Chapter 15 Translation

1. Translation machinery

2. translation process

3. Regulation of Translation

Published: 17 May 2017

Global translational reprogramming is a fundamental layer of immune regulation in plants

Guoyong Xu, George H. Greene, Heejin Yoo, Lijing Liu, Jorge Marqués, Jonathan Motley & Xinnian Dong

Nature **545**, 487–490 (2017) | Cite this article

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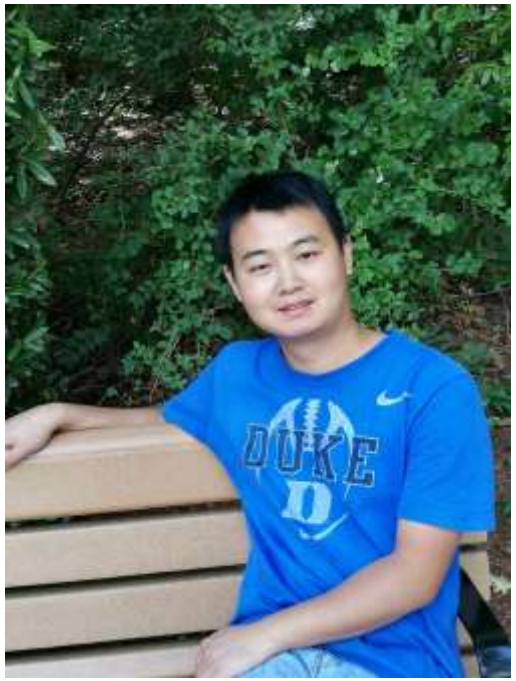

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Article | Published: 16 February 2023

Plant HEM1 specifies a condensation domain to control immune gene translation

Yulu Zhou, Ruixia Niu, Zhijuan Tang, Rui Mou, Zhao Wang, Sitao Zhu, Hongchun Yang, Pingtao Ding & Guoyong Xu

Nature Plants **9**, 289–301 (2023) | Cite this article

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Translation:

The genetic information contained within the order of nucleotides in messenger RNA (mRNA) is interpreted to generate the linear sequences of amino acids in proteins.

Translation is among the most highly conserved across all organisms and among the most energetically costly for the cell.

In rapidly growing bacterial cells, up to 80% of the cell's energy and 50% of the cell's dry weight are dedicated to protein synthesis.

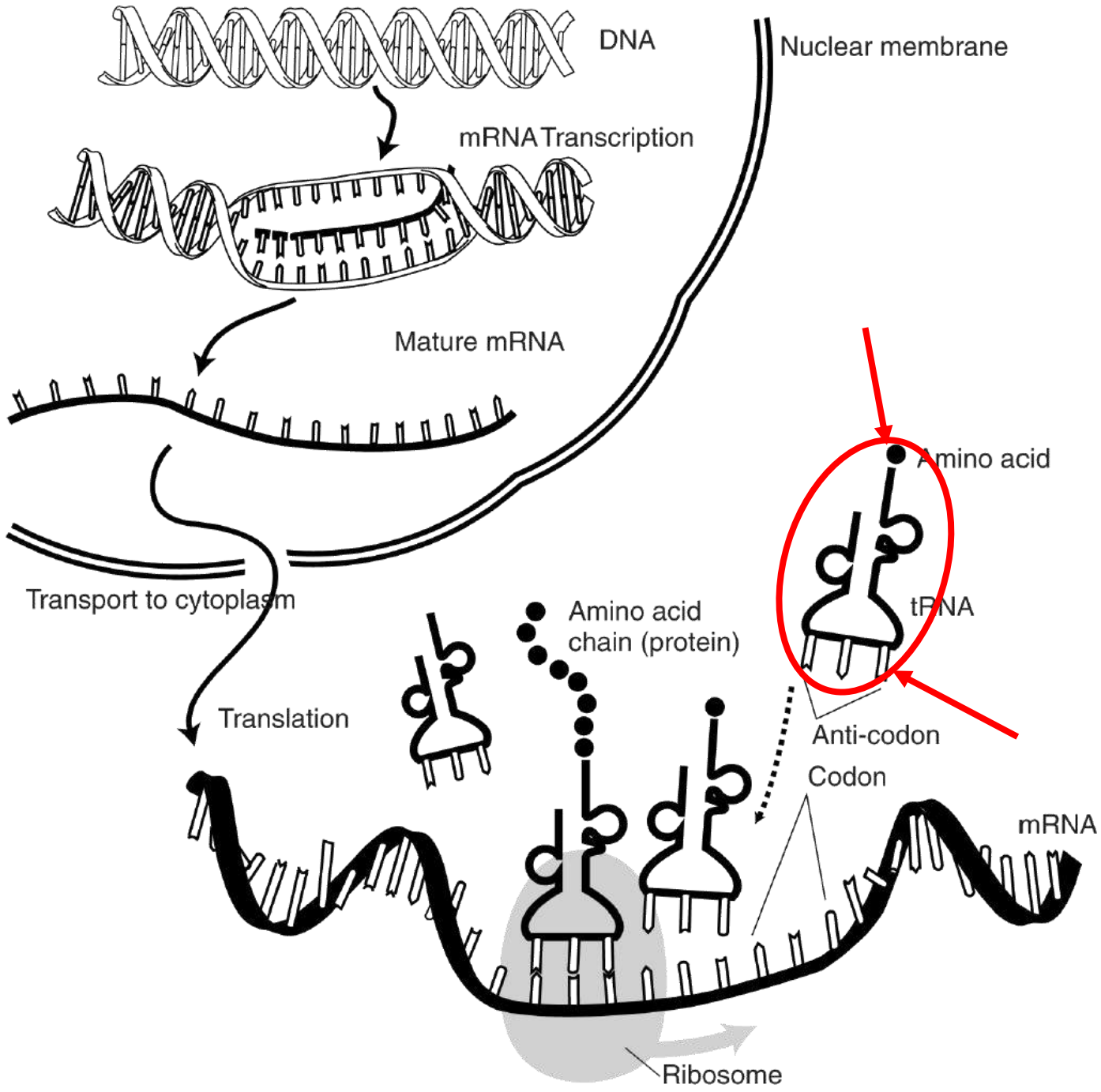
Translation:

Translation is a much more formidable challenge in information transfer than the transcription of DNA into RNA.

In 1955 Francis H. Crick

a special adaptor molecule

Transfer RNA - tRNA



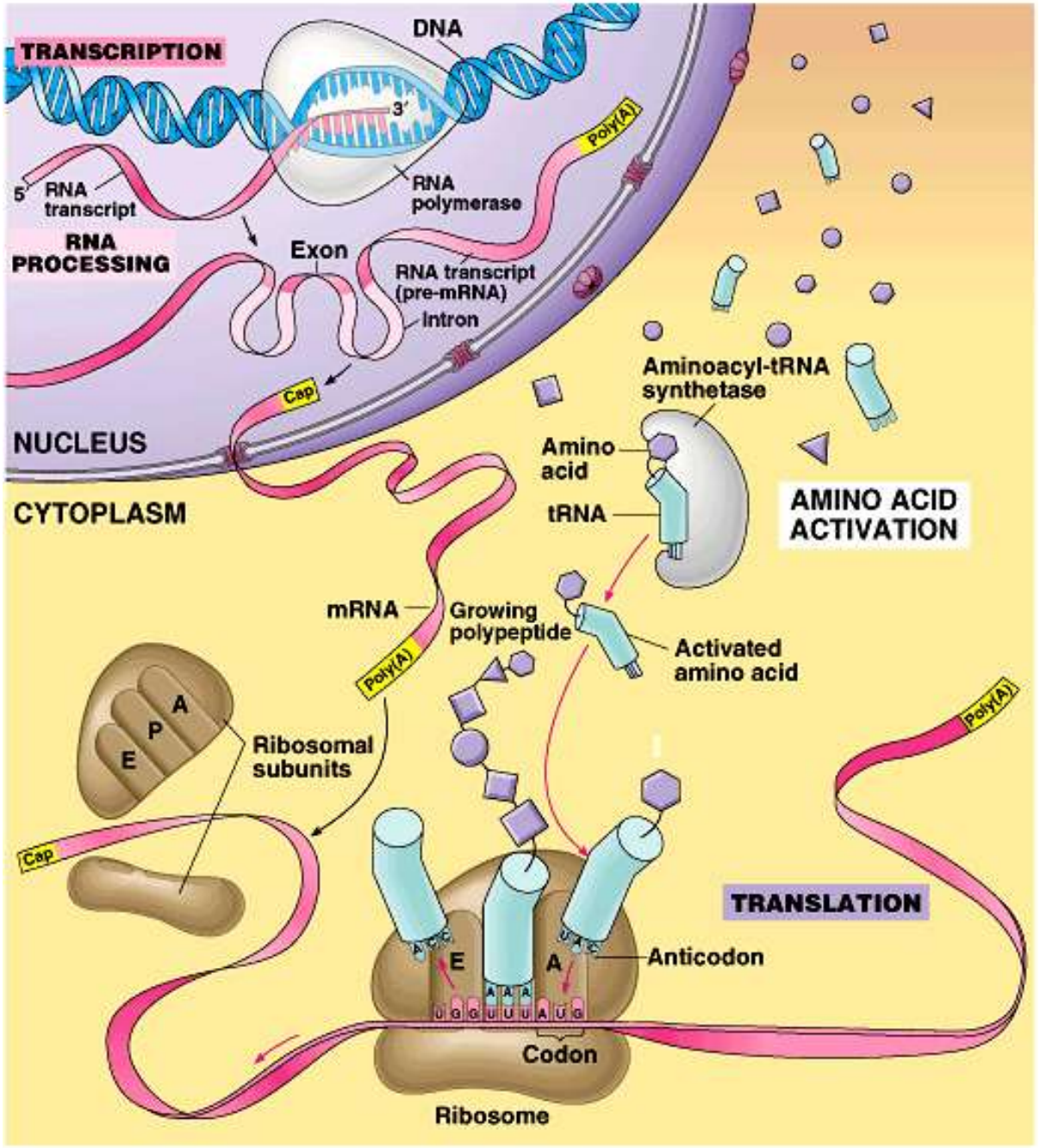
1. The translation machinery

- Messenger RNA
  Template

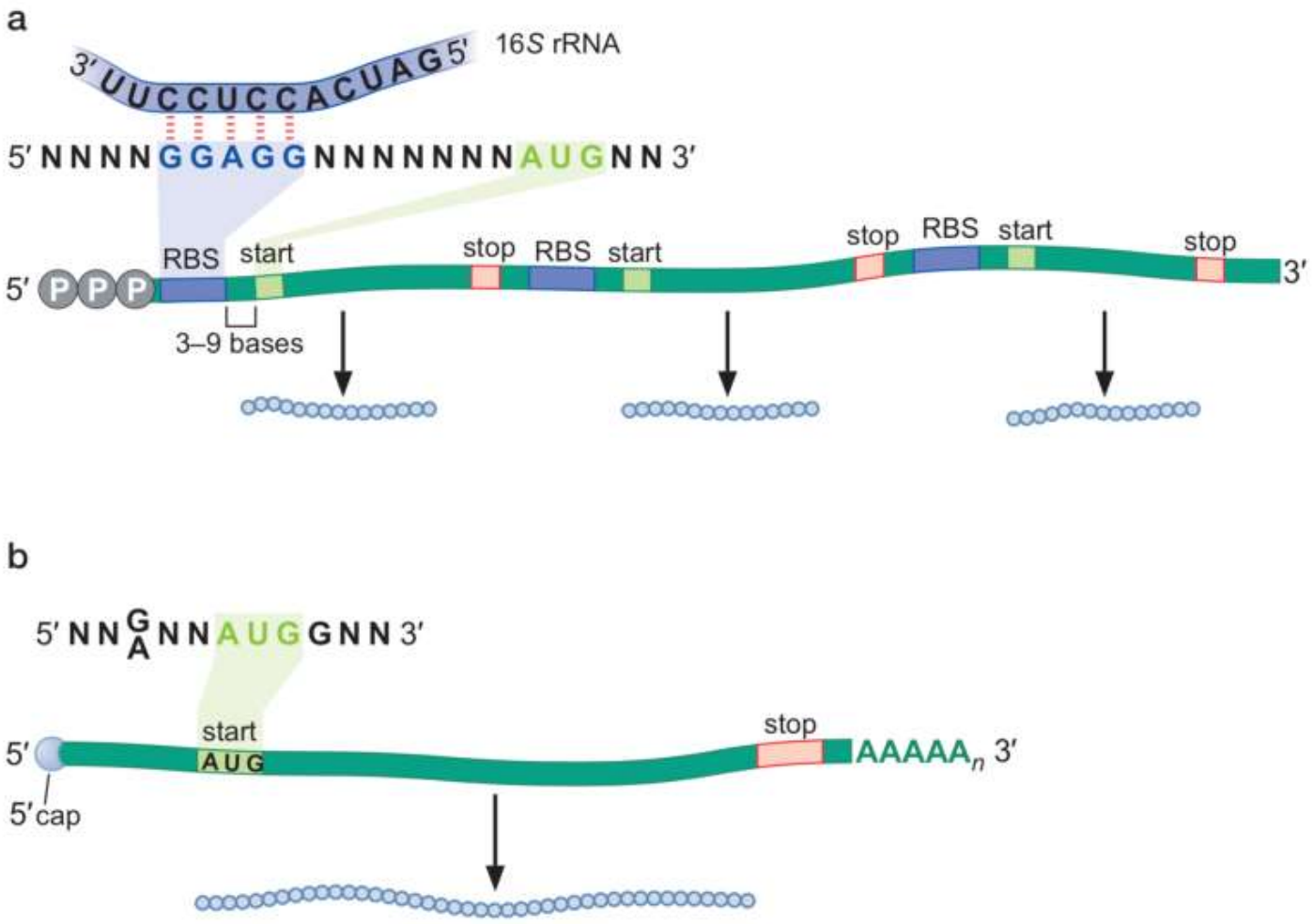
- Transfer RNA
  Adaptor

- Aminoacyl-tRNA Synthetases
  Add amino acids to specific RNAs

- The Ribosome
  correct recognition of the mRNA
  peptide-bond formation



1.1 Messenger RNA （信使RNA）

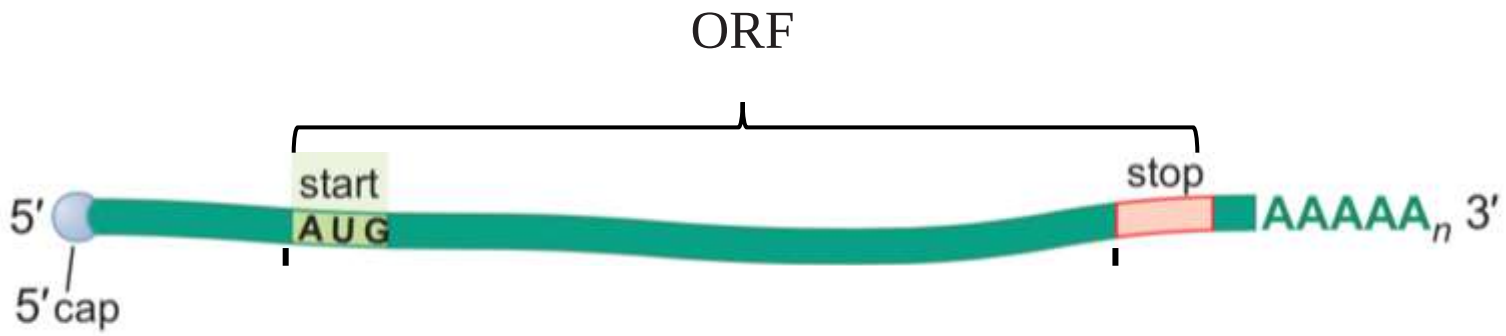


Prokaryotes

Eukaryotes

Open Reading Frame (ORF) 开放阅读框

ORF: The protein-coding region(s) of each mRNA.



A portion of each mRNA

ORF:

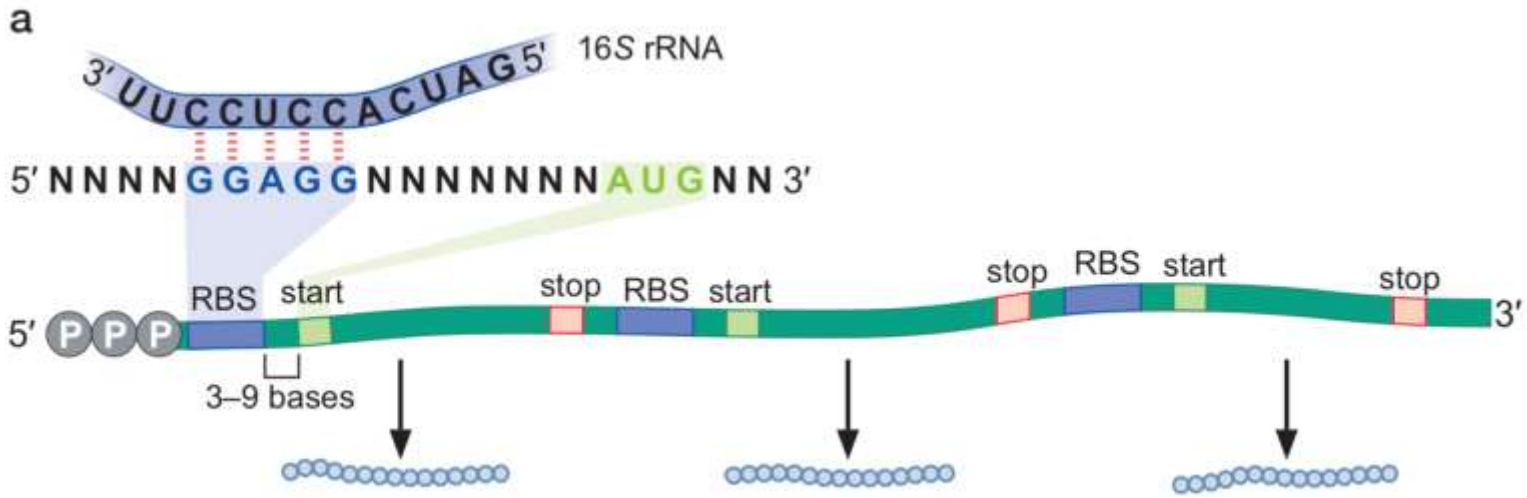
- ◆Starts with a start codon
- ◆ends with a stop codon

Start codon:

- ◘In bacteria: AUG, GUG or UUG;
- ◘In eukaryotes: AUG

Stop codon:

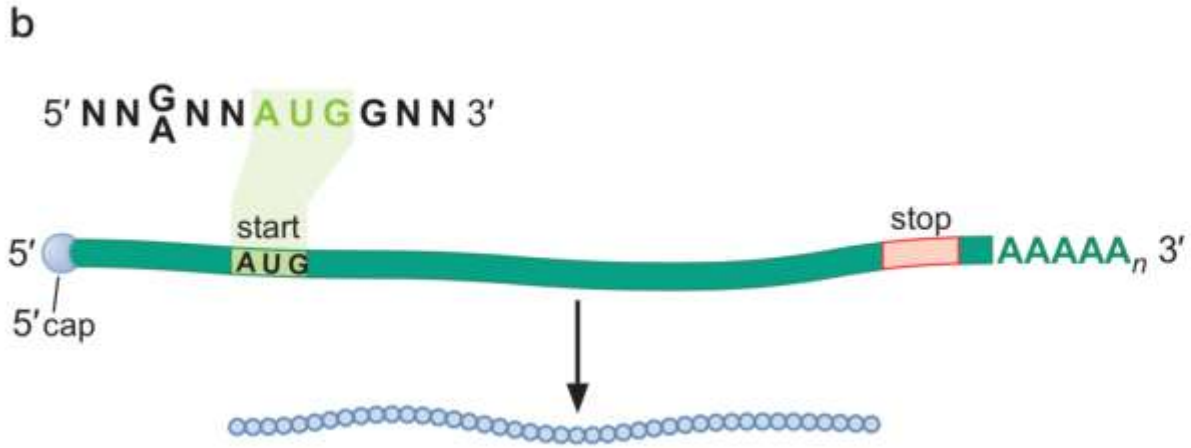
- ◘UAG, UGA, UAA



Prokaryotes

polycistronic mRNAs

多顺反子



Eukaryotes

monocistronic mRNAs

单顺反子

Ribosome-Binding Site - RBS

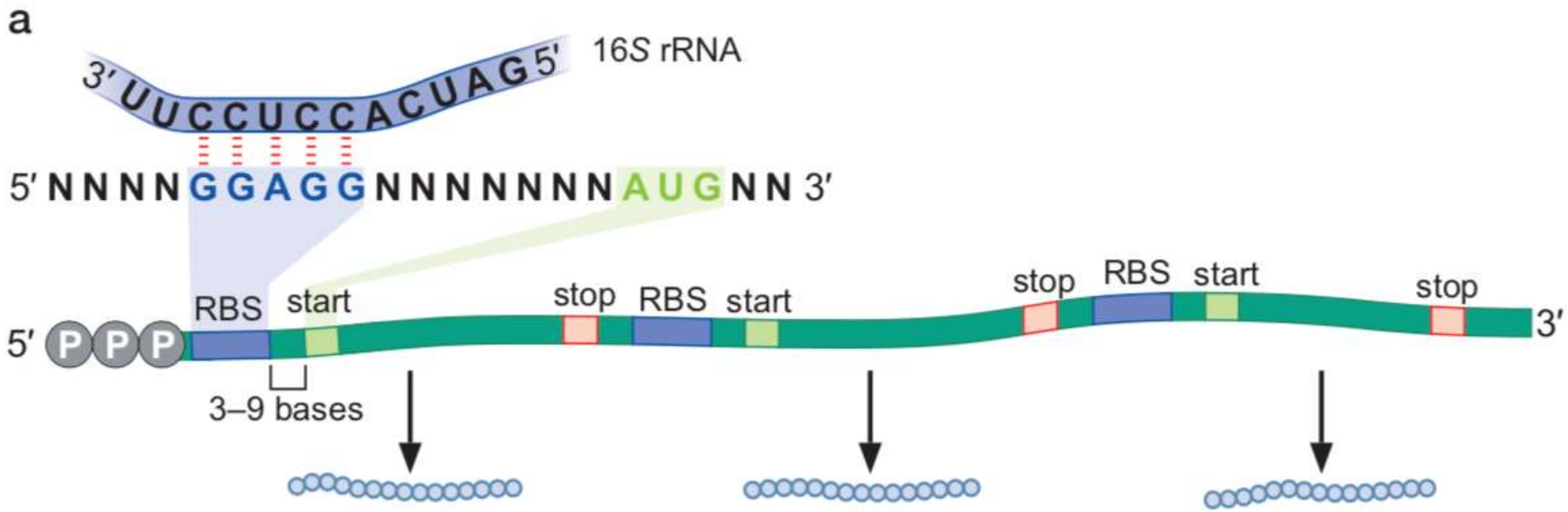
Shine-Dalgarno S.D seq

Prokaryotic mRNAs Have a Ribosome-Binding Site That Recruits the Translational Machinery

How to recruit the translational machinery?

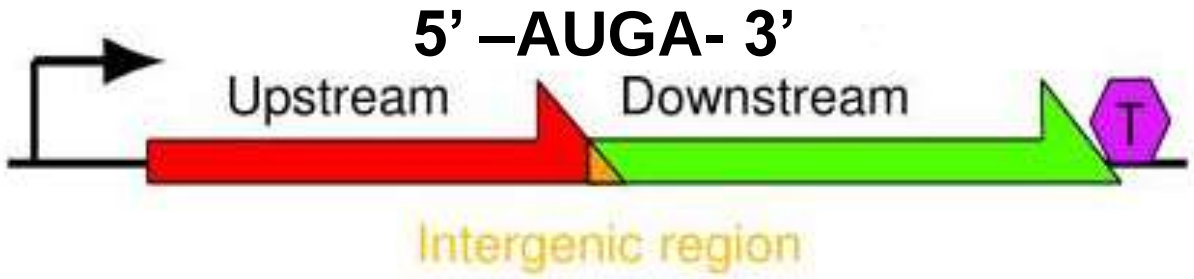
Translation initiation efficiency:

- Complementarity
- Spacing

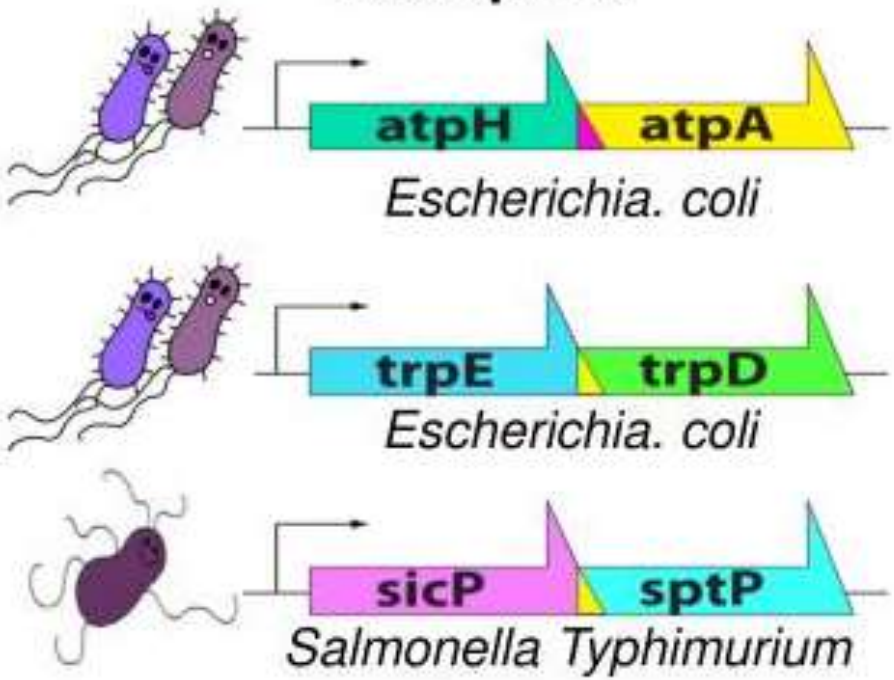


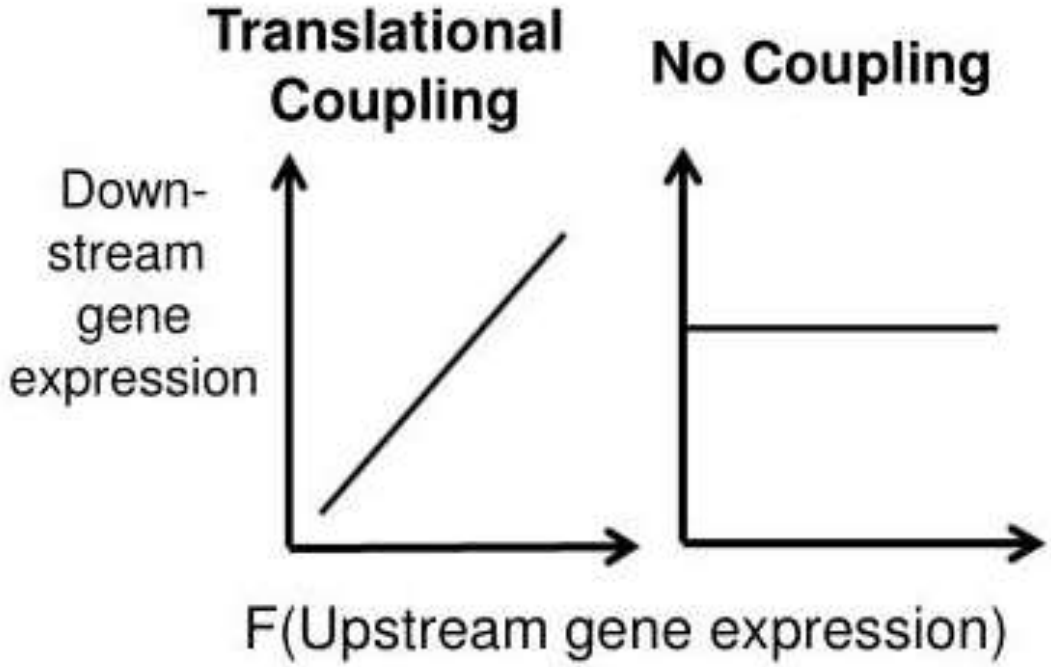
Translation coupling

Some prokaryotic ORFs lack a strong RBS but are nonetheless actively translated.



Examples:



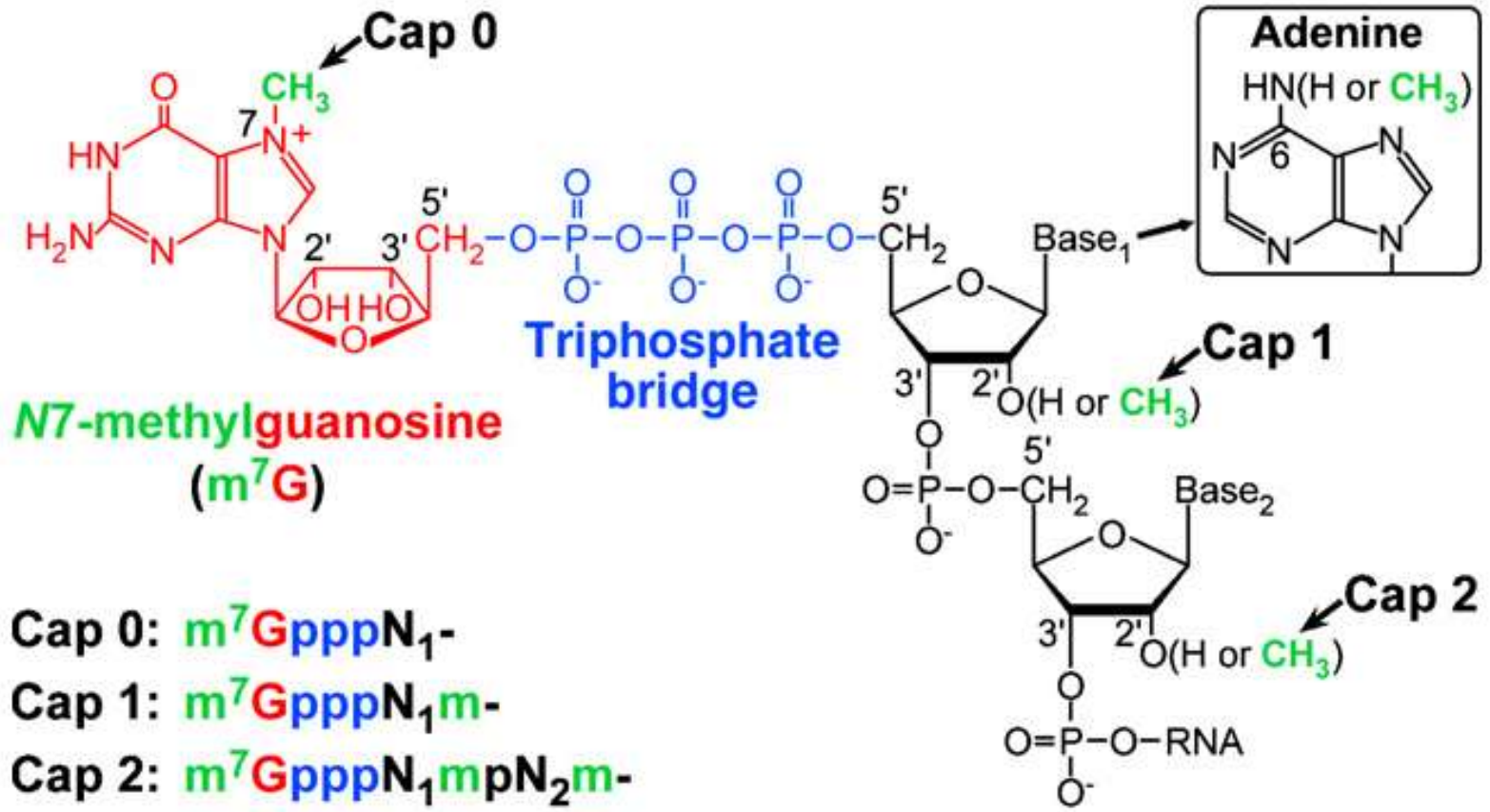


Translation of the downstream ORF requires translation of the upstream ORF.

Eukaryotic mRNAs Are Modified at Their 5’ and 3’ Ends to Facilitate Translation

Cap:

Eukaryotic mRNAs recruit ribosomes using a specific chemical modification called the 5′ cap.



Eukaryotic mRNAs Are Modified at Their 5’ and 3’ Ends to Facilitate Translation

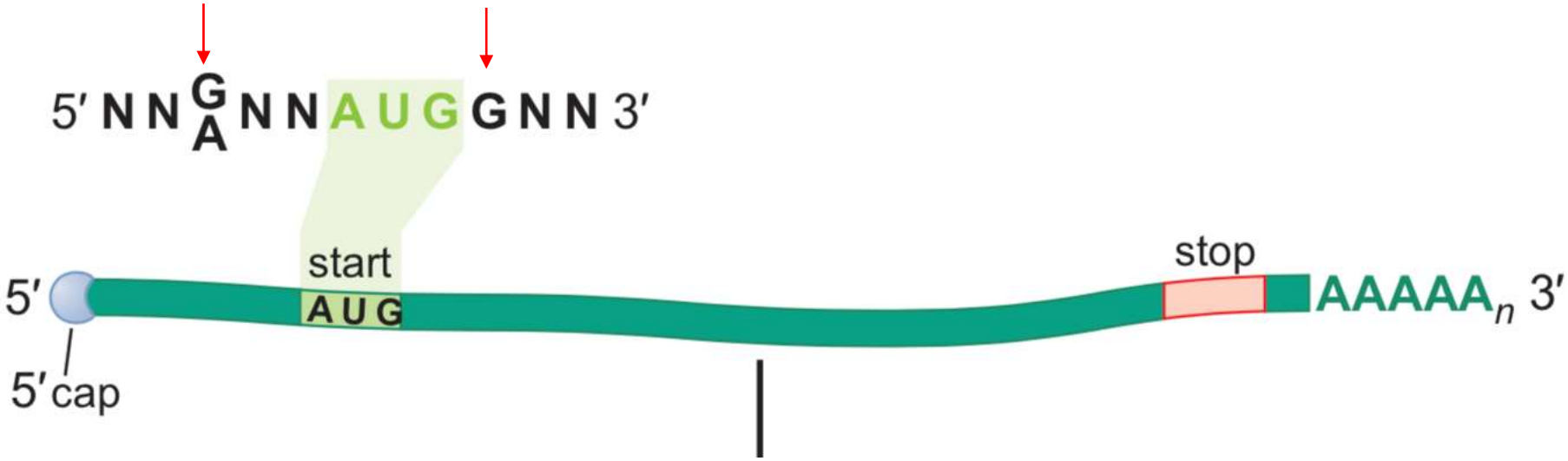
Scanning:
Once bound to the mRNA, the ribosome moves in a 5′→3′ direction until it encounters a 5′-AUG-3′ start codon.

Scanning: an important regulatory step!

Two other features of eukaryotic mRNAs stimulate translation

Kozak sequence :

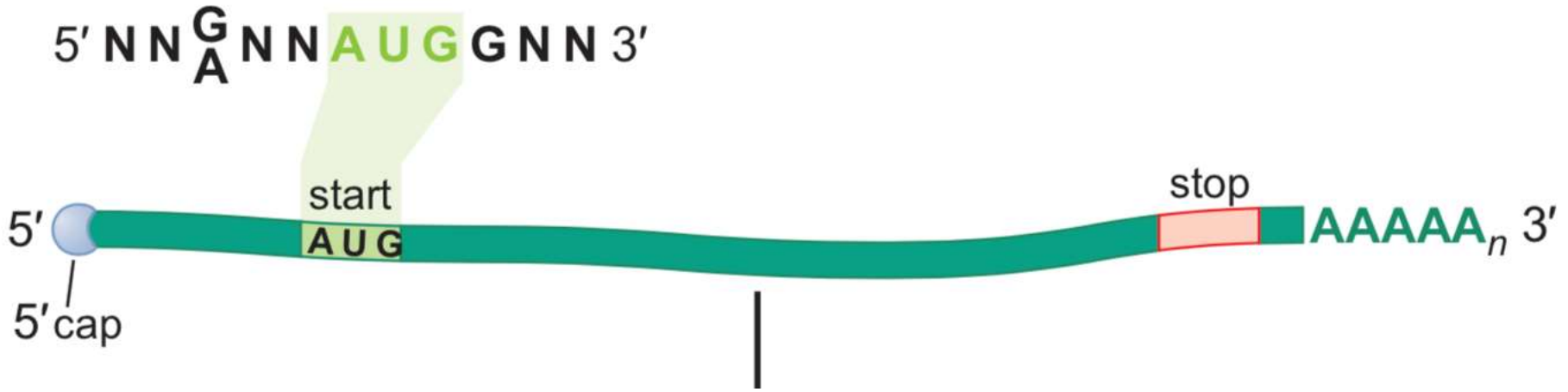
- increases the efficiency of translation;
- Recognized by the initiator tRNA



Two other features of eukaryotic mRNAs stimulate translation

The poly-A tail:

- enhances the level of translation of RNA
- by promoting the recruitment of key translation initiation factors.



1.2 Transfer RNA (tRNA) – 转运RNA

tRNAs Are Adaptors （适配器）between Codons and Amino Acids

The heart of protein synthesis is the “translation” of nucleotide sequence information (in the form of codons) into amino acids.

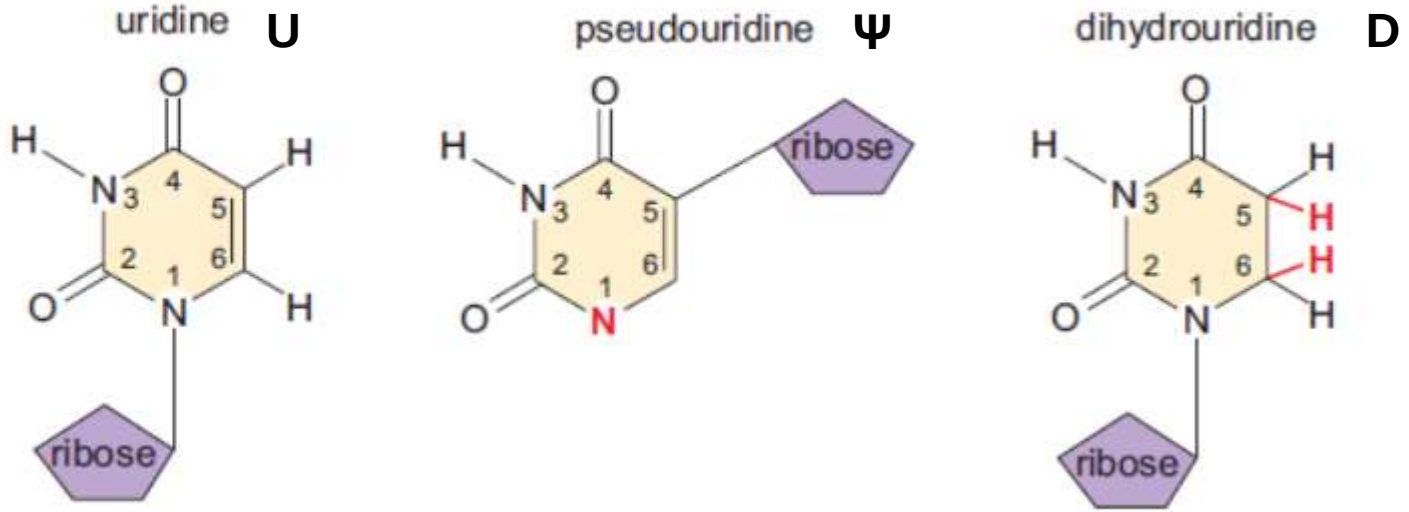
This is accomplished by tRNA molecules, which act as adaptors between codons and the amino acids they specify.

There are many different types of tRNA molecules, but each is attached to a specific amino acid, and each recognizes a particular codon/codons.

Primary structure of tRNAs

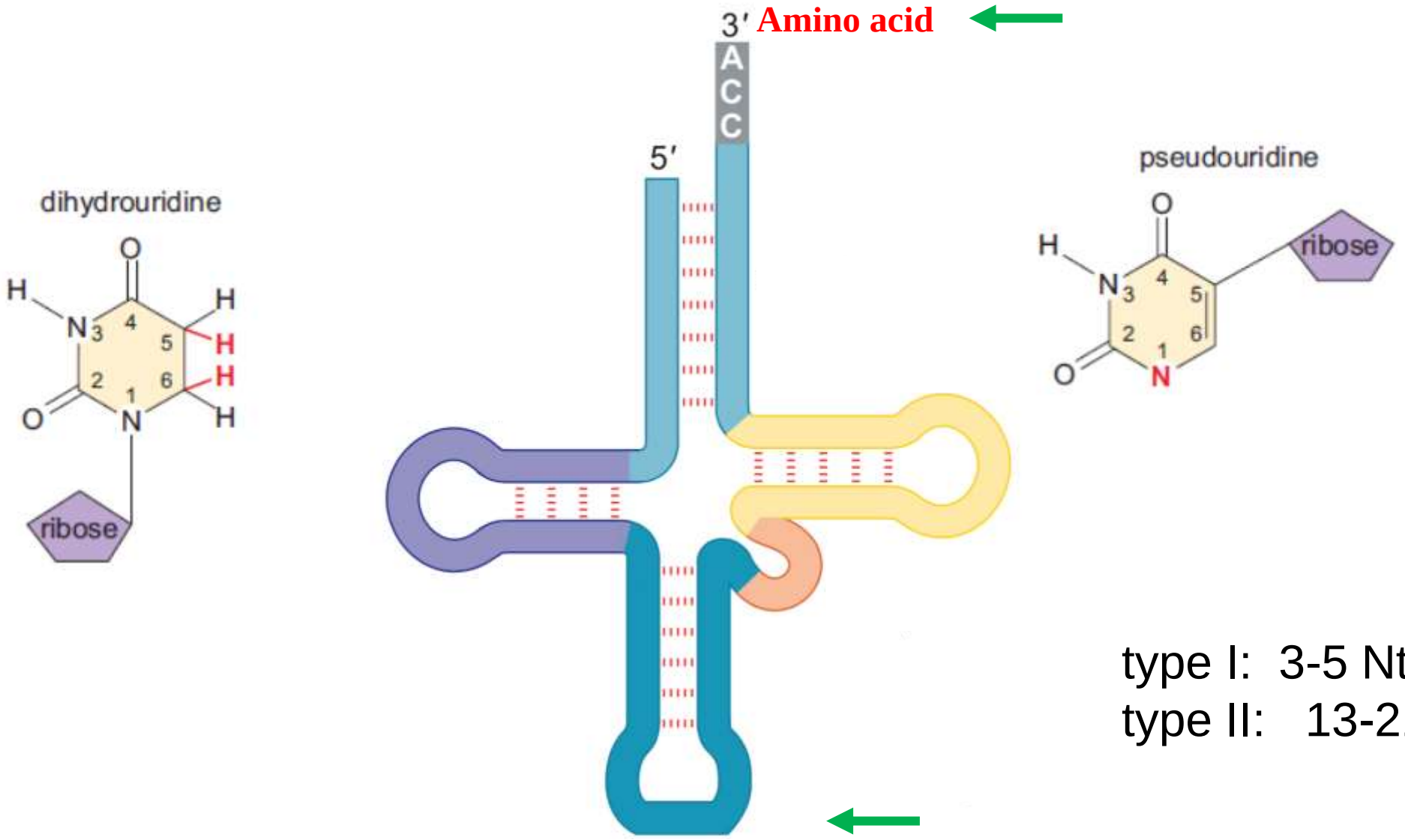
```
H.sapiens       AGCAGAGUGG CGCAGC--GG AAGCG-UGCU GGGCCCAUAA CCCAGAGGUC GAUGGAUCUA AACCAUCCUC UGCUACCA
M.musculus      AGCAGAGUGG CGCAGC--GG AAGCG-UGCU GGGCCCAUAA CCCAGAGGUC GAUGGAUCGA AACCAUCCUC UGCUACCA
X.laevis        AGCAGAGUGG CGCAGC--GG AAGCG-UGCU GGGCCCAUAA CCCAGAGGUC GAUGGAUCGA AACCAUUCUC UGCUACCA
D.melanogaster  AGCAGAGUGG CGCAGU--GG AAGCG-UGCU GGGCCCAUAA CCCAGAGGUC CGAGGAUCGA AACCUUGCUC UGCUACCA
A. thaliana     AGCAGAGUGG CGCAGC--GG AAGCG-UGGU GGGCCCAUAA CCCACAGGUC CCAGGAUCGA AACCUGGCUC UGCUACCA
Z. mays         AUCAGAGUGG CGCAGU--GG AAGCG-UGGU GGGCCCAUAA CCCACAGGUC CCAGGAUCGA AACCUGGCUC UGAUACCA
S. Cerevisiae   AGCGCCGUGG CGCAGU--GG AAGCG-CGCA GGGCUCAUAA CCCUGAUGUC CUCGGAUCGA AACCGAGCGG CGCUACCA
N. crassa       AGCTGCAUGG CGCAGC--GG AAGCG-CGC- GGGCUCAUAA CCCGGAGGUC ACUCGAUCGA AACGAGUUGC AGCUACCA
E. coli         AGCGGGGUGG AGCAGCCUGG UAGCU-CGUC GGGCUCAUAA CCCGAAGGUC GUCGGUUCAA AUCCGGCCCC CGCUACCA
```

- 75 and 95 nt in length
- Two common features:
  - 5'-CCA-3'
  - Highly modified bases



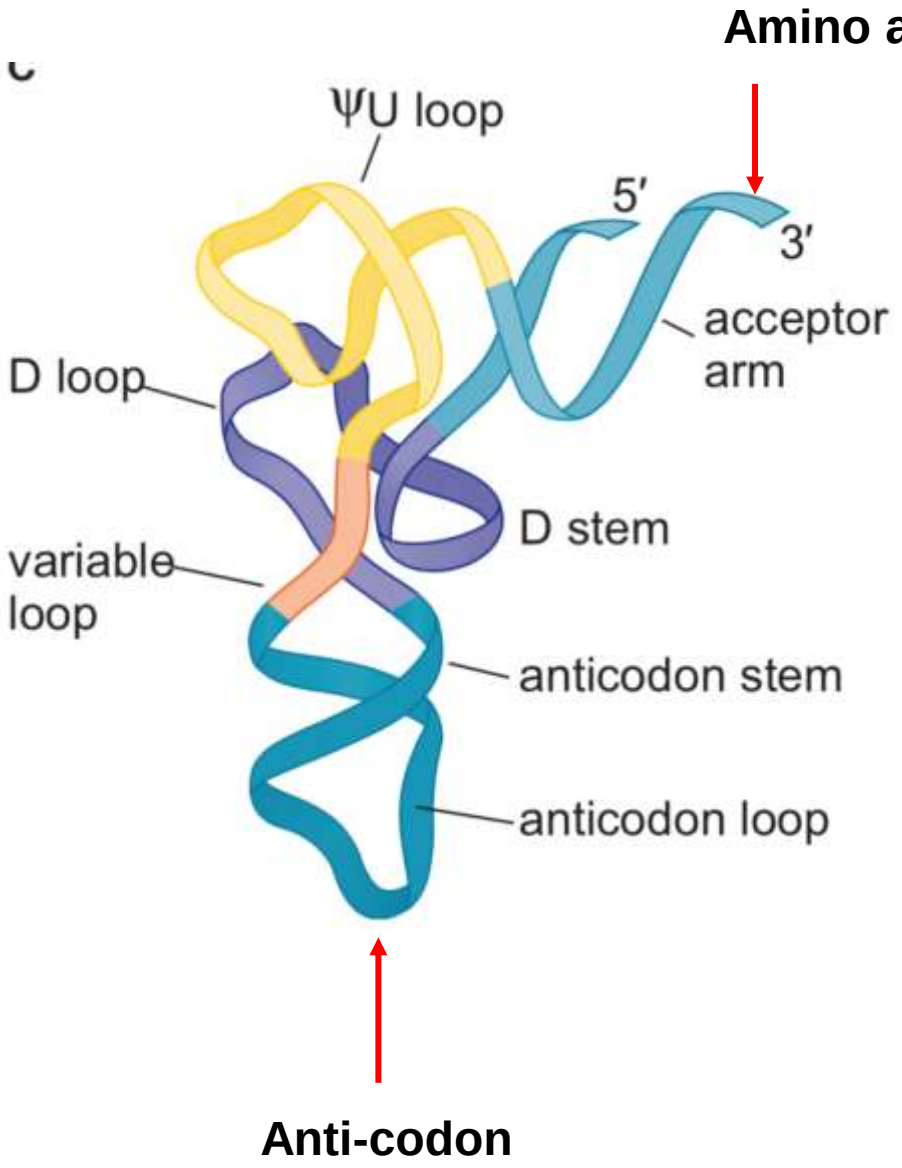
What are the functions of these modified bases ?

Secondary Structure - a Cloverleaf - 三叶草



type I: 3-5 Nt
type II: 13-21 Nt

tRNAs Have an L-Shaped Three-Dimensional Structure



Three-dimensional (3D) configuration of tRNA:
the terminus of the acceptor stem is at one end of the molecule and the anticodon loop is 70A˚ away at the other end

Three kinds of interactions stabilize this L-shaped structure

1, base-stacking interactions

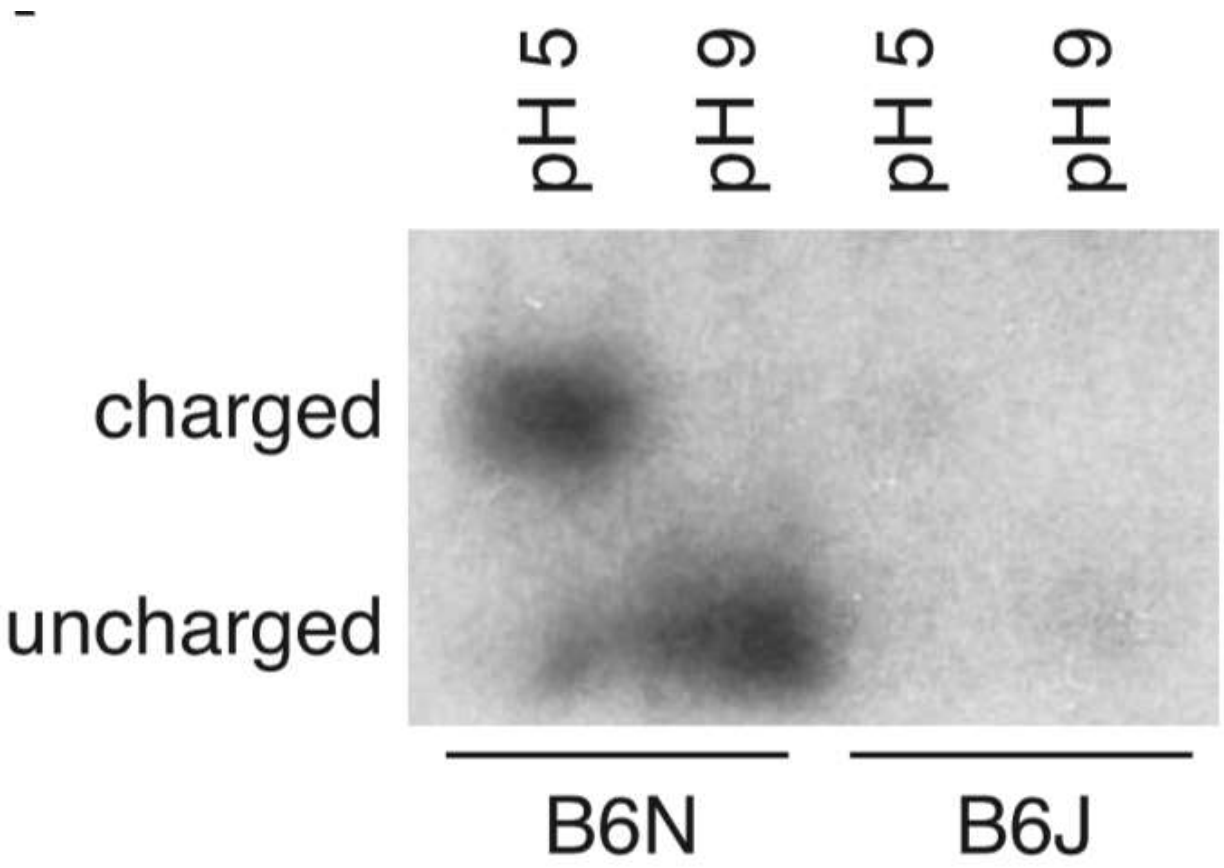
2, hydrogen bonds are formed between bases in different helical regions

3, interactions between the bases and the sugar–phosphate backbone

1.3 Aminoacyl-tRNA Synthetases（氨酰tRNA合成酶）

ATTACHMENT OF AMINO ACIDS TO tRNA

Charged and uncharged tRNA



charged tRNAs （负载的）

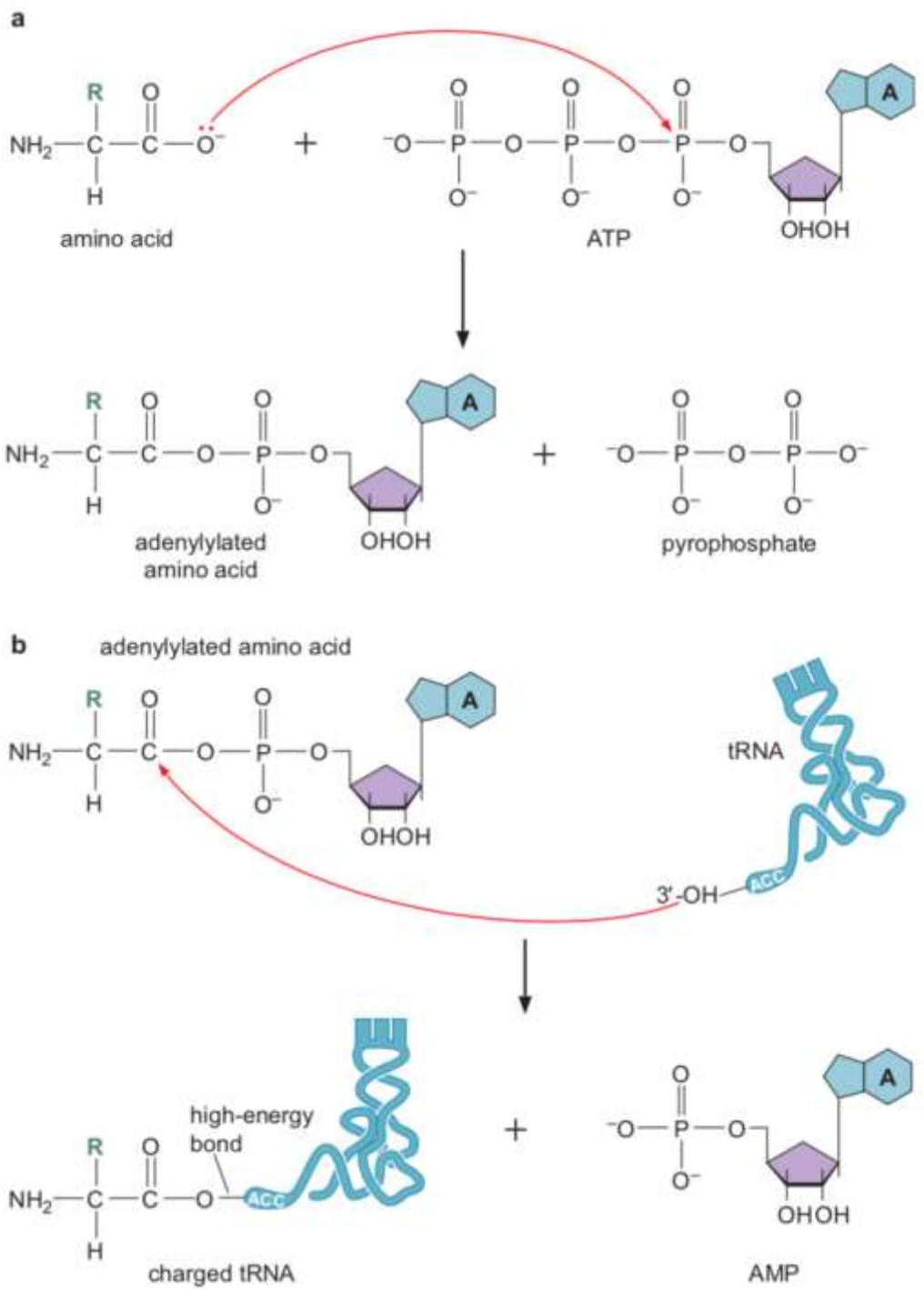
uncharged tRNAs （空载的）

Aminoacyl-tRNA Synthetases Charge tRNAs in Two Steps

adenylylation

腺苷酰化

tRNA charging



There are two classes of tRNA synthetases

TABLE 15-1 Classes of Aminoacyl-tRNA Synthetases

Class II	Quarternary Structure	Class I	Quarternary Structure
Gly	$(\alpha_2\beta_2)$	Glu	(α)
Ala	(α_4)	Gln	(α)
Pro	(α_2)	Arg	(α)
Ser	(α_2)	Cys	(α_2)
Thr	(α_2)	Met	(α_2)
His	(α_2)	Val	(α)
Asp	(α_2)	Ile	(α)
Asn	(α_2)	Leu	(α)
Lys	(α_2)	Tyr	(α)
Phe	$(\alpha_2\beta_2)$	Trp	(α)

Class I enzymes: 2’-OH of the tRNA, monomeric.
Class II enzymes: 3’-OH of the tRNA, dimeric or tetrameric.

Once released from the synthetase, the amino acid rapidly equilibrates between attachment at the 3’-OH and the 2’-OH .

Each Aminoacyl-tRNA Synthetase Attaches a Single Amino Acid to One or More tRNAs

- Each of the 20 amino acids is attached to the appropriate tRNA by a single, dedicated tRNA synthetase.
- One synthetase can recognize and charge more than one tRNA .
- Thus, one and only one tRNA synthetase attaches each amino acid to all of the appropriate tRNAs.

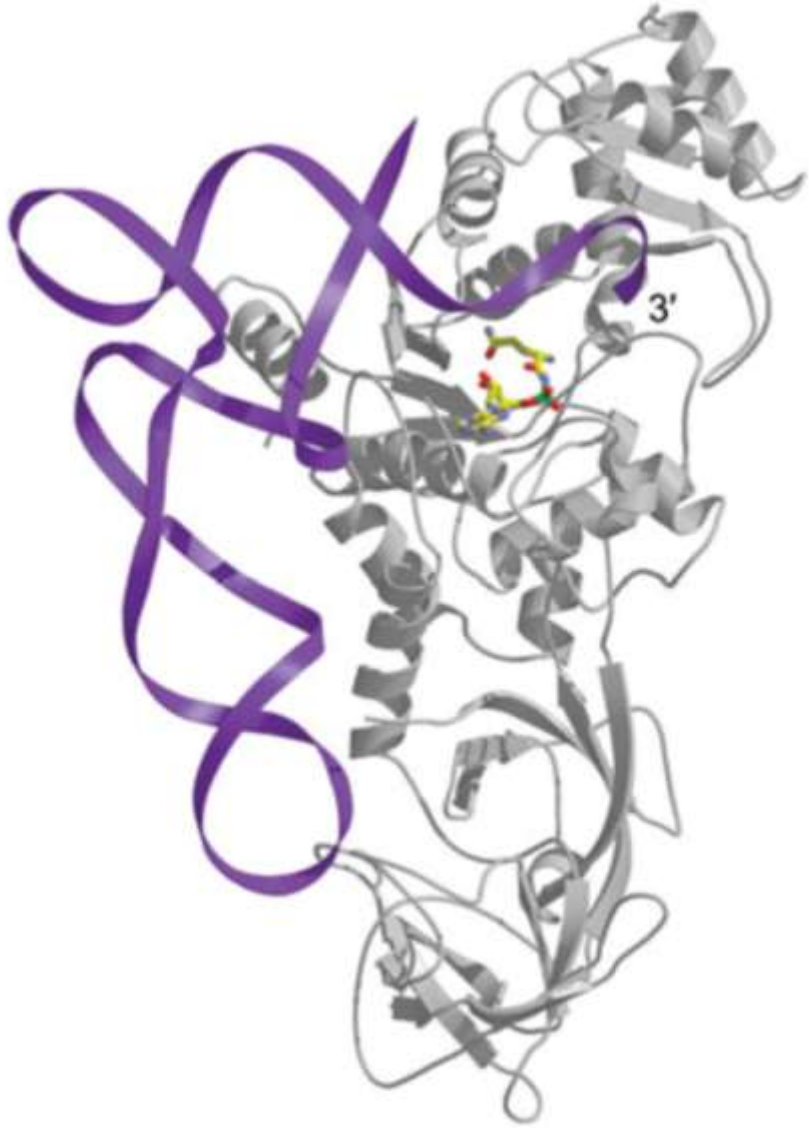
tRNA Synthetases Recognize Unique Structural Features of Cognate tRNAs

How does tRNA synthetase recognize all the iso-accepting tRNAs?

The acceptor stem

The anticodon loop

} **Paracodon**



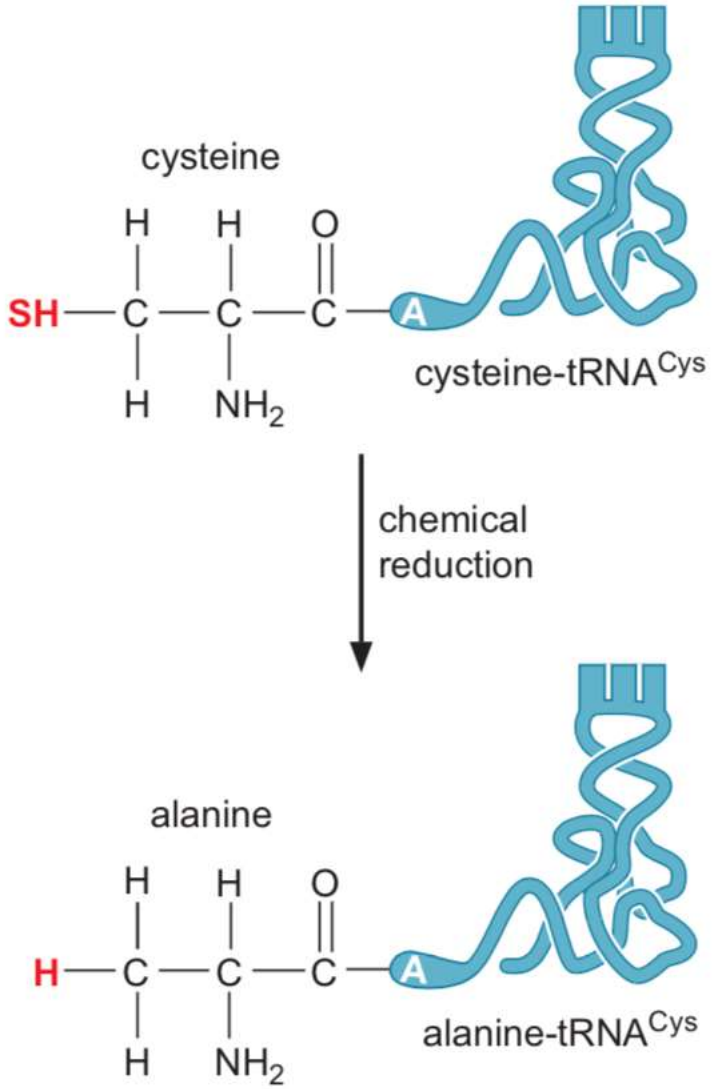
Cocrystal glutaminyl aminoacyl-tRNA synthetase with $tRNA^{Gln}$

Aminoacyl-tRNA Formation Is Very Accurate

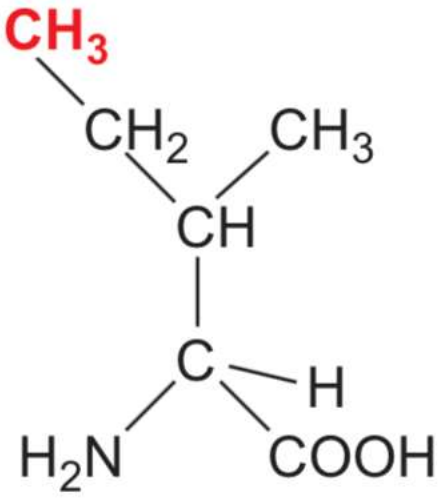
The Ribosome Is Unable to Discriminate between Correctly and Incorrectly Charged tRNAs

The responsibility for the correct coupling of the proper amino acid with its cognate tRNA falls on aminoacyl-tRNA synthetases.

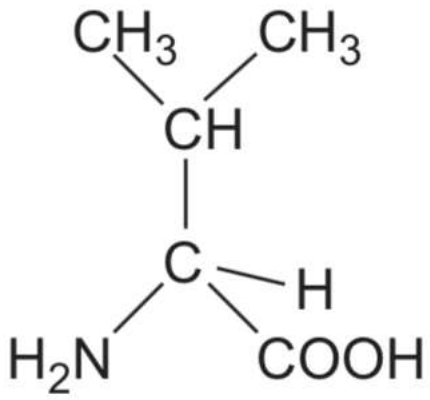
Chemical reduction of cysteine-tRNA^{Cys} to alanine-tRNA^{Cys}



Aminoacyl-tRNA Formation Is Very Accurate



isoleucine



valine

Challenges

- **Small size**
- **Structure similarity**
- **Similar concentration in cell**

***In vitro* C(Val): C(Ile) = 1:1**

Error rate:1/200

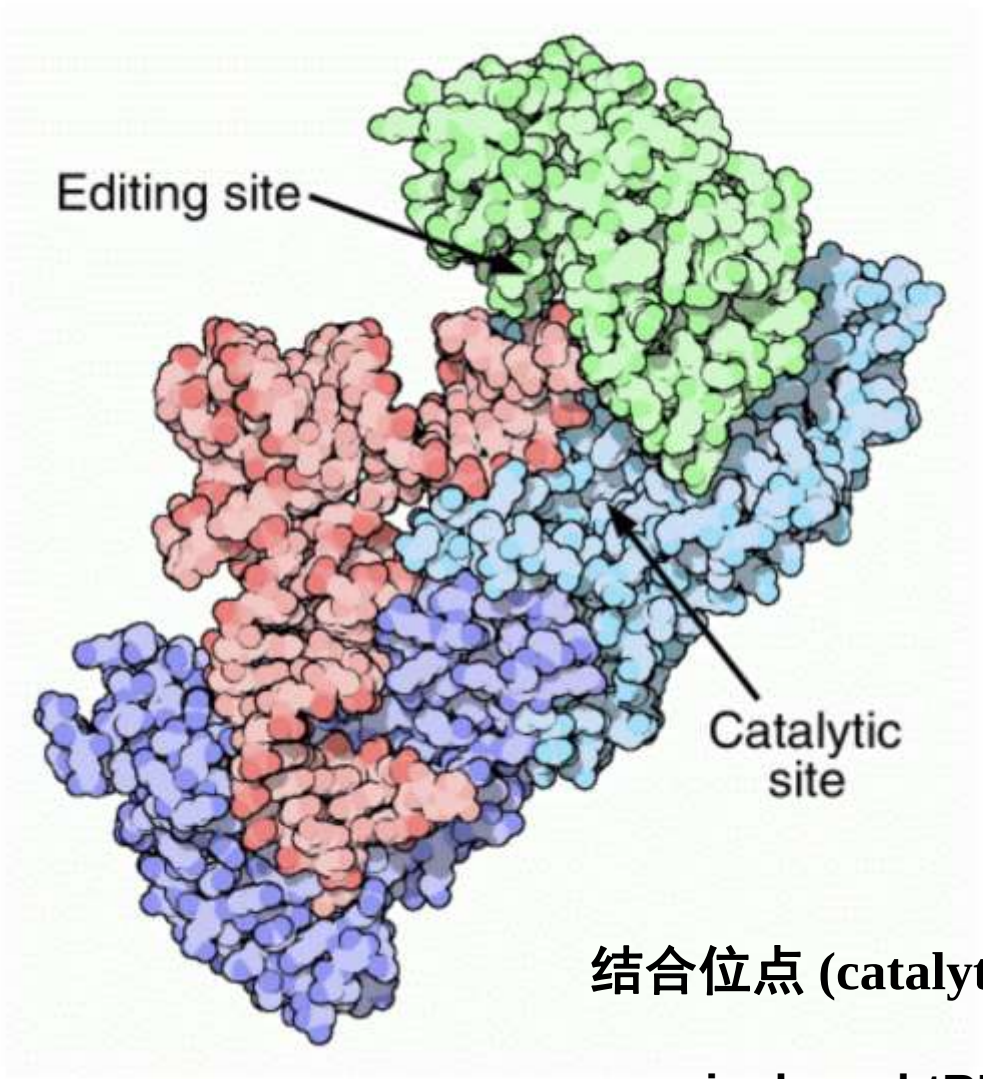
***In vivo* C(Val): C(Ile) = 5:1**

In theory, error rate is 1/40 !!

Actual error rate is less than 1/3000.

Some Aminoacyl-tRNA Synthetases Use an Editing Pocket to Charge tRNAs with High Accuracy

水解位点 (Hydrolytic Site or Editing Site)

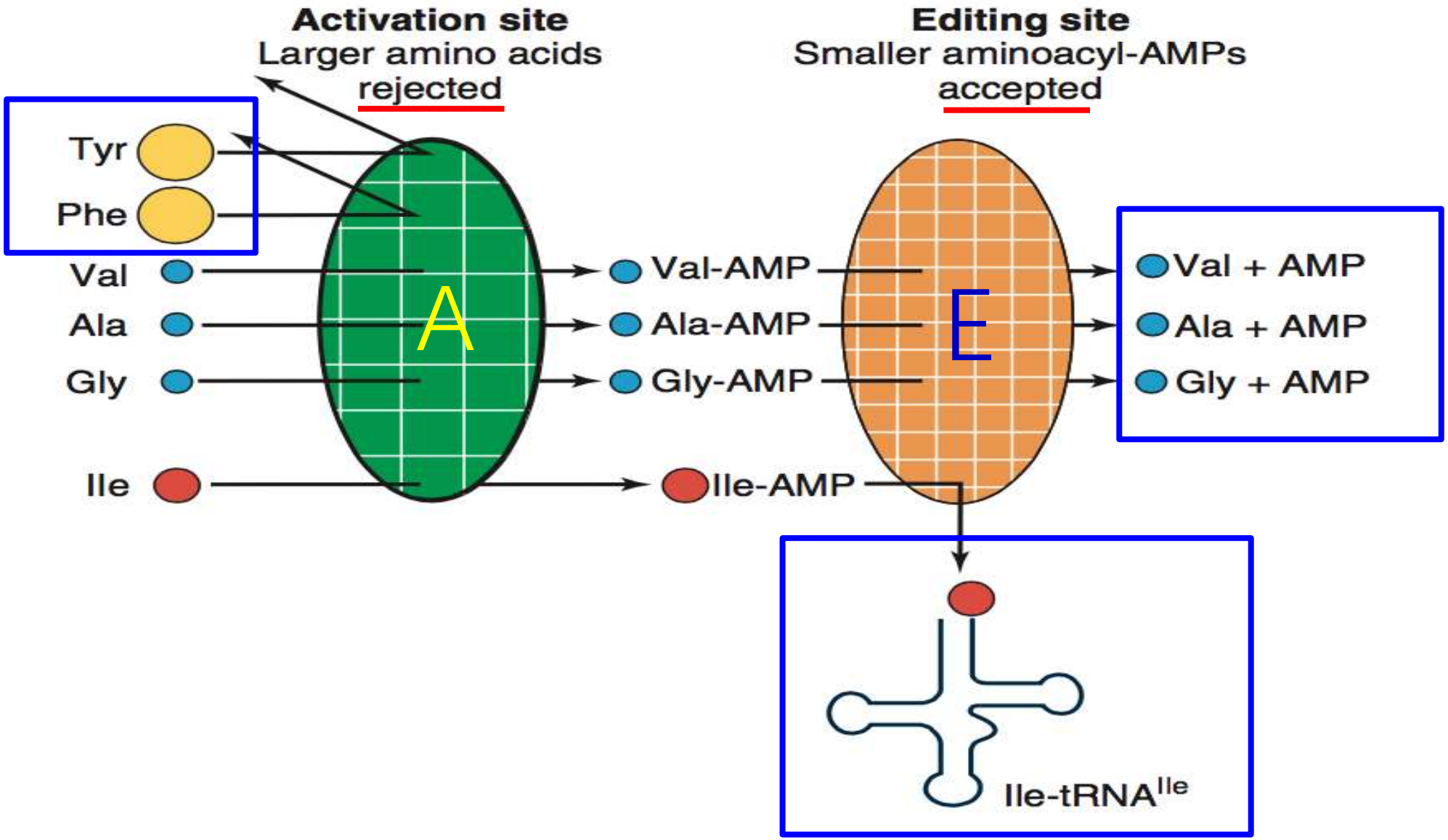


结合位点 (catalytic Site or Activation Site)

The red one is tRNA

isoleucyl-tRNA synthetase

Double sieve effect



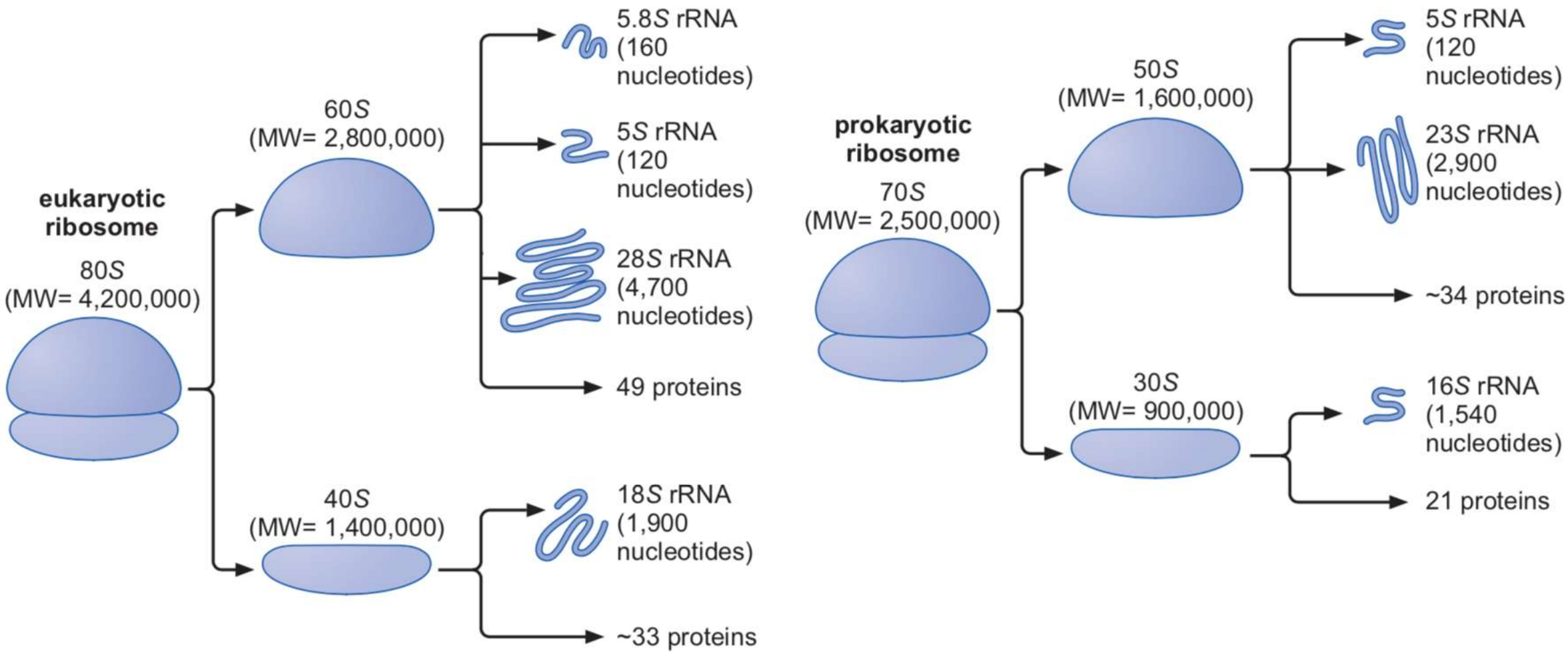
1.4 the Ribosome （核糖体）

The ribosome is the macromolecular machine that directs the synthesis of proteins.

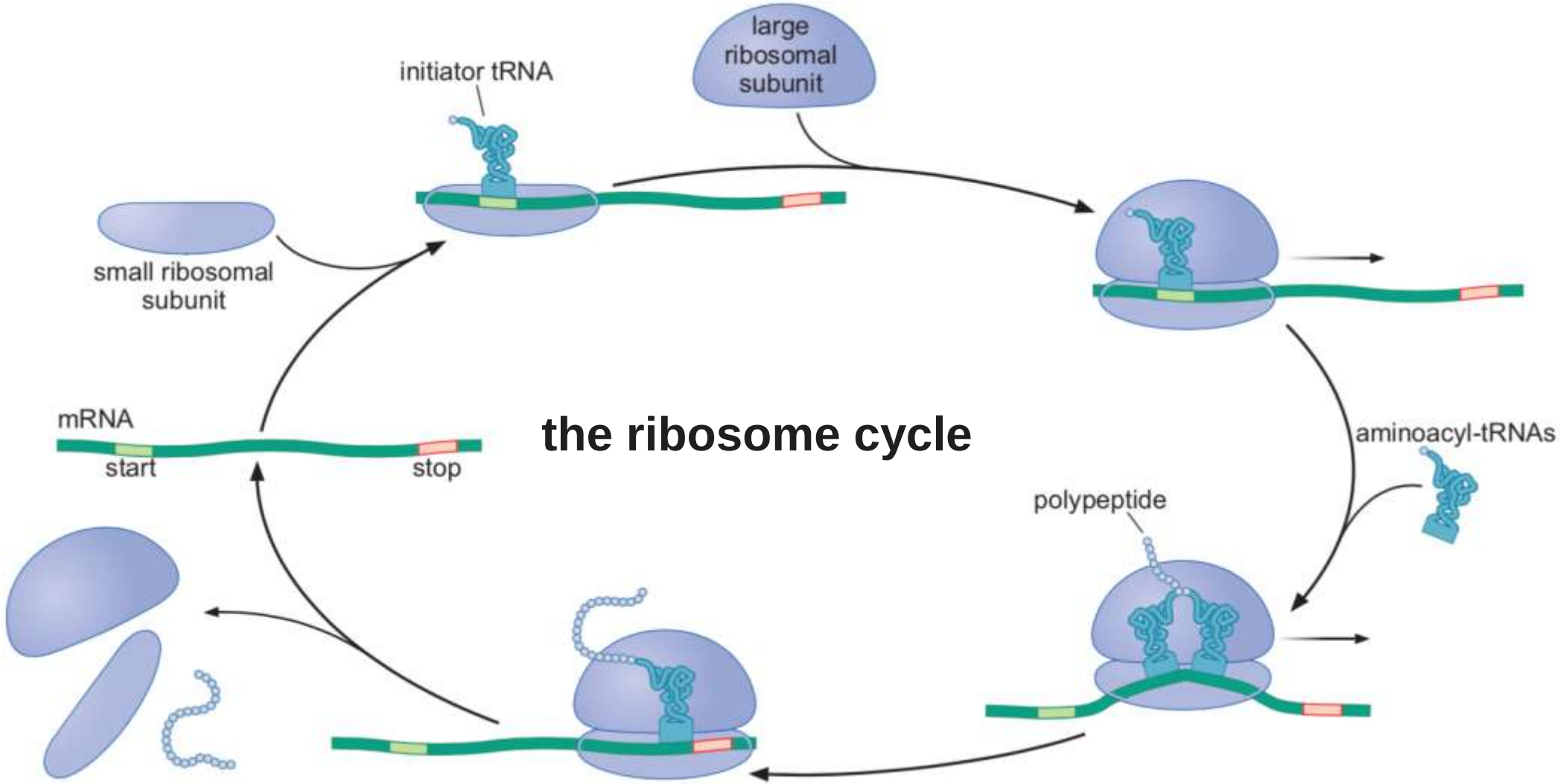
The machinery for polymerizing amino acids is composed of at least three RNA molecules and more than 50 different proteins, with an overall molecular mass of >2.5 MDa.

Compared with the speed of DNA replication—200–1000 nucleotides per second—translation takes place at a rate of only 2 to 20 amino acids per second.

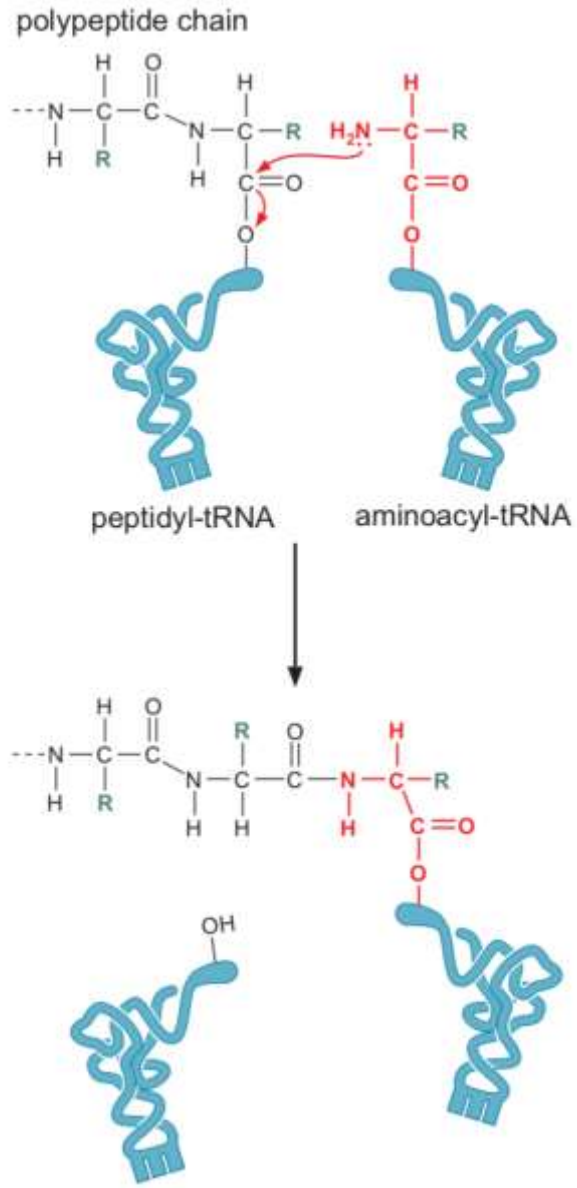
The Ribosomes are made of two subunits of RNAs and proteins



The Large and Small Subunits Undergo Association and Dissociation during Each Cycle of Translation



Peptide Bonds Are Formed by Transfer of the Growing Polypeptide Chain from One tRNA to Another



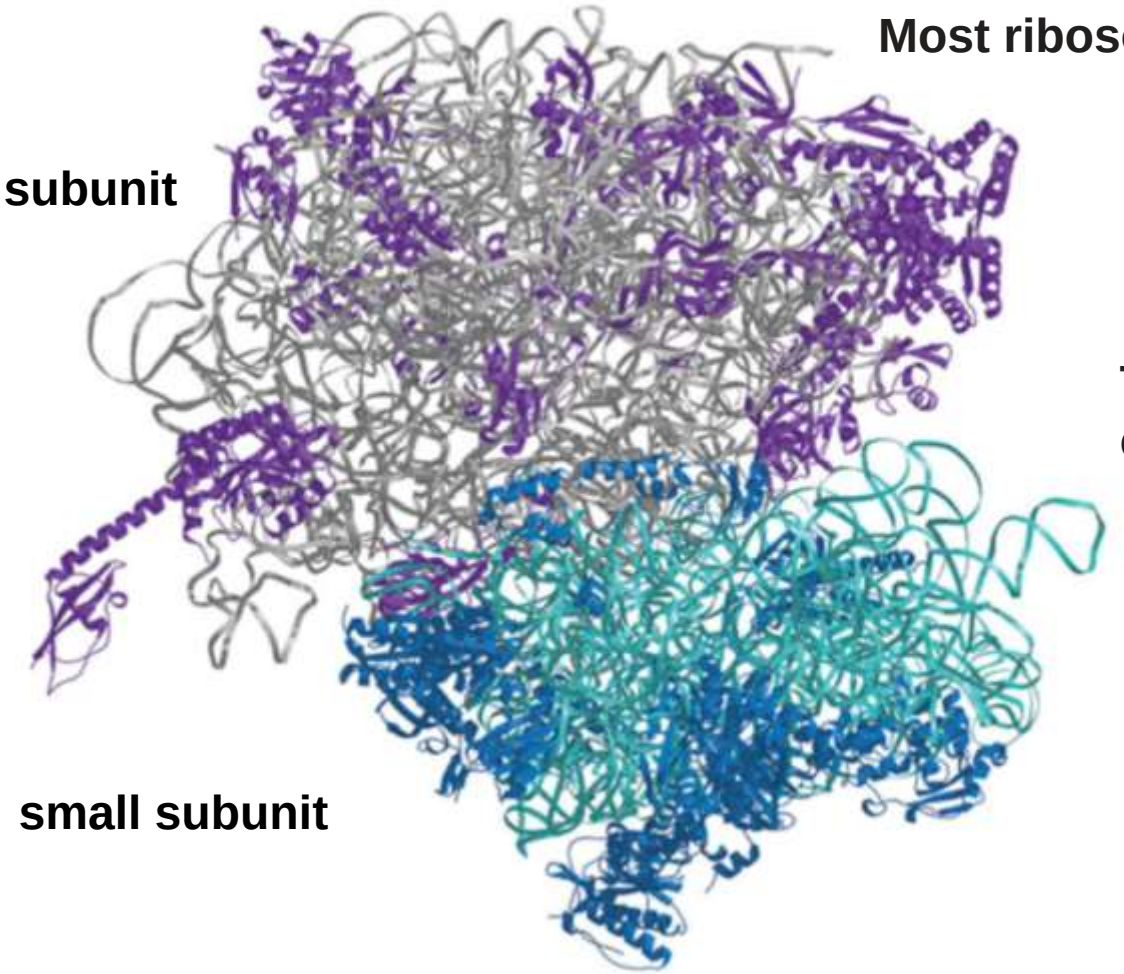
The amino acid attached to aminoacyl-tRNA to attack the carbonyl group of the most carboxy-terminal amino acid attached to the peptidyl-tRNA and form a new peptide bond.

The peptide-bond formation is driven by breaking the high-energy acyl bond that joins the growing polypeptide chain to the tRNA.

Ribosomal RNAs Are Both Structural and Catalytic Determinants of the Ribosome

Most ribosomal proteins are on the periphery of the ribosome

large subunit



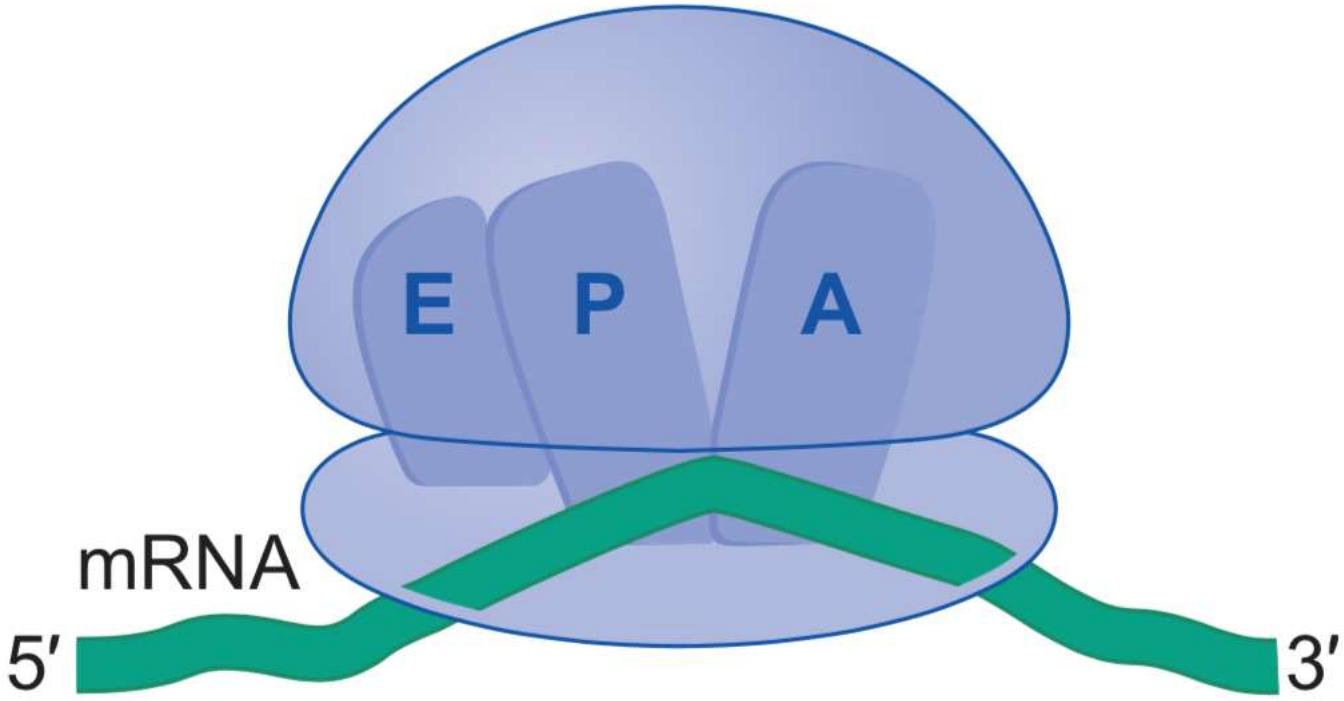
small subunit

The peptidyl transferase center and the decoding center are composed mostly from RNA.

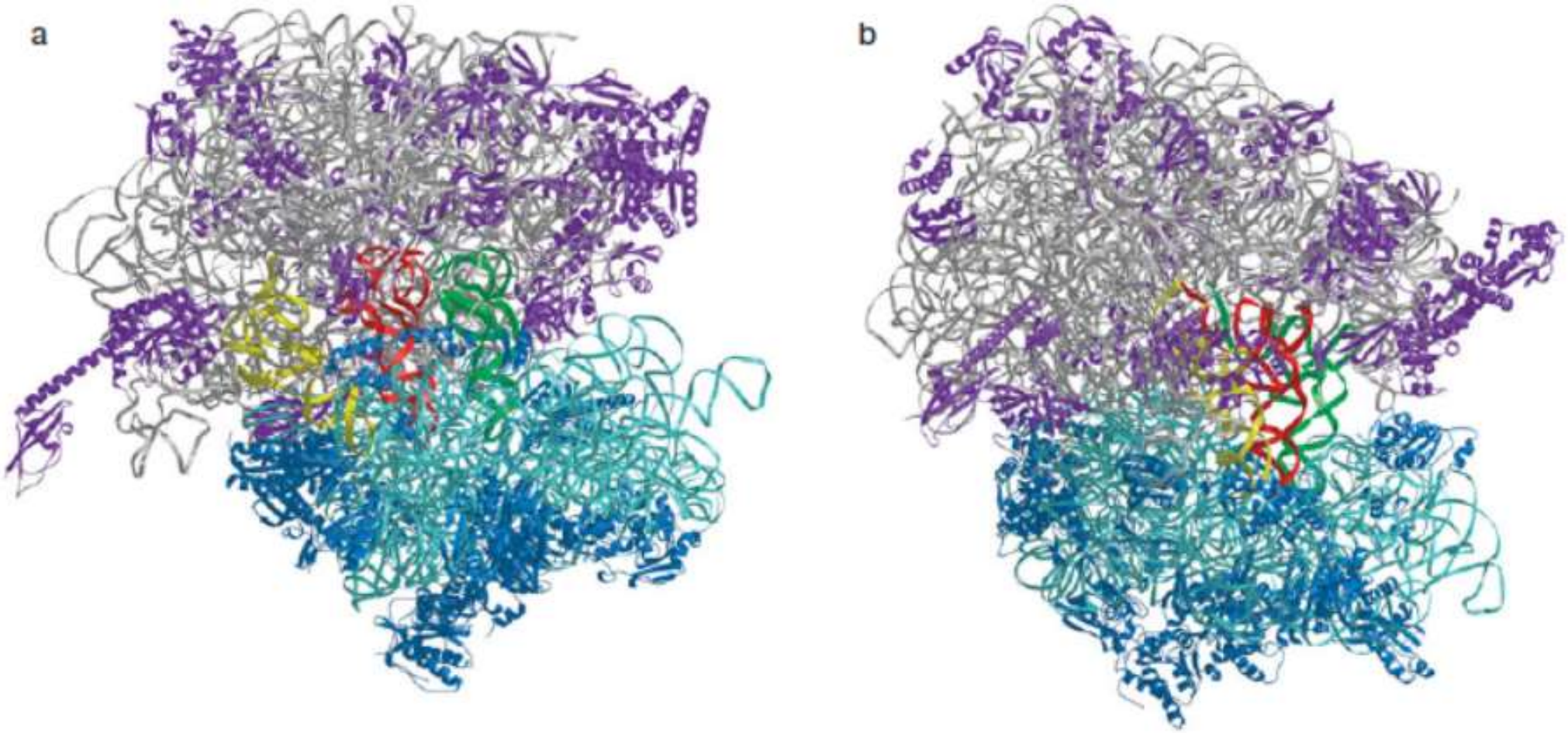
50S: RNA gray, protein purple;
30S: light blue, dark blue.

The Ribosome Has Three Binding Sites for tRNA

- A-site: the aminoacyl-tRNA
- P-site: the peptidyl-tRNA
- E-site: the uncharged tRNA (E is for “exiting”)



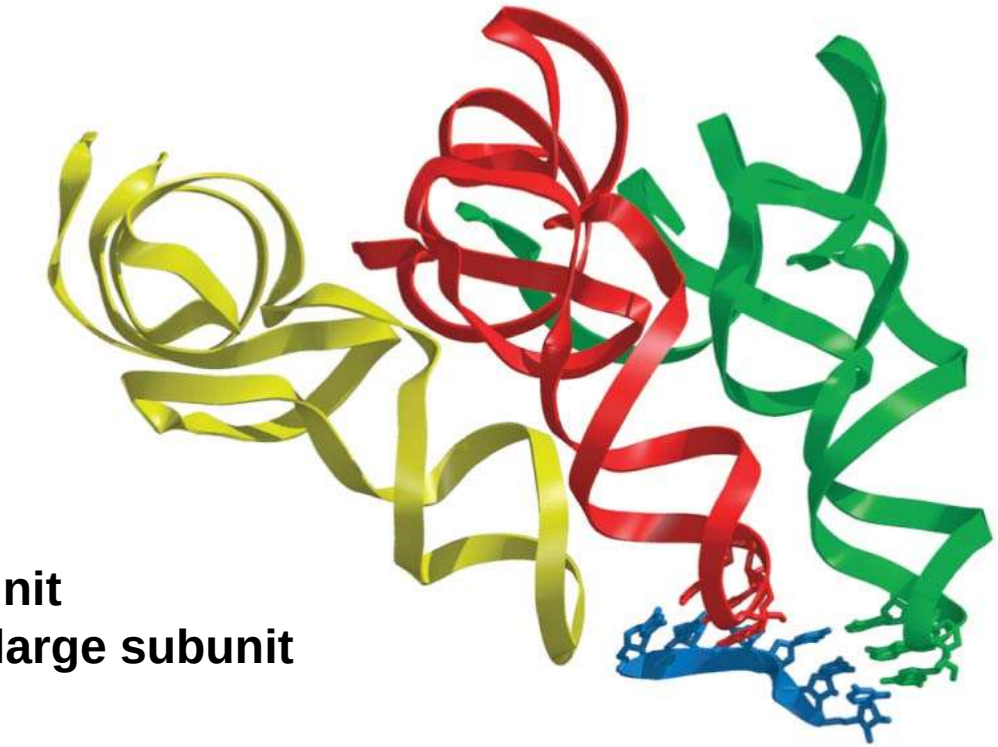
The Ribosome Has Three Binding Sites for tRNA

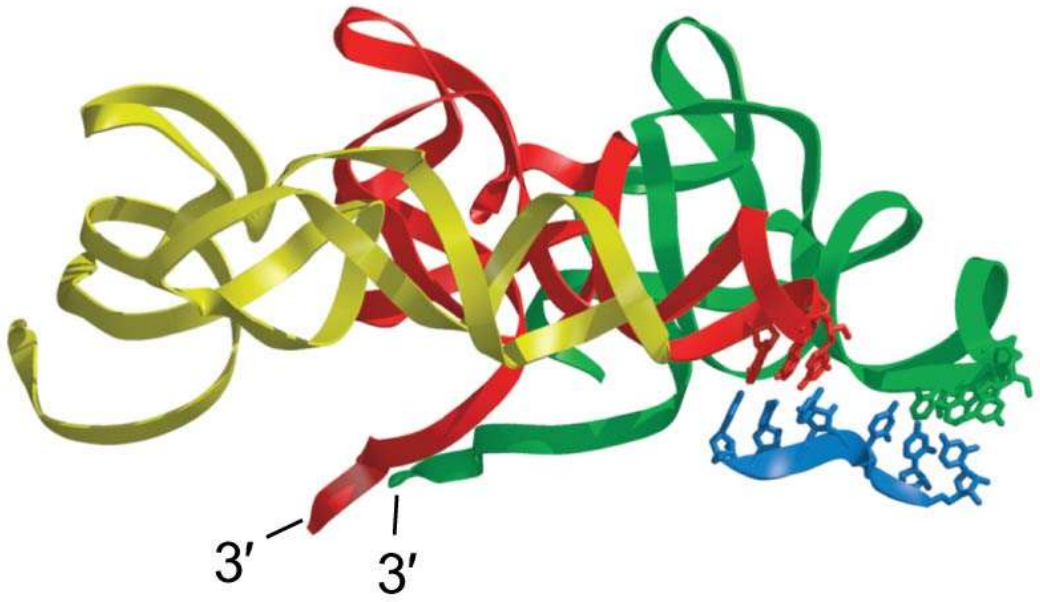


The E-, P-, and A-site tRNAs are shown in yellow, red, and green, respectively

Two Centers

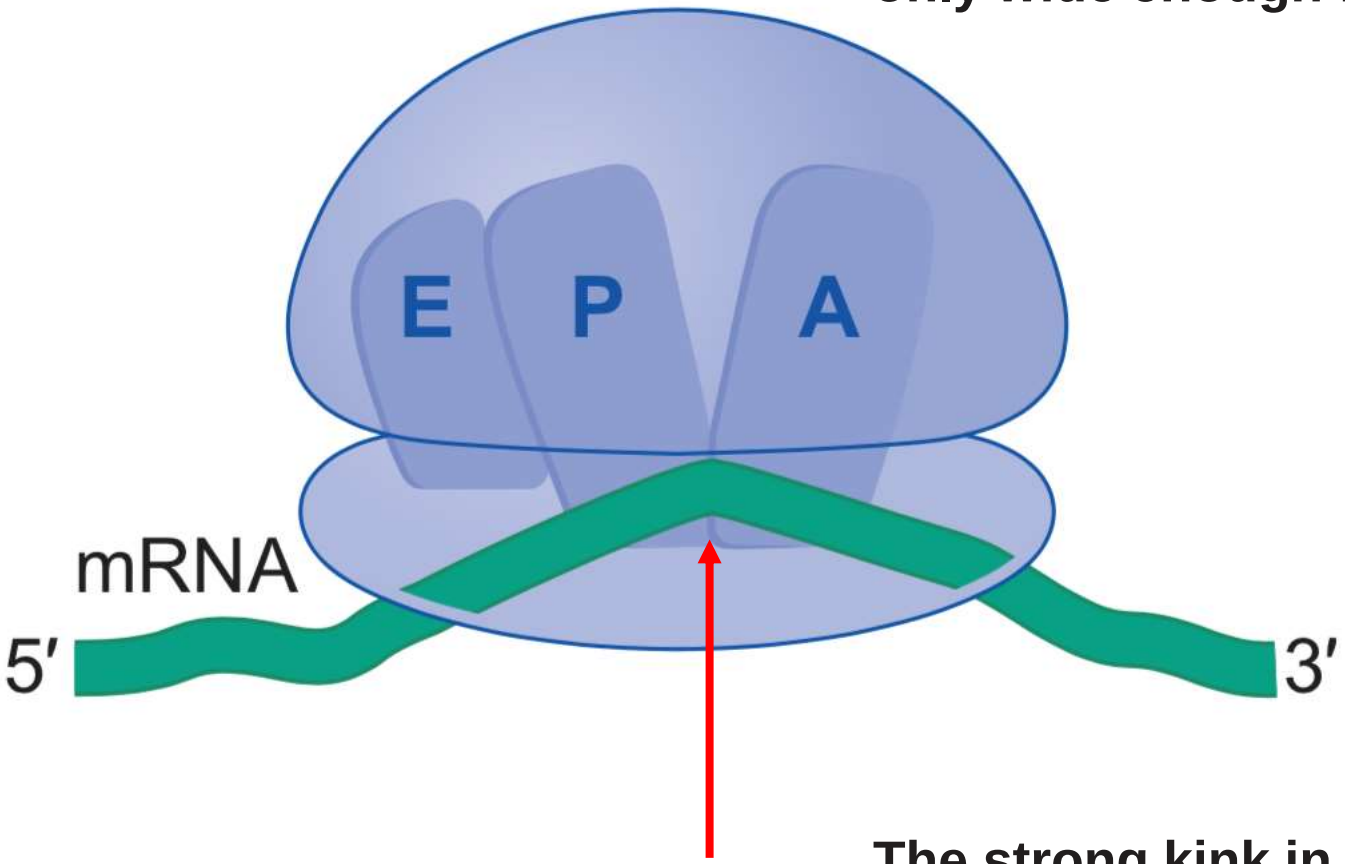
- **Decoding center – small subunit**
- **Peptidyl transferase center – large subunit**





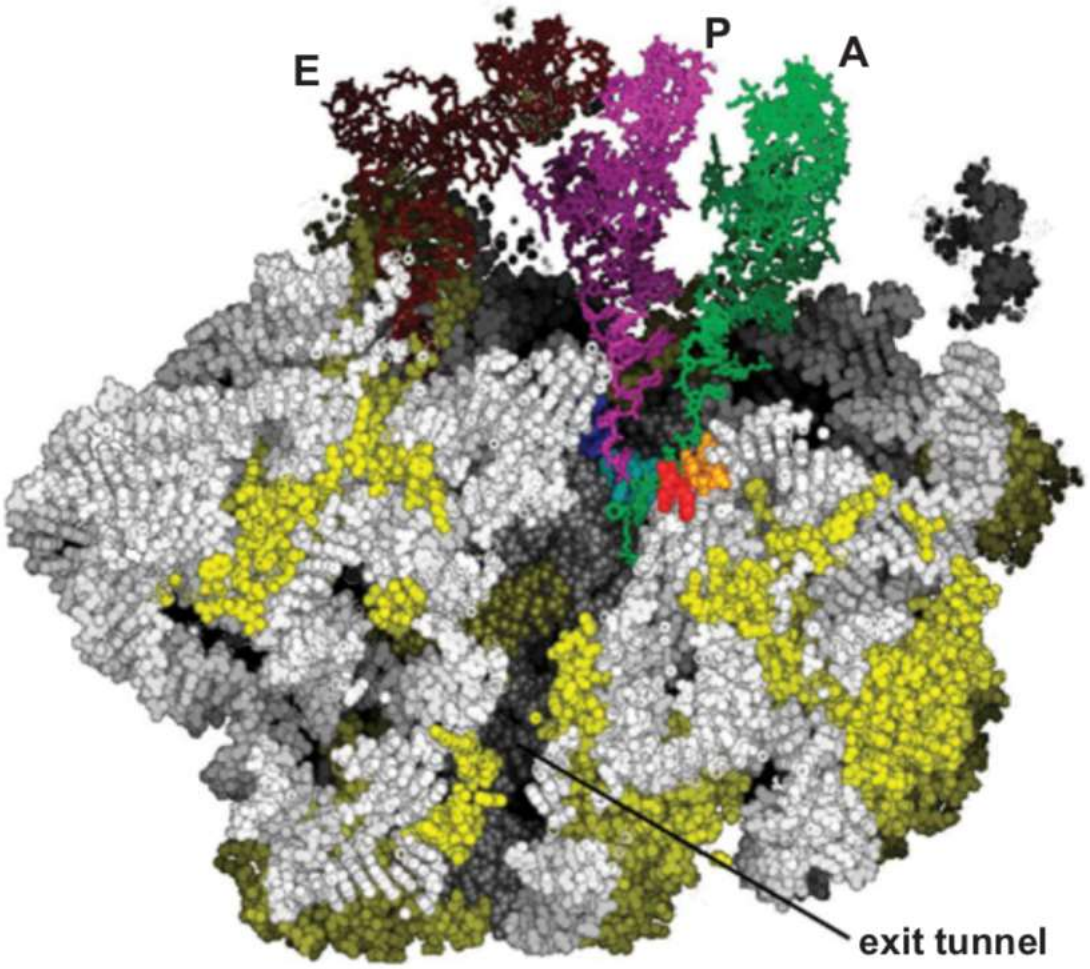
Channels for mRNA

The mRNA enters and exits the decoding center through two narrow channels in the small subunit. The entry channel is only wide enough for unpaired RNA to pass through.



The strong kink in the mRNA clearly distinguishes between the A-site and P-site codons.

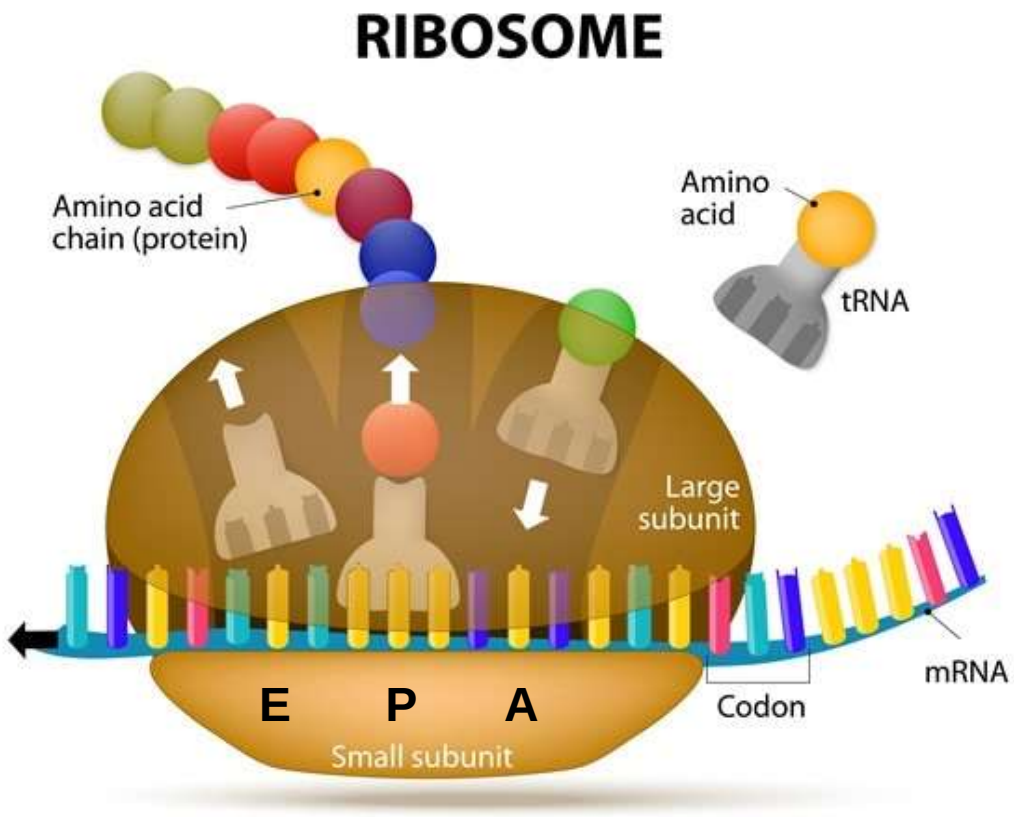
The polypeptide exit tunnel



Located in the large subunit

30 aa long

Narrow, only a-helix is allowed



Three Binding Sites for tRNA

A site - amino acid
P site – polypeptide
E site - exit

Two Centers

- **Decoding center – small subunit**
- **Peptidyl transferase center – large subunit**

Two Channels

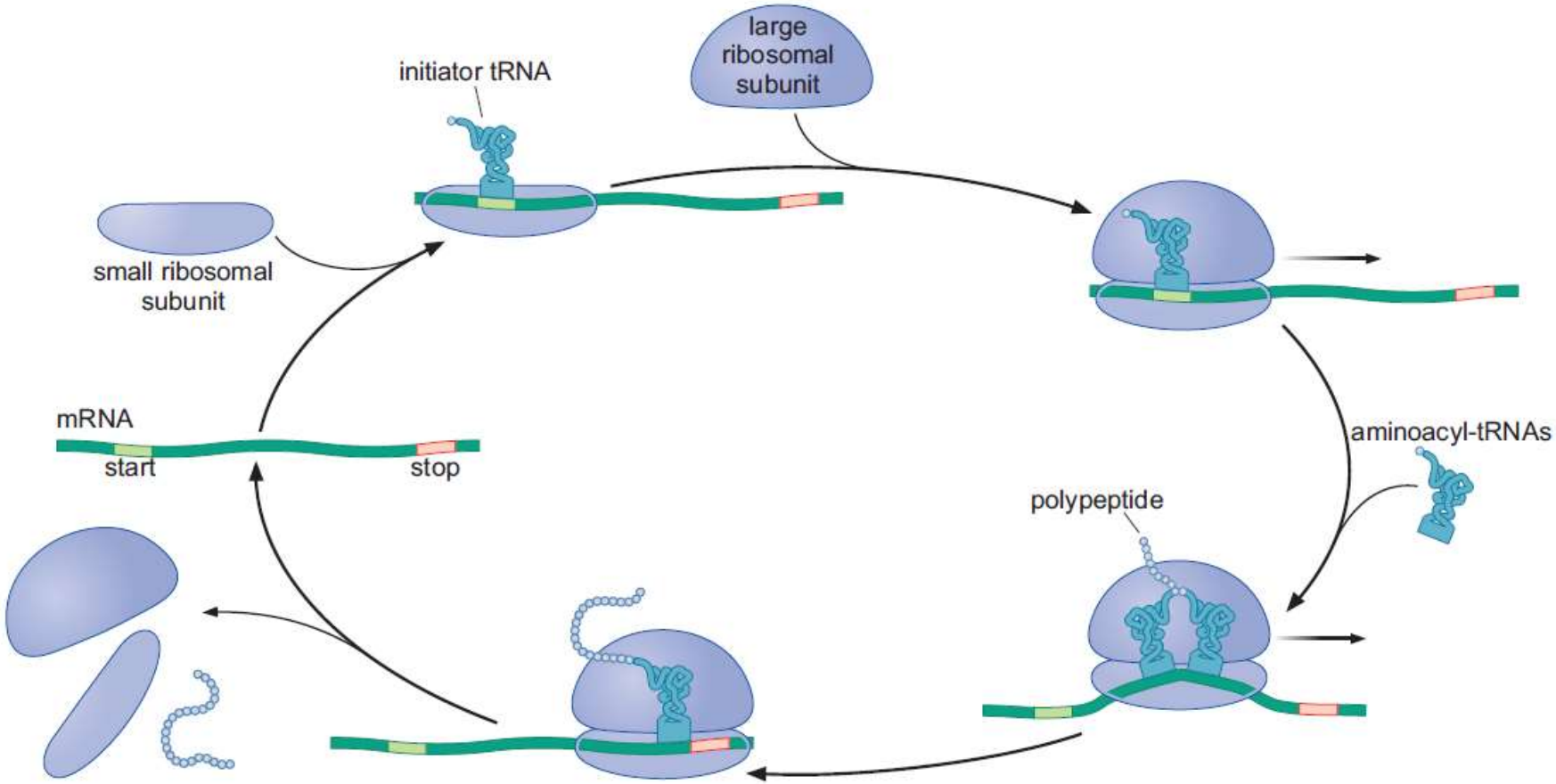
- **mRNA chanel**
- **Exit tunel**

1. Translation machinery

- Messenger RNA
- Transfer RNA
- Aminoacyl-tRNA Synthetases
- The Ribosome

15.2 Translation process 翻译过程

Initiation
翻译起始



Elongation
翻译延伸

Termination
翻译终止

Prokaryote VS Eukaryote

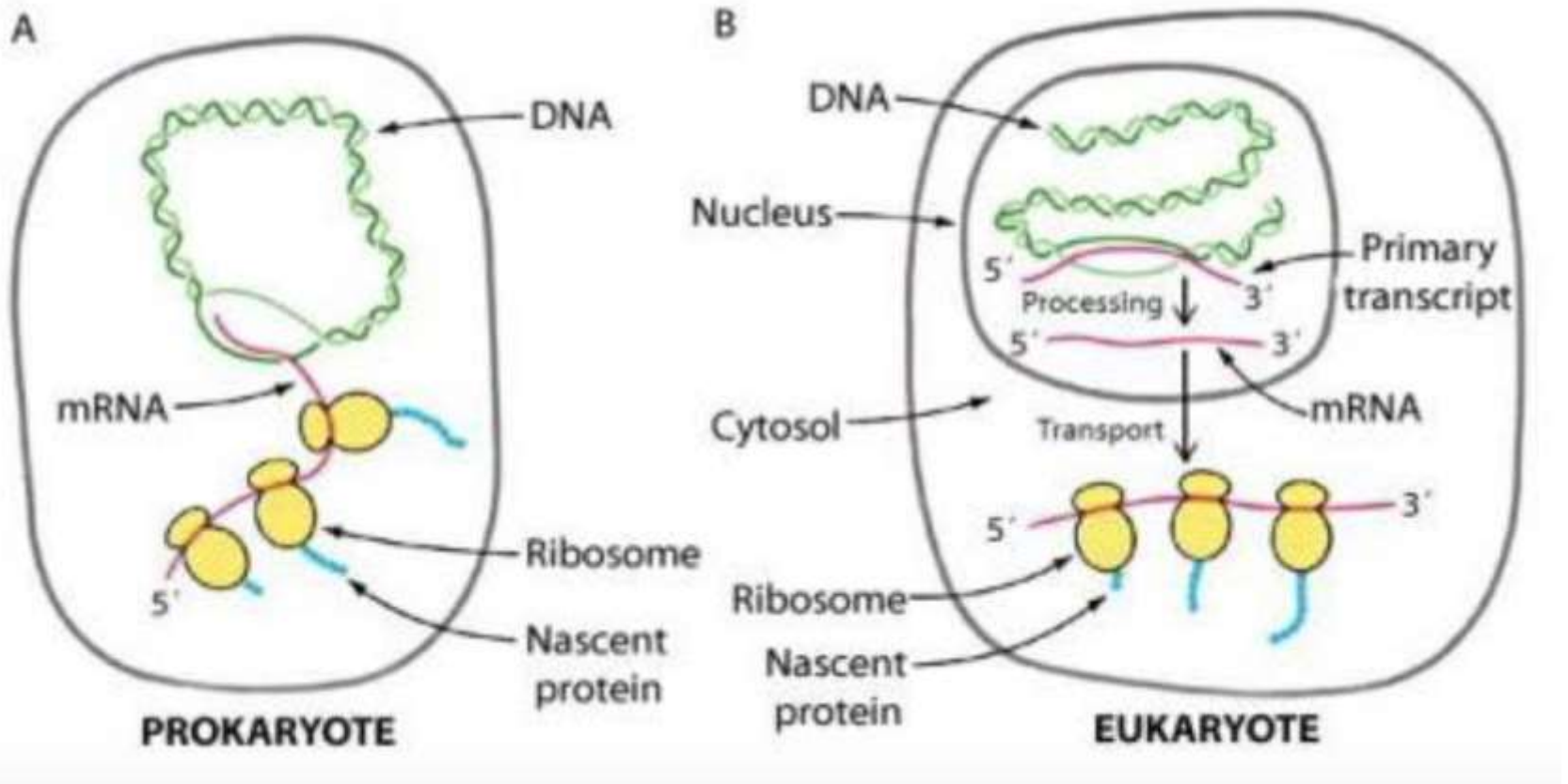
similarities and differences

Coupled with transcription

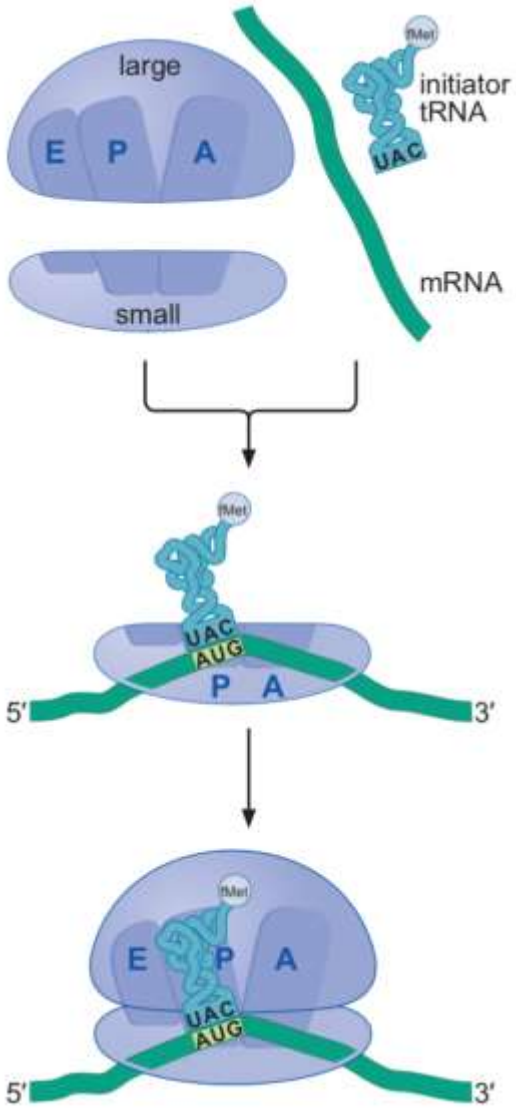
20 aa per second

Separated from transcription

2-4 aa per second



2.1 Translation initiation

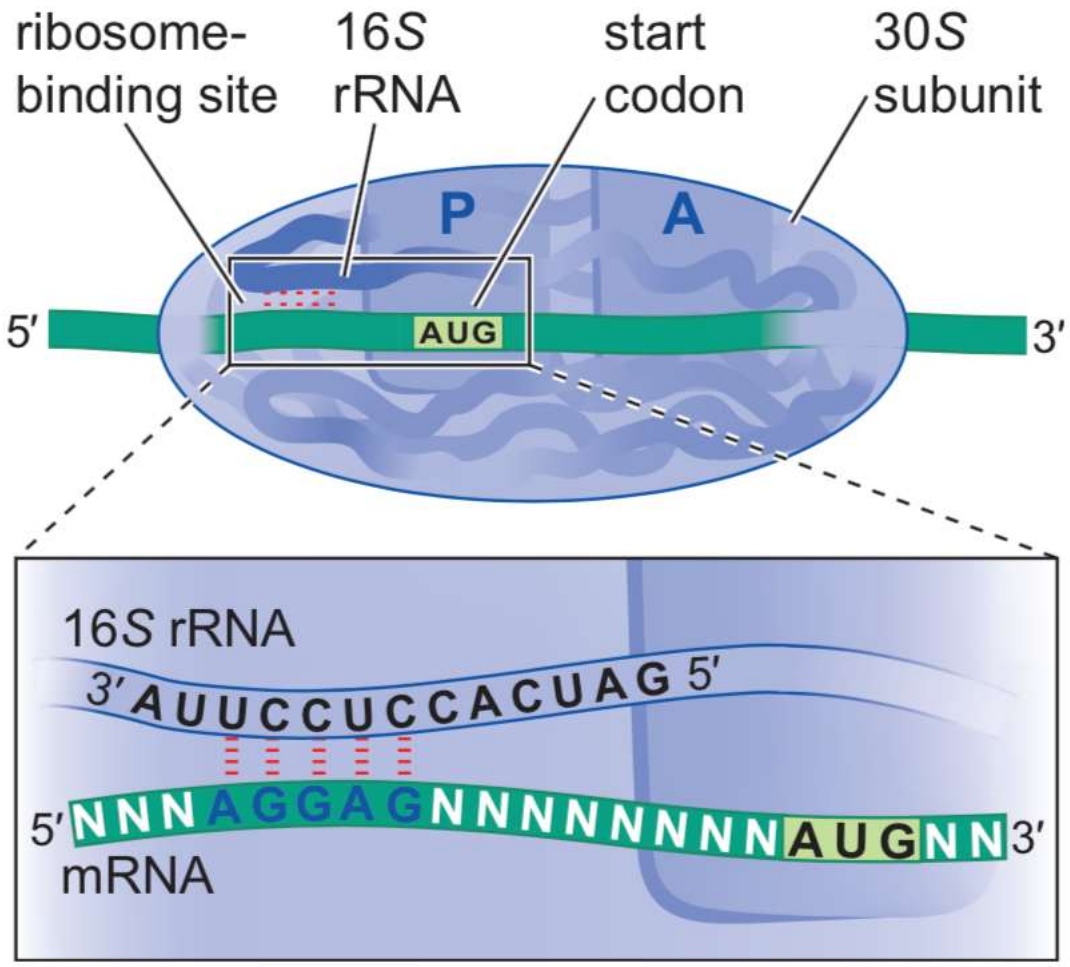


An overview of the events of translation initiation

- the ribosome must be recruited to the mRNA;
- a charged tRNA must be placed into the P-site of the ribosome;
- the ribosome must be precisely positioned over the start codon.

The correct positioning of the ribosome over the start codon is critical because this establishes the reading frame for the translation of the mRNA.

Prokaryotic mRNAs Are Initially Recruited to the Small Subunit by Base Pairing to rRNA



The assembly of the ribosome on an mRNA occurs one subunit at a time.

The small subunit associates with the mRNA first.

The association of the small subunit with the mRNA is mediated by base-pairing interactions between the RBS and the 16S rRNA.

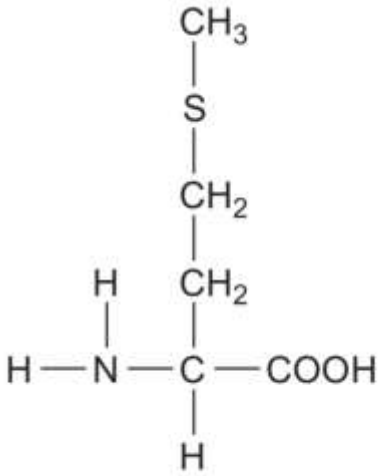
For ideally positioned RBSs, the start codon will be in the P-site.

The large subunit joins its partner only at the very end of the initiation process.

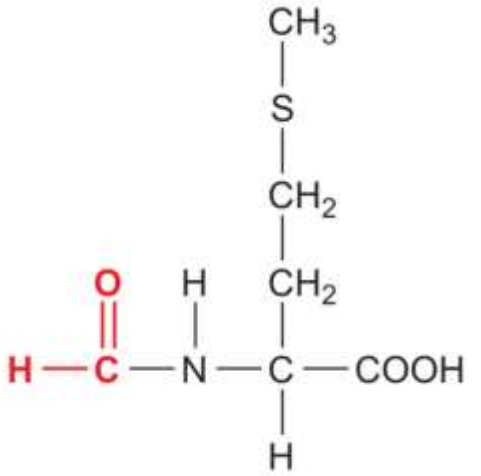
Prokaryote

A Specialized tRNA Charged with a Modified Methionine Binds Directly to the Prokaryotic Small Subunit.

Prokaryote



methionine



N-formyl methionine (fMet)

N-甲酰甲硫氨酸

Translation initiation is the only time a tRNA binds to the P-site without previously occupying the A-site.

initiator tRNA

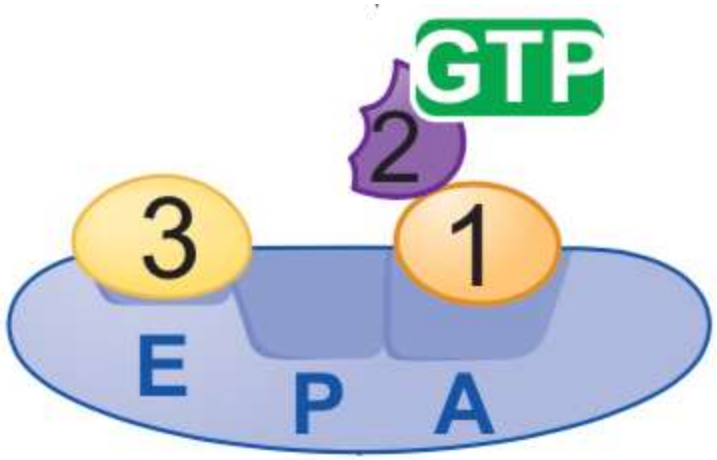
The initiator tRNA is first charged with a methionine, a formyl group is rapidly added to the methionine amino group by Met-tRNA transformylase.

The charged initiator tRNA - **$fMet\text{-}tRNA_i^{fMet}$**

Deformylase removes the formyl group from the amino terminus during or after the synthesis of the polypeptide chain.

Three Initiation Factors Direct the Assembly of an Initiation Complex

Prokaryote



IF1 prevents tRNAs from binding to the portion of the small subunit that will become part of the A-site.

IF2 is a GTPase that interacts with three key components of the initiation machinery: the small subunit, IF1, and the charged initiator tRNA (fMet-$tRNA_i^{fmet}$).

IF2 facilitates the association of fMet-$tRNA_i^{fmet}$ with the small subunit and prevents other charged tRNAs from associating with the small subunit.

IF3 binds to the small subunit and blocks it from re-associating with a large subunit.

Each of the initiation factors binds at, or near, one of the three tRNA- binding sites on the small subunit. Only the P-site is capable of binding a tRNA in the presence of the initiation factors.

The recruitment of mRNA and tRNA to the small subunit

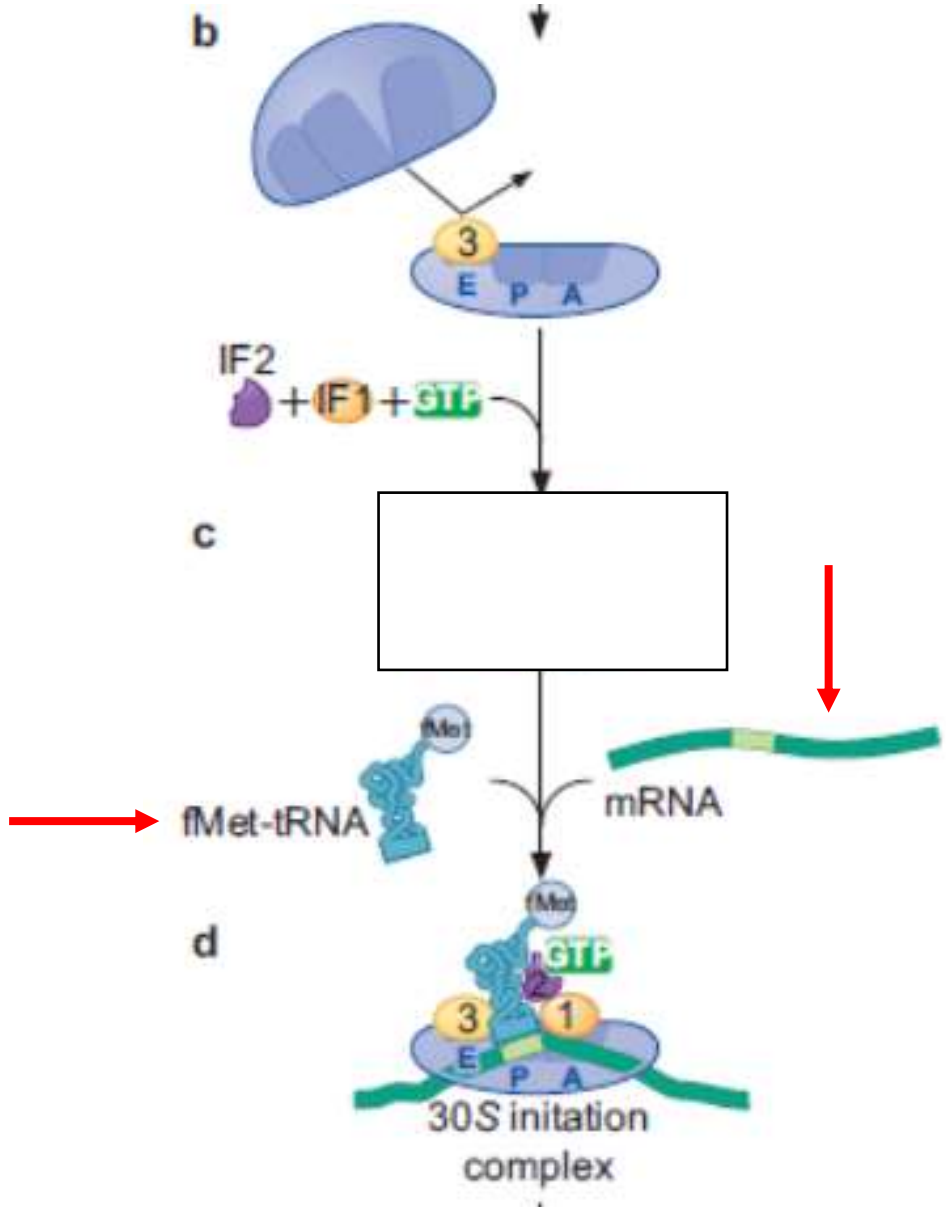
With all three initiation factors bound, the small subunit is prepared to bind to the mRNA and the initiator tRNA

These two RNAs can bind in either order and independently of each other.

The mRNA binds with the small subunit by base pairing between the RBS and the 16S rRNA.

fMet-tRNA$_i^{fmet}$ binds to the small subunit by interacting with IF2 bound to GTP and base pairing between the anticodon and the start codon of the mRNA.

Base pairing between the fMet-tRNA$_i^{fmet}$ f Met and the mRNA serves to position the start codon in the P-site.



The association of large subunit

a change in conformation and results in the release of IF3.

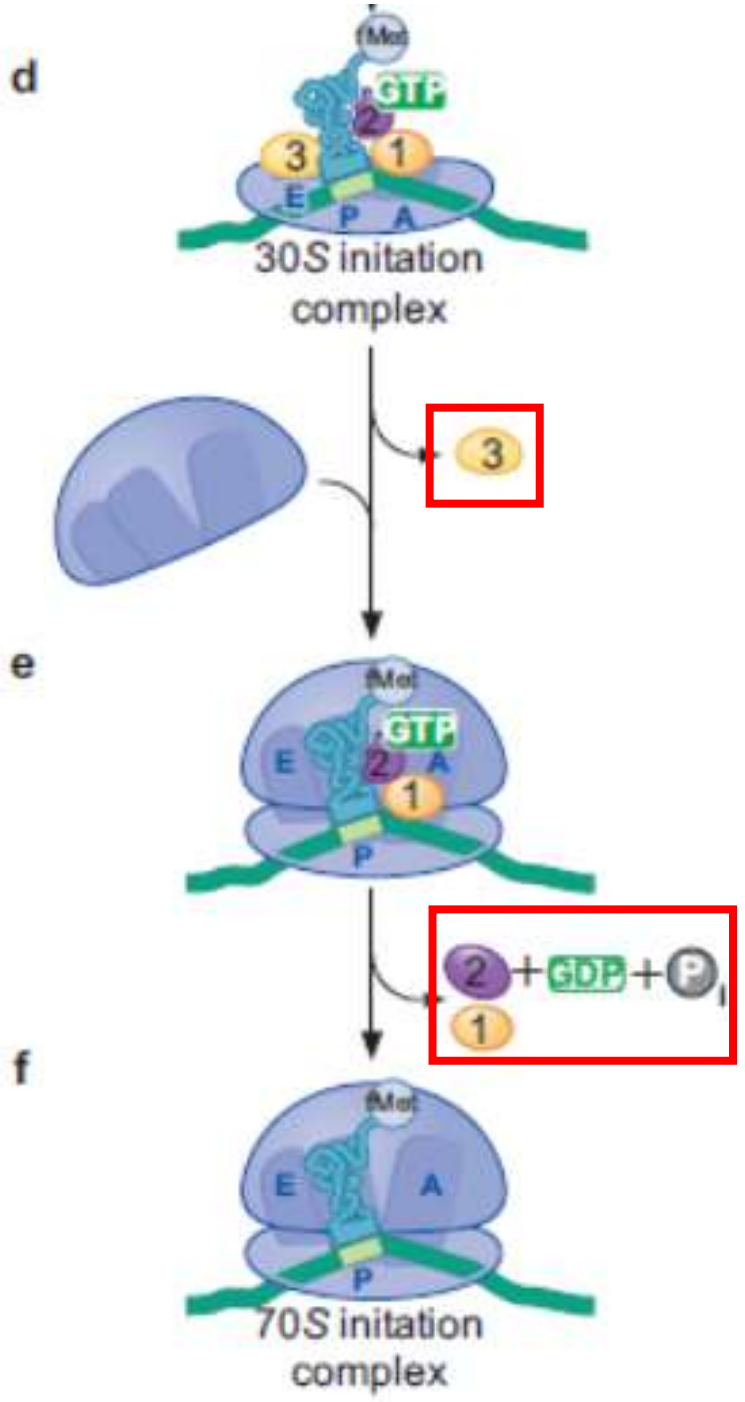
The large subunit binds to the small subunit with its cargo of IF1 IF2, mRNA, and fMet-tRNA$_i^{fmet}$

this interaction subsequently stimulates the GTPase activity of IF2.GTP.

the release of IF2.GDP as well as IF1 from the ribosome.

the formation of an intact (70S) ribosome
assembled at the start site of the mRNA with fMet-tRNAfiMet in the P-site and an empty A-site.

Conformational change and GTP hydrolysis



Translation initiation in eukaryotes

Similarity:

- Both use a start codon and a dedicated initiator tRNA,
- Both use initiation factors to form a complex with the small ribosomal subunit that assembles on the mRNA before addition of the large subunit.

Difference:

- Eukaryotes use a fundamentally distinct method to recognize the mRNA and the start codon.
- More complex translation initiation factors.

Eukaryotic Ribosomes Are Recruited to the mRNA by the 5′ Cap

- In eukaryotes, the small subunit is already associated with an initiator tRNA when it is recruited to the capped 5’ end of the mRNA.

- It then “scans” along the mRNA in a 5’ to 3’ direction until it reaches the first 5’-AUG-3’ which it recognizes as the start codon.

Translation initiation in eukaryotes

4 steps

First, binding of the initiator tRNA to the small subunit always precedes association with the mRNA.

Second, a separate set of auxiliary factors mediates the recognition of the mRNA.

Third, the small ribosomal subunit bound to the initiator tRNA scans the mRNA for the first AUG sequence.

Finally, the large subunit of the ribosome is recruited after the initiator tRNA base-pairs with the start codon.

The formation of the 43 S preinitiation complex

Initiation factors bind to the small subunit to prevent both large subunit binding and tRNA binding.

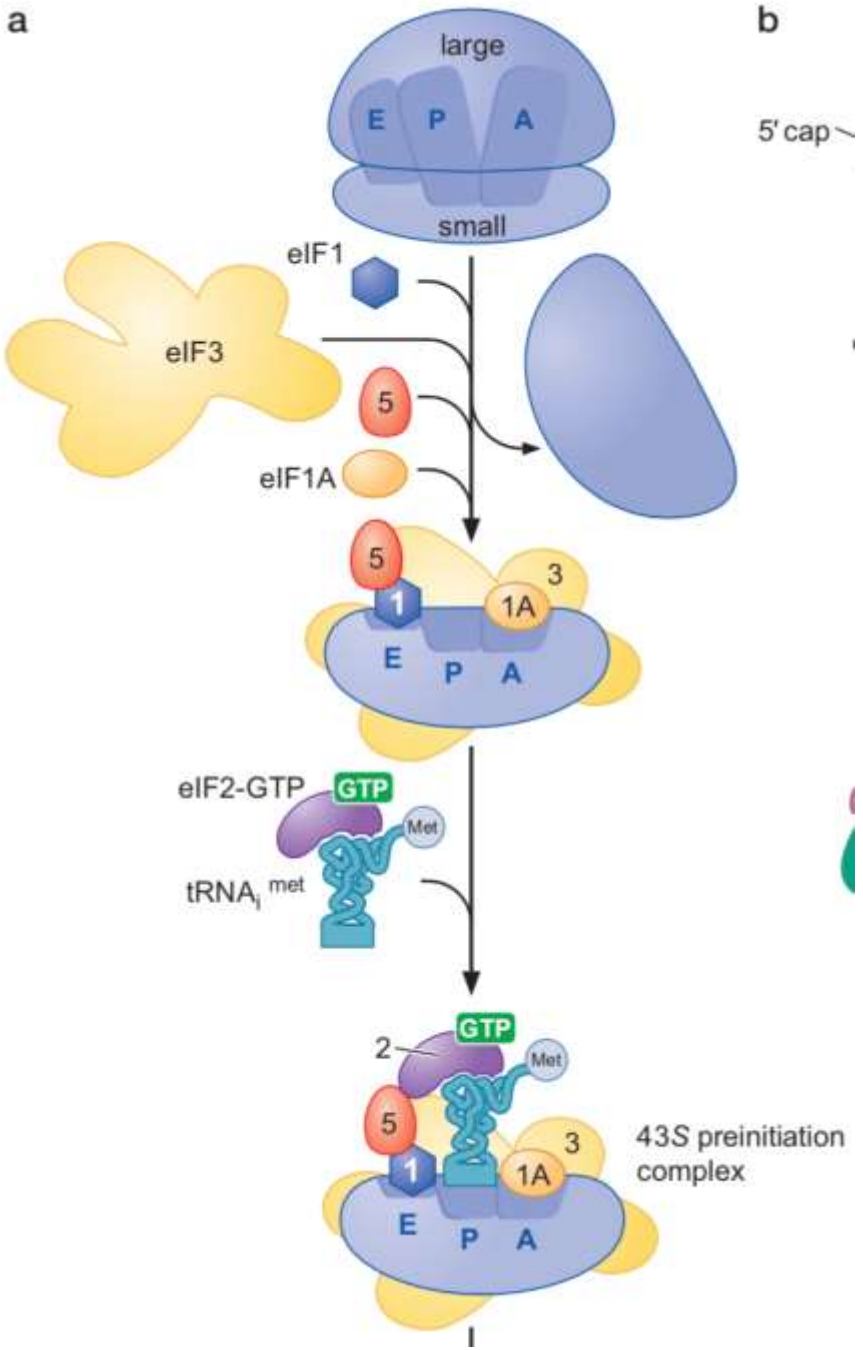
eIF3 - platform

eIF1A – A site

eIF1 eIF5 – E site

GTP-bound eIF2 binds to the initiator tRNA and forms the ternary complex (TC).

TC positions the Met-$tRNA_i^{met}$ in the P-site of the small subunit.



The recognition of mRNA

eIF4E – 5’ cap (cap-binding protein)

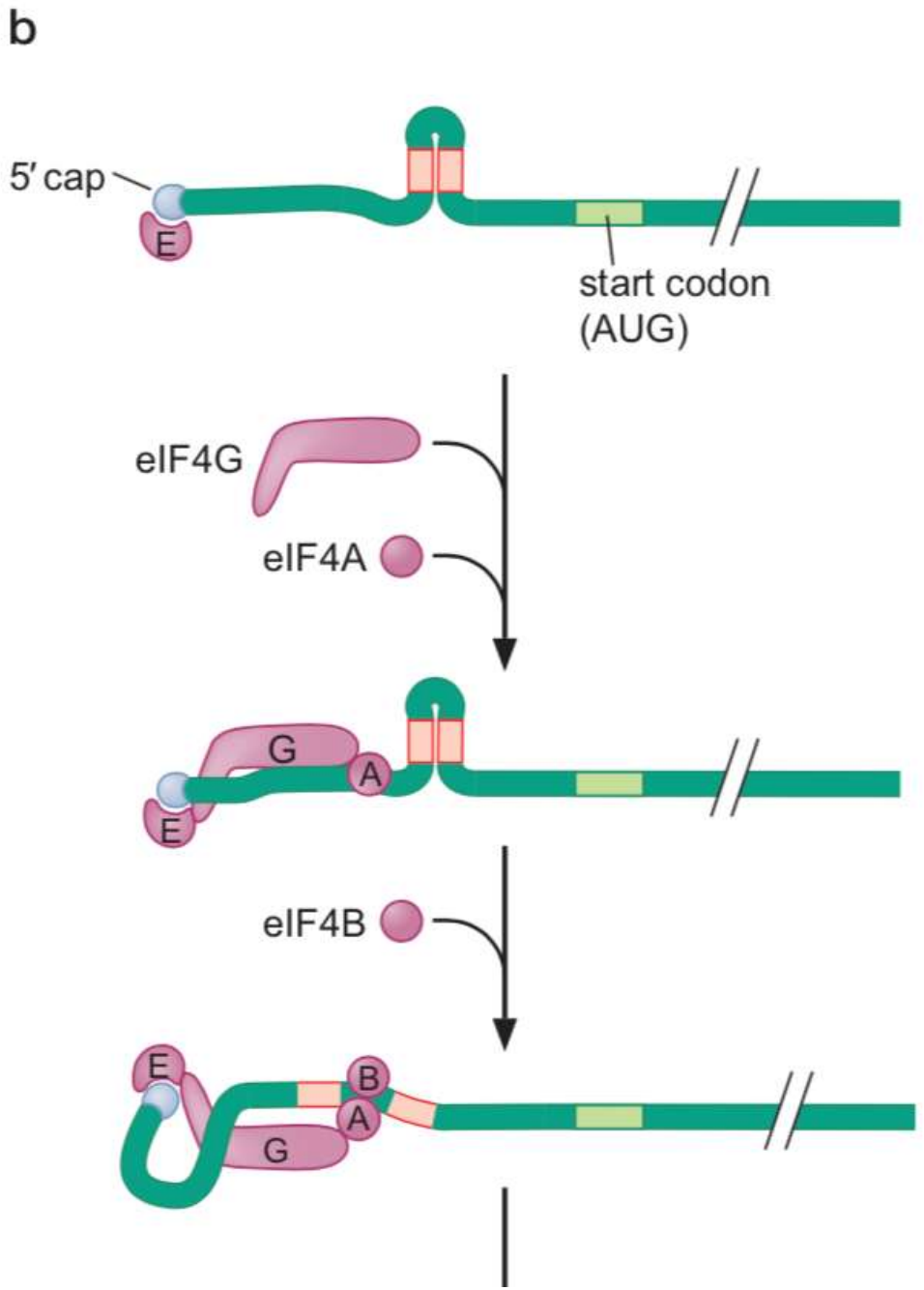
eIF4G - eIF4E and the mRNA

eIF4A - eIF4G and the mRNA

eIF4B - activates the RNA helicase activity of eIF4A

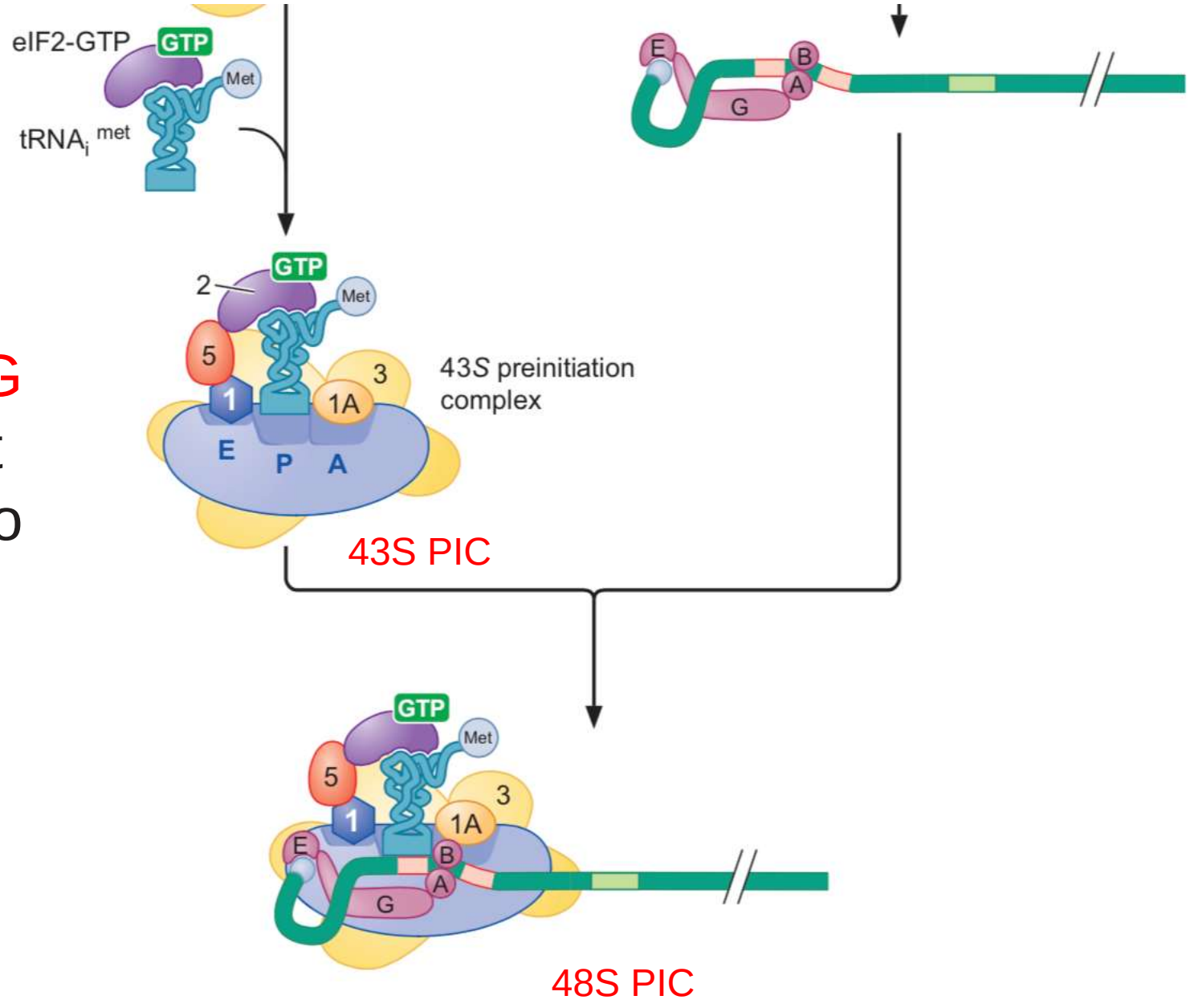
The association of eIF4G with eIF4E is particularly important—the overall level of translation in the cell is controlled at this step

The helicase eIF4A unwinds any secondary structures, which is required for the binding with small subunit.



The formation of the 48 S preinitiation complex

Interactions between the eIF4G and the initiation factors recruit the 43S preinitiation complex to the mRNA to form the 48S preinitiation complex.



Identification initiating AUG by the 48S PIC

Scaning - in an ATP- dependent process

The start codon is recognized by base pairing between the anticodon of the initiator tRNA and the start codon

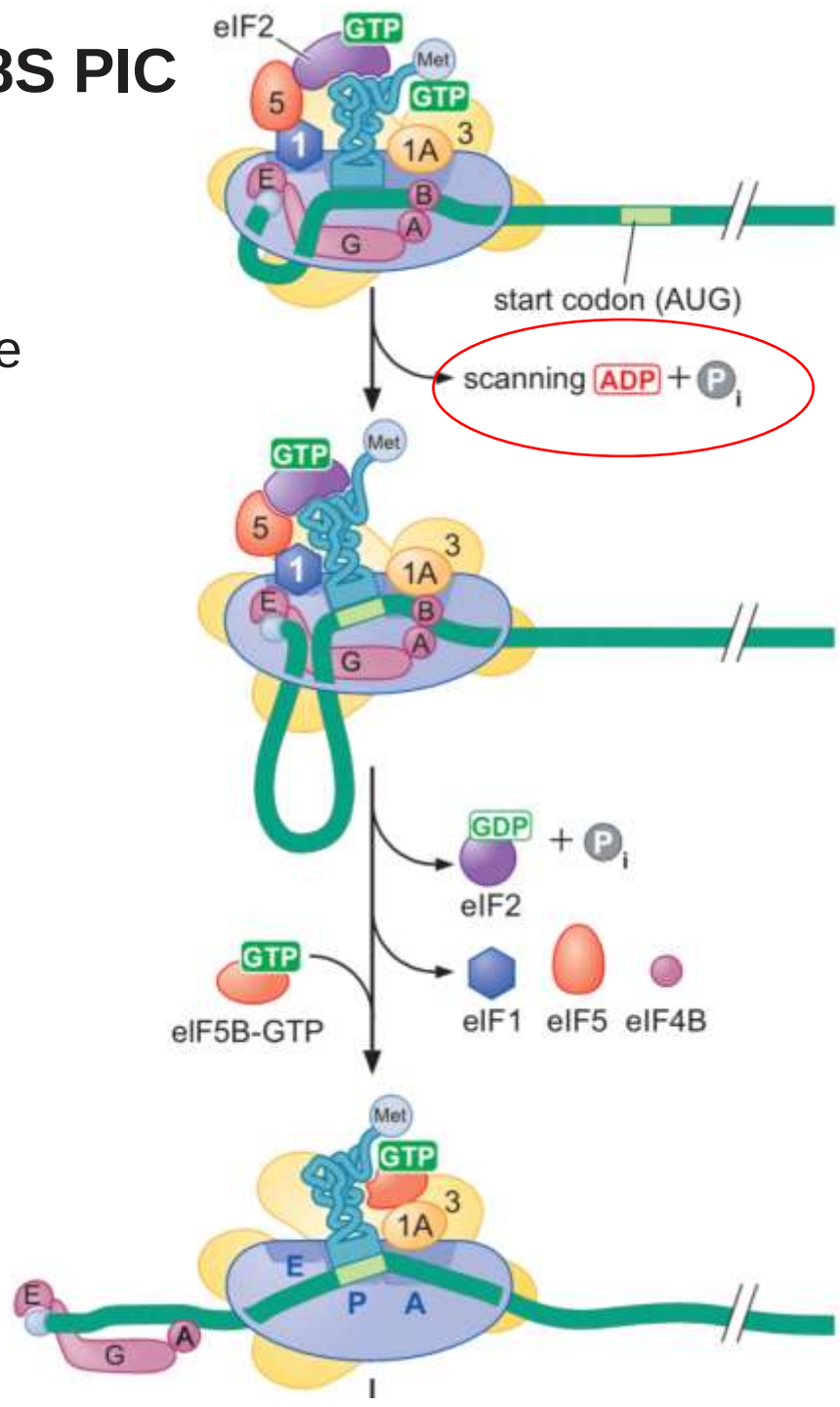
Conformation change of the 48S PIC

Release of eIF1 and a change in conformation of eIF5

eIF2: GTP to GDP

Release of eIF2 and eIF5

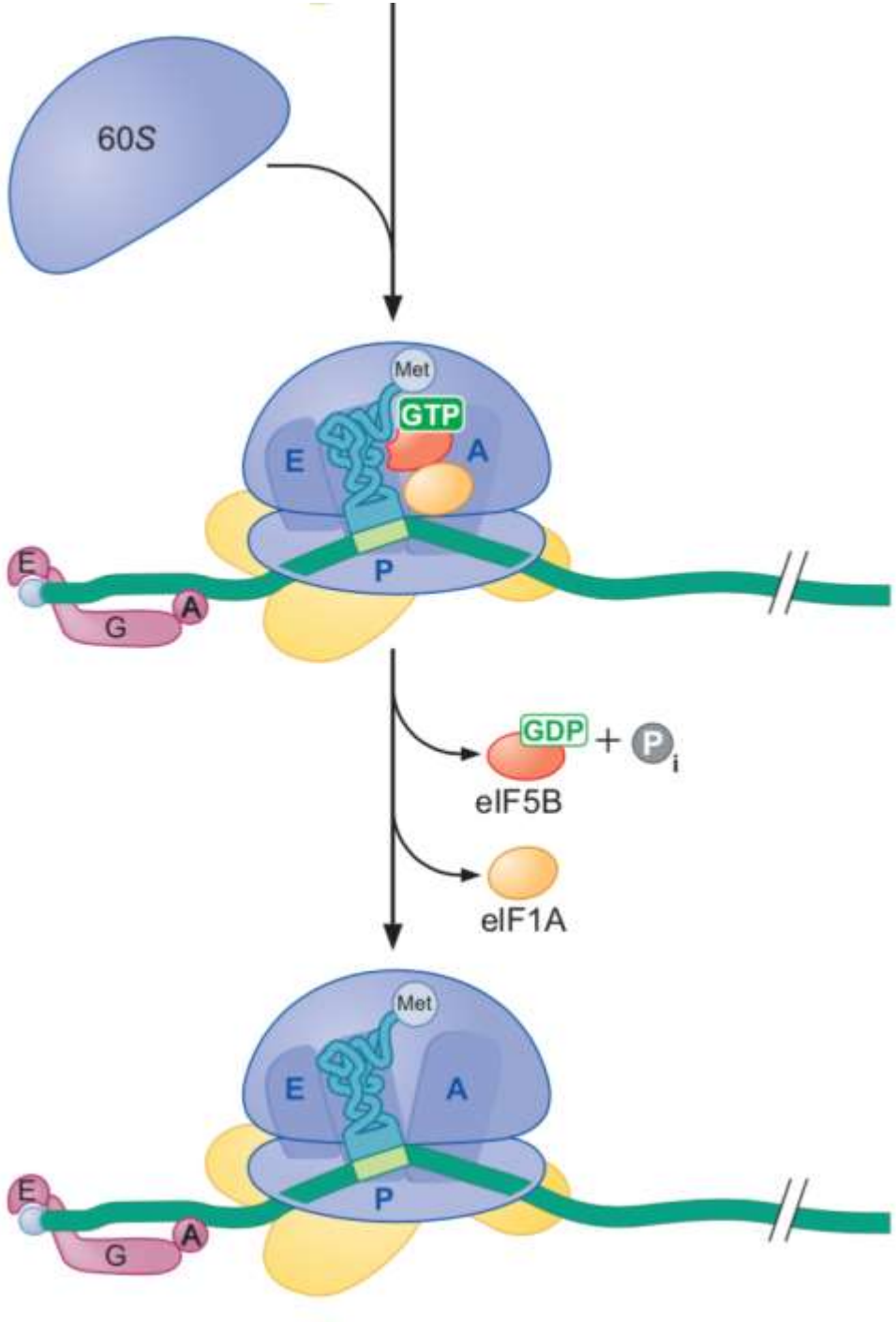
Binding of eIF5B



The formation of the 80S initiation complex

Association of the 60S subunit

Release of the remaining initiation factors by stimulating GTP hydrolysis by eIF5B



Comparison of translation initiation in prokaryote and eukaryote

	prokaryote	eukaryote
E site	IF3	eIF1 eIF5
P site	IF2	eIF2 eIF5B
A site	IF1	eIF1A

P site

↓

E site

↓

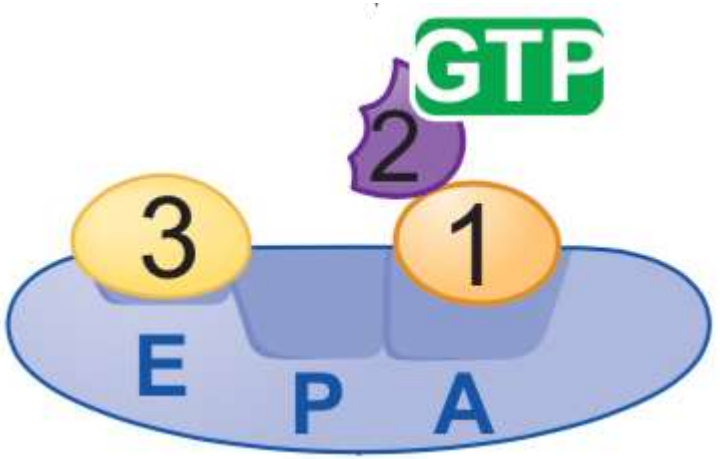
Large subunit

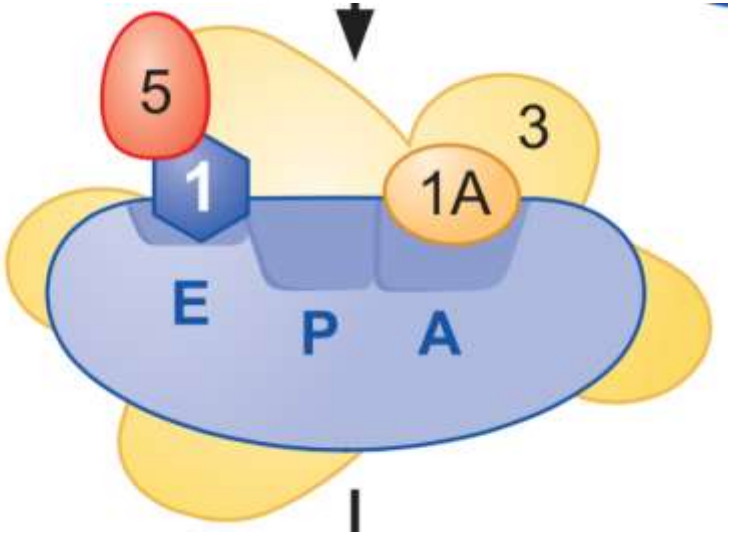
↓

GTP to GDP

↓

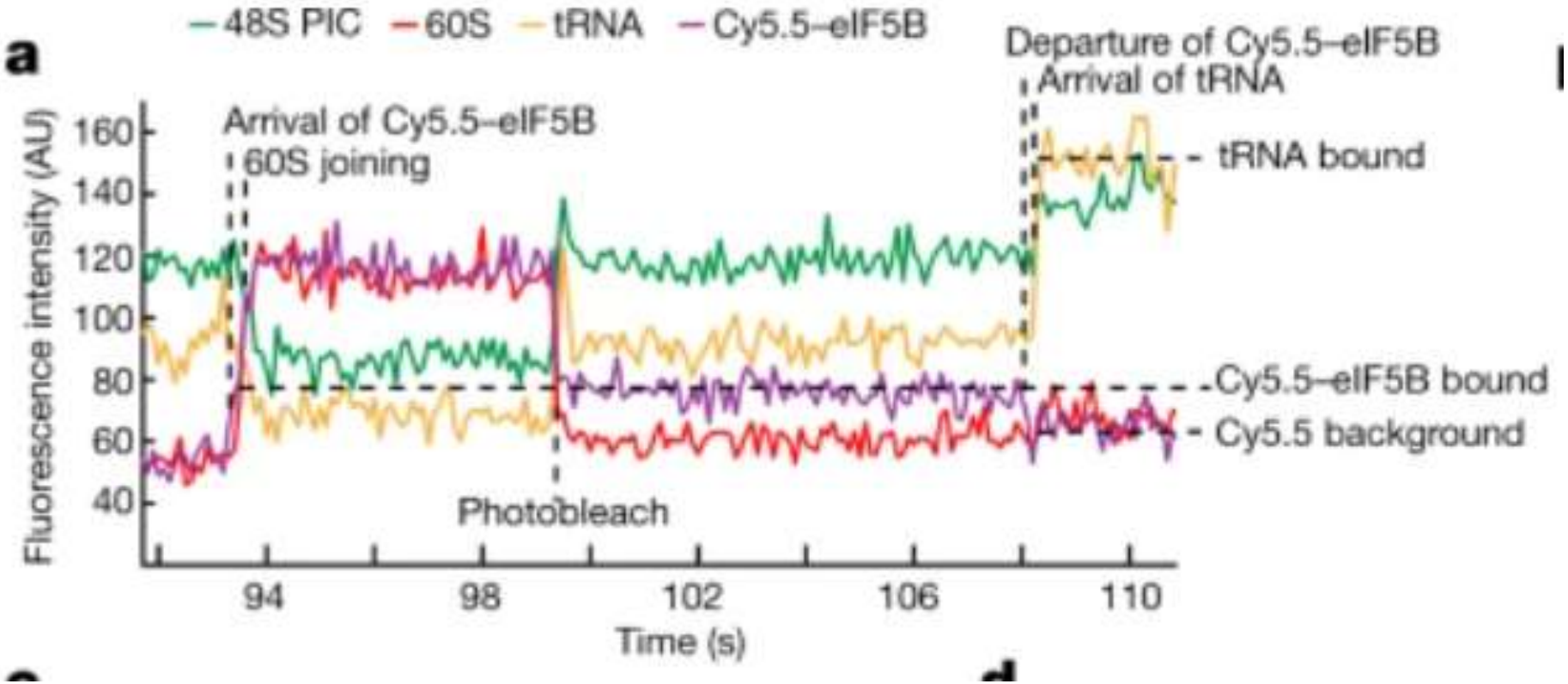
A site





eIF5B gates the transition from translation initiation to elongation

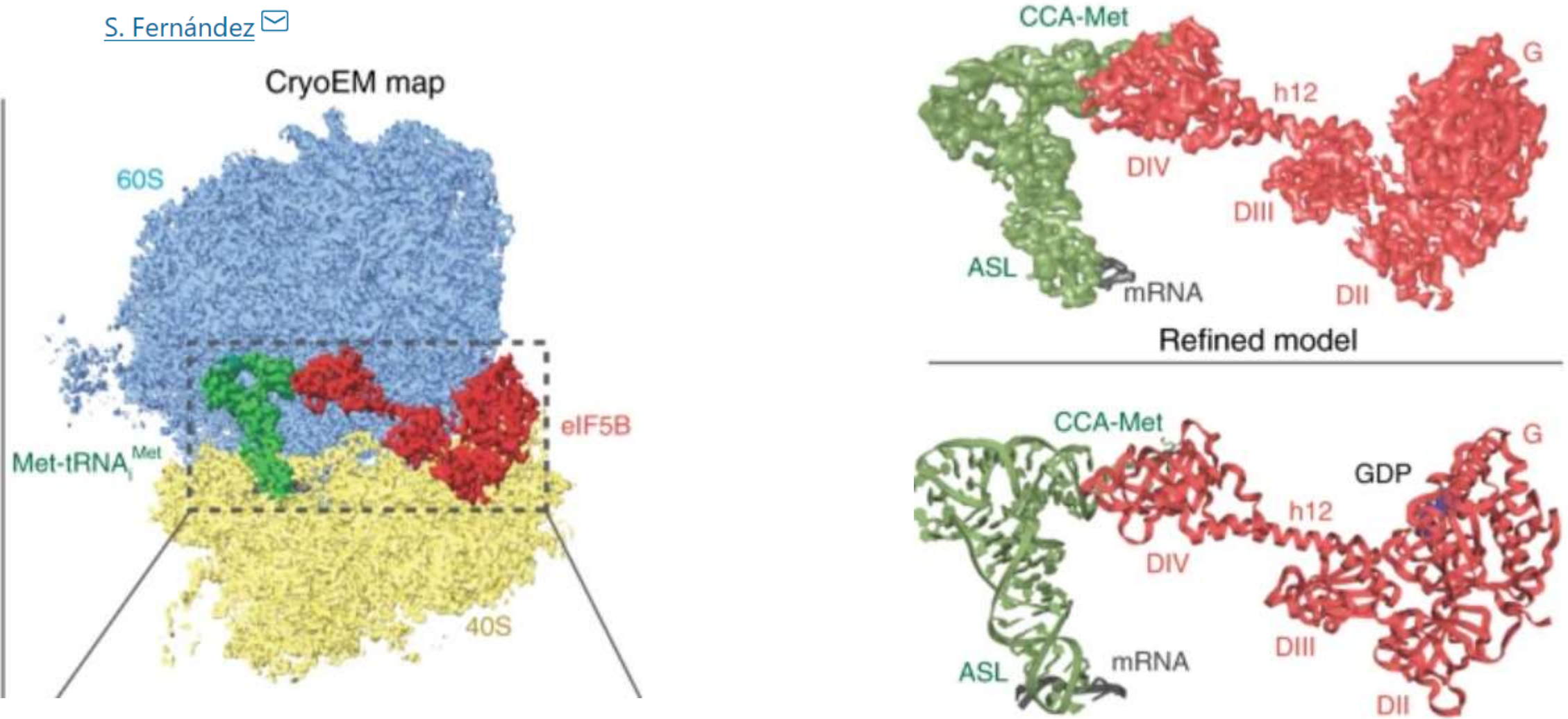
Jinfan Wang, Alex G. Johnson, Christopher P. Lapointe, Junhong Choi, Arjun Prabhakar, Dong-Hua Chen, Alexey N. Petrov & Joseph D. Puglisi



Article | Open Access | Published: 06 October 2020

Structural basis for the transition from translation initiation to elongation by an 80S-eIF5B complex

Jinfan Wang, Jing Wang, Byung-Sik Shin, Joo-Ran Kim, Thomas E. Dever, Joseph D. Puglisi & Israel S. Fernández



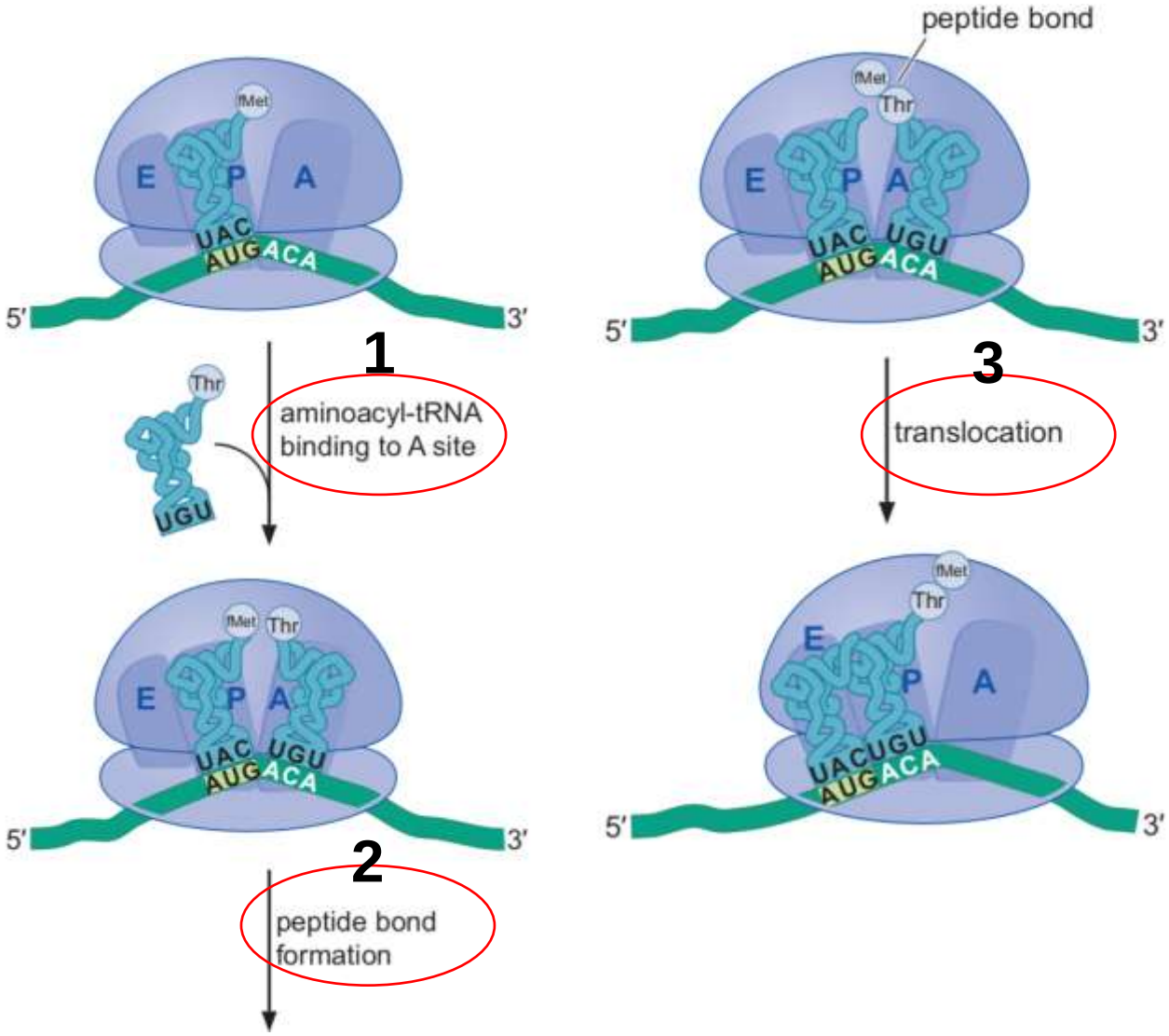
2.2 Translation elongation

Once the ribosome is assembled with the charged initiator tRNA in the P-site, polypeptide synthesis can begin.

Three steps:

- The loading of correct aminoacyl-tRNA to A-site
- Peptide bond formation
- translocation

Two auxiliary proteins known as elongation factors control these events (EF-Tu and EF-G).



The mechanism of elongation is highly conserved between prokaryotic and eukaryotic cells.

Aminoacyl-tRNAs Are Delivered to the A-Site by Elongation Factor EF-Tu

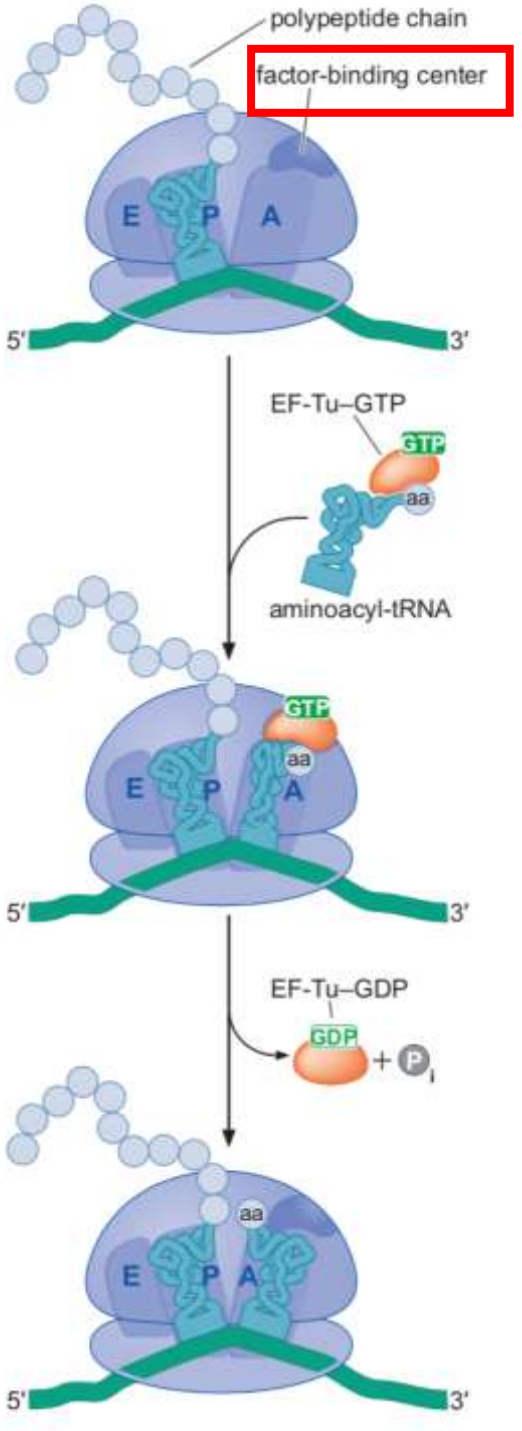
1. EF-Tu-GTP binds to aminoacyl-tRNAs .

2. Deliver a charged tRNA to A site;

3. Once a correct codon–anticodon match is made, EF-Tu interacts with the factor-binding center and activates its GTPase activity.

4. After the hydrolyzation of GTP, EF-Tu is released from the ribosome.

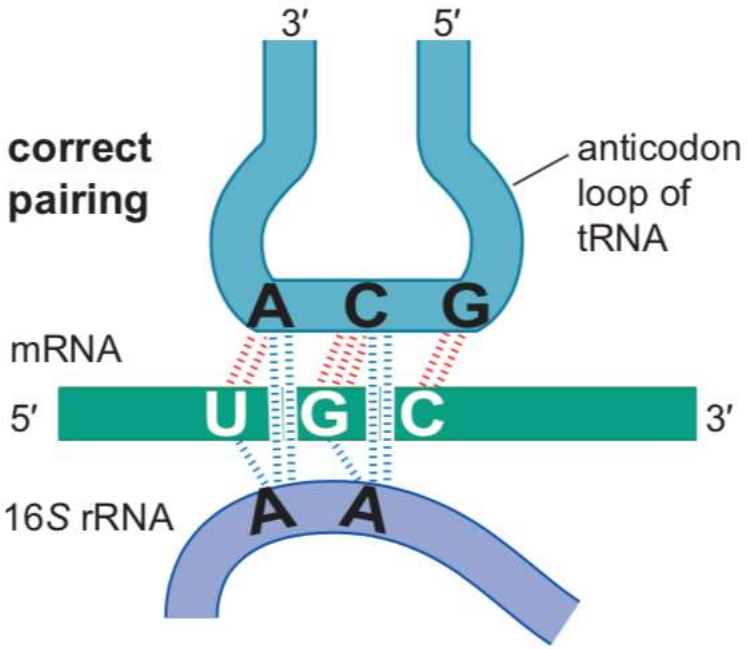
Factor binding center – 因子结合中心



The Ribosome Uses Multiple Mechanisms to Select against Incorrect Aminoacyl-tRNAs

The error rate of translation is between 10^{-3} and 10^{-4}.

The ultimate basis for the selection of the correct aminoacyl-tRNA is the **base pairing** between codon and anticodon.

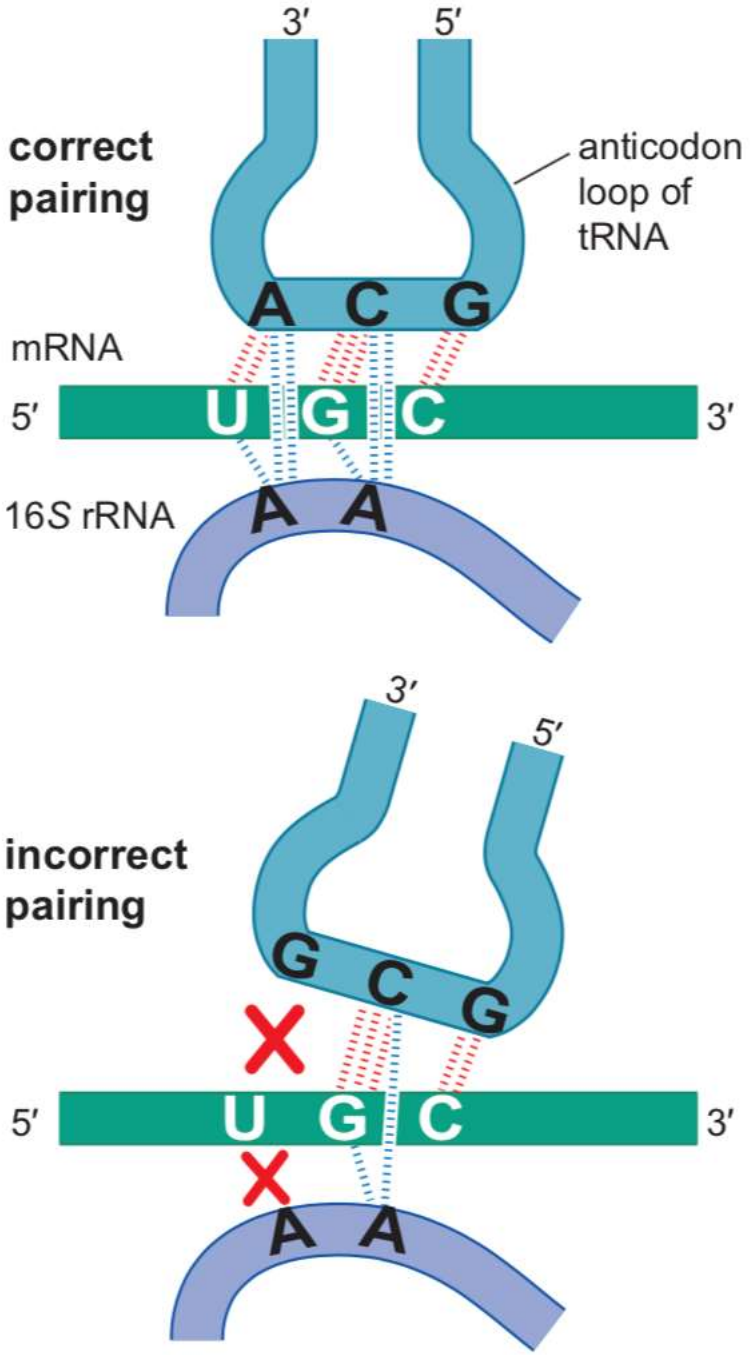


Three mechanisms to ensure correct pairing between the tRNA and the mRNA.

1. Additional hydrogen bonds:

Two adjacent adenine residues in the 16S rRNA form **hydrogen bonds** with the minor groove of each correct base pair formed between the anticodon and the codon in the A-site

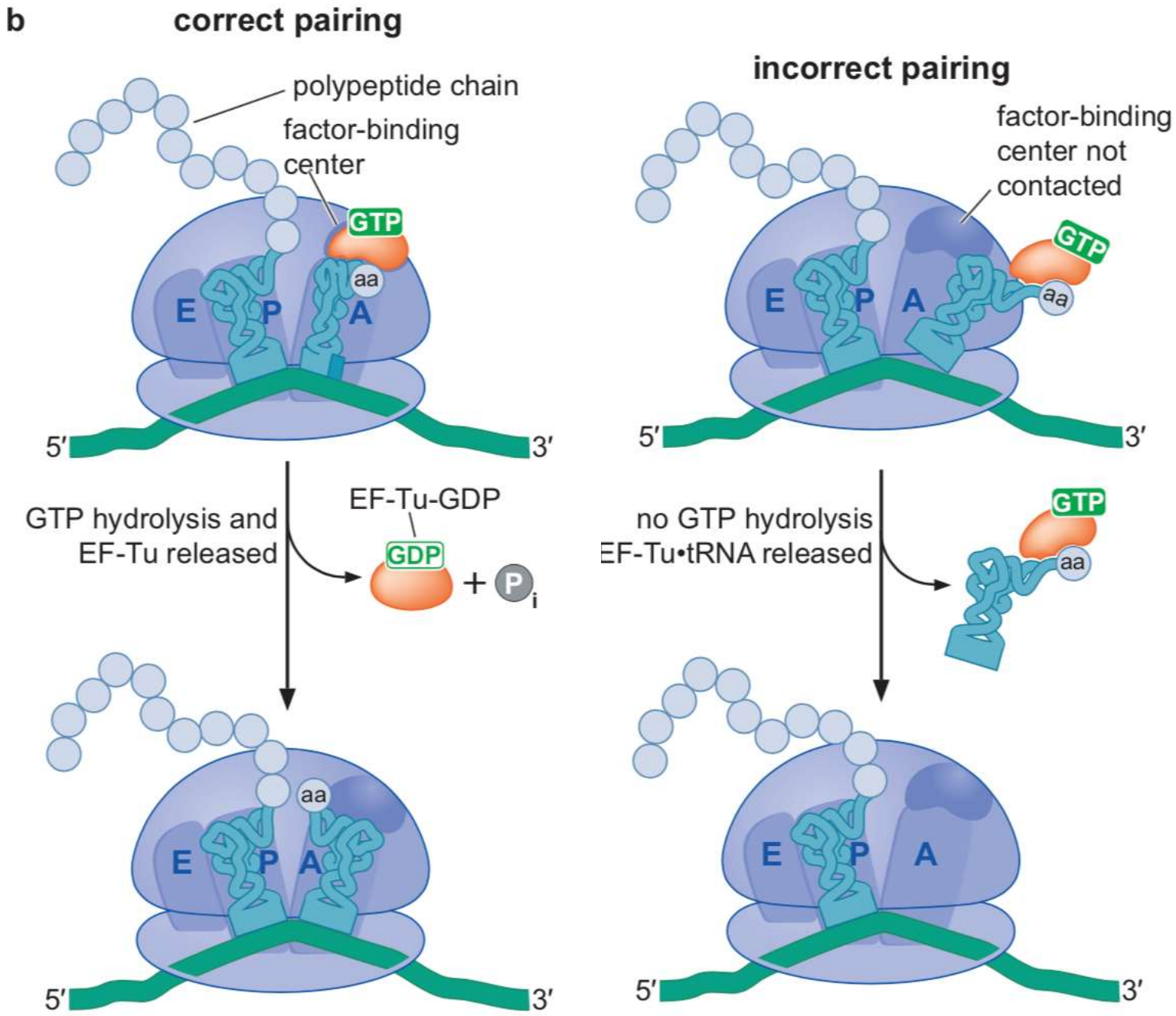
Correctly paired tRNAs show a much lower rate of dissociation from the ribosome than do incorrectly paired tRNAs.



2. the GTPase activity of EF-Tu

GTP hydrolysis is highly sensitive to correct codon – anticodon base pairing.

Even a single mismatch in the codon–anticodon base pairing alters the position of EF-Tu, reducing its ability to interact with the factor-binding center.

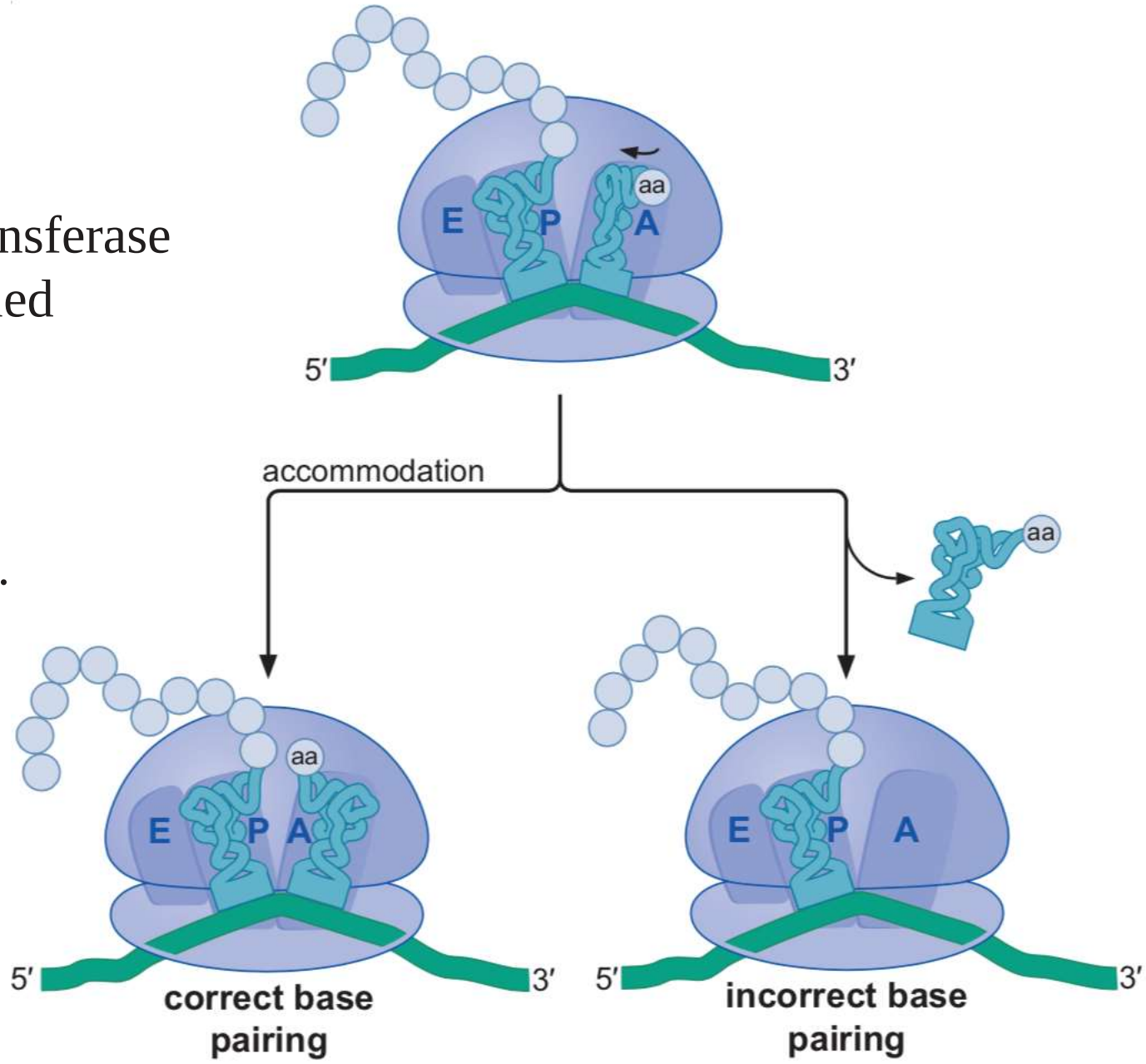


3. Accommodation (入位)

The tRNA must rotate into the peptidyl transferase center of the large subunit in a process called accommodation

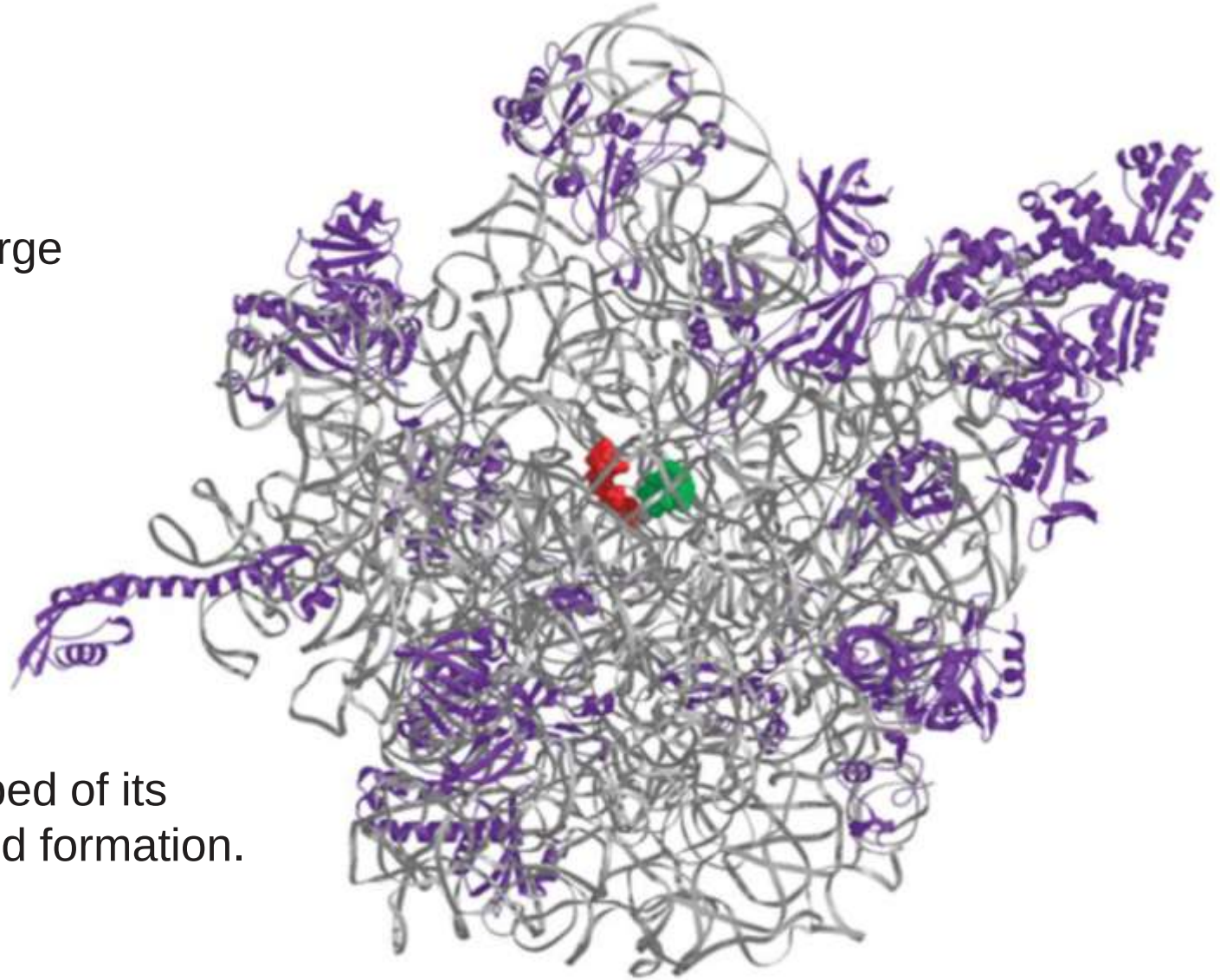
During accommodation, the 3’ end of the aminoacylated tRNA moves almost 70 A˚.

Incorrectly paired tRNAs frequently dissociate from the ribosome during accommodation.



The Ribosome Is a Ribozyme

Peptide-bond formation is catalyzed by RNA, specifically the 23S rRNA component of the large subunit.



The 3D structure of the bacterial 50S subunit:
RNAs (gray),
the ribosomal proteins (purple),
3’ end of the A-site tRNA green,
3’ end of the P-site tRNAs red.

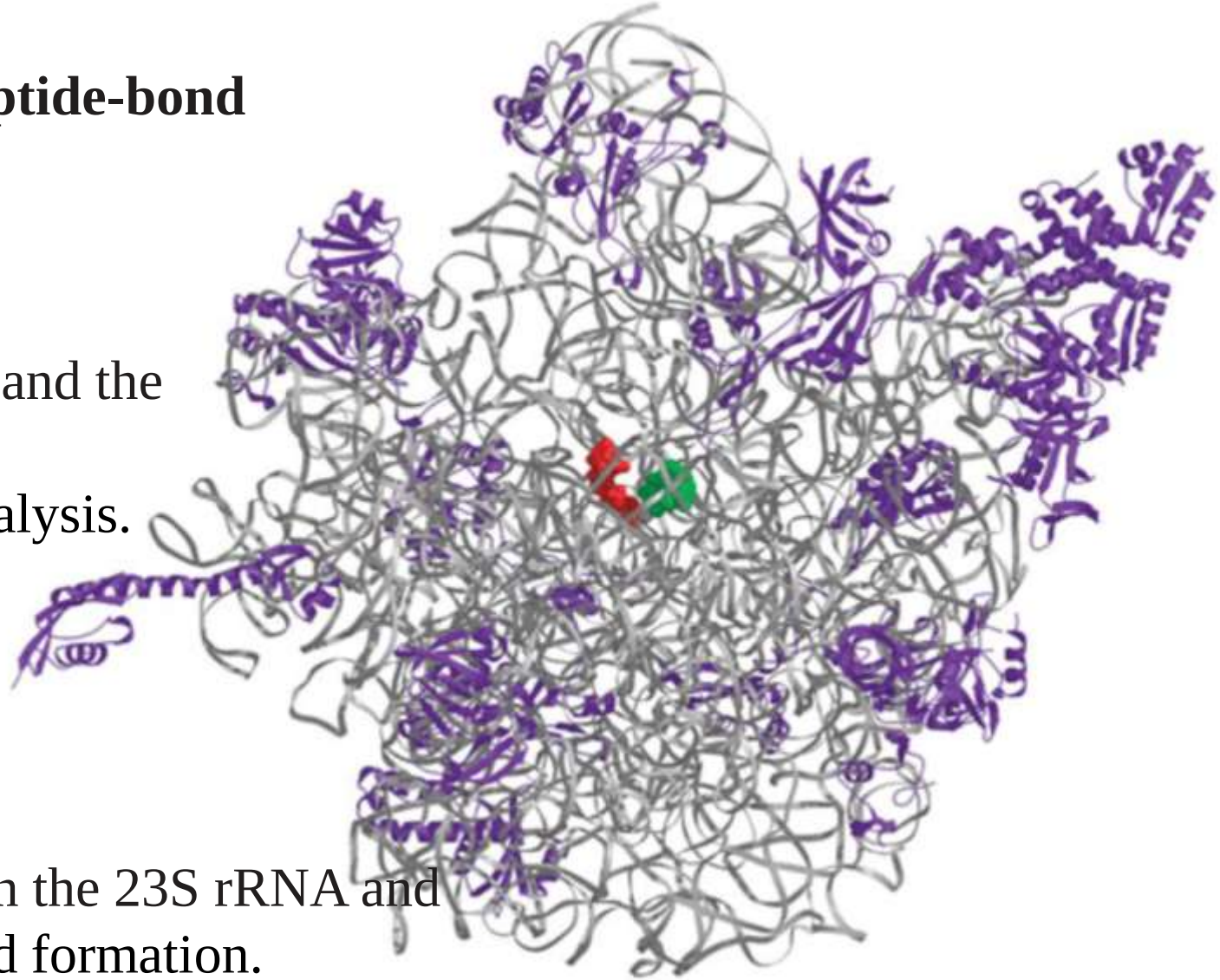
Evidence

- A large subunit that had been largely stripped of its proteins was still able to direct peptide bond formation.
- No amino acid within 18 A˚ of the active site

How does the 23S rRNA catalyze peptide-bond formation?

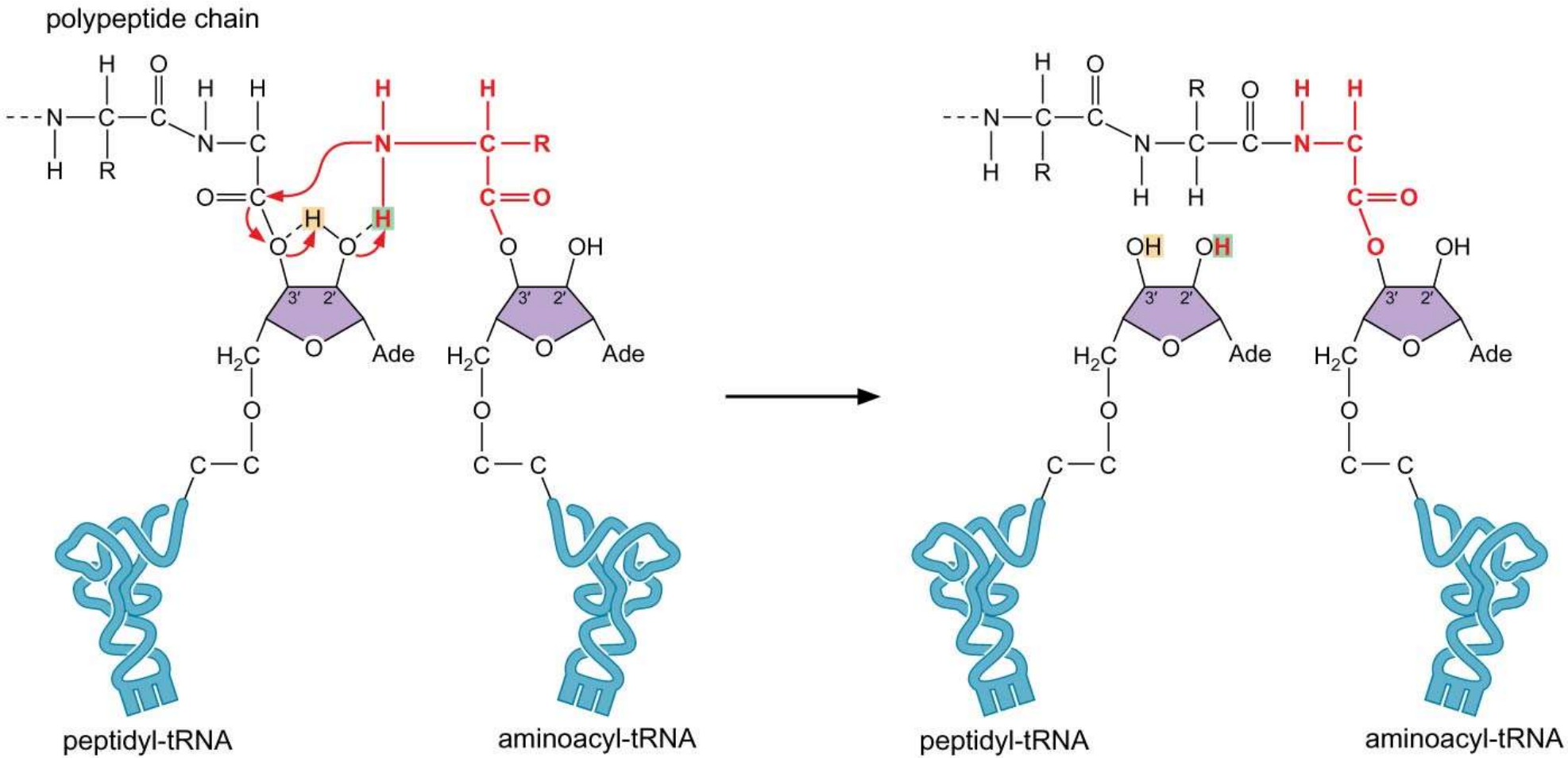
Base pairing between the 23S rRNA and the CCA ends of the tRNAs bringing the substrates together and stimulates catalysis.

2'-OH of a highly conserved residue in the 23S rRNA and the P-site tRNA promote peptide-bond formation.



The 3D structure of the bacterial 50S subunit:
RNAs (gray),
the ribosomal proteins (purple),
3' end of the A-site tRNA green,
3' end of the P-site tRNAs red.

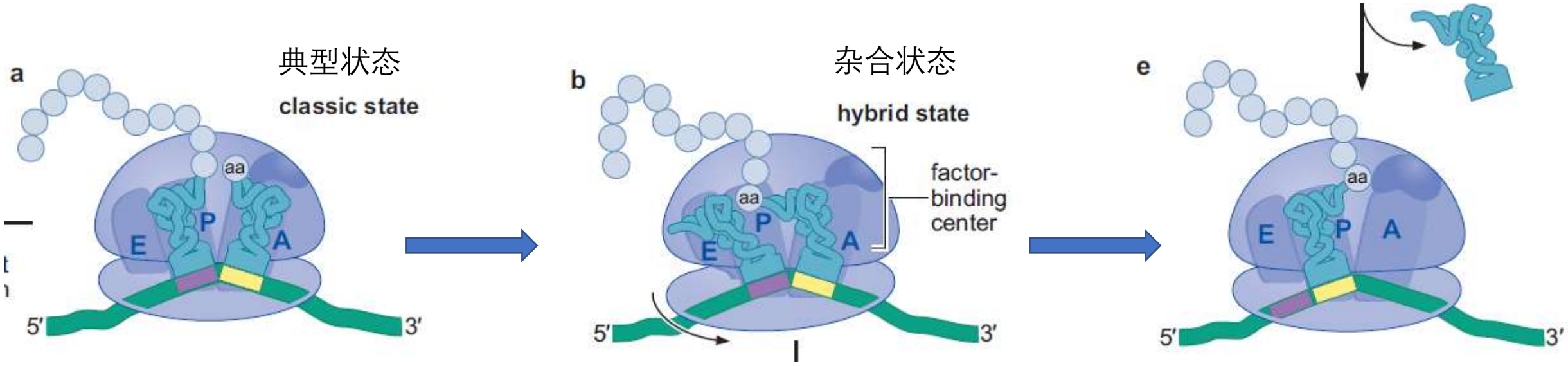
Proposed role for the 2'- OH of the P-stie tRNA in peptide –bond formation



Peptide-Bond Formation Initiates Translocation in the Large Subunit

Translocation 易位

the P-site tRNA must move to the E-site,
the A-site tRNA must move to the P-site,
the mRNA must move by three nucleotides.



The initial steps of translocation are coupled to the peptidyl transferase reaction.

The completion of translocation requires the action of a second elongation factor called EF-G.

EF-G Drives Translocation by Stabilizing Intermediates in Translocation

EF-G – GTP binds to and stabilizes the ribosome in the rotated, hybrid state.

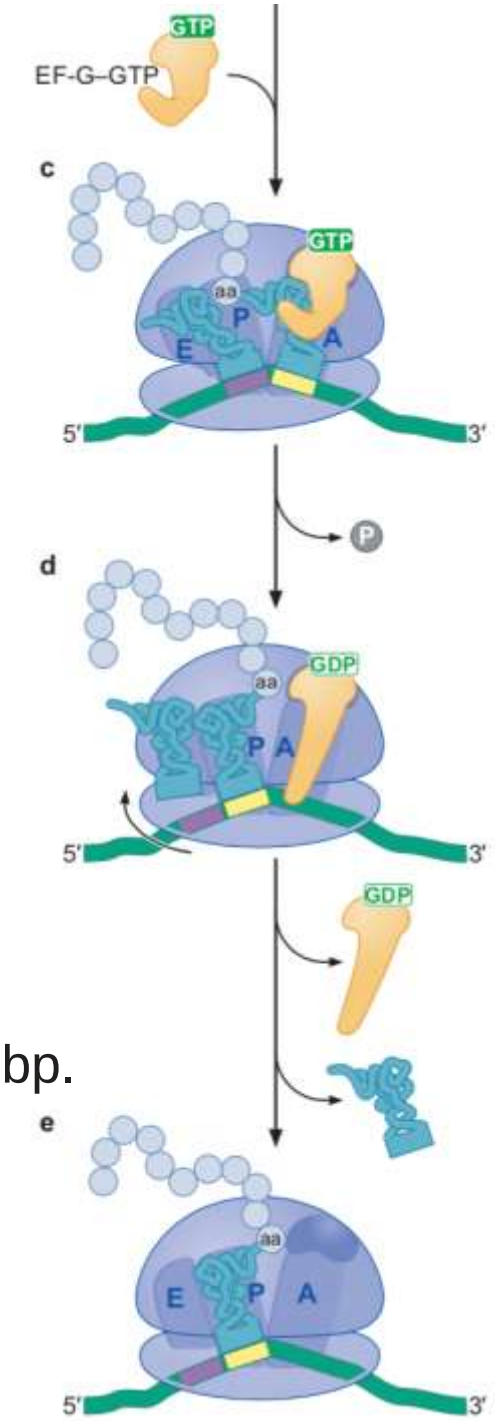
EF-G–GTP contacts the factor-binding center of the large subunit and hydrolyzes GTP.

Two consequences:

- Interactions between EF-G–GDP and the ribosome “unlock” the ribosome.
- The changed EF-G – GDP conformation binds to the A-site of the decoding center.

Base pairing between the tRNAs and the mRNA causes the mRNA to move by 3 bp.

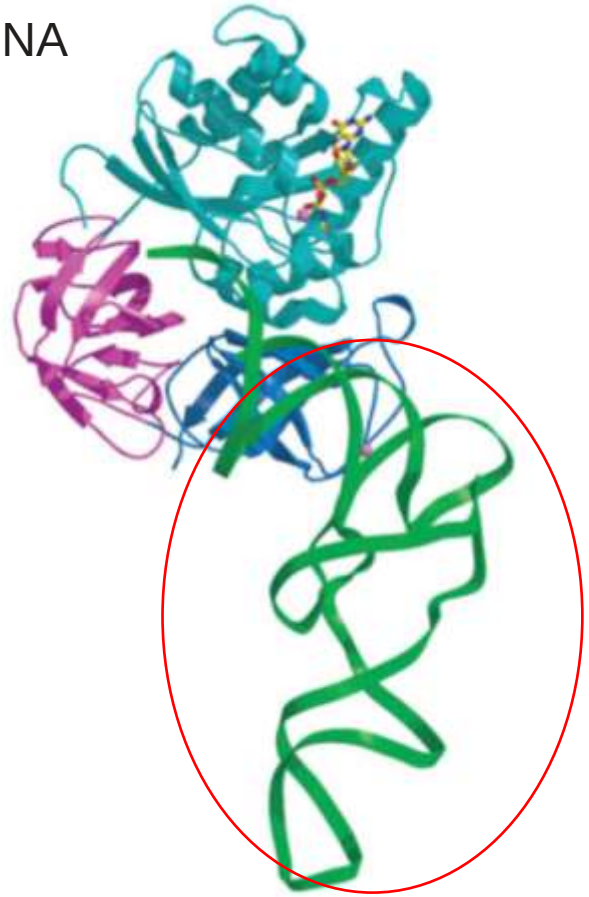
Release of EF-G after subunit rotation, and results in the return of the ribosome to a “locked” state.



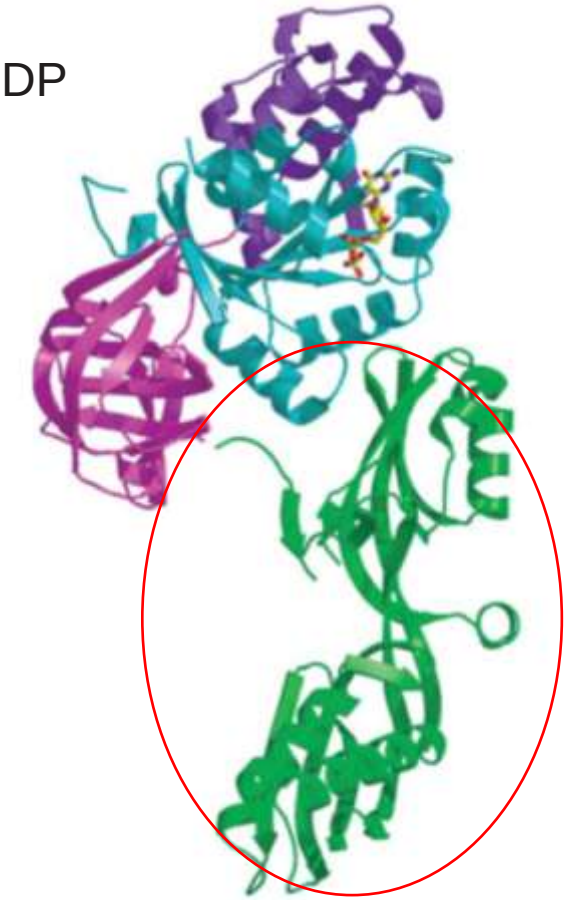
How does EF-G–GDP interact with the A-site of the decoding center so effectively?

EF-G–GDP and EF-Tu–GTP–tRNA have a very similar structure.

EF-Tu – GDPNP – Phe–tRNA



EF-G – GDP

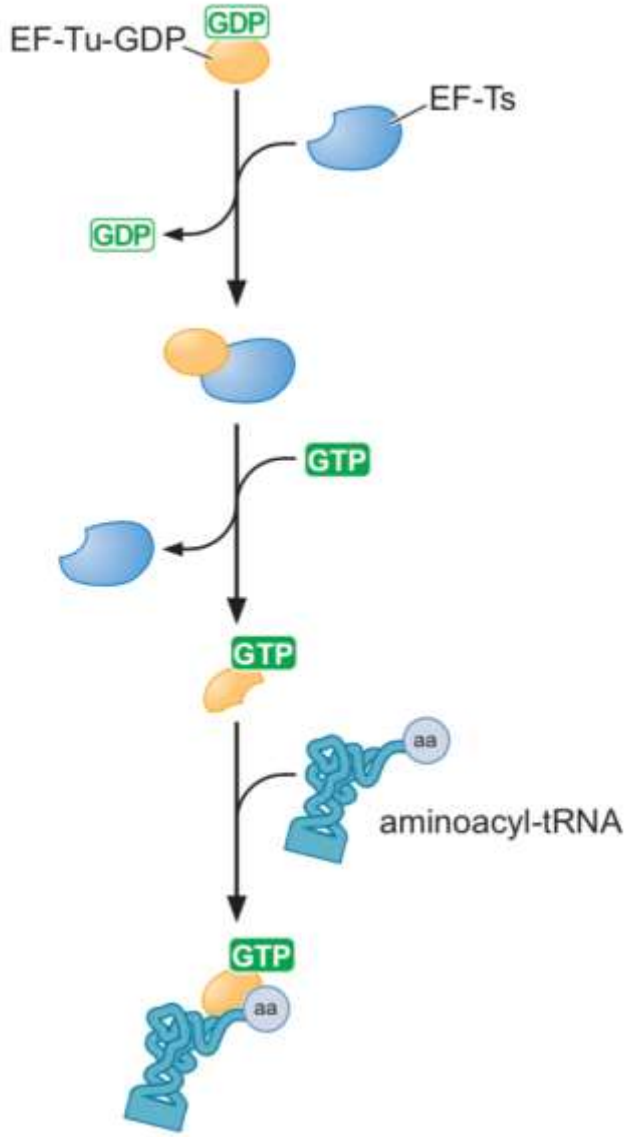


Even though EF-G is composed of a single polypeptide, its structure mimics that of a tRNA bound to a protein.

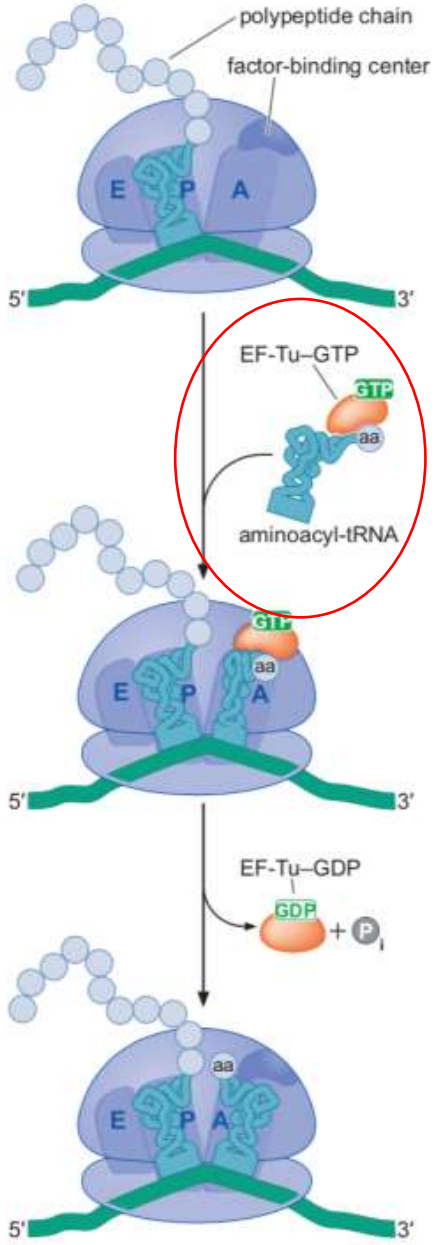
EF-Tu–GDP and EF-G–GDP Must Exchange GDP for GTP before Participating in a New Round of Elongation

GDP has a lower affinity for EF-G than does GTP.

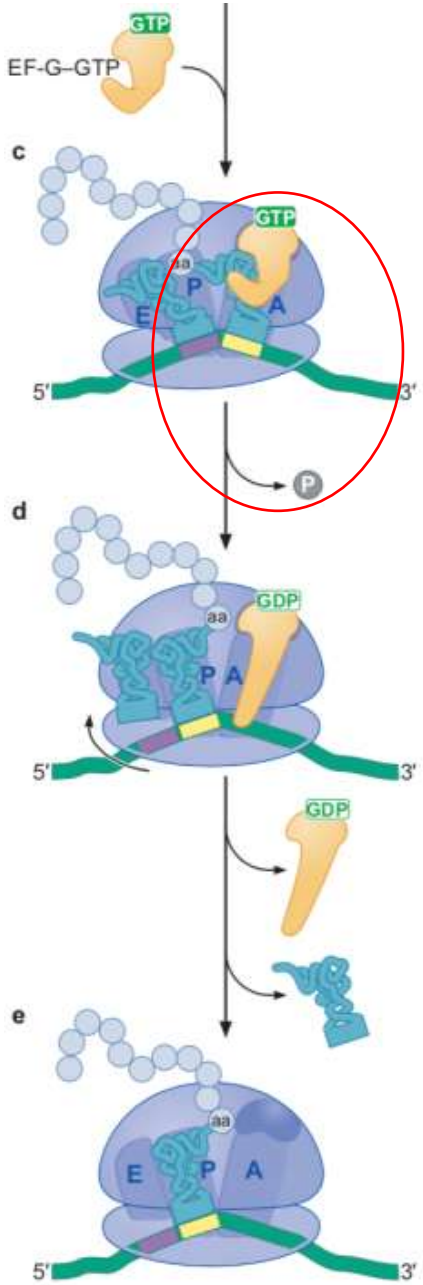
The elongation factor EF-Ts acts as a GTP exchange factor for EF-Tu.



Aminoacyl-tRNAs delivery

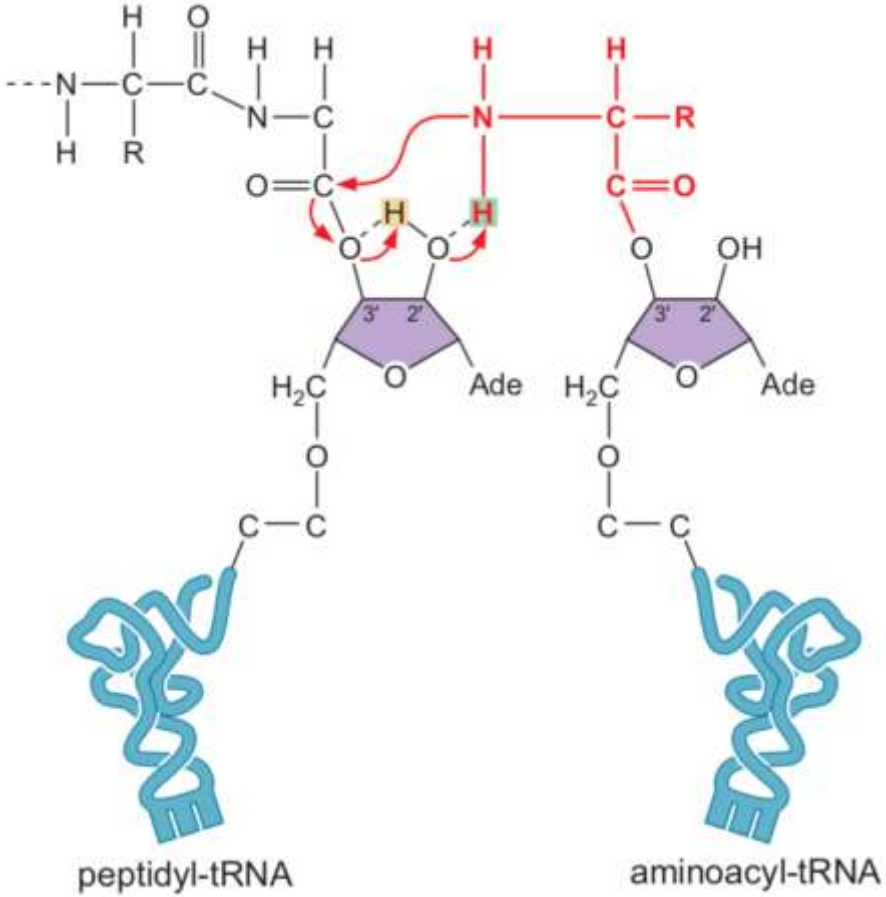


Translocation



A Cycle of Peptide-Bond Formation Consumes Two Molecules of GTP and One Molecule of ATP

Peptide formation



Elongation factors required for translation elongation in eukaryote

prokaryote	eukaryote
EF-Tu	eEF1
EF-G	eEF2

2.3 Translation termination

Where and Who?

- The ribosome's cycle of aminoacyl-tRNA binding, peptide-bond formation, and translocation continues until one of the three stop codons enters the A-site.
- Stop codons are recognized by proteins called release factors (RFs) that activate the hydrolysis of the polypeptide from the peptidyl-tRNA.

release factors (RFs) – 释放因子

Release Factors Terminate Translation in Response to Stop Codons

Stop codons are recognized by release factors (RFs)

Class I release factors

Recognize the stop codons and trigger hydrolysis of the peptide chain from the tRNA

Prokaryote
- RF1: UAG UAA
- RF2: UGA UAA

Eukaryote eRF1: UAG UGA UAA

Class II release factors

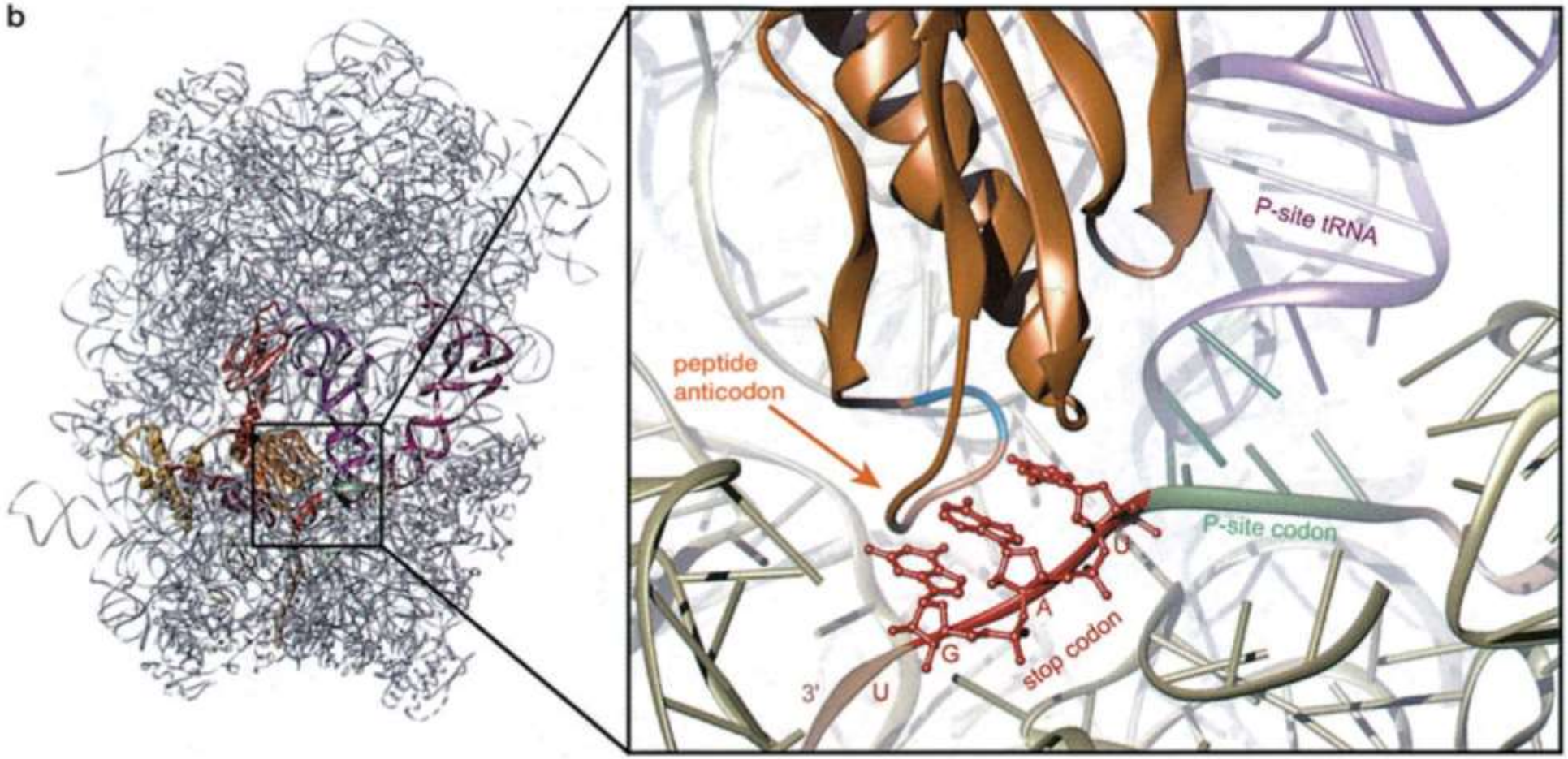
Stimulate the dissociation of the class I factors from the ribosome

Prokaryote	Eukaryote:
RF3	eRF3

The peptide anticodon of Class I Release Factors Recognize Stop Codons

How do release factors recognize stop codons?

Peptide anticodon – 肽反密码子:
a three-amino-acid sequence interact with and recognize stop codons



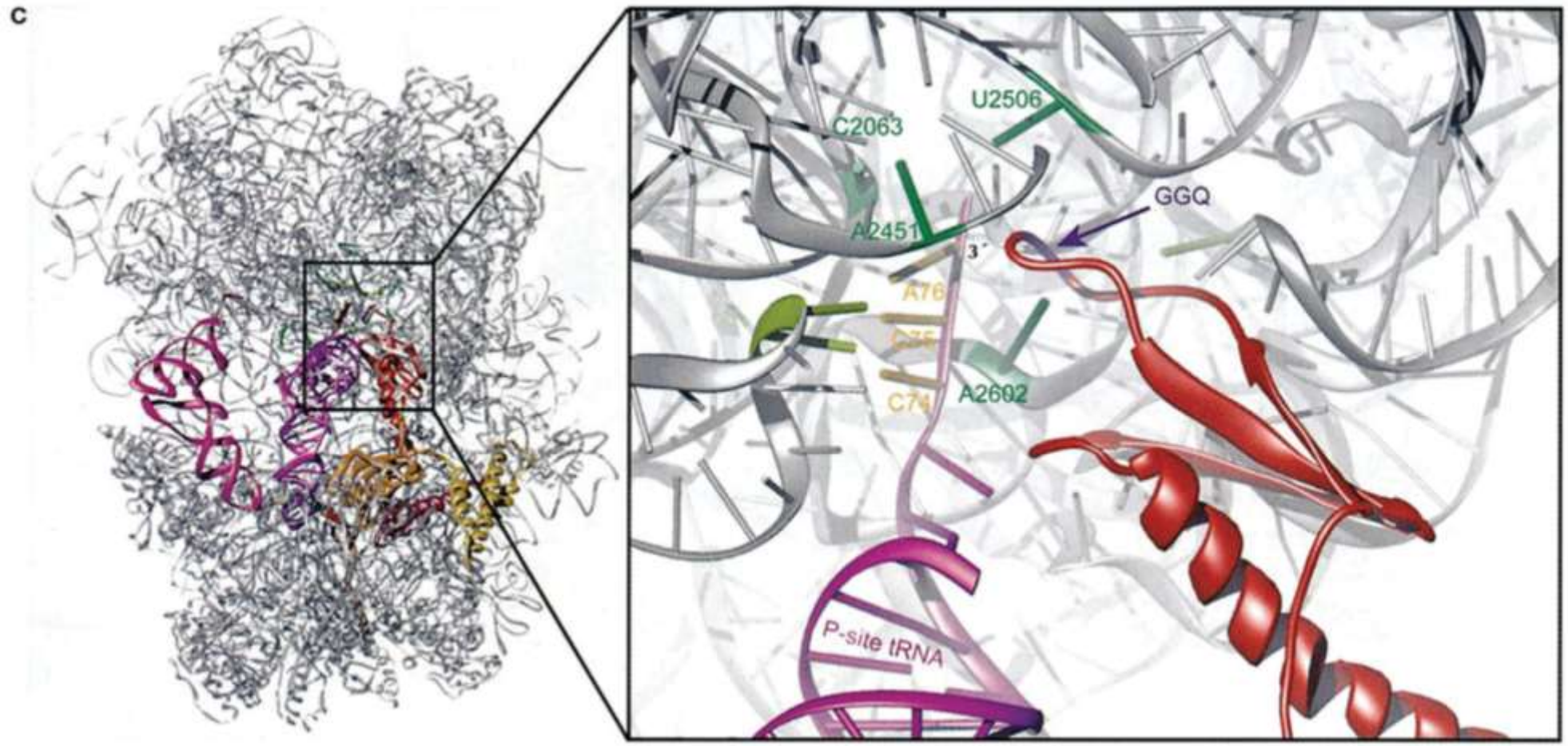
Prokaryote

- RF1: UAG UAA
- RF2: UGA UAA

GGQ motif within Class I Release Factors triggers polypeptide release

A conserved three-amino-acid sequence (GGQ) is essential for polypeptide release

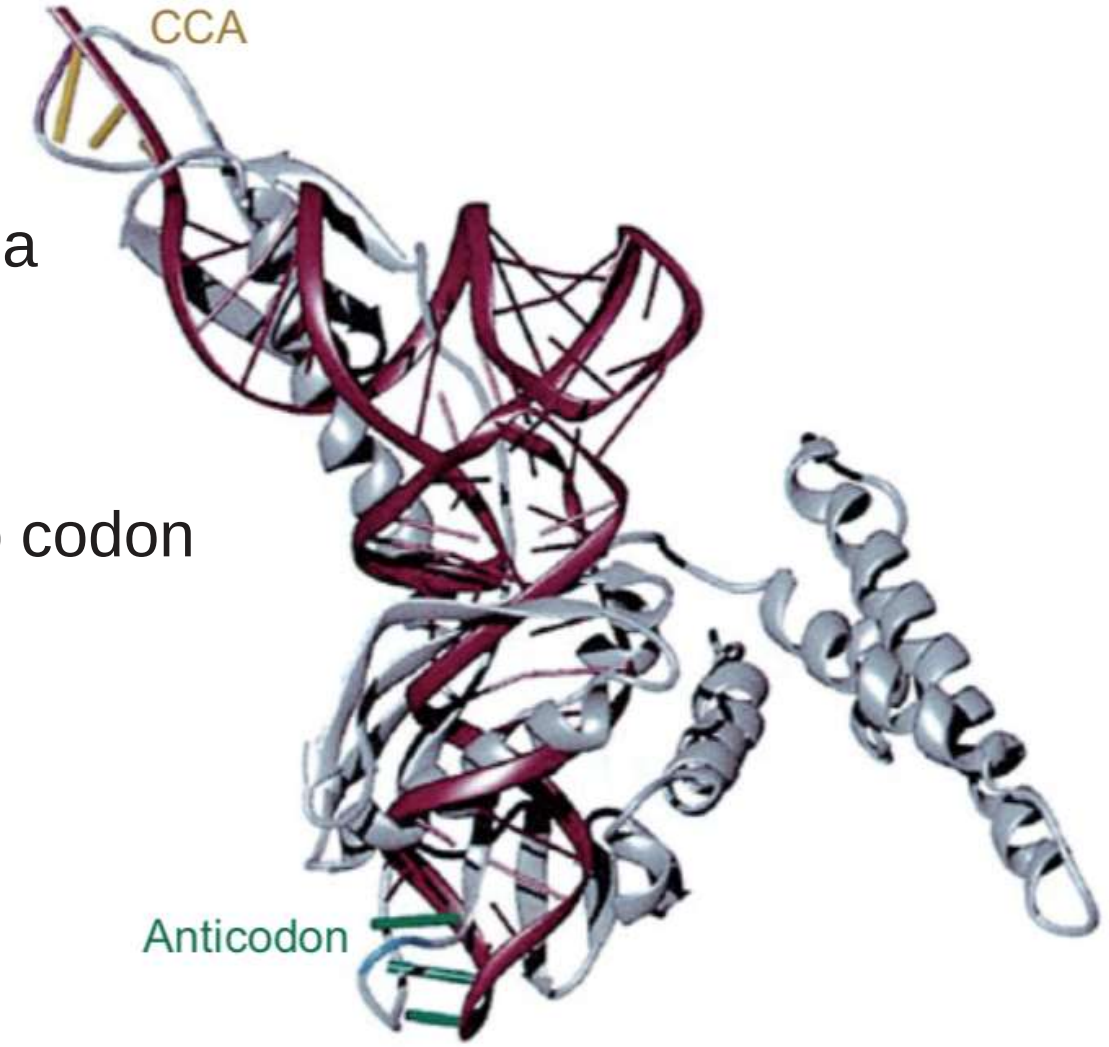
GGQ motif is located in close proximity to the peptidyl transferase center



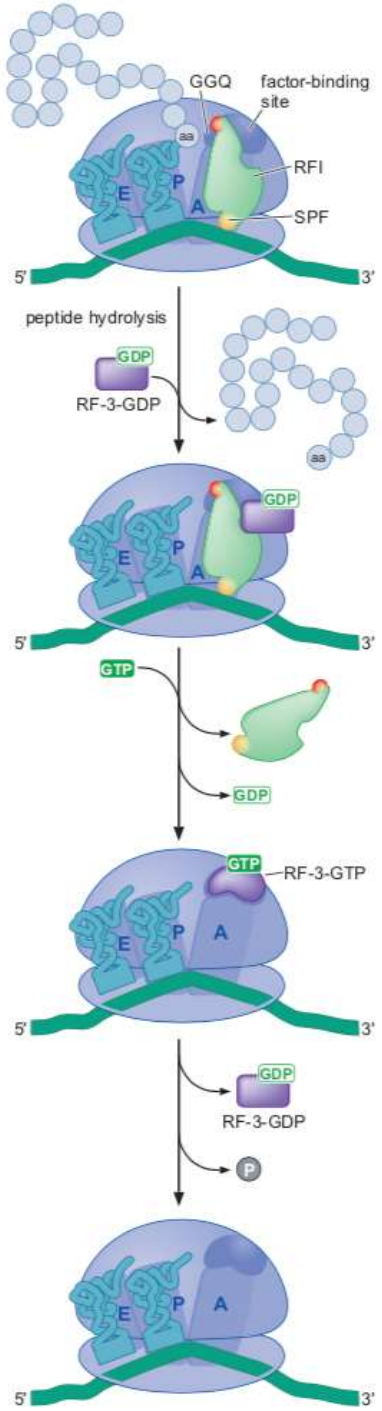
Class I release factors functionally mimic a tRNA

The peptide anticodon recognize the stop codon

The GGQ motif reaches into the peptidyl transferase center and promotes peptide bond hydrolysis



tRNA - dark red
RF1 - gray



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

RF3 has a higher affinity for GDP than GTP

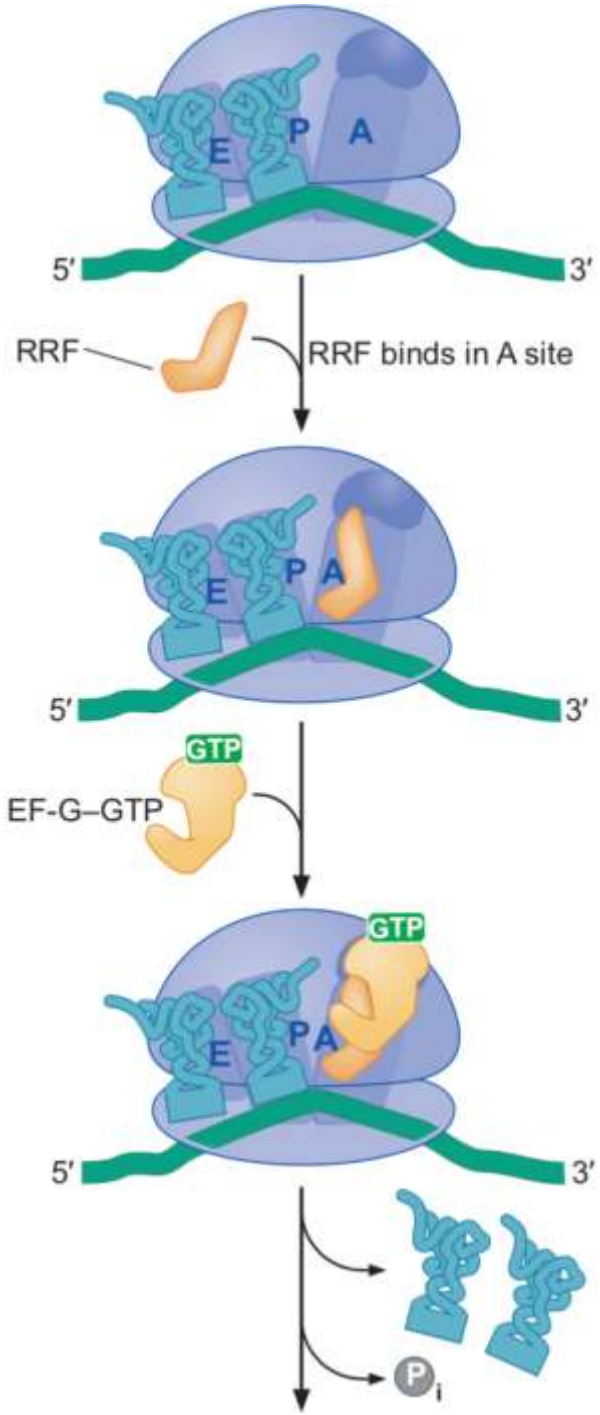
RF3-GDP binds to the ribosome in a manner that depends on the presence of RF1.

a change in the conformation of the ribosome and RF1 stimulates RF3 to exchange its bound GDP for a GTP.

The binding of GTP to RF3 leads to conformation change of the ribosome and displaces the class I factor from the ribosome.

RF3 associates with the factor-binding center of the large subunit and hydrolyzes of GTP.

In the absence of a bound class I factor, the resulting RF3.GDP has a low affinity for the ribosome and is released.



RRF and EF-G combine to stimulate the release of tRNA and mRNA from a terminated ribosome

ribosome recycling – 核糖体循环:

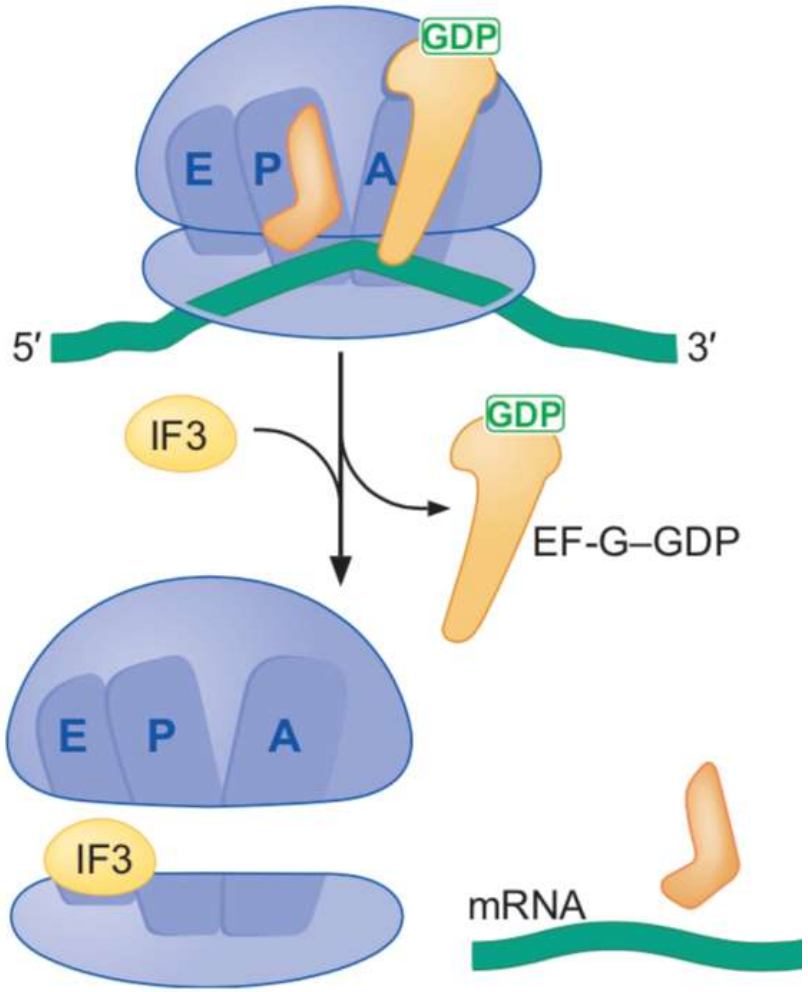
ribosome recycling factor (RRF) cooperates with EF-G and IF3 to recycle ribosomes after polypeptide release

RRF binds to the empty A-site of the ribosome

RRF also recruits EF-G – GTP to the ribosome

EF-G stimulates the release of the uncharged tRNAs bound in the P- and E-sites.

How exactly EF-G stimulates the release is not known now.



Once the tRNAs are removed, EF-G–GDP and RRF are released from the ribosome along with the mRNA.

IF3 is required to separate the two ribosomal subunits from each other.

The final outcome is a small subunit bound to IF3 and a free large subunit.

15.3 REGULATION OF TRANSLATION

Although the expression of many genes is regulated at the level of mRNA transcription, many genes are also regulated at the level of protein synthesis.

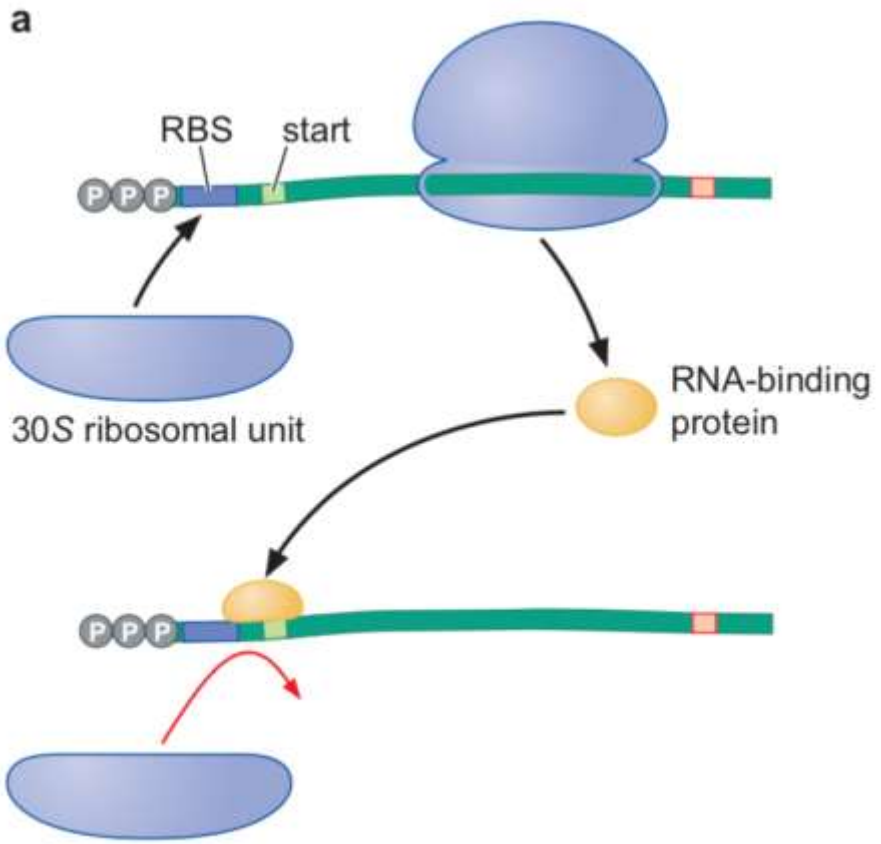
One advantage of control of translation over transcription is the ability to respond very rapidly to external stimuli.

Regulation at the level of protein synthesis eliminates the time required to alter the levels of mRNA transcription

As with other types of regulation, translational control typically functions at the level of initiation.

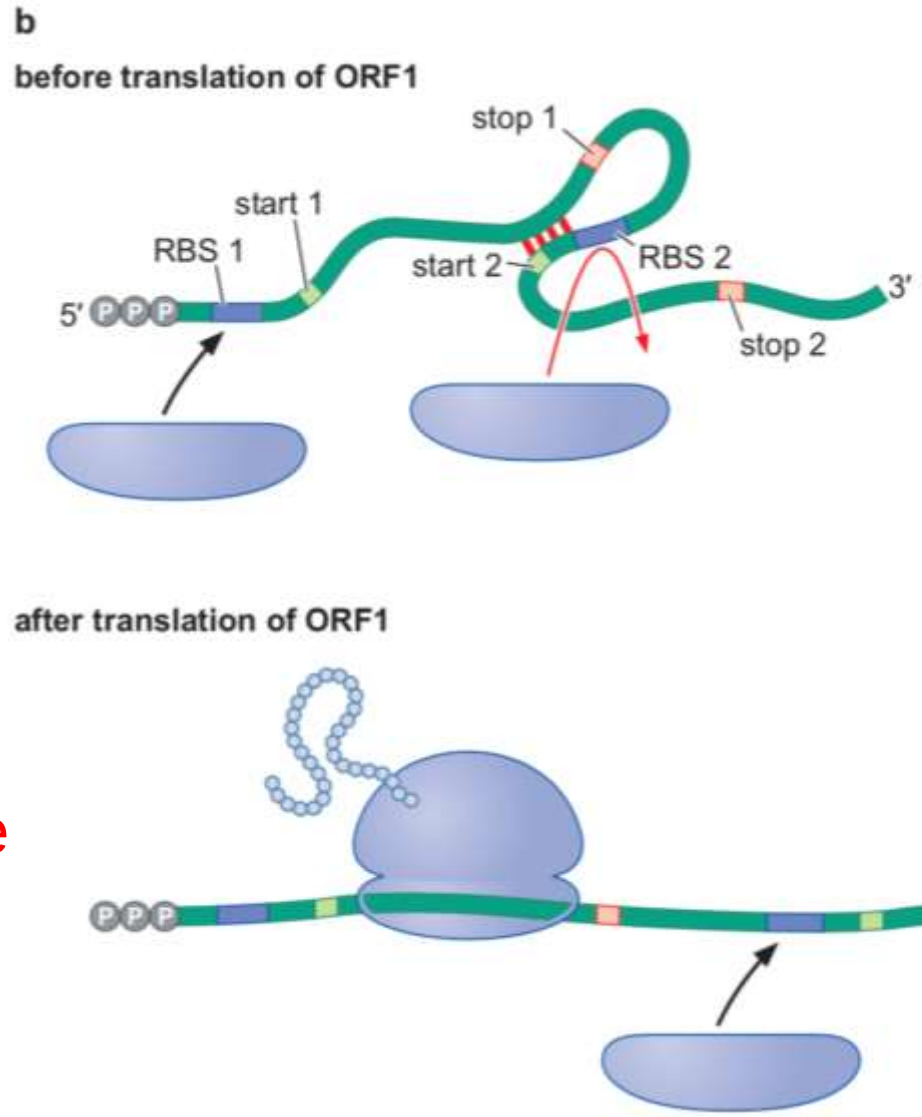
3.1 Translational regulation in prokaryote

- RNA-binding proteins that recognize RNA structures that form adjacent to the RBS.



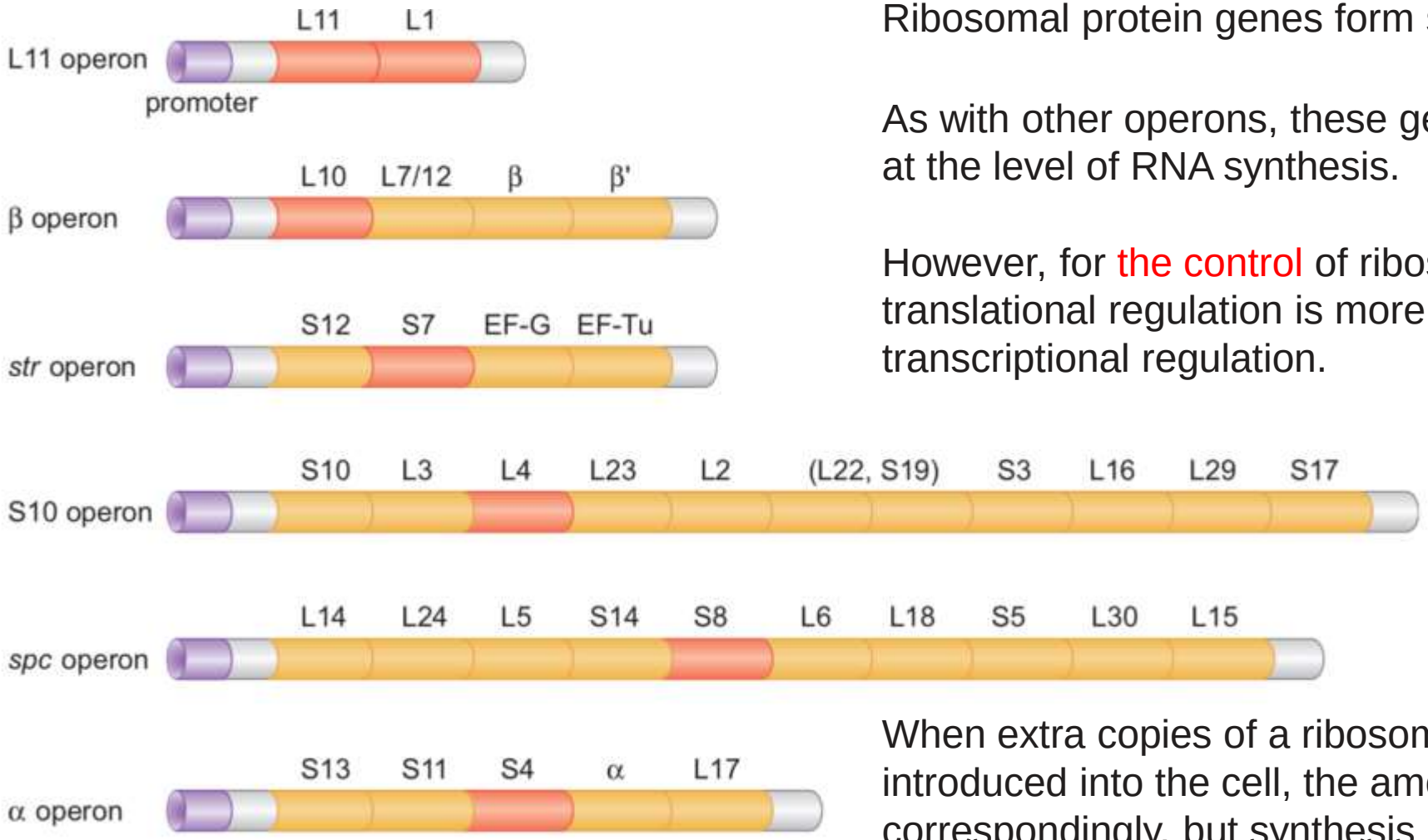
3.1 Translational regulation in prokaryote

- RNA molecules can also act as inhibitors, when an mRNA base-pairs with itself to mask one or more RBSs



The primary target is to interfere with the recognition of the RBS by the 30S subunit.

How the cell controls correct expression of ribosomal protein genes?



Ribosomal protein genes form several operons.

As with other operons, these gene clusters are regulated at the level of RNA synthesis.

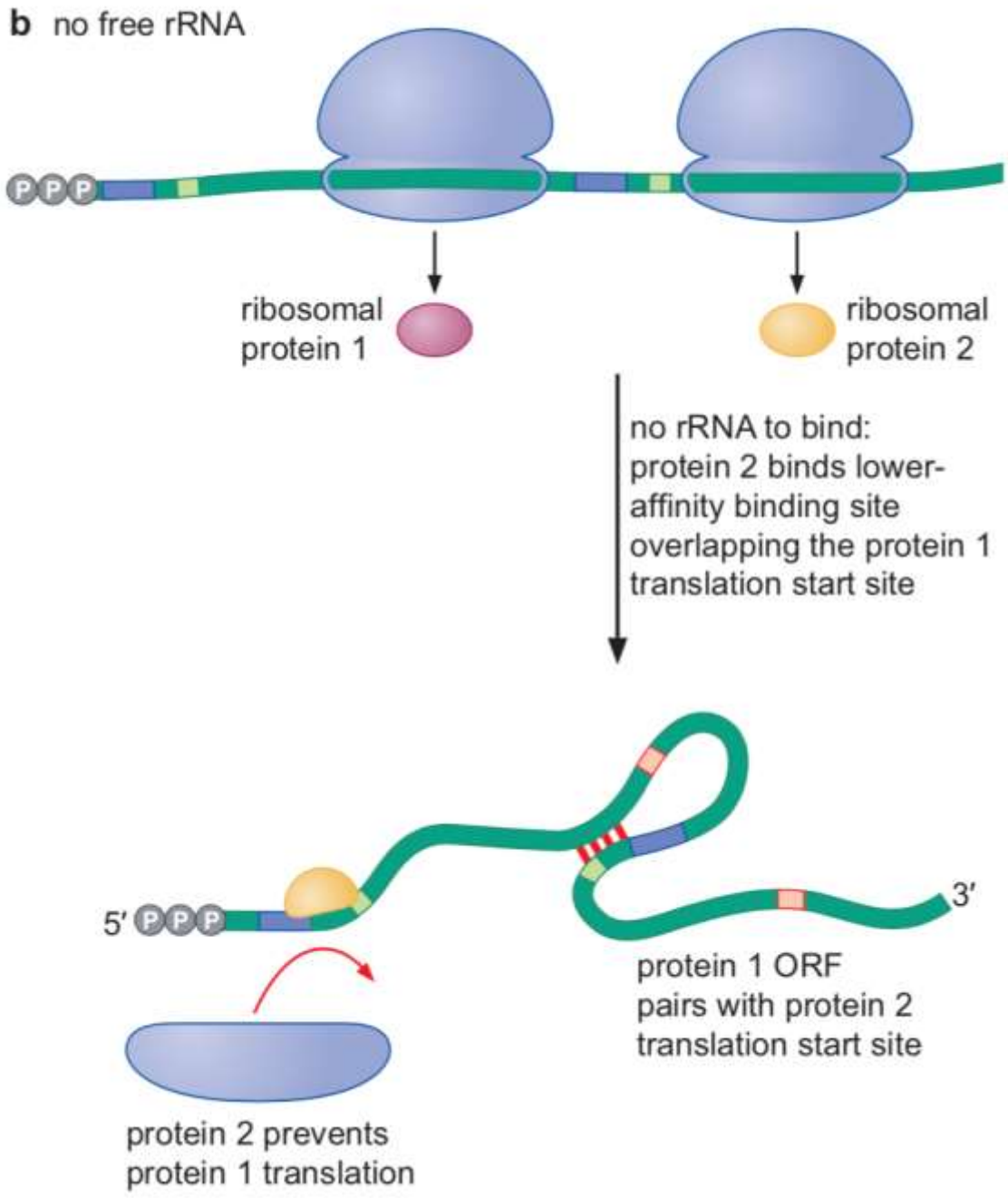
However, for the control of ribosomal protein synthesis, translational regulation is more important than transcriptional regulation.

When extra copies of a ribosomal protein operon are introduced into the cell, the amount of mRNA increases correspondingly, but synthesis of ribosomal proteins stays nearly the same.

How to regulate the translation of ribosomal proteins?

Initial genes: autorepression – 自我抑制

Binding of the ribosomal protein sterically inhibits association of the ribosomal small subunit with the nearby RBS, thereby inhibiting translation initiation.



How is expression of the ribosomal proteins coupled to the amount of rRNA in the cell?

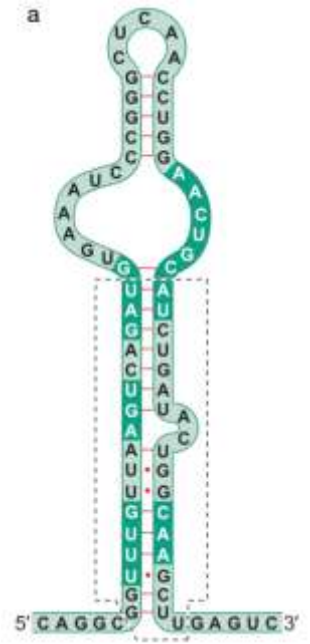
The regulatory ribosomal protein that binds the mRNA also recognizes a very strong binding site on the appropriate rRNA

Only when the ribosomal protein is present in excess to its target rRNA will it bind its own mRNA.

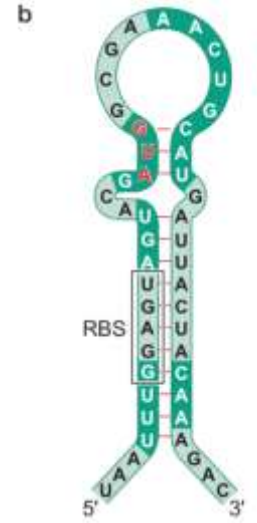
This simple competitive binding event ensures that ribosomal protein synthesis is inhibited only when the regulatory ribosomal protein is in excess.

Ribosomal protein S8

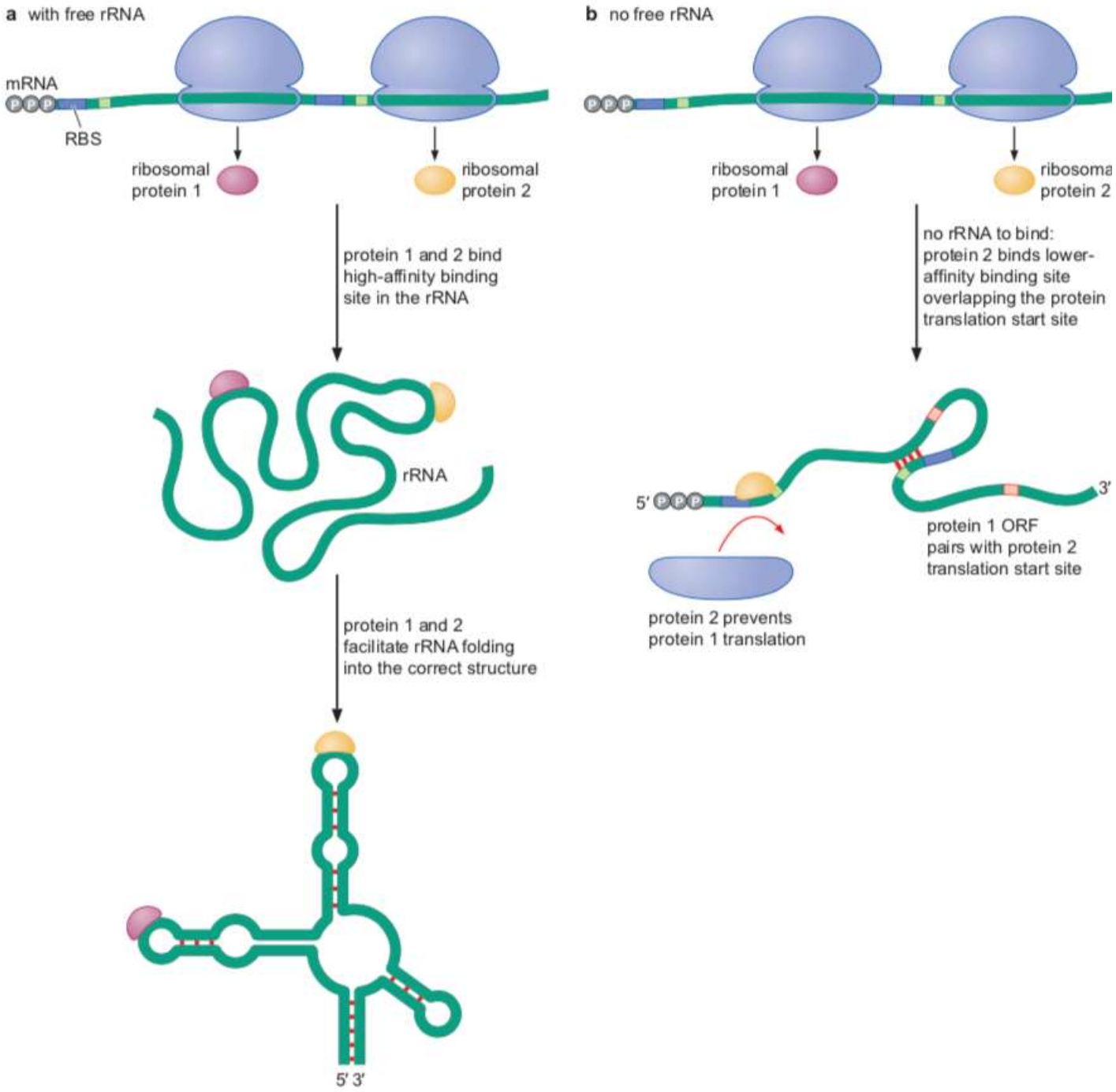
16S rRNA



mRNA



Regulation of ribosomal protein expression



3.2 Translational regulation in eukaryote

Global Regulators of Eukaryotic Translation Target Key Factors Required for mRNA Recognition and Initiator tRNA Ribosome Binding

Under conditions of reduced nutrients or other cellular stresses, it is often useful for eukaryotic cells to reduce translation globally.

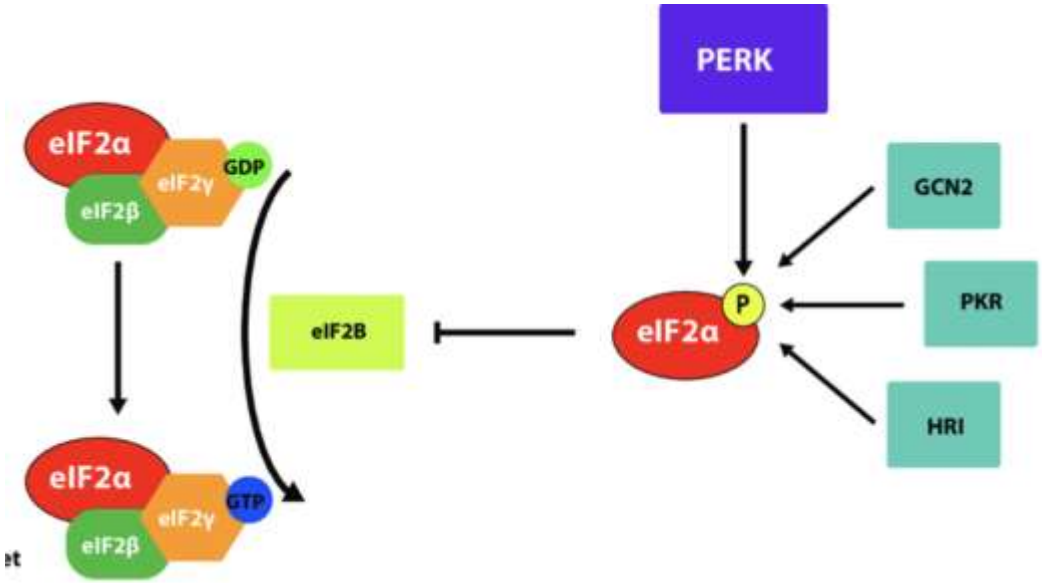
Two early steps in eukaryotic translation initiation are targeted for inhibition:

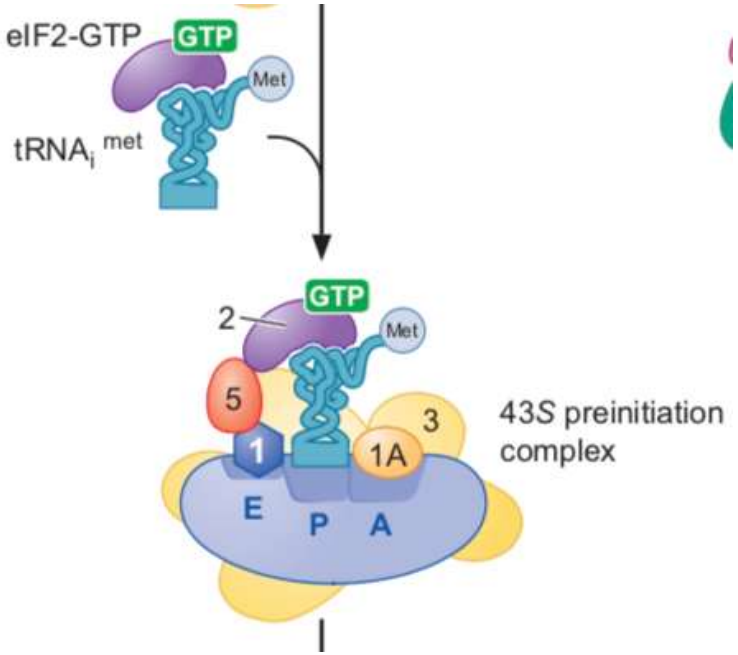
- recognition of the mRNA
- initiator tRNA binding to the 40S subunit.

These events occur independently of one another, but inhibition of either eliminates new protein synthesis.

In each case, the mechanism of inhibition is controlled by phosphorylation.

Translation initiation is globally regulated by the phosphorylation of eIF2a





eIF2B, a a GTP-exchange factor, stimulates eIF2 – GDP to release its bound GDP and bind GTP

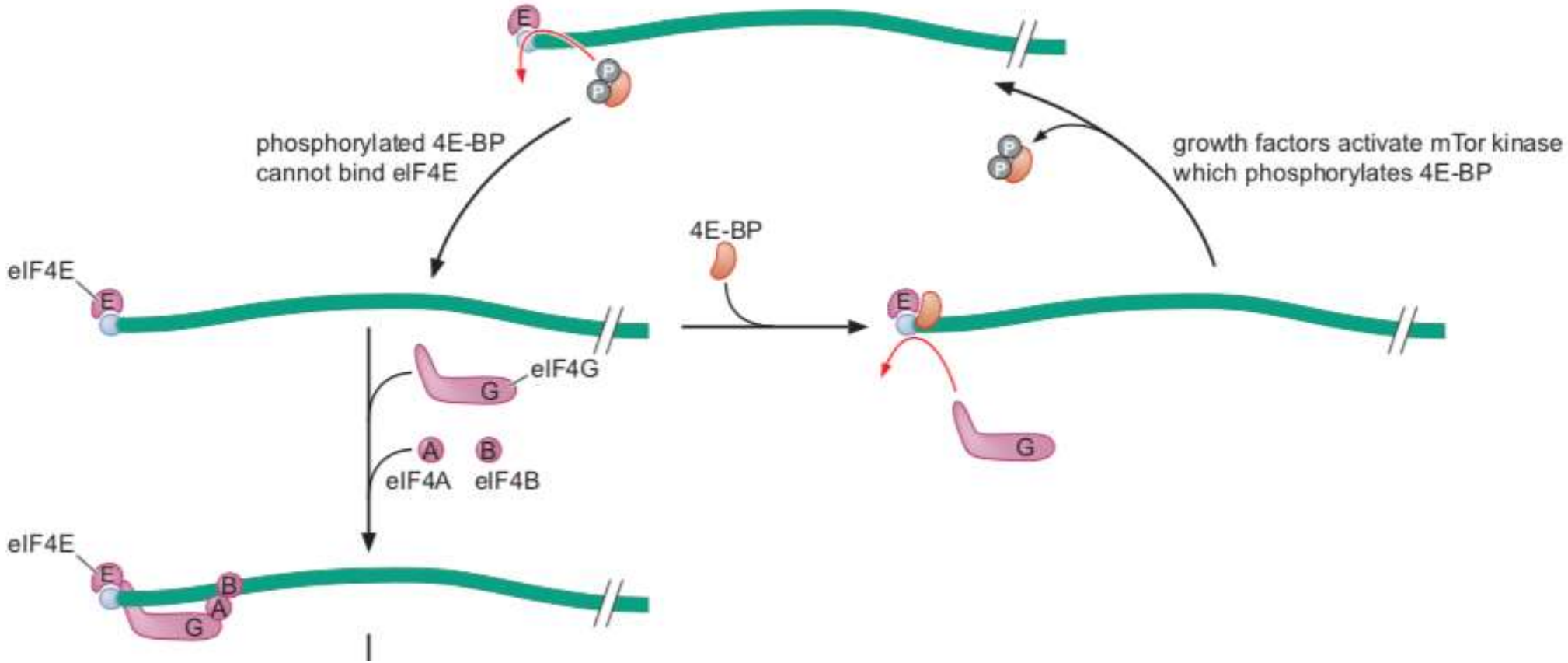
Phosphorylation of eIF2a inhibits the action of eIF2B, and leads to reduced levels of eIF2 – GTP.

Because eIF2 bound to GTP is required to escort the initiator tRNA to the 40S subunit, reduced levels of eIF2–GTP limit initiation of translation.

The known eIF2a kinases are activated by several different cellular conditions including amino acid starvation, viral infection, and elevated temperature.

Initiation of eukaryotic translation is globally regulated by eIF4E- binding proteins (4E-BPs)

4E-BPs compete with eIF4G for binding to eIF4E and therefore act as general inhibitors of translation initiation .



Phosphorylation of 4E-BPs by mTor inhibits their binding to eIF4E

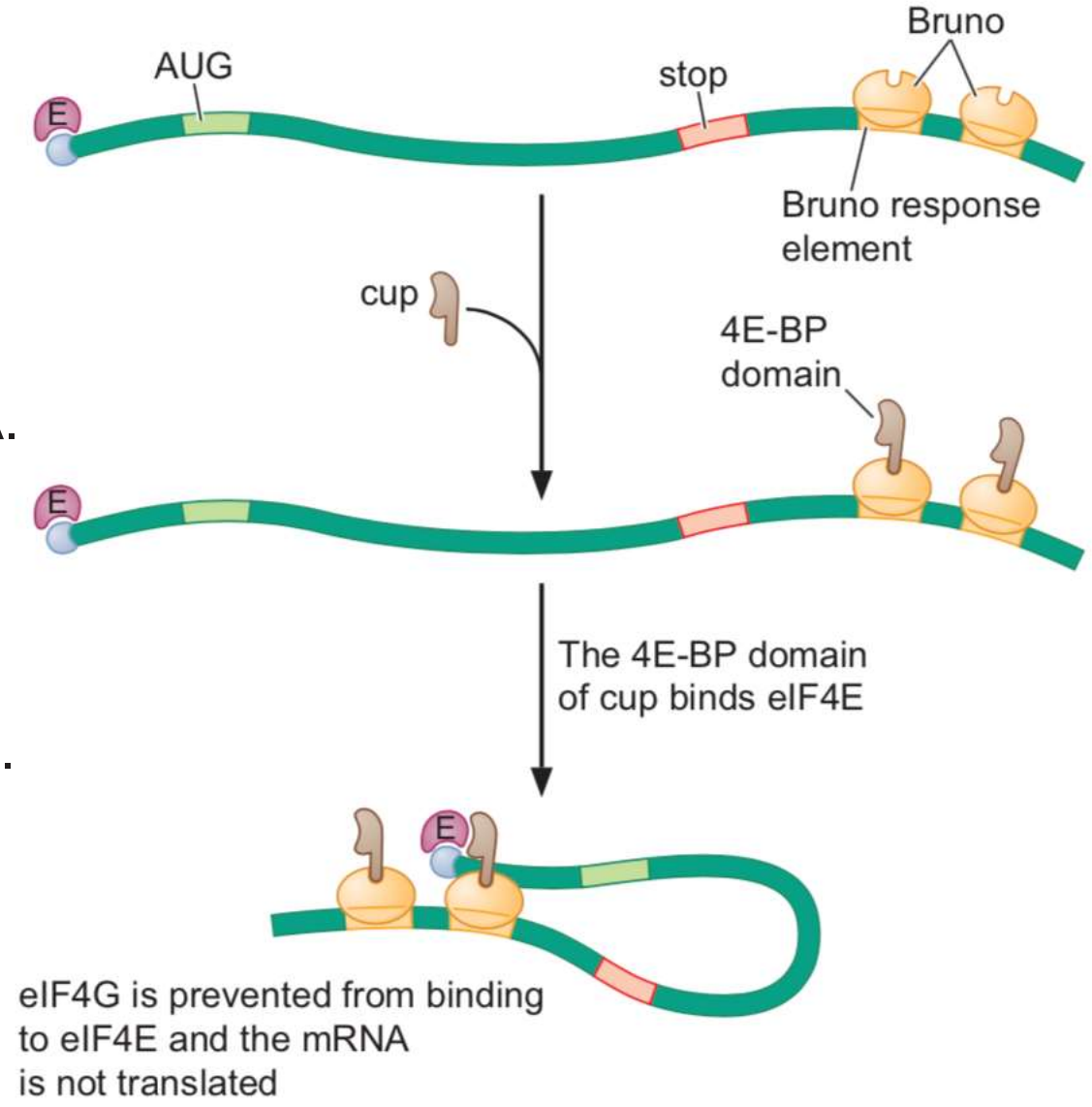
Spatial Control of Translation by mRNA-Specific 4E-BPs

Oskar mRNA is transported from the anterior to the posterior region of the oocyte and its translation is repressed during the transportation

Bruno binds specifically to the the 3’-UTR Oskar mRNA.

Bruno recruits Cup to Oskar mRNA to inhibit translation.

Cup is not abundant enough to act generally on all translation as do the global 4E-BPs.



Key Points of Chapter 15

- The major challenge of translaton compared with transcription.
- The components of translational machinery.
- Three steps of translation in prok and euk.
- Translational regulation in prok and euk.