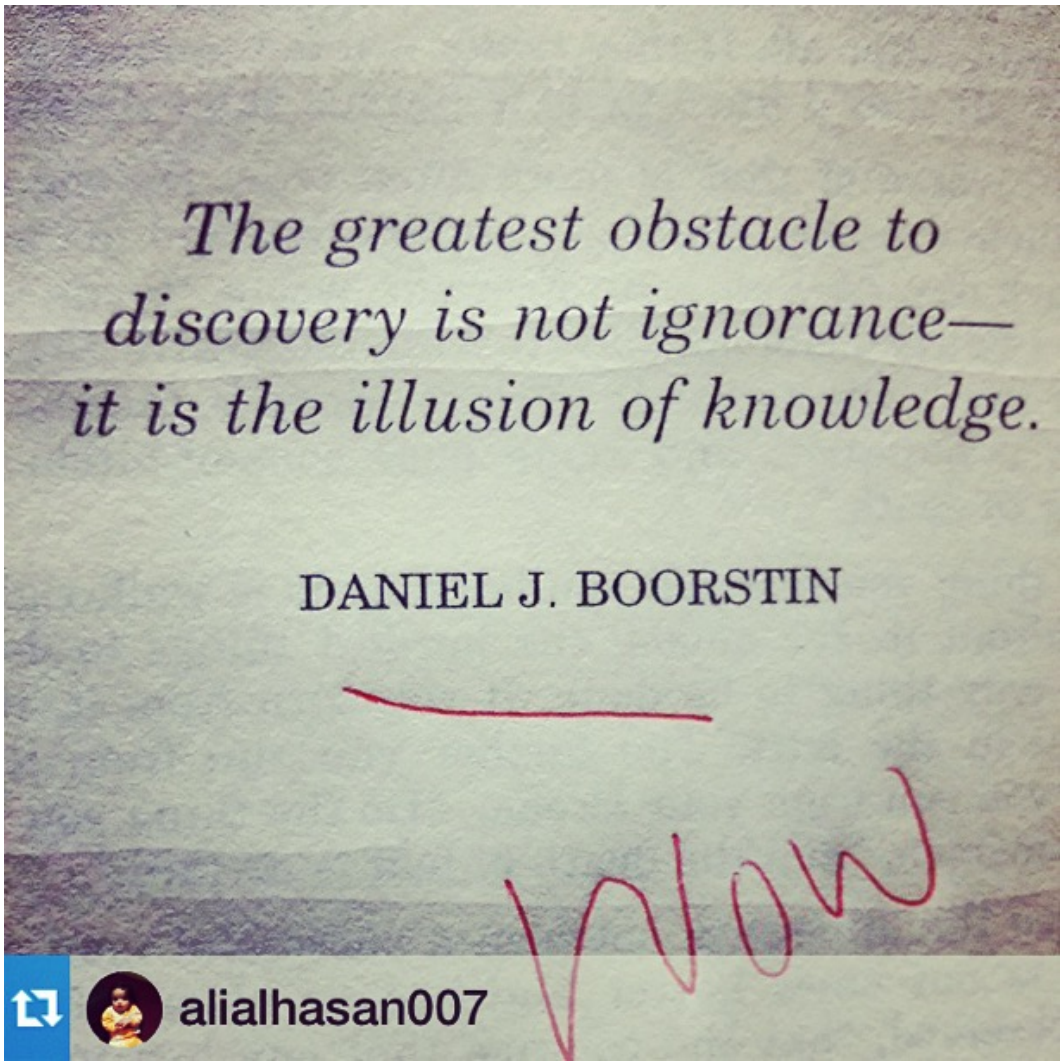
Lecture 22:

Translation in prokaryotes

Course 371

Lessons for life

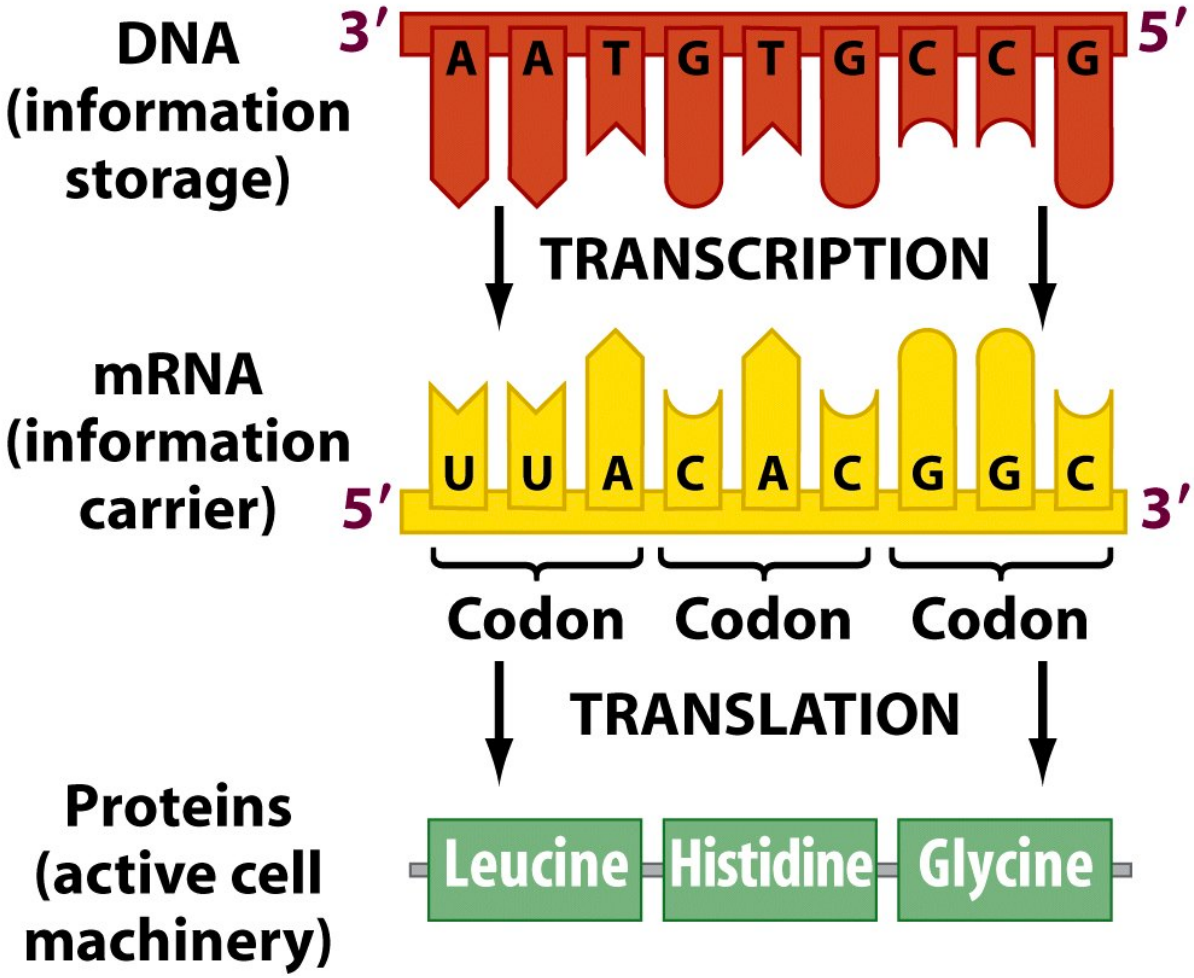


AIMS

- Understand the process of translation in prokaryotes.
- Understand the molecular requirements to translate a prokaryotic mRNA into a protein.
- Understand the sequence of events in prokaryotic translation.

Review

Information flows from DNA to RNA to proteins.

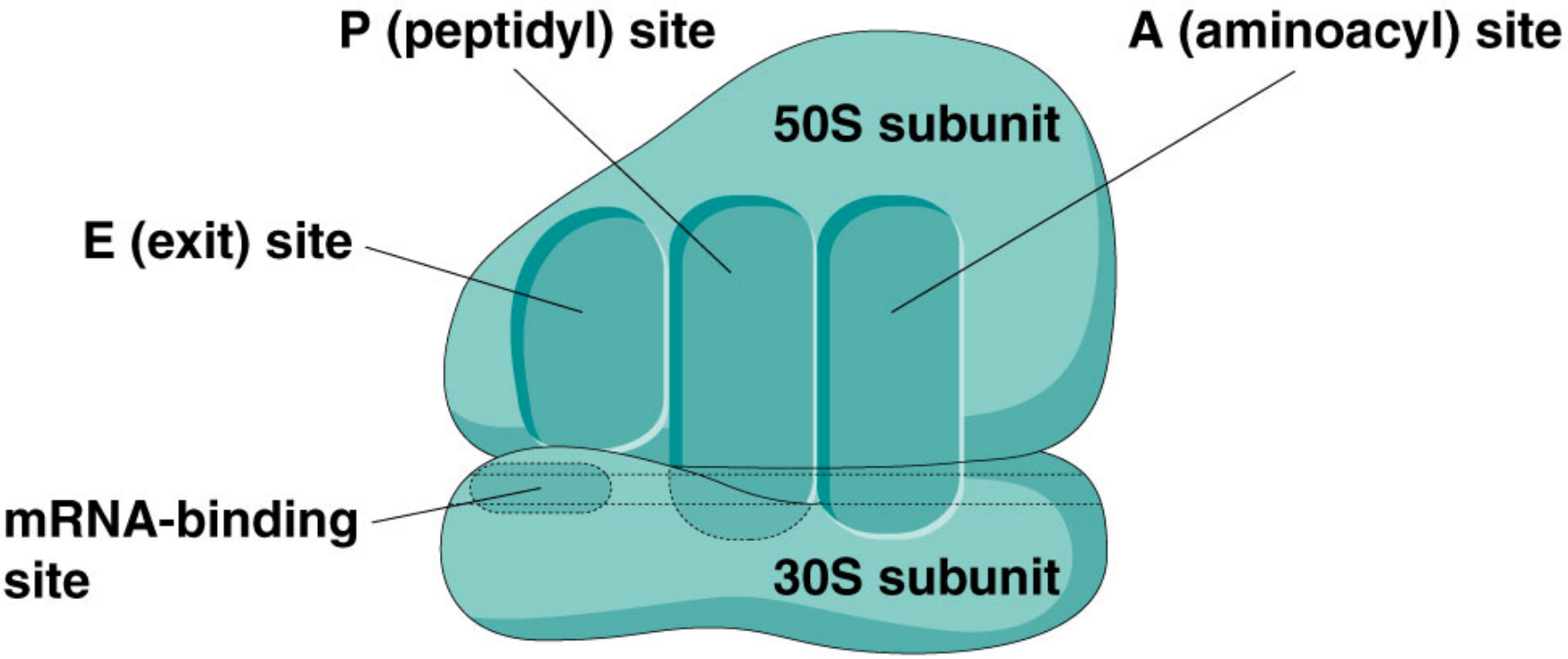


The ribosome revisited

Three sites in the two subunits are associated with translation:

- **A site (aminoacyl site):** the site where the aminoacyl tRNA binds.
- **P site (peptidyl site):** the site where the peptide bond is formed between two amino acids.
- **E site (Exit site):** the site where the tRNA leaves the ribosome.

The ribosome revisited



Translation process

What are the stages of translation (protein synthesis)?

(1) Initiation

(2) Elongation

(3) Termination

Translation initiation

Initiation involves all the steps before the formation of the peptide bond between the first two amino acids in the peptide chain.

What molecules involved in translation initiation?

1. mRNA
2. Ribosome
3. Specific initiator tRNA (start codon!)
4. Initiation factors
5. Energy (GTP guanine triphosphate)

Translation initiation in bacteria

Simply ☺

1. The small ribosome finds the mRNA
2. Finding the start codon
3. Place the start tRNA (Met tRNA) in its correct location
4. Assembly of the entire ribosome (large and small) and the start tRNA (tRNA Met)

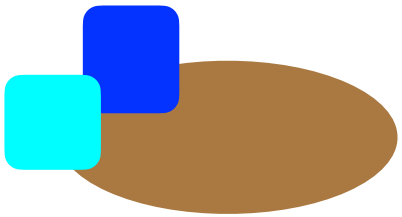
We need scientific details. Don't we?

Translation initiation in bacteria

Translation initiation in bacteria starts with (Finding the mRNA):

1.The interaction between the small ribosomal subunit (30S) and two initiation factors (**IF 1 and IF 3**).

2.The complex (30S ribosomal subunit + IF1 + IF 3) bind to the mRNA at a specific location.

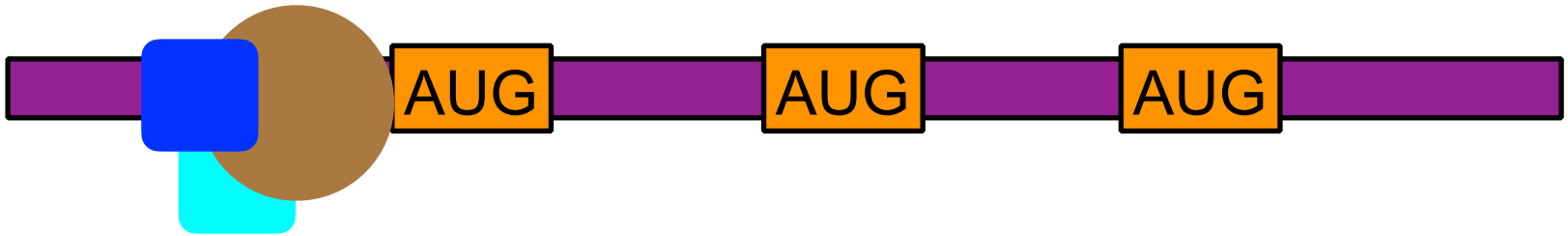


Translation initiation in bacteria

Where in the mRNA this complex binds?

What is unique about the beginning of mRNA?

Translation initiation in bacteria



Translation initiation in bacteria

The ribosome binding site in mRNA

1.The binding site in mRNA is not only the start codon (AUG). **WHY?**

2.A sequences upstream of the start codon are essential for specific binding of ribosome to the correct location.

•**The ribosome binding site (RBS)** in prokaryotic mRNA is called **Shine-Dalgarno sequence.**

Translation initiation in bacteria

Shine-Dalgarno sequence

- 8-12 specific nucleotide sequence upstream of the start codon (of each gene/transcript).
- The sequence interacts with the complementary sequence in 16S rRNA in the small ribosomal subunit.
- Interacts specifically with the small ribosomal subunit 30S.

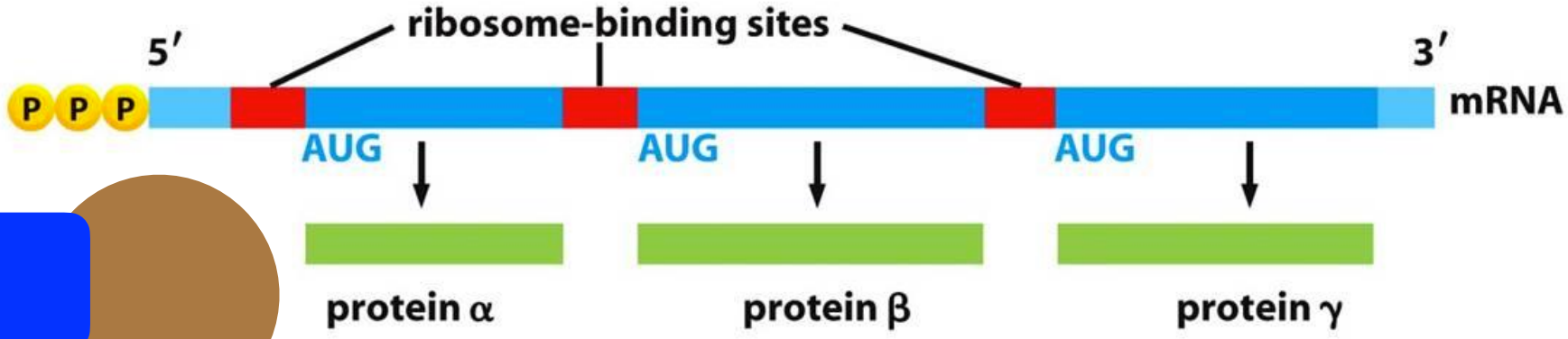
Translation initiation in bacteria

Shine-Dalgarno sequence

- This ensures **specificity** of where the ribosome assembles and start translating.
- This sequence helps translating a polycistronic transcript and each gene therein independently!

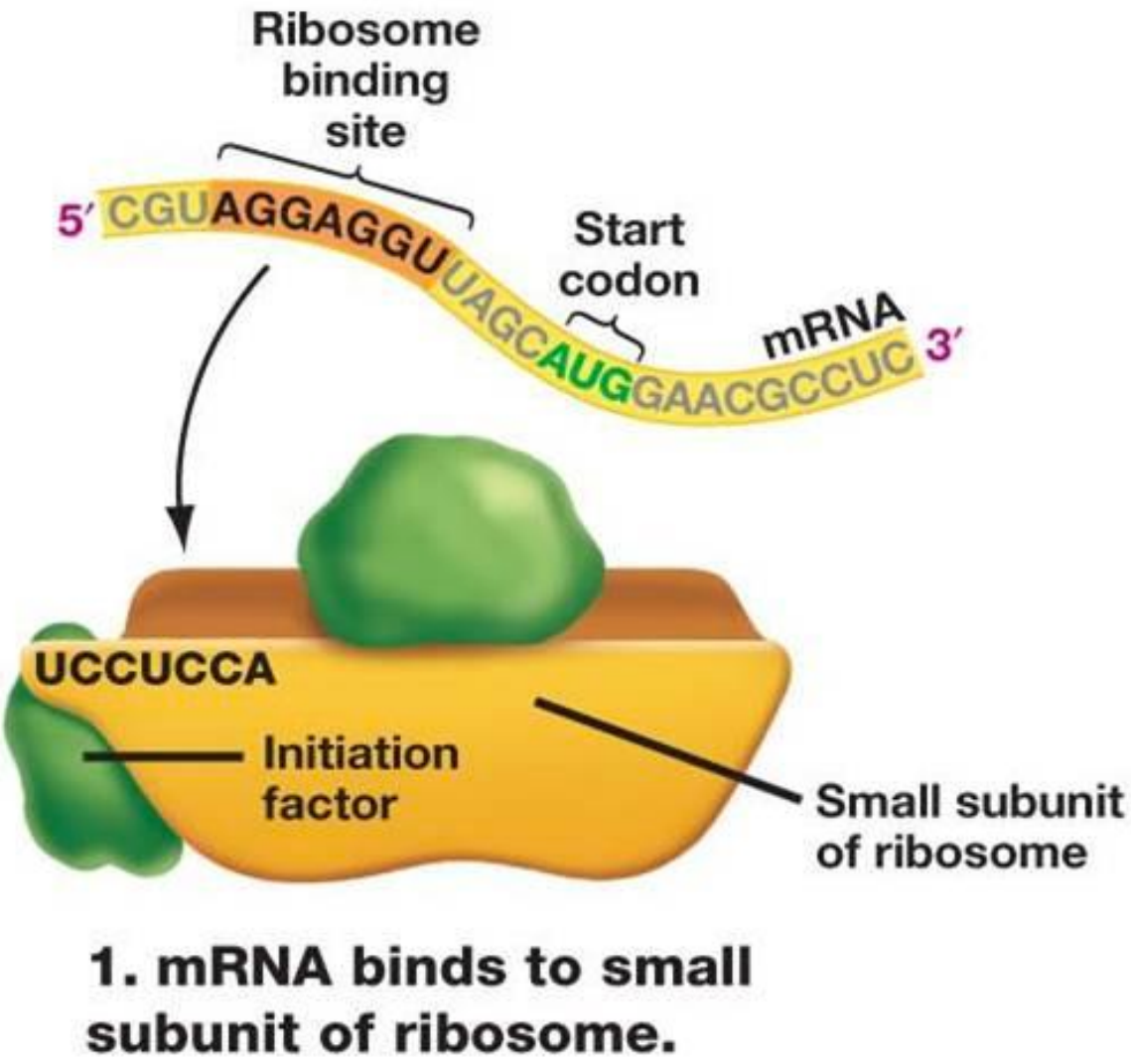
Translation initiation in bacteria

Before each protein-coding gene in a polycistronic transcript, a ribosome-binding site exists



Translation initiation in bacteria

Shine-Dalgarno sequence



1. mRNA binds to small subunit of ribosome.

Translation initiation in bacteria

1. The interaction between the small ribosomal subunit (30S) and two initiation factors (**IF 1 and IF 3**).

2. The complex (30S ribosomal subunit + IF1 + IF 3) bind to the mRNA at a specific location.

3. A special initiator tRNA binds to the 30S ribosome and mRNA at the start codon.

Translation initiation in bacteria

What is the start codon?

What is the start codon tRNA?

Translation initiation in bacteria

Initiator tRNA in bacteria

1.The initiator tRNA in bacteria recognizes the start codon with a complementary anti-codon sequence of:

5’ CAU 3’

Isn’t the start codon AUG?

What is happening?

Translation initiation in bacteria

Initiator tRNA in bacteria

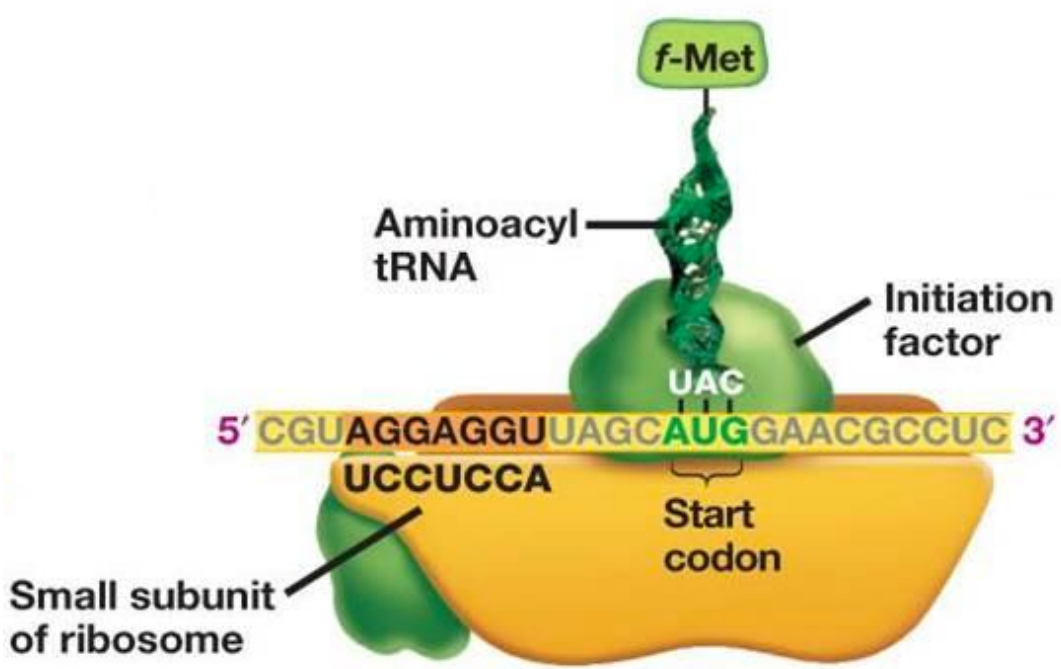
• The initiator tRNA carries a specific modified amino acid called **formyl-methoionine (fMet-tRNA).** It is a methoionine with a formyl group added.

• When AUG is in the middle of a transcript another tRNA is used. It is called **Met-tRNA.**

Translation initiation in bacteria

Initiator tRNA in bacteria

1.The initiator tRNA (fMet-tRNA) gets carried to the complex (30S ribosome + IF1 + IF 3) by initiation factor IF2 using GTP.

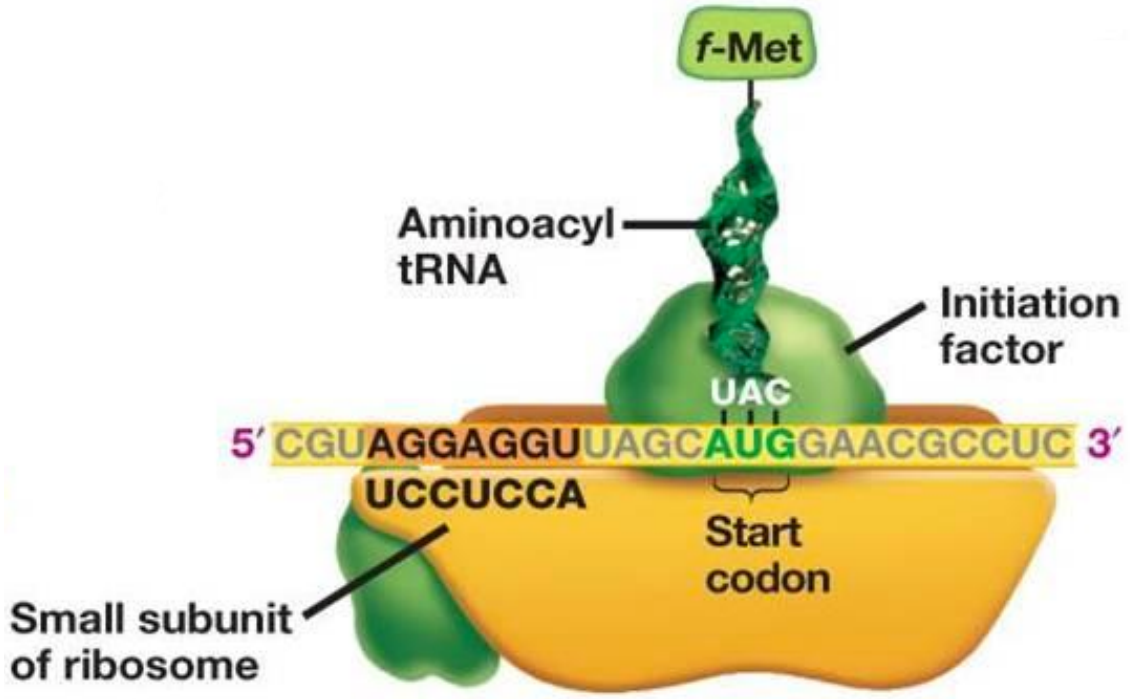


2. f-Met tRNA binds.

Translation initiation in bacteria

Initiator tRNA in bacteria

2.Initiator tRNA binds to a specific site in the ribosome (P site).



2. f-Met tRNA binds.

Translation initiation in bacteria

Functions of translation initiation factors

- **IF 1:**
  - Blocks the A site in the ribosome so that only P site is available for initiator tRNA is available to bind.

Translation initiation in bacteria

Functions of translation initiation factors

- **IF 2:**
  - Carries the initiator tRNA to the small ribosomal subunit and places it in the P site.

Translation initiation in bacteria

Functions of translation initiation factors

- **IF 3:**
  - Binds to the mRNA in ribosomal binding site.
  - Prevent the binding of the 50S ribosomal large subunit to the small one.

Translation initiation in bacteria

1. The interaction between the small ribosomal subunit (30S) and two initiation factors (**IF 1 and IF 3**).

2. The complex (30S ribosomal subunit + IF1 + IF 3) bind to the mRNA at a specific location.

3. A special initiator tRNA binds to the 30S ribosome and mRNA at the start codon.

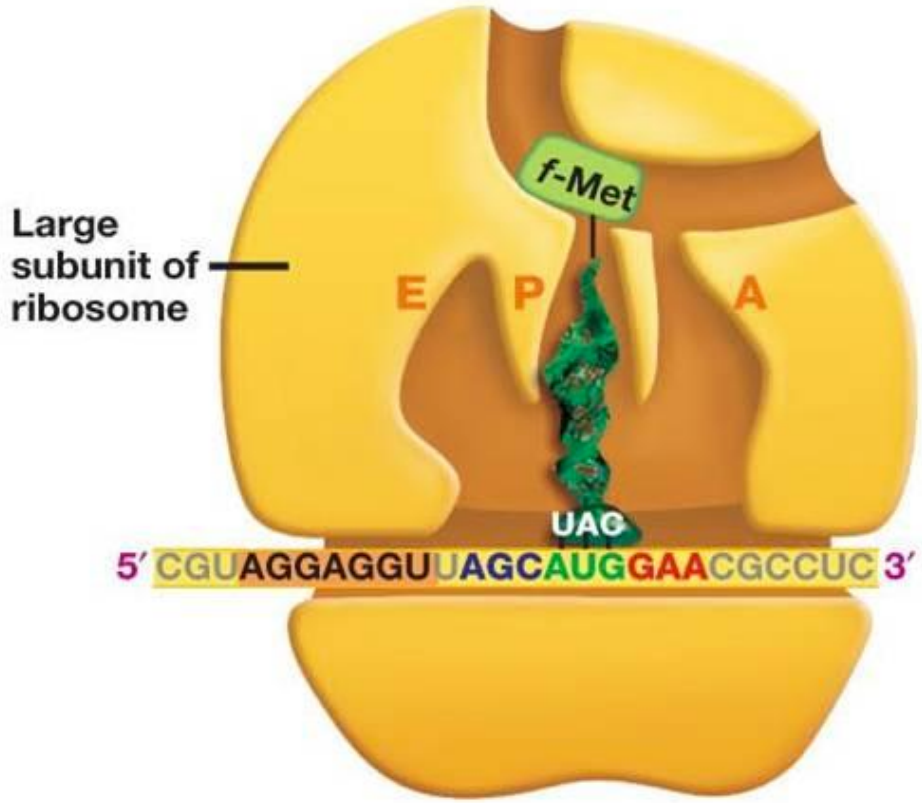
4. The 50S ribosomal subunit binds to the (30S + mRNA + fMet-tRNA) using GTP as a source of energy.

Translation initiation in bacteria

What about IFs?

Translation initiation in bacteria

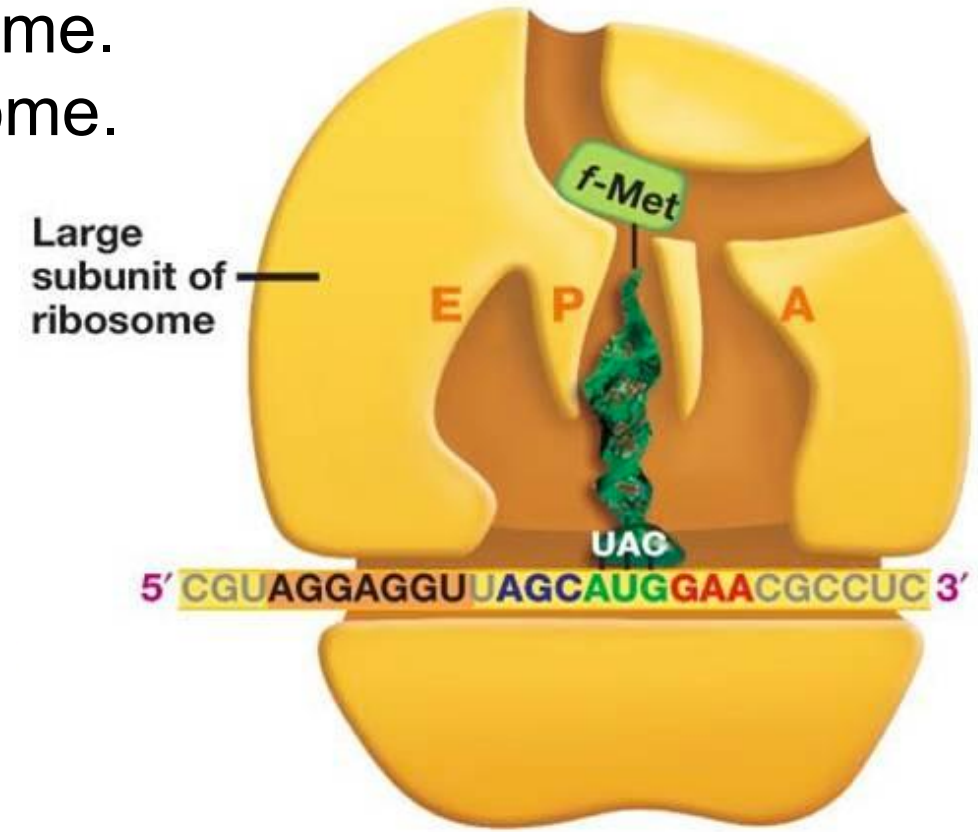
- The initiation factors (IF1 and IF3) gets released and the resulting complex is called **the initiation complex.**



3. Large subunit binds.

Translation initiation in bacteria

- **Initiation complex includes:**
    1. fMet-tRNA.
    2. mRNA.
    3. Small ribosome.
    4. Large ribosome.



3. Large subunit binds.

Translation initiation in bacteria

Summary

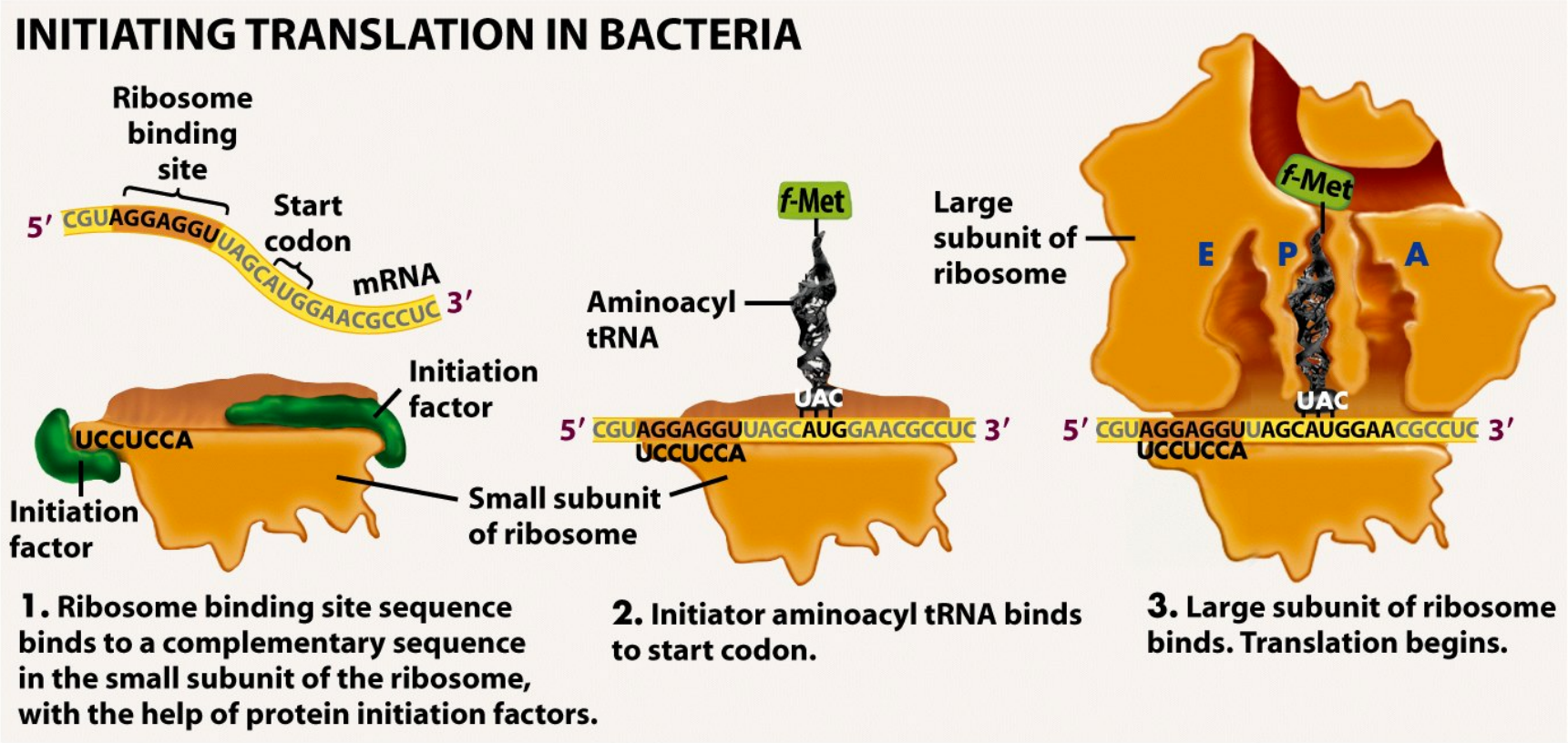


Figure 16-14 Biological Science, 2/e

Translation elongation in bacteria

Elongation is adding more amino acids carried by tRNA to Met (the start amino acid).

What are the steps in translation elongation?

1. Amino-acyl tRNA (charged tRNA) binds to the ribosome's **A site.**

2. Peptide bond forms.

3. Ribosome moves (translocate) one codon downstream.

Translation elongation in bacteria

What is needed for elongation?

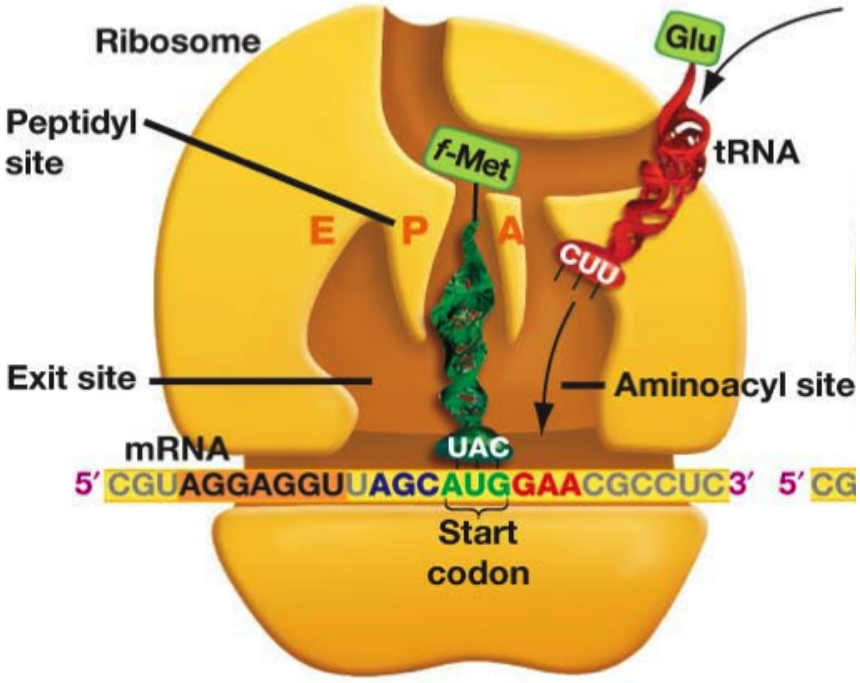
1. Charged tRNA
2. Elongation factors (EF)
3. GTP

Translation elongation in bacteria

Elongation process:

1. fMet tRNA is bound to the AUG codon at P site.

2. Next codon is positioned in the A site.



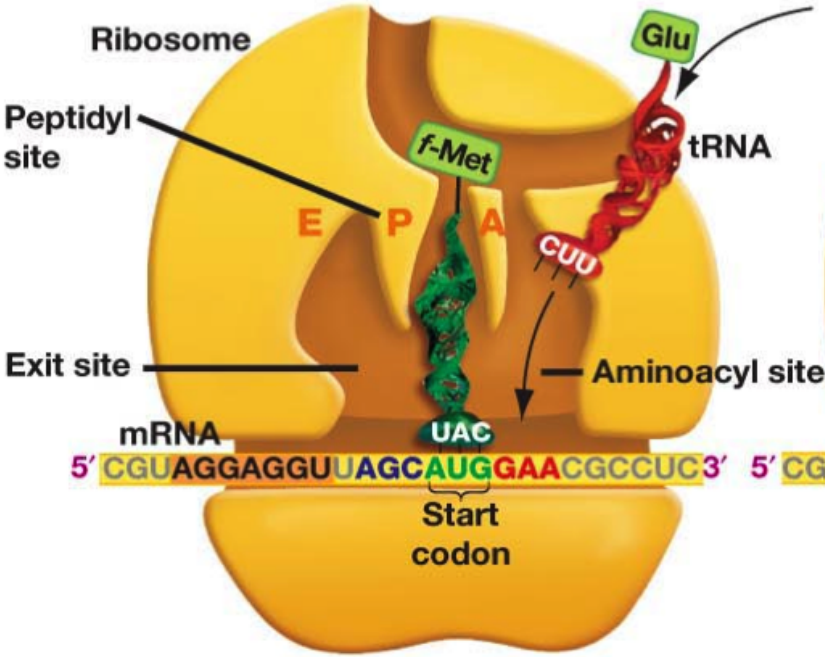
1. Incoming aminoacyl tRNA

Translation elongation in bacteria

Elongation process:

3. Appropriate amino-acyl tRNA binds to the A site.

4.The charged tRNA is brought to the ribosome by **elongation factors (EF and GTP)**.



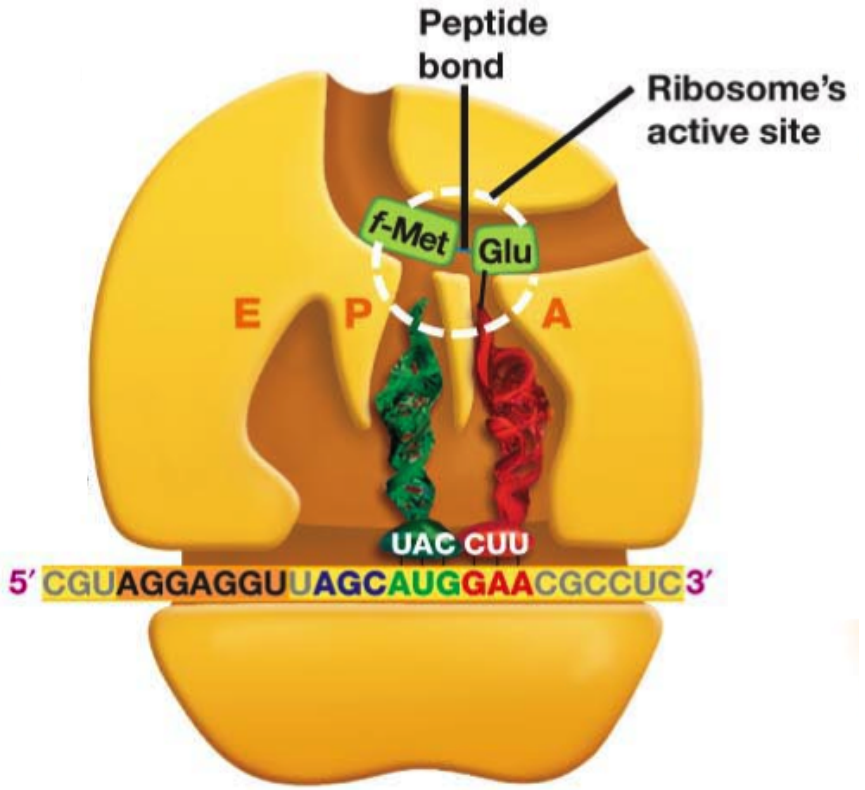
1. Incoming aminoacyl tRNA

Translation elongation in bacteria

Elongation process:

5. Two amino-acyl tRNAs are in positions P and A and a peptide bond is formed between the two amino acids.

6. The bond between the amino acid and tRNA at P site is broken.



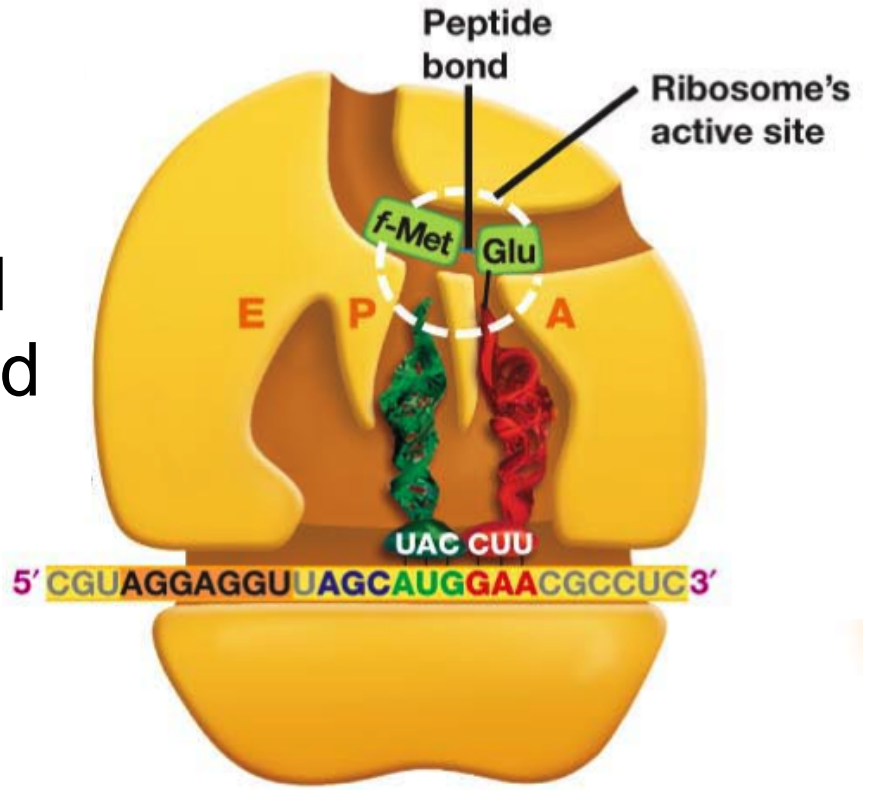
2. Peptide bond formation

Translation elongation in bacteria

Elongation process:

7. A peptide bond is formed between the free amino acid from the P site and the one at the A site by:

Peptidyl Transferase



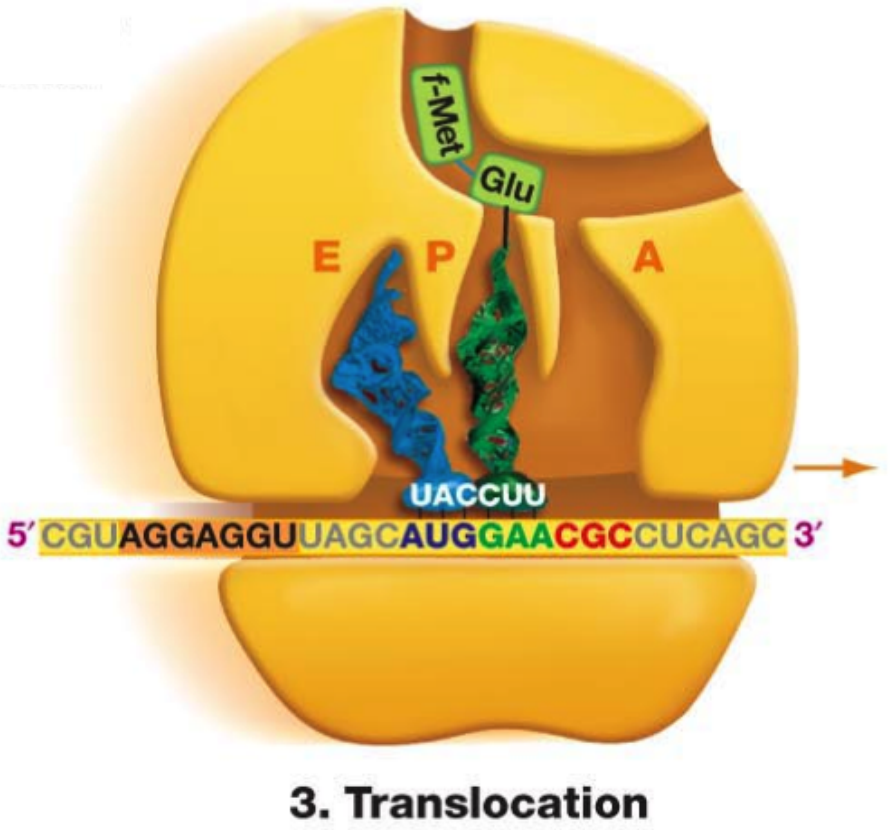
2. Peptide bond formation

Translation elongation in bacteria

Elongation process:

8.When a peptide bond is formed the free tRNA is in site P and the tRNA at site A has two amino acids.

9.Ribosome moves one codon downstream (3').



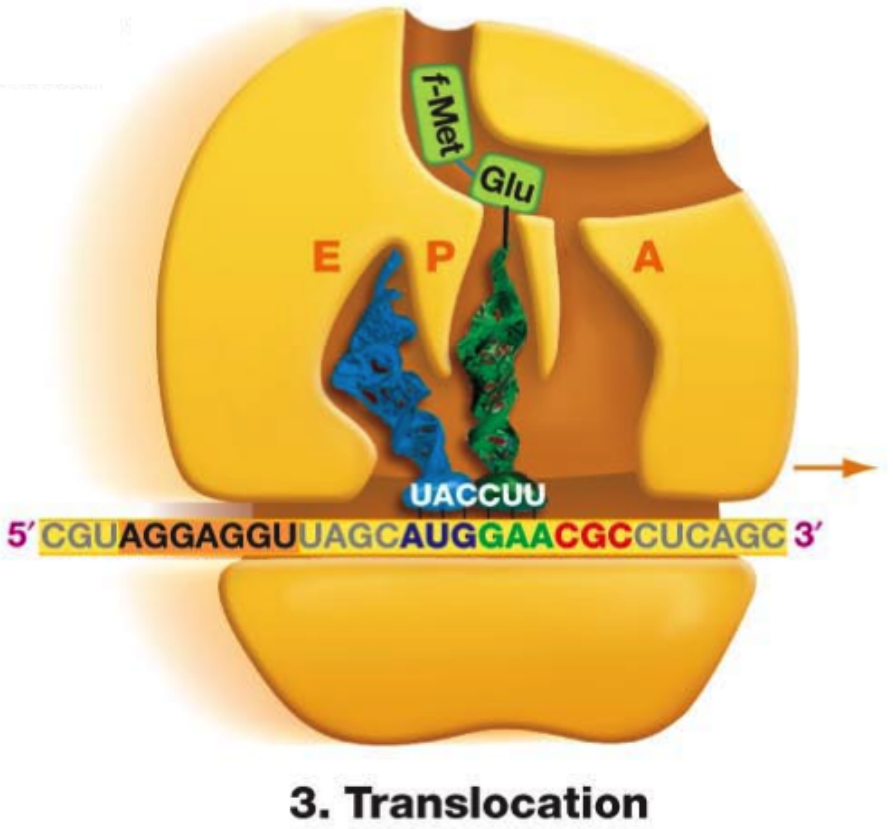
3. Translocation

Translation elongation in bacteria

Elongation process:

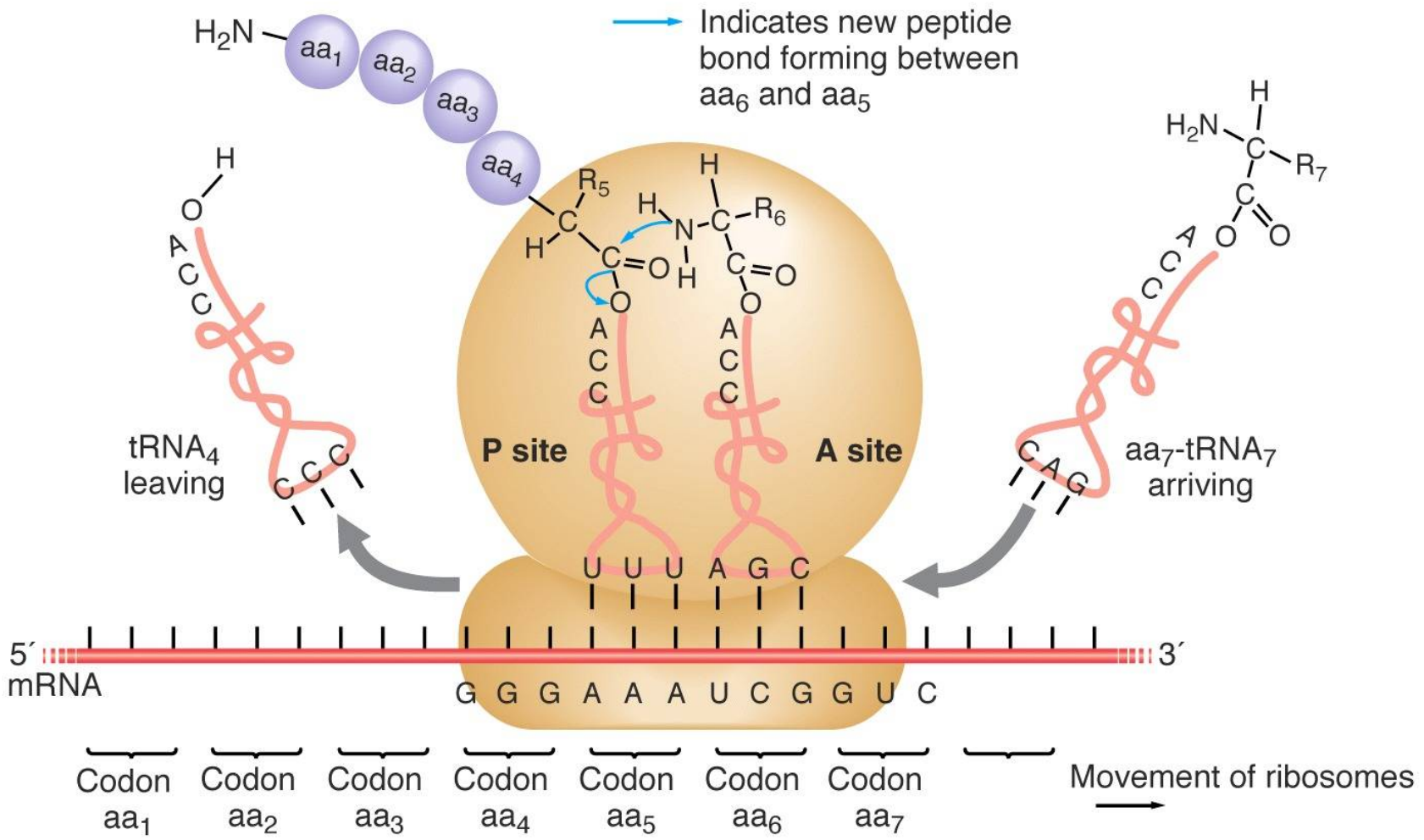
10. Free tRNA moves to the E site.

11. A new charged tRNA gets to the A site and the cycle repeats.



3. Translocation

Peptide bond formation



Translation elongation in bacteria

Summary 1

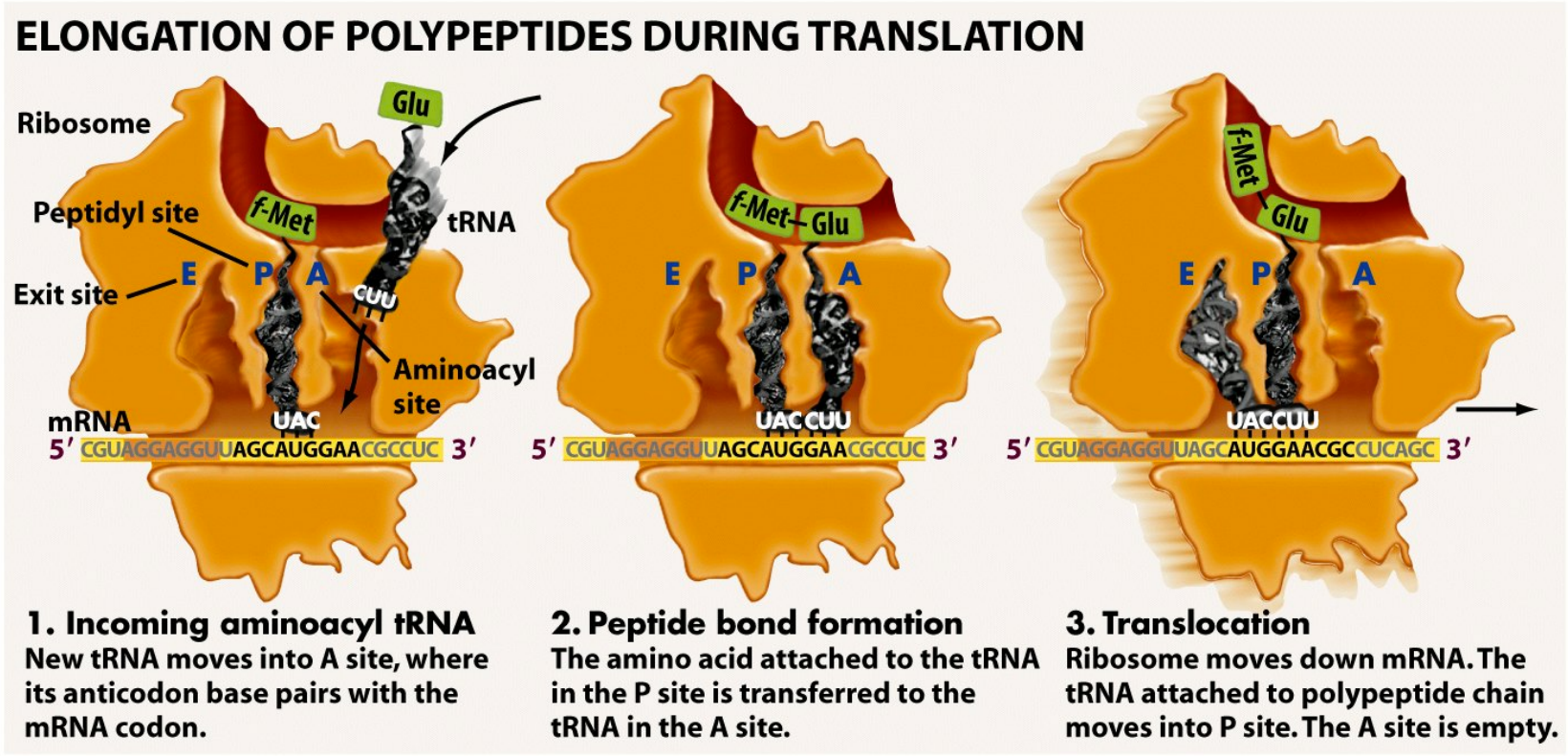
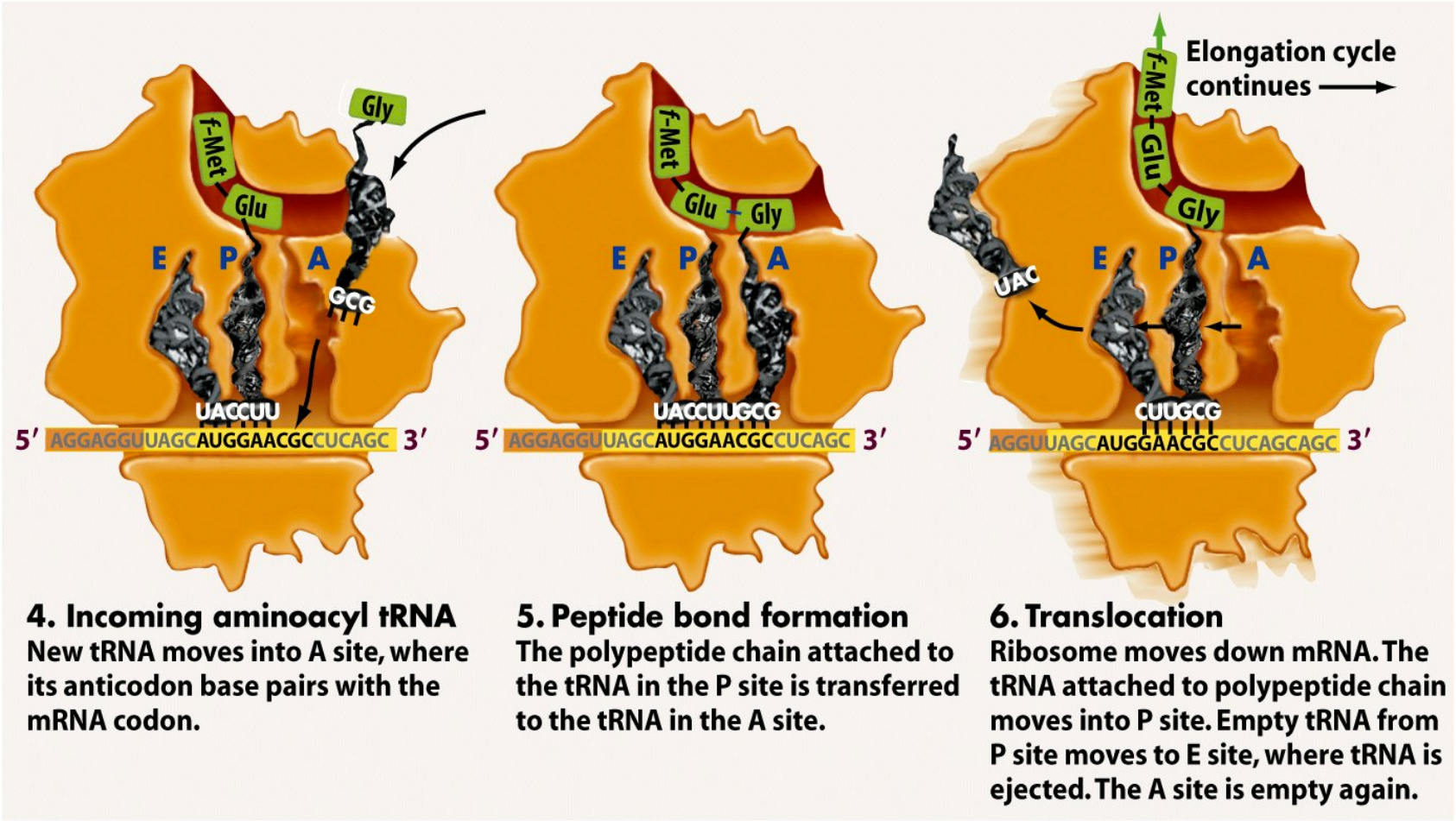


Figure 16-15 part 1 Biological Science, 2/e

Translation elongation in bacteria

Summary 2



4. Incoming aminoacyl tRNA
New tRNA moves into A site, where its anticodon base pairs with the mRNA codon.

5. Peptide bond formation
The polypeptide chain attached to the tRNA in the P site is transferred to the tRNA in the A site.

6. Translocation
Ribosome moves down mRNA. The tRNA attached to polypeptide chain moves into P site. Empty tRNA from P site moves to E site, where tRNA is ejected. The A site is empty again.

Figure 16-15 part 2 Biological Science, 2/e

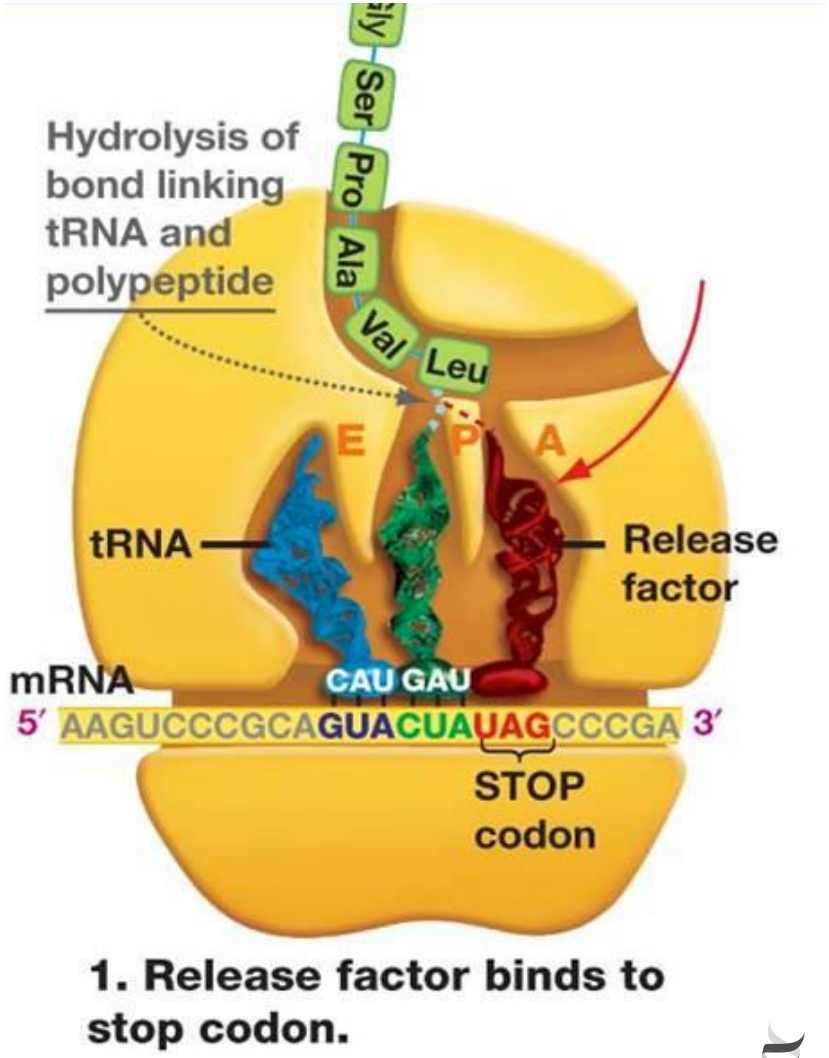
Translation termination in bacteria

Termination is signaled by a stop codon.

1.Stop codons **DO NOT** code for amino acids and thus **DO NOT** have tRNAs.

2.**Release Factors (RF)** which looks like tRNA binds to the A site.

3.Peptide is cleaved by peptidyl transferase at the P site.

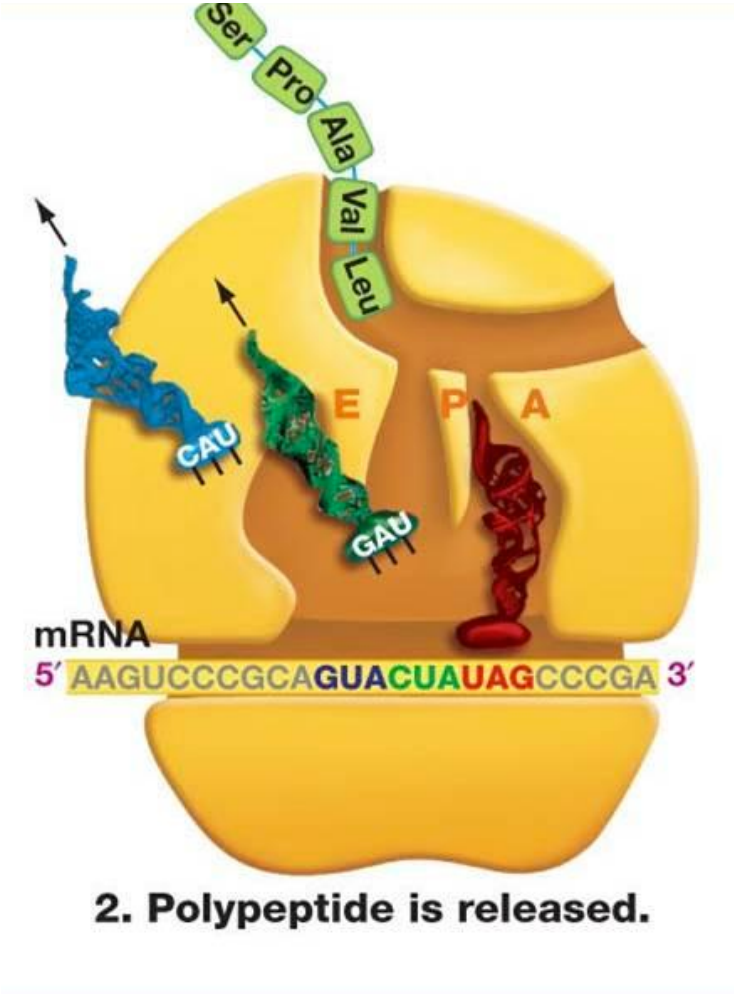


1. Release factor binds to stop codon.

Translation termination in bacteria

4. Amino acid chain is released.

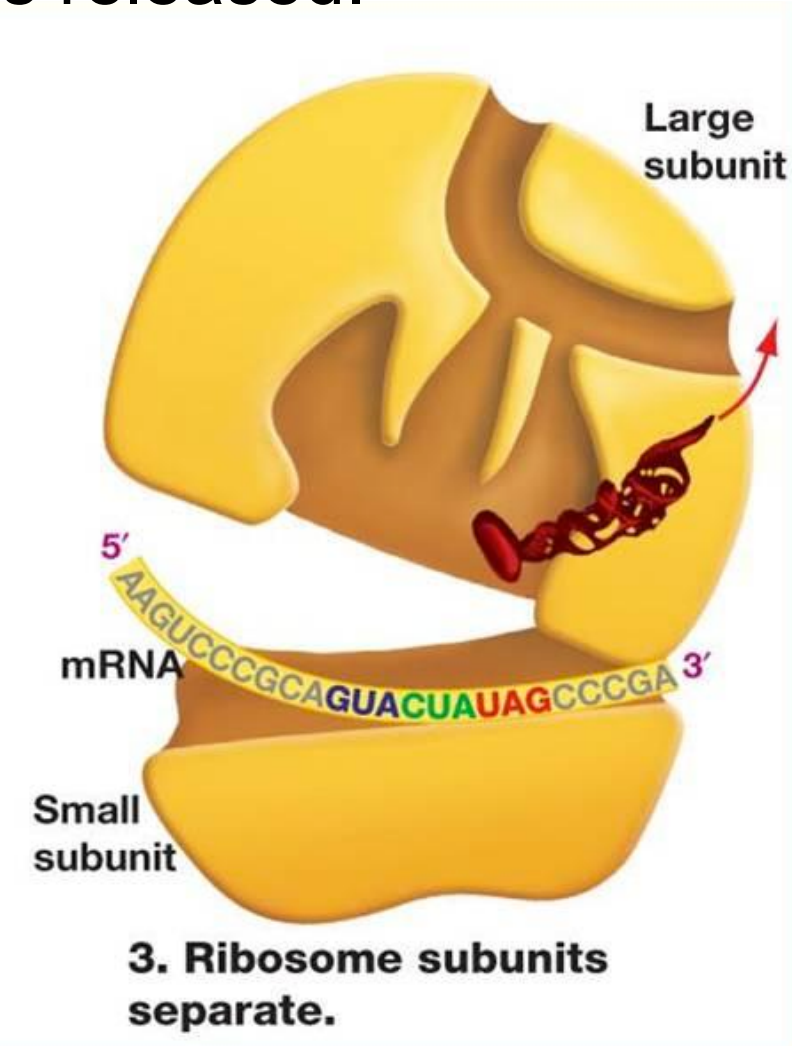
5. tRNA at E site and P site are released.



2. Polypeptide is released.

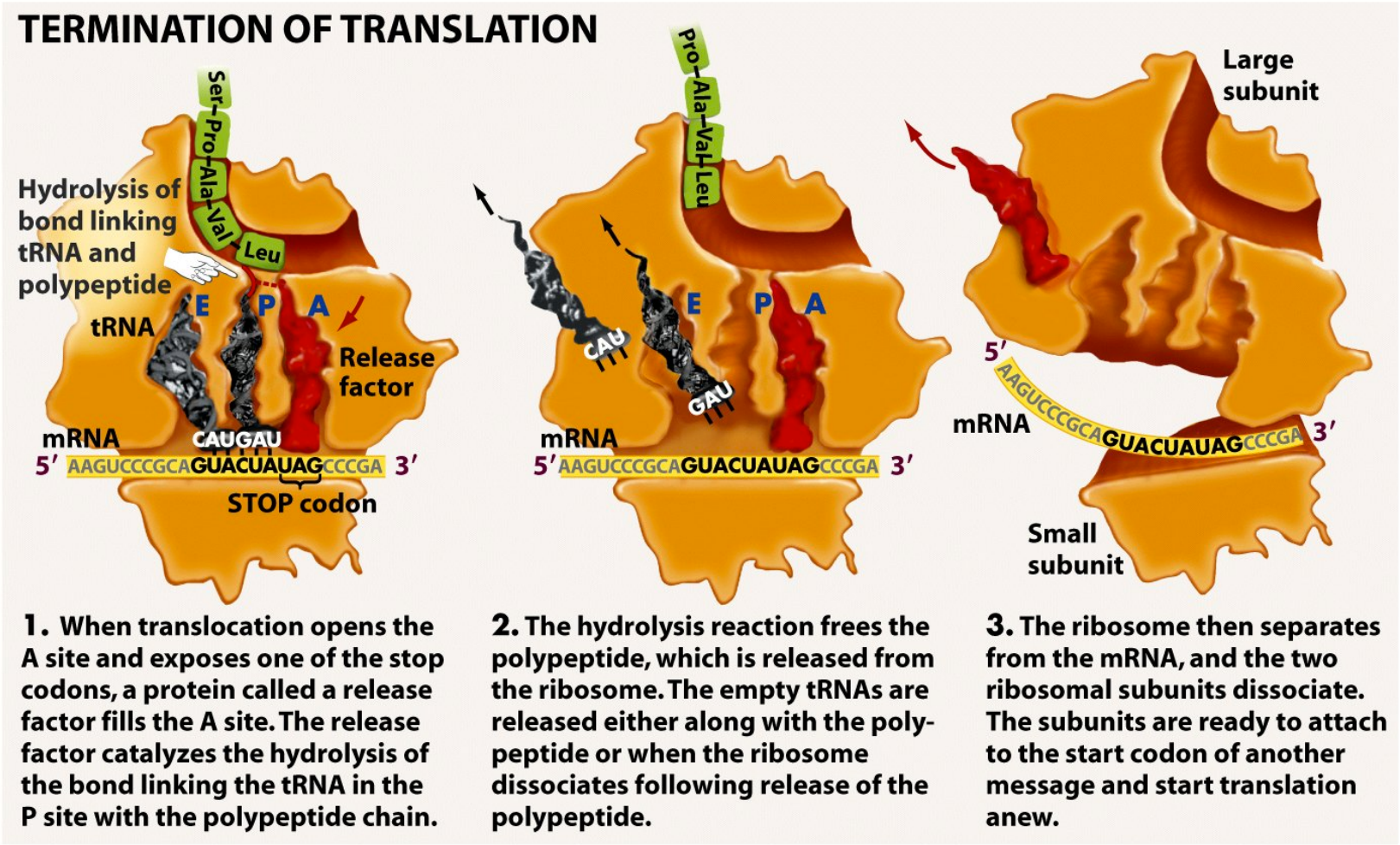
Translation termination in bacteria

6. Ribosome two units break free from the mRNA and RF is released.



Translation termination in bacteria

Summary



Stuff to know

Amino-acyl site

IF 1

AUG

Translation initiation

EF

P site

Start codon

70S subunit

RF

Translation elongation

IF 2

50S subunit

Elongation factors

A site

fMet-tRNA

Peptidyl site

Peptidyl transferase

Initiation factors

Stop codons

Exit site

Ribosome

Translation termination

Release factor

30S subunit

E site

IF 3

Shine-Dalgarno sequence

Expectations

- You know how translation process occurs in prokaryotes.
- You know how ribosome finds the correct location to start translation.
- You know the molecules needed in every step of translation.
- You the sequence of events in prokaryotic translation.

For a smile