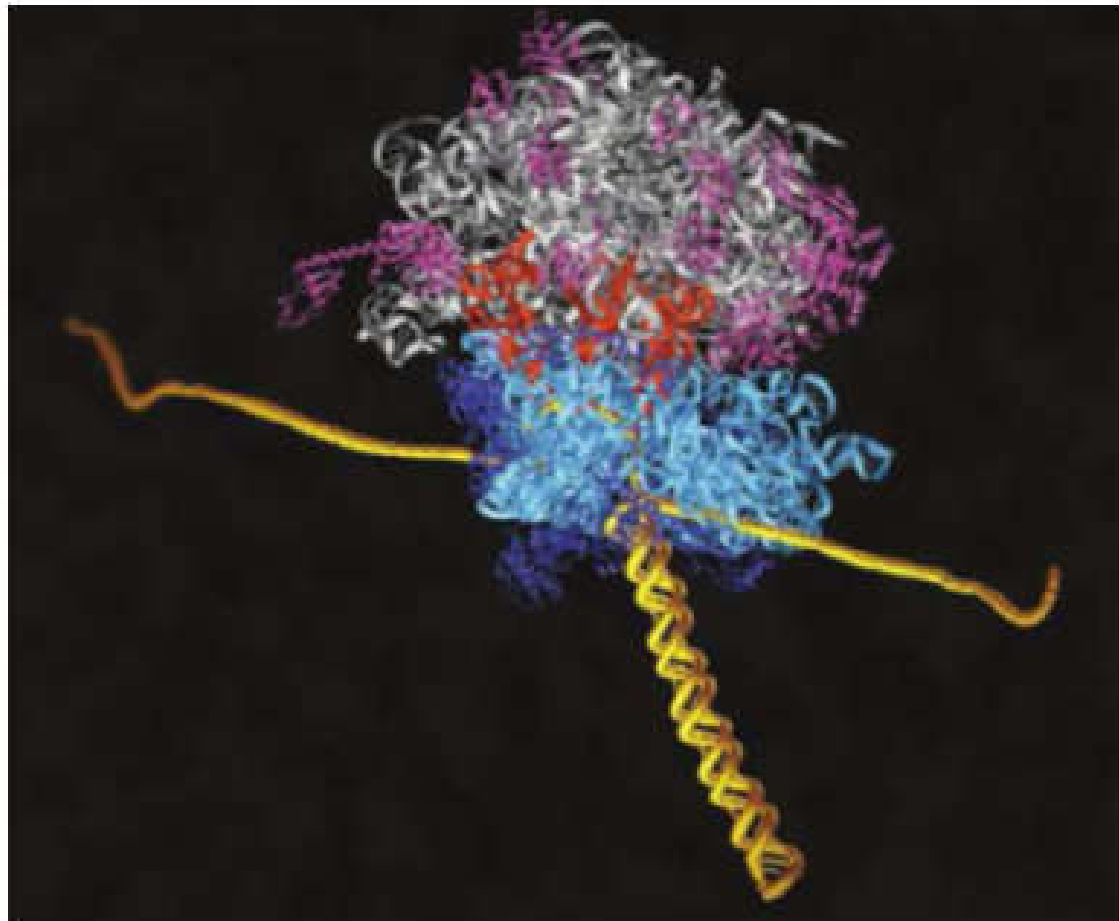


# Chapter 8 Protein biosynthesis (Translation)

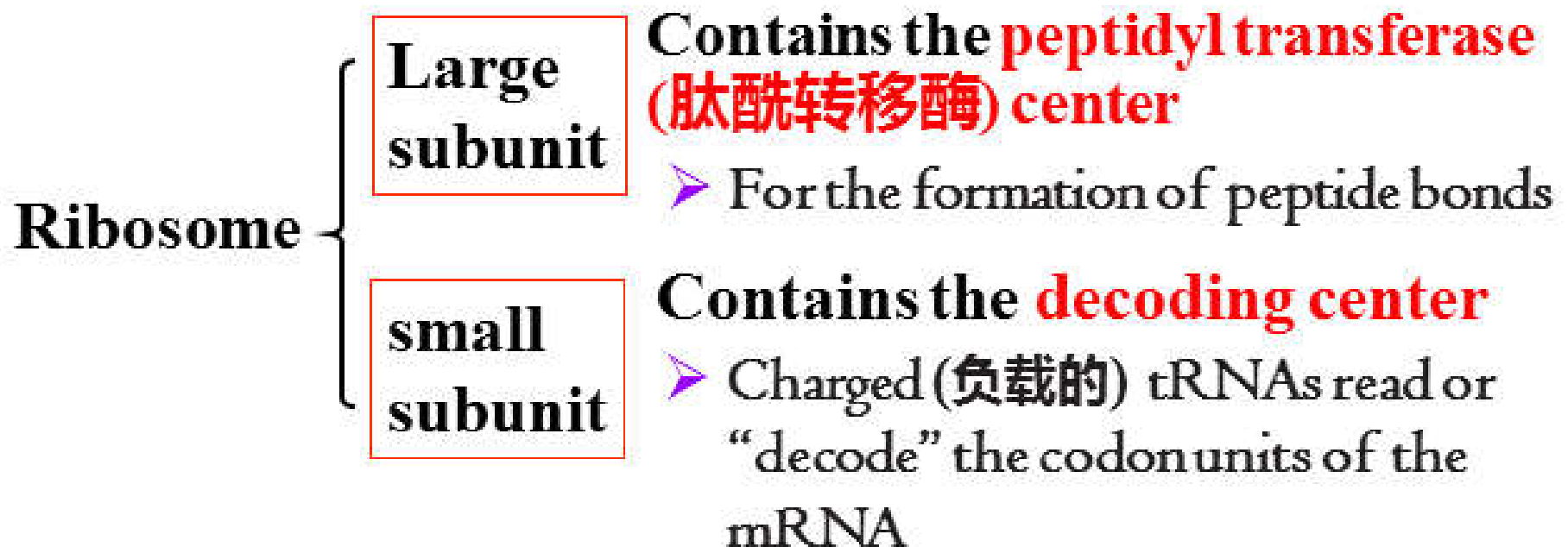




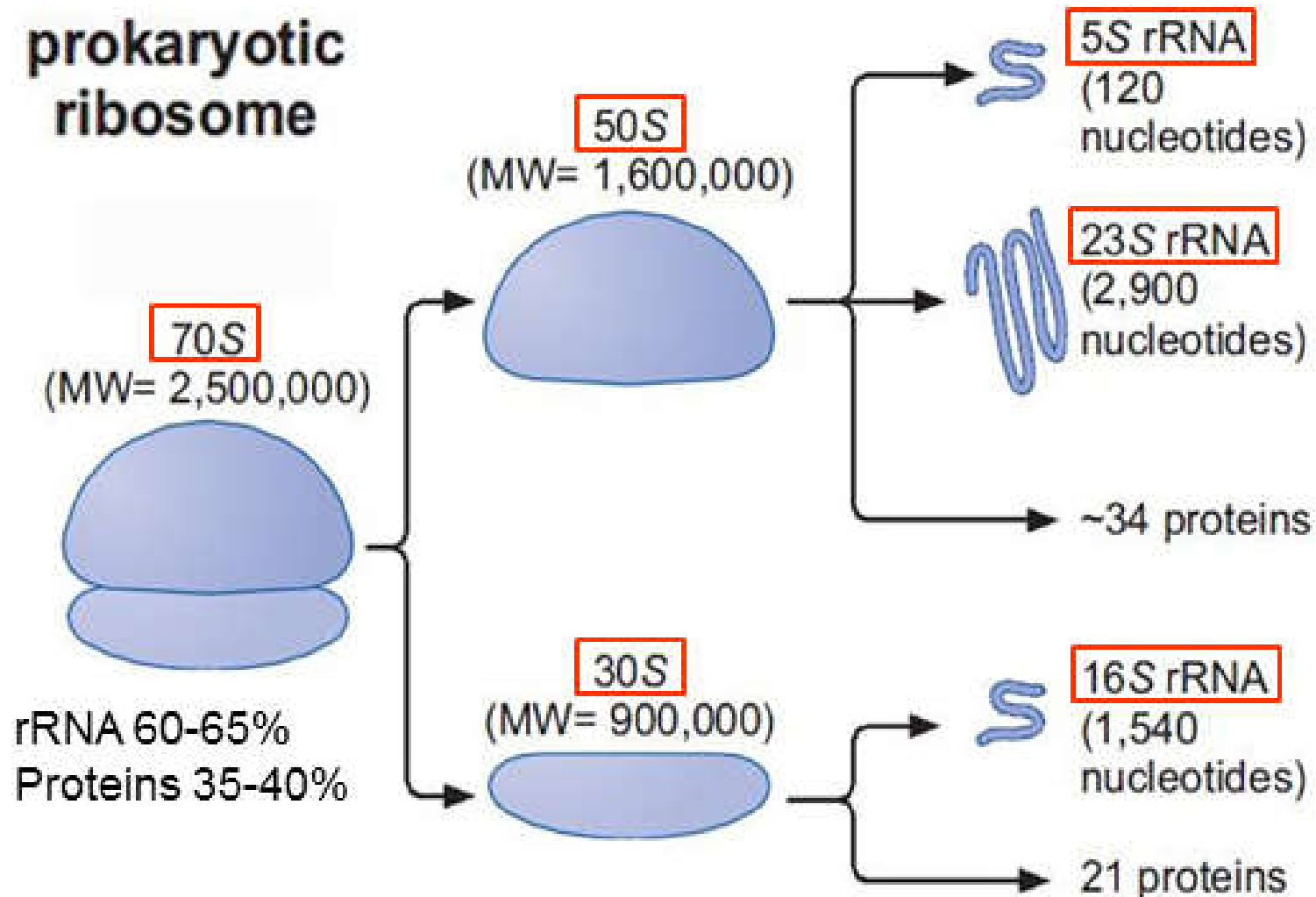
# 1. Ribosome (核糖体)

- The ribosome is the machine that directs the synthesis of proteins.
- The ribosome is the largest and most complex RNPs.

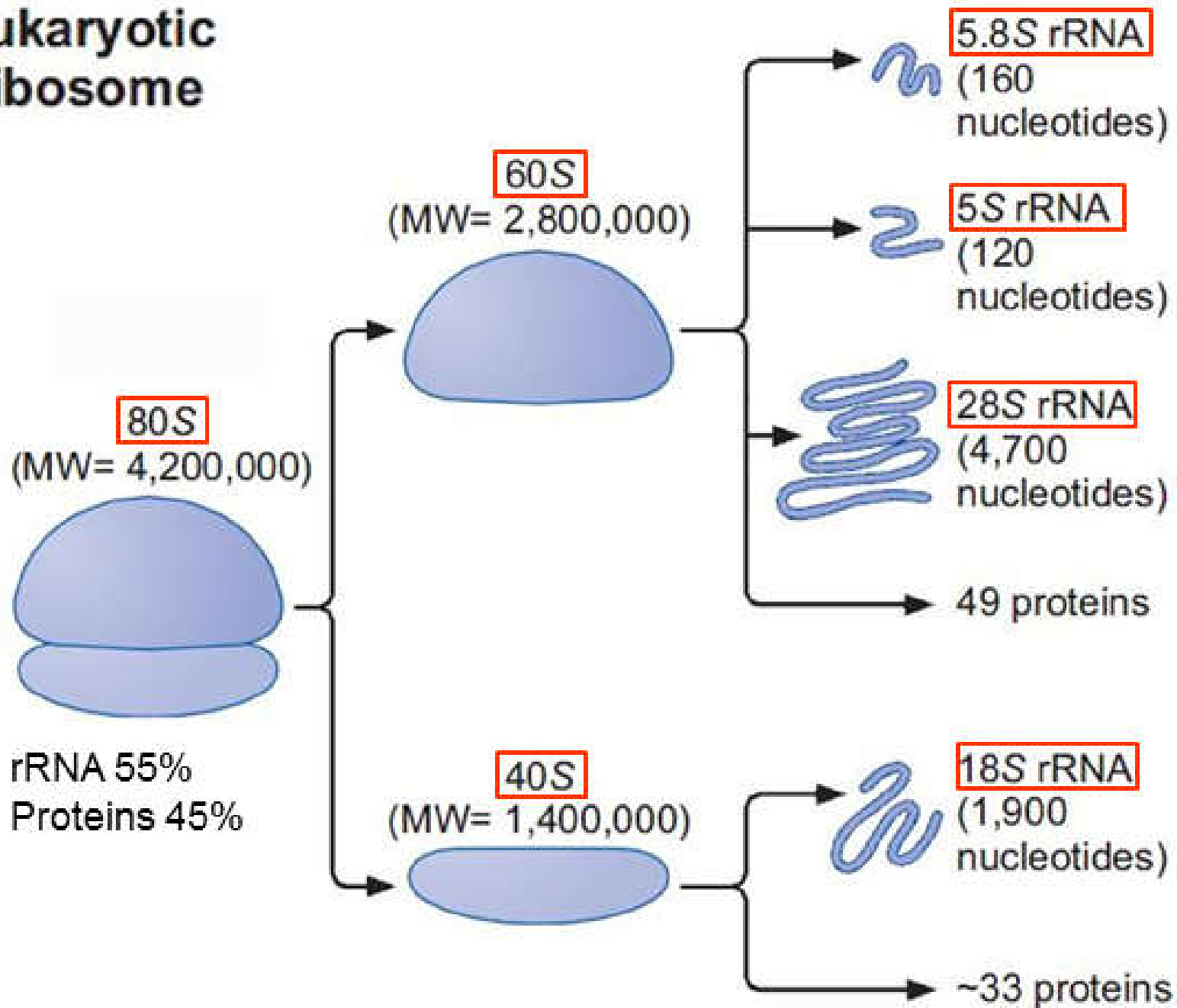
## 1.1 Ribosome Composition



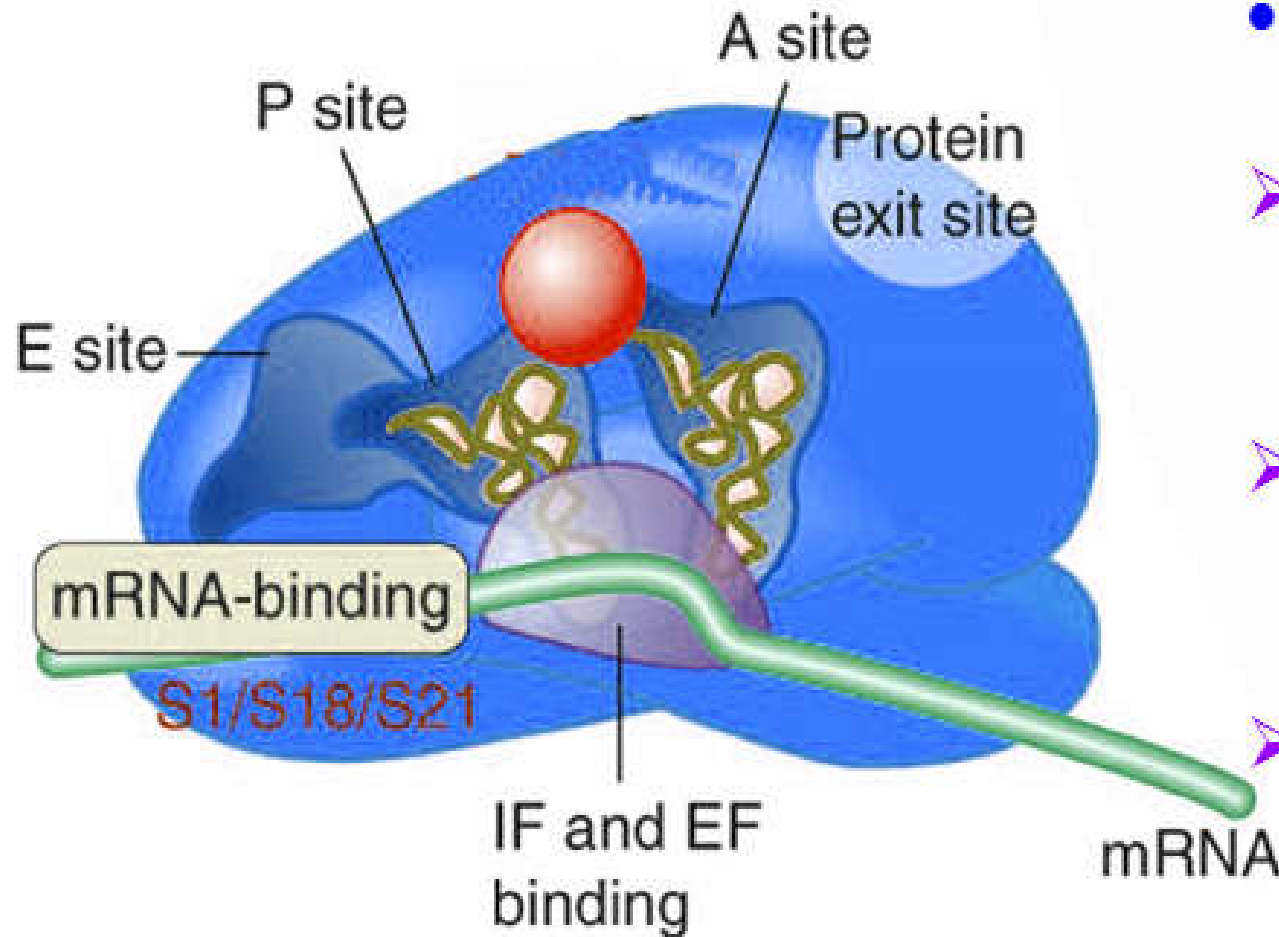
## prokaryotic ribosome



# eukaryotic ribosome



## 1.2 Active centers of ribosome

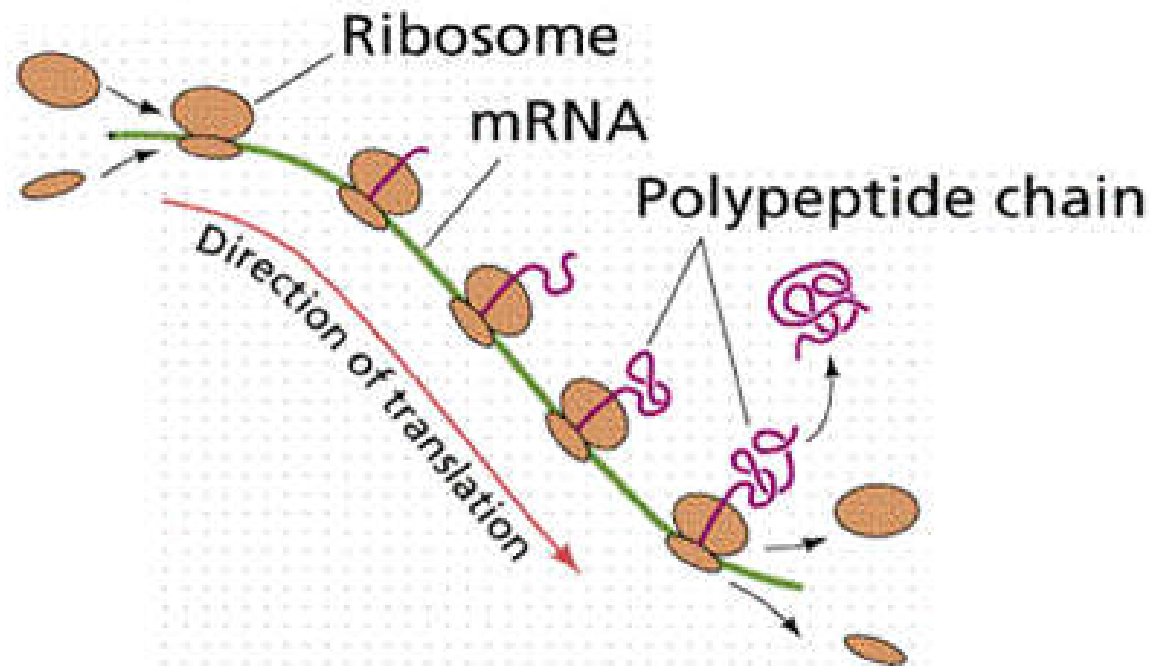


- Three tRNA-binding sites:
  - An **aminoacyl-tRNA** enters the **A site**.
  - **Peptidyl-tRNA** is bound in the **P site**.
  - **Deacylated tRNA** exits *via* the **E site**.

### 1.3 Polyribosome or polysome (多聚核糖体)

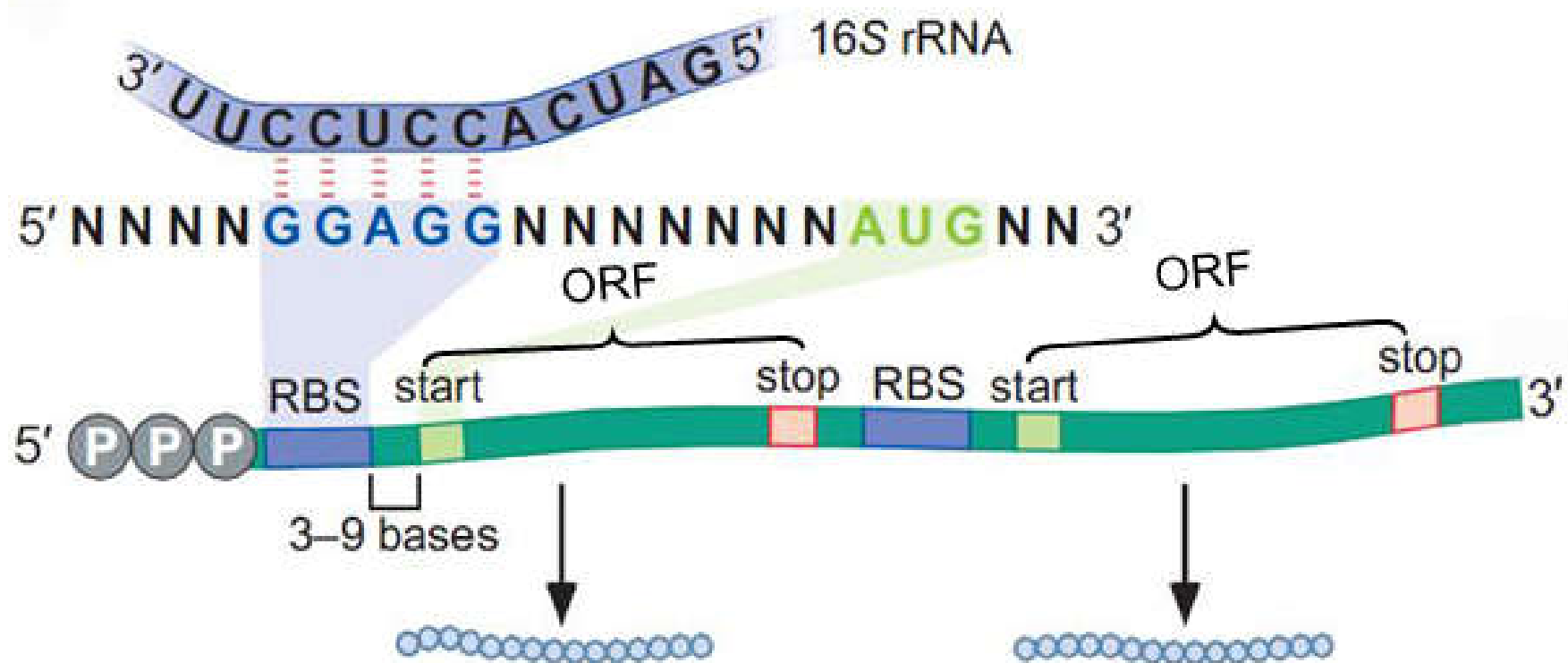
- Most mRNAs are translated by more than one ribosome at a time.
- **Polysome** is a structure in which **many ribosomes** translate an mRNA in tandem.

许多核糖体串联翻译mRNA的结构称为**多核糖体**



## 2. mRNA

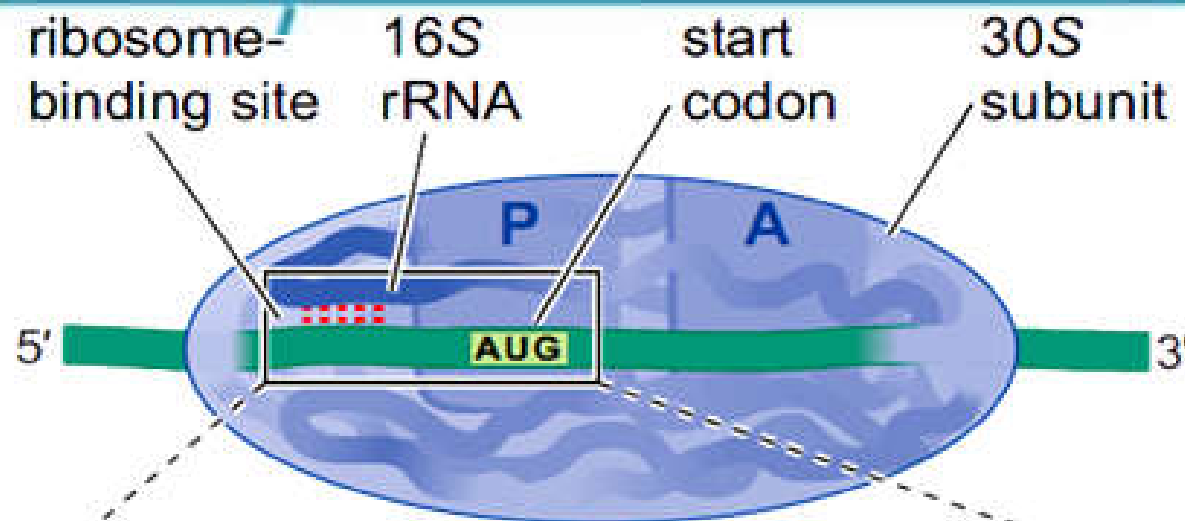
### 2.1 Prokaryotic mRNA



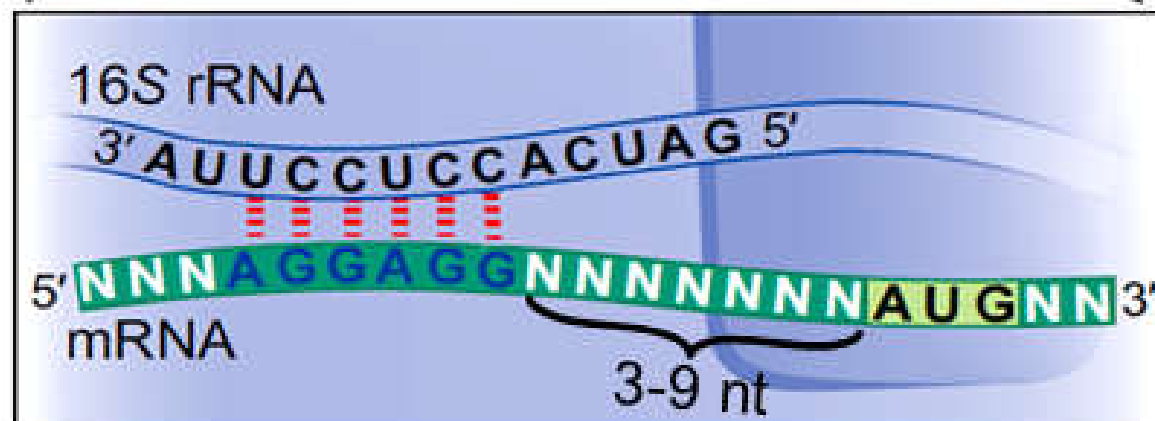
## 2.1.1 Prokaryotic RBS (核糖体结合位点)

Ribosome binding site (RBS) / SD(Shine-Dalgarno)序列

5'... AGGAGG... 3', 与16S rRNA配对



- Typically located 3-9 nt on the 5' side of the start codon
- Complementary to a sequence of the 16S rRNA







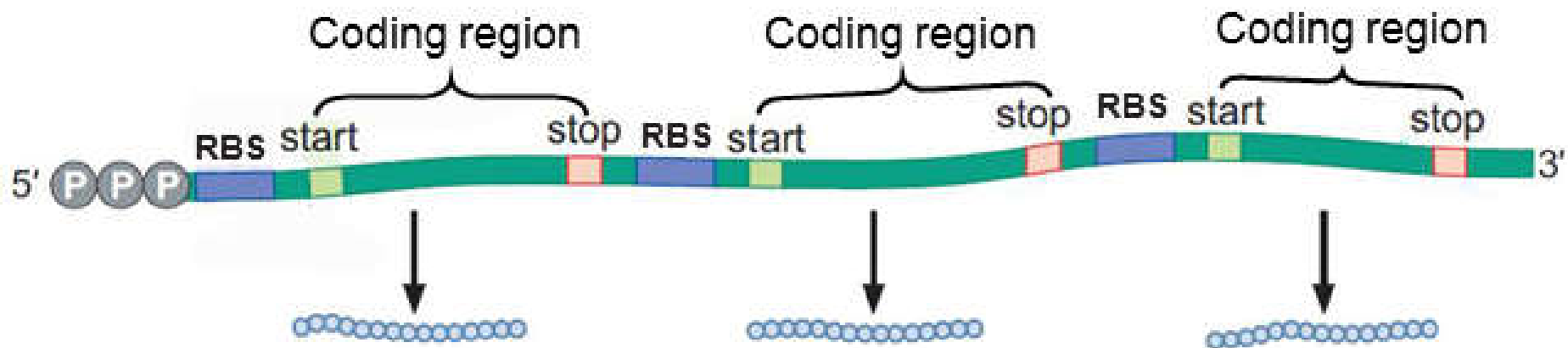
## 2.1.2 ORF (开放阅读框) and coding region

- **Open reading frames (ORFs)** are **suspected coding regions** usually identified by computer in DNA. They are **continuous** groups of adjacent codons following a **start codon** and ending at a **stop codon**.

**开放阅读框**是指DNA序列中由计算机辨认出的可能编码区，它是从**起始密码子**到**终止密码子**的一段**连续的**密码子区域。

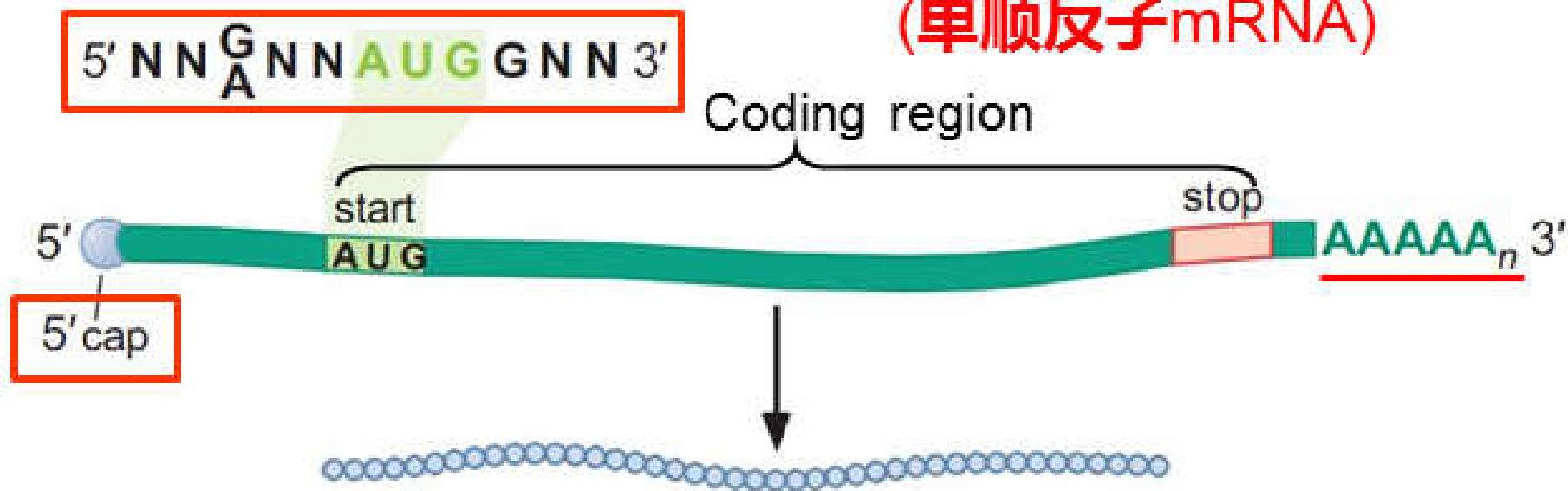
- When a particular ORF is known to encode a certain protein, the ORF is usually referred to as a coding region.

- Prokaryotic mRNAs frequently contain **two or more coding regions**.
- mRNAs containing multiple coding regions are known as **polycistronic mRNAs (多顺反子mRNA)**.
- polycistronic mRNAs encode proteins that perform **related functions**.



## 2.2 Eukaryotic mRNA

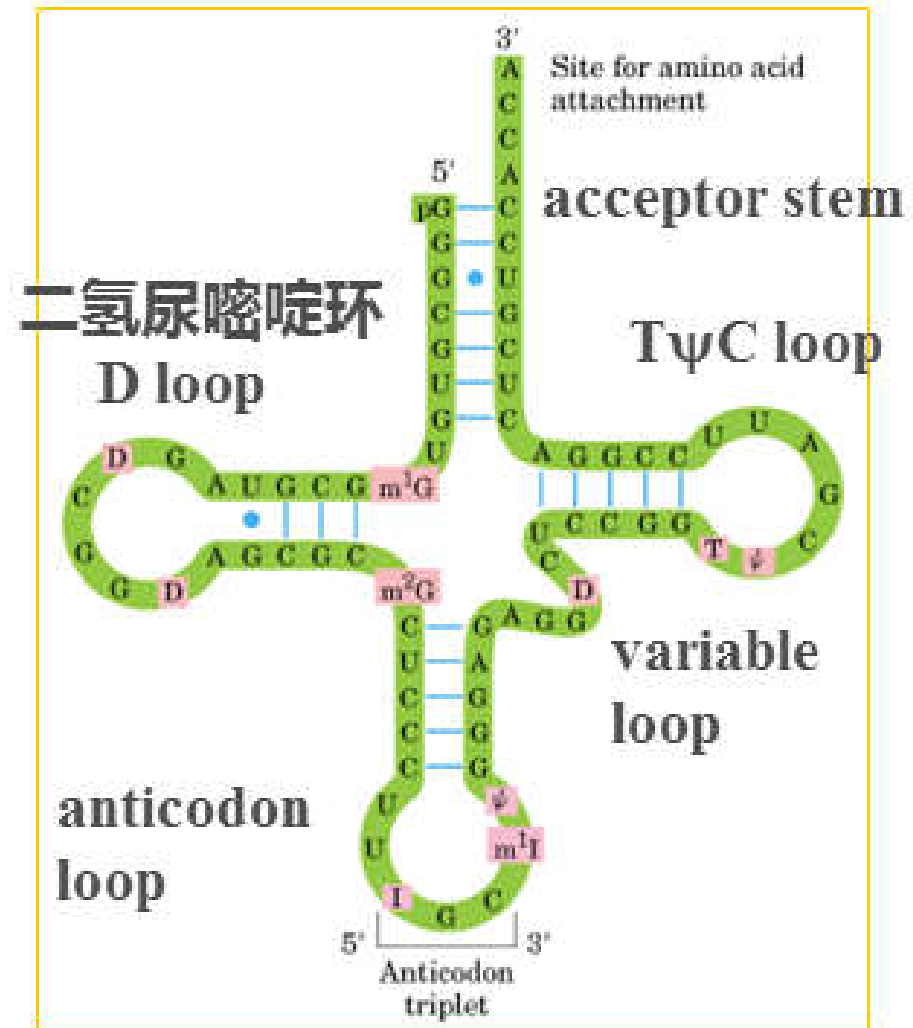
- Eukaryotic mRNA has two features recognized by ribosomes: **5' cap** and **Kozak sequence** near the start codon.
- Eukaryotic mRNAs almost always contain **a single coding region**. Monocistronic mRNA (单顺反子mRNA)



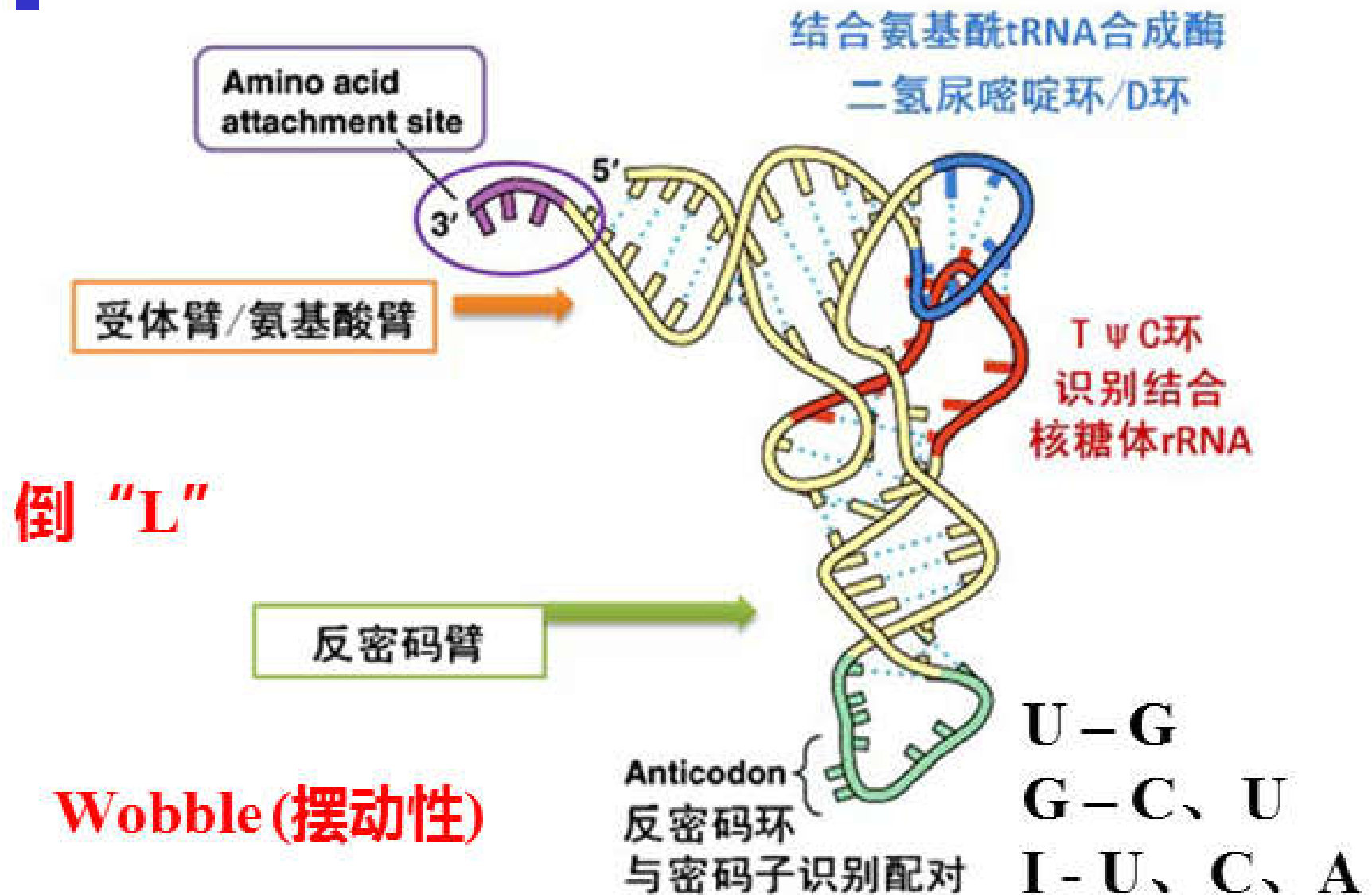
## 3. tRNA

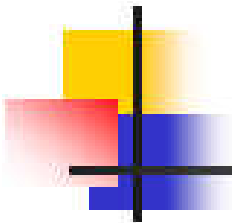
### 3.1 Secondary structure of tRNA

四环四臂/茎  
( Cloverleaf,  
三叶草 )



## 3.2 Tertiary structure of tRNA



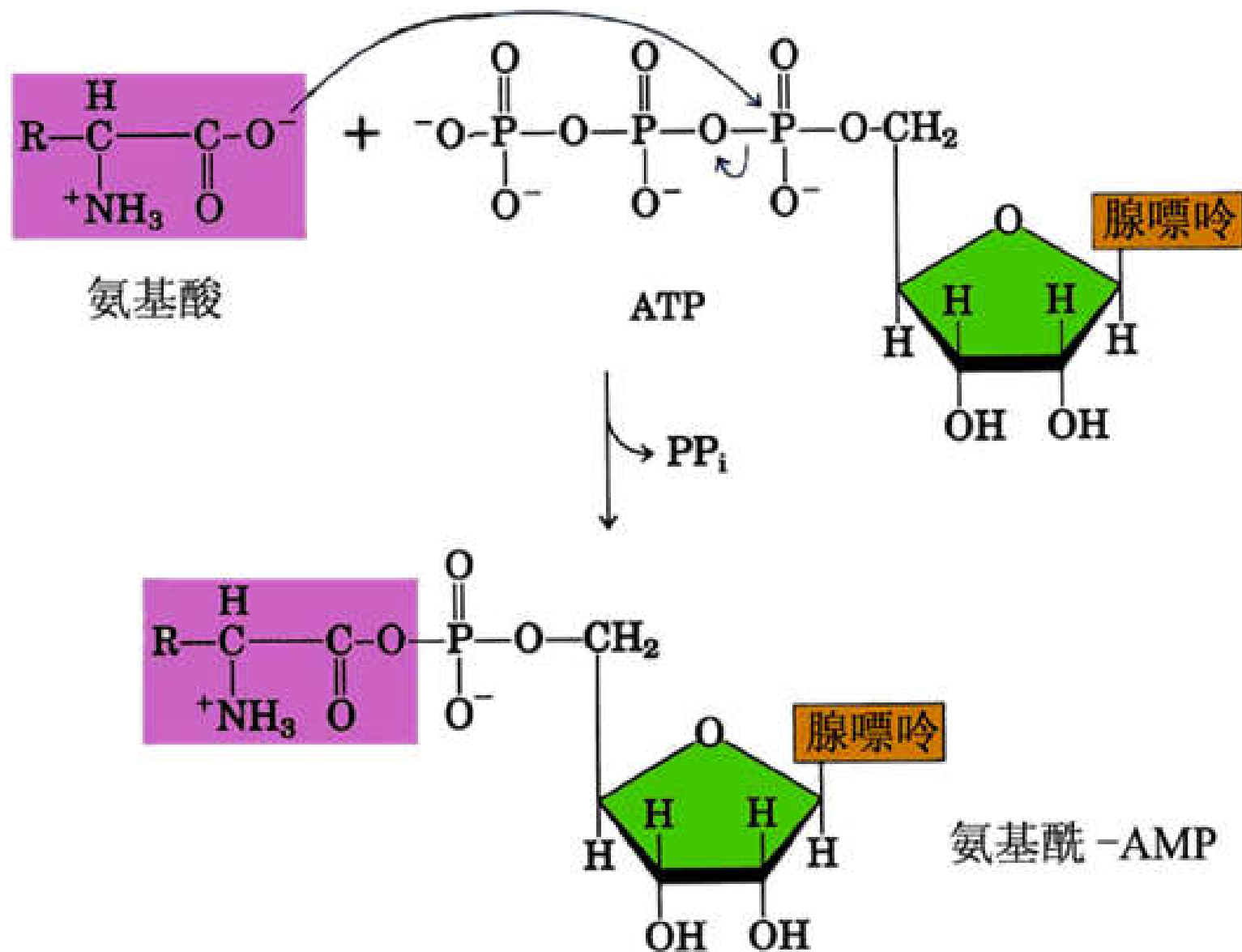


### 3.3 Aminoacyl-tRNA synthetase

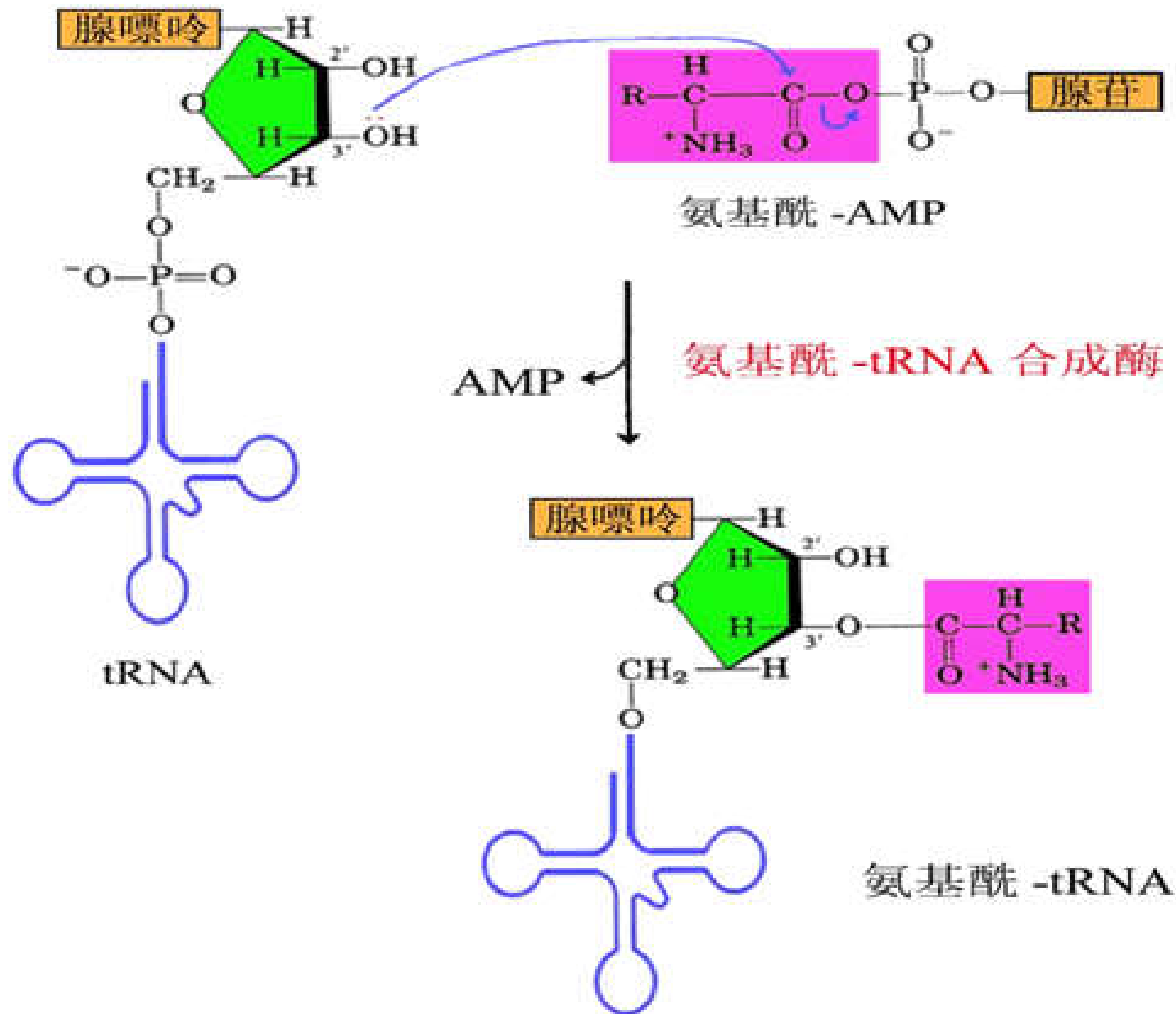
- Aminoacyl-tRNA synthetases (氨酰tRNA合成酶) charge tRNAs by the attachment of an amino acid to the 3'-terminal adenosine nucleotide via a high-energy acyl linkage (酰基键).
- **Each aminoacyl-tRNA synthetase attaches a single amino acid to one or more tRNAs. (20种氨酰tRNA合成酶)**
- Aminoacyl-tRNA synthetases charge tRNAs in two steps.

## (1) Adenylation (腺苷酰化)

## 氨基酸活化



## (2) tRNA charging

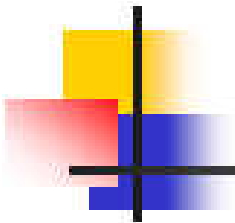






## 3.4 Initiator tRNA

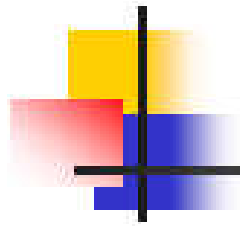
- Initiator tRNA is a special tRNA that recognizes the start codon.
- Prokaryotic initiator tRNA – **N-formylmethionine-tRNA (fMet-tRNA<sub>i</sub>, N-甲酰甲硫氨酰-tRNA<sub>i</sub>)**
  - First charged with methionine by methionyl-tRNA synthetase
  - The methionine residue is then converted to N-formylmethionine by **transformylase (转甲酰酶)**
- Eukaryotic initiator tRNA – **methionyl-tRNA (Met-tRNA<sub>i</sub>, 甲硫氨酰-tRNA<sub>i</sub>).**



## 4. Genetic code

- A **genetic code** is a set of rules that translates an mRNA sequence in groups of three nucleotides into an amino acid sequence of a protein.

**遗传密码**是一组规则，将mRNA序列以三个核苷酸为一组转译为蛋白质的氨基酸序列。  
遗传密码还可称为**三联体密码(triplet code)**或**密码子(codon)**。



## 4.1 Characteristics of genetic code

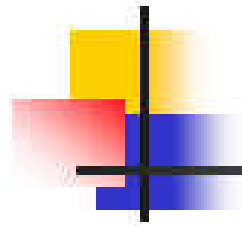
- (1) Direction of codon: 5' → 3'**
- (2) Nonoverlapping / adjacent (不重叠/相邻的)**
- (3) Comma-less (无逗号, 连续性)**
- (4) Degeneracy (简并性, synonymous codons)**
- (5) Universality (通用性)**
  - Some deviations occur in mitochondria and some unicellular organisms (单细胞生物)



## 4.2 Start codon and stop codon

### 4.2.1 Start codon

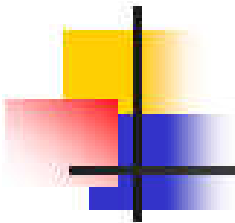
- **AUG -真核/原核**，真核中起始为Met、原核中起始为fMet，翻译中间为Met。
- **GUG -原核**，起始为fMet，翻译中间为Val。极少数还用到UUG和AUU。



## 4.2.2 Stop codon

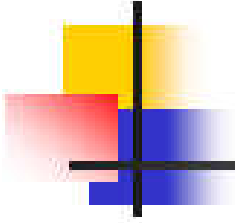
- **UAG** (amber 琥珀)
- **UAA** (ochre 赭石)
- **UGA** (opal 卵白石)

In bacteria, frequencies: UAA>UGA>UAG

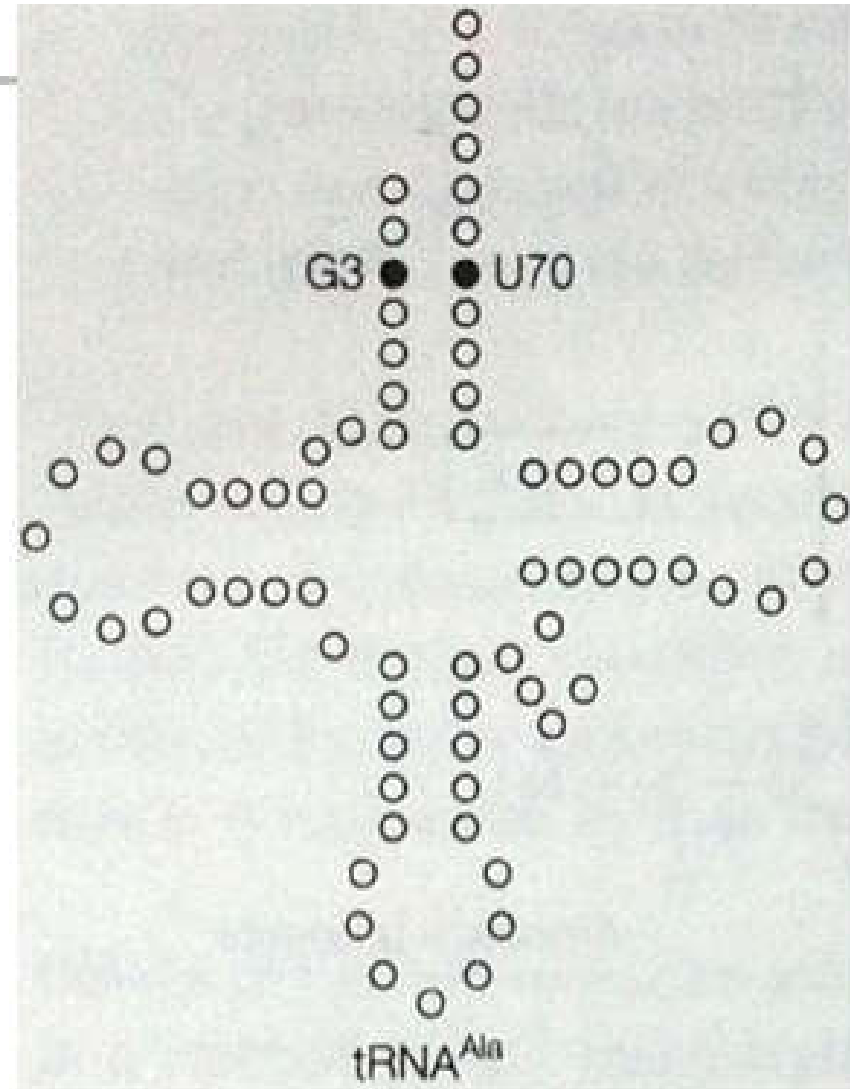
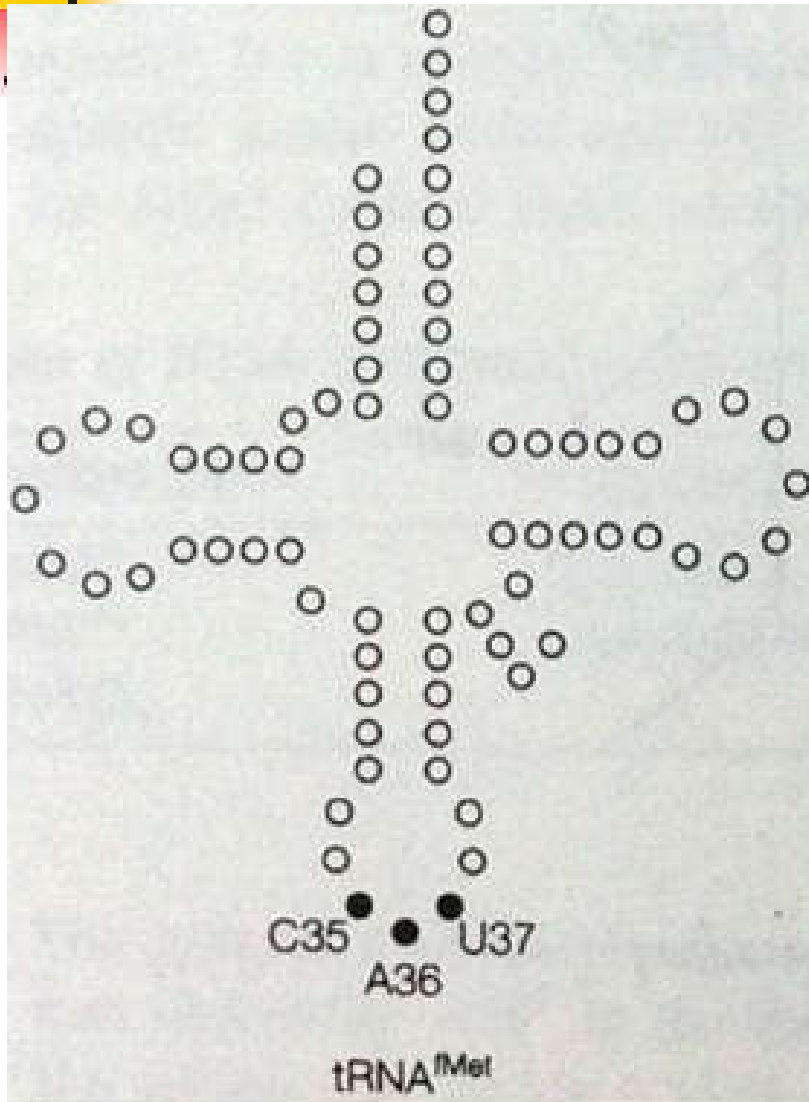


## 4.3 The second set of genetic codes

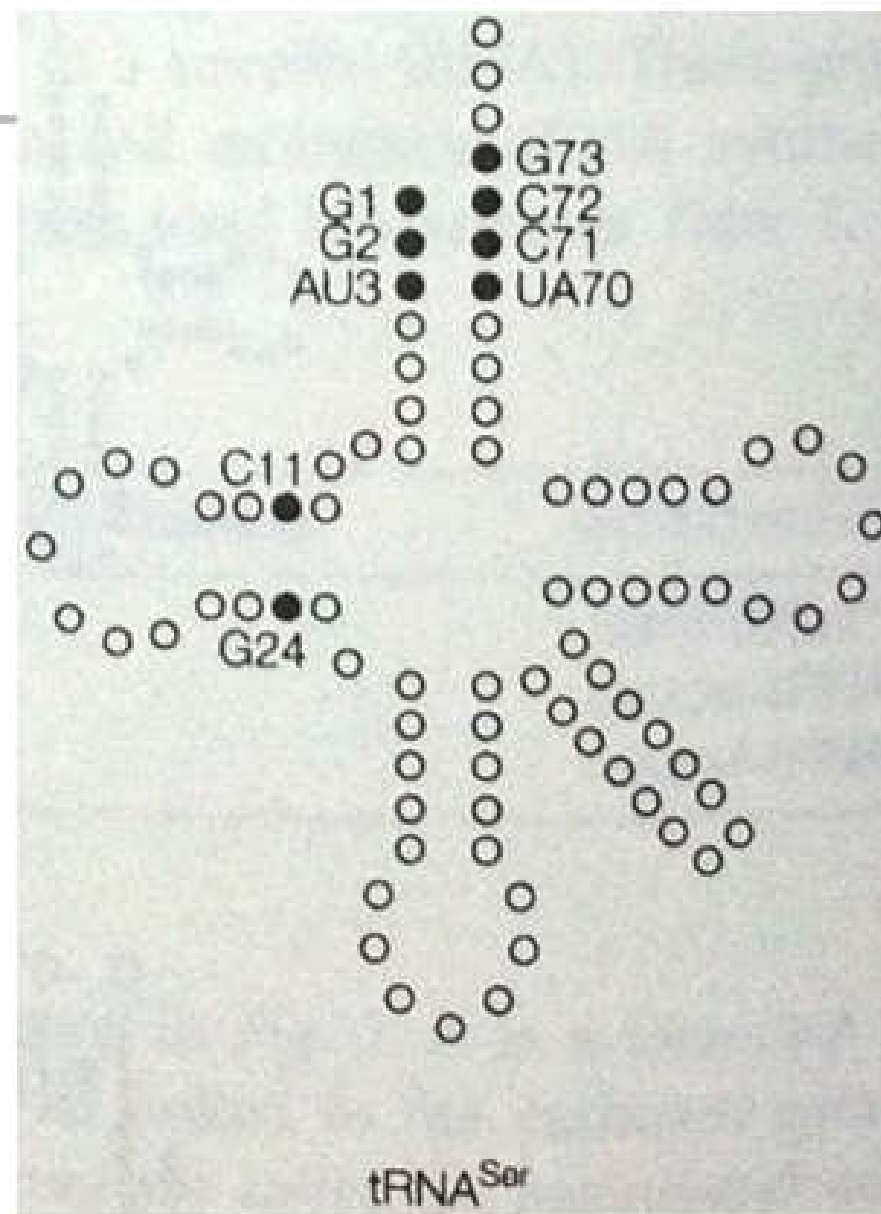
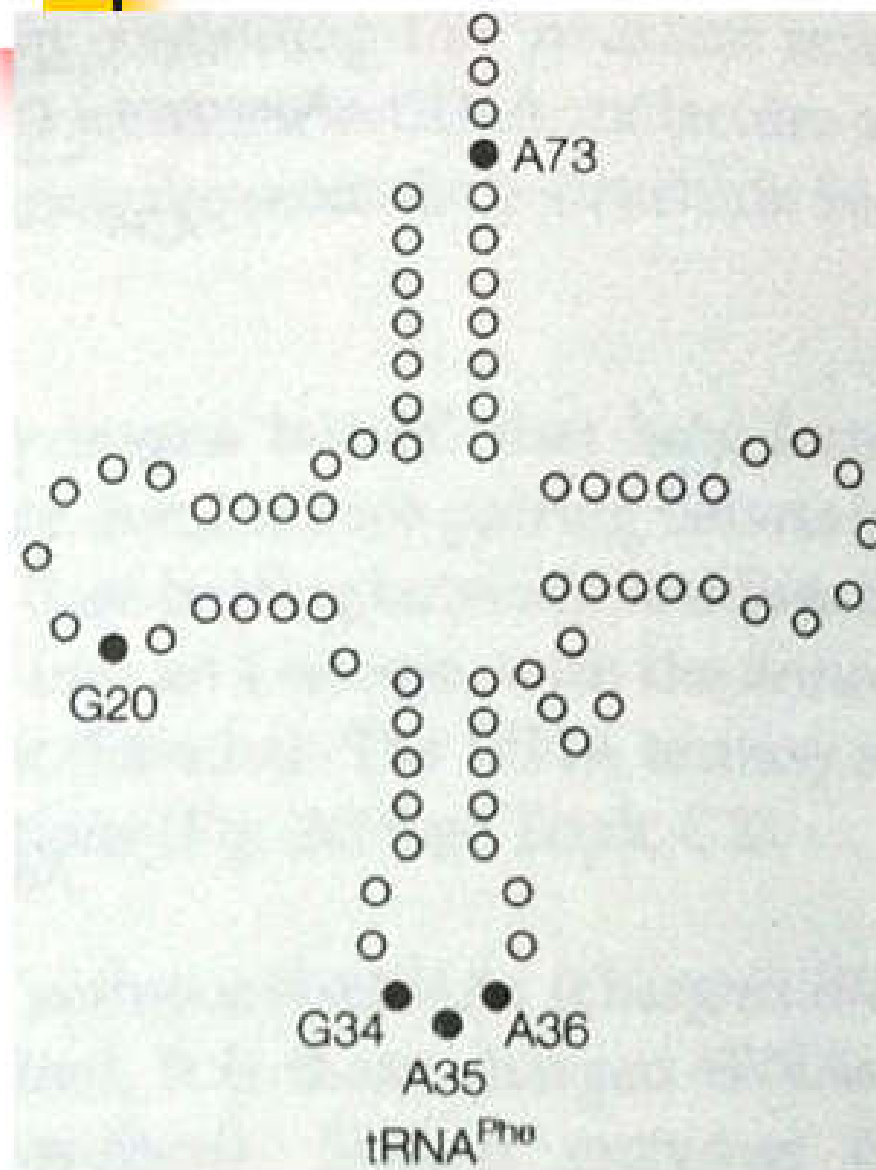
- The ribosome is unable to distinguish between correctly and incorrectly charged tRNAs.
- Aminoacyl-tRNA synthetases recognize unique structural features of cognate (相应的) tRNAs
- **The second set of genetic codes** guarantees the correct binding of amino acids to cognate RNA.

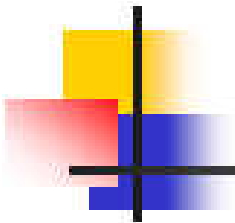


- In the structure of the **aminoacyl-tRNA synthetase**
- **Identity elements (鉴别元件)** in tRNA molecules









## 5. Prokaryotic protein synthesis

- **Protein synthesis system:**
  - **Template:** mRNA
  - **Materials:** amino acids
  - **Vehicle:** tRNAs
  - **Site:** Ribosome
  - **Enzymes:** aminoacyl-tRNA synthetase, peptidyl transferase, translocase (移位酶)
  - **Cofactors:**  $Mg^{2+}/K^{+}$
  - **Protein factors:** IF, EF, RF
  - **Energy supply:** ATP, GTP



## 5.1 Initiation of translation

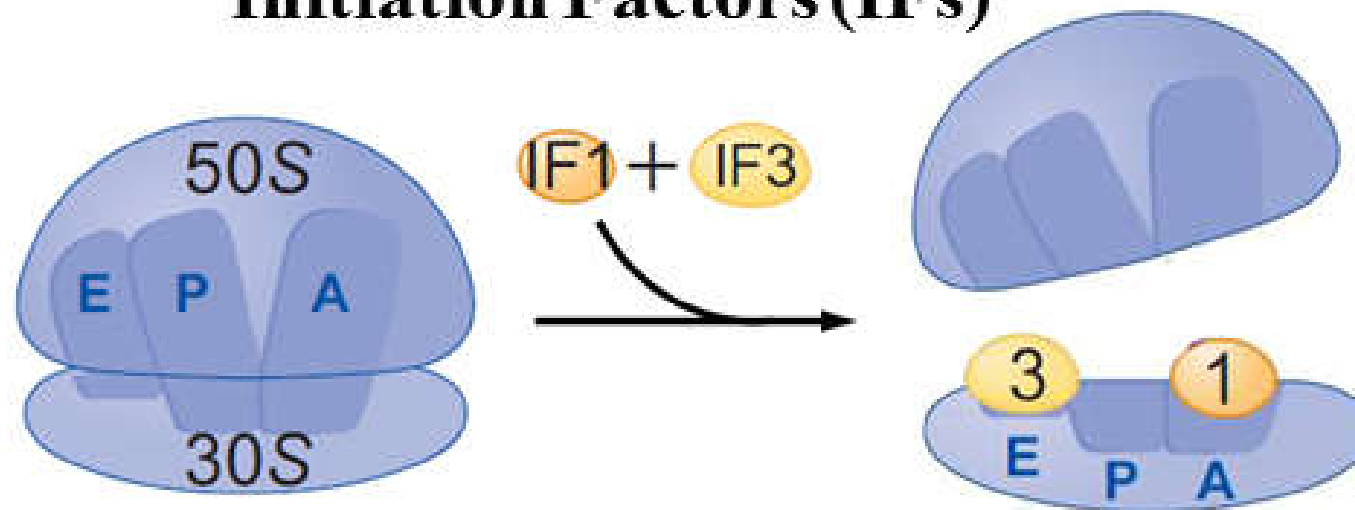
### (1) tRNA charging

**Aminoacyl-tRNA synthetases** link amino acids to their cognate tRNAs.

- amino acid + **ATP** → aminoacyl-AMP + pyrophosphate (**PP<sub>i</sub>**)
- aminoacyl-AMP + tRNA → aminoacyl-tRNA + AMP

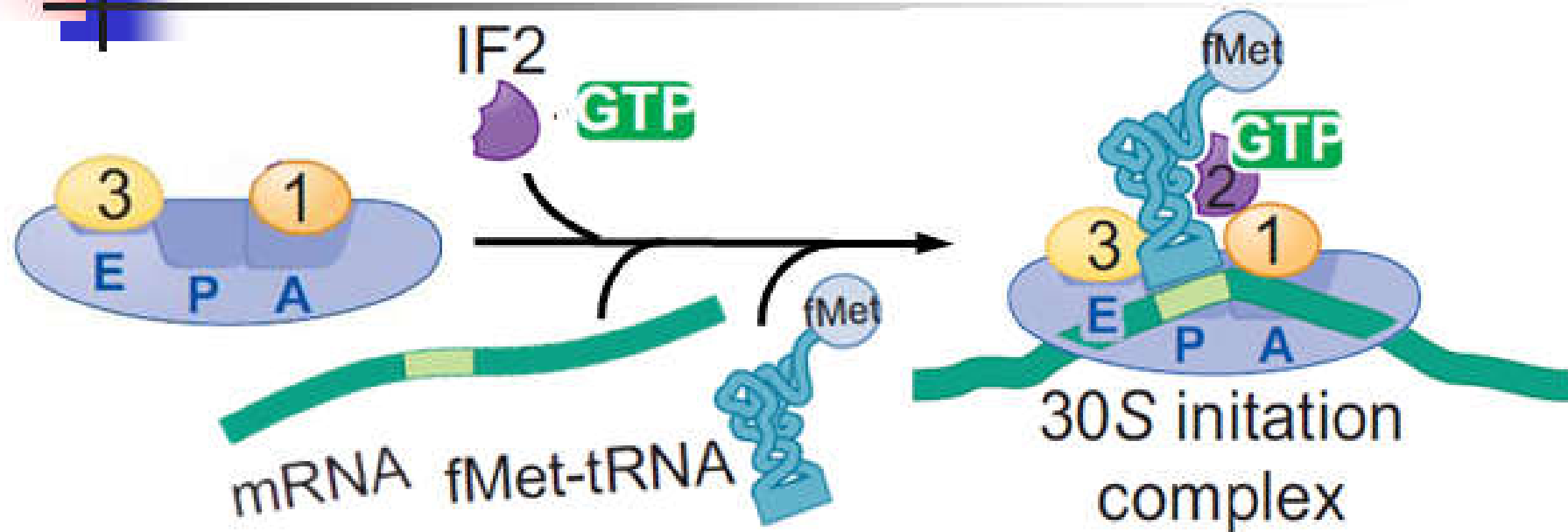
## (2) Dissociation of ribosomes

### Initiation Factors (IFs)



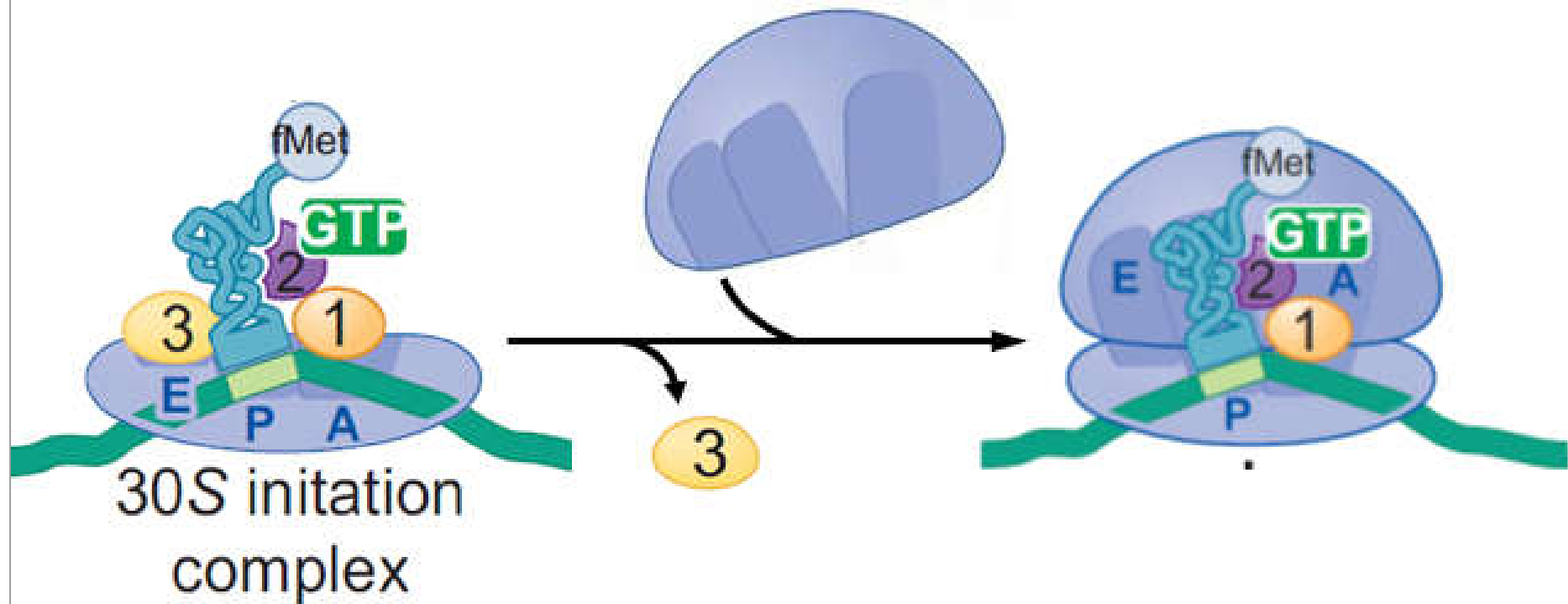
- **IF1** prevents tRNAs from binding to the **A site** of the small subunit.
- **IF3** binds to the small subunit (**E site**) and blocks it from reassociating with a large subunit.

### (3) Formation of the 30S initiation complex

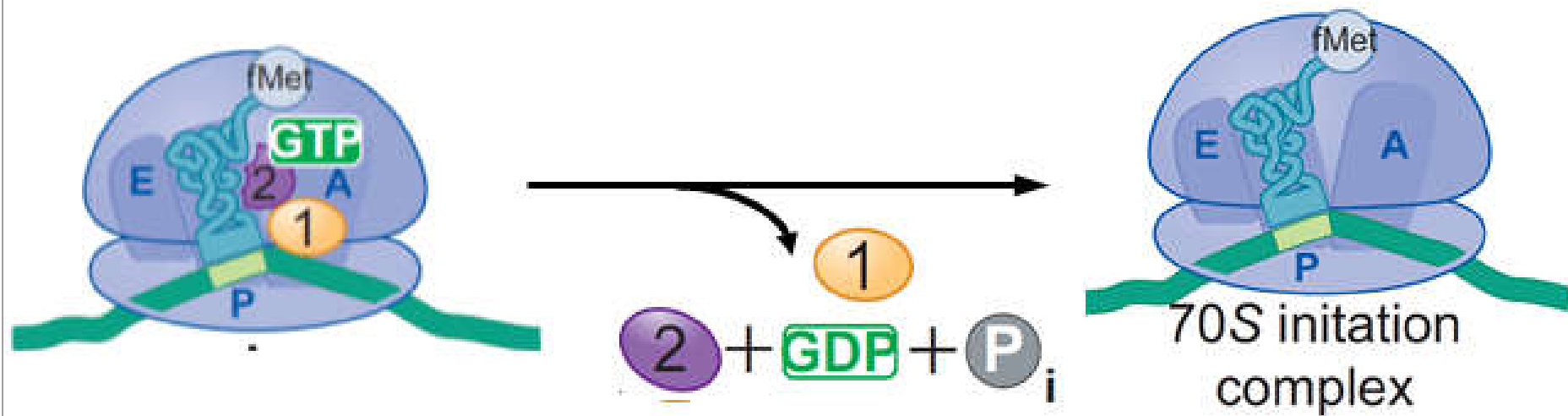


- **IF2** with **GTP** binds to IF1 and facilitates the association of **fMet-tRNA<sub>i</sub>** with the **P site** of the small subunit.
- The small subunit with all three initiation factors recognizes and binds to the **RBS (SD sequence)**. Thus, the **30S initiation complex** is formed.

## (4) Formation of the 70S initiation complex



- **IF3 releases**, and the **large subunit** is free to bind to the small subunit.

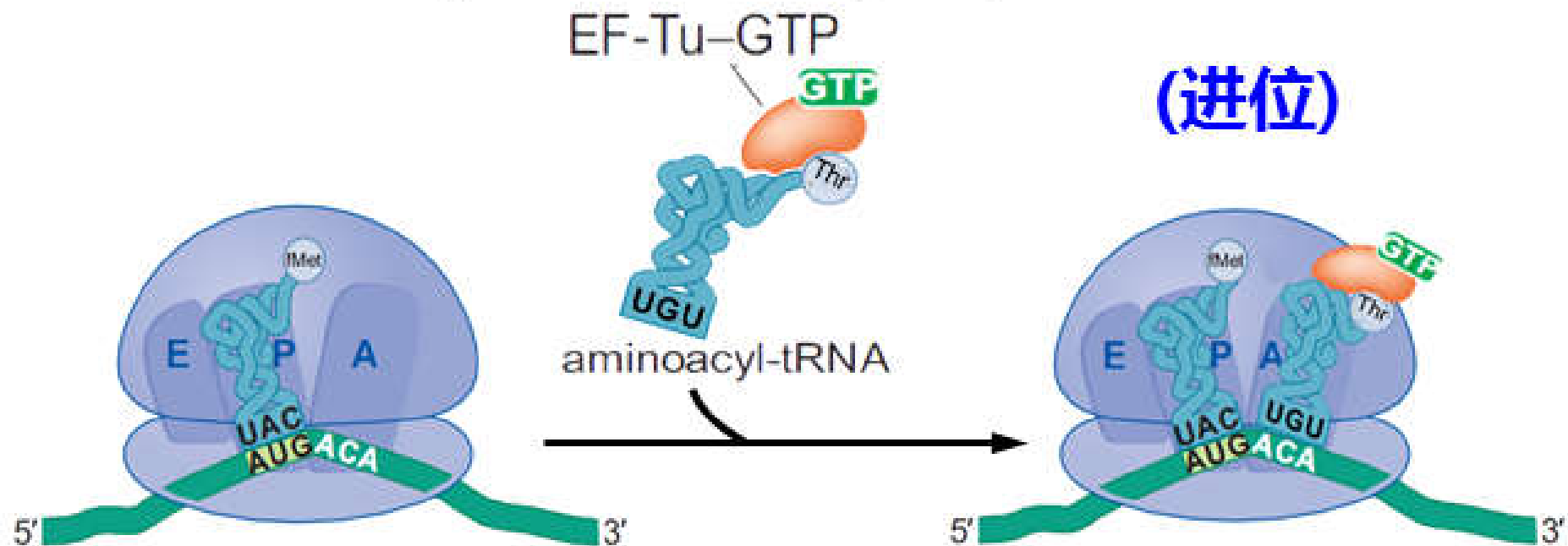


- GTP is cleaved (GTPase activity of IF2), which leading to the **release of IF2•GDP** as well as **IF1** from the ribosome.
- **70S initiation complex** is assembled at the start site of the mRNA with **fMet-tRNA<sub>i</sub>** in the P site and an **empty A site**.

## 5.2 Elongation of translation

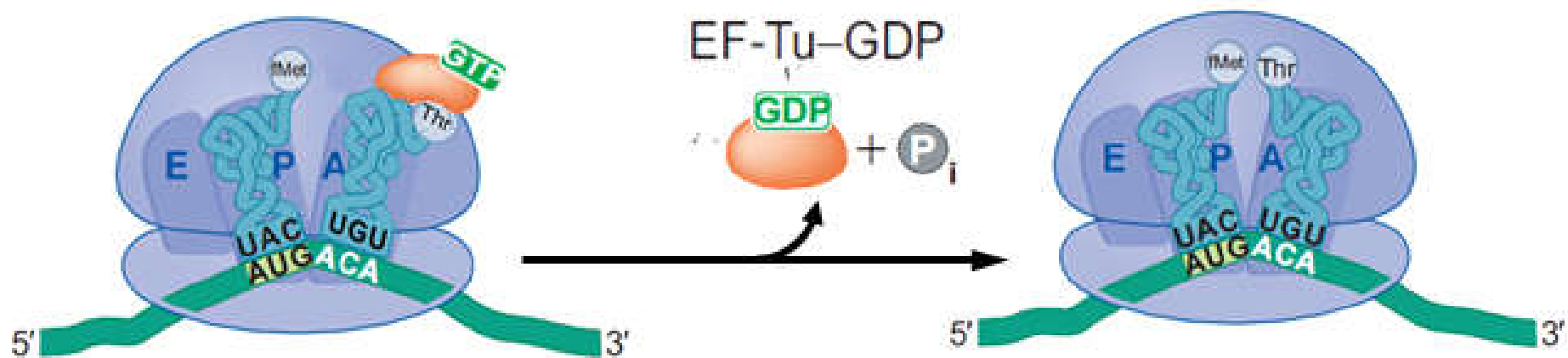
### (1) Aminoacyl-tRNA binding

#### Elongation Factors (EFs)



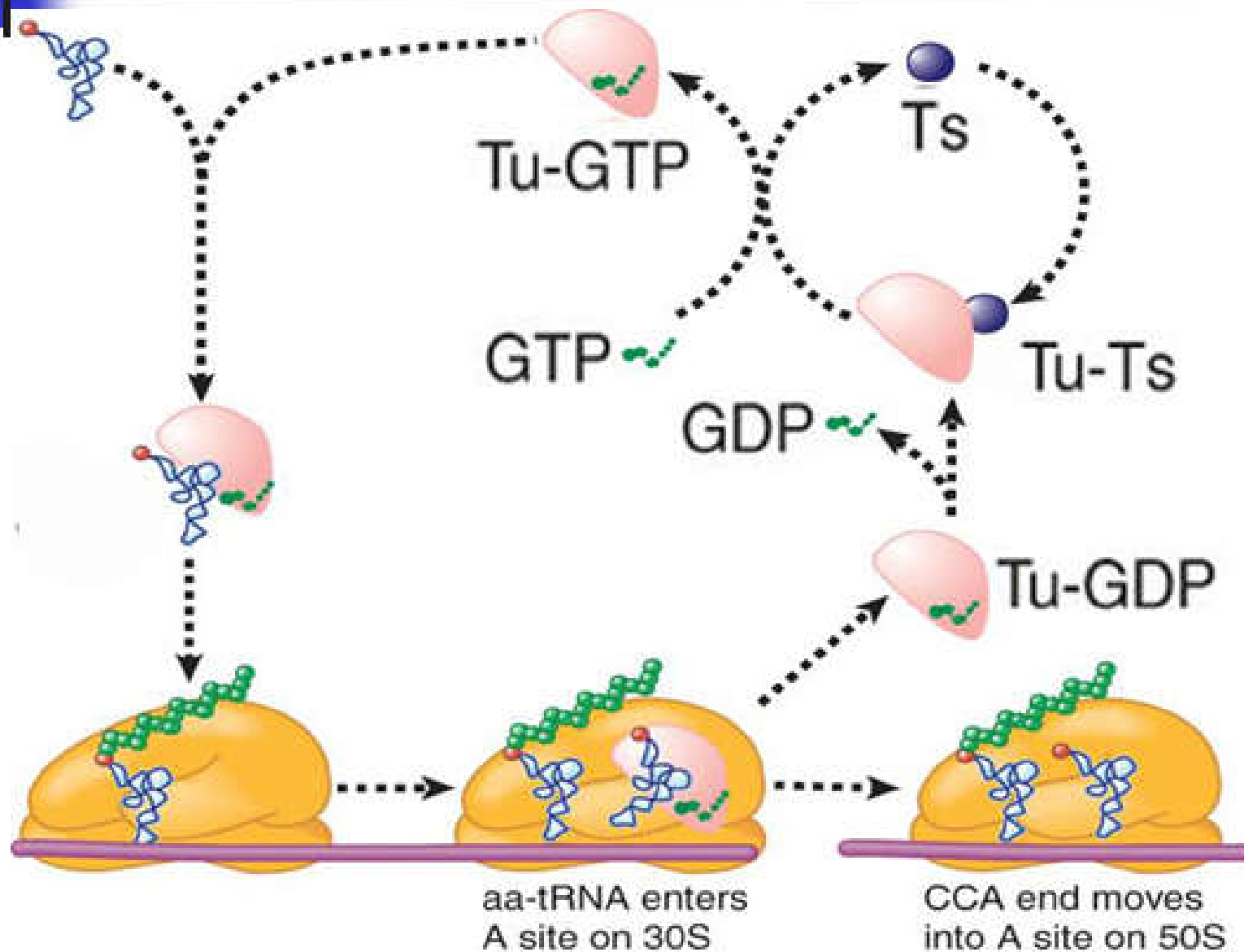
- Charged tRNA bound to **EF-Tu-GTP** interact with the **A-site** of the ribosome.





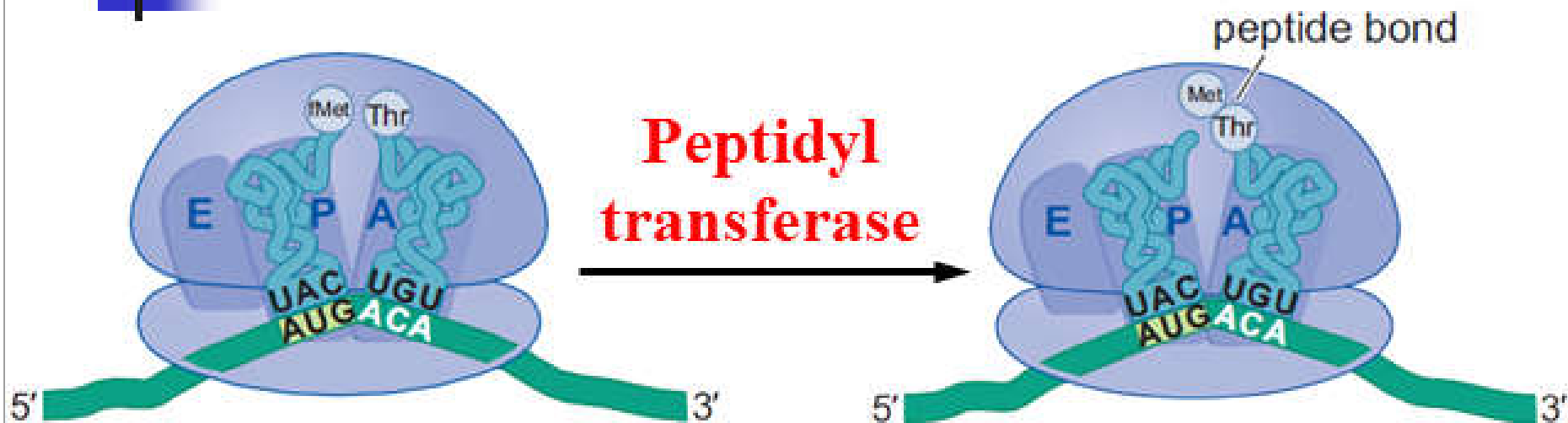
- When the correct codon–anticodon interaction occurs, EF-Tu hydrolyzes its bound GTP, and is released from the tRNA and the ribosome.
- After EF-Tu release, the tRNA rotates into the peptidyl transferase center of the ribosome.

## EF-Tu-**EF-Ts** exchange cycle



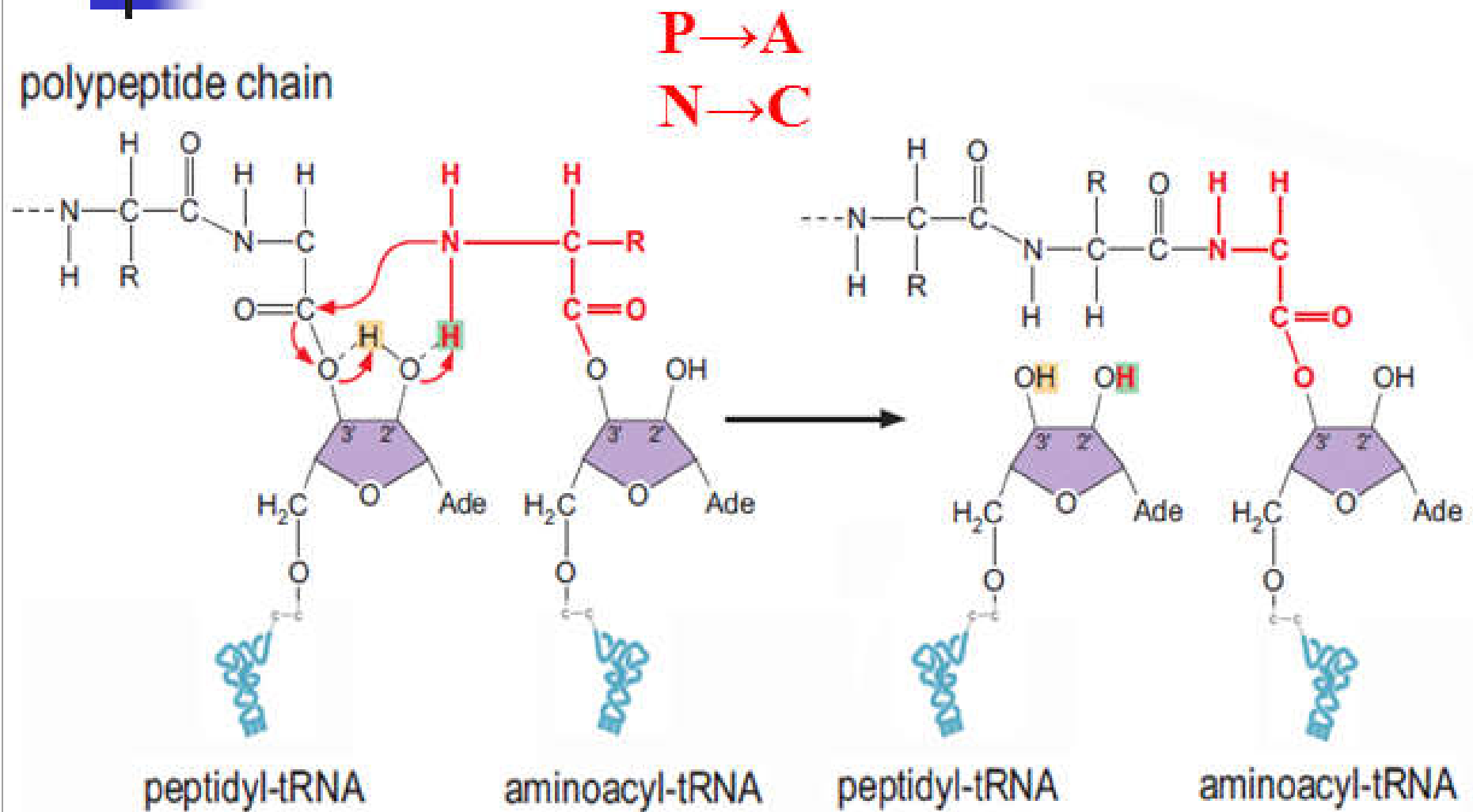
## (2) Peptide bond formation

(转肽)

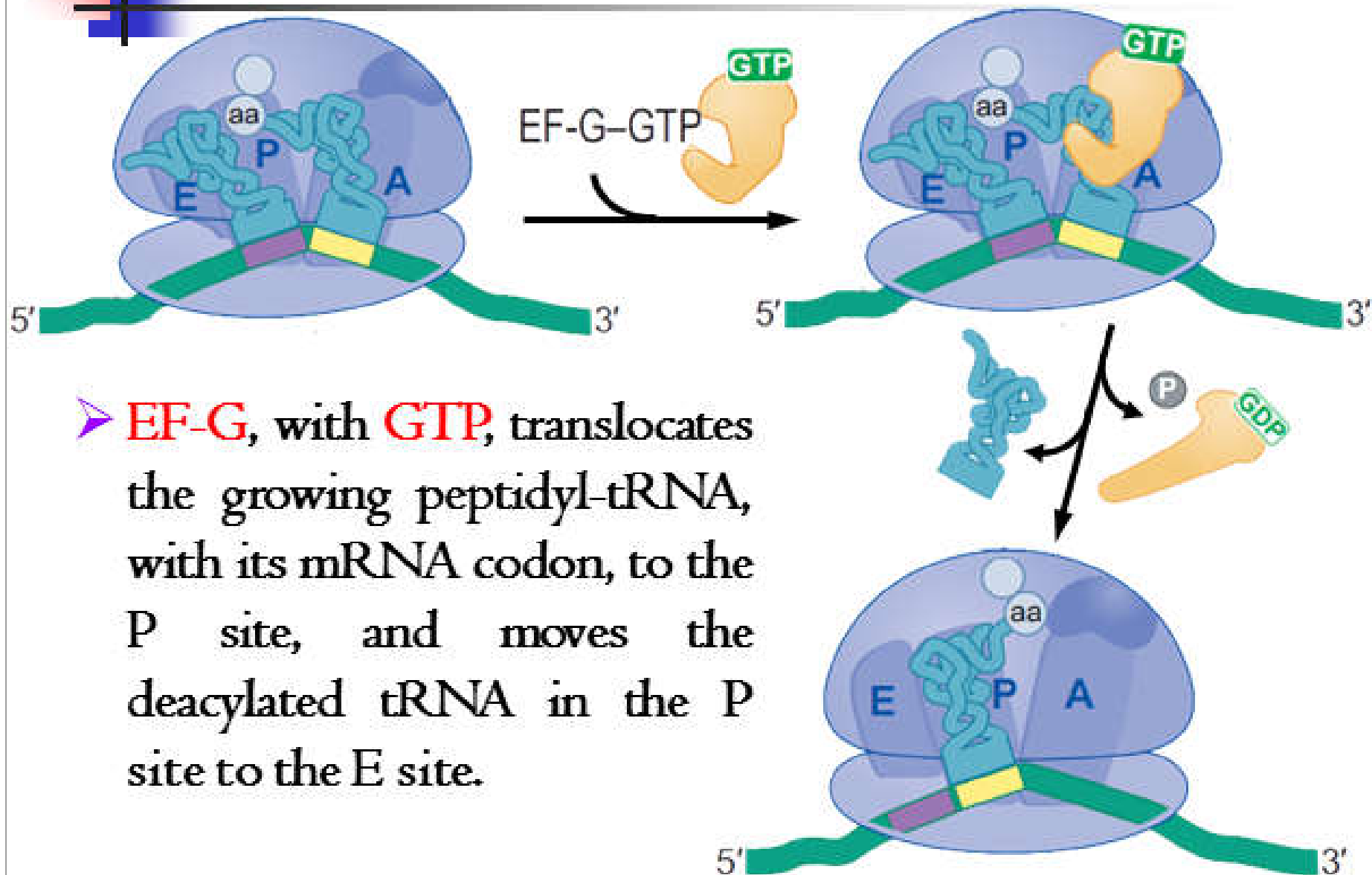


- The 50S subunit has peptidyl transferase activity as provided by an **rRNA ribozyme**.
- The nascent polypeptide chain is **transferred from** peptidyl-tRNA in the **P site to** newly arrived aminoacyl-tRNA in the **A site**.

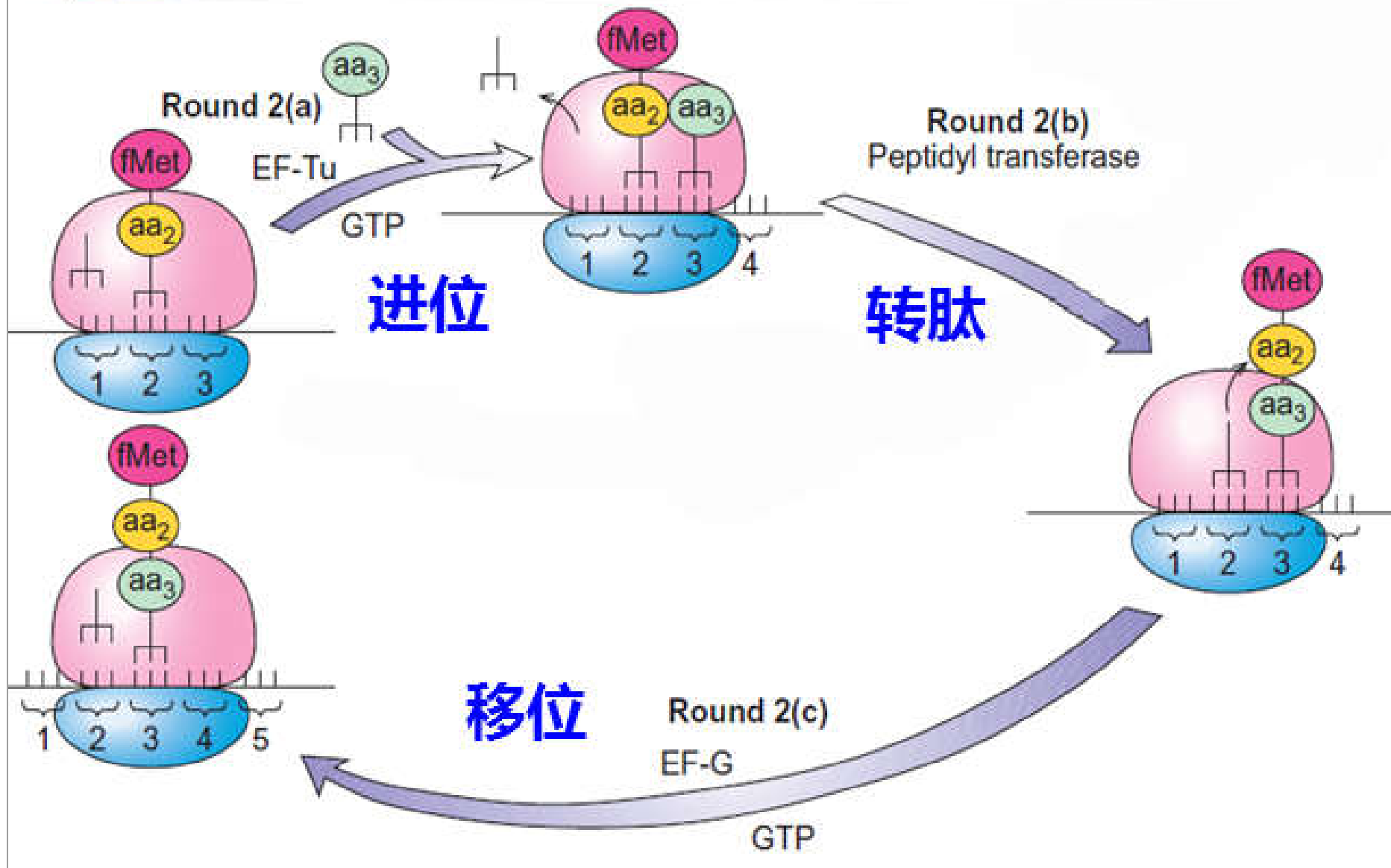
# The mechanism of peptide bond formation



### (3) Translocation (移位)



# The Elongation Cycle





## 5.3. Termination of translation

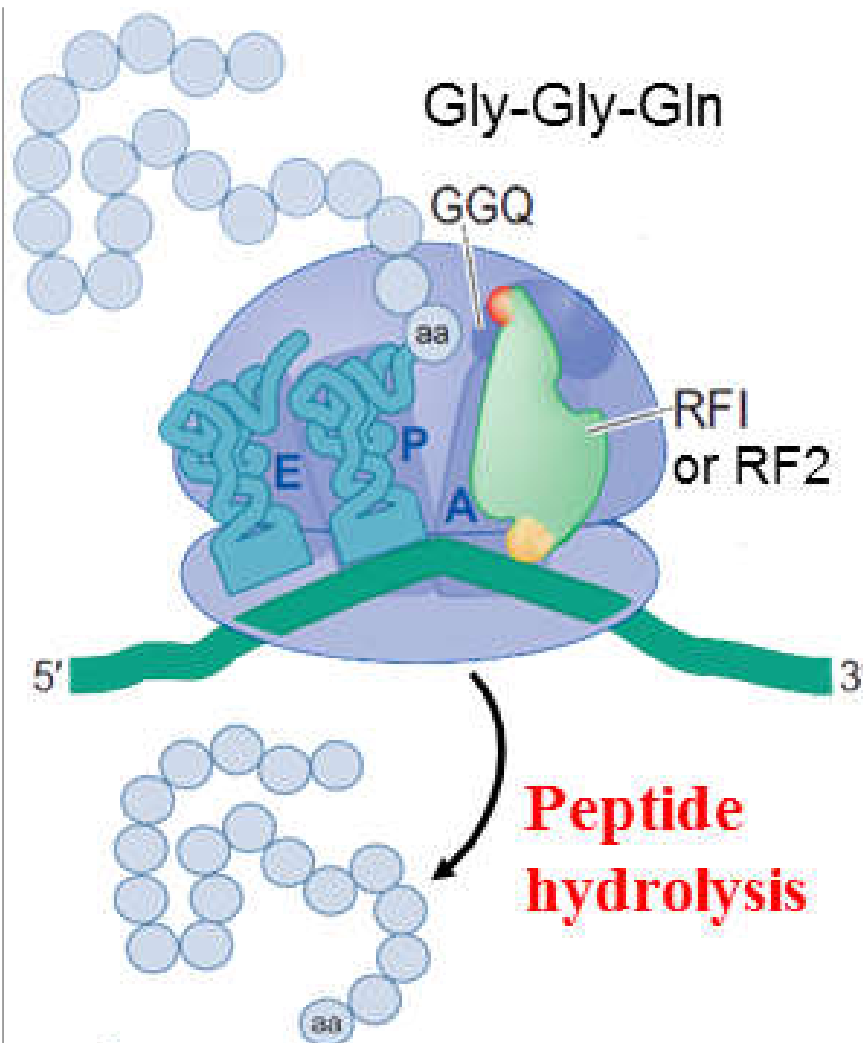
### (1) Release factors (RFs)

Class I RFs { RF1: UAA, UAG  
RF2: UAA, UGA

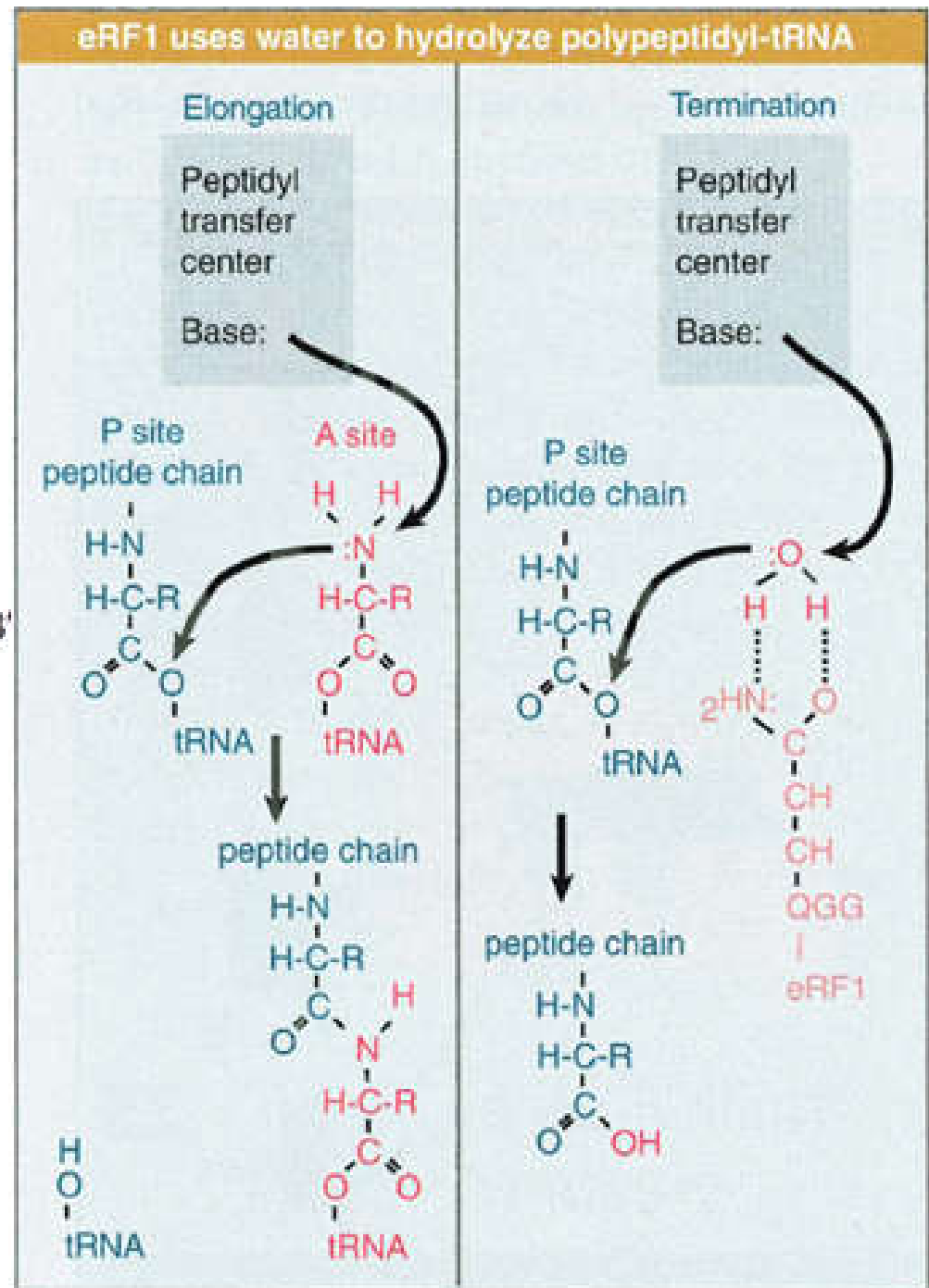
Class I RFs recognize the stop codons and trigger hydrolysis of the peptide chain from the tRNA in the P site.

Class II RF RF3

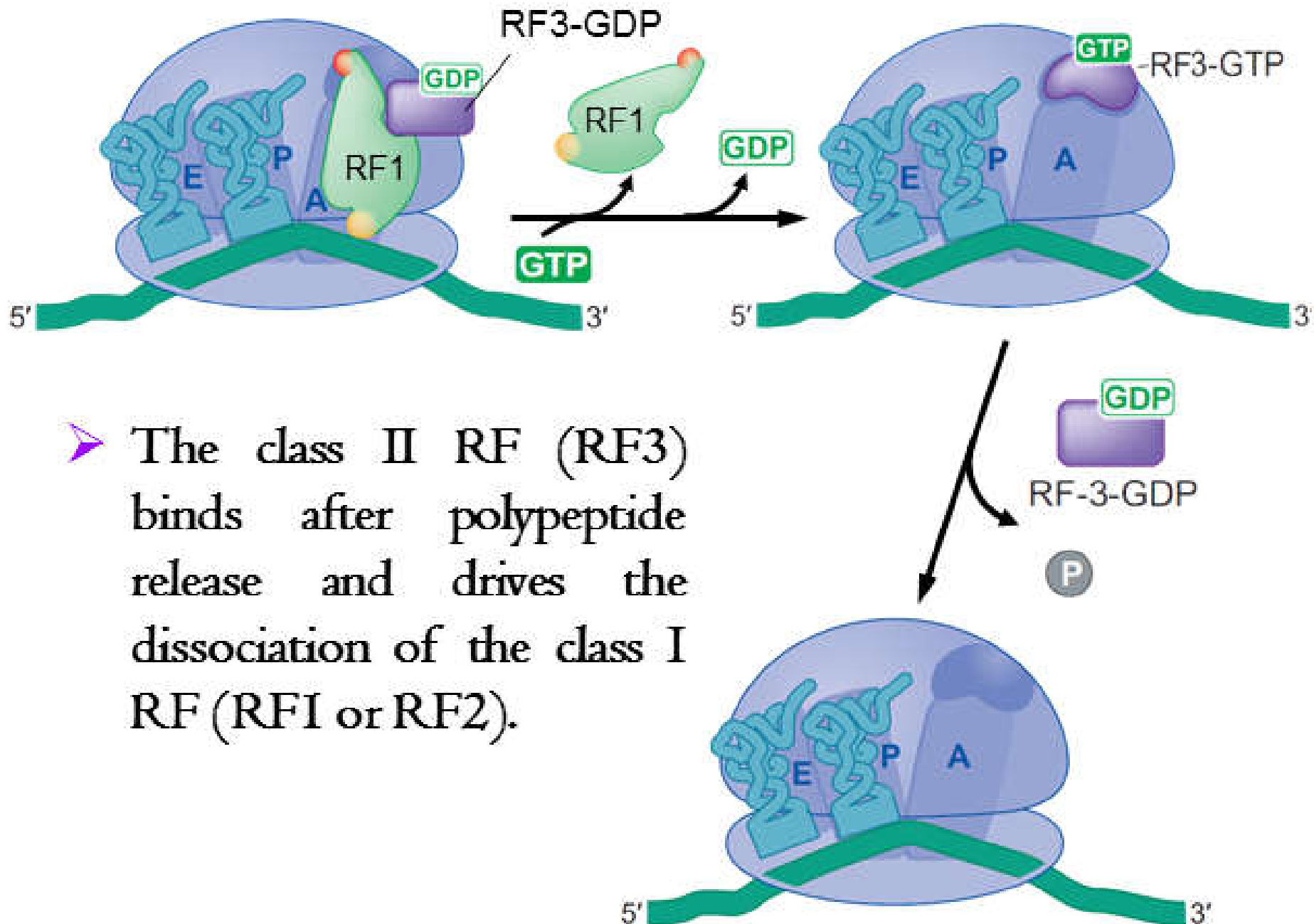
Class II release factors stimulate the dissociation of the class I factors from the ribosome after release of the polypeptide chain.



➤ 转肽反应将肽链转移到A位点结合的I类RF顶端GGQ序列中的Gln侧链结合的 $H_2O$ 上，释放肽段。

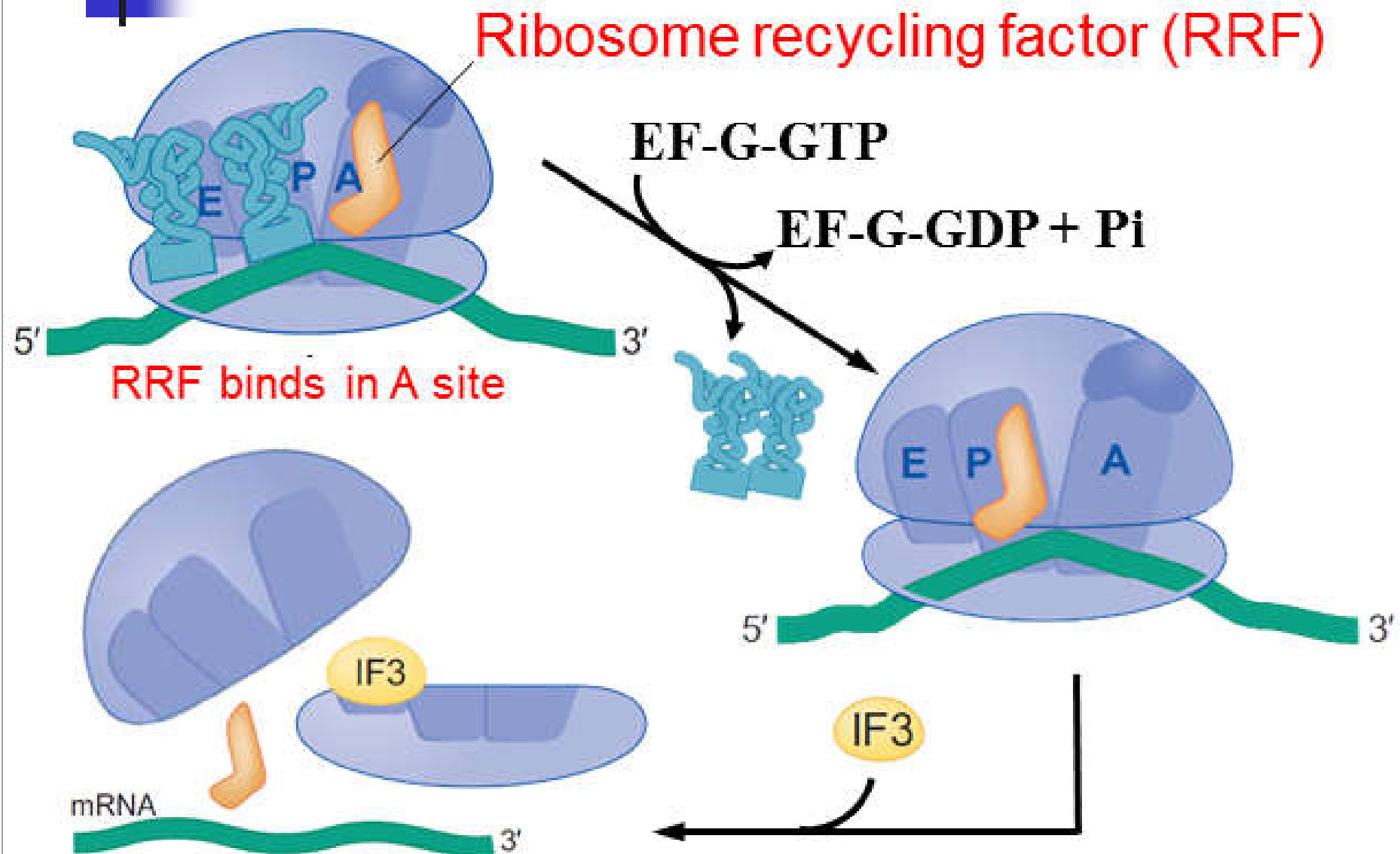






- The class II RF (RF3) binds after polypeptide release and drives the dissociation of the class I RF (RF1 or RF2).

## (2) Ribosome dissociation





## **6. Differences between prokaryotic and eukaryotic protein synthesis**

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- (1) Ribosomes**
- (2) Initiation codon and initiator tRNA**
- (3) 5' structure of mRNA (cap and Kozak sequence)**
- (4) Monocistronic mRNA**
- (5) Translation is not coupled to transcription**
- (6) Translation factors**

Initiation Factors			
Prokaryotic	Eukaryotic	General Function	Notes
IF-1	eIF1A	Blocks A site	eIF1A assists eIF2 in promoting Met-tRNA <sup>Met</sup> to binding to 40S; also promotes subunit dissociation eIF2 is a GTPase eIF3 stimulates formation of the ternary complex, its binding to 40S, and binding and scanning of mRNA eIF5B is involved in initiator tRNA entry and is a GTPase eIF4 complex functions in cap binding
IF-2*†	eIF2, eIF3, eIF5B*	Entry of initiator tRNA	
IF-3	4A, 4B, 4E, 4G eIF1, eIF4 complex, eIF3	Small subunit binding to mRNA	
Elongation Factors			
Prokaryotic	Eukaryotic	General Function	
EF-Tu†‡, EF-G† EF-Ts EF-G§	eEF1α‡ eEF1β, eEF1γ eEF2§	GTP-binding GDP-exchanging Ribosome translocation	
Release Factors			
Prokaryotic	Eukaryotic	General Function	
RF1 RF2 RF3†	eRF1 eRF1 eRF3	UAA/UAG recognition UAA/UGA recognition Stimulation of other RF(s)	

\* IF-2 and eIF5B have sequence homology.

† IF-2, EF-Tu, EF-G, and RF3 have sequence homology.

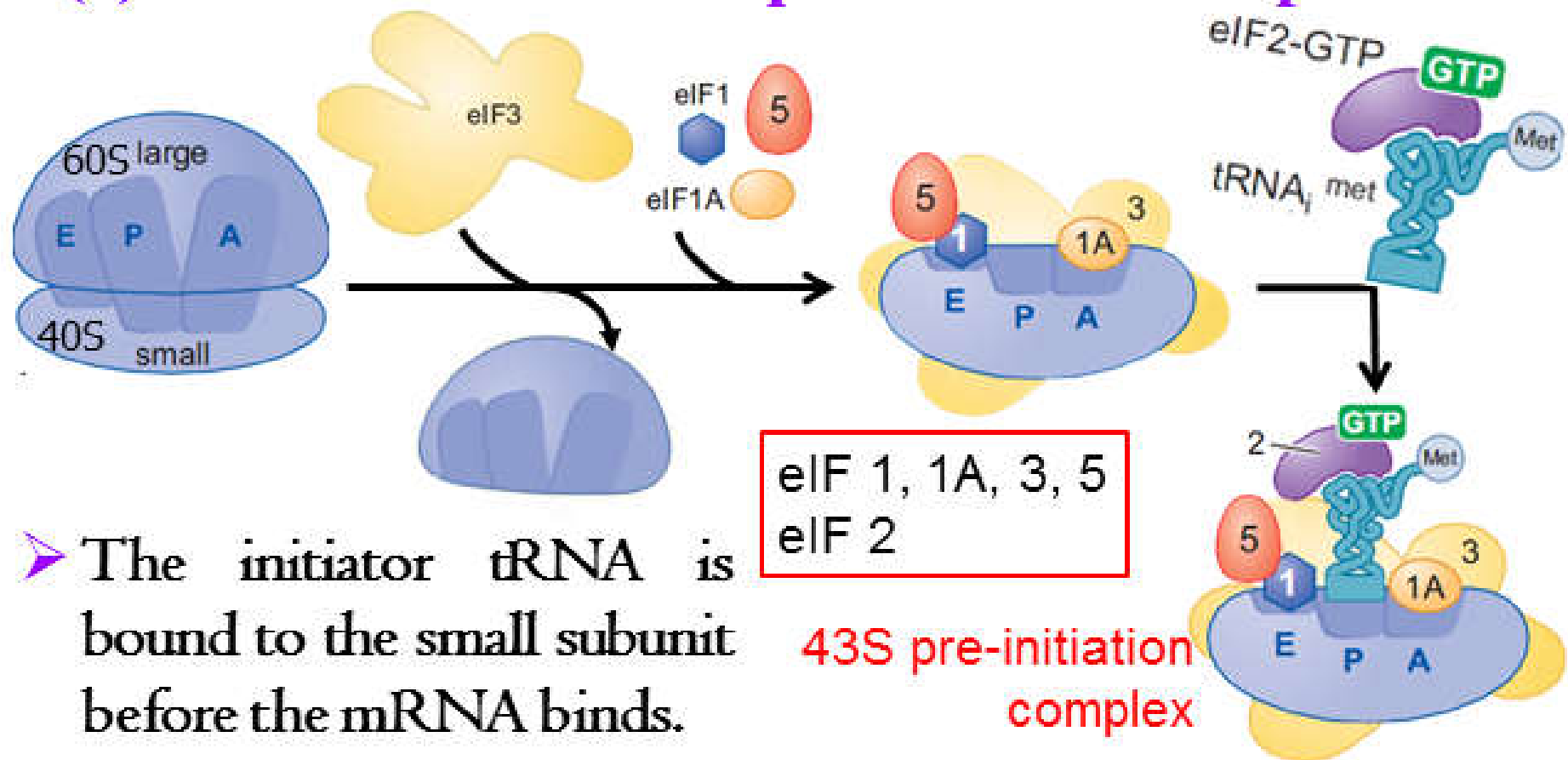
‡ EF-Tu and eEF1α have sequence homology.

§ EF-G and eEF2 have sequence homology.

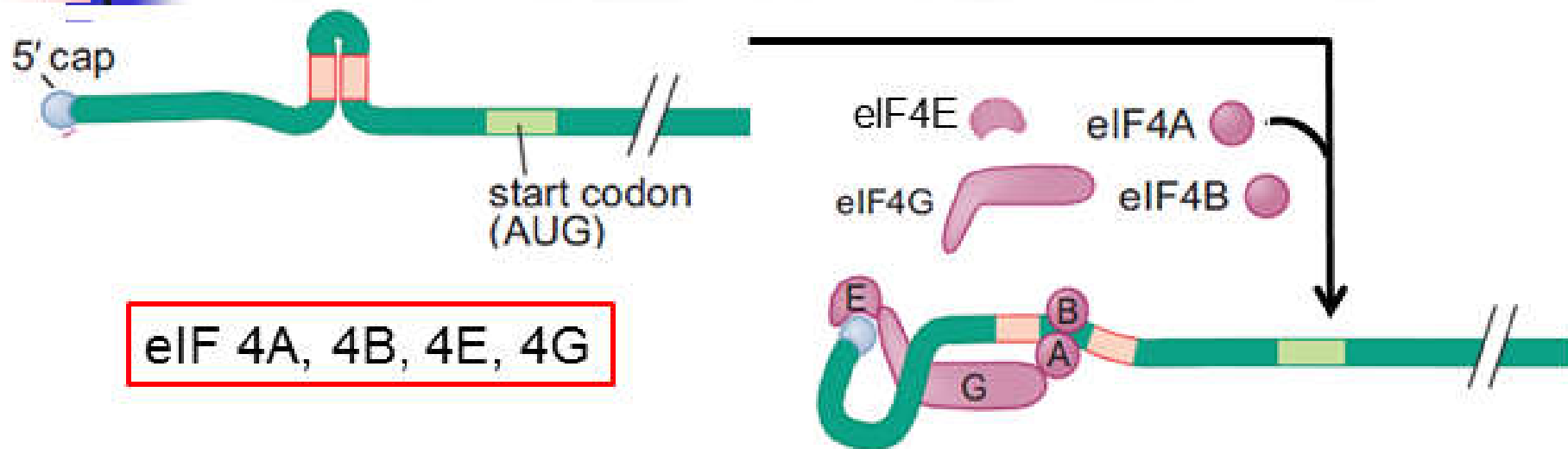
## 7. Initiation of translation in eukaryotes

### 7.1 Formation of the 48S pre-initiation complex

#### (1) Formation of the 43S pre-initiation complex

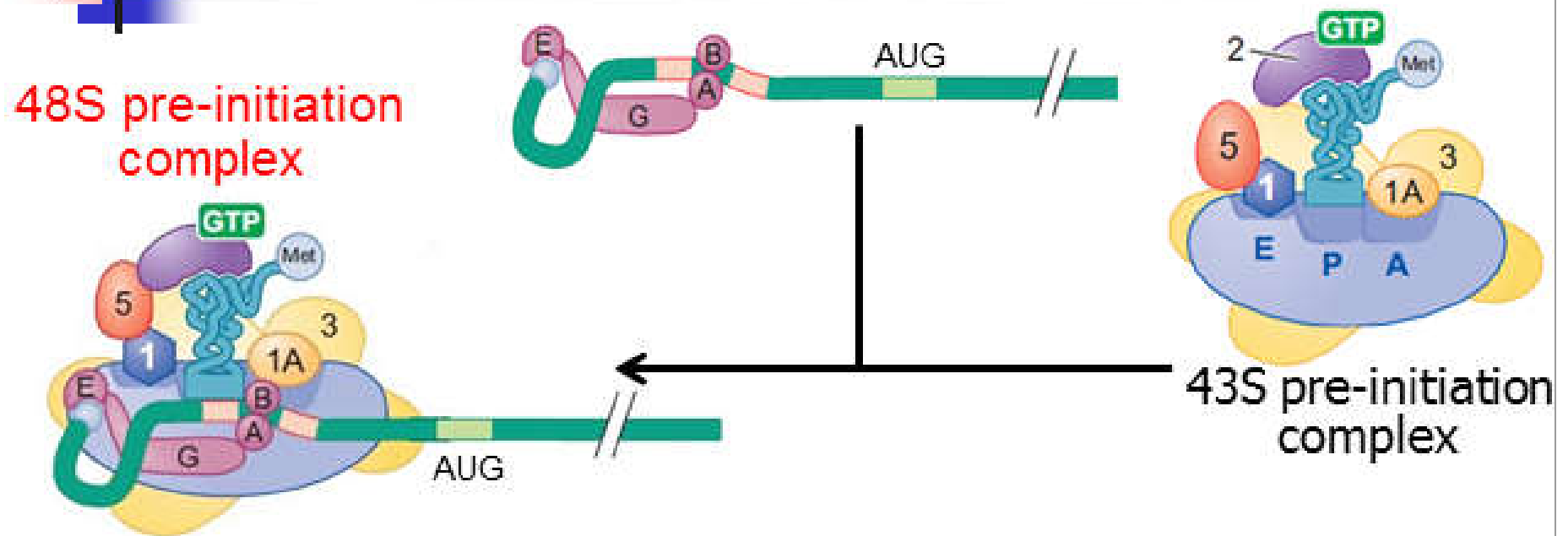


## (2) mRNA is prepared for recognition by the 40S subunit



- **eIF4E** – cap binding protein
- **eIF4A** – RNA helicase which binds the mRNA unwinds any secondary structures formed at the end of the mRNA.
- **eIF4B** – activates the activity of eIF4A
- **eIF4G** – scaffold protein which binds to both eIF4E and eIF4A

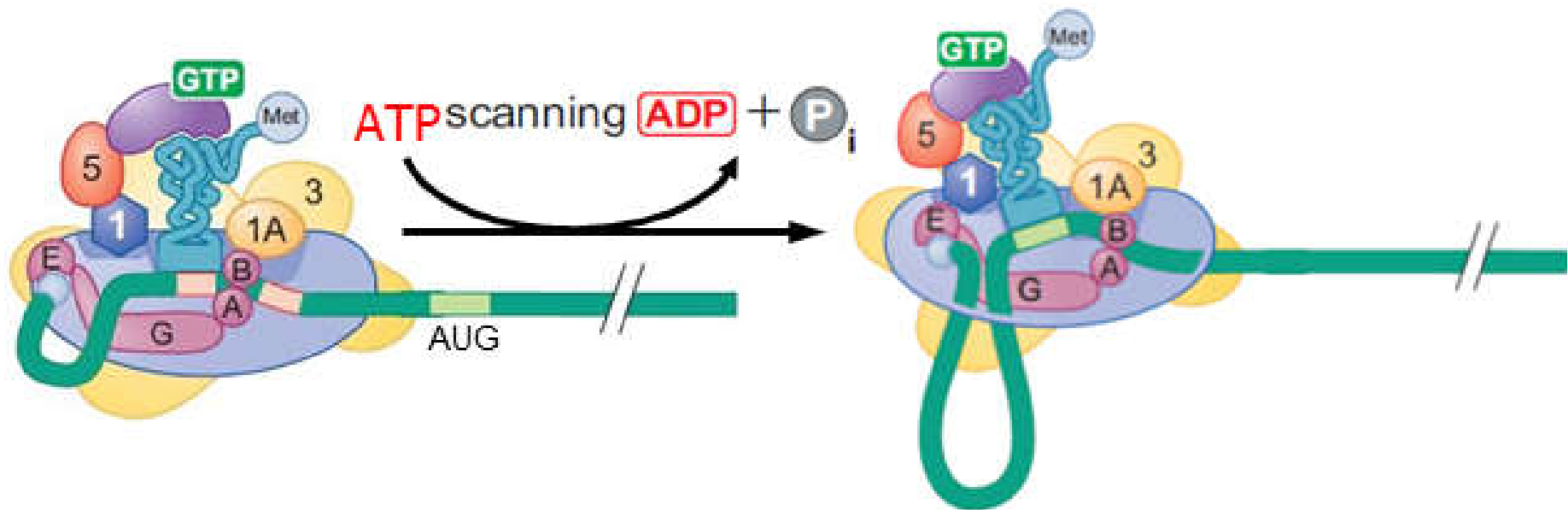
### (3) Recruit the 43S preinitiation complex to the mRNA



- 43S pre-initiation complex binds to the mRNA complex via the interactions between eIF4G and the initiation factors (particularly eIF3) bound to the small subunit to form the 48S pre-initiation complex.

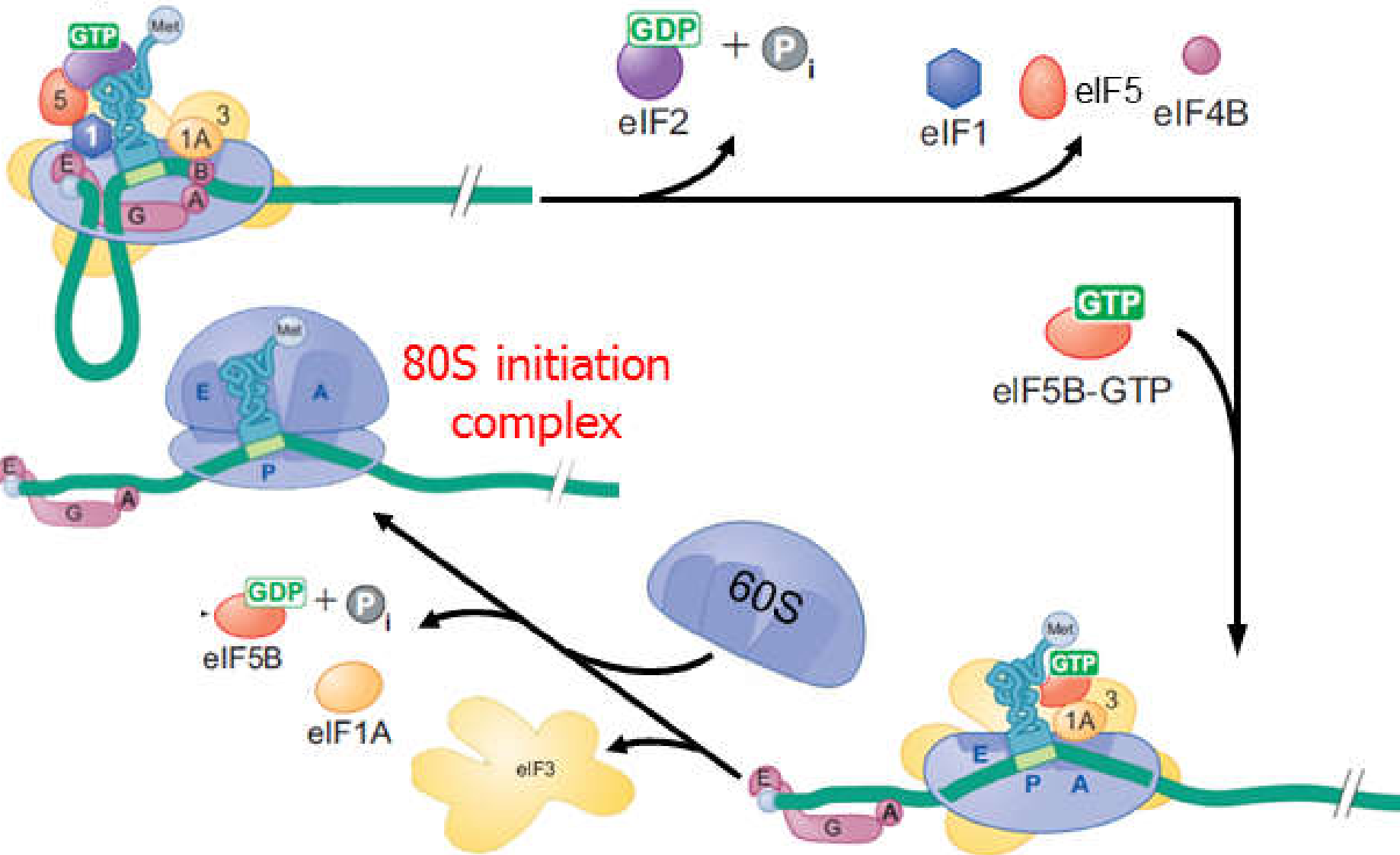
## 7.2 Formation of the 80S initiation complex

### (1) Scanning



- The small subunit first binds to methylated cap, and then “scans” the mRNA for the first start codon.





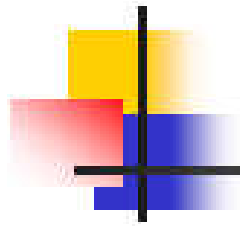


## 8. Post-translational events

### 8.1 Post-translational processing

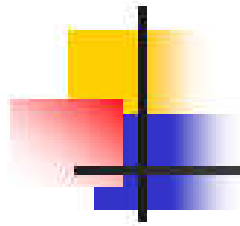
#### (1) Cleavage

- To remove signal peptides
- To release mature fragments from polyprotein
  - Polyprotein – mRNA are translated to a single polypeptide chain that is cleaved subsequently by specific proteases to produce multiple mature protein from one translation product.
- To remove internal peptides as well as
- To trim both N- and C-termini.



## (2) Covalent modification

- Acetylation (乙酰化)
- Methylation (甲基化)
- Hydroxylation (羟基化)
- Phosphorylation (磷酸化)
- Glycosylation (糖基化)
- Addition of nucleotides

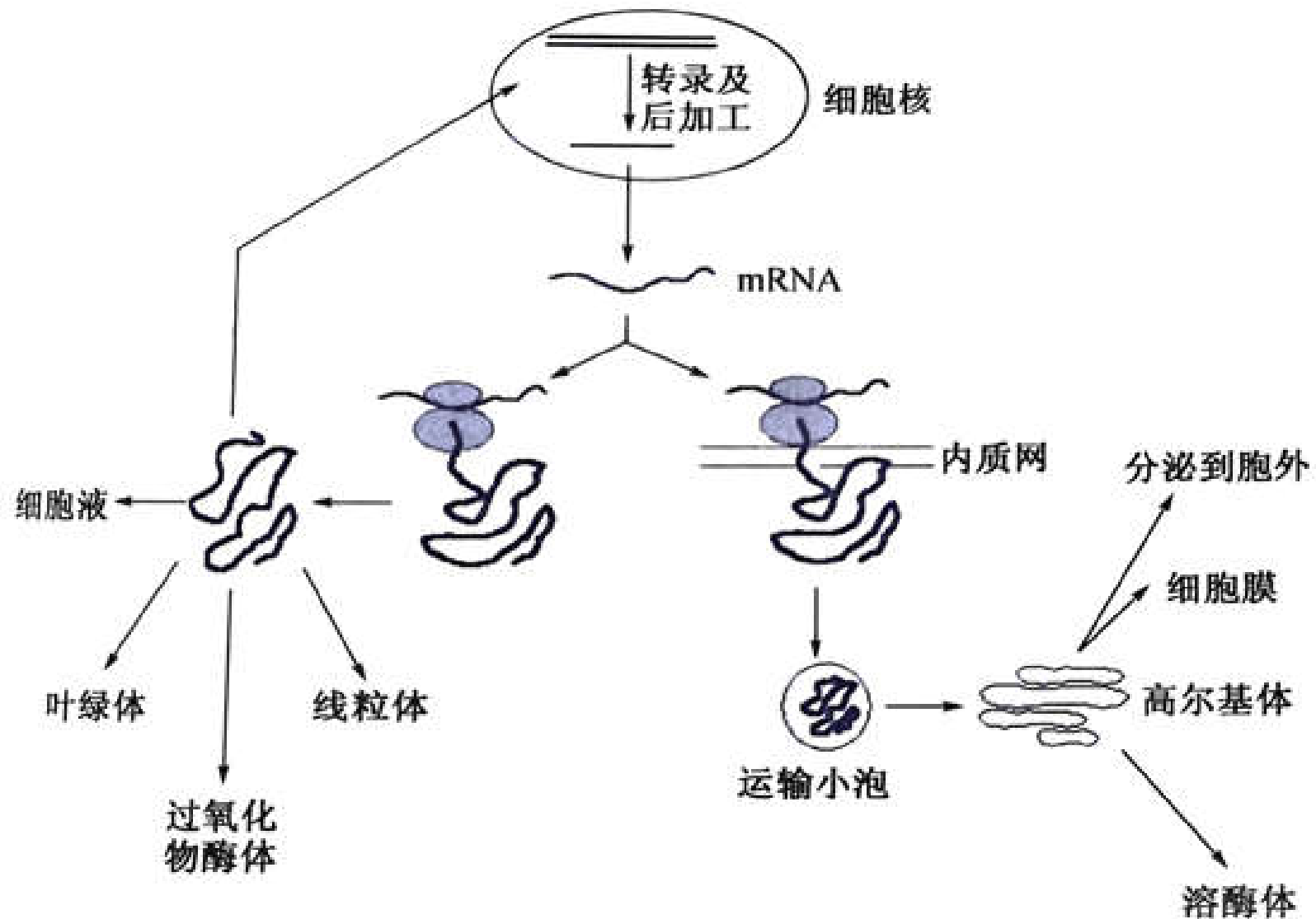


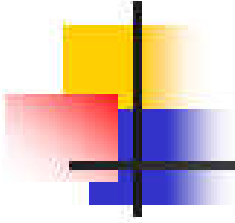
### (3) Folding

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- Formation of the three-dimensional structure
- With the help of Molecular chaperones  
(分子伴侣)

## 8.2 Protein targeting





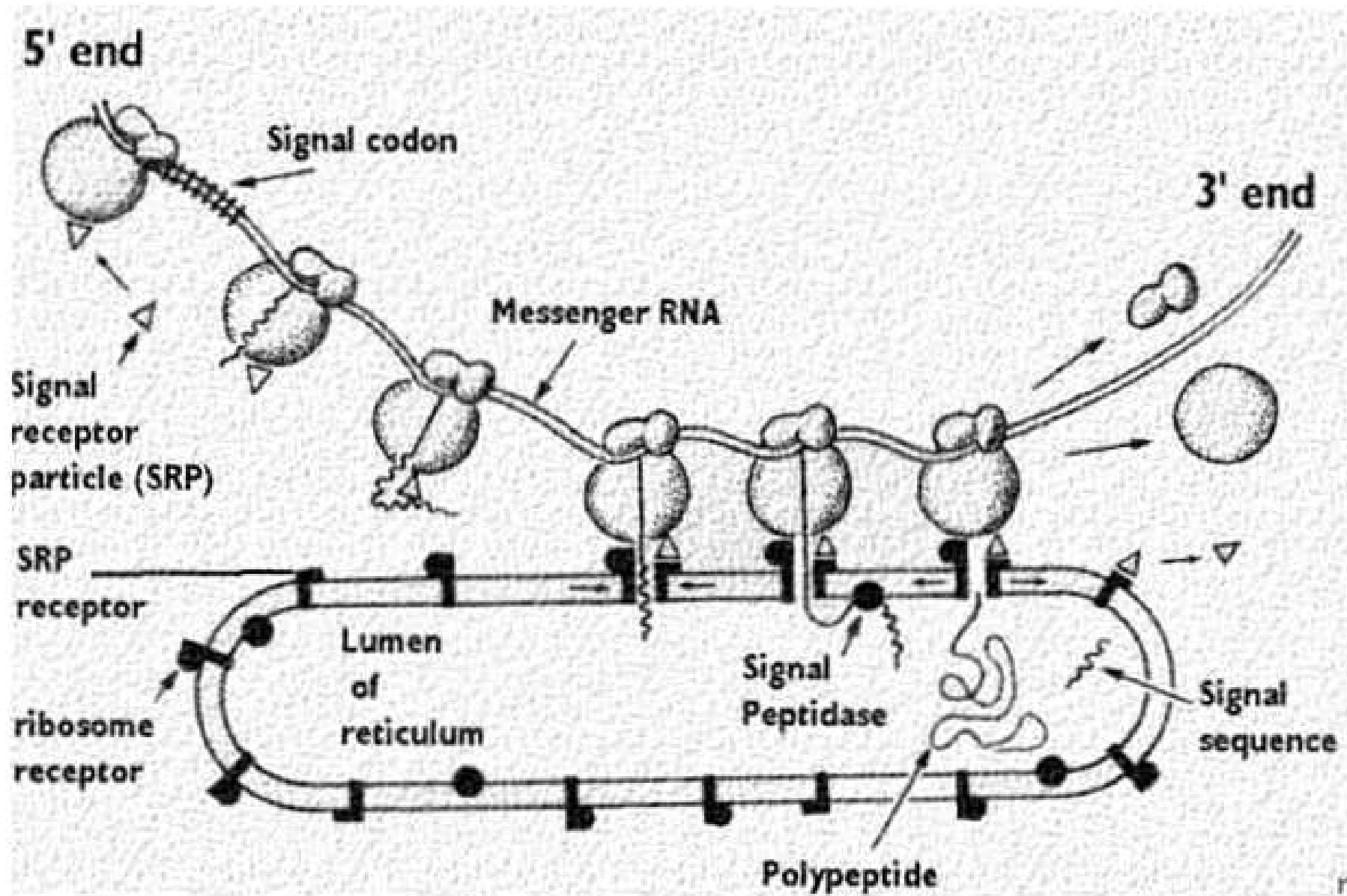
1975, Blobel & Dobberstein

- **Signal hypothesis** – the ultimate cellular location of proteins is often determined by specific, relatively short, amino acid sequences within the proteins themselves.

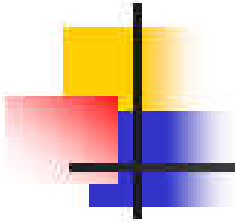


## 8.2.1 Co-translation pathway

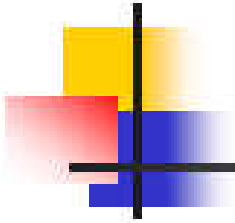
- **Proteins of endoplasmic reticulum (ER), Golgi apparatus, lysosome, cell membrane, and secreted proteins.**
- **N-signal peptide**
  - Composed of 13-16 amino acids
  - Have at least 1 positively charged residue
  - A hydrophobic core of 10-15 residues
  - Neutral residue (often Ala) at C-terminal
  - **Binds to signal recognition particle (SRP)**







- **Signal recognition particle (SRP)**
  - Recognize ribosomes with signal peptide of the nascent chain.
- **SRP receptor (docking protein)**
  - SRP with the arrested ribosome can bind to SRP receptor on the cytosolic side of the ER.

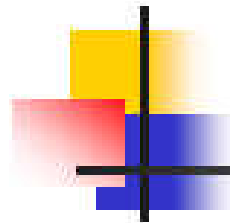


- **Ribosome receptor (protein translocator complex)**
  - Ribosome with SRP attaches to ribosome receptor on the ER.
  - SRP is released and can be re-used.
  - The ribosome is able to continue translation, and the nascent polypeptide chain is pushed through into the lumen of the ER.



## 8.2.2 Post-translation pathway

- **Proteins of mitochondria, chloroplast and nuclear**
- **Signal peptide**
  - Mitochondrial protein – leader peptide sequence (导肽序列)
  - Chloroplast protein – transit peptide (输送肽)
  - Nuclear protein – nuclear localization signal (NLS)

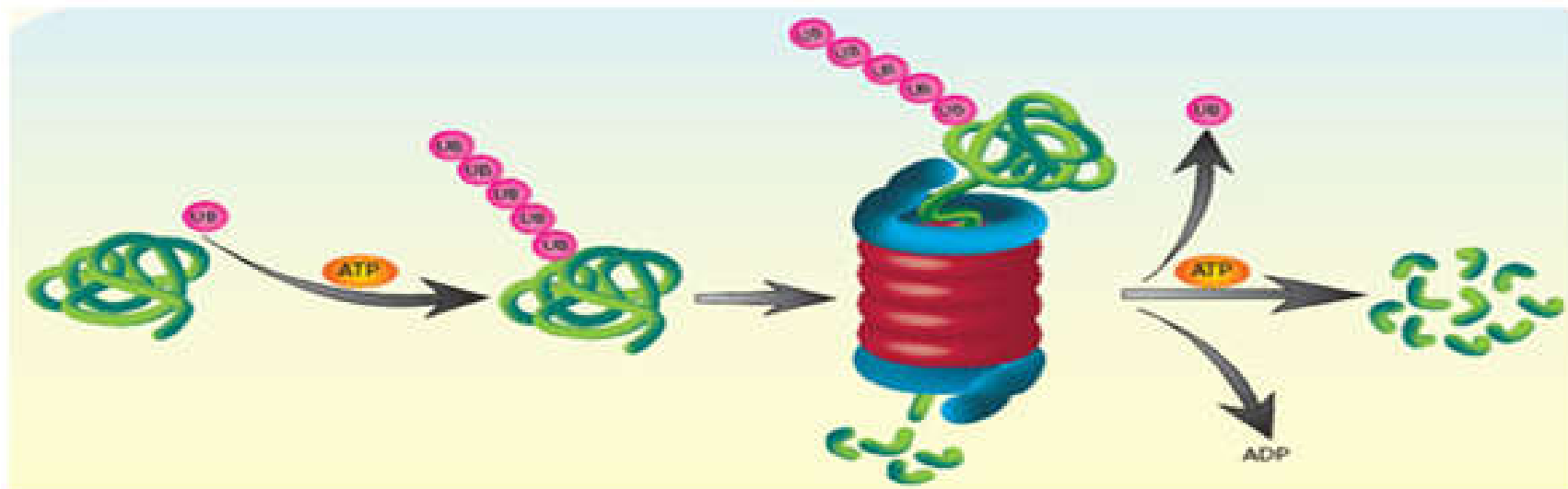


## 8.3 Protein degradation

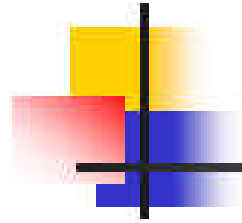
### 8.3.1 Proteasome (蛋白酶体) pathway

- Degradation of **endogenous proteins**
- In eukaryotes, **N-terminal residue plays a critical role in inherent stability:**
  - $t_{1/2} > 20$  hours: Ala, Cys, Gly, Met, Pro, Ser, Thr, Val;
  - $t_{1/2} 2 \sim 30$  min: Arg, His, Ile, Leu, Lys, Phe, Trp, Tyr;
  - Destabilizing: Asn, Asp, Gln, Glu.

- N-terminal residue becomes **ubiquitinated** (泛素化) by covalent linkage of ubiquitin via its C-terminal Gly, to lysine residues in the protein.
- The ubiquitinated protein is digested by a 26S protease complex in a reaction **that requires ATP** and releases intact ubiquitin for re-use.



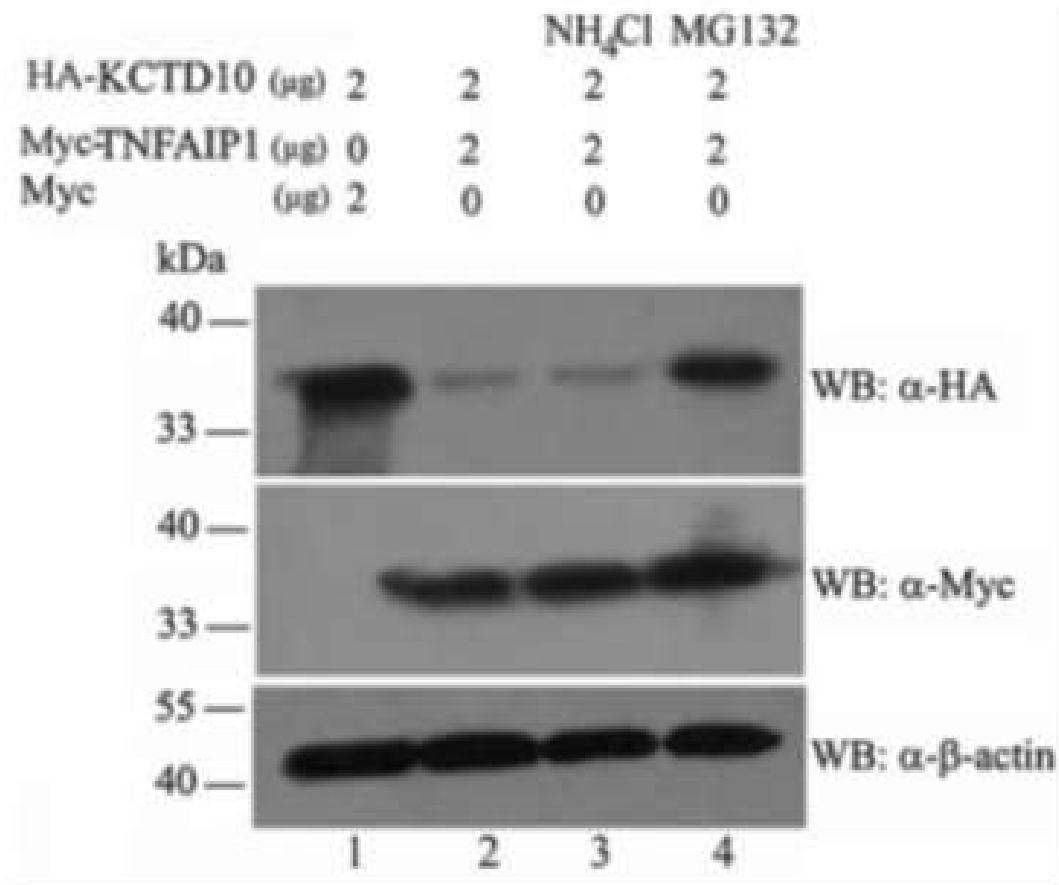
**26S protease complex - Proteasome**

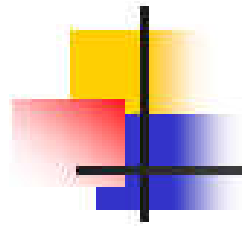


## 8.3.2 Lysosome (溶酶体) pathway

- Degradation of the **extracellular proteins** taken into the cell and the intracellular proteins taken into the autophagosomes (自噬体).
- Receptor-mediated endocytosis into endosome

- **NH<sub>4</sub>Cl** : 溶酶体降解途径抑制剂
- **MG132** : 蛋白酶体降解途径抑制剂

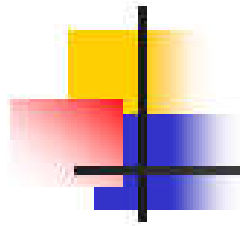




### 8.3.3 Results of protein degradation

- Reduced to **amino acids** that can be used to make new proteins
- **Random peptide fragments** of 9 amino acids in length are attached to peptide receptors – major histocompatibility complex class I molecules (MHC I, I类主要组织相容性复合物)





## 9. Inhibitors of protein synthesis

### 9.1 Antibiotics

- **Target cells:** Prokaryotic cells

#### 9.1.1 Streptomycin (链霉素)

- **Molecular target:** 16S rRNA of the **30S subunit**
- **Consequence:** interfering with the binding of formylmethionyl-tRNA to the 30S subunit.

### 9.1.2 Kanamycin (卡那霉素)

- **Molecular target:** 30S subunit
- **Consequence:** Mistranslation and indirectly inhibits translocation during protein synthesis

### 9.1.3 Neomycin (新霉素)

- **Molecular target:** 30S subunit
- **Consequence:** prevent assembly of the small subunit

### 9.1.4 Tetracyclines (四环素类)

- **Molecular target:** A-site of **30S subunit**
- **Consequence:** Inhibits aminoacyl-tRNA binding to A-site

### 9.1.5 Chloramphenicol (氯霉素)

- **Molecular target:** Peptidyl transferase center of **50S subunit**
- **Consequence:** Blocks correct positioning of A-site aminoacyl-tRNA for peptidyl transfer reaction

## 9.2 Diphtheria toxin (白喉毒素)

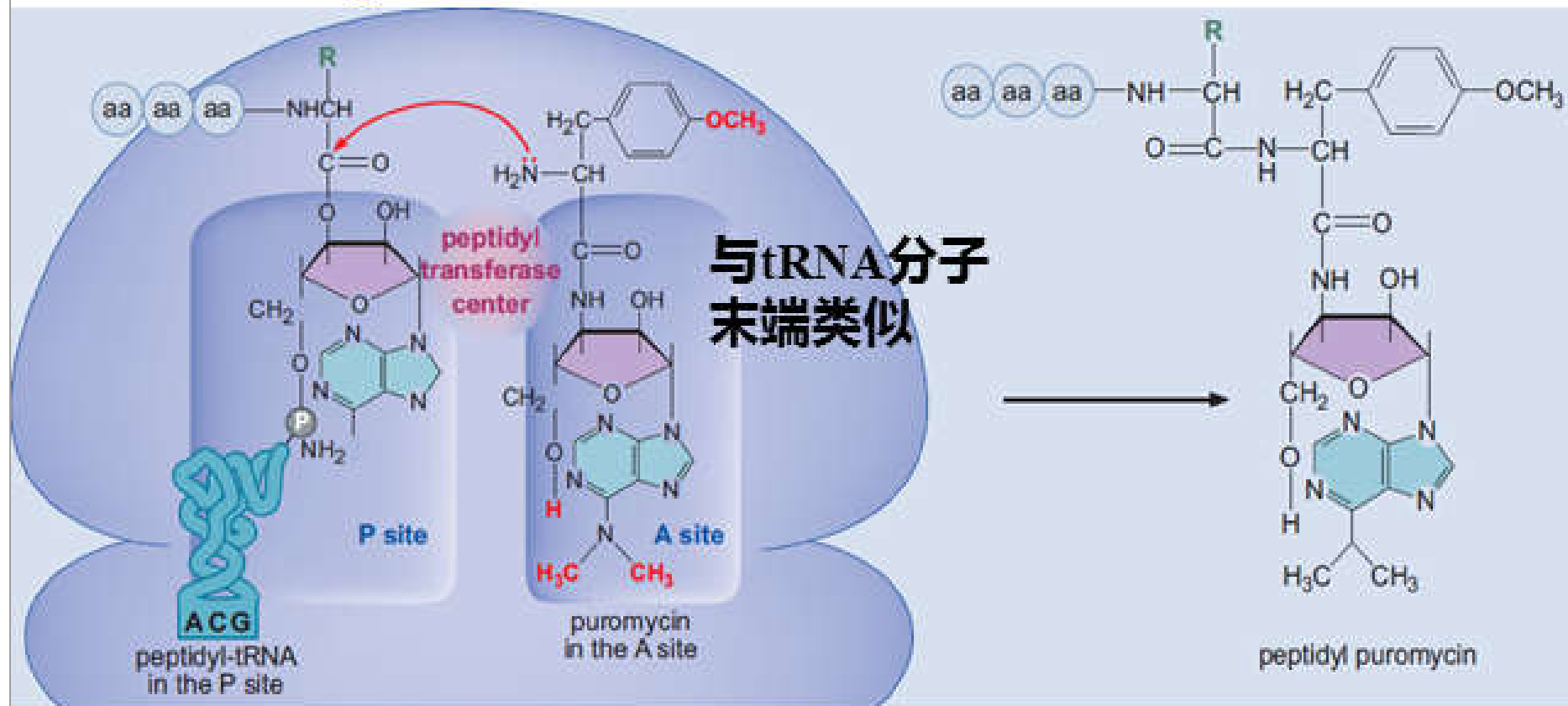
- **Target cells:** Eukaryotic cells
- **Molecular target:** Chemically modifies **eEF2**
- **Consequence:** Inhibits **eEF2** function (translocation)

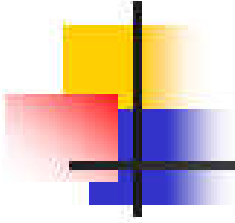
## 9.3 Cycloheximide (放线菌酮)

- **Target cells:** Eukaryotic cells
- **Molecular target:** Peptidyl transferase center of **60S subunit**
- **Consequence:** Inhibits peptidyl transferase activity

## 9.4 Puromycin (嘌呤霉素)

- **Target cells:** Prokaryotic and eukaryotic cells
- **Molecular target:** Peptidyl transferase center of **large ribosomal subunit**
- **Consequence:** Chain terminator





# Summary

- 1. Structure features and function of ribosome, mRNA and tRNA**
- 2. Features of genetic code**
- 3. Mechanisms of protein biosynthesis (especially the functions of each translation factors in prokaryotes and the differences between prokaryotic and eukaryotic protein synthesis)**
- 4. The ways of post-translational processing**
- 5. Inhibitors of protein synthesis**