

# **The Translation 3**

## **Ribosomes**

### **Translation initiation, elongation, termination**

#### **(Chapter 15)**

LMR05.001 – 18

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Inst Mol & Cell Biol

# What acts as a template for protein synthesis?

- Initially, **ribosomal RNA was thought to be the template for proteins**
  - 85% of cellular RNA is in ribosomes
  - tRNA 15% of cellular DNA
  - mRNA – all amount it's in nucleus

It is the template for getting information from DNA

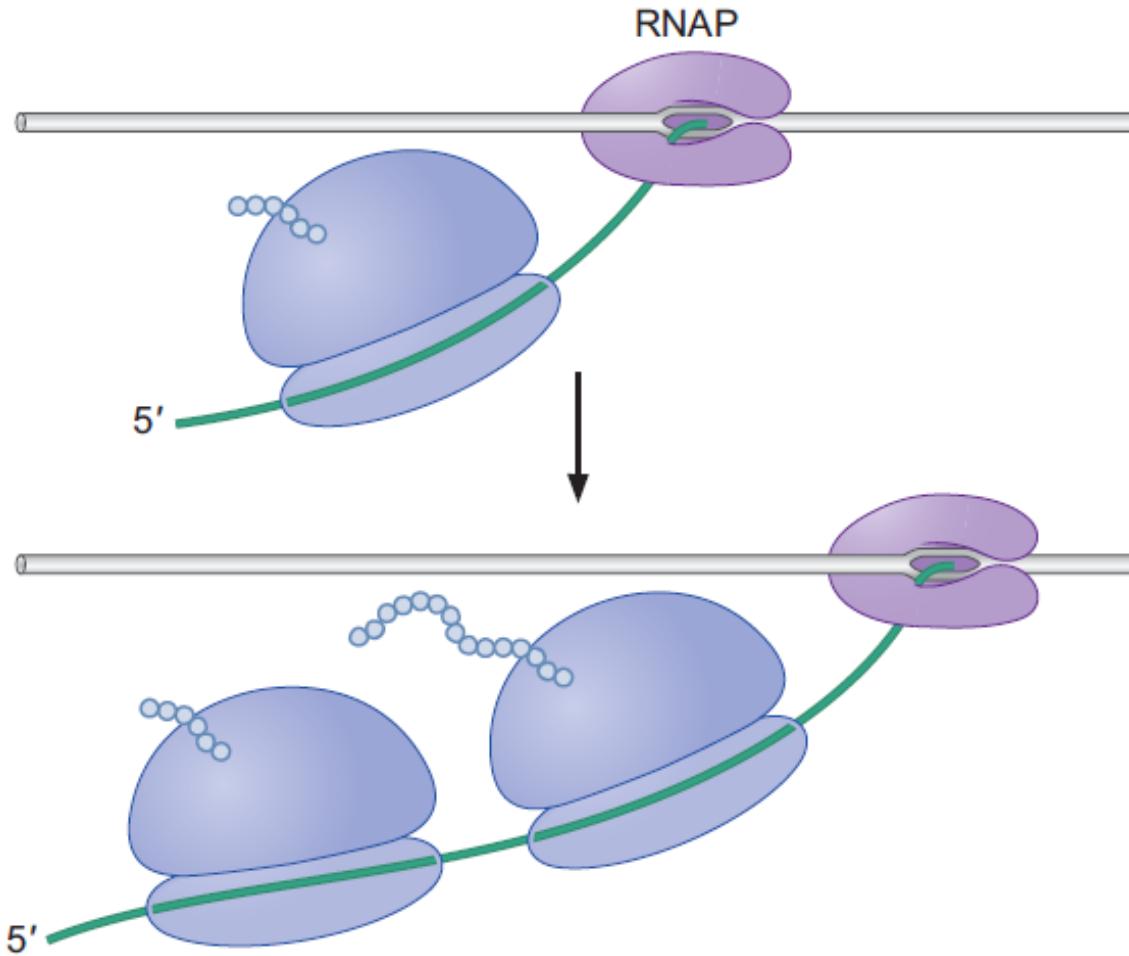


**FIGURE 2-13** Electron micrograph of ribosomes attached to the endoplasmic reticulum. This electron micrograph (105,000x) shows a portion of a pancreatic cell. The upper right portion shows a portion of the mitochondrion and the lower left shows a large number of ribosomes (small circles of electron density) attached to the membranous endoplasmic reticulum. Some ribosomes exist free in the cytoplasm; others are attached to the membranous endoplasmic reticulum. (Courtesy of K.R. Porter.)

# Translation machinery

- mRNAs
  - tRNAs
  - Aminoacyl-tRNA synthetases
  - Ribosome
- 
- 4 nucleotides - > 20 amino acids

# Ribosomes don't act alone in bacteria



**FIGURE 15-11** Prokaryotic RNA polymerase and ribosomes at work on the same mRNA.

# Translation machinery speed

- Prokaryotic ribosome – 20 amino acids/sec
  - RNA polymerase – 50-100 nucleotides/sec
- Eukaryotic ribosome – 2-4 amino acids/sec

# What is the ribosome?

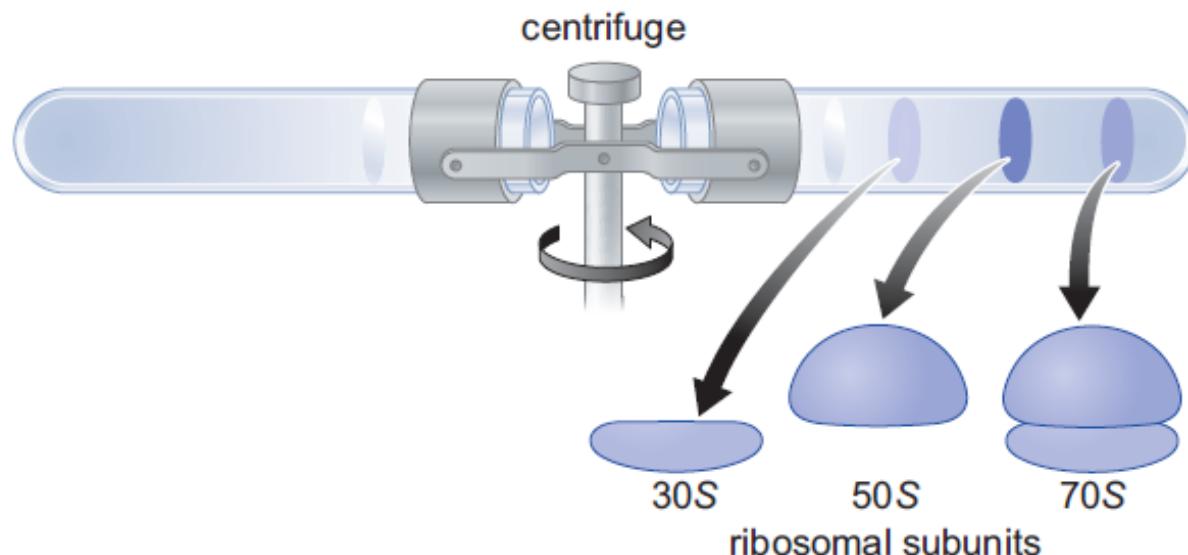
- Two subunits
  - Large (50S/60S) – peptidyl transferase center
  - Small (30S/40S) – decoding center

The sedimentation coefficient (*s*) of a particle characterizes its sedimentation during centrifugation. It is defined as the ratio of a particle's **sedimentation velocity to the applied acceleration** causing the sedimentation.

$$s = v_t / a$$

*V<sub>t</sub>* = sedimentation speed in m/s

*a* – applied acceleration - is the rate of change of velocity – in m/s<sup>2</sup>



**FIGURE 15-12** Sedimentation by ultracentrifugation separates the bacterial ribosome subunits from the full ribosome.

The **sedimentation coefficient** has units of time, expressed in **svedbergs**. One svedberg is exactly  $10^{-13}$  s.

Ribosomes are typically identified by their sedimentation coefficient. For instance, the **70 S ribosome** from bacteria has a sedimentation coefficient of **70 svedberg**, although it **is composed of a 50 S subunit and a 30 S subunit**

**A Svedberg unit or svedberg (symbol S, sometimes Sv) is a non-SI metric unit for sedimentation coefficients.**

**The Svedberg unit offers a measure of a particle's size indirectly based on its sedimentation rate under acceleration (i.e. how fast a particle of given size and shape settles to the bottom of a solution).**

**The svedberg is a measure of time, defined as exactly  $10^{-13}$  seconds (100 fs)**

Heavier particles sediment faster and have higher svedberg, or s values.

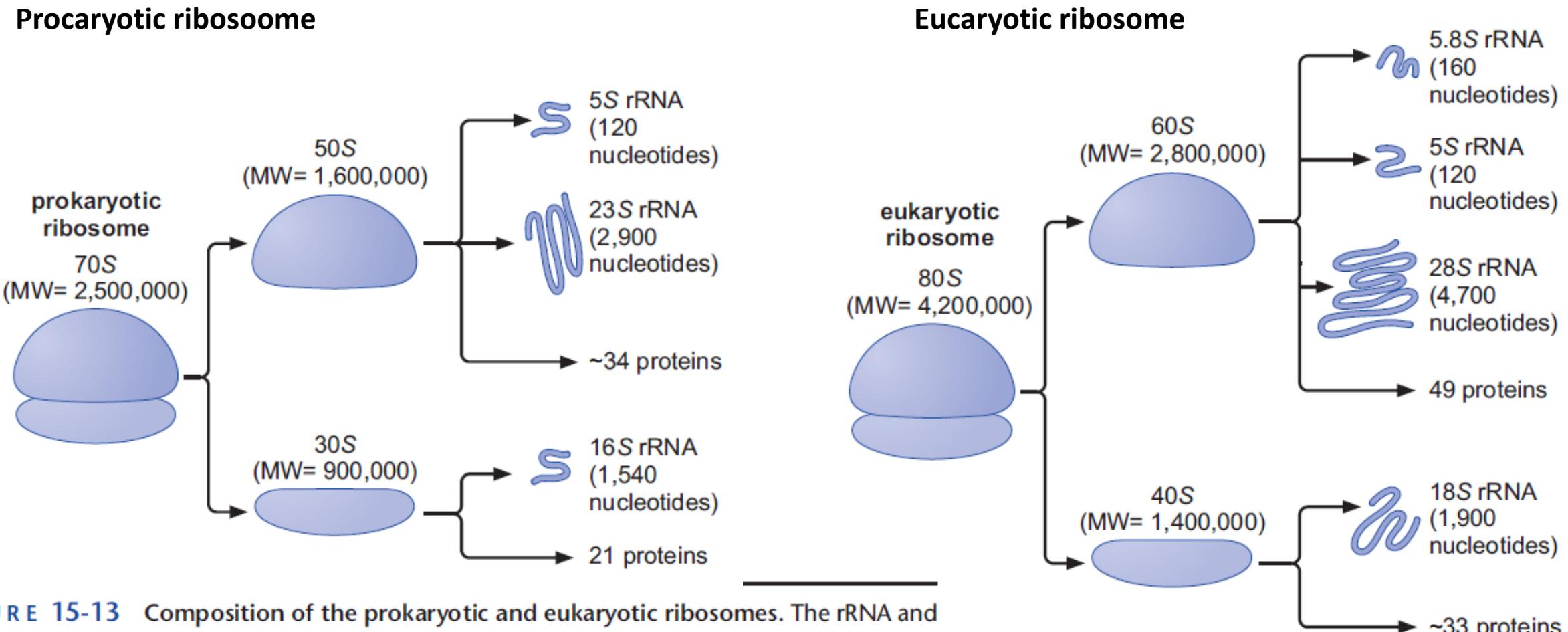
Sedimentation coefficients are, however, not additive.

When two particles bind together, they have reduced surface area.

Thus, when measured separately they may have svedberg values that do not total that of the bound particle.

# Ribosome

- Two subunits
  - Large (50S/60S) – peptidyl transferase center
  - Small (30S/40S) – decoding center



**FIGURE 15-13** Composition of the prokaryotic and eukaryotic ribosomes. The rRNA and protein composition of the different subunits are indicated. The length of the rRNA and the number of ribosomal proteins are indicated for each subunit.

# The ribosome cycle

- Sequence of association and dissociation of ribosomal subunits
  - Binding of mRNA and initiator tRNA to the small ribosomal subunit
  - Recruiting large ribosomal subunit
  - Elongation – protein synthesis
  - Release of polypeptide
  - Dissociation of ribosome subunits

# The ribosome cycle

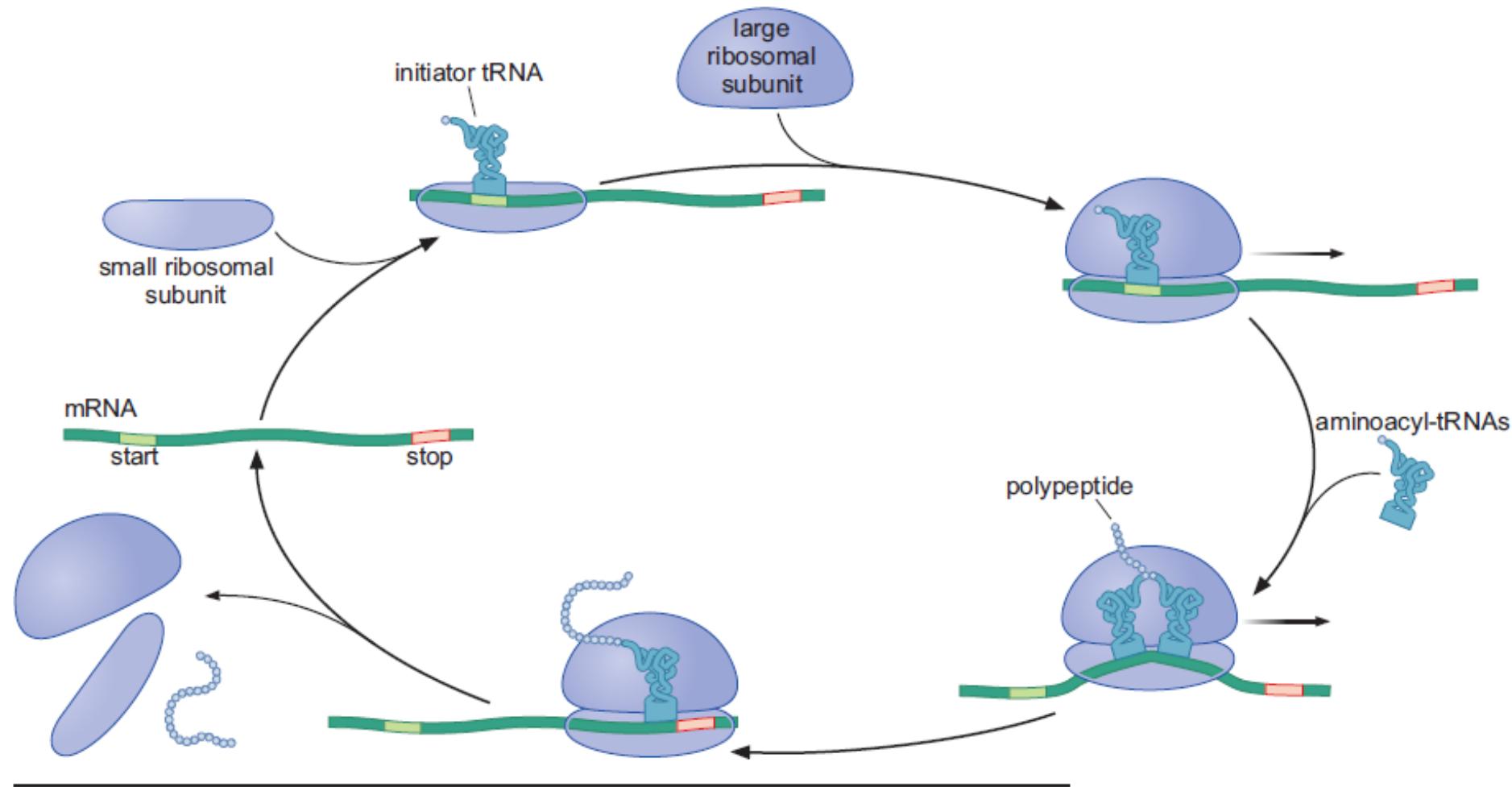


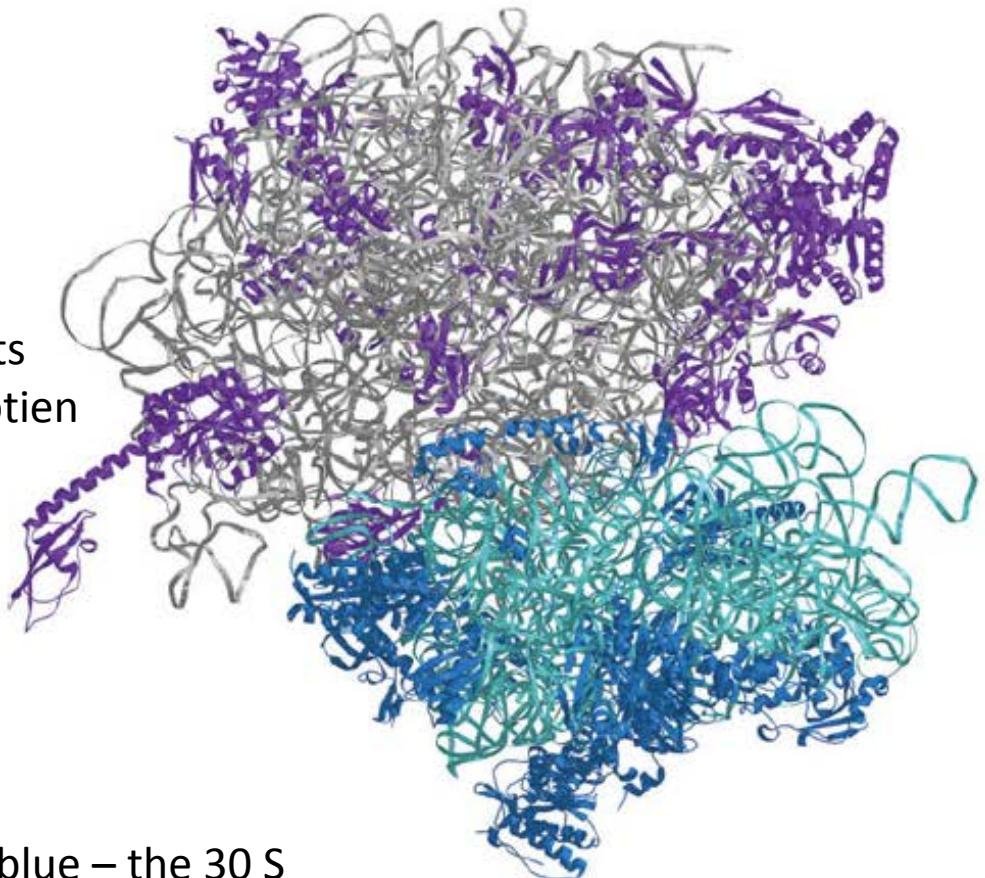
FIGURE 15-14 Overview of the events of translation: the ribosome cycle.

# Ribosome

50 S

Gray – the 50 S  
RNA components

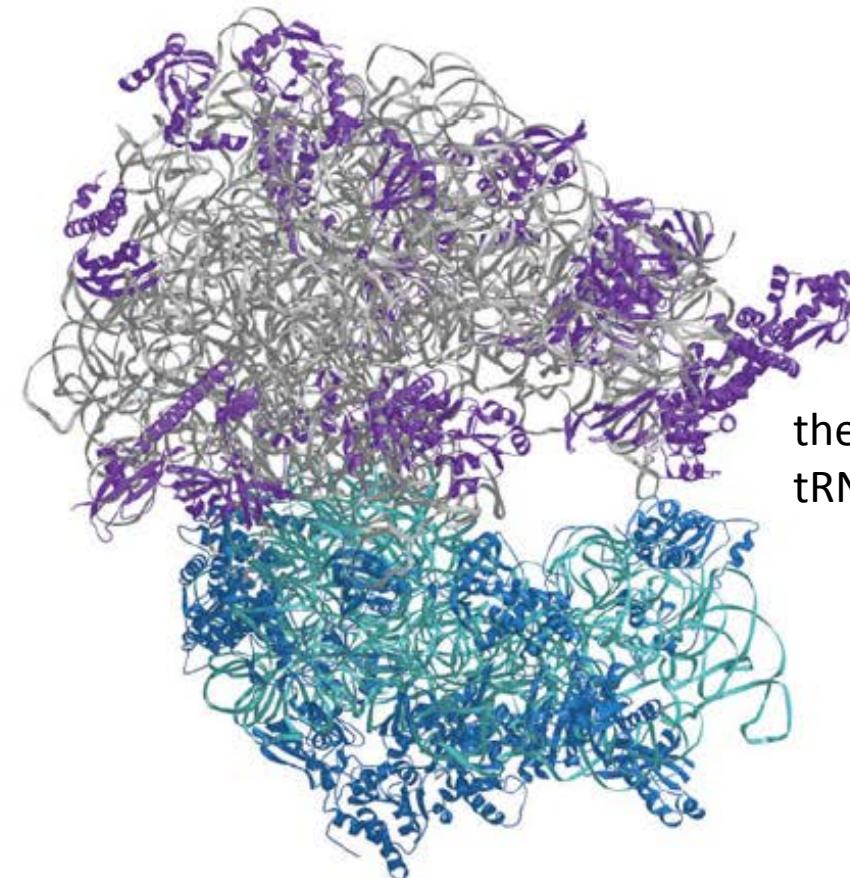
Purple – the protein  
components



30 S

Light blue – the 30 S  
RNA components

Dark blue – the protein  
components



**FIGURE 15-17** Two views of the ribosome. The 50S subunit is above the 30S subunit in both views. The cavity between the 50S and 30S subunits in the right-hand image represents the site of tRNA association (see Fig. 15-19b). The RNA component of the 50S subunit is shown in gray; the protein component of the 50S subunit is shown in purple; the RNA component of the 30S subunit is shown in light blue; the protein component of the 30S subunit is shown in dark blue. (Yusupov M.M. et al. 2001. *Science* 292: 883–896.) Images prepared with MolScript, BobScript, and Raster3D.

Many ribosomes on the same mRNA make polysomes (poly ribosomes)

- Single ribosomes contacts ~30 nt of RNA but covers ~80 nt
  - Still space for more than one ribosome on any given mRNA

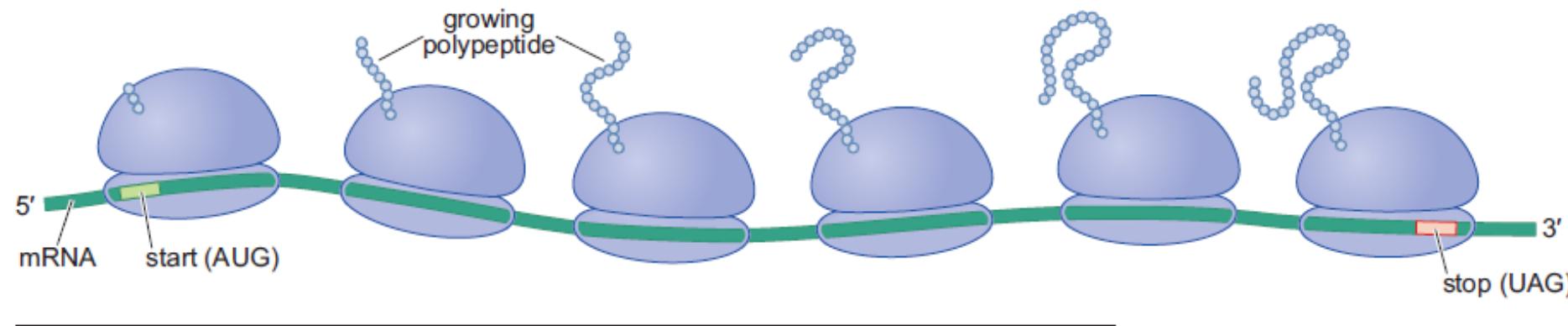


FIGURE 15-15 A polyribosome.

The ribosome catalyzes a single chemical reaction—the formation of a peptide bond.

- The reaction to form a new peptide bond is called the **peptidyl transferase reaction**

- Aminoacyl-tRNA
- Peptidyl-tRNA

- From N to C!
- No additional ATP required (high-energy acyl bond!)

This charging reaction involves the hydrolysis of a molecule of ATP!!!

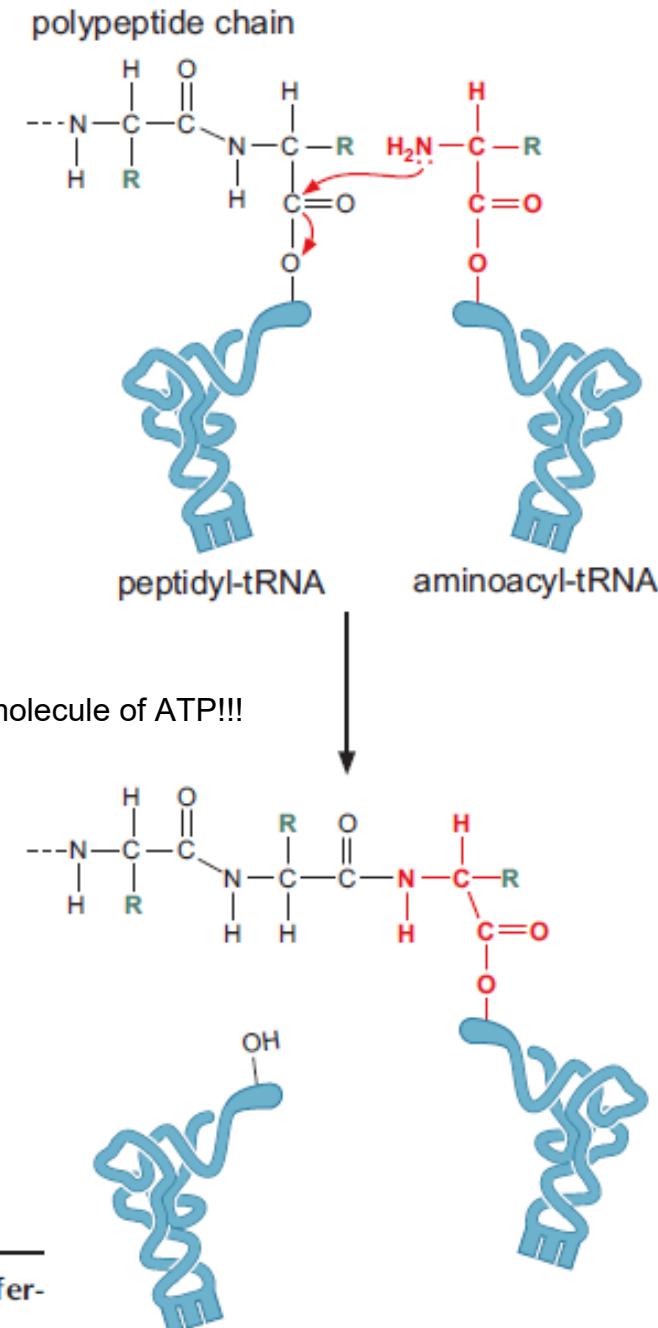
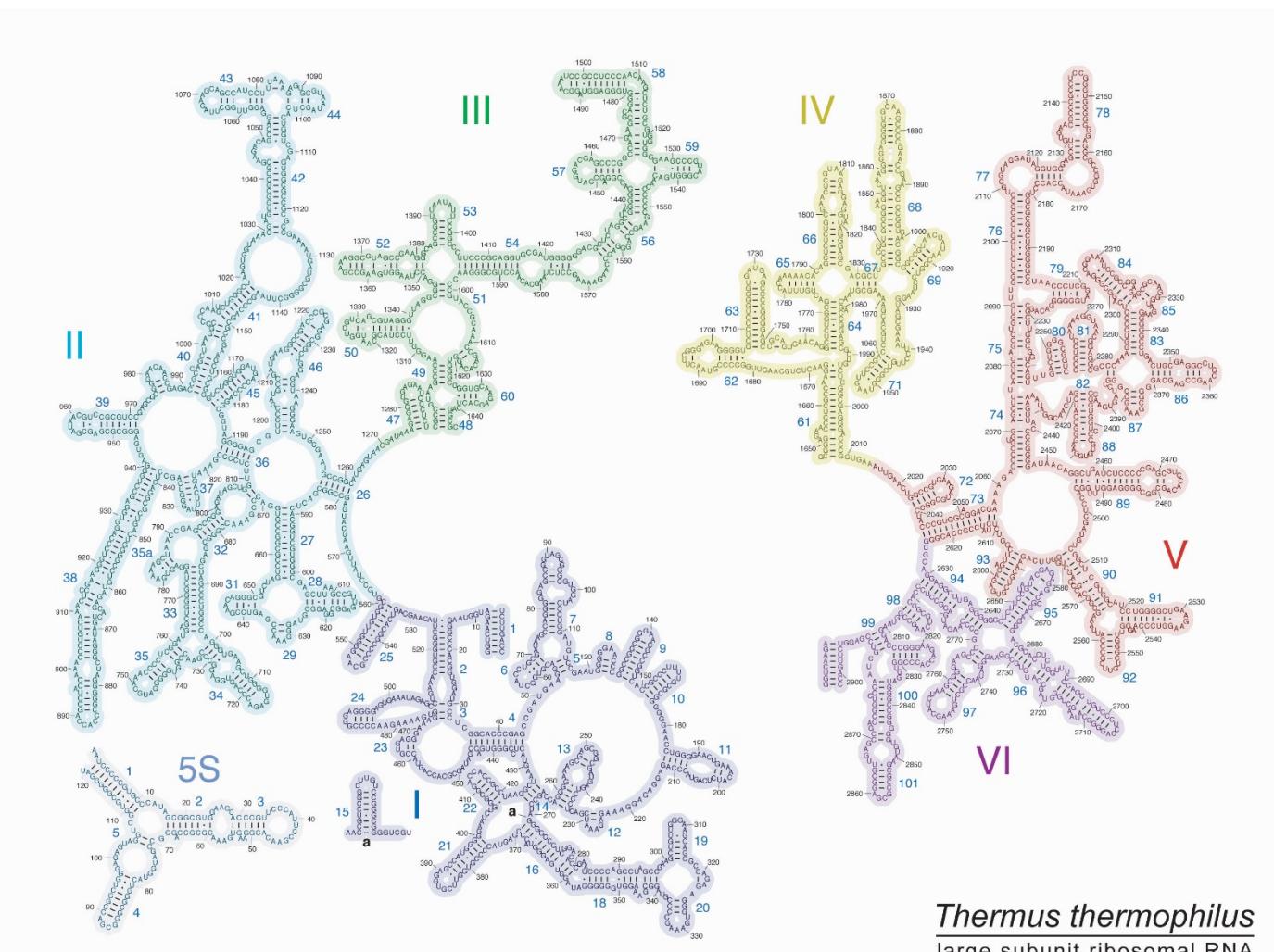
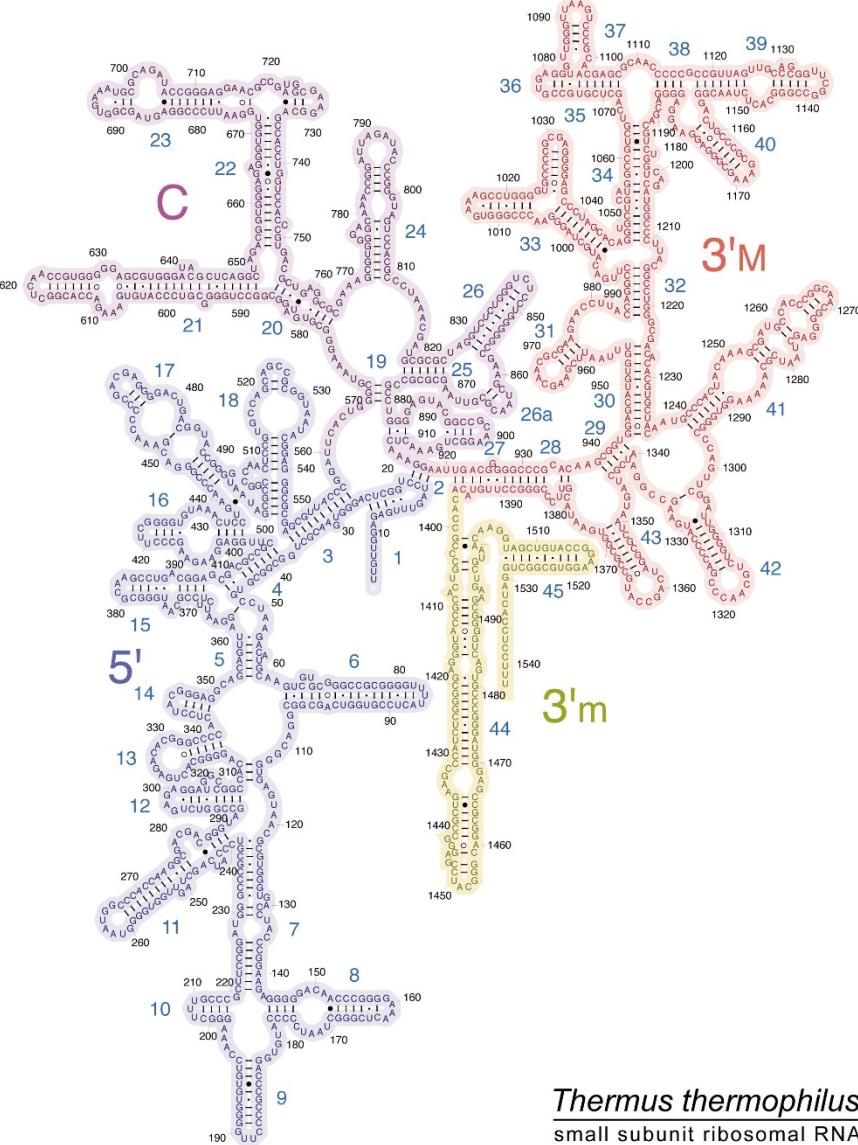
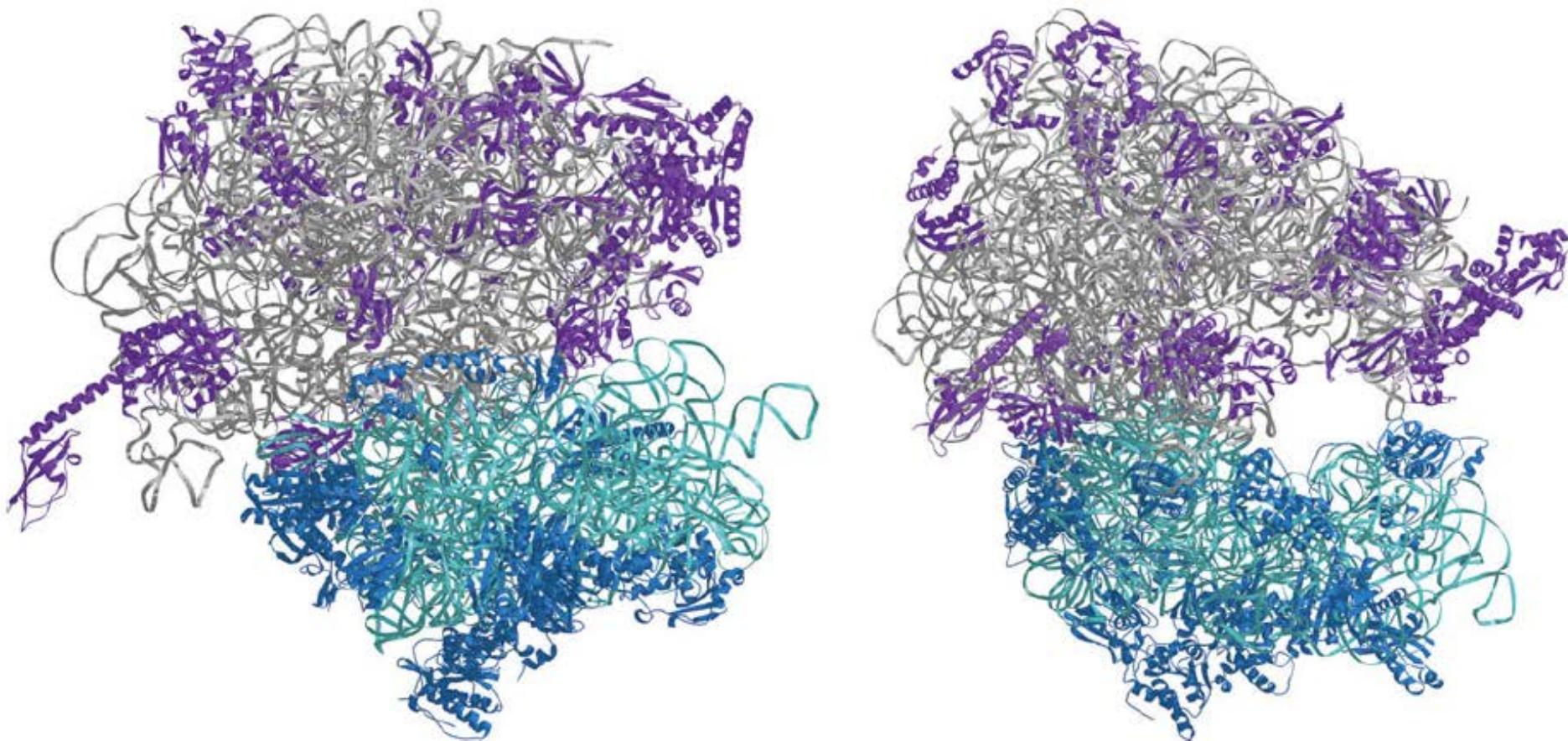


FIGURE 15-16 The peptidyl transferase reaction.

# Small and large subunit of ribosomal RNA



# Ribosome: 50 S and 30 S subunits

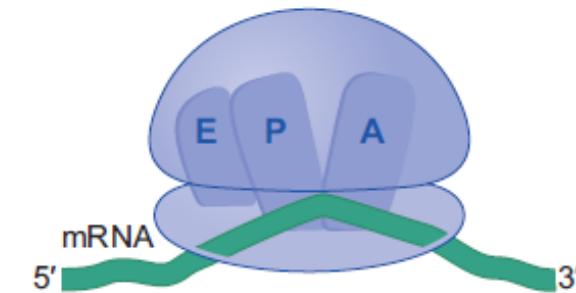


**FIGURE 15-17** Two views of the ribosome. The 50S subunit is above the 30S subunit in both views. The cavity between the 50S and 30S subunits in the right-hand image represents the site of tRNA association (see Fig. 15-19b). The RNA component of the 50S subunit is shown in gray; the protein component of the 50S subunit is shown in purple; the RNA component of the 30S subunit is shown in light blue; the protein component of the 30S subunit is shown in dark blue. (Yusupov M.M. et al. 2001. *Science* 292: 883–896.) Images prepared with MolScript, BobScript, and Raster3D.

- Main structural components of the ribosome
- Catalytic determinants of the ribosome
  - Peptidyl transferase
  - Decoding center
- Most ribosomal proteins are on the outside of the ribosome

# The Ribosome Has Three Binding Sites for tRNA

- A site
  - Aminoacyl-tRNA
- P site
  - Peptidyl-tRNA
- E site
  - Exit binding site for the tRNA that is released after the growing polypeptide chain has been transferred to the aminoacyl-tRNA



**FIGURE 15-18** The ribosome has three tRNA-binding sites. The schematic illustration of the ribosome shows the three binding sites (E, P, and A) that each spans the two subunits.

To perform the peptidyl transferase reaction, the ribosome must be able to bind at least two tRNAs simultaneously.

# Bend the ribbon!

- Kink in the mRNA helps to distinguish between A- and P-site codon – its important to facilitate maintenance of the correct reading frame

mRNA –blue

E-site – yellow

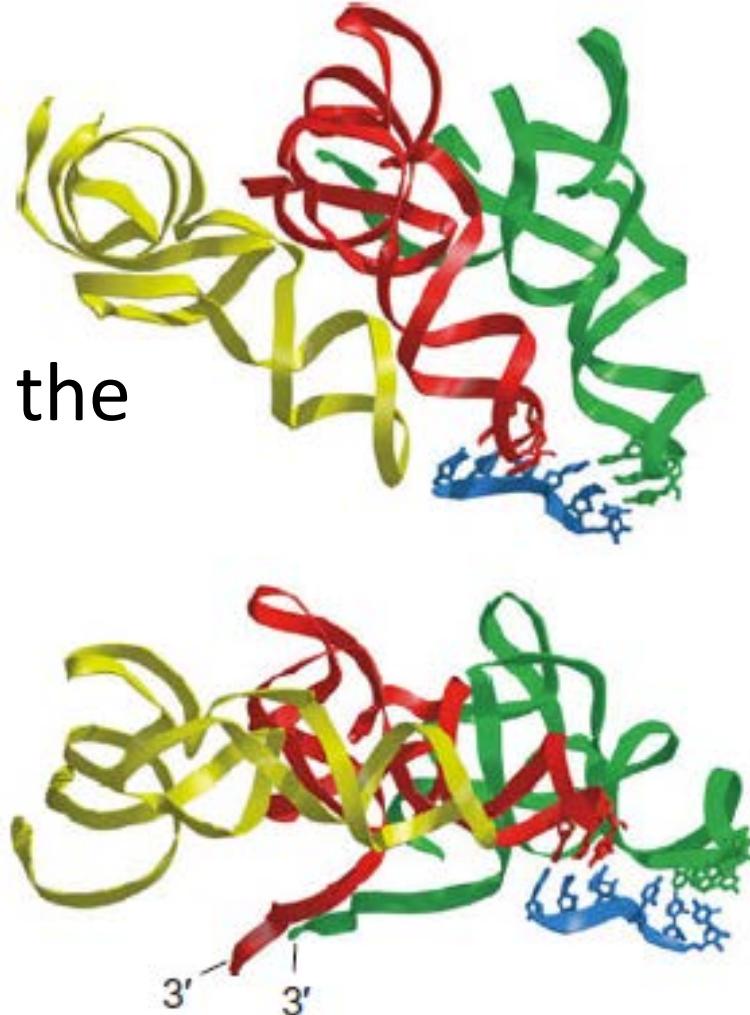
P-site – red

A-site – green

codon- anticodon interaction

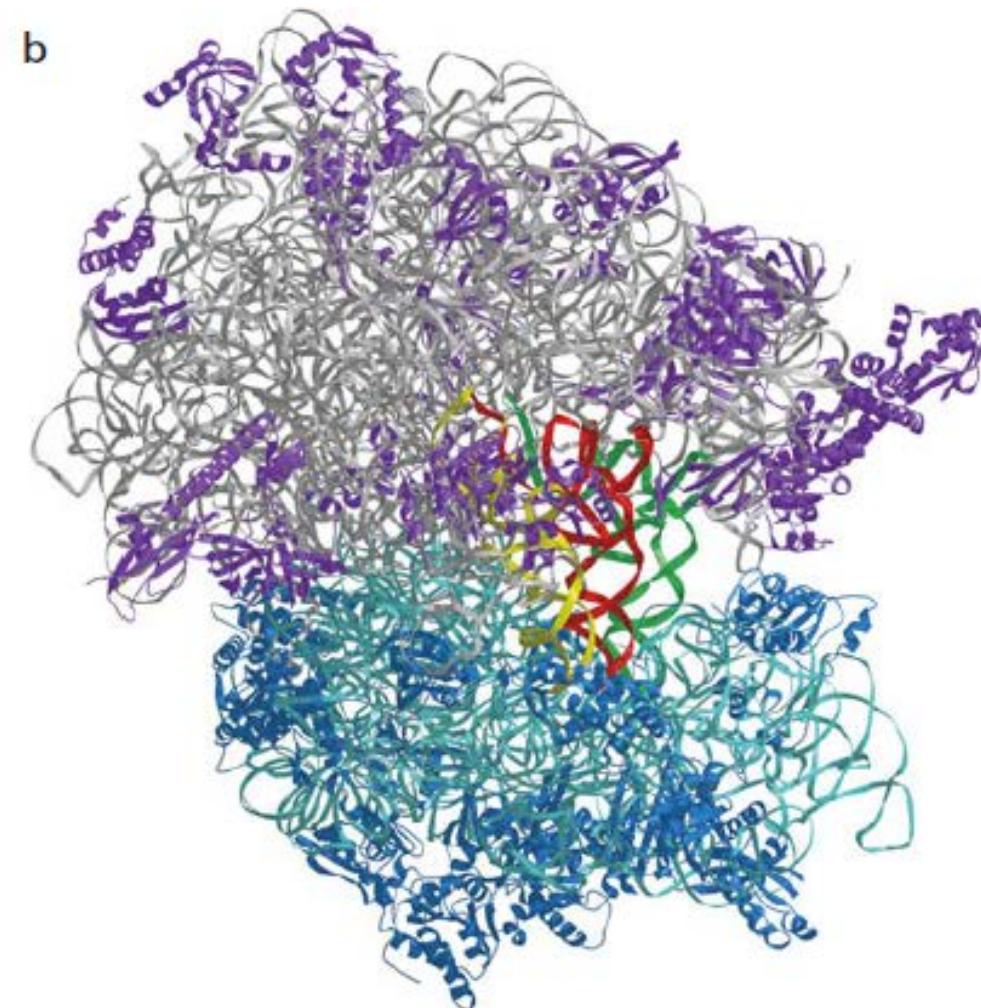
No mRNA

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**.



**FIGURE 15-20** The interaction between the A-site and P-site tRNAs and the mRNA within the ribosome. Two views of the structure of the mRNA and tRNAs are shown as they are found in the ribosome. For clarity, the ribosome is not shown. The E-, P-, and A-site tRNAs are shown in yellow, red, and green, respectively, and the mRNA is shown in blue. Only the bases involved in the codon–anticodon interaction are shown. The strong kink in the mRNA clearly distinguishes between the A-site and P-site codons. The close proximity of the 3' ends of the A-site and P-site tRNAs can be seen in the lower image. (Yusupov M.M. et al. 2001. *Science* 292: 883–896.) Image prepared with Mol-Script, BobScript, and Raster3D.

# Three sites for tRNAs



**FIGURE 15-19** Views of the 3D structure of the ribosome including three bound tRNAs. The E-, P-, and A-site tRNAs are shown in yellow, red, and green, respectively. The colors representing the RNA and protein components of the small and large subunits are the same as those in Figure 15-17. (a,b) Two views of the ribosome bound to the three tRNAs in the E-, P-, and A-sites. Note that the left (a) and right (b) views shown here correspond to those views of the ribosome shown in Figure 15-17. (c) The isolated 50S subunit with tRNAs as seen in the full ribosome. (This view is as if you were looking up at the large subunit from the small subunit.) The peptidyl transferase center is circled. (d) The isolated 30S subunit with tRNAs as seen in the full ribosome. The decoding center is circled. (Yusupov M.M. et al. 2001. *Science* 292: 883–896.) Images prepared with MolScript, BobScript, and Raster3D.

# Holes in the ribosome

- Necessary components must be able to enter and exit the ribosome
- mRNA
  - **Two narrow channels on the small subunit**
  - **mRNA must be single-stranded to fit through!**
- tRNA
  - A region between the two channels
- Polypeptide
  - Channel through the large subunit – **exit path** for the polypeptide

# Bend the ribbon!

- Kink in the mRNA helps to distinguish between A- and P-site codon – its important to facilitate maintenance of the correct reading frame

mRNA –blue

E-site – yellow

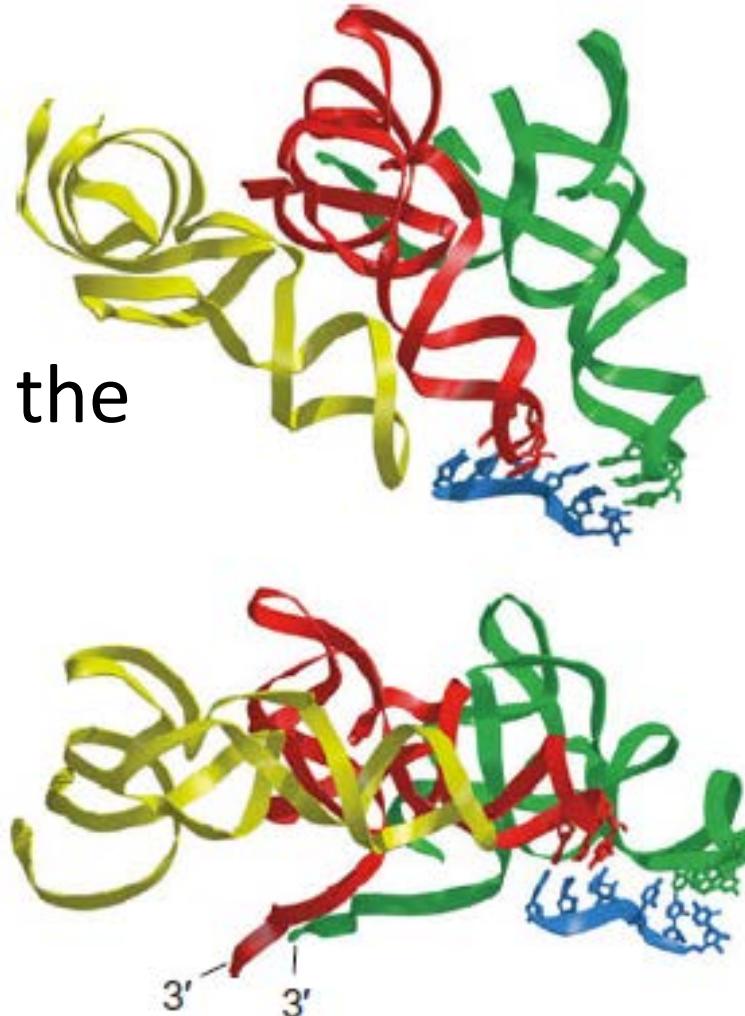
P-site – red

A-site – green

codon- anticodon interaction

NO 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.

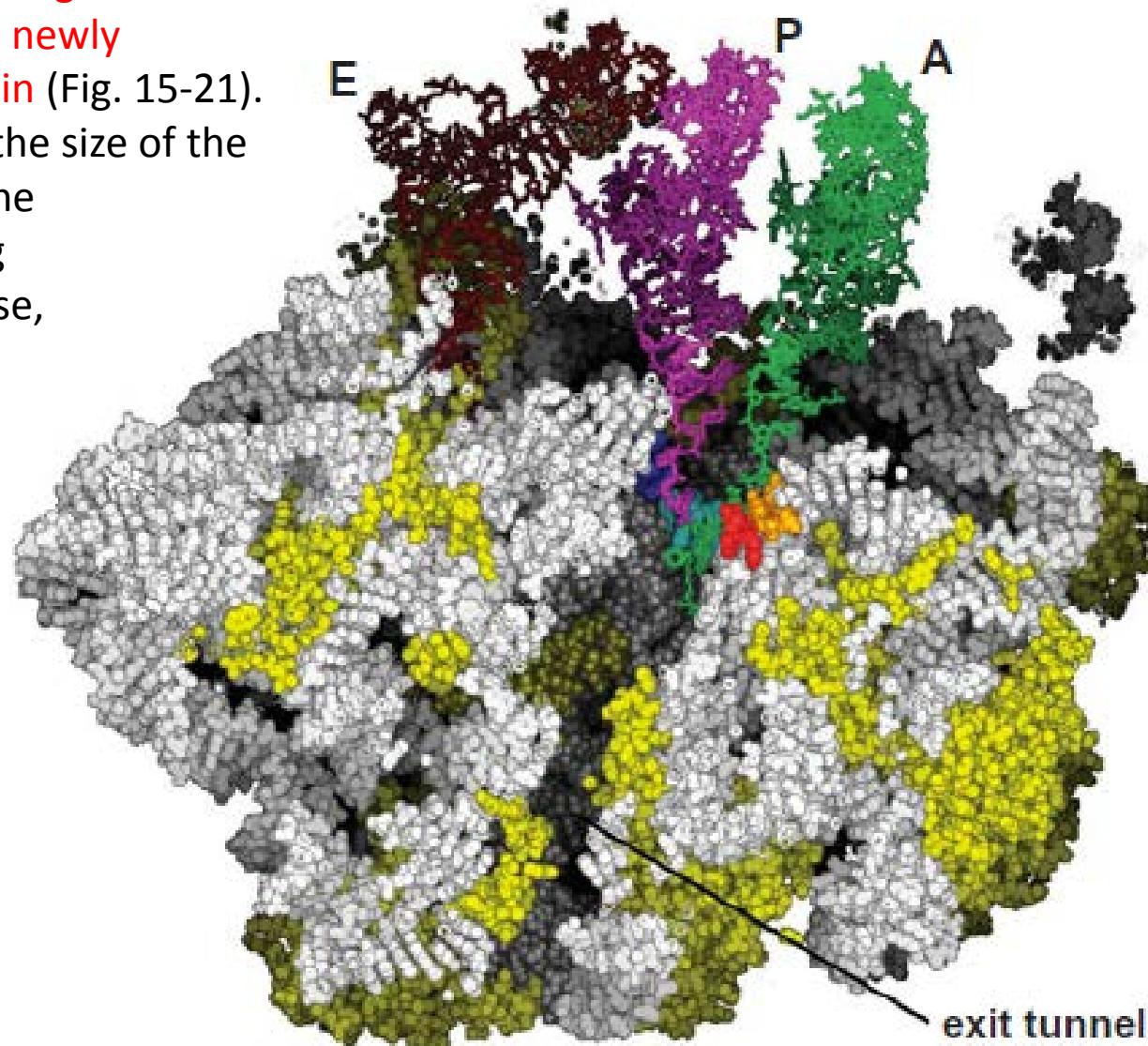


**FIGURE 15-20** The interaction between the A-site and P-site tRNAs and the mRNA within the ribosome. Two views of the structure of the mRNA and tRNAs are shown as they are found in the ribosome. For clarity, the ribosome is not shown. The E-, P-, and A-site tRNAs are shown in yellow, red, and green, respectively, and the mRNA is shown in blue. Only the bases involved in the codon–anticodon interaction are shown. The strong kink in the mRNA clearly distinguishes between the A-site and P-site codons. The close proximity of the 3' ends of the A-site and P-site tRNAs can be seen in the lower image. (Yusupov M.M. et al. 2001. *Science* 292: 883–896.) Image prepared with Mol-Script, BobScript, and Raster3D.

# Holes in the ribosome

Second channel through the large subunit provides an exit path for the newly synthesized polypeptide chain (Fig. 15-21).

As with the mRNA channel, the size of the peptide exit channel limits the conformation of the growing polypeptide chain. In this case, a polypeptide can form an α helix within the channel, but other secondary structures (such as β sheets) and tertiary interactions can form only after the polypeptide exits the large ribosomal subunit.

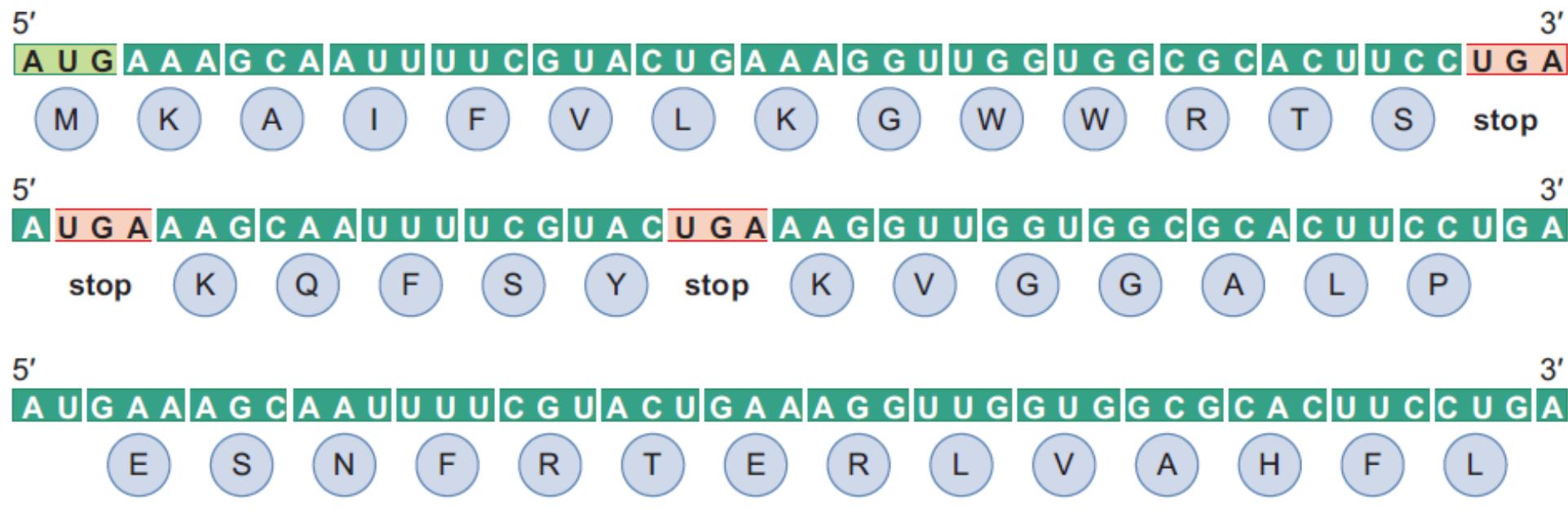


RNA – white  
Ribosomal proteins – yellow  
Peptidyl transferase center – red and gold parts

**FIGURE 15-21** The polypeptide exit tunnel. In this image, the 50S subunit is cut in half to reveal the polypeptide exit tunnel. The rRNA is white; the ribosomal proteins are yellow. The three bound tRNAs are colored as follows: E-site (brown), P-site (purple), and A-site (green). The red and gold parts of the rRNA adjacent to the A-site tRNA are components of the peptidyl transferase center. (Courtesy of T. Martin Schmeing and Thomas Steitz; adapted, with permission, from Schmeing T.M. et al. 2002. *Nat. Struct. Biol.* 9: 225–230. © Macmillan.)

# mRNA

- Three potential reading frames



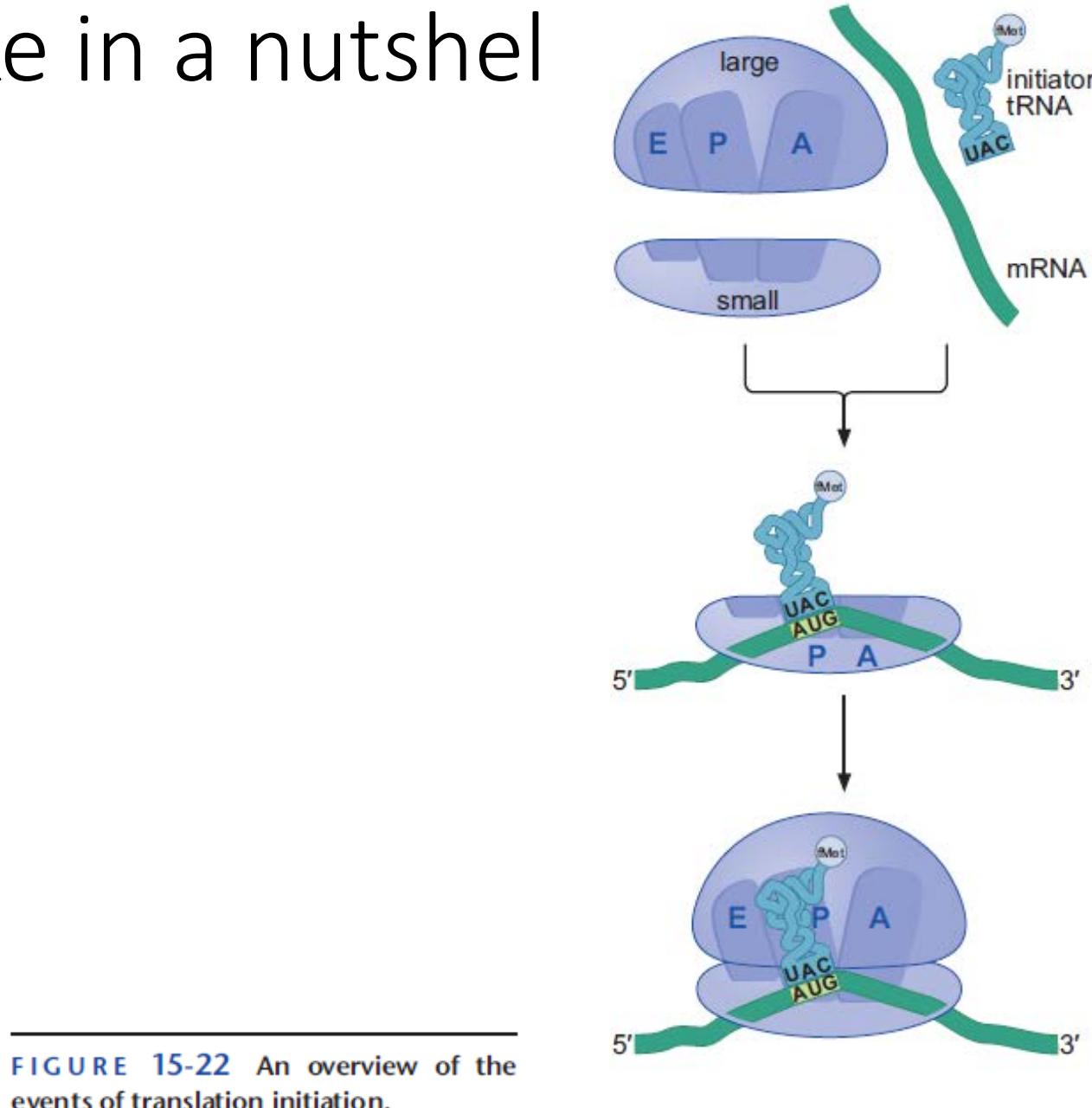
**FIGURE 15-1** Three possible reading frames of the *Escherichia coli trp* leader sequence. Start codons are shaded in green, and stop codons are shaded in red. The amino acid sequence encoded by each reading frame is indicated in the single-letter code below each codon.

# Translation initiation

- Ribosome must be recruited to the mRNA
- Charged tRNA must be placed in the P-site
- Ribosome must be precisely positioned over the start codon
  - Frameshift will mostly do harm!

# Translation like in a nutshell - initiation

- Initiation
- Elongation
- Termination



# Bacterial translation initiation in a nutshell

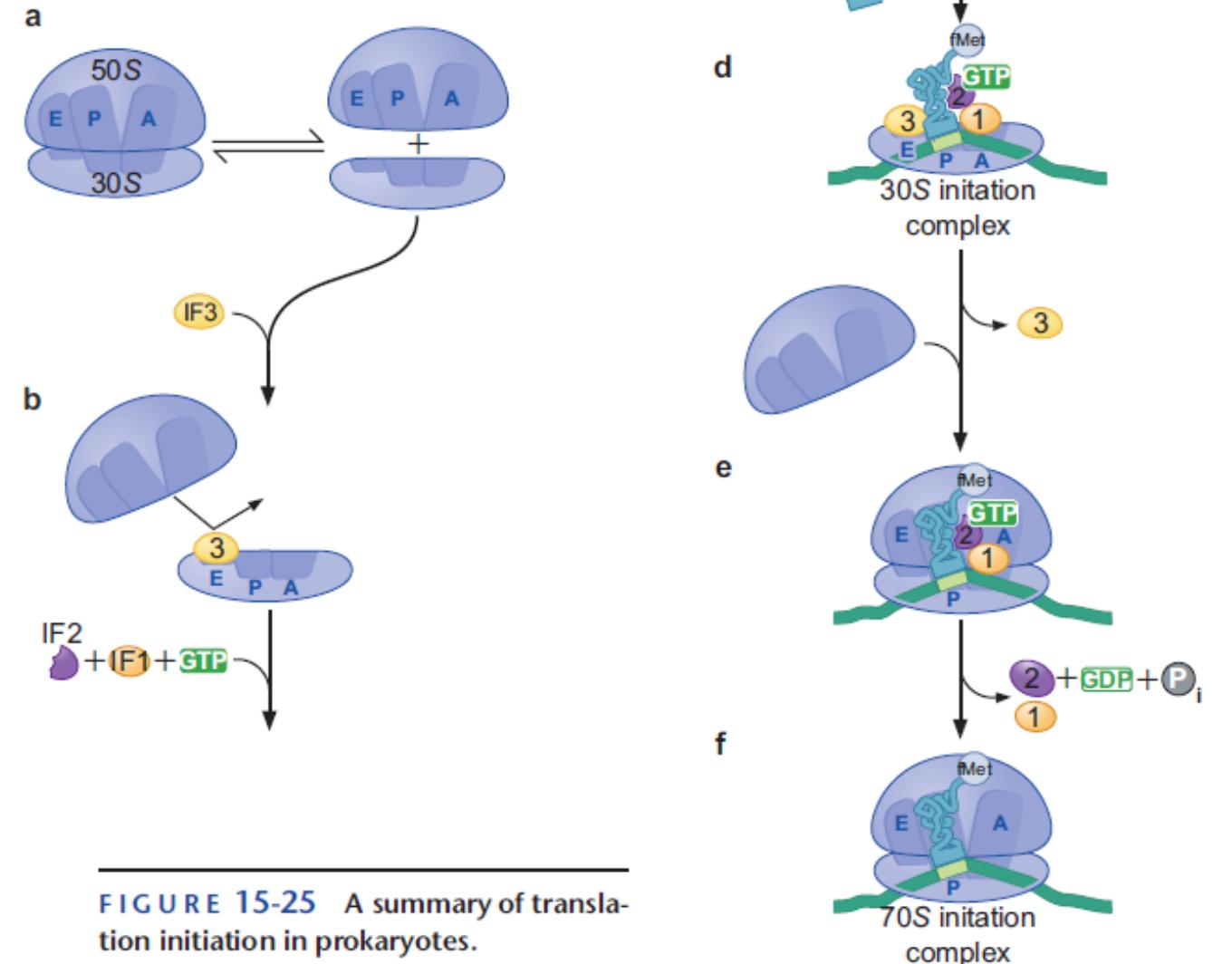
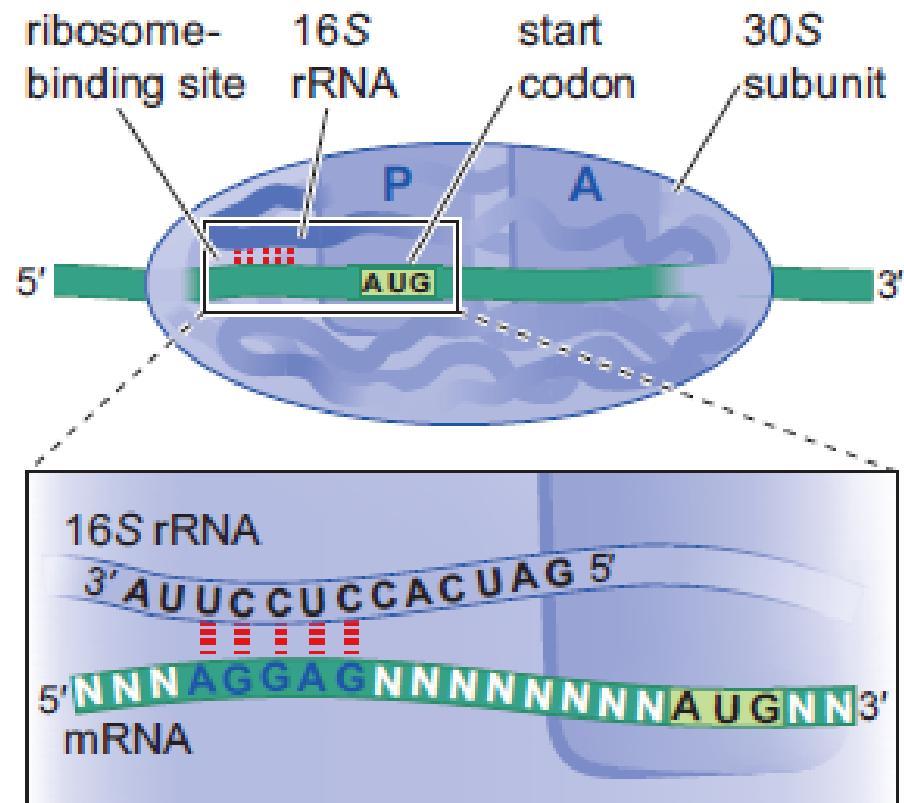


FIGURE 15-25 A summary of translation initiation in prokaryotes.

# 1- Step one of initiation

- Find and recognize mRNA

Translation initiation is the only time a tRNA binds to the P-site without previously occupying the A-site. This event requires a special tRNA known as the initiator tRNA, which base-pairs with the start codon—usually AUG or GUG.



**FIGURE 15-23** The 16S rRNA interacts with the RBS to position the AUG in the P-site. This illustration shows an mRNA with the ideal separation between the RBS and the initiating AUG. This spacing places the AUG in the region of the P-site. Many mRNAs have non-ideal spacings leading to a reduced rate of translation initiation. Other mRNAs lack an RBS completely and recruit the ribosome by distinct mechanisms.

# 2- Step two – of initiation

- Bind initiator tRNA to the P-site
  - fMet-tRNA

A deformylase removes the formyl group from the amino terminus during or after the synthesis of the polypeptide chain

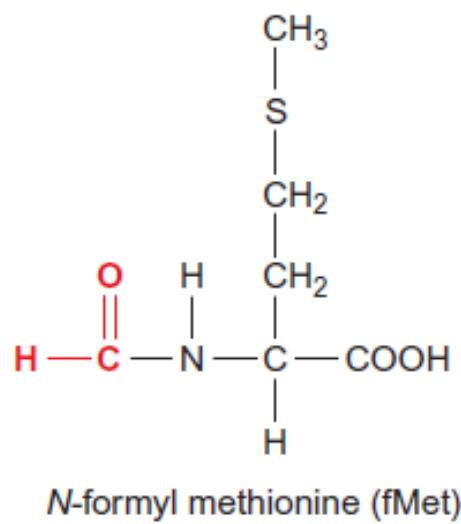
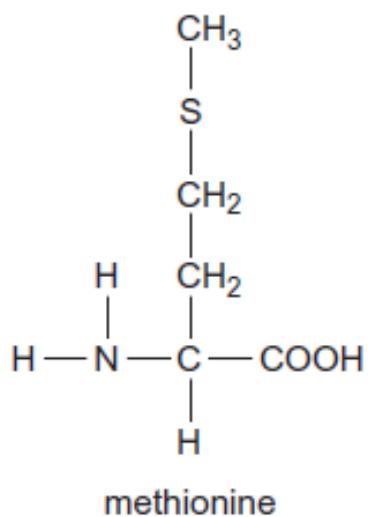


FIGURE 15-24 Methionine and *N*-formyl methionine.

# 3 -Step three of initiation– assembly of initiation complex (mRNA + initiator tRNA)

- IF1
  - Prevents tRNA binding to the A-site
- IF2
  - GTPase, facilitates the association of the initiator tRNA with the small subunit and prevents other charged tRNAs from binding
- IF3
  - Blocks reassociation with the large subunit
- All IFs bind at, or near, one of the three tRNA-binding sites

# Eukaryotic initiation

( see the previous lecture p. 5)

- Similar to prokaryotic initiation in many ways
- Four steps
  - Small subunit is associated with an initiator tRNA before being recruited to the capped 5' end of the mRNA
  - Set of factors to recognize mRNA
  - AUG scanning (one start codon)
  - Recruitment of the large subunit

# Eukaryotic initiation

- Four steps
  - Small subunit is associated with an initiator tRNA before being recruited to the capped 5' end of the mRNA
  - A separate set of factors to recognize mRNA
  - First AUG scanning (the small subunit of ribosome binds to the initiator mRNA)
  - Recruitment of the large subunit after the initiator RNA base-pairs with the start codon

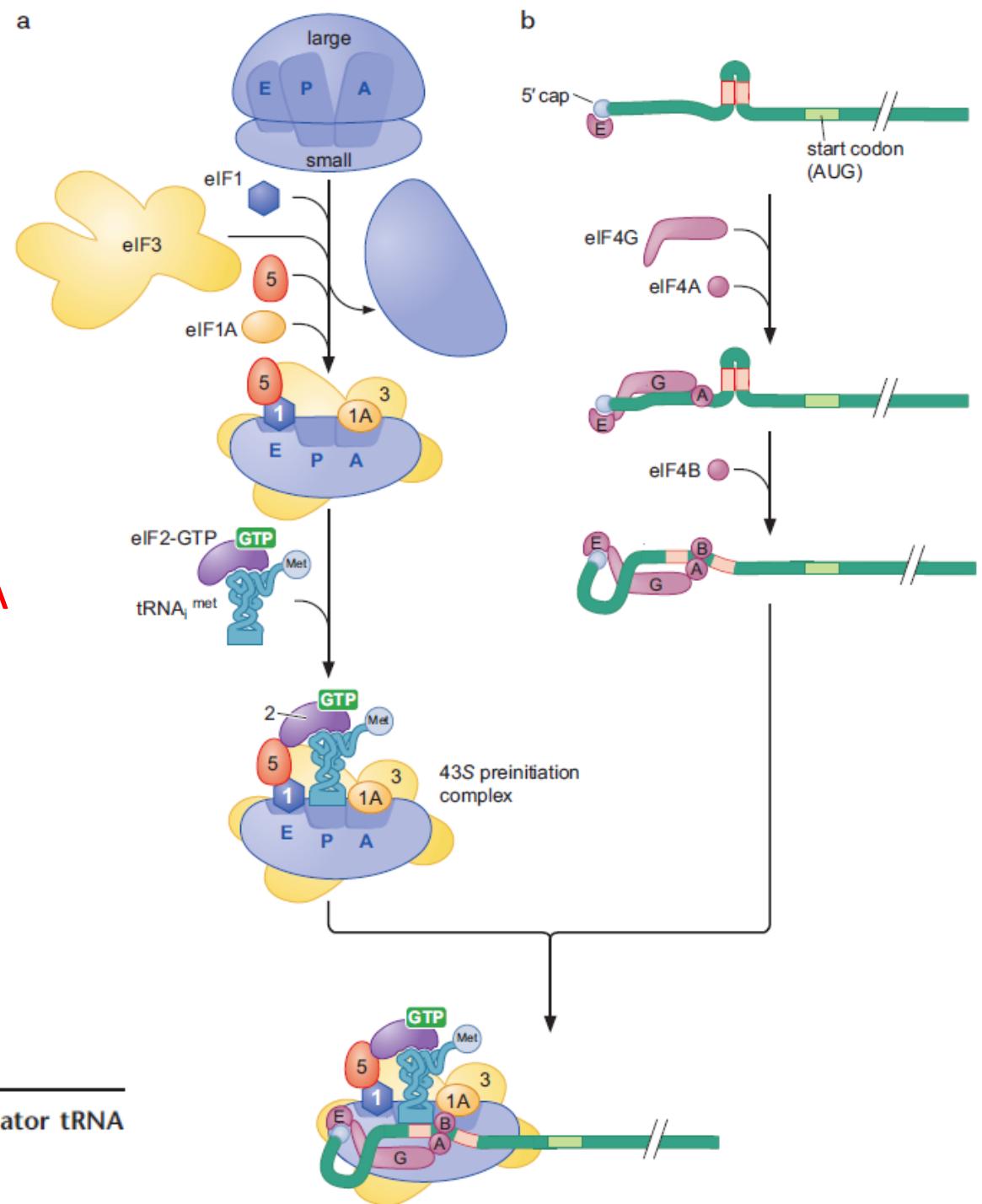


FIGURE 15-26 Assembly of the eukaryotic small ribosomal subunit and initiator tRNA onto the mRNA.

# Eukaryotic initiation - ribosome small subunit

- Ribosome subunits are dissociated
- eIF1, eIF1A, eIF3, and eIF5 bind to the small subunit
  - They act similar to IF1 and IF3 in bacteria
- G-protein **eIF2 brings in initiator tRNA**
  - Only in GTP-bound state!
  - Called **ternary complex (TC)**
- eIF2 positions Met-tRNA<sub>i</sub><sup>Met</sup> in the P-site, forms **43S PIC (preinitiation complex)**

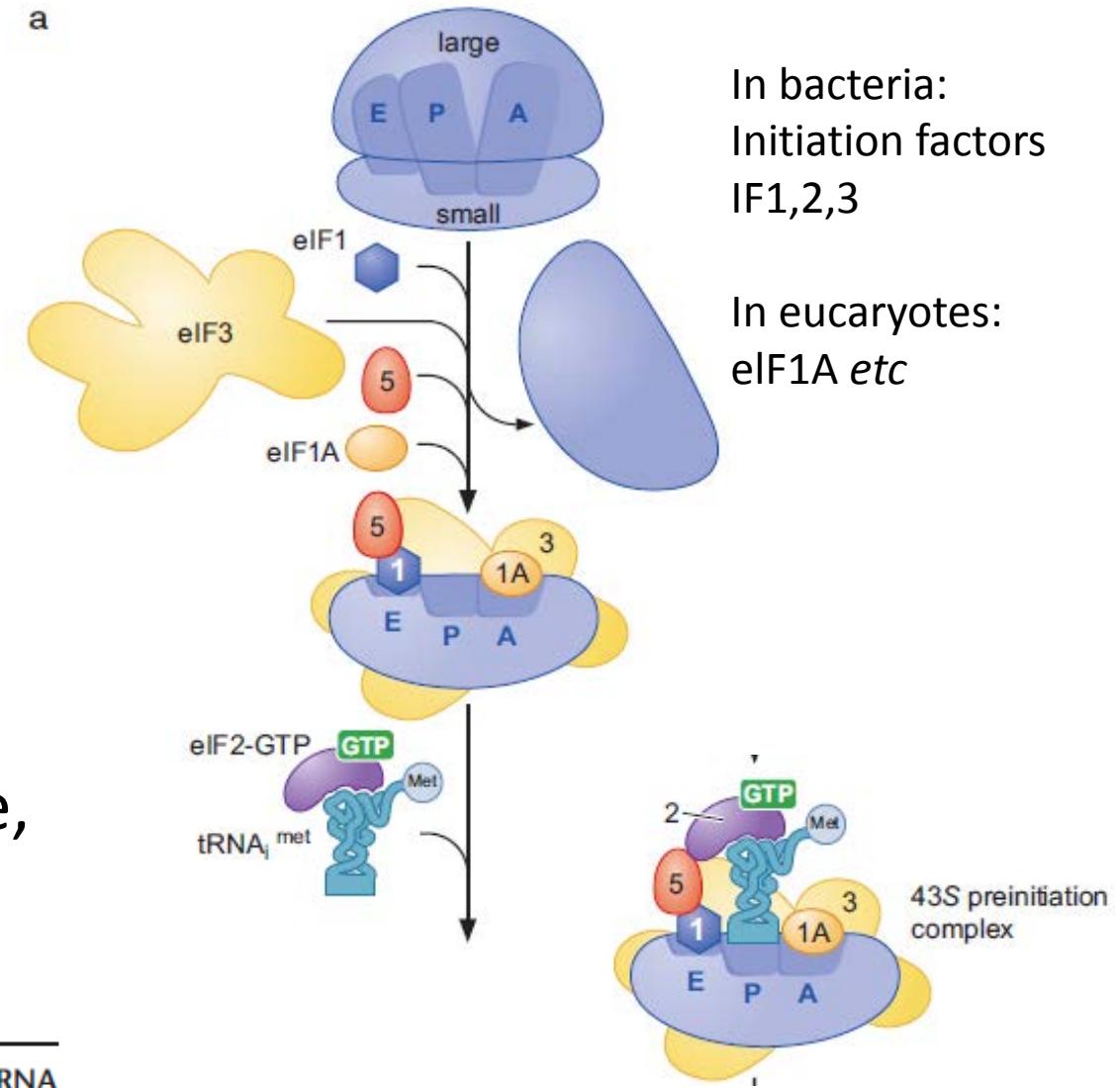


FIGURE 15-26 Assembly of the eukaryotic small ribosomal subunit and initiator tRNA onto the mRNA.

# Eukaryotic initiation - mRNA

- 5' cap is recognized by the cap-binding protein **elf4E**
- **elf4G** binds to the **elf4E** and mRNA
- **elf4A** binds to the **elf4G** and mRNA
  - the overall level of translation is controlled at this step by **elf4E**-binding proteins that compete with **elf4G**
- **elf4B** activates **helicase activity** of **elf4A**

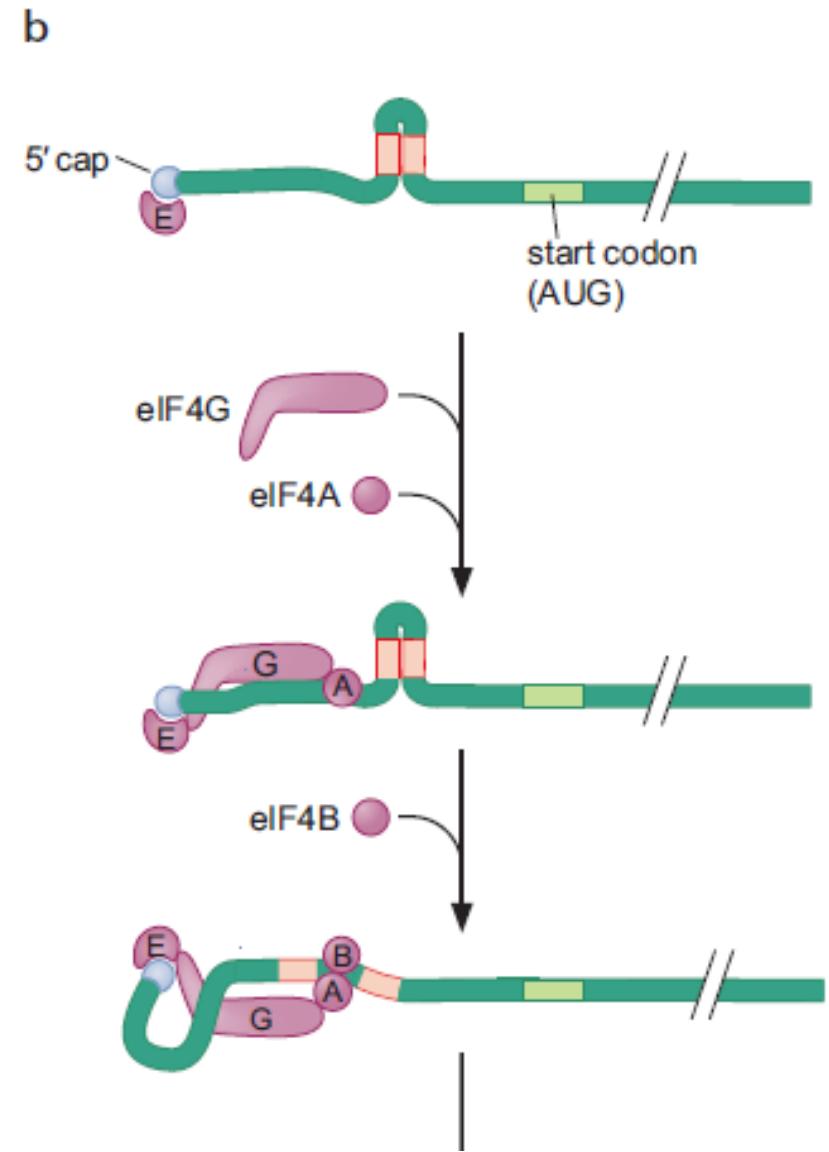


FIGURE 15-26 Assembly of the eukaryotic small ribosomal subunit and initiator tRNA onto the mRNA.

# Eukaryotic initiation – final step

- Interactions between eIF4G bound to the (unstructured) mRNA and the initiation factors (particulary eIF3) recruit the 43S PIC to the mRNA
- 48S pre-initiation complex (PIC) is formed

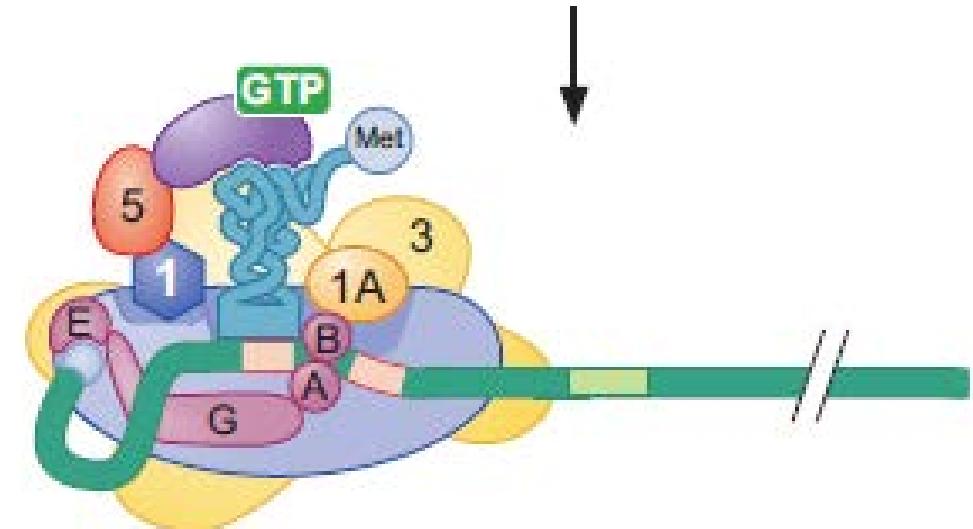


FIGURE 15-26 Assembly of the eukaryotic small ribosomal subunit and initiator tRNA onto the mRNA.

# Circle of mRNA

- **elf4G (mediated circulation)**

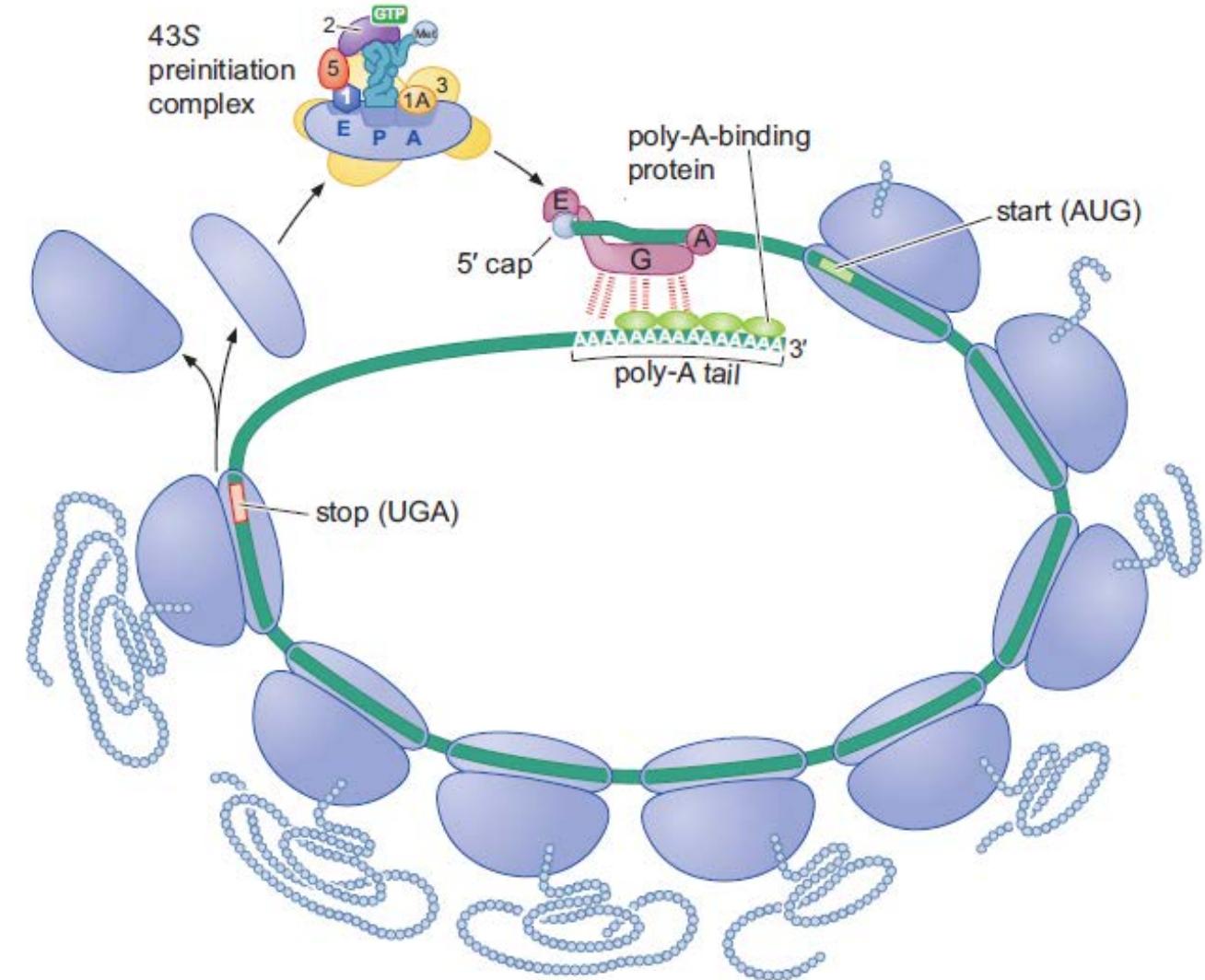
- Binds to the 5' end of the mRNA
- Binds to the poly-A-binding protein of the 3' end of the same mRNA

- **Enhances several steps of initiation**

(Importantly, the interactions of elf4G and the poly-A-binding protein with the mRNA are maintained through **multiple rounds of translation**)

- **Enhances reinitiation** (this mRNA confirmation has the added benefit of locating recently terminated ribosomes near the AUG, presumably enhancing reinitiation)

## Translation Initiation Factors Hold Eukaryotic mRNAs in Circles

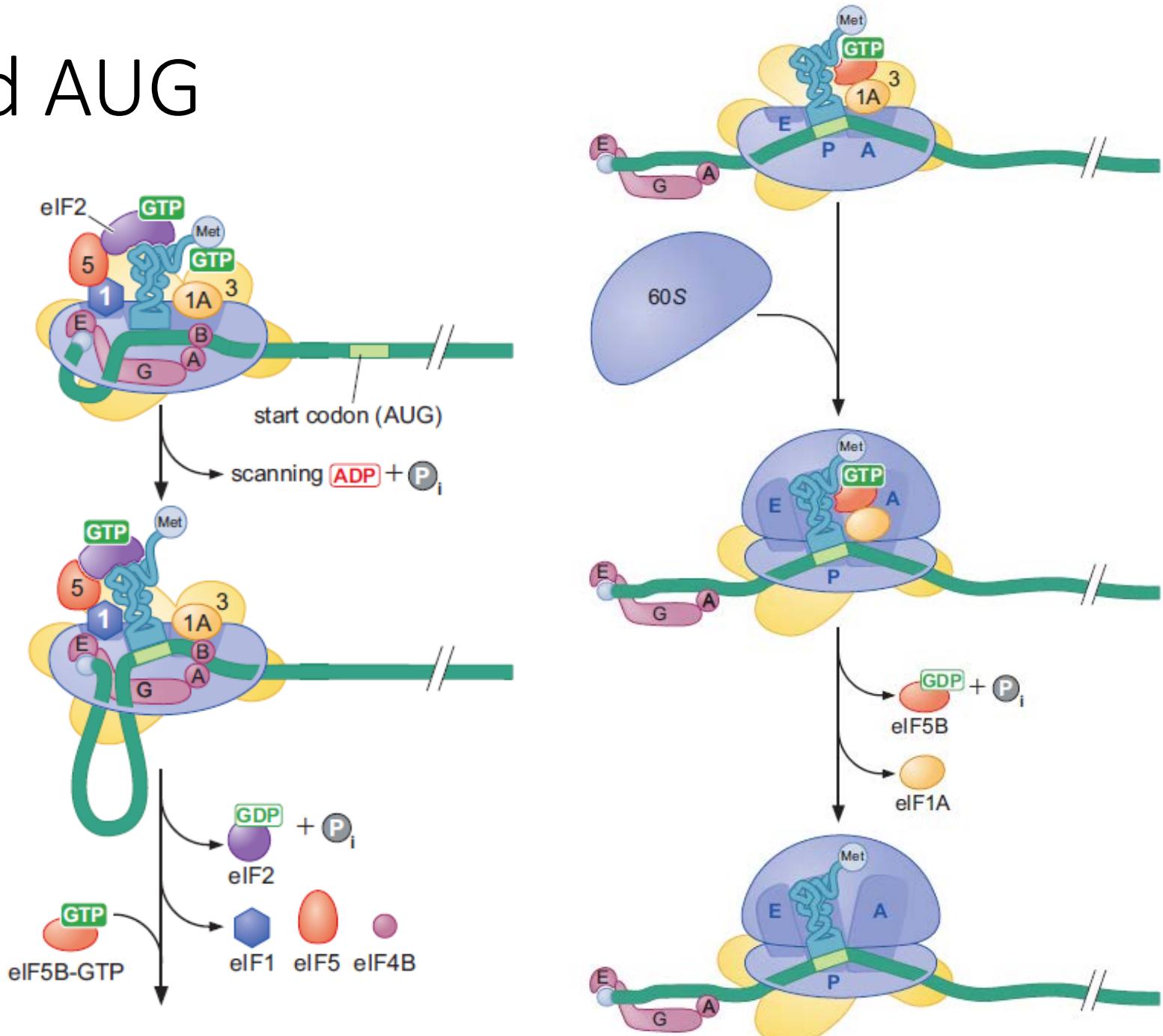


**FIGURE 15-27** A model for the circularization of eukaryotic mRNA. Circularization is mediated by interactions between elf4G, the poly-A-binding protein, and the poly-A tail.

# Scan and find AUG

- **Small subunit (48S PIC) scans mRNA in a 5' -> 3' direction**
  - ATP-dependent, stimulated by RNA helicase
- **Start codon recognized through base pairing**
  - Correct basepairing changes the conformation of 48S PIC
  - Release of eIF1, conformational change of eIF5
  - GTP hydrolysis by eIF2, release of eIF2 and eIF5
- **Loss of eIF2 leads to the binding of eIF5B:GTP with initiator tRNA, stimulates association of 60S subunit to the small subunit 40S and mRNA**
- Remaining initiation factors are released, formation of **80S initiation complex**

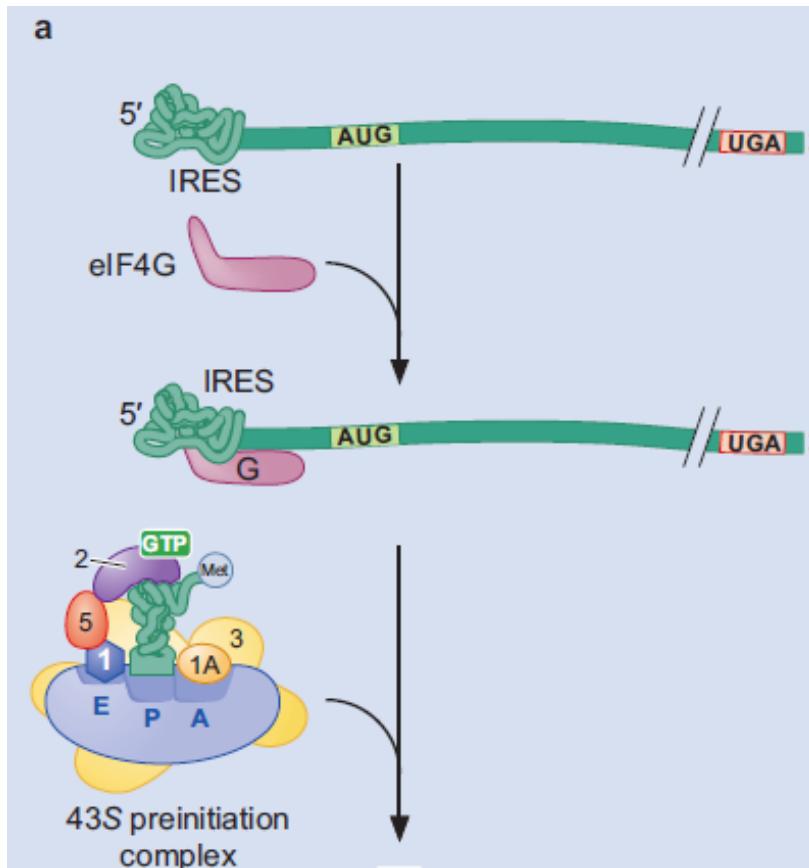
# Scan and find AUG



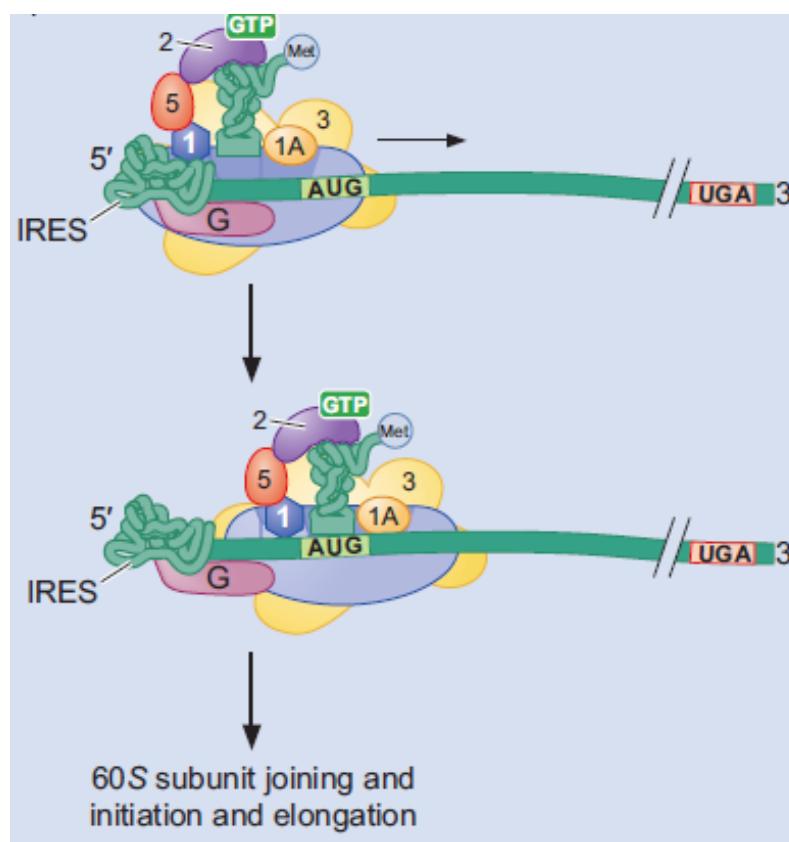
**FIGURE 15-28** Identification of the initiating AUG by the 48S PIC and large subunit joining during eukaryotic translation initiation. See the text for a complete description.

# IRES – forget the factors!

The poliovirus IRES bypasses the requirement for the 5' cap by directly binding to eIF4G.



The Cricket paralysis virus encodes an mRNA with an elaborate IRES that folds to resemble a tRNA that binds directly to the P-site of the 40S and 60S ribosomal subunits.



## Internal ribosome entry site (IRES) –

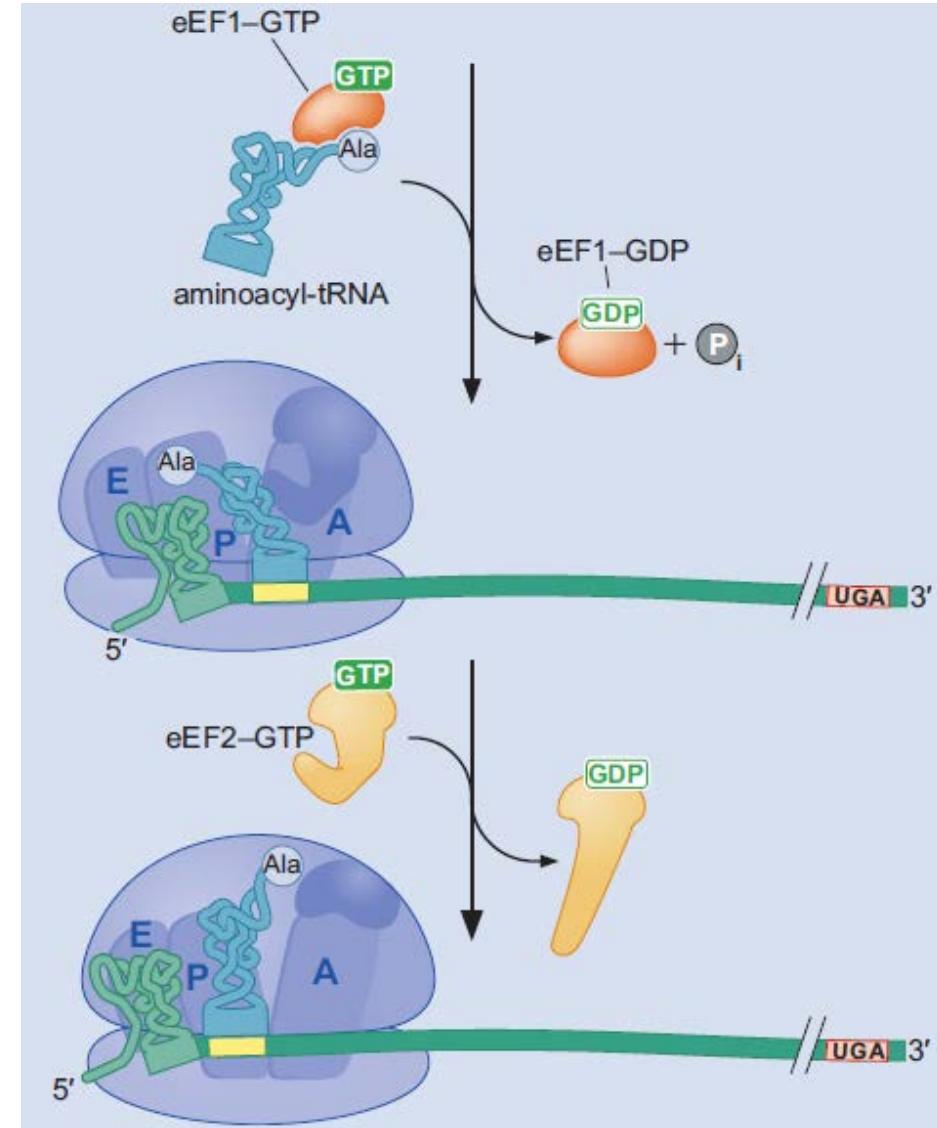
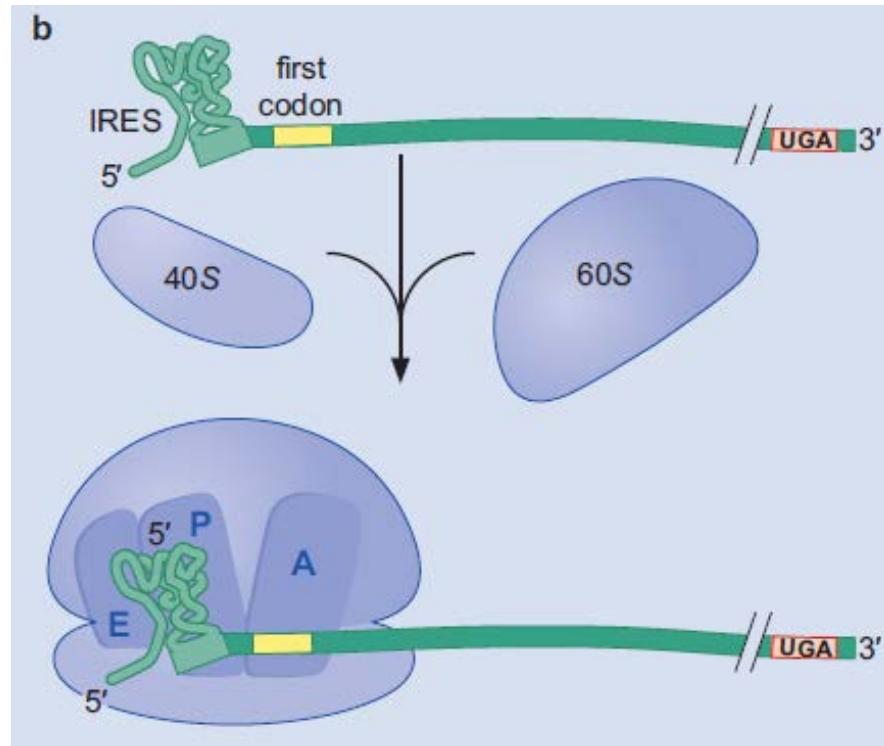
A more extreme example of initiating translation at sites downstream from the most 5'-proximal AUG.

IRESs are often encoded in viral mRNAs that lack a 5'-cap end and have a need to exploit the sequences of their genome maximally. By using an IRES, a viral mRNA can encode more than one protein, reducing the need for extended transcriptional regulatory sequences for each protein-coding sequence.

**IRESs are RNA sequences that function like prokaryotic RBSs.**

**BOX 15-3 FIGURE 2** IRESs bypass normal requirements for initiation of translation. Viruses frequently encode IRES sequences that fold into RNA structures that bypass the need for one or more eukaryotic translation factors. (a) The poliovirus IRES bypasses the requirement for the 5' cap by directly binding to eIF4G. (b) The Cricket paralysis virus encodes an mRNA with an elaborate IRES that folds to resemble a tRNA that binds directly to the P-site of the 40S and 60S ribosomal subunits. The downstream mRNA is placed into the A-site and encodes for an Ala as the first amino acid.

# IRES – forget the factors!



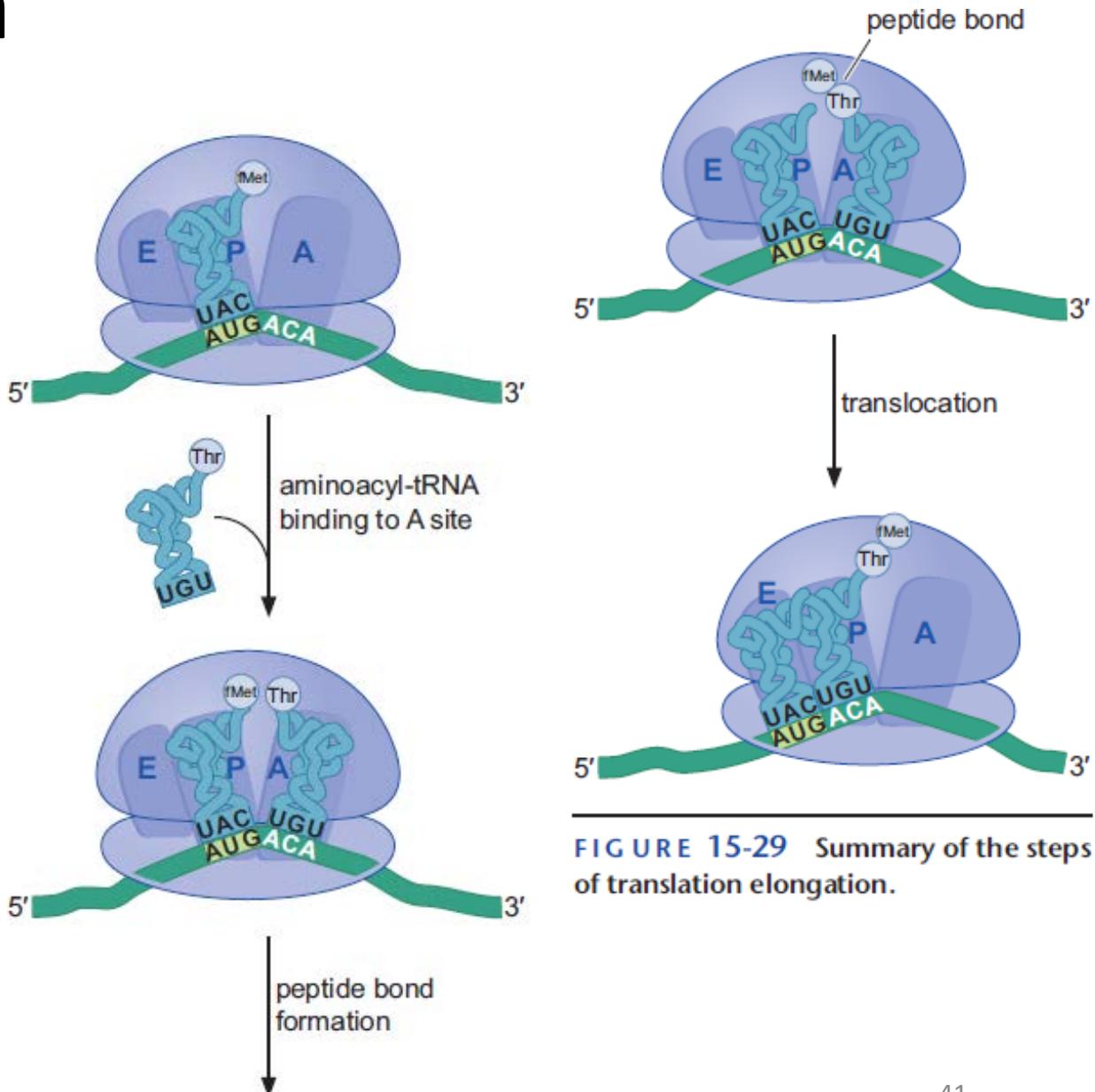
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# Translation in a nutshell - elongation

- Initiation
- **Elongation**
- Termination

# Moving on - elongation

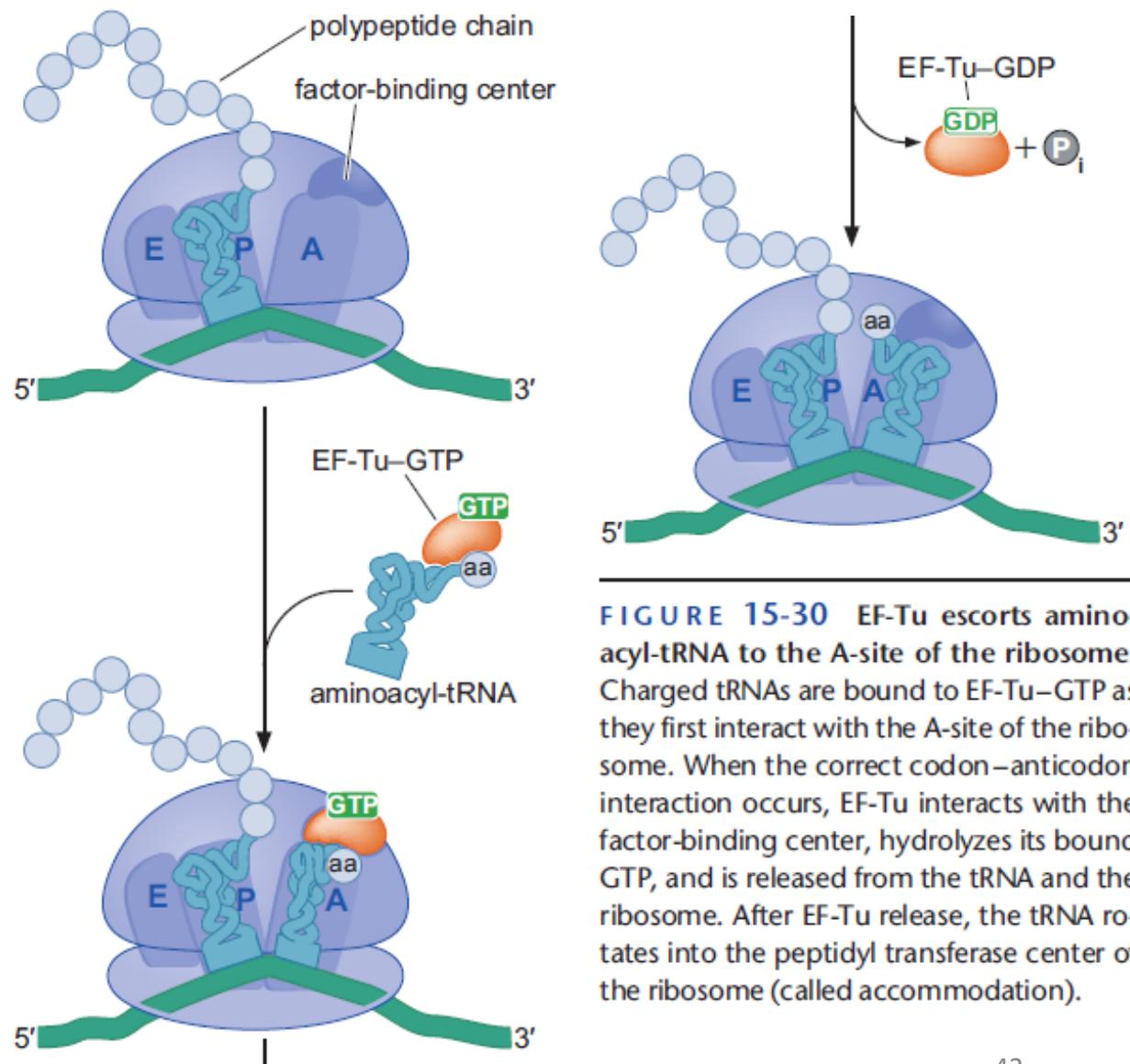
- Loading correct aminoacyl-tRNA into the A-site
- Peptide bond formation
- Translocation of ‘new’ peptidyl-tRNA from the A-site to the P-site
- Elongation factors



**FIGURE 15-29** Summary of the steps of translation elongation.

# Who are you, EF-Tu?

- EF-Tu elongation factor needed to “escort” tRNAs to the ribosome
  - Masks amino acid, prevents peptide bond formation
  - GTPase
- GTPase activated when it associates with large subunit factor-binding center
  - Interacts only after tRNA enters the A-site and correct codon-anticodon match is made
  - EF-Tu hydrolyzes its bound GTP and is released from the ribosome



**FIGURE 15-30** EF-Tu escorts aminoacyl-tRNA to the A-site of the ribosome. Charged tRNAs are bound to EF-Tu–GTP as they first interact with the A-site of the ribosome. When the correct codon–anticodon interaction occurs, EF-Tu interacts with the factor-binding center, 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 (called accommodation).



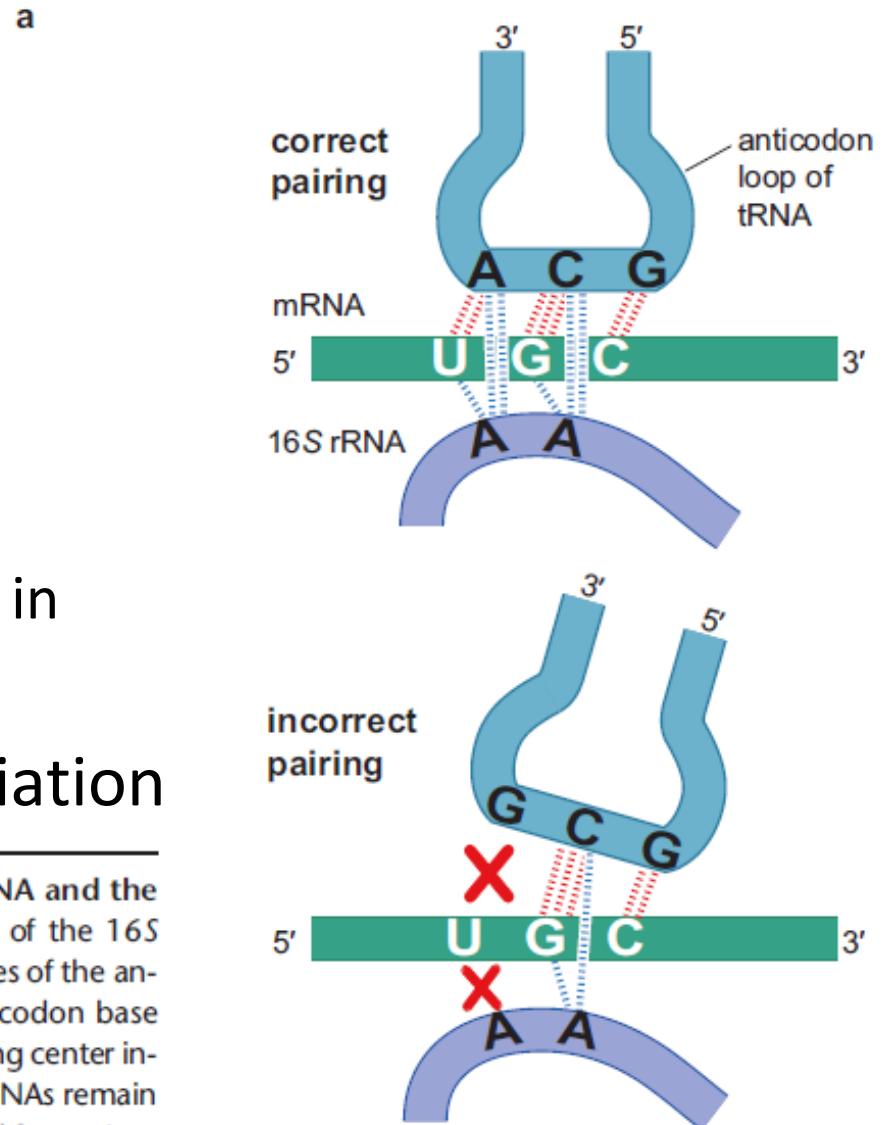
# Ribosome has means to be accurate

- Error rate of translation is  $10^{-3}$  to  $10^{-4}$   
no more than one in every 1000 amino acids incorporated into protein is incorrect
- Energy differences in anticodon-codon basepairing not enough
- Three mechanisms

# Ribosome has means to be accurate - one

- Two bases in the 16S rRNA form hydrogen bonds
  - Minor groove of the anticodon-codon pair
  - Watson-Crick base pairs can form H-bonds
  - Minor groove from non-Watson-Crick base pairs or mismatched base pairs not recognized, resulting in significantly reduced affinity for incorrect tRNAs
- **Correctly paired tRNAs have low rate of dissociation**

**FIGURE 15-31** Three mechanisms to ensure correct pairing between the tRNA and the mRNA. (a) Additional hydrogen bonds are formed between two adenine residues of the 16S rRNA and the minor groove of the anticodon–codon pair only when the first two bases of the anticodon–codon pair form correct Watson–Crick base pairs. (b) Correct codon–anticodon base pairing facilitates EF-Tu bound to the aminoacyl-tRNA to interact with the factor-binding center inducing GTP hydrolysis and EF-Tu release. (c) Only correctly base-paired aminoacyl-tRNAs remain associated with the ribosome as they rotate into the correct position for peptide-bond formation. This rotation is referred to as tRNA accommodation.



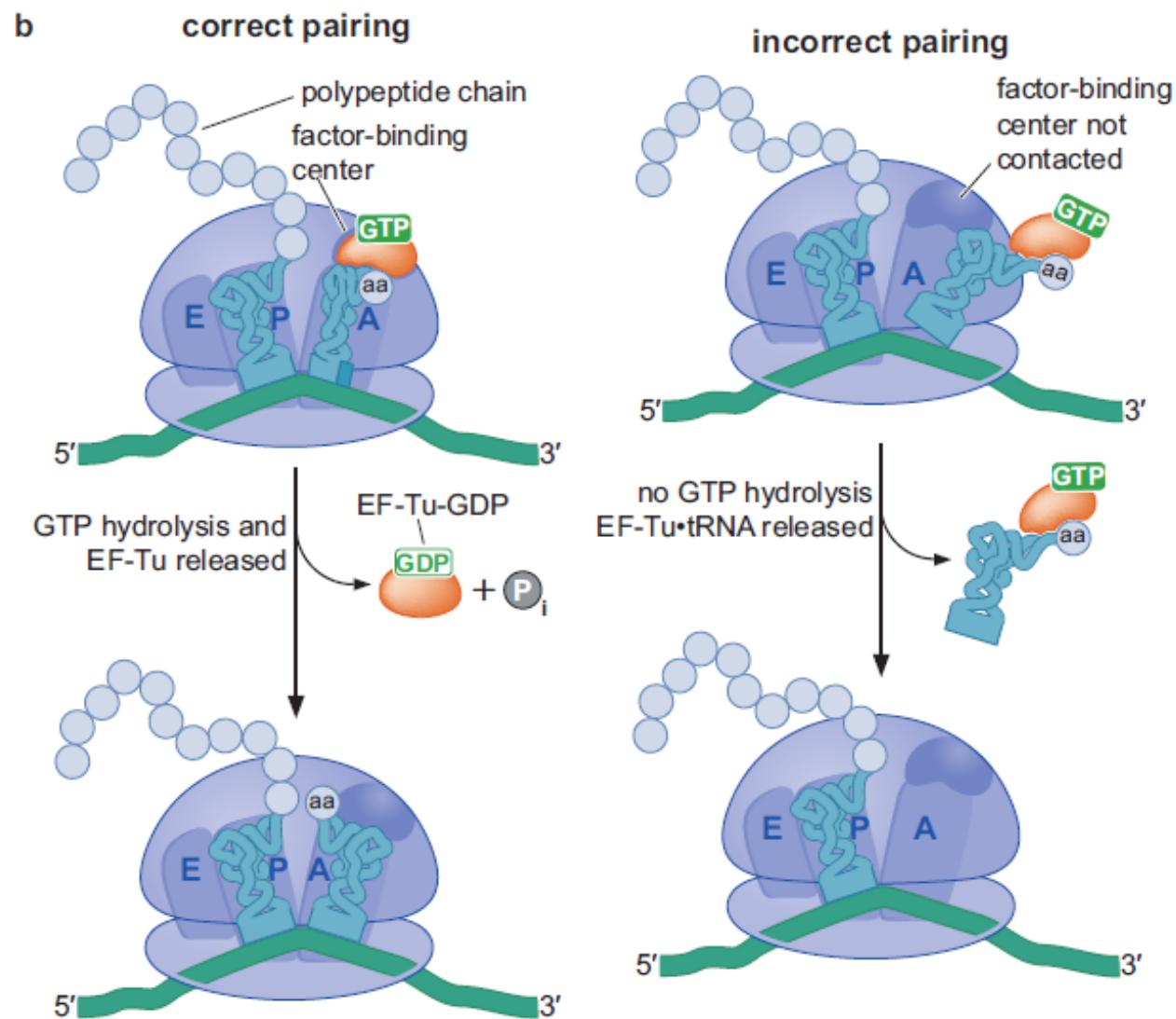
# Ribosome has means to be accurate - two

- GTPase activity of EF-Tu
  - Highly sensitive to correct pairing

**Correct codon–anticodon base pairing** facilitates EF-Tu bound to the aminoacyl-tRNA to interact with the factor-binding center **inducing GTP hydrolysis** and EF-Tu release

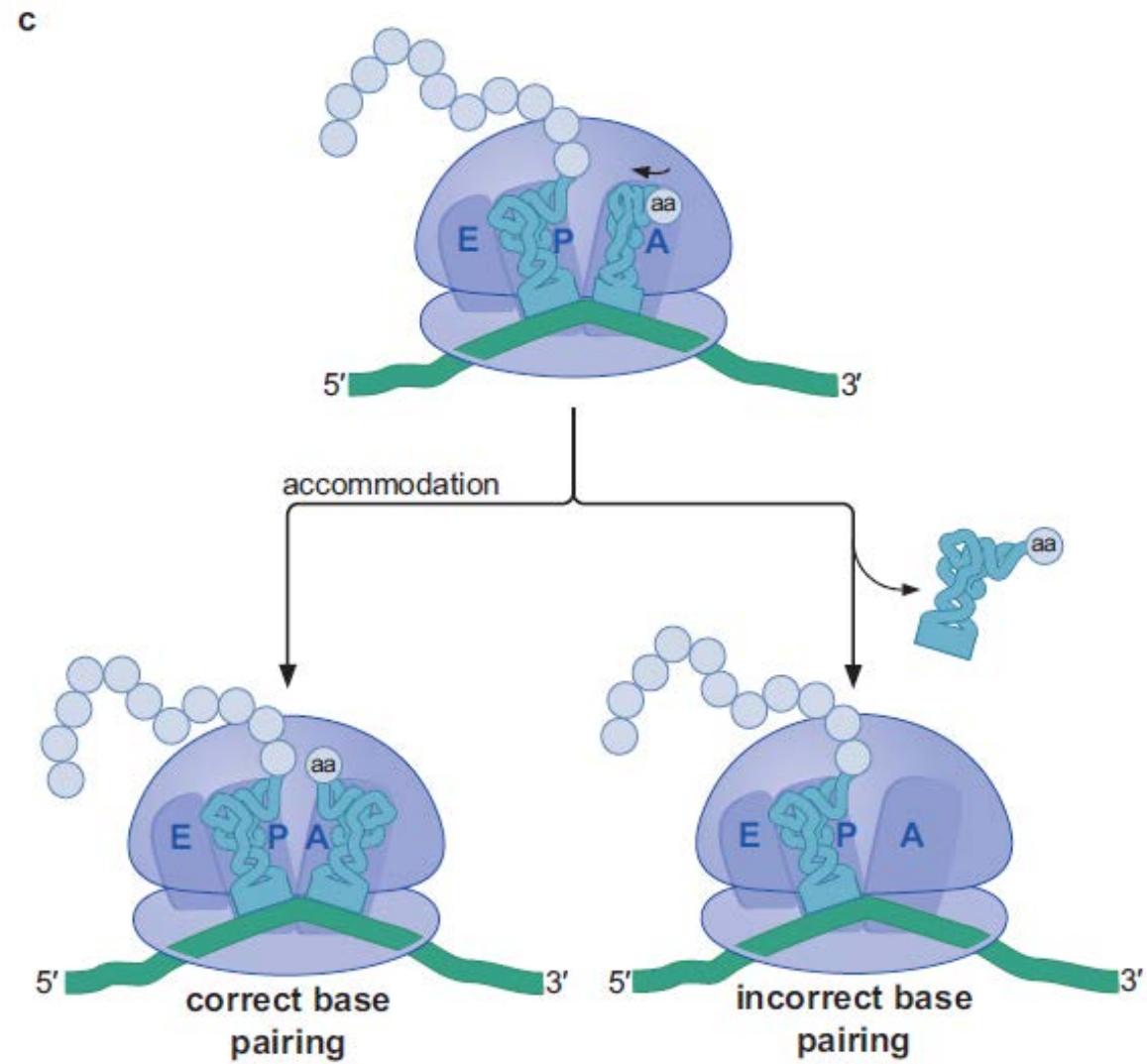
As described above, release of EF-Tu from the tRNA requires **GTP hydrolysis**, which is **highly sensitive to correct codon– anticodon base pairing**. Even a single mis-match in the codon– anticodon base pairing alters the position of EF-Tu, reducing its ability to interact with the factor-binding center.

**FIGURE 15-31** Three mechanisms to ensure correct pairing between the tRNA and the mRNA. (a) Additional hydrogen bonds are formed between two adenine residues of the 16S rRNA and the minor groove of the anticodon–codon pair only when the first two bases of the anticodon–codon pair form correct Watson–Crick base pairs. (b) Correct codon–anticodon base pairing facilitates EF-Tu bound to the aminoacyl-tRNA to interact with the factor-binding center inducing GTP hydrolysis and EF-Tu release. (c) Only correctly base-paired aminoacyl-tRNAs remain associated with the ribosome as they rotate into the correct position for peptide-bond formation. This rotation is referred to as tRNA accommodation.



# Ribosome has means to be accurate - three

- **Proofreading** after EF-Tu release
  - **Accommodation** – rotation of tRNA 3' end into the peptidyl transferase center
  - **Incorrectly paired tRNA dissociate** – the rotation of the tRNA places a strain on the codon–anticodon interaction



**FIGURE 15-31** Three mechanisms to ensure correct pairing between the tRNA and the mRNA. (a) Additional hydrogen bonds are formed between two adenine residues of the 16S rRNA and the minor groove of the anticodon–codon pair only when the first two bases of the anticodon–codon pair form correct Watson–Crick base pairs. (b) Correct codon–anticodon base pairing facilitates EF-Tu bound to the aminoacyl-tRNA to interact with the factor-binding center inducing GTP hydrolysis and EF-Tu release. (c) Only correctly base-paired aminoacyl-tRNAs remain associated with the ribosome as they rotate into the correct position for peptide-bond formation. This rotation is referred to as tRNA accommodation.

In Prokaryotes, the **50S (23S component) ribosome subunit contains the peptidyl transferase component and acts as a ribozyme**. The peptidyl transferase center on the 50S subunit lies at the lower tips (acceptor ends) of the A- and P- site tRNAs.

In Eukaryotes, the **60S (28S component) ribosome subunit contains the peptidyl transferase component and acts as the ribozyme**.

Peptidyl transferases are not limited to translation, but there are relatively few enzymes with this function.

In 1967, Carl Woese, Francis Crick, and Leslie Orgel were the first to suggest that RNA could act as a catalyst - as ribozyme.

Ribozymes (ribonucleic acid enzymes) are RNA molecules that have the ability to catalyze specific biochemical reactions, including RNA splicing in gene expression, similar to the action of protein enzymes. The 1982 discovery of ribozymes demonstrated that RNA can be both genetic material (like DNA) and a biological catalyst (like protein enzymes), and contributed to the RNA world hypothesis, which suggests that RNA may have been important in the evolution of prebiotic self-replicating systems. The most common activities of natural or in vitro-evolved ribozymes are the cleavage or ligation of RNA and DNA and peptide bond formation.<sup>2</sup> For example, the smallest ribozyme known (G<sub>4</sub>GGC-3') can aminoacylate a G<sub>4</sub>C<sub>3</sub>-3' sequence in the presence of PheA<sup>+</sup>.<sup>3</sup> Within the ribosome, ribozymes function as part of the large subunit ribosomal RNA to link amino acids during protein synthesis. They also participate in a variety of RNA processing reactions, including RNA splicing, viral replication, and transfer RNA biosynthesis.

In summary, in addition to the codon–anticodon interactions,

the ribosome used minor groove interactions and two phases of proofreading to ensure that a correct aminoacyl-tRNA binds in the A-site.

Each of these three additional selectivity mechanisms inhibits retention of aminoacyl tRNAs that do not form correct codon–anticodon interactions.

# Peptide bond formation – actions of an ribozyme

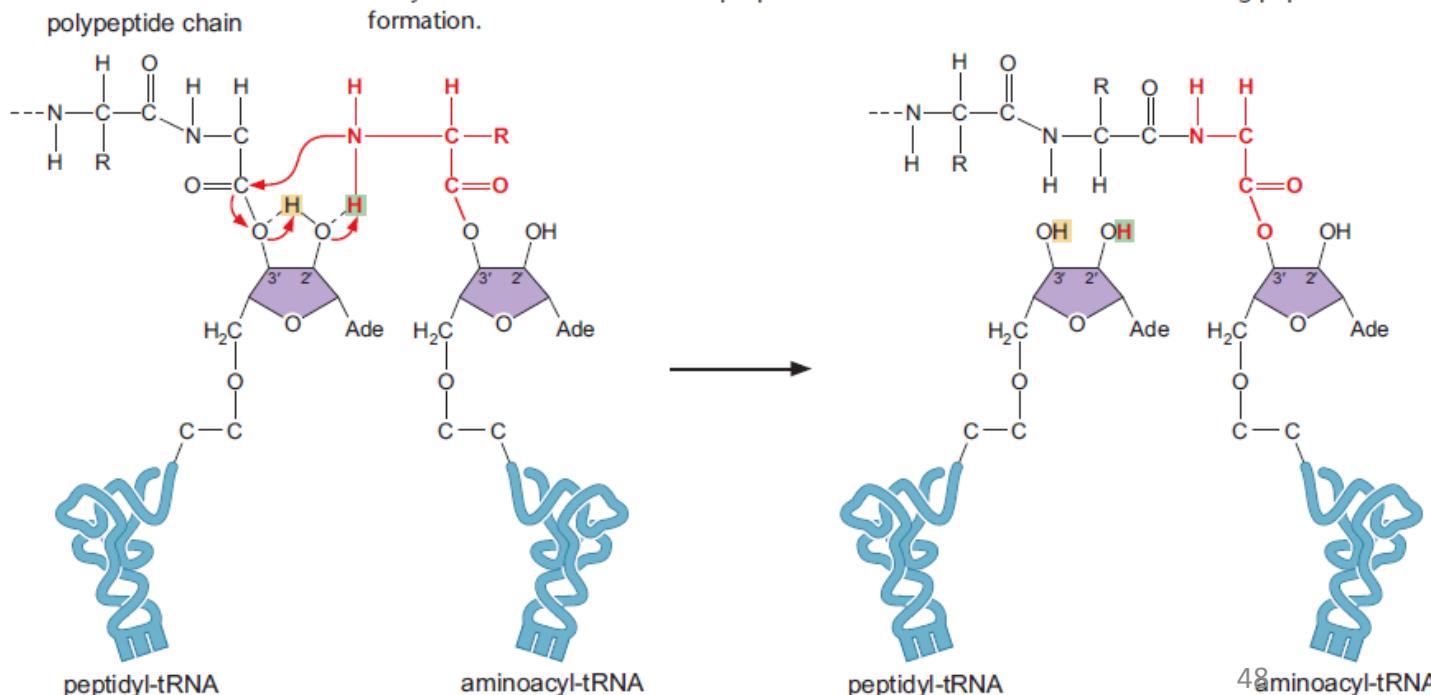
- Entropic catalysis – bring the substrates together to stimulate catalysis

- Other RNA elements contribute

- Basepairing between 23S rRNA and tRNA CCA end
- E. coli 23S rRNA (A2451) (10-fold reduced)
- 2'-OH of P-site tRNA residue (10e6-fold reduced)

Consistent of CCA end (the 3' end of tRNA) is absolutely conserved – this is the site that is attached to the cognate amino acid!!!

CCA End - The CCA tail is a cytosine-cytosine-adenine sequence at the 3' end of the tRNA molecule.



# Translocation

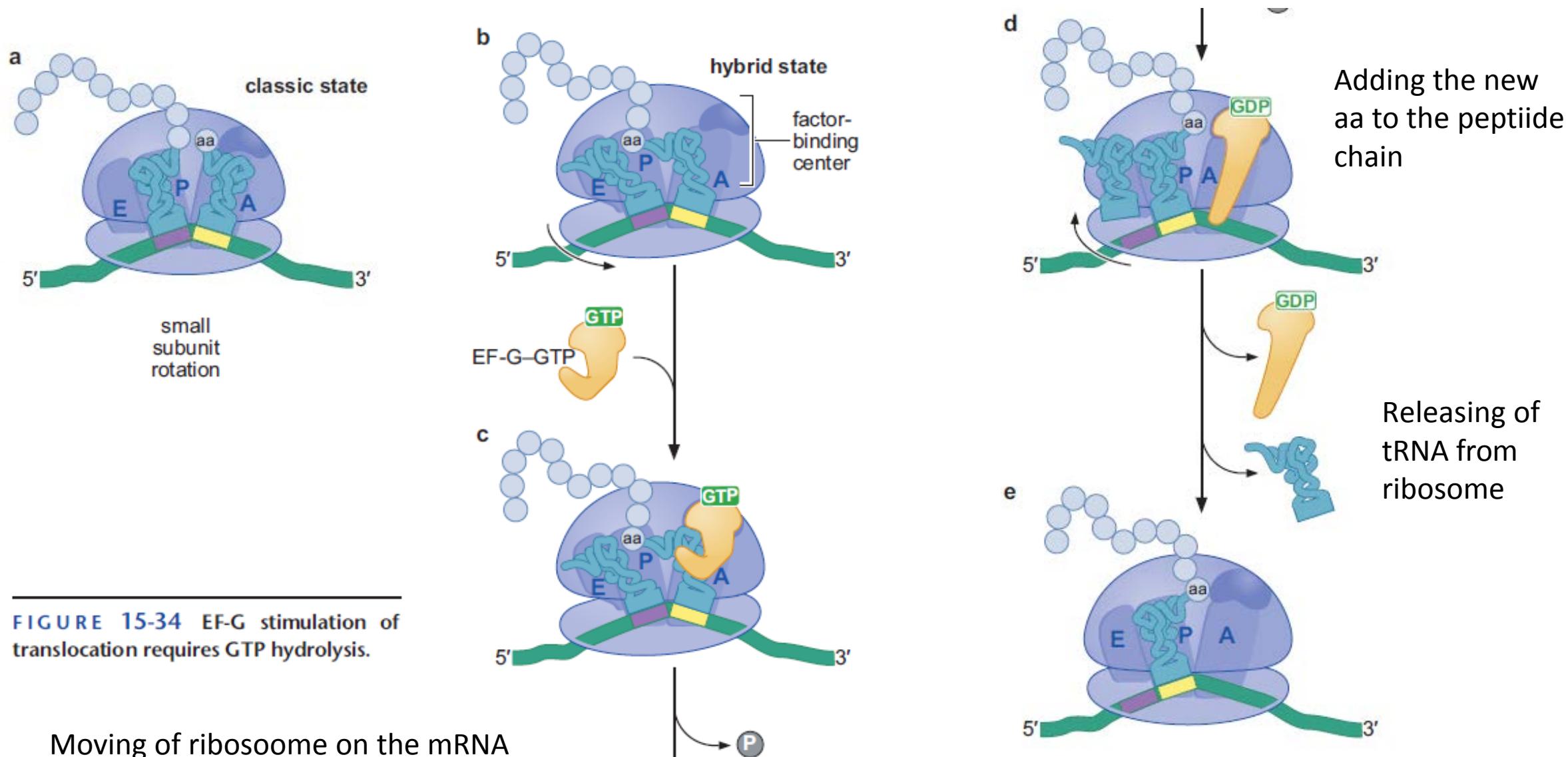
Peptide-Bond Formation Initiates **translocation** in the Large Subunit

- P-site deacetylated tRNA -> E-site
- A-site peptidyl-tRNA -> P-site
- mRNA must move by three nucleotides
- **Translocation is initiated in the large subunit** before the small subunit, tRNAs are in “hybrid states”



# Translocation with EF-G

Translocation is initiated in the large subunit before the small subunit, and the tRNAs are said to be in “hybrid states.”



**FIGURE 15-34** EF-G stimulation of translocation requires GTP hydrolysis.

Moving of ribosome on the mRNA

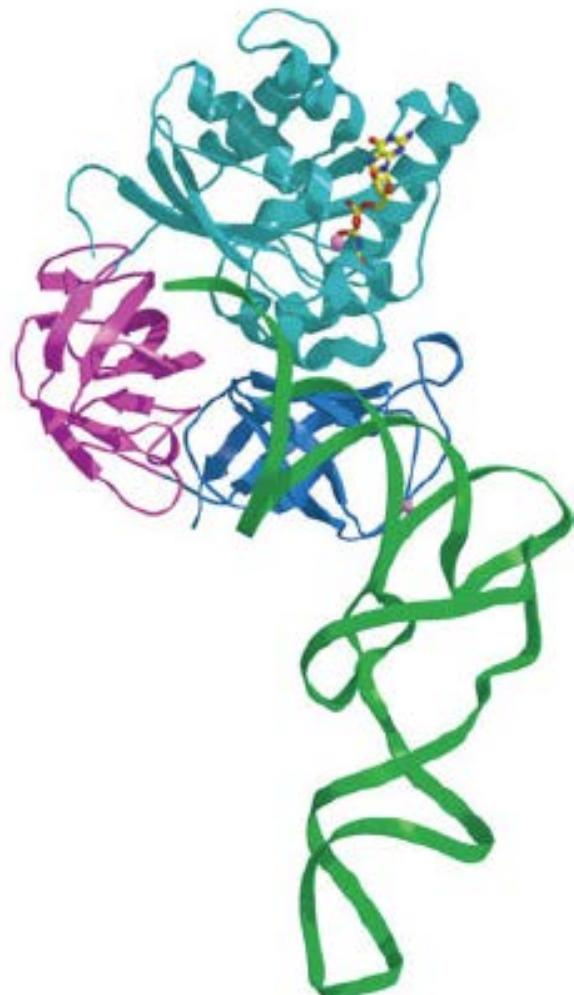
# EF-G actions

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

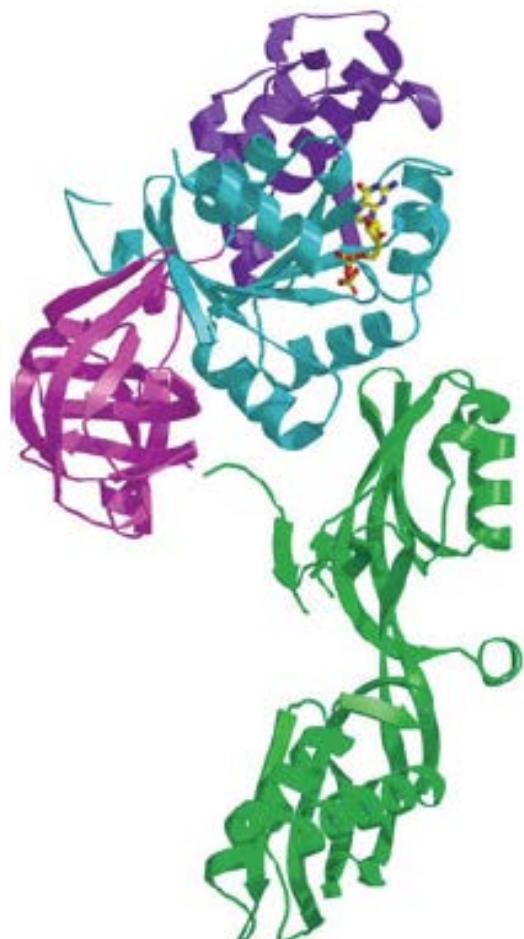
- GTPase
  - After peptidyl transferase reaction, EF-G:GTP binds to the ribosome
  - Stabilizes the rotated, hybrid state
- GTP hydrolysis changes the conformation of EF-G
  - “gates” are opened, A-site tRNA moves to the P-site
  - EF-G:GDP binds to the A-site
  - Basepairing between the tRNAs and the mRNA cause the mRNA to move by 3 nt
  - Small subunit rotates back to its starting position
  - EF-G:GDP is released

# EF-Tu versus EF-G – molecular mimicry

EF-Tu–GDPNP–  
Phe–tRNA



EF-G–  
GDP



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

What is most remarkable about this similarity is that even though EF-G is composed of a single polypeptide, its structure mimics that of a tRNA bound to a protein. This is an example of “molecular mimicry” in which a protein takes on the appearance of a tRNA to facilitate association with the same binding site.

**FIGURE 15-35** Structural comparison of elongation factors. EF-Tu–GDPNP–Phe–tRNA is shown on the top and EF-G–GDP is shown on the bottom. GDPNP is an analog of GTP that cannot be hydrolyzed that is used to lock the molecule in the GTP-bound conformation during the determination of the 3D structure. Note the similarity between the structure of the green domain in EF-G and the tRNA bound to EF-Tu (also shown in green). (Top structure: Nissen P. et al. 1995. *Science* 270: 1464–1472. Bottom structure: al-Karadaghi S. et al. 1996. *Structure* 4: 555–565.) Images prepared with MolScript, BobScript, and Raster3D.

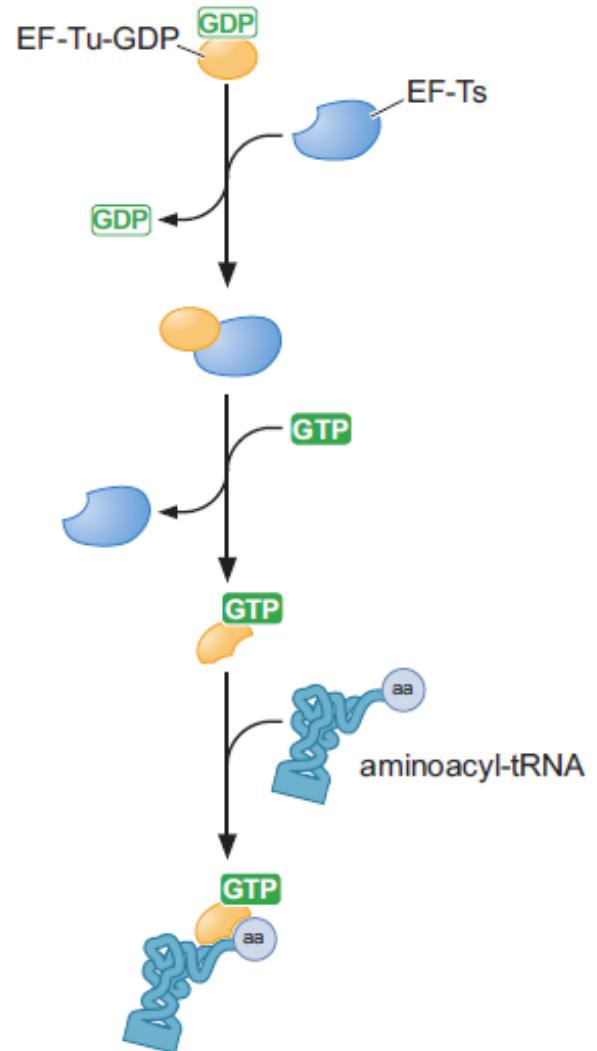
# New bullet, please - EF-Ts loads EF-Tu

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

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

- GTP exchange factor for EF-Tu
  - EF-Tu:GDP binds EF-Ts, GDP is released
  - GTP binds to EF-Tu:EF-Ts complex, EF-Ts is released
  - EF-Tu:GTP binds aminoacyl-tRNA
- Elongation cycle starts again

**FIGURE 15-36** EF-Ts stimulates release of GDP from EF-Tu. GDP bound to EF-Tu is released very slowly in isolation. EF-Ts binds EF-Tu–GDP and causes the rapid release of GDP. GTP binding to EF-Tu in the EF-Tu–EF-Ts complex displaces EF-Ts and leaves EF-Tu–GTP, which can then bind a new aminoacyl-tRNA for delivery to the ribosome.

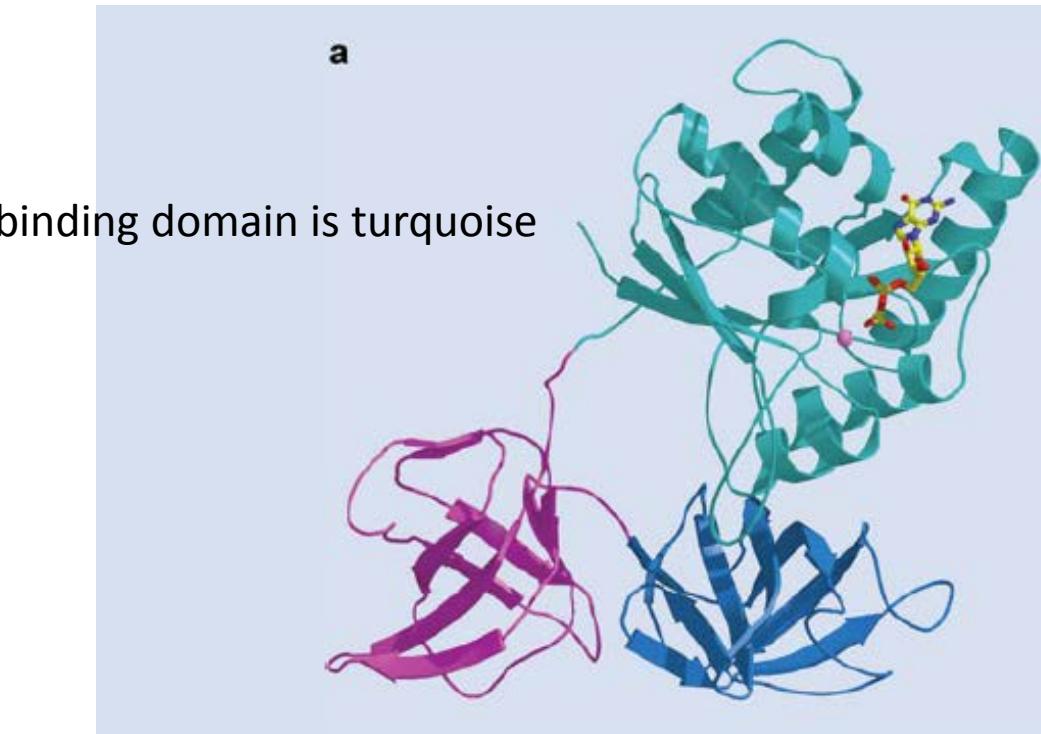


# How much energy is required for one peptide bond formation?

- One ATP molecule (where?)
  - Drives peptide bond synthesis (peptidyl transferase reaction) – Fig. 15 - 6
- Two GTP molecules (where?) - correct codon-anticodon base pairing
  - Accuracy – Fig. 15-31

# GTP versus GDP

EF-Tu bound to GDP



GTP-binding domain is turquoise

EF-Tu bound to GTP



The formation of a strong tRNA-binding site when GTP is bound

**BOX 15-4 FIGURE 1** Comparison of EF-Tu bound to GDP and GTP. (a) EF-Tu bound to GDP. (b) EF-Tu bound to GTP. The GTP-binding domain is turquoise. The rotation of the magenta domain and the changes in the structure of the turquoise and blue domains lead to the formation of a strong tRNA-binding site when GTP is bound (see Fig. 15-35). GTP is depicted in stick representation. (a, Polekhina G. et al. 1996. *Structure* 4: 1141–1151; b, Kjeldgaard M. et al. 1993. *Structure* 1: 35–50.) Images prepared with MolScript, BobScript, and Raster3D.

# Translation in a nutshell – the last step - termination

- Initiation
- Elongation
- **Termination**

# Release me, please!

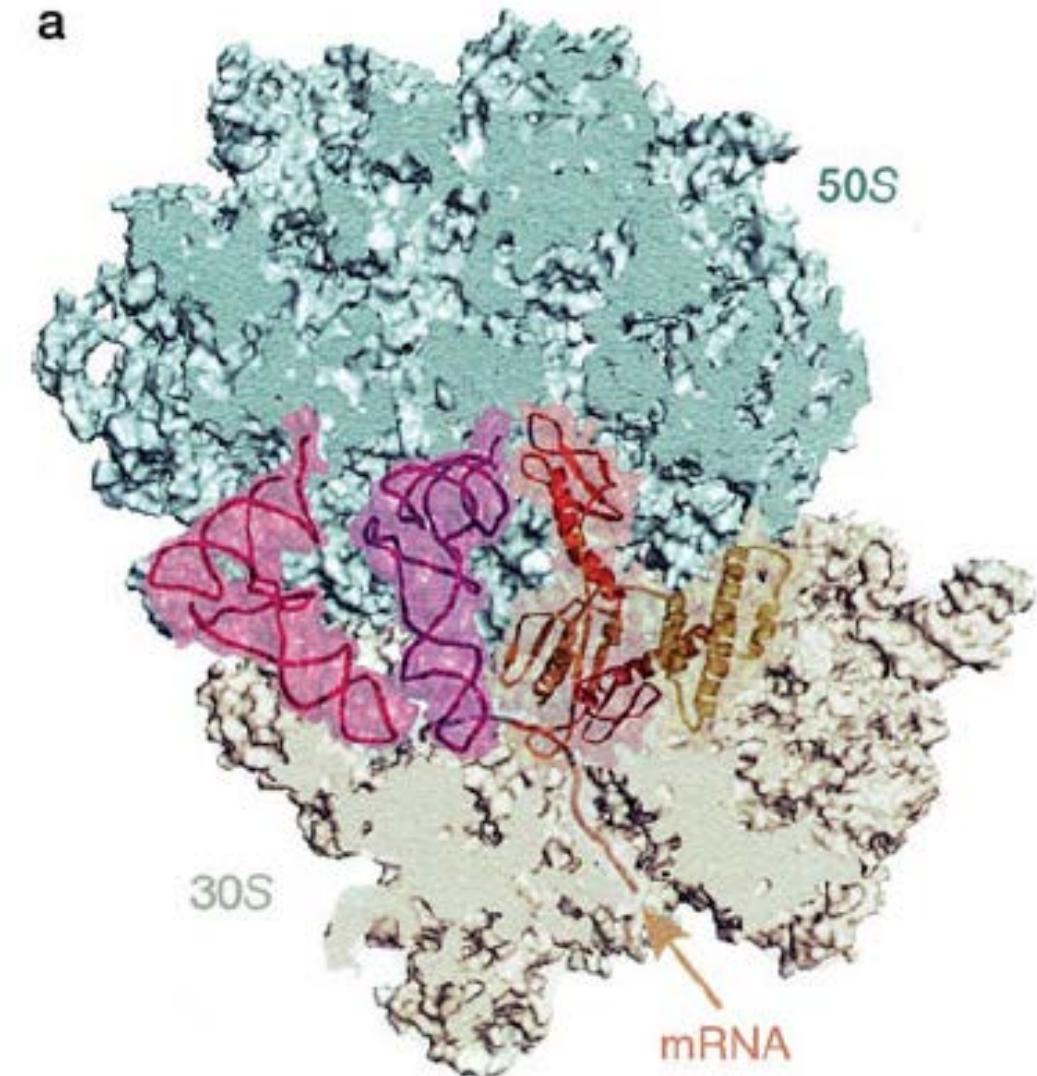
- If there is an initiator tRNA, is there a terminator tRNA?
- Release factors
  - Class I – recognize stop codons and trigger hydrolysis of peptide bond
    - RF1 – UAG, UAA
    - RF2 – UGA, UAA
    - eRF1 – all three stop codons
  - Class II – stimulate the dissociation of class I factors from the ribosome (GTPase)
    - RF3
    - eRF3

# Stop codon recognition - Release Factors

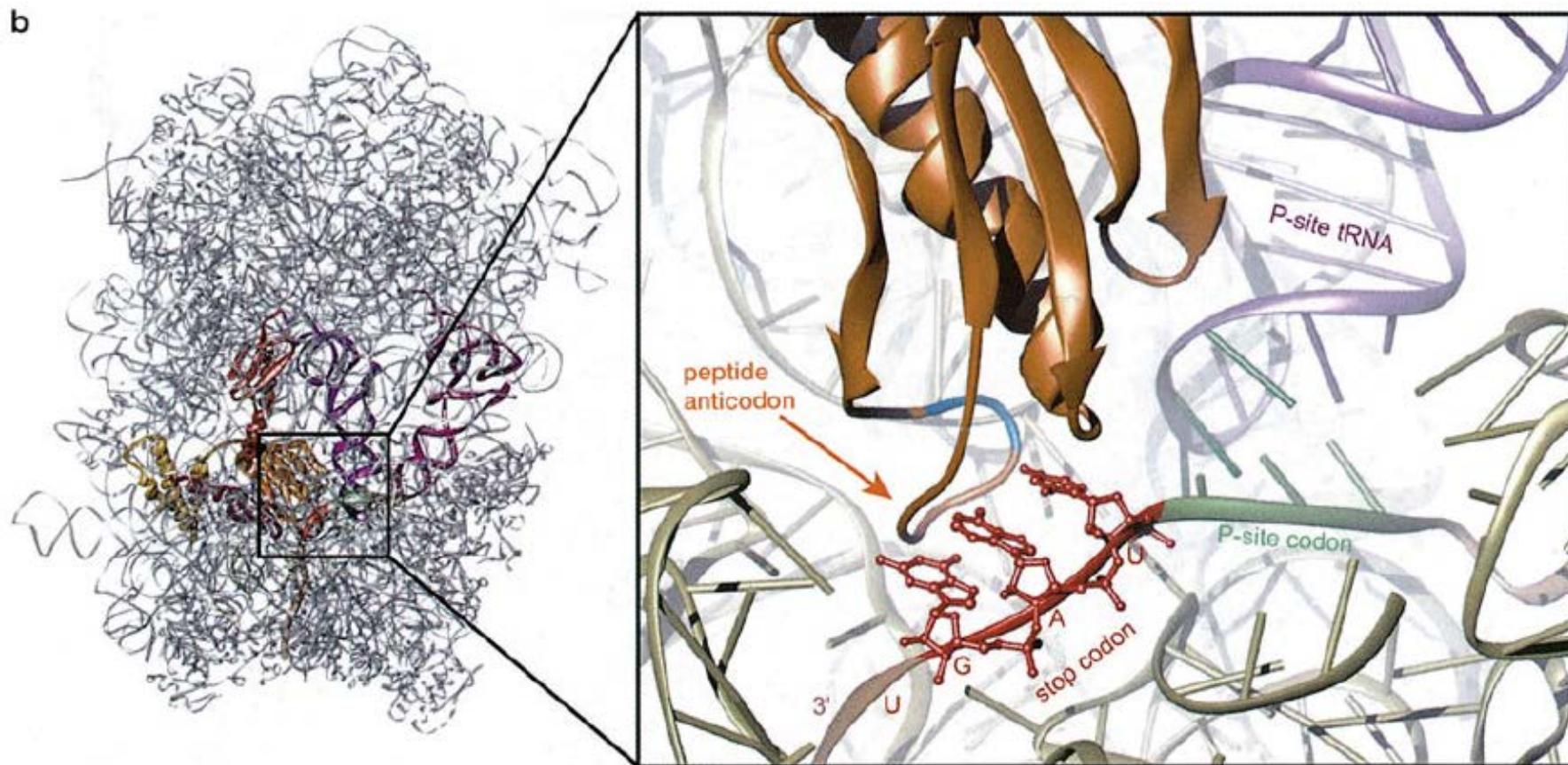
## Terminate Translation in Response to Stop Codons

- No RNA involved
- Peptide anticodon – three amino acids
- Stop codons are recognized by proteins called release factors (RFs) that activate the hydrolysis of the polypeptide from the peptidyl-tRNA.

**FIGURE 15-37** 3D structures of RF1 bound to the ribosome. (a) This view shows RF1 binding to the A-site of the ribosome. (b) This structure shows that the peptide anticodon is located very near the anticodon. (c) In this view, the structure of RF1 bound to the ribosome shows the GGQ motif located close to the 3' end of the P-site tRNA and the peptidyl transferase center. (Adapted, with permission, from Petry et al. 2005. *Cell* 123: 1255–1266. © Elsevier.)



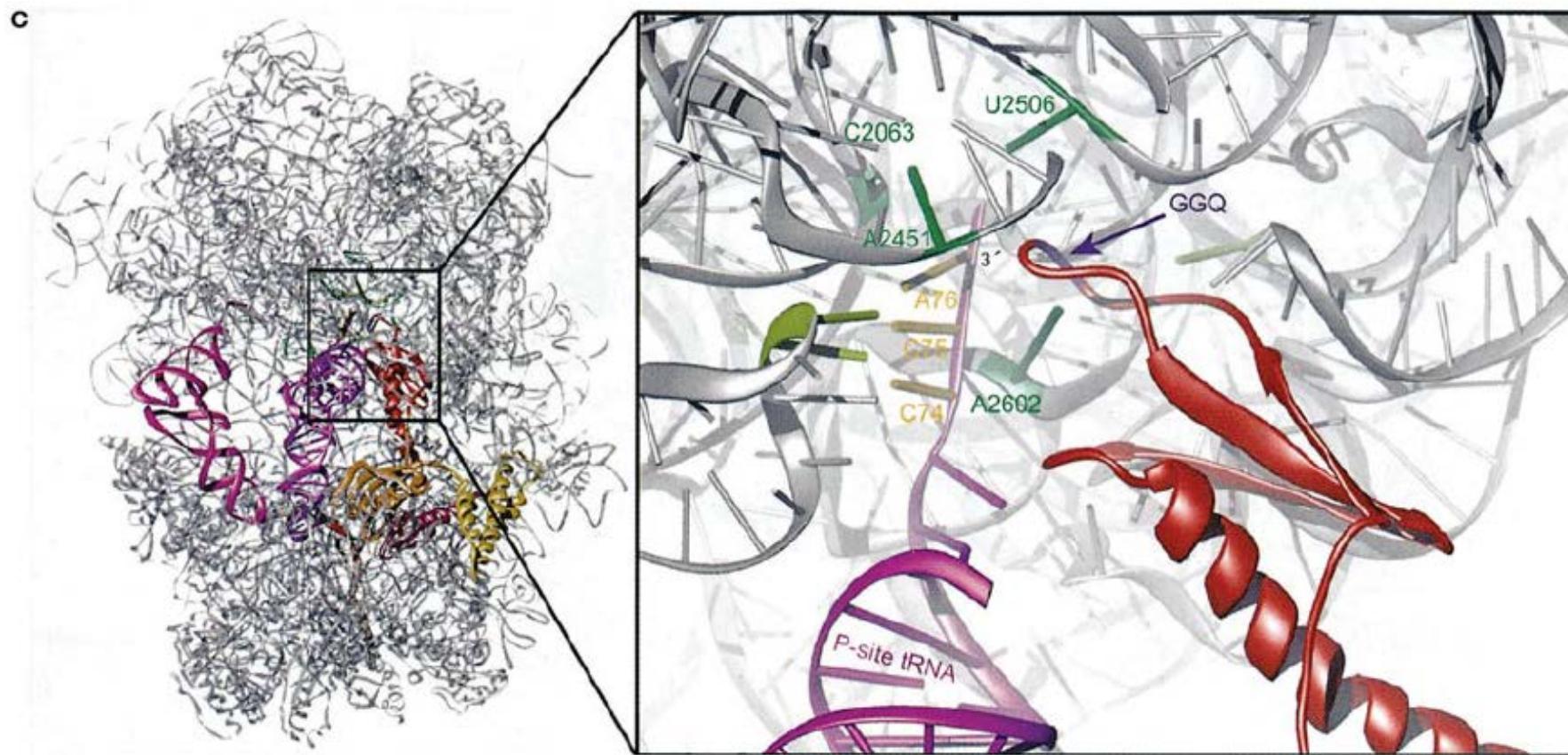
# Stop codon recognition



**FIGURE 15-37** 3D structures of RF1 bound to the ribosome. (a) This view shows RF1 binding to the A-site of the ribosome. (b) This structure shows that the peptide anticodon is located very near the anticodon. (c) In this view, the structure of RF1 bound to the ribosome shows the GGQ motif located close to the 3' end of the P-site tRNA and the peptidyl transferase center. (Adapted, with permission, from Petry et al. 2005. *Cell* 123: 1255–1266. © Elsevier.)

# Peptidyl-tRNA hydrolysis

**FIGURE 15-37** 3D structures of RF1 bound to the ribosome. (a) This view shows RF1 binding to the A-site of the ribosome. (b) This structure shows that the peptide anticodon is located very near the anticodon. (c) In this view, the structure of RF1 bound to the ribosome shows the GGQ motif located close to the 3' end of the P-site tRNA and the peptidyl transferase center. (Adapted, with permission, from Petry et al. 2005. *Cell* 123: 1255–1266. © Elsevier.)



# Two-step stopping

- Stop codon recognition and polypeptide release
- Dissociation of RF1/RF2

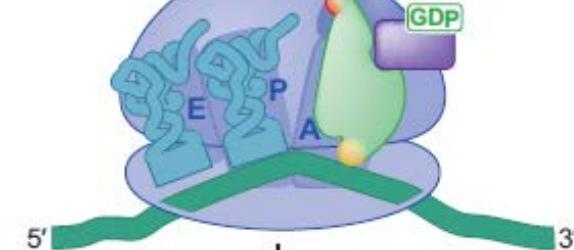
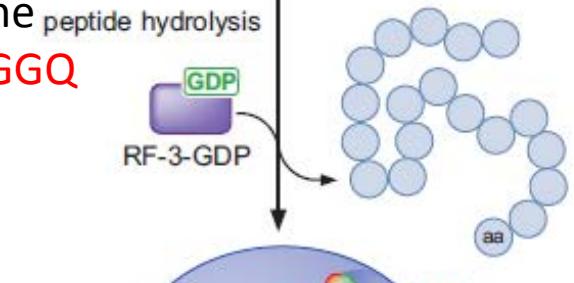
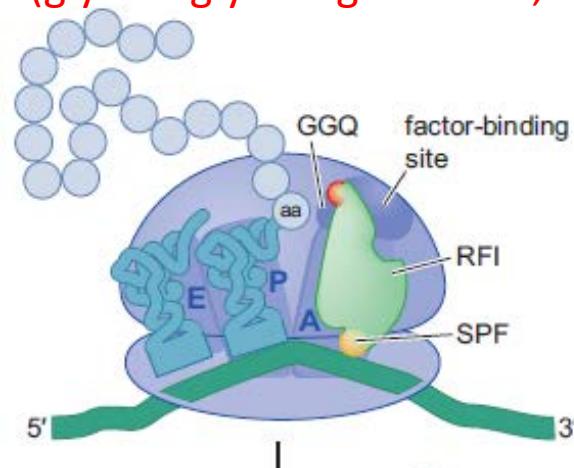
The class I release factor (shown here as RF1) recognizes the stop codon and stimulates polypeptide release through a **GGQ motif** that is localized to the peptidyl transferase center

The class II release factor (RF3) binds only after polypeptide release and drives the dissociation of the class I release factor

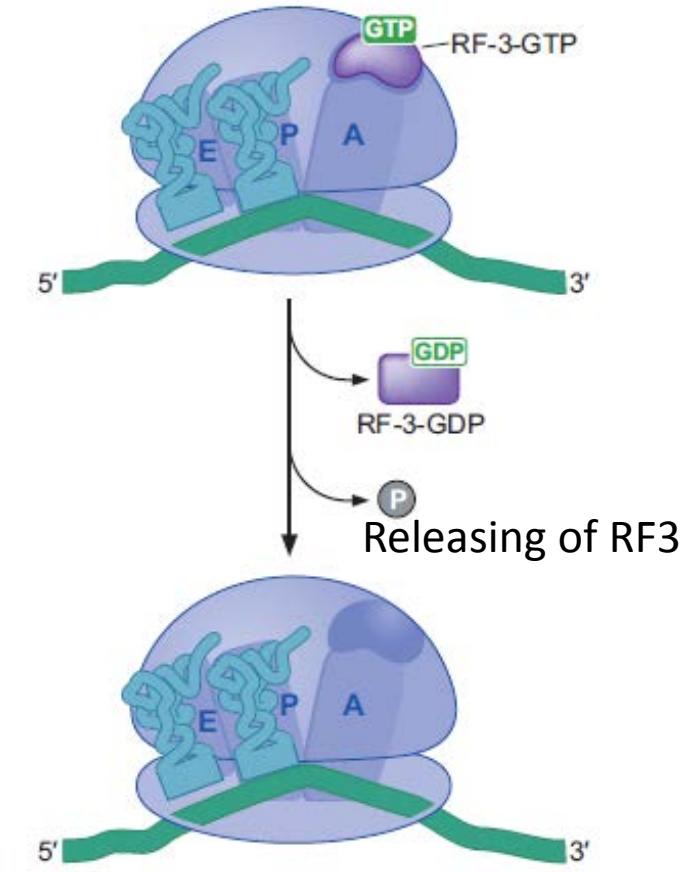
As with other GTP-binding proteins involved in translation, this interaction stimulates the hydrolysis of GTP. In the absence of a bound class I factor, the resulting **RF3-GDP** has a low affinity for the ribosome and is released.

GTP energy

A conserved three-amino-acid sequence (glycine glycine glutamine, GGQ)



Releasing of RF1



**FIGURE 15-39** Polypeptide release is catalyzed by two release factors. The class I release factor (shown here as RF1) recognizes the stop codon and stimulates polypeptide release through a GGQ motif that is localized to the peptidyl transferase center. The class II release factor (RF3) binds only after polypeptide release and drives the dissociation of the class I release factor.

# Even ribosomes recycle

To participate in a new round of polypeptide synthesis (the tRNAs and the mRNA must be removed from the ribosome, and the ribosome Must dissociate into its large and small subunits) – we need RRF

- Ribosome recycling factor (RRF) – mimics a tRNA
  - Binds to the empty A-site, mimics a tRNA
  - Recruits EF-G:GTP, stimulates the release of uncharged tRNAs

In prokaryotic cells, a factor known as the ribosome recycling factor (RRF) cooperates with EF-G and IF3 to recycle ribosomes after polypeptide release

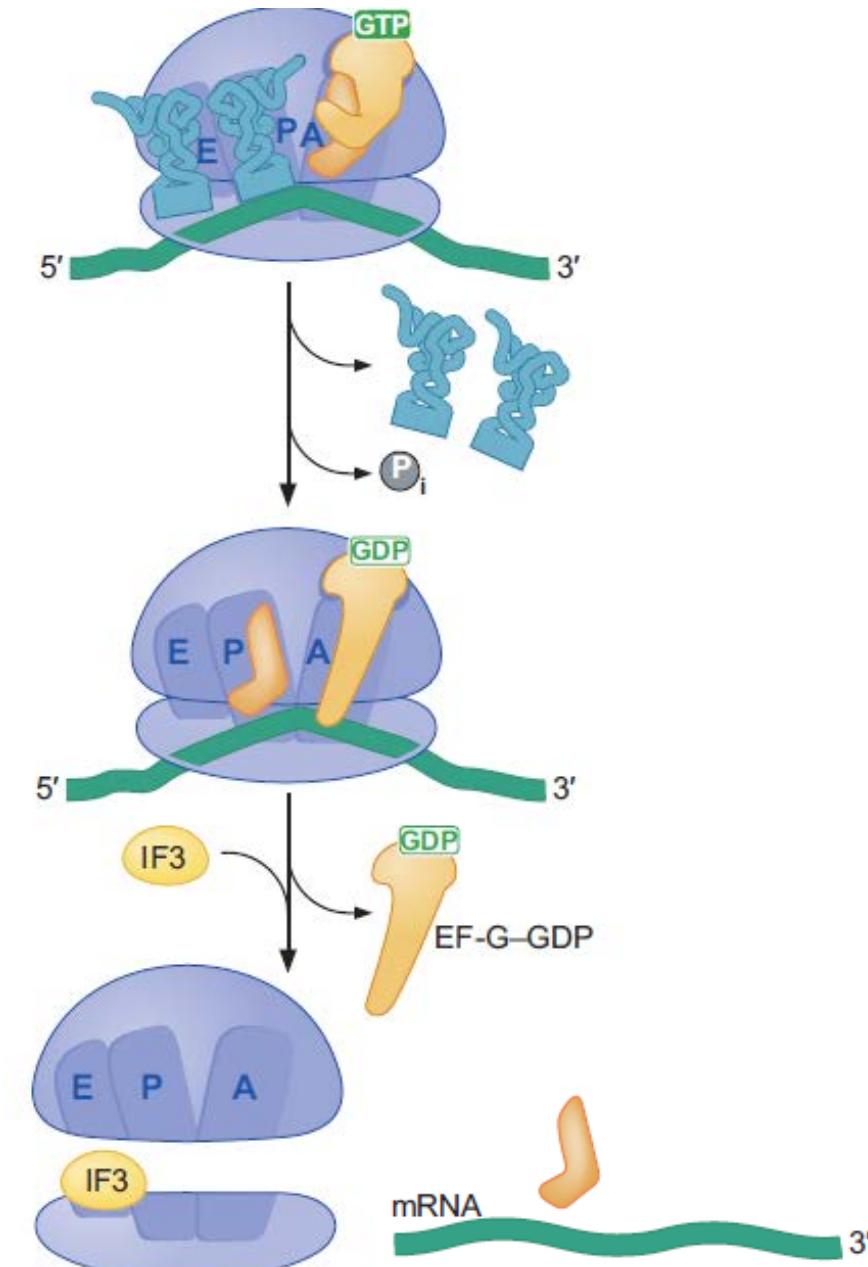
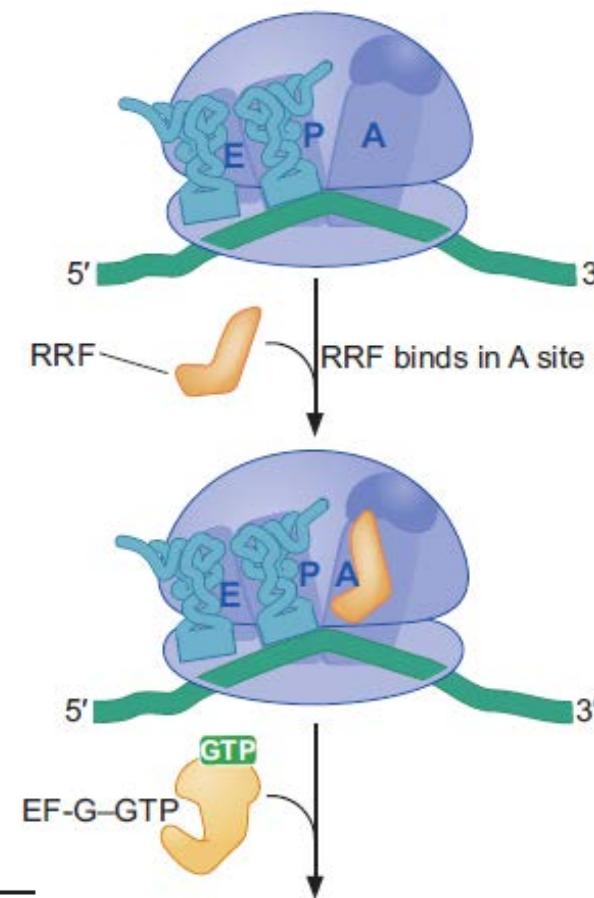


FIGURE 15-40 RRF and EF-G combine to stimulate the release of tRNA and mRNA from a terminated ribosome.

# Termination and recycling in eukaryotes

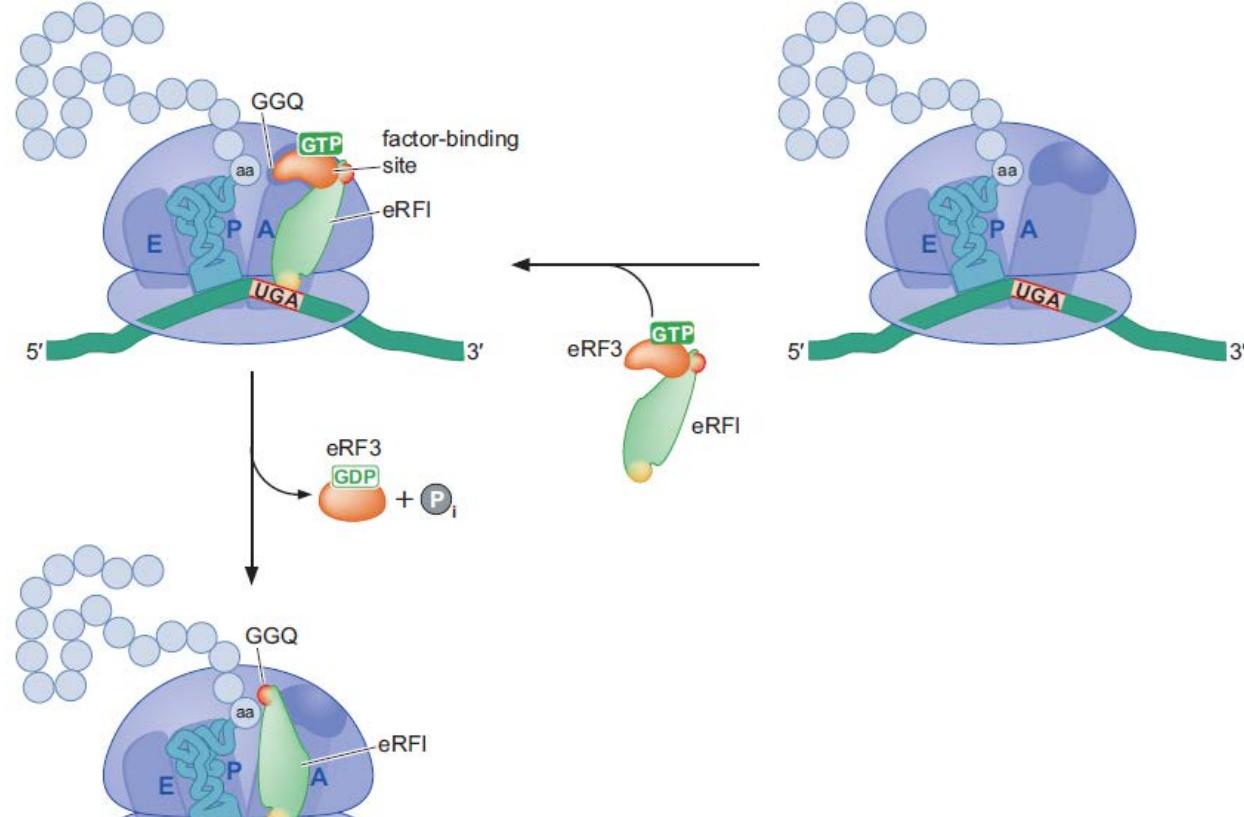
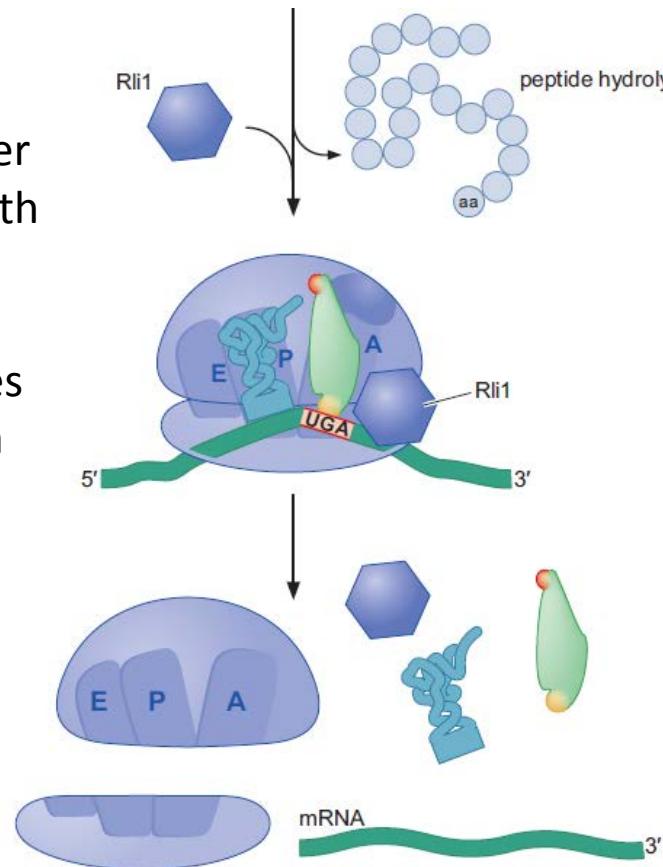
- Eukaryotic and prokaryotic release factors have different structure and amino acid sequence
  - eRF1 functions similar to RF1 and RF2
  - eRF3 delivers eRF1 to the ribosome
- No known recycling factor in eukaryotes
  - eEF2 (eukaryotic EF-G) does not participate in recycling

# Termination and recycling in eukaryotes

Rli1 = eRF1 after conjunction with an ATPase

eRF1 recognizes the stop codon

Rli1 = translation initiation factor 1 in eukaryotes (*Saccharomyces cerevisiae*)



**FIGURE 15-41** Eukaryotic translation termination and ribosome recycling.