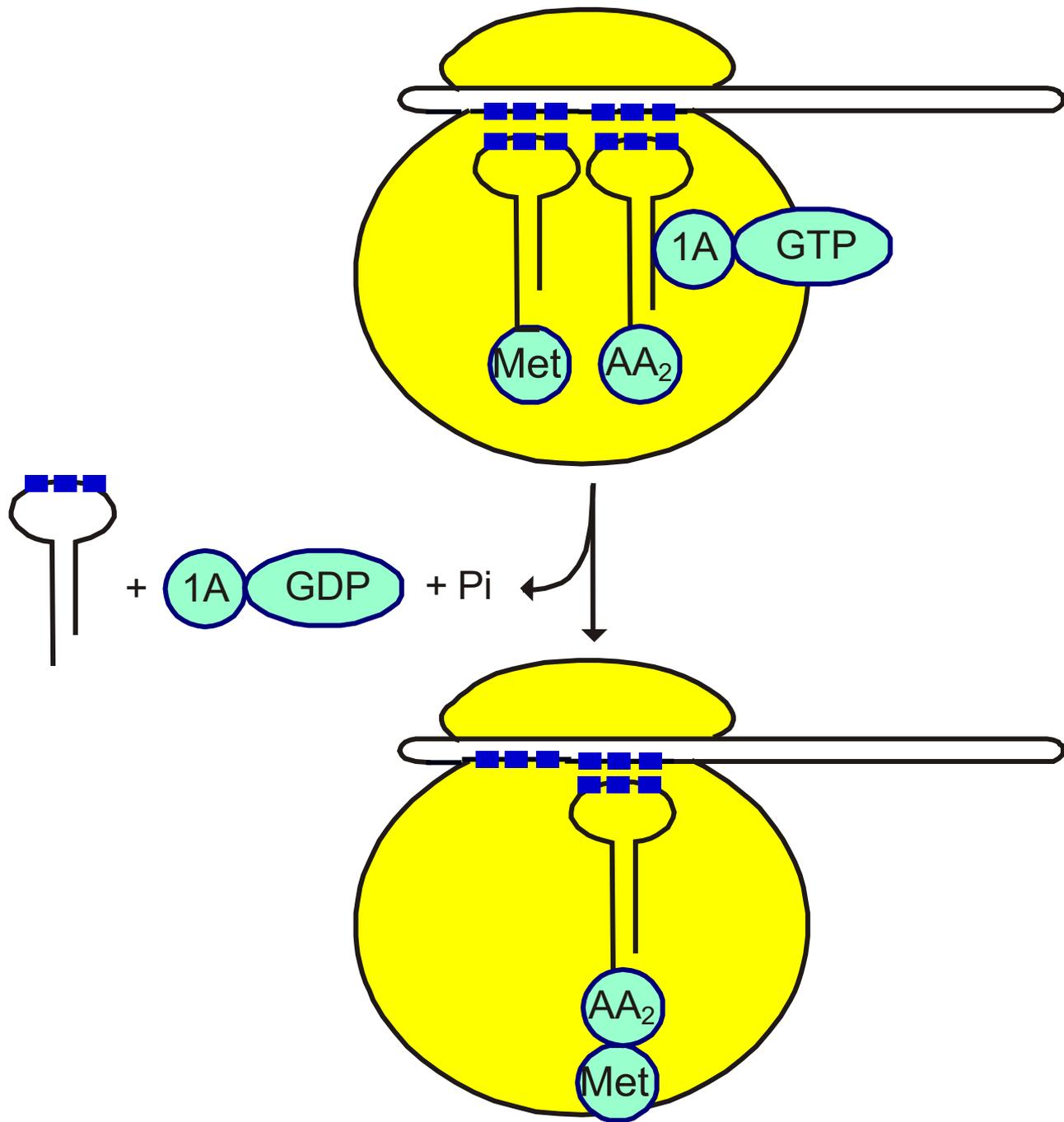
The dipeptide that is formed is attached to the second tRNA

The first tRNA, which is now free, is released

The P site becomes vacant

P
site

AA$_2$

Met

RCG
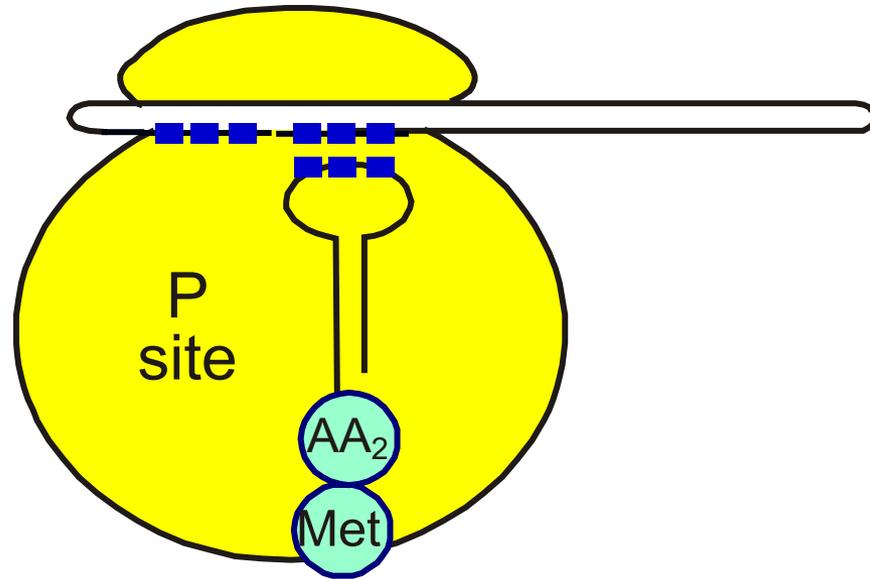
eEF-2 translocates the mRNA along the ribosome by one-codon distance

The dipeptide moves to the P site, and the A site becomes vacant

Hydrolysis of GTP into GDP and Pi provides the energy for translocation

P
site

AA$_2$

Met

2 GTP

2 GDP + Pi

A
site

AA$_2$

Met

RCG
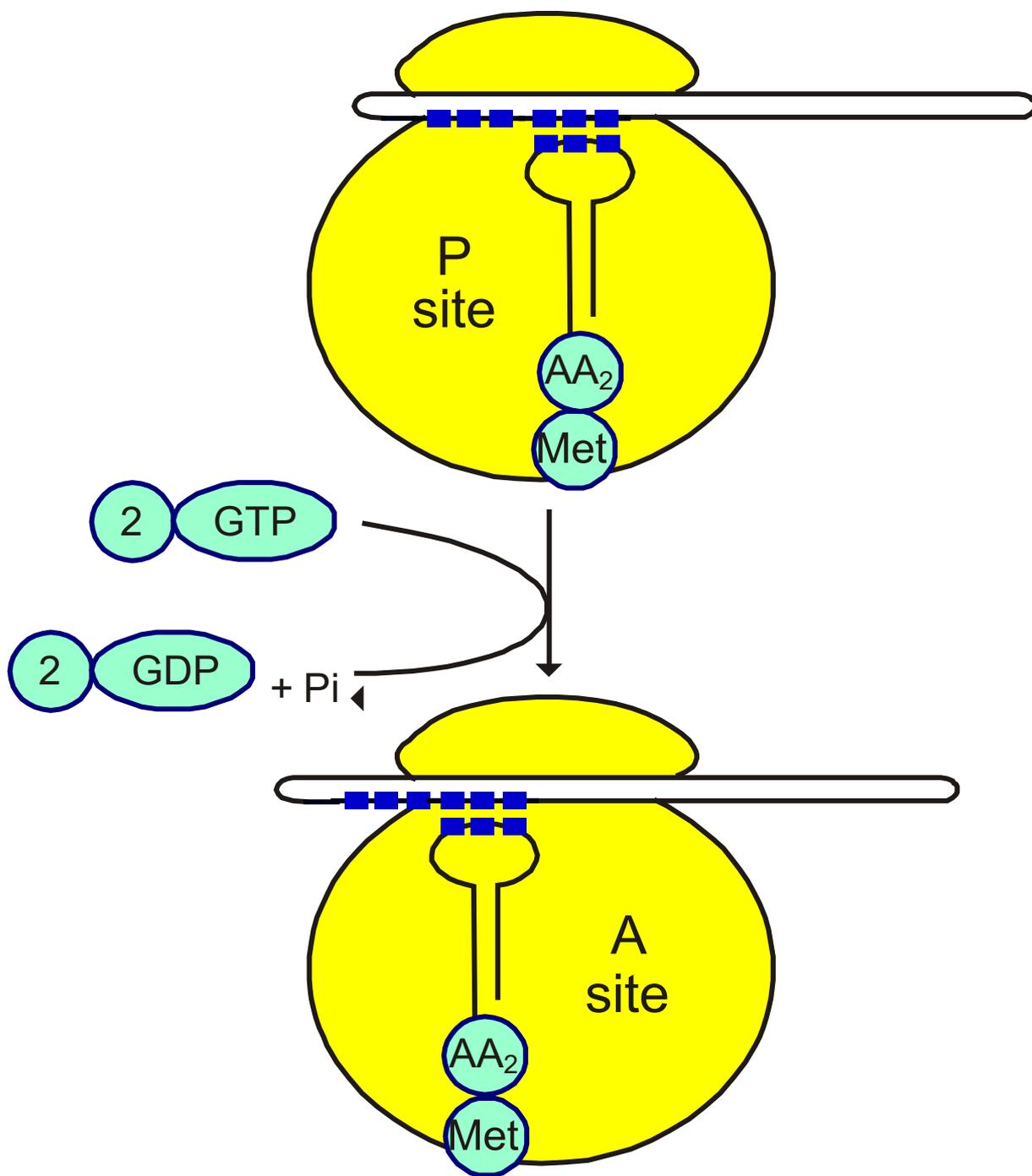
A new cycle of elongation begins

The dipeptide is converted into a tri-peptide

This process continues until all the codons on mRNA have been translated

Four ATP equivalents are spent for forming each peptide bond:

Two for charging of tRNA

Two for each cycle of elongation

Termination

Termination occurs when a nonsense codon appears on the mRNA

Nonsense codons have no comple-mentary anticodons

Nonsense codon cannot be recognized by any tRNA

When there is a nonsense codon opposite A site, the A site cannot be occupied by any amino acyl tRNA

Instead, this site is occupied by a eukaryotic releasing factor (eRF) and GTP

In the presence of eRF and GTP, peptidyl transferase has a different catalytic activity

It hydrolyses the bond between the carboxyl group of the last amino acid and the tRNA

The polypeptide and the mRNA are released from the ribosome

GTP is hydrolysed into GDP and Pi, which are released

The eRF and the last tRNA are released

The ribosome becomes free

STOP

GTP + eRF

STOP

eRF
GTP

eRF + GDP + Pi ← → Polypeptide + tRNA + Ribosome

mRNA

RCG
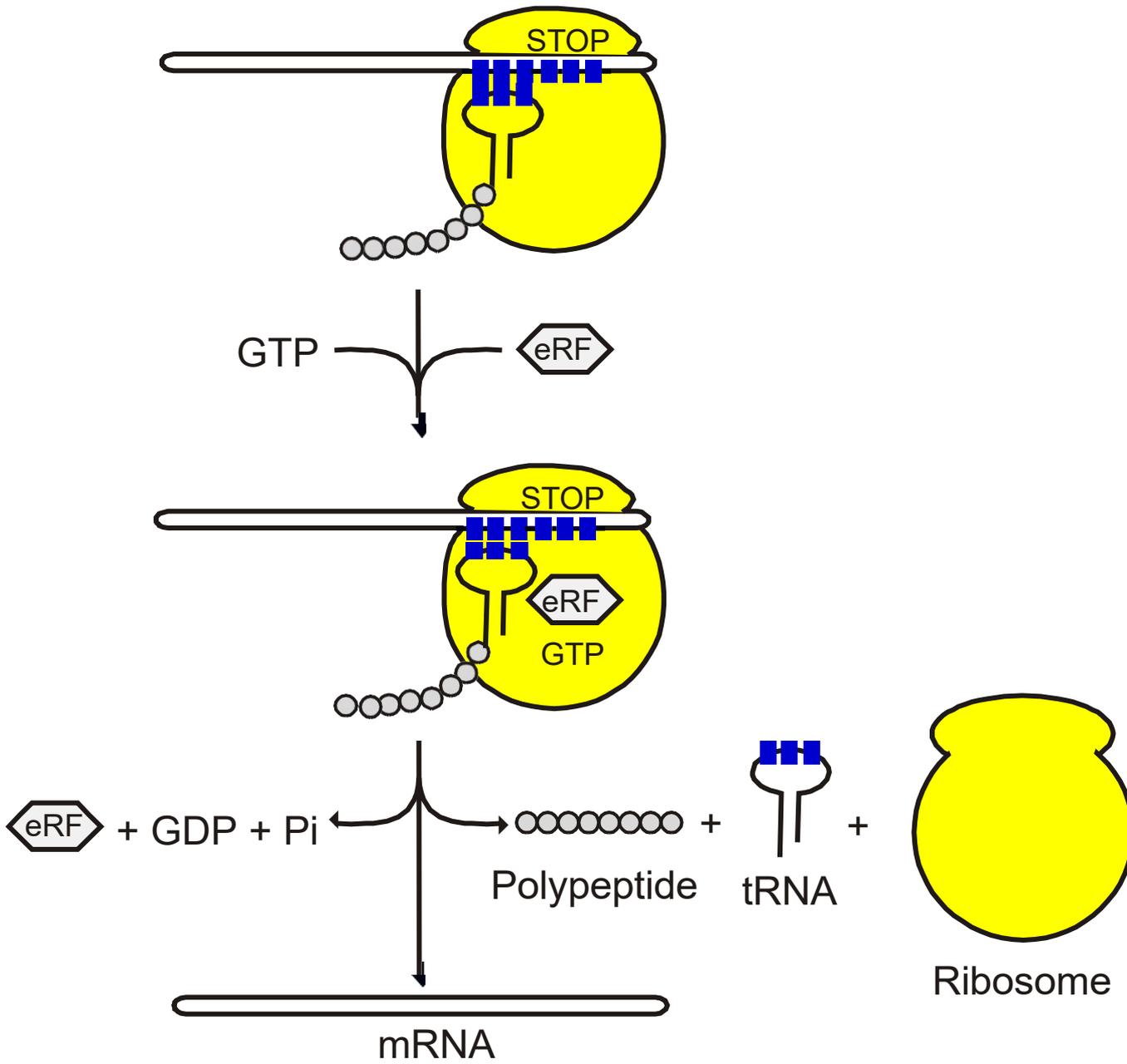
Polysome

As chain elongation occurs, the 5'-end of mRNA emerges from the ribosome

A new ribosome can attach to it

Thus, several ribosomes can translate the mRNA simultaneously

A number of ribosomes attached to a mRNA constitute a polyribosome or polysome

5'   **3'**
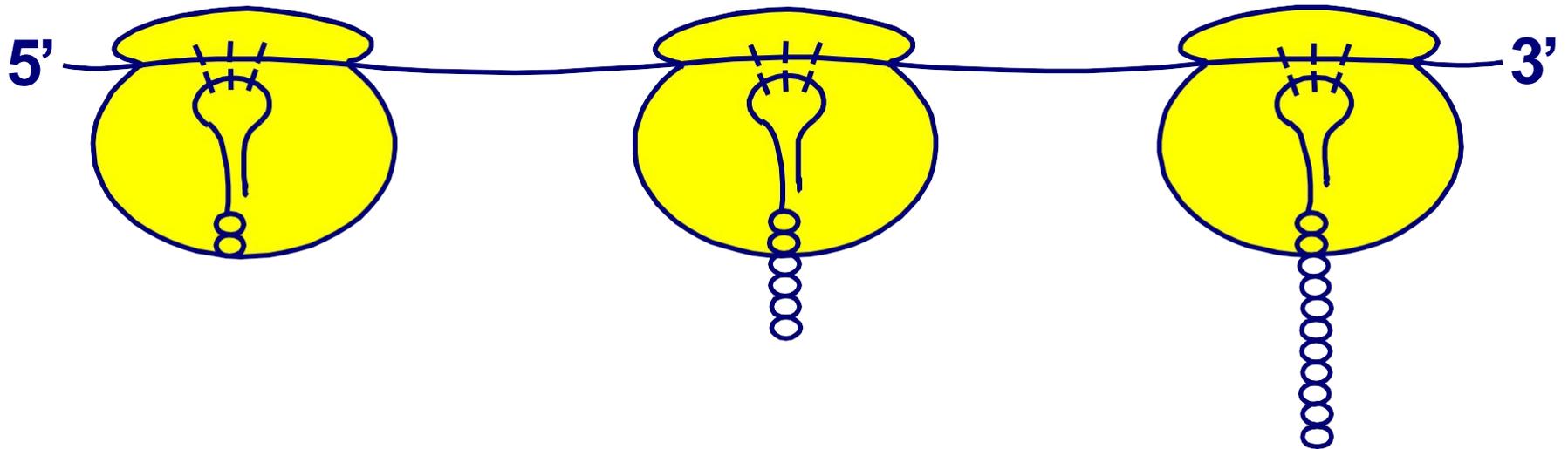
Polysome

Post-translational modifications

Many newly-synthesized proteins are functionally inactive

They require some modifications in their structure before they become active

These modifications are known as post-translational modifications

Common post-translational modifications are:

- Cleavage
- Hydroxylation
- Carboxylation
- Phosphorylation
- Glycosylation
- Addition of other prosthetic groups

Cleavage

The nascent protein may contain some extra amino acids which are removed

An example is removal of connecting peptide from proinsulin to form insulin
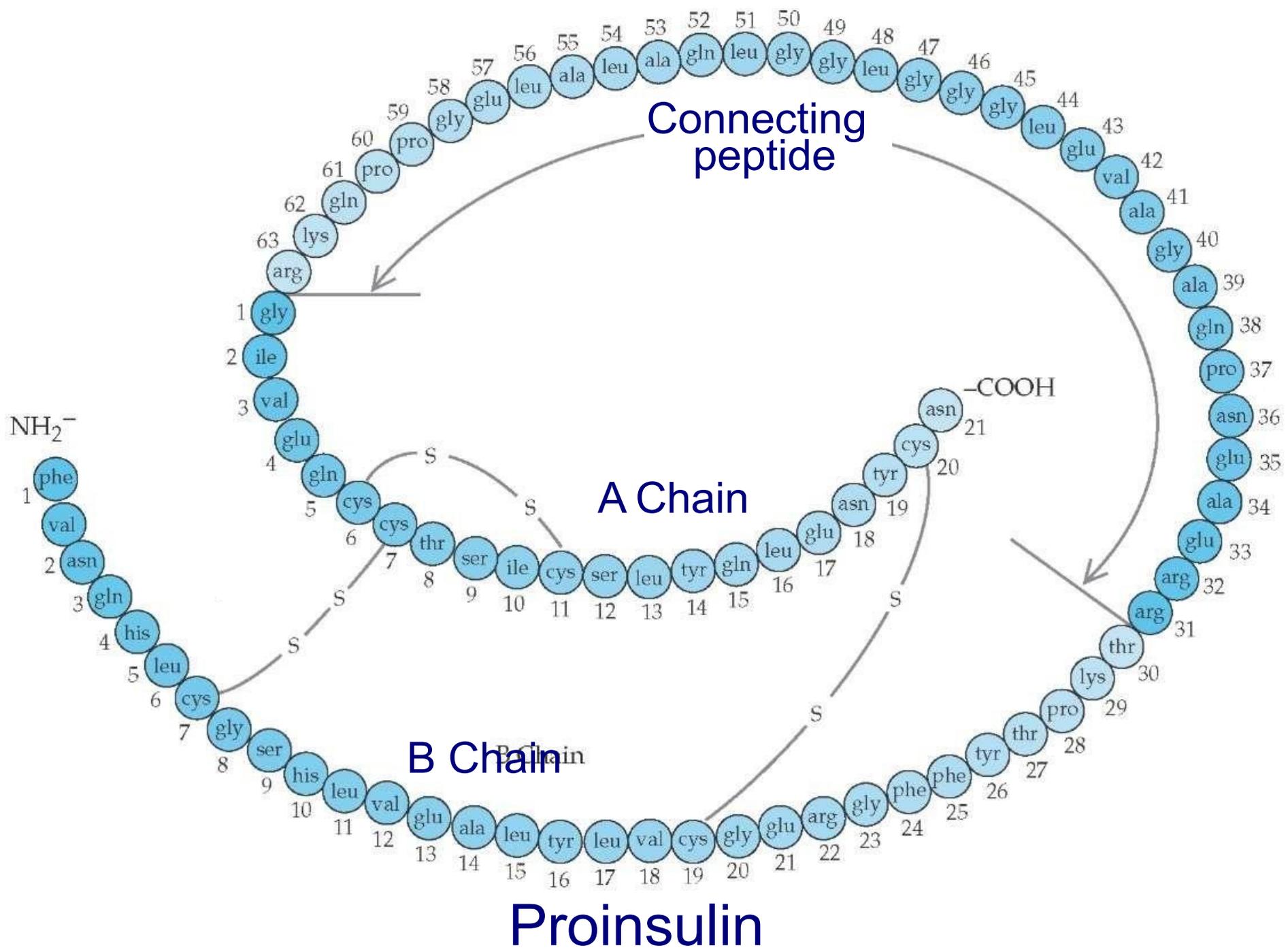
Connecting peptide

A Chain

B Chain

Proinsulin

S—S

COOH

S

S

H₂N

S

S

Proinsulin

A-chain

S—S

S

S

B-chain

S

S
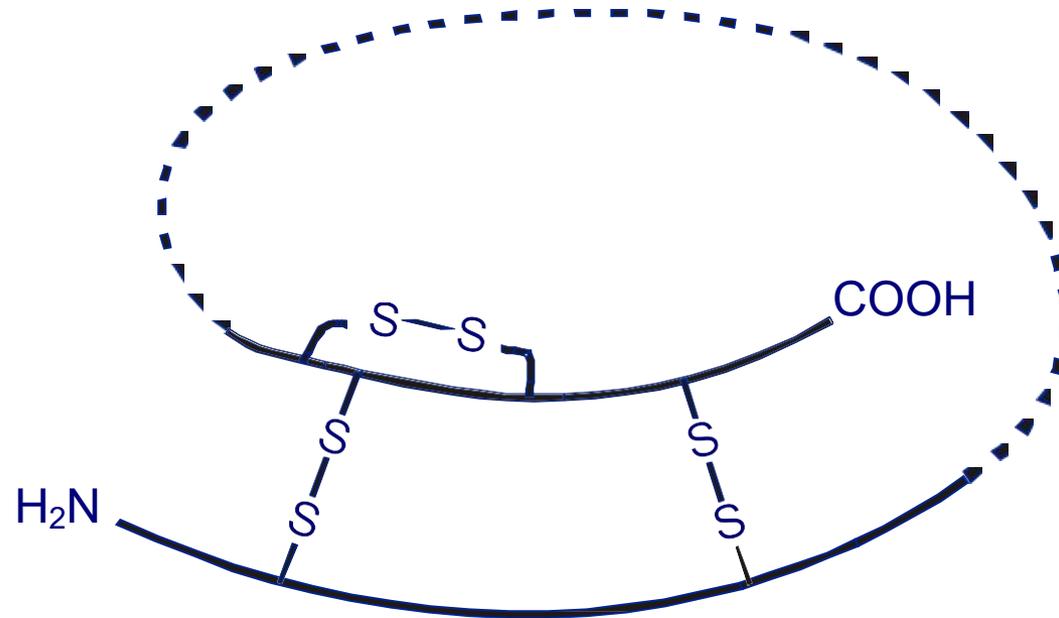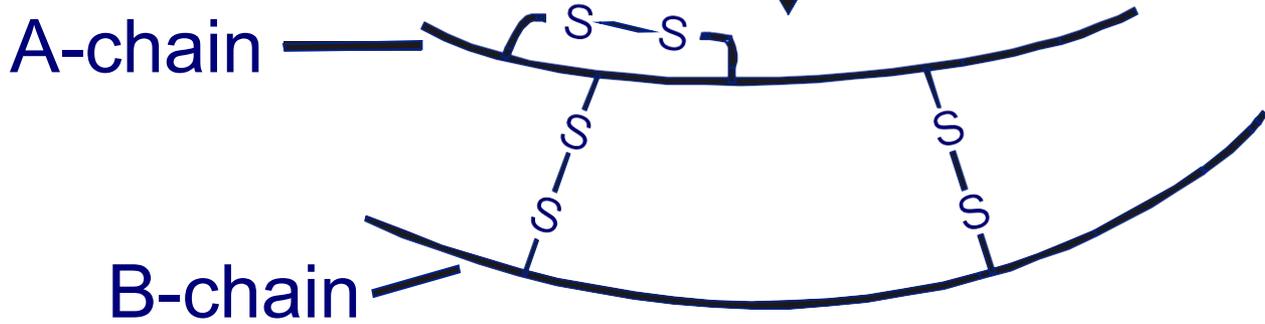
Insulin

Another example of cleavage is pro-opio-melano-cortin (POMC)

Alternative cleavage of POMC yields eight different peptides

Hydroxylation

Some amino acid residues, e.g. proline and lysine, may be hydroxylated after translation

Hydroxylation of proline and lysine residues is important in the conversion of pro-collagen into collagen

Carboxylation

Glutamate residues of pre-prothrombin are carboxylated after translation

This converts pre-prothrombin into prothrombin

Glutamate residues of several other proteins are also carboxylated

Phosphorylation

Serine, threonine and tyrosine residues of some proteins can be phosphorylated

Phosphorylation is a method used for regulating the activity of some enzymes

Phosphorylation of tyrosine residues is important in intracellular signaling

Glycosylation

Carbohydrate prosthetic groups are added to many proteins

Examples are mucin, immunoglobulins, hCG etc

These can form O-linked, N-linked and GPI-linked glycoproteins

The linkage between carbohydrate portion and protein occurs through:

Hydroxyl group of serine in O-linked glycoproteins

Amide group of asparagine in N-linked glycoproteins

Phosphatidyl inositol in GPI-linked glycoproteins

Addition of other prosthetic groups

Several other groups are added to proteins after translation e.g.

Haem

Flavin nucleotides

Biotin

Retinal

Metals

Prokaryotic translation

Prokaryotic translation differs from eukaryotic translation in:

- Primary transcript
- Number of cistrons
- Number of initiation sites
- Initiator amino acid
- Initiation factors
- Elongation factors
- Releasing factors

Primary transcript

In prokaryotes, the primary transcript is mRNA which is directly translated

Translation can begin even before the transcription is complete

Ribosomes can bind to partially synthe-sized mRNA , and protein synthesis can begin

Number of cistrons

A cistron is the coding unit for one polypeptide

Some of the prokaryotic mRNAs are polycistronic

Therefore, a number of polypeptides can be formed from one mRNA

Number of initiation sites

If the mRNA is polycystronic, it will have more than one initiation sites

AUG is the initiator codon in pro-karyotes also

Besides initiator AUG, there may be AUG codons for internal methionine residues

AUG codons can be distinguished by Shine-Dalgarno sequence which is:

A short purine-rich sequence

Present just upstream of initiator AUG

Shine-Dalgarno sequence

5'------AGGAGGU–NNNNN–AUG---

Shine-Dalgarno sequence is present on mRNA

A 3-9 base complementary sequence is present in the prokaryotic16S rRNA

The 16S rRNA binds to the Shine-Dalgarno sequence of mRNA

It positions the mRNA correctly on the ribosome for initiation of translation

Shine-Dalgarno sequence / Initiator codon

mRNA 5'------AGGAGGU—NNNNN—AUG---

16S rRNA 3'—AUUCCUCCACUAGGGAGGU---

In prokaryotes as well as eukaryotes, initiator amino acid is methionine

But in prokaryotes, initiator methionine is formylated

It becomes formylmethionine

Formylation of methionine is catalysed by formyl transferase

The formyl group is provided by N^{10}-formyl-tetrahydrofolate

Formylation occurs after methionine has been bound to its tRNA

Initiation factors

There are only three initiation factors in prokaryotes

These are IF-1, IF-2 and IF-3

Elongation factors

There are three elongation factors in prokaryotes

These are EF-ts, EF-tu and EF-G

EF-G is analogous to eEF-2 of eukaryotes

Releasing factors

There are three releasing factors in prokaryotes

These are RF-1, RF-2 and RF-3

Either RF-1 and RF-3 or RF-2 and RF-3, are required to terminate translation

Protein folding

The newly formed primary structure has to acquire:

Secondary structure

Tertiary structure

Sometimes quaternary structure

This requires extensive folding of the polypeptide chain

If a correct primary structure has been formed:

- The nascent protein will fold by itself
- It will attain higher orders of structure
- It will attain the correct conformation

However, spontaneous folding is a slow process

Enzymes involved in protein folding

Protein disulphide isomerase	It ensures that the disulphide bonds are formed between the correct cysteine residues
Peptidyl prolyl *cis-trans* isomerase	It ensures that the bonds involving proline residues are *cis* or *trans* as required

The chaperone proteins are:

Heat shock proteins 40 and 70
(HSP 40 and HSP 70) in cytosol

Heat shock proteins 10 and 60
(HSP 10 and HSP 60) in mitochondria

Calnexin and calreticulin
in endoplasmic reticulum

The chaperonins include:

Binding immunoglobulin Protein (BiP)

TCP-1 Ring Complex (TRiC)

Protein targeting

The proteins synthesized on ribosomes have different destinations such as:

- Mitochondria
- Lysosomes
- Nucleus
- Cell membrane
- Export from the cell

The signal that directs the protein to its destination is inbuilt in the protein molecule
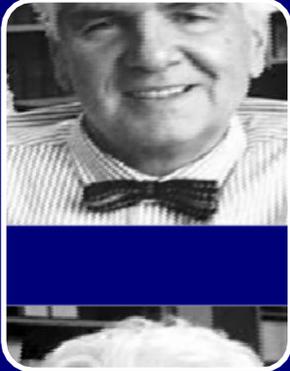
This signal is like an address written on the protein molecule

Addition of mannose-6-phosphate to a protein directs it to lysosomes

If mannose-6-phosphate is not added, lysosomal enzymes fail to reach the lysosomes

This results in inclusion cell disease

Signal hypothesis

Gunter Blobel showed how proteins are exported from the cell

He proposed the signal hypothesis which is now proven

Presence of signal sequence directs the protein to cell membrane or export outside the cell

Signal sequence is also known as leader peptide or signal peptide

It is a sequence of 15-30 amino acids at the N-terminus of the protein

When signal sequence emerges from the ribosome, it is recognized by signal recognition particle

Signal recognition particle (SRP) is a complex of 7S RNA and six polypeptides

SRP binds the signal sequence

SRP also binds to SRP receptor on the endoplasmic reticulum (ER)

SRP receptor is also known as the docking protein

It is present on the external side of ER

The ribosome synthesizing the protein is bound to a ribosome receptor on ER

Ribosome receptor is present on the external surface of ER

The signal sequence is directed into ER through translocon

Translocon is a protein-conducting channel

The signal sequence is split off by signal peptidase present in ER

The nascent protein is usually glycosyl-ated and transferred to Golgi apparatus

From there, it is either directed to cell membrane or is exported from the cell

Signal sequence

Ribosomes

SRP

SRP receptor

Signal peptidase

Ribosome receptor

Endoplasmic reticulum
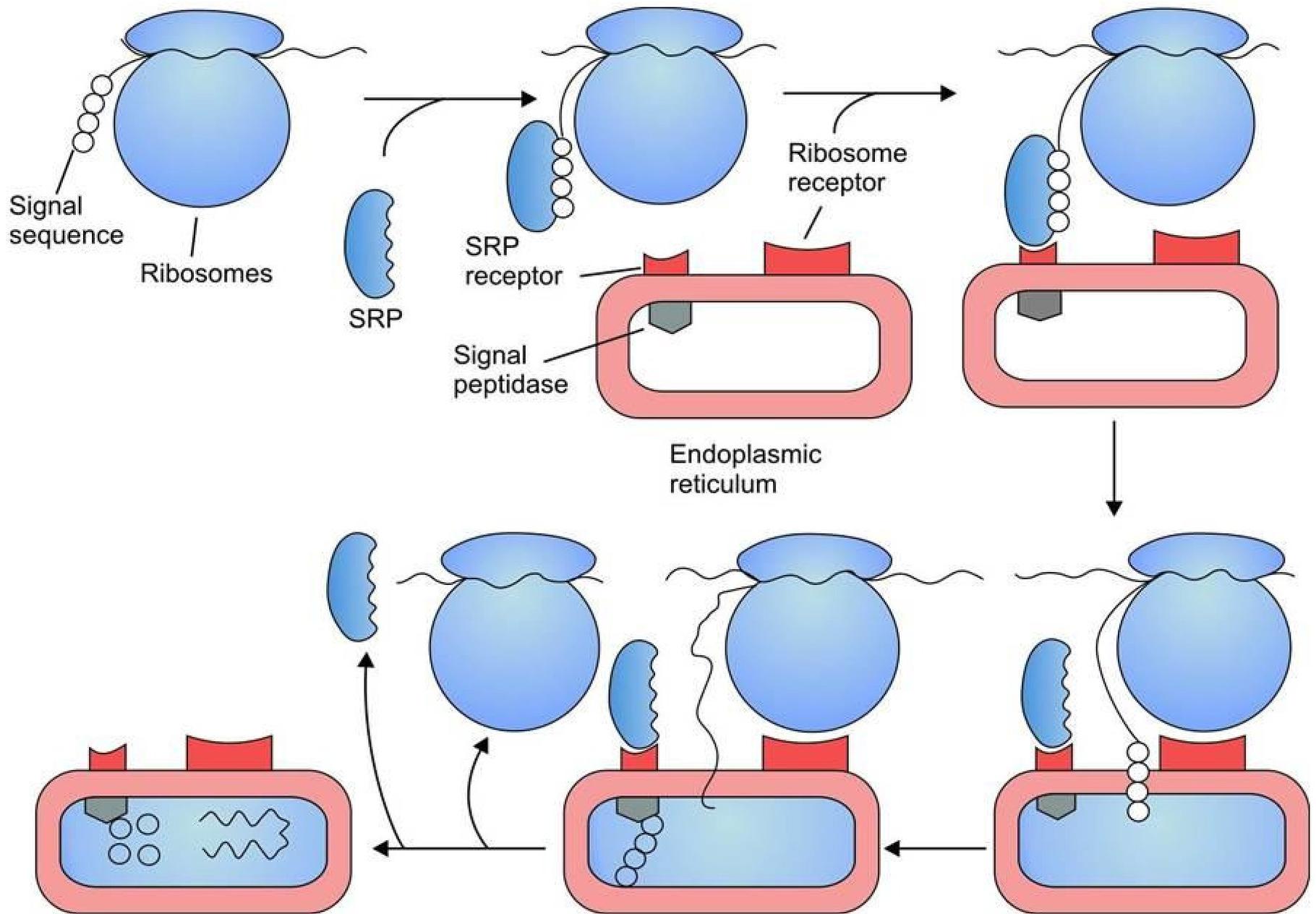
Role of signal sequence

EMB-RCG

Inhibitors of translation

- Translation can be inhibited by a number of compounds
- Inhibitors may act only on prokaryotes or eukaryotes or on both
- Inhibitors acting only on prokaryotes can be used as antibiotics

Inhibitors of prokaryotic translation used as antibiotics are:

Streptomycin

Tetracyclines

Chloramphenicol

Erythromycin

Streptomycin

Prokaryotic translation begins with the binding of formylmethionyl tRNA to 30S ribosomal subunit

Streptomycin inhibits this binding and prevents initiation

It also causes misreading of codons on mRNA

Some other antibiotics acting like streptomycin are:

- Neomycin
- Kanamycin
- Gentamycin

Tetracyclines

Tetracyclines bind to 50S ribosomal subunit of prokaryotes

They prevent binding of amino acyl tRNA to the A site

Chloramphenicol

Chloramphenicol is an inhibitor of peptidyl transferase activity of 23S rRNA

This rRNA is present in 50S ribosomal subunit in prokaryotes

Inhibition of peptidyl transferase prevents the formation of peptide bonds

Erythromycin

Erythromycin is an inhibitor of EF-G, the analogue of eukaryotic eEF-2

Inhibition of EF-G prevents translocation of mRNA along the ribosome

This blocks elongation