Steps in protein synthesis process

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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:

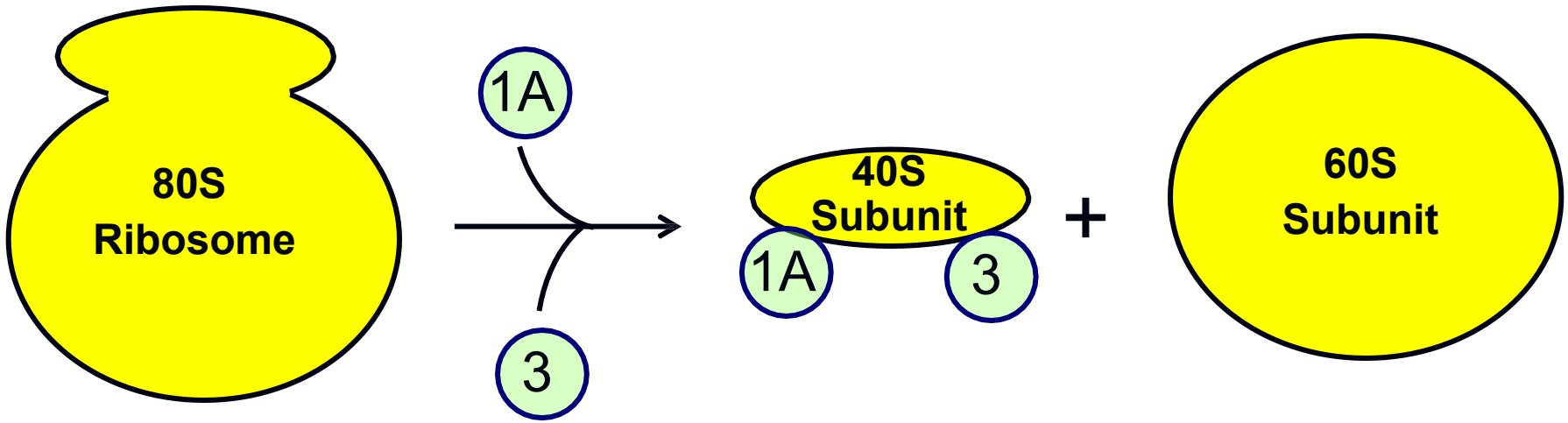
- Dissociation of ribosomal subunits
- Formation of 43S pre-initiation complex
- Formation of 48S initiation complex
- Formation of 80S initiation complex

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

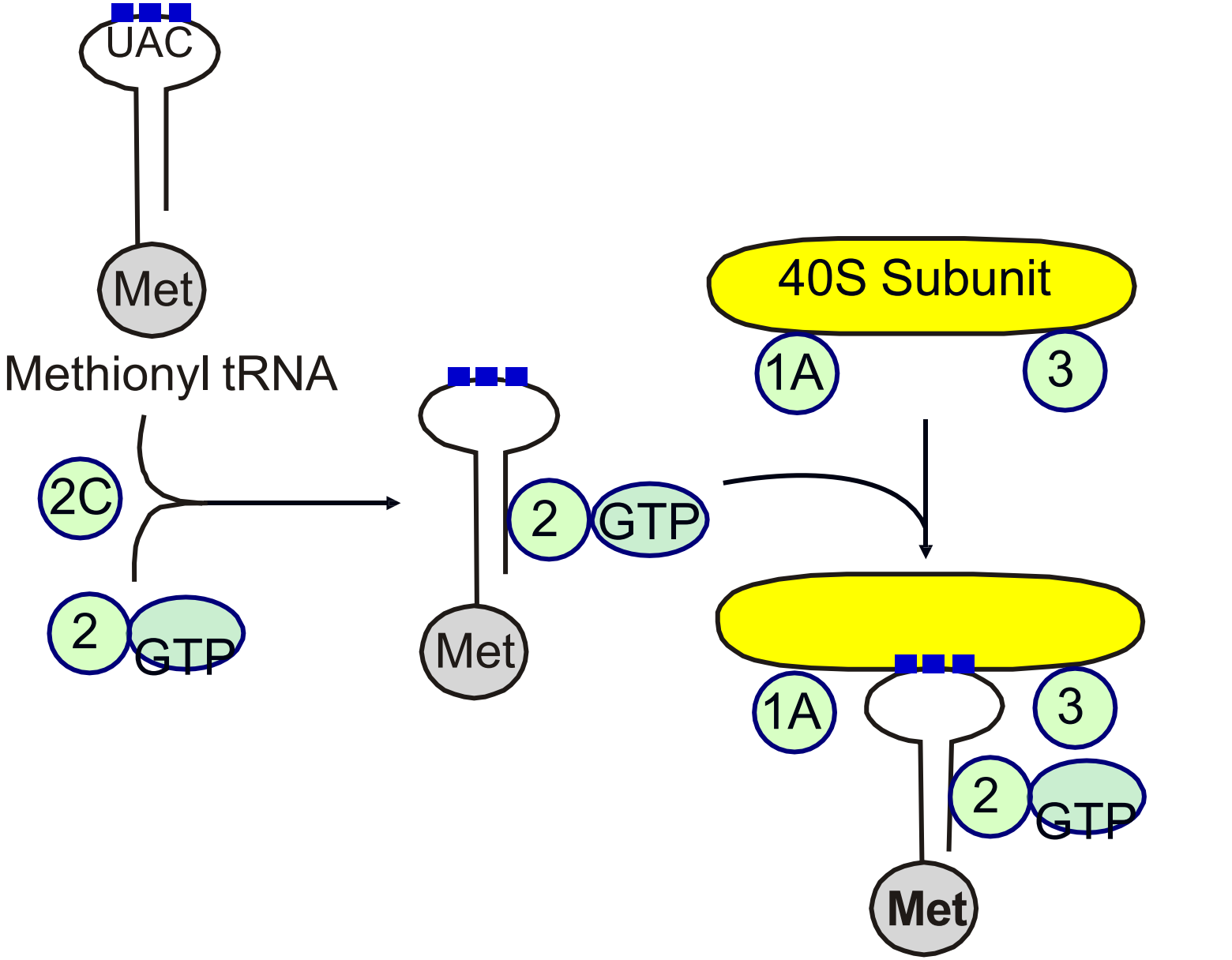


Formation of 43S pre-initiation complex

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
Met
2
GTP
40S Subunit
1A
3
1A
3
2
GTP
Met
43S Pre-initiation complex

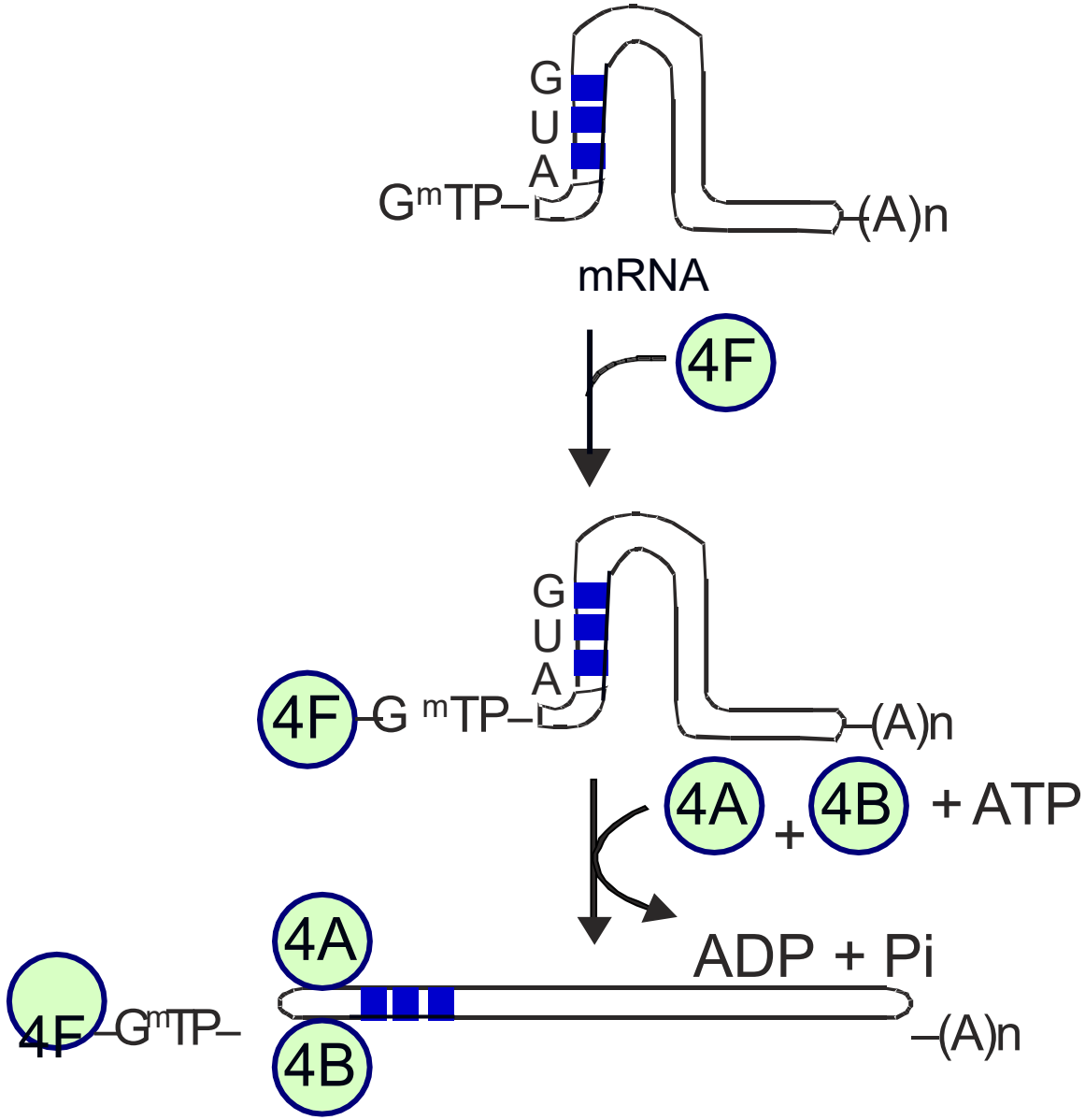
Formation of 48S initiation complex

eIF-4F binds to the 5’ cap of mRNA

eIF-4A and eIF-4B bind to mRNA in the presence of ATP

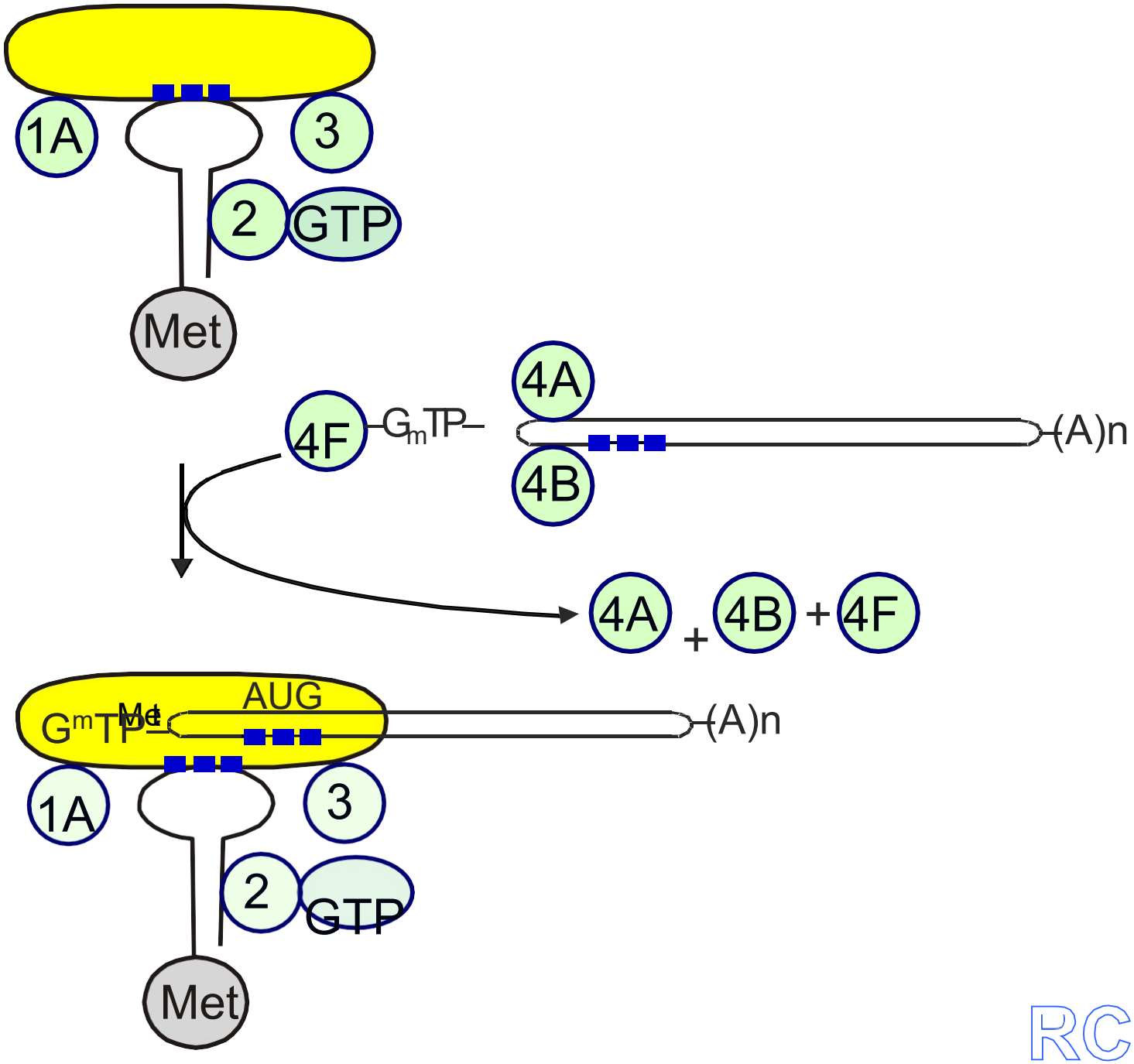
IF-4A hydrolyses ATP into ADP and Pi

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



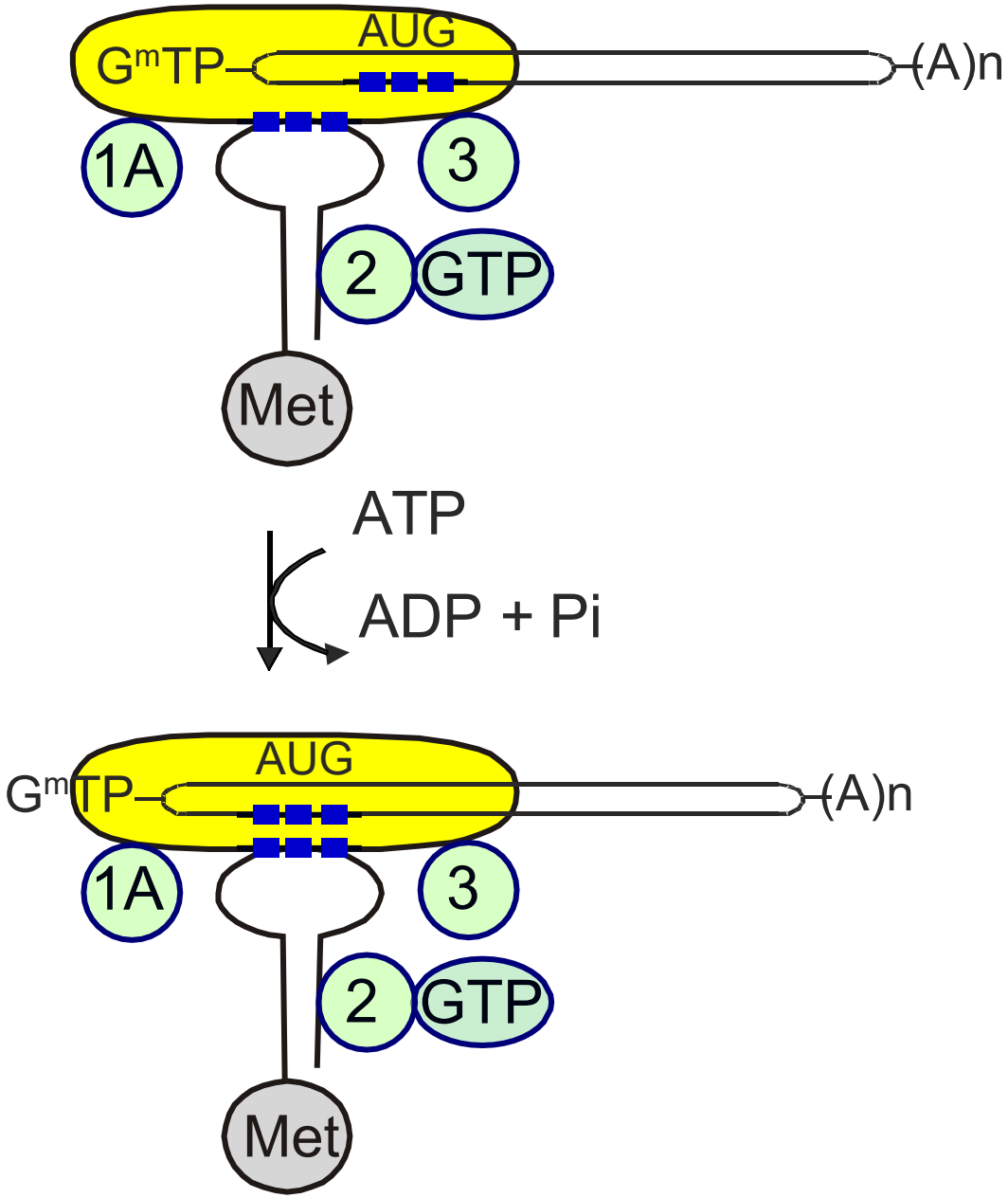
The mRNA binds to the 40S ribosomal subunit

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


1A
3
2
GTP
Met
4A
4F
GmTP–
(A)n
4B
4A
+
4B
+
4F
AUG
GmTP–
Met
(A)n
1A
3
2
GTP
Met

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

This complex is known as the 48S initiation complex



48S Initiation complex

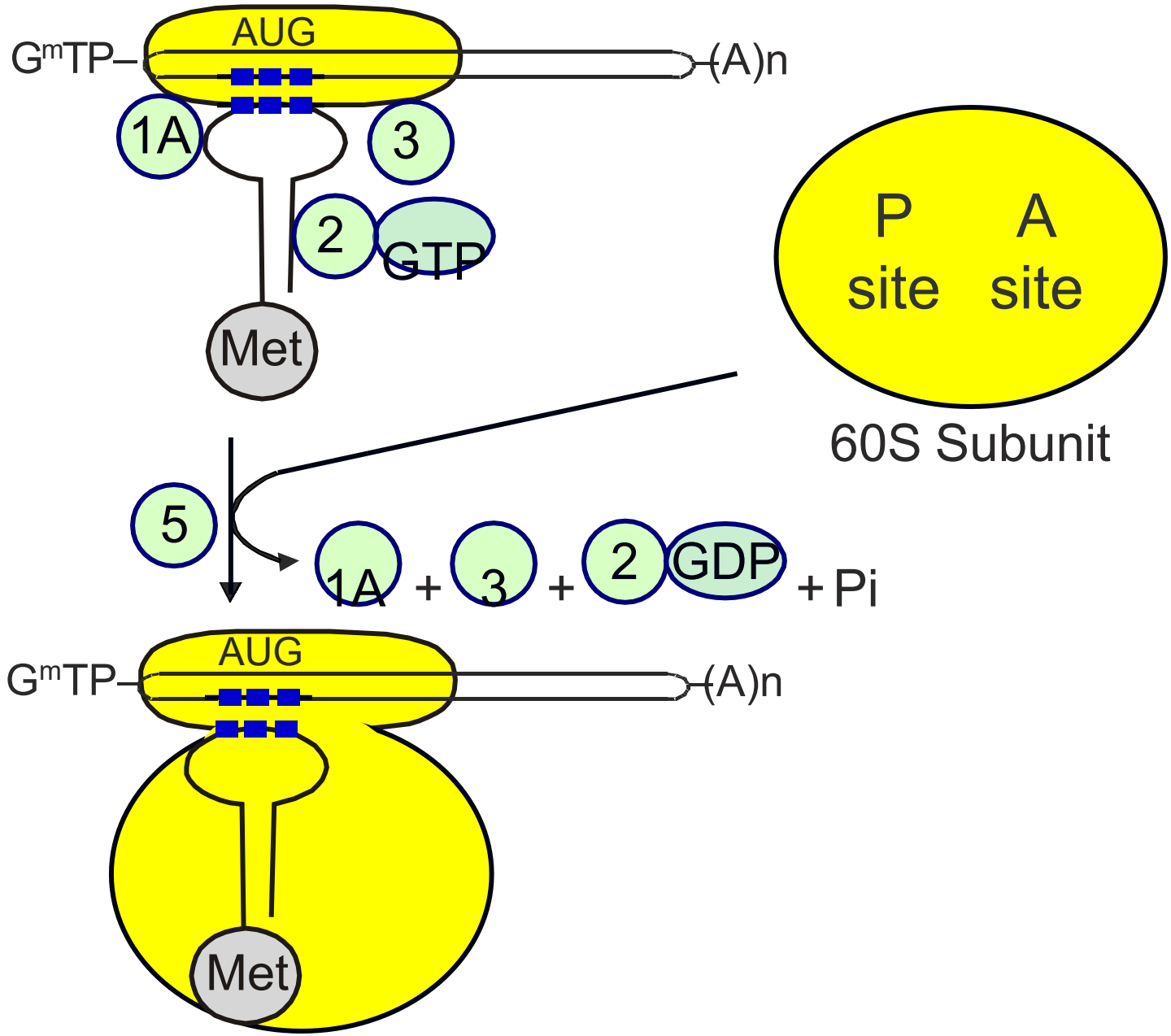
Formation of 80S initiation complex

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



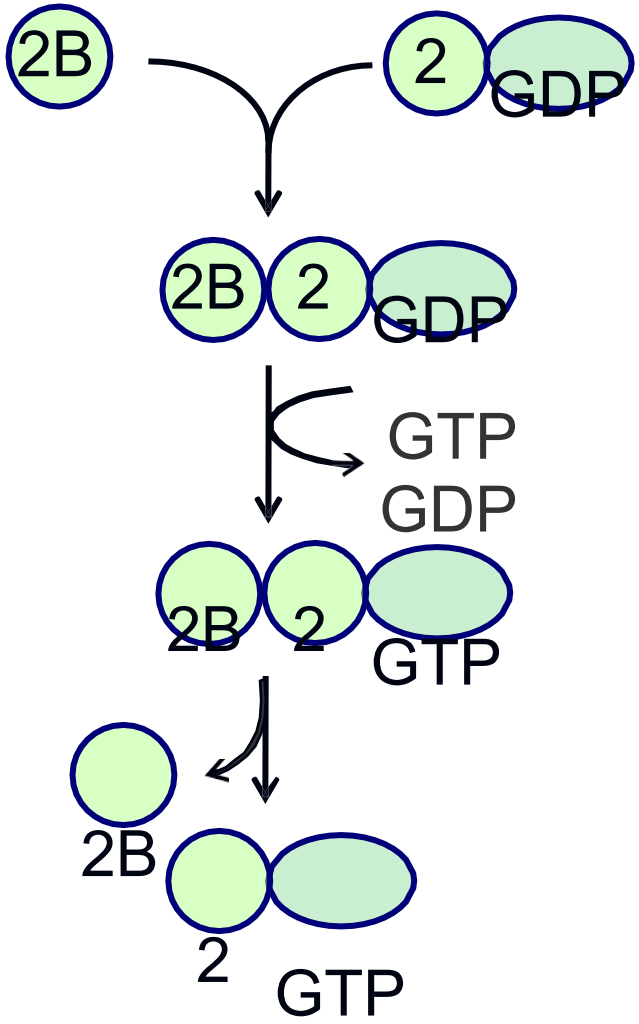
80S Initiation complex

RCG

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

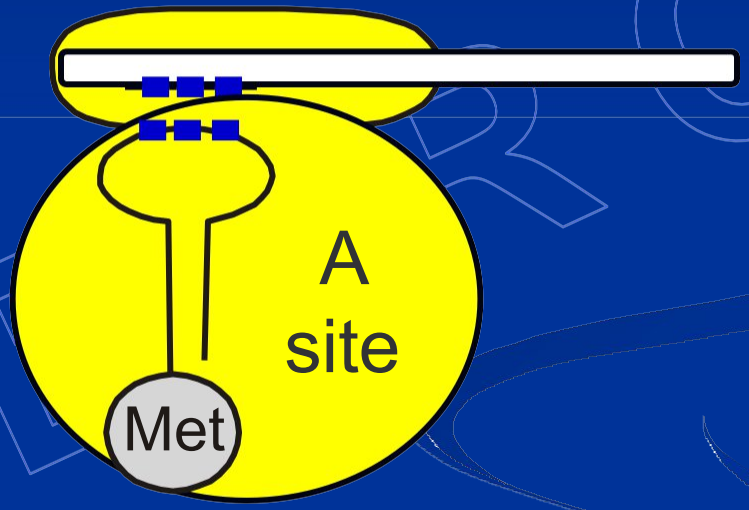
Elongation requires:

- 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

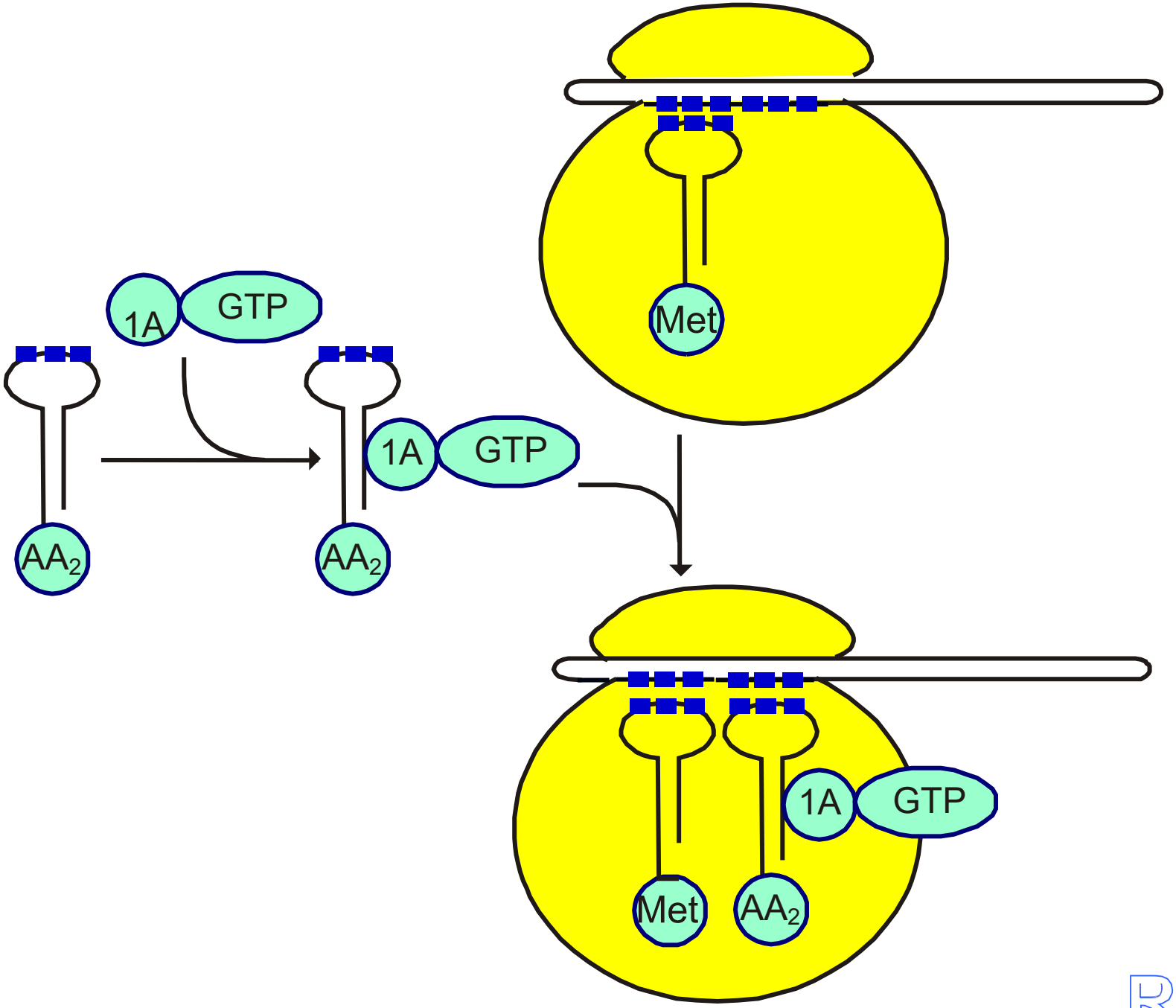


After initiation, P site is occupied by methionyl amino acyl tRNA; A site is vacant

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


1A
GTP
Met
1A
GTP
AA2
AA2
1A
GTP
Met
AA2

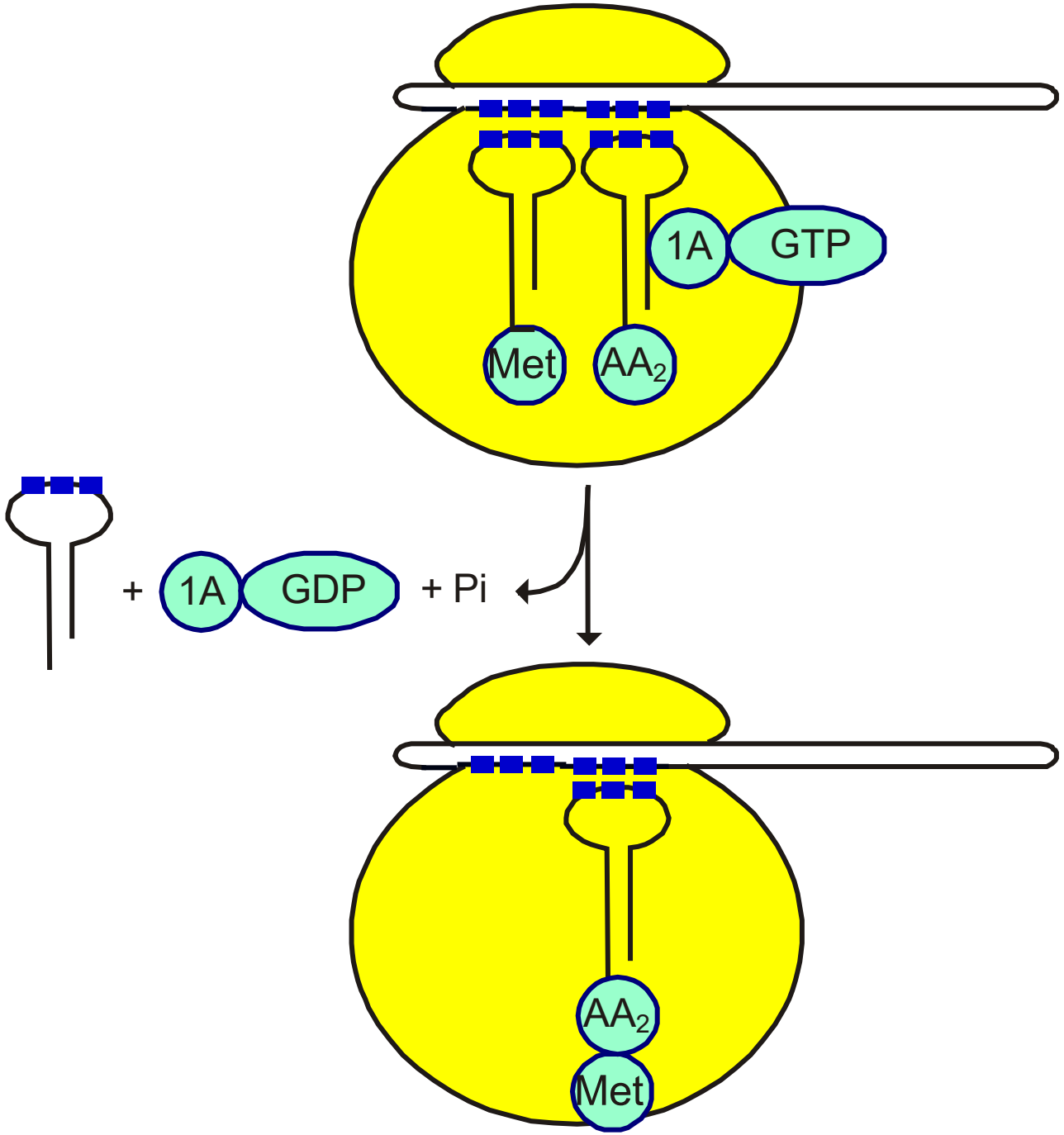
After binding of the second amino acyl tRNA to ribosome:

- 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

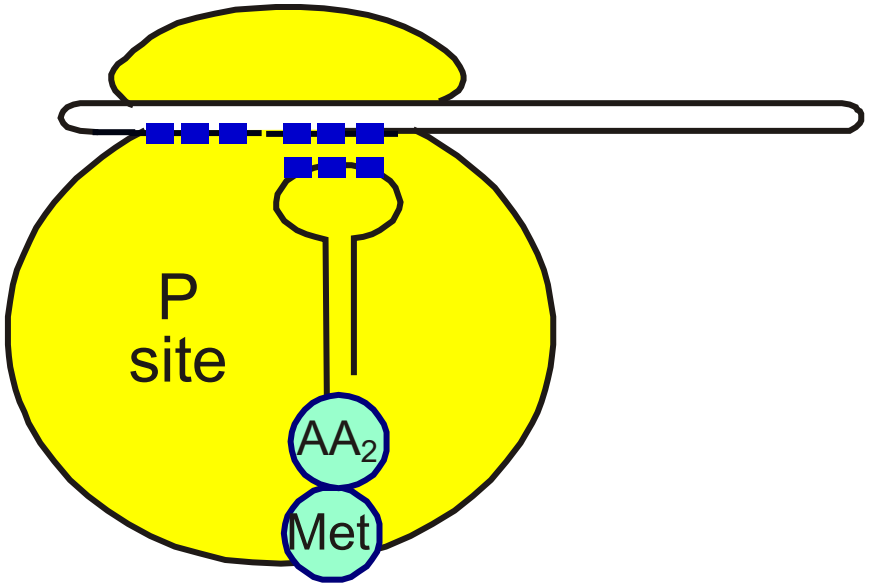
This enzyme forms a peptide bond between carboxyl group of first amino acid and amino group of second amino acid


1A
GTP
Met
AA2
+
1A
GDP
+ Pi
AA2
Met

The dipeptide that is formed is attached to the second tRNA

The first tRNA, which is now free, is released

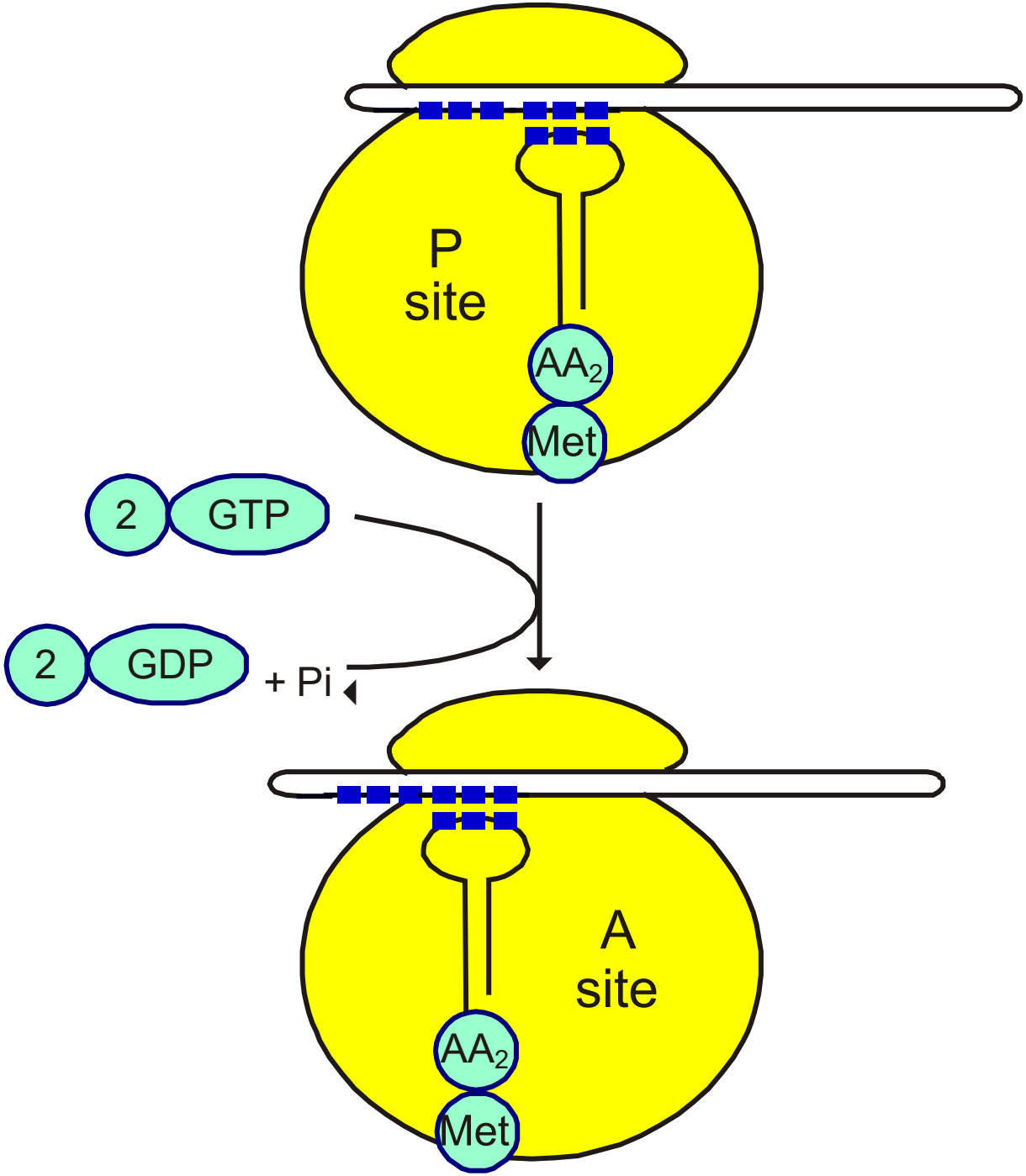
The P site becomes vacant



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



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

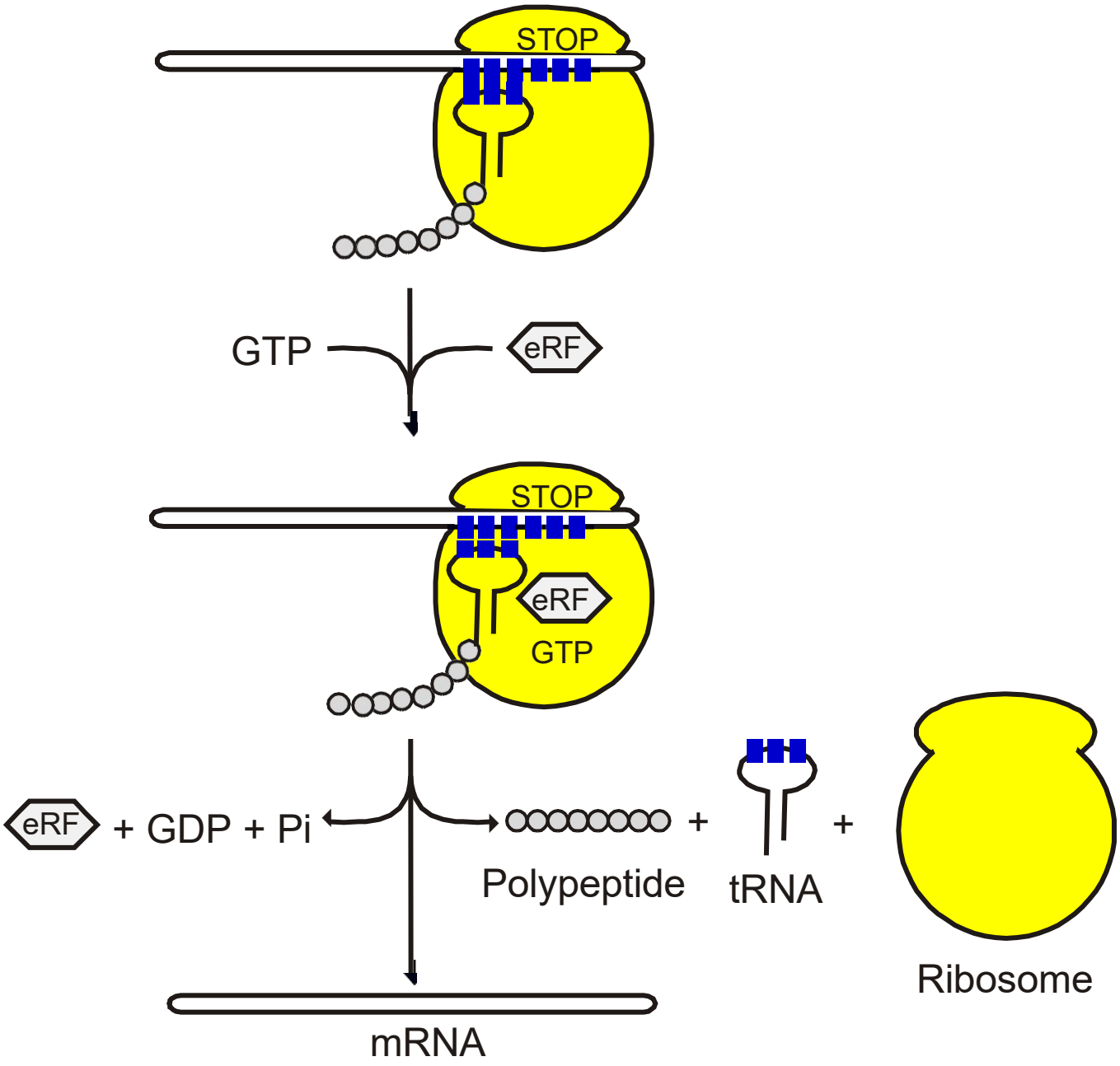
It hydrolyses the bond between the carboxyl group of the last amino acid and the tRNA

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

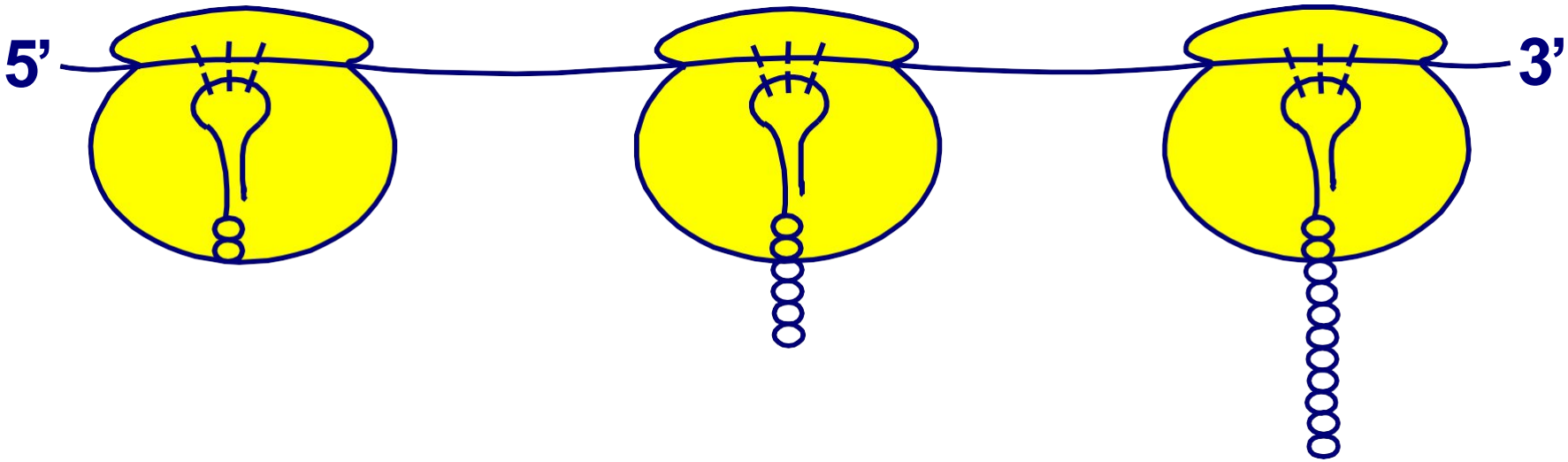
Polysome

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



Polysome

Thank you