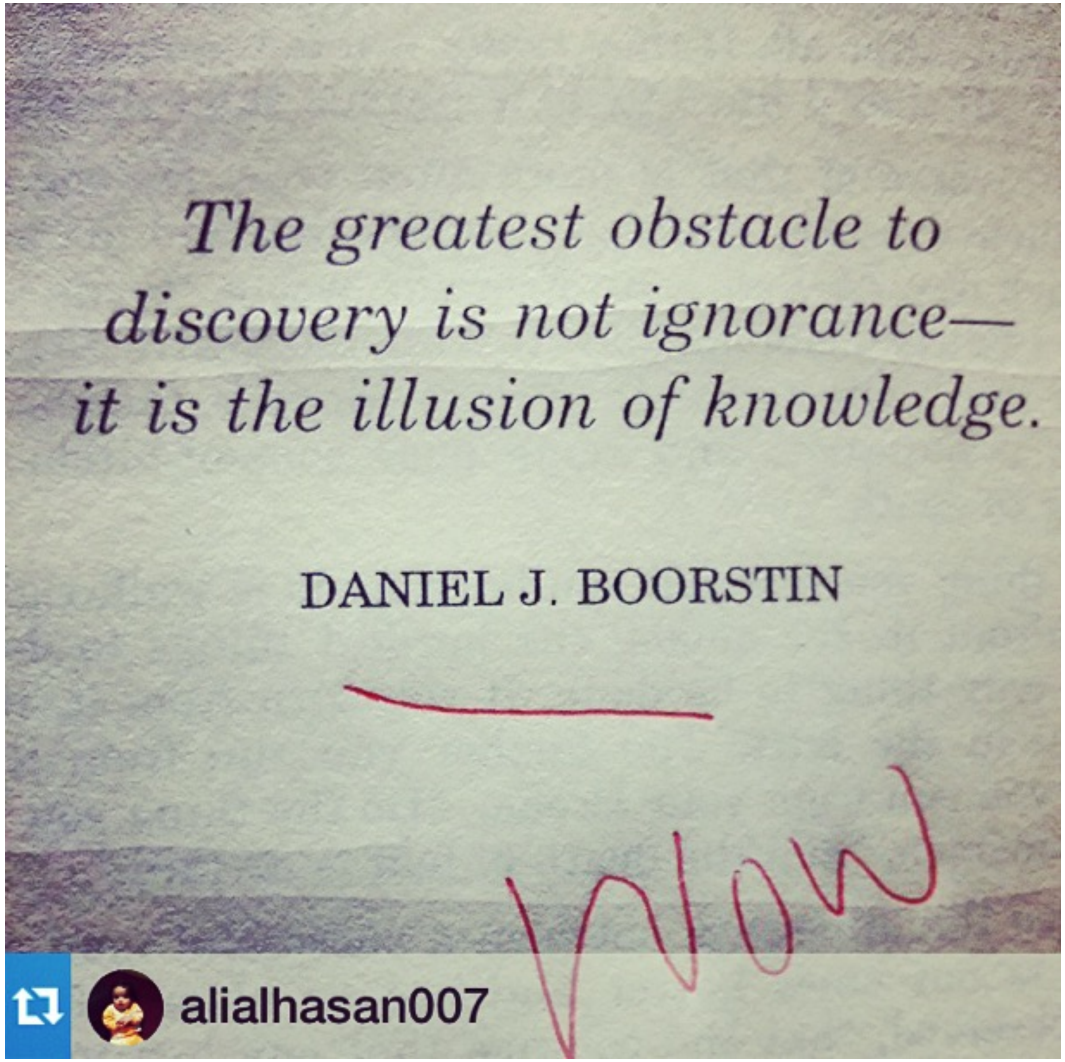
Lecture 17:

Translation in prokaryotes

Course 281

Lessons for life

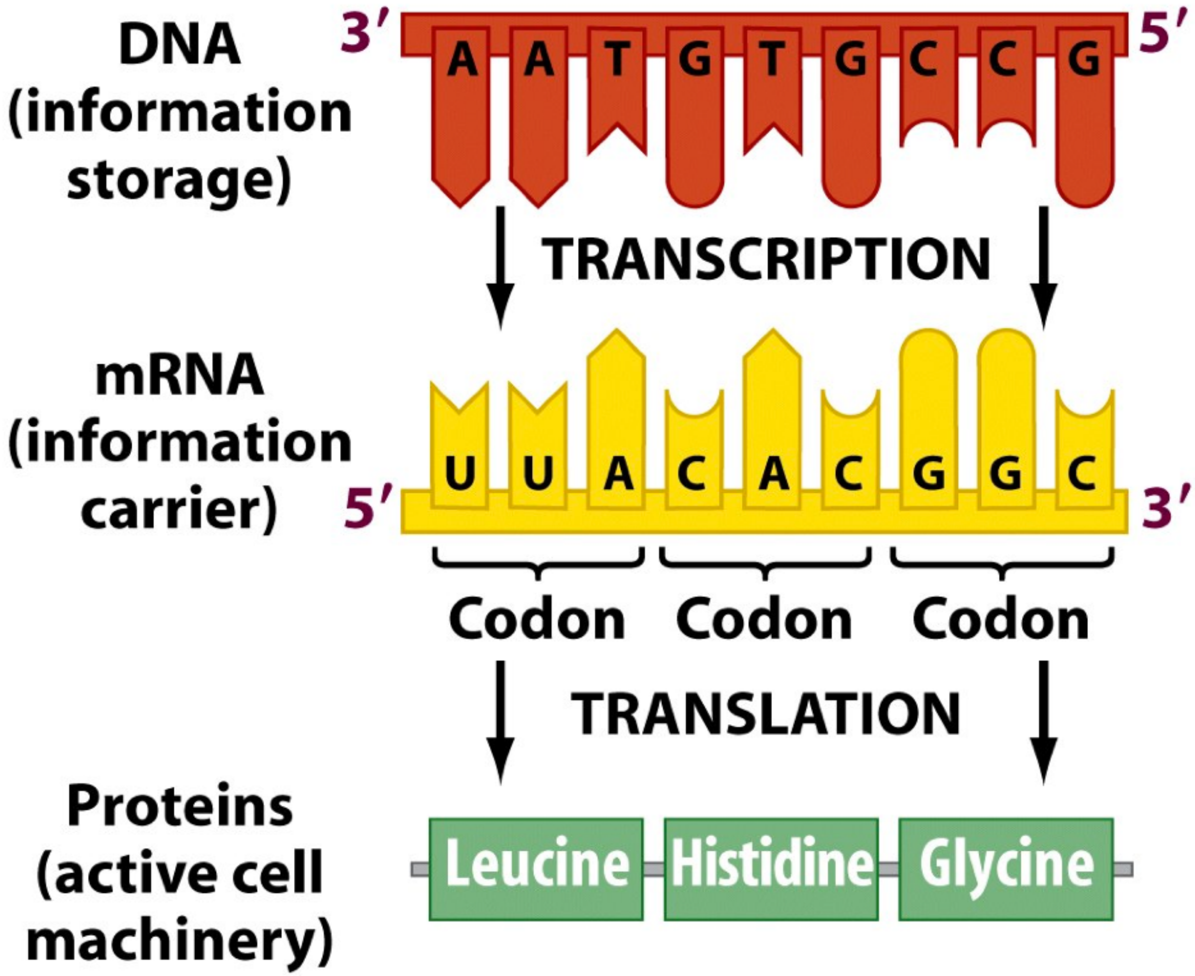


AIMS

- 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

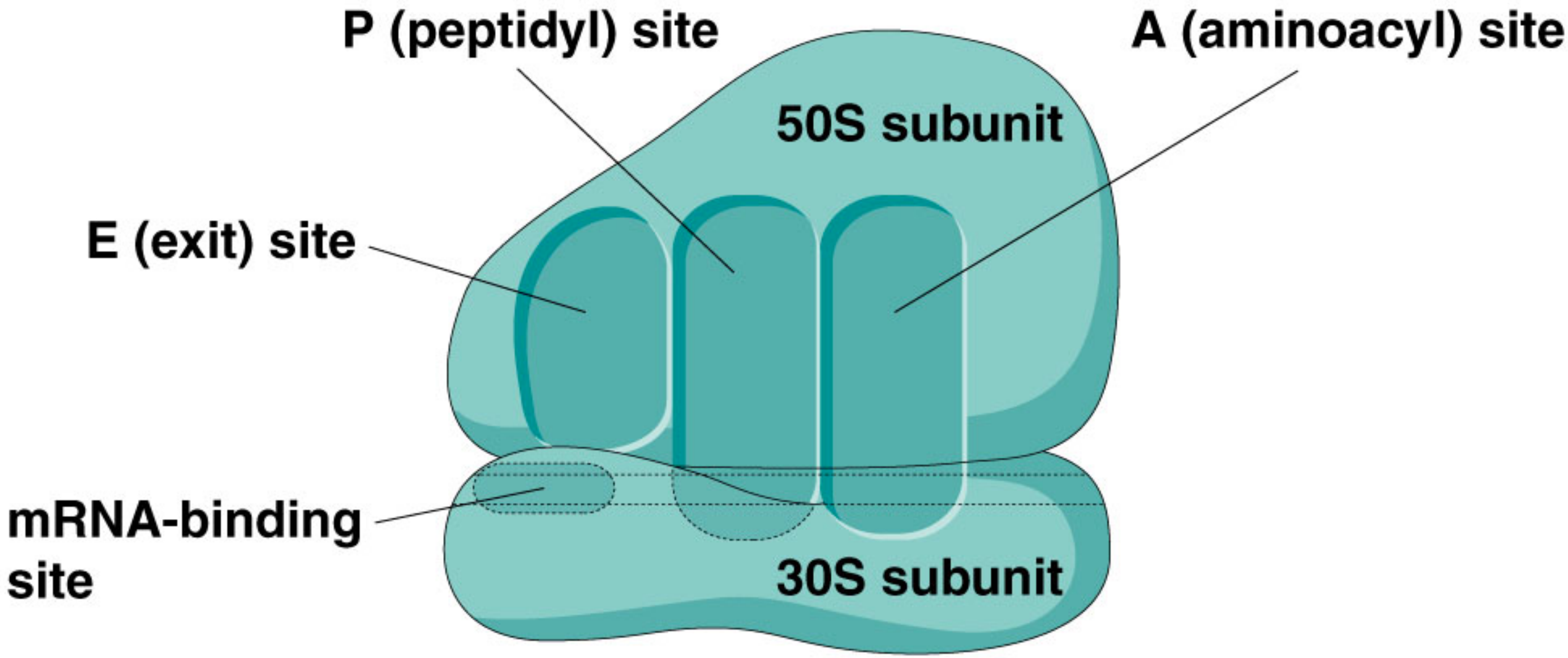
Three sites in the two subunits are associated with translation:

- **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**).

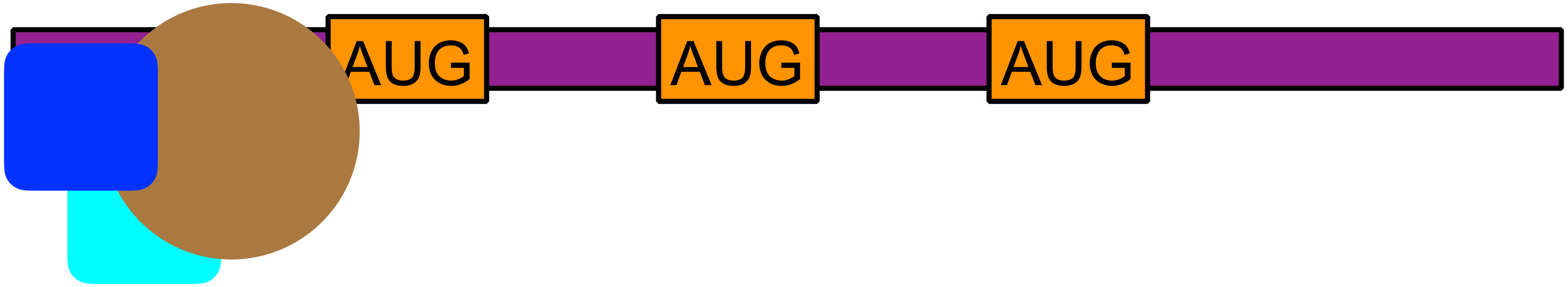
2. The complex (30S ribosomal subunit + IF1 + IF 3) bind to the mRNA at a specific location.

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.

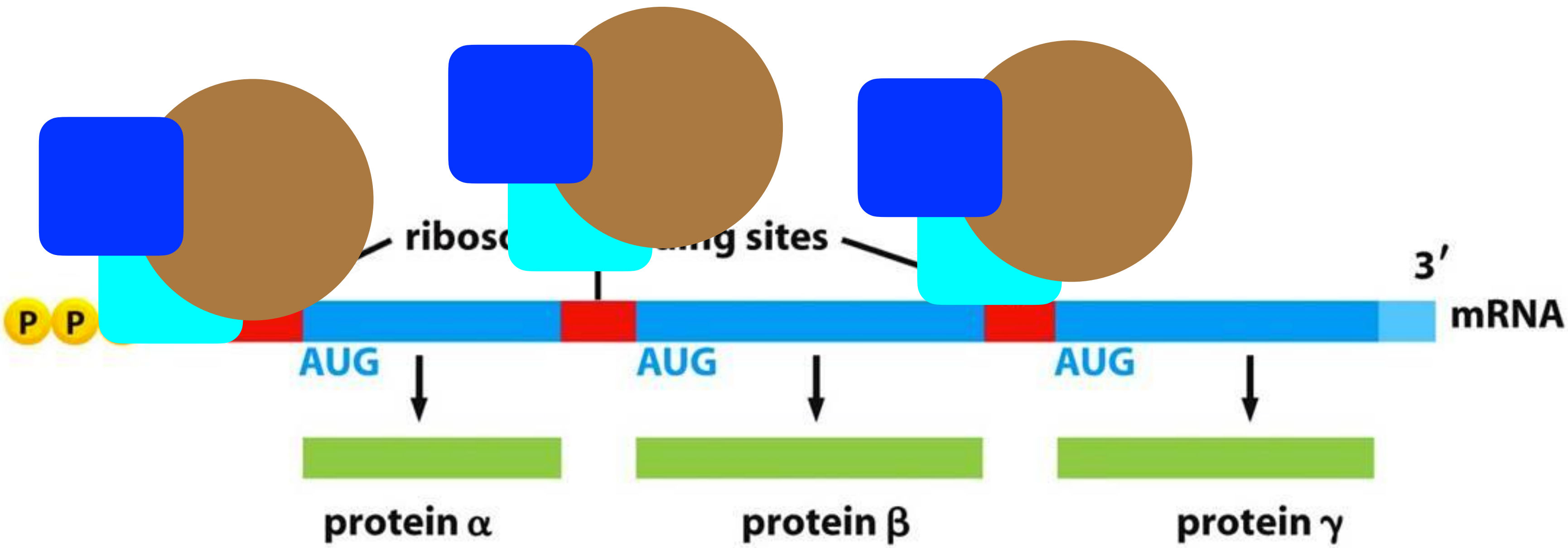
Translation initiation in bacteria

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!

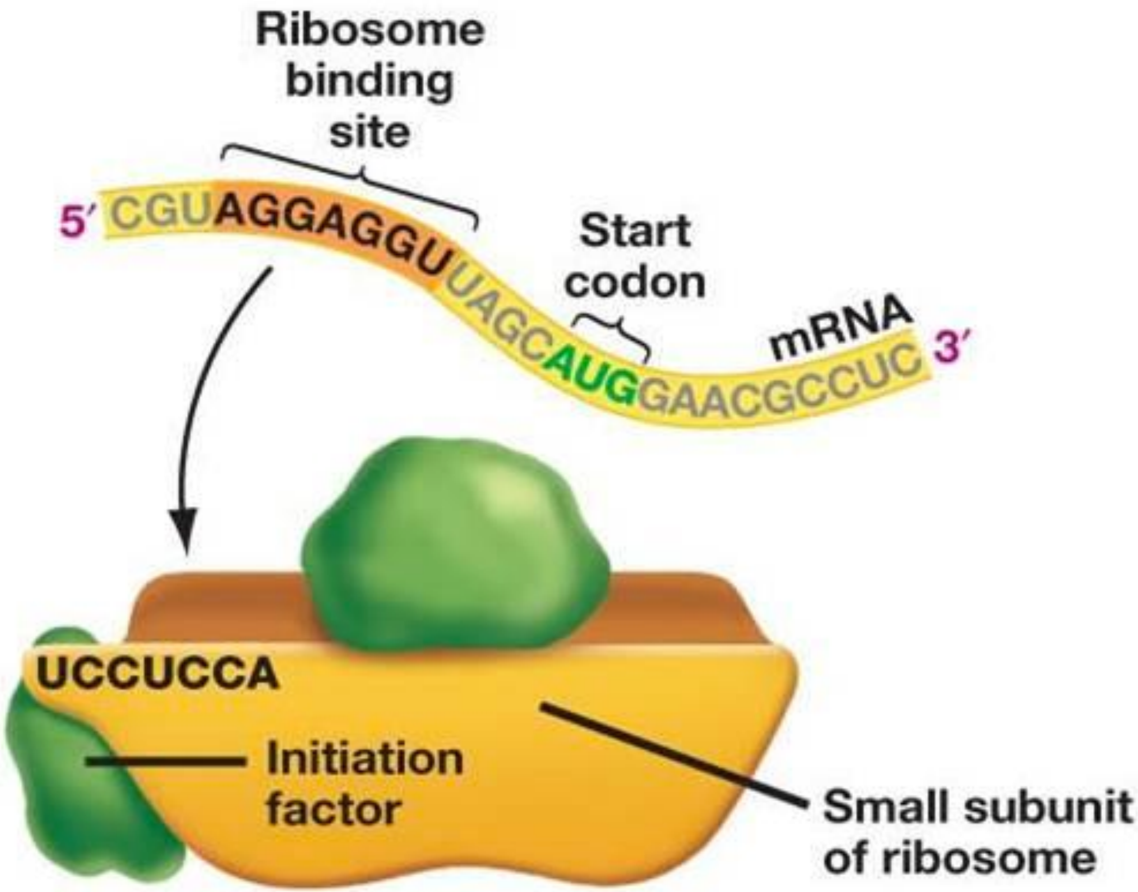
Translation initiation in bacteria

Before each protein coding gene in a polycistronic transcript, a ribosome binding site exist.



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

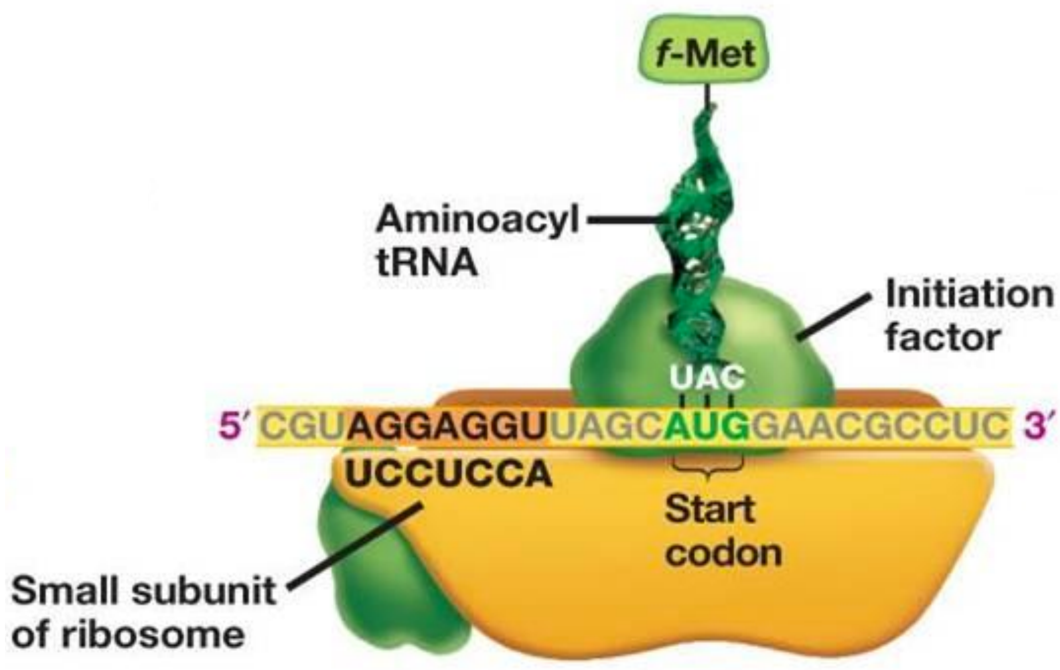
• The initiator tRNA carries a specific modified amino acid called **formyl-methoioinine (fMet-tRNA).** It is a methoionine with a formyl group added.

• 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.

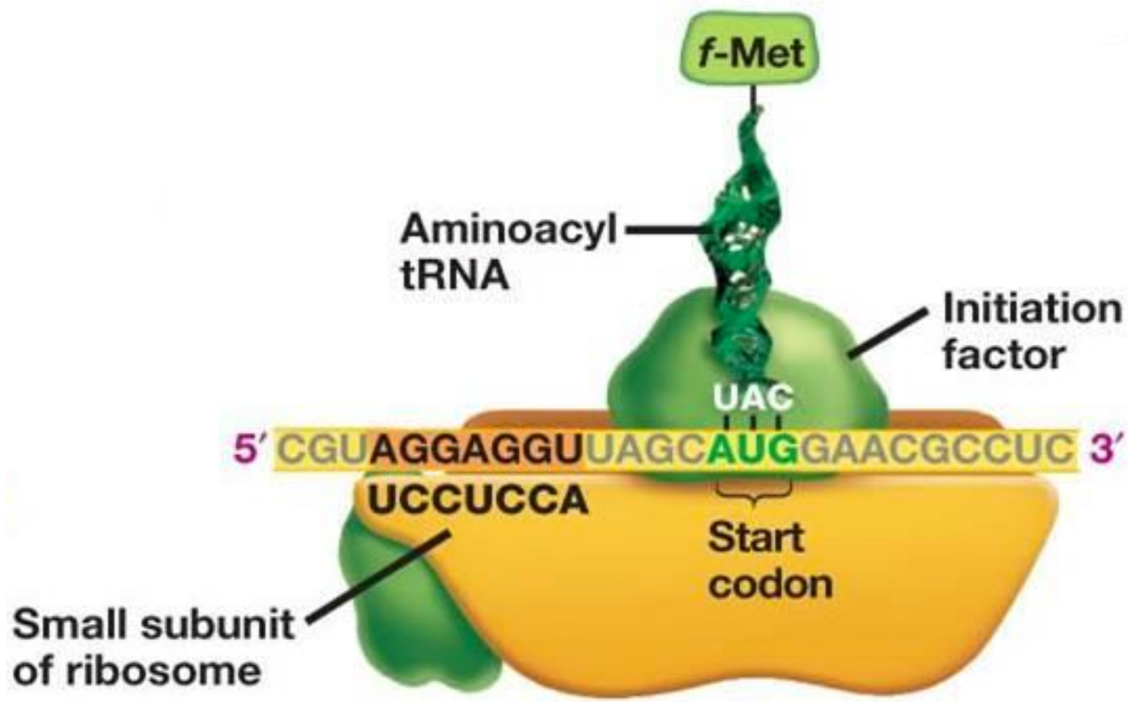


2. f-Met tRNA binds.

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.

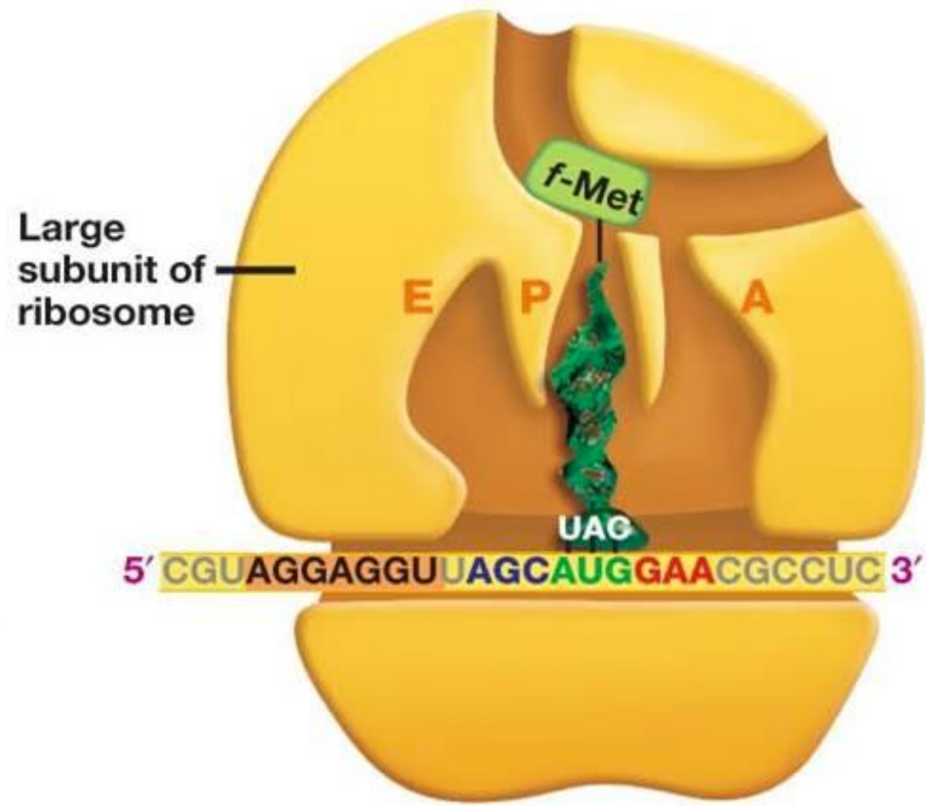
4. The 50S ribosomal subunit binds to the (30S + mRNA + fMet-tRNA) using GTP as a source of energy.

Translation initiation in bacteria

What about IFs?

Translation initiation in bacteria

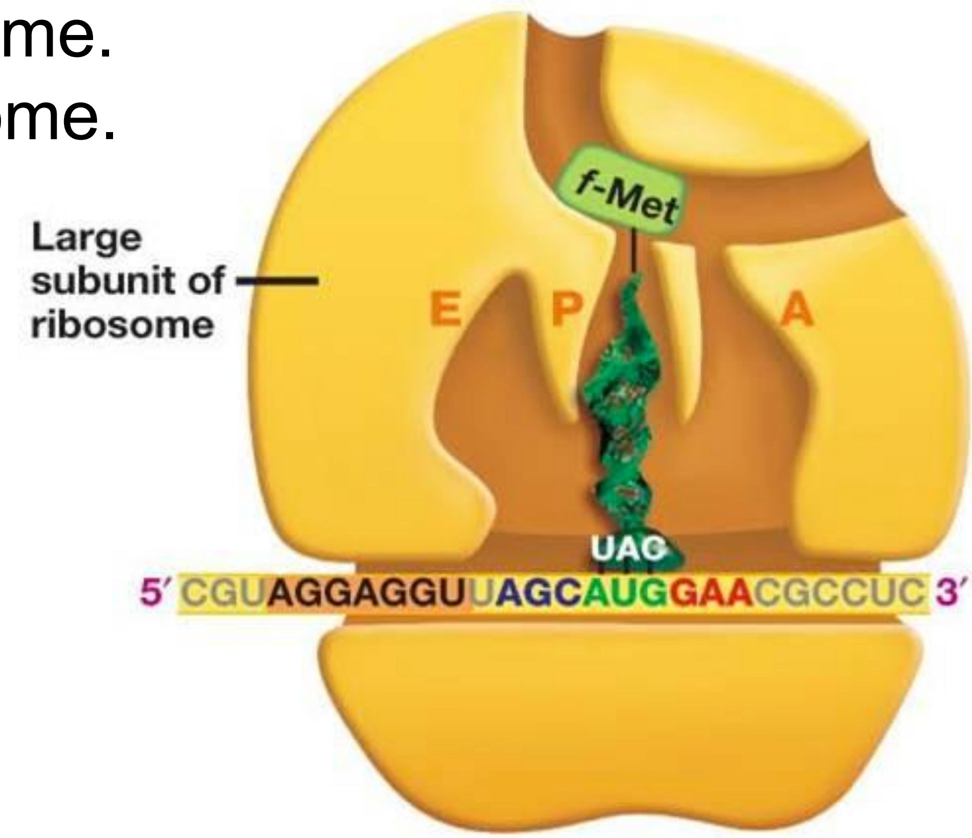
- The initiation factors (IF1 and IF3) gets released and the resulting complex is called **the initiation complex.**



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

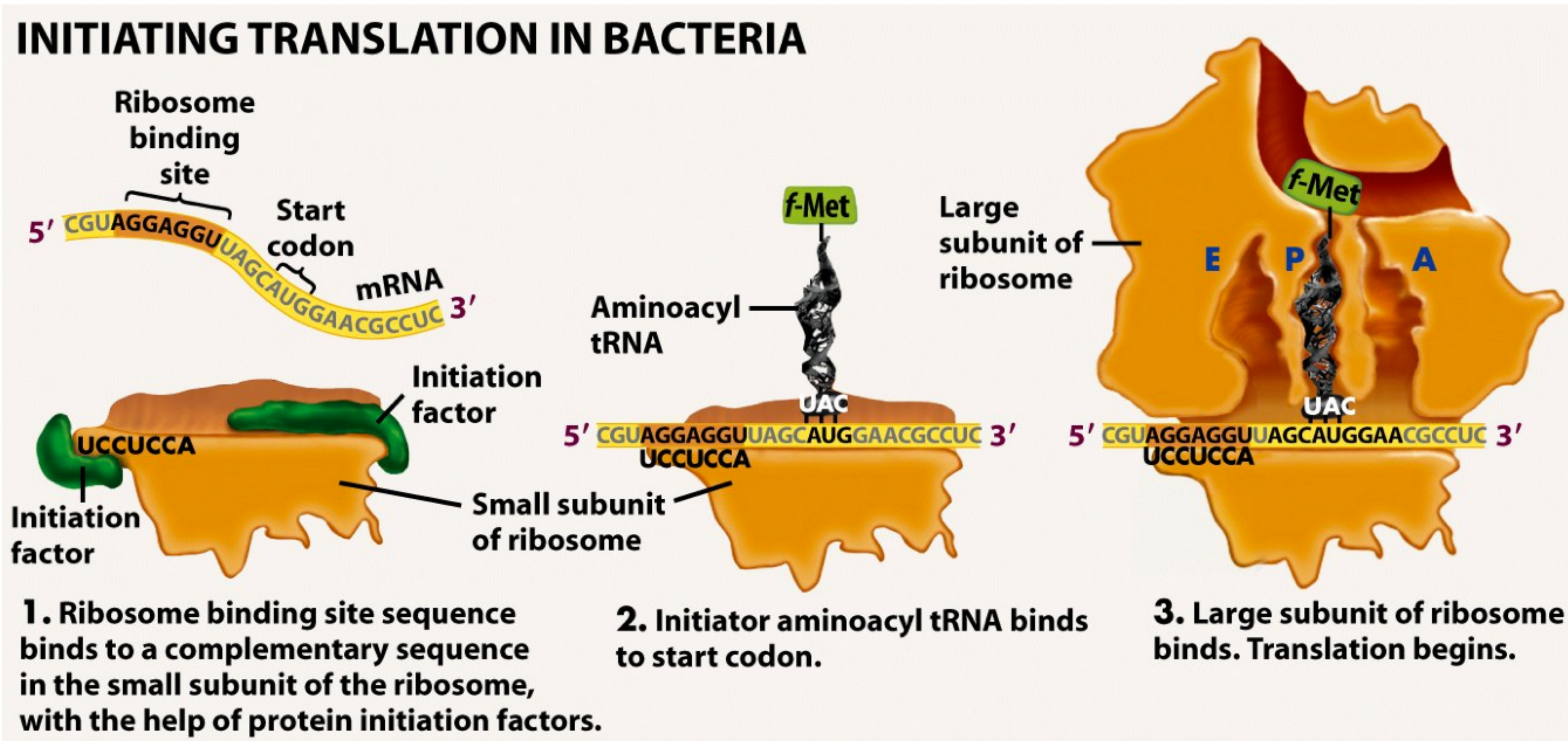
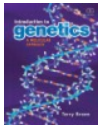


Figure 16-14 Biological Science, 2/e

- The nucleotide sequence of an mRNA is translated into a protein.
- Translation is only the first stage in protein synthesis
- Before becoming functional in the cell, the linear amino acid sequence that results from translation must be folded into its correct secondary, tertiary, and, possibly, quaternary structures



- In some cases protein must also undergo processing, possibly by:
  - Chemical modification
  - Removal of some segments of the polypeptide chain

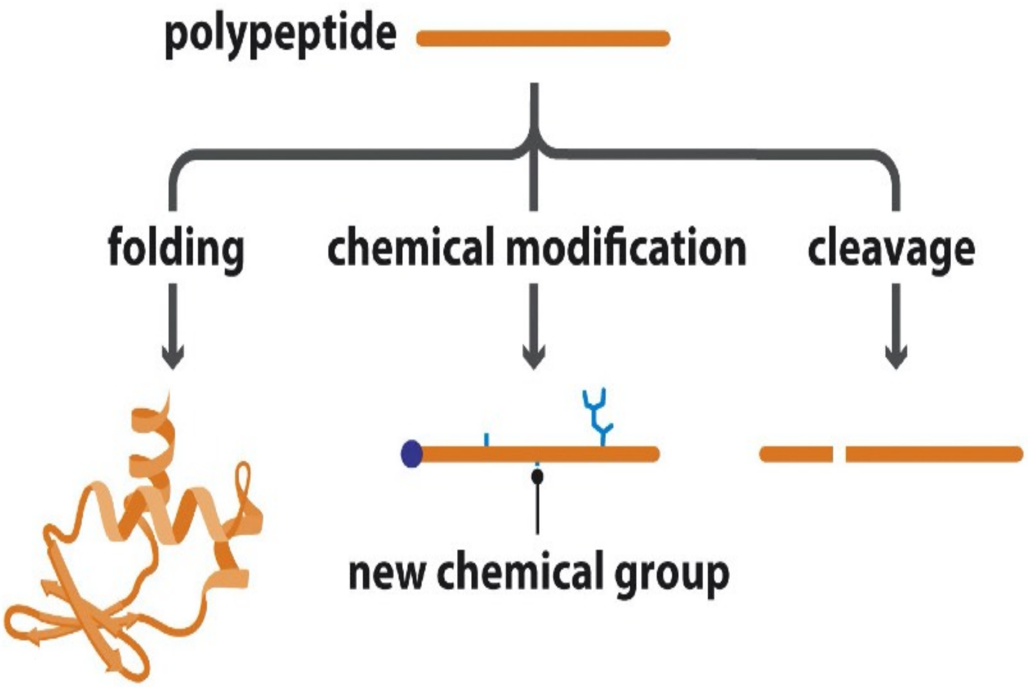
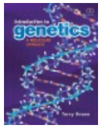
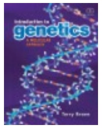


Figure. A summary of protein processing events.



- These post-translational events are essential steps in the final stage of gene expression
- To allow the biological information contained in the gene fully available to the cell
- The collection of proteins in a cell is called the **proteome**
- Within the proteome, the identity and relative abundance of individual proteins represent a balance between the synthesis of new proteins and the degradation of existing ones



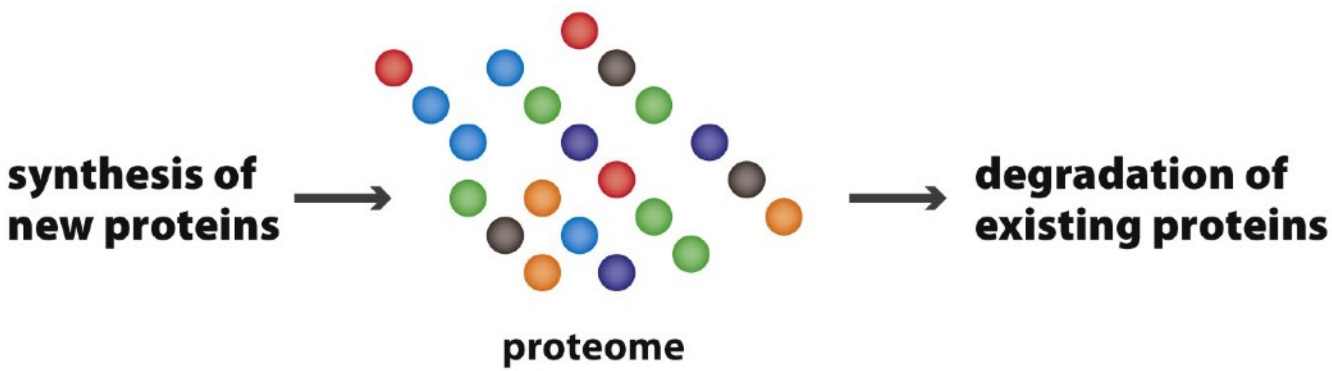
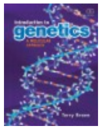
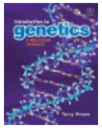


Figure. The composition of a proteome reflects the balance between synthesis of new proteins and degradation of existing ones.

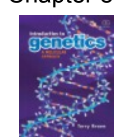


- Degradation is needed so that individual proteins can be down- as well as up-regulated by changing their rate of synthesis
- The terminal step in the expression pathway for a gene is therefore the process by which its protein, when no longer needed, is removed from the proteome.



The role of the ribosome in protein synthesis

- Messenger RNAs are translated within the structures called ribosomes.
- Events of translation:
  - Initiation of translation
  - Elongation of the polypeptide chain
  - Termination of translation
- These events are similar in bacteria and eukaryotes, though the details are different



Initiation

- Initiation in bacteria requires an internal ribosome binding site
- In bacteria the translation initiation complex is built up directly at the initiation codon
  - The point at which protein synthesis will begin
- Eukaryotes use a more indirect process for locating the initiation point

Initiation in Bacteria

- The process initiates when a small subunit attaches to the **ribosome binding site** (also called the **Shine– Dalgarno sequence**) on the mRNA
- This is a short sequence, consensus 5’-AGGAGGU-3’ in *E. coli*
- It is located about 3 to 10 nucleotides upstream of the initiation codon, the point at which translation will begin
- The ribosome binding site is complementary to a region at the 3’ end of the 16S rRNA present in the small subunit of the ribosome

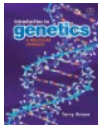
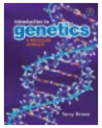


TABLE 8.1 EXAMPLES OF RIBOSOME BINDING SITE SEQUENCES IN *E. COLI*

Gene	Codes for	Ribosome binding sequence	Nucleotides from the initiation codon
E. coli consensus	—	5′-AGGAGGU-3′	3–10
Lactose operon	Lactose utilization enzymes	5′-AGGA-3′	7
galE	Hexose-1-phosphate uridyltransferase	5′-GGAG-3′	6
rplJ	Ribosomal protein L10	5′-AGGAG-3′	8

Table 8.1 Introduction to Genetics (© Garland Science 2012)



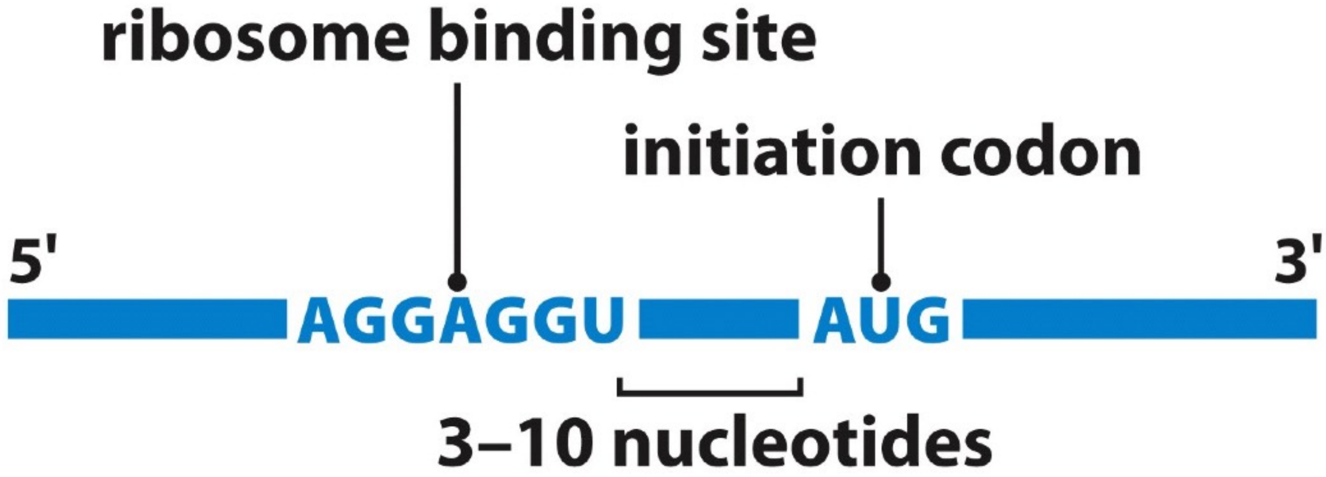
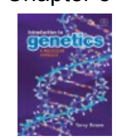


Figure. The ribosome binding site for bacterial translation

- Base pairing between the two is involved in the attachment of the small subunit to the mRNA
- Attachment to the ribosome binding site positions the small subunit of the ribosome over the initiation codon 5’-AUG-3’, which codes for methionine.
- Although 5’-GUG-3’ and 5’-UUG-3’ are sometimes used.
- All three codons can be recognized by the same initiator tRNA, the last two by wobble



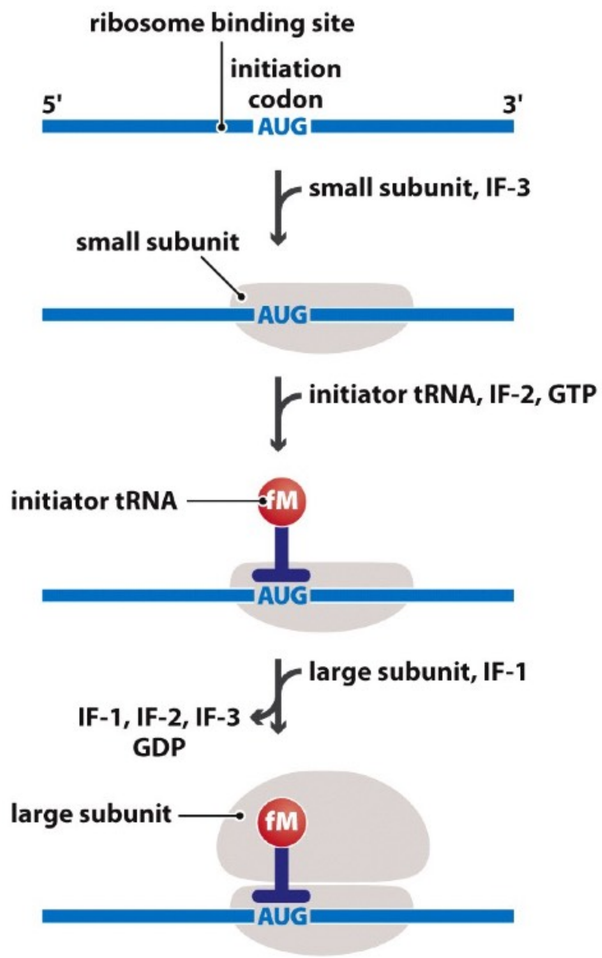
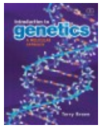
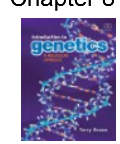


Figure. Initiation of translation in *E. coli*.



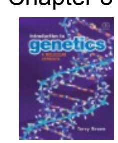
- The initiator tRNA (tRNAi) joins the small subunit of the ribosome along with a molecule of GTP
  - which will be used as an energy source for the final step of initiation.
- This initiator tRNA is aminoacylated with methionine and subsequently modified by conversion of the methionine to ***N*-formylmethionine**
  - The modification attaches a formyl group (–COH) to the amino group
  - Only the carboxyl of the initiator methionine is free to participate in peptide bond formation



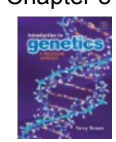
- This ensures that polypeptide synthesis can take place only in the

  N C direction

- The formyl group remains attached until translation has proceeded into the elongation phase
- Then it is removed from the growing polypeptide
- Either on its own or along with the rest of the initial methionine



- The $tRNA_i^{Met}$ is able to decode only the initiation codon
- It cannot enter the complete ribosome during the elongation phase of translation
- During elongation, the internal 5’-AUG-3’ codons are recognized by a different $tRNA^{Met}$ carrying an unmodified methionine

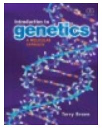


- Initiation also requires three proteins, called **initiation factors**
- These are not permanent components of the ribosome
- They attach at the appropriate times in order to perform their functions

TABLE 8.2 FUNCTIONS OF INITIATION FACTORS IN BACTERIA

Initiation factor	Function
IF-1	Unclear. Might cause conformational changes that prepare the small subunit for attachment to the large subunit, or might prevent premature entry of the second aminoacyl-tRNA.
IF-2	Directs the initiator tRNA to its correct position in the initiation complex.
IF-3	Prevents premature reassociation of the large and small subunits of the ribosome.

Table 8.2 Introduction to Genetics (© Garland Science 2012)



- The initiation phase of translation is completed when the large subunit of the ribosome attaches to the small subunit
- forming a complete ribosome positioned over the initiation codon
- Attachment of the large subunit requires energy
- Energy generated by hydrolysis of the GTP that was bound earlier during initiation
- This results in release of the initiation factors

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?

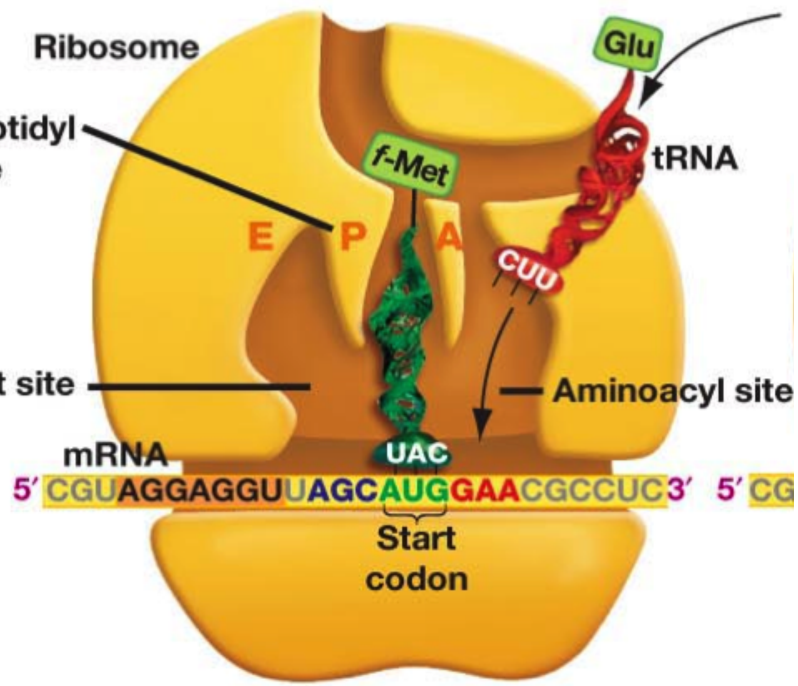
1. Charged tRNA
2. Elongation factors (EF)
3. GTP

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.



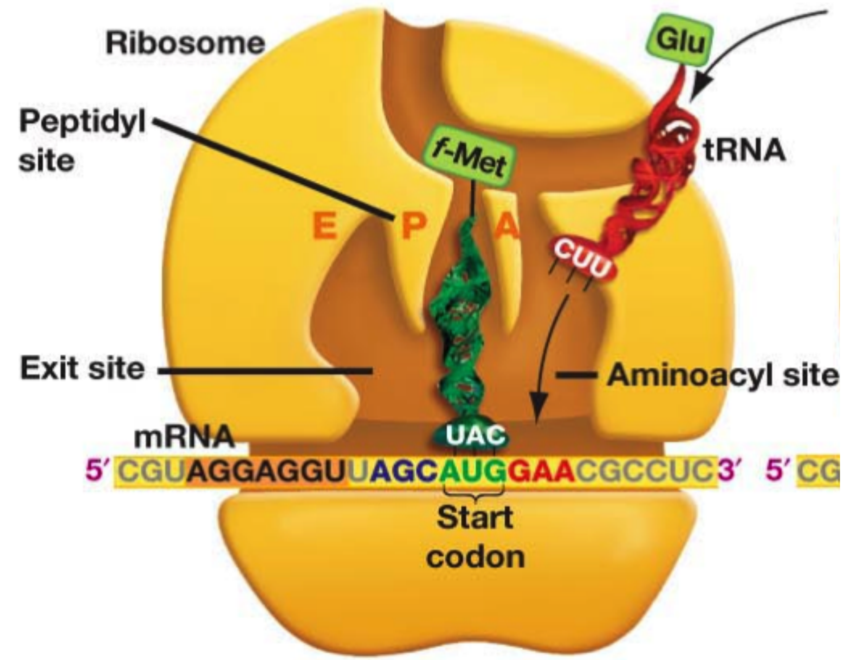
1. Incoming aminoacyl tRNA

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)**.



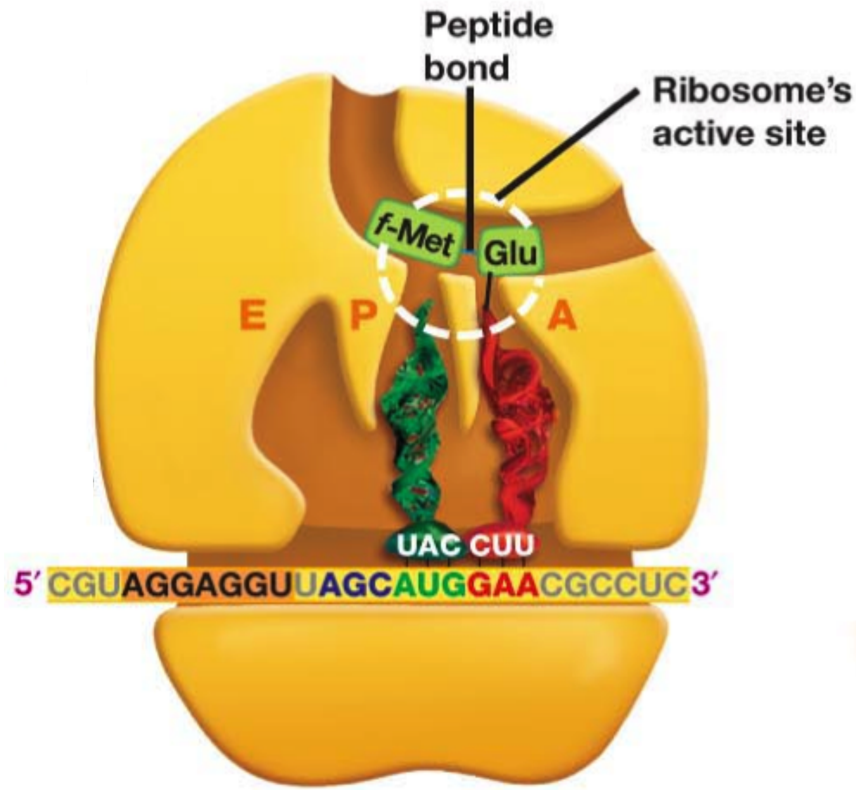
1. Incoming aminoacyl tRNA

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.



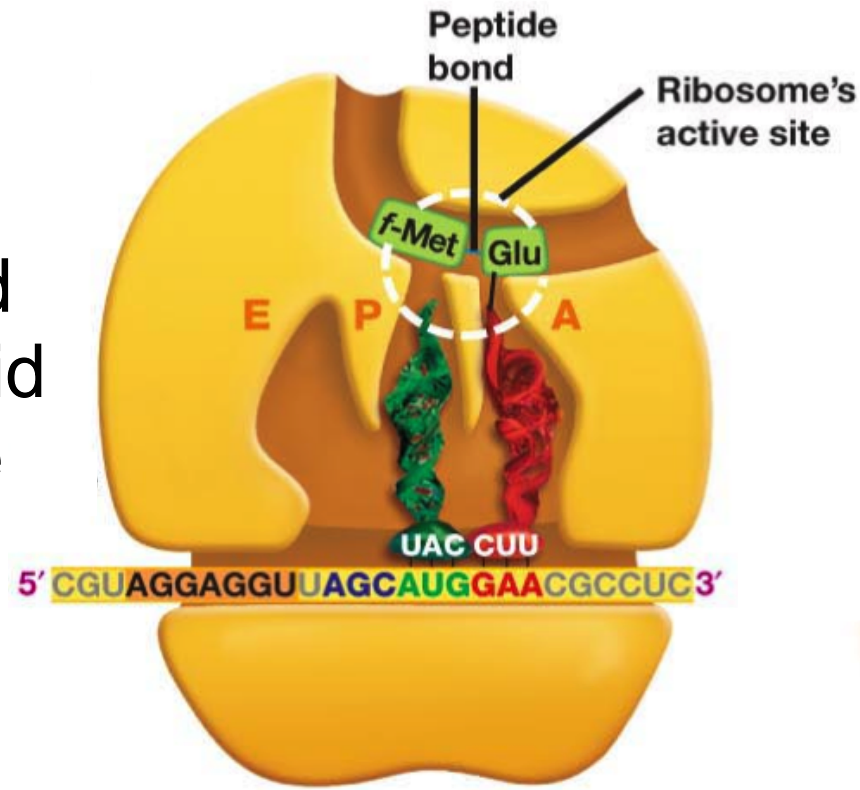
2. Peptide bond formation

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



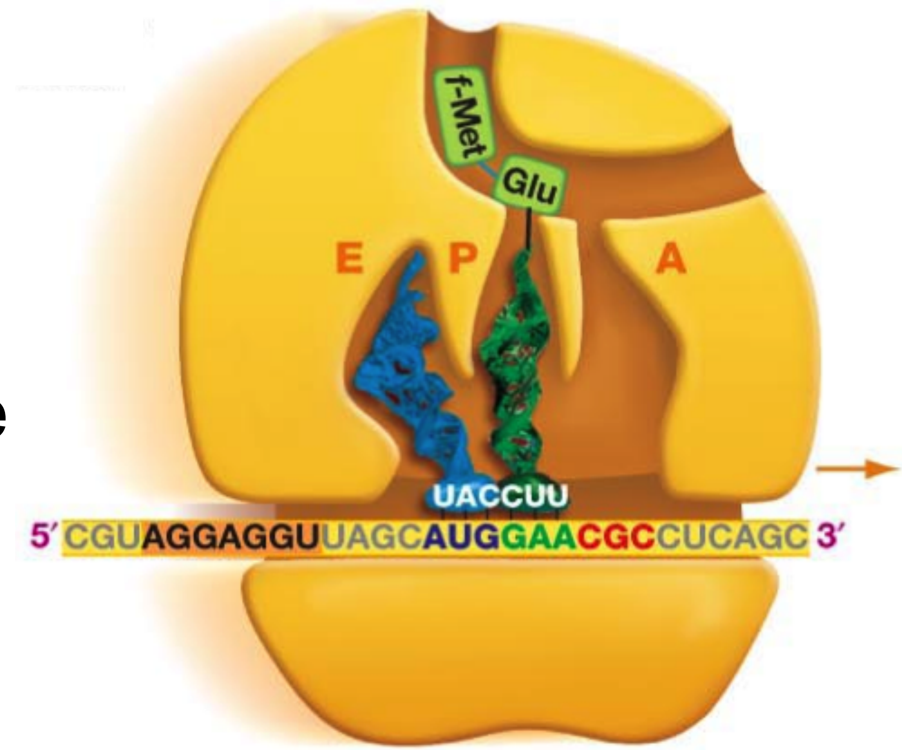
2. Peptide bond formation

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’).



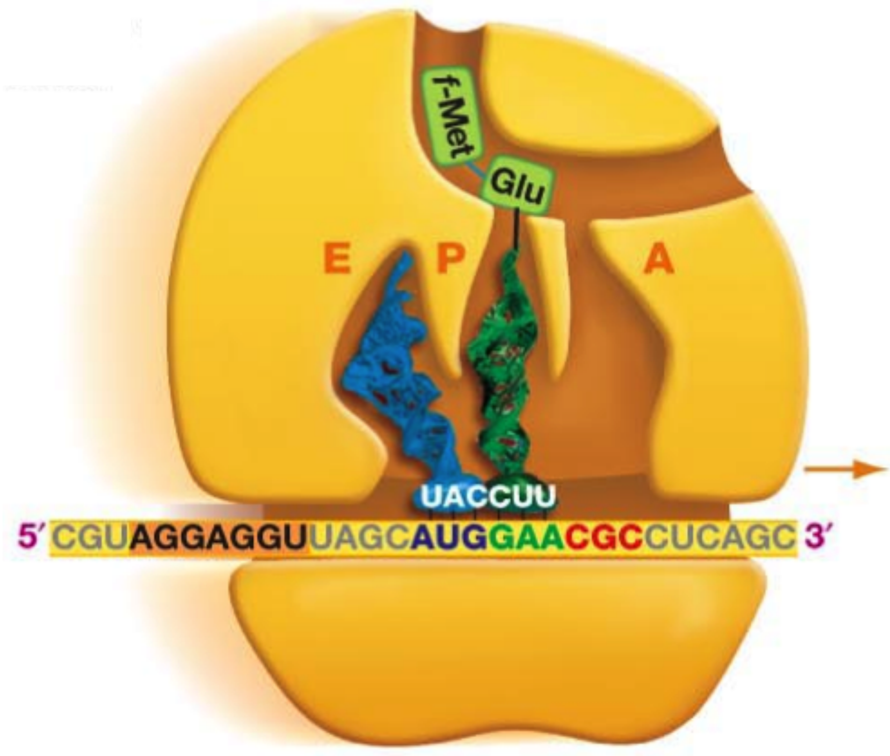
3. Translocation

Translation elongation in bacteria

Elongation process:

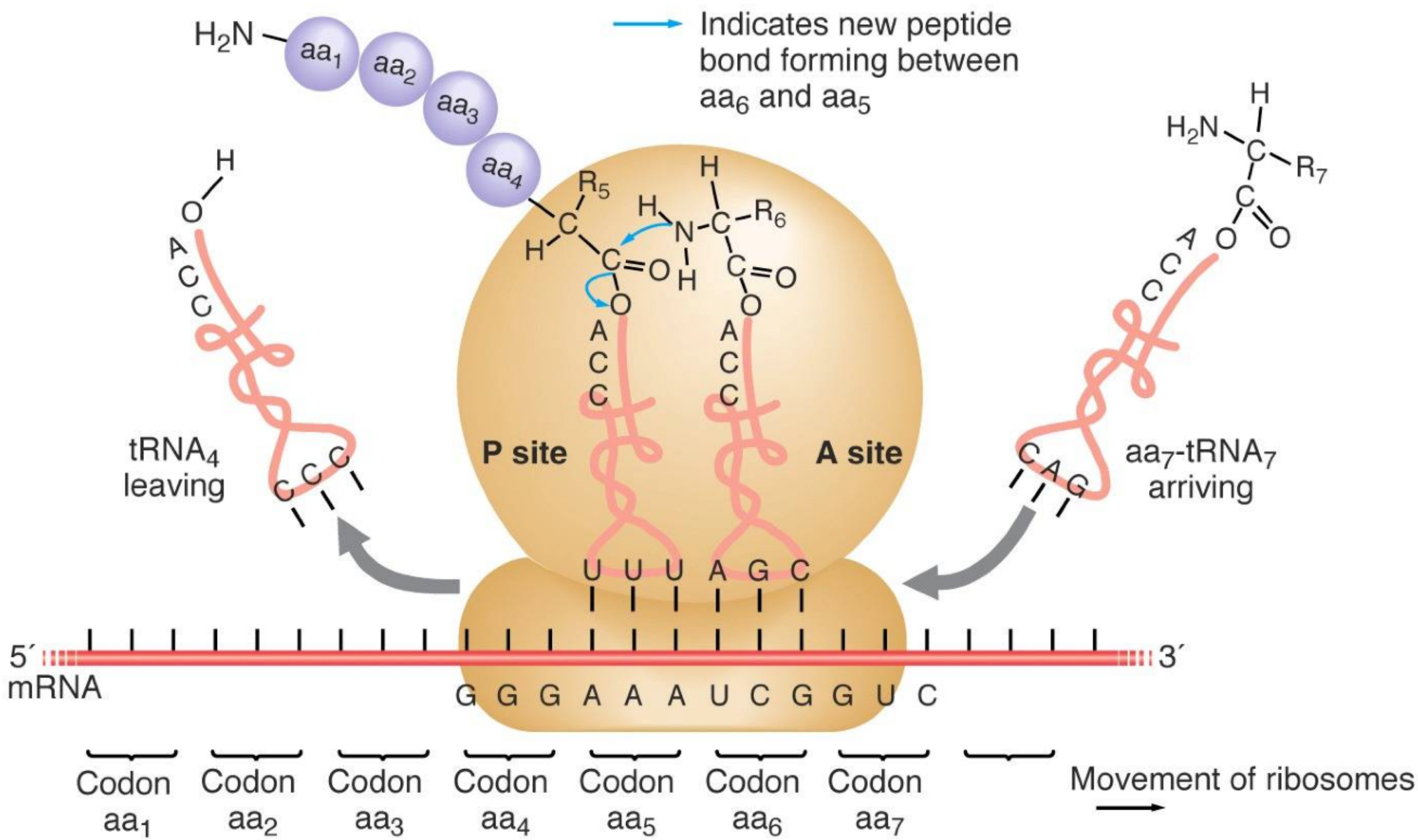
10. Free tRNA moves to the E site.

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



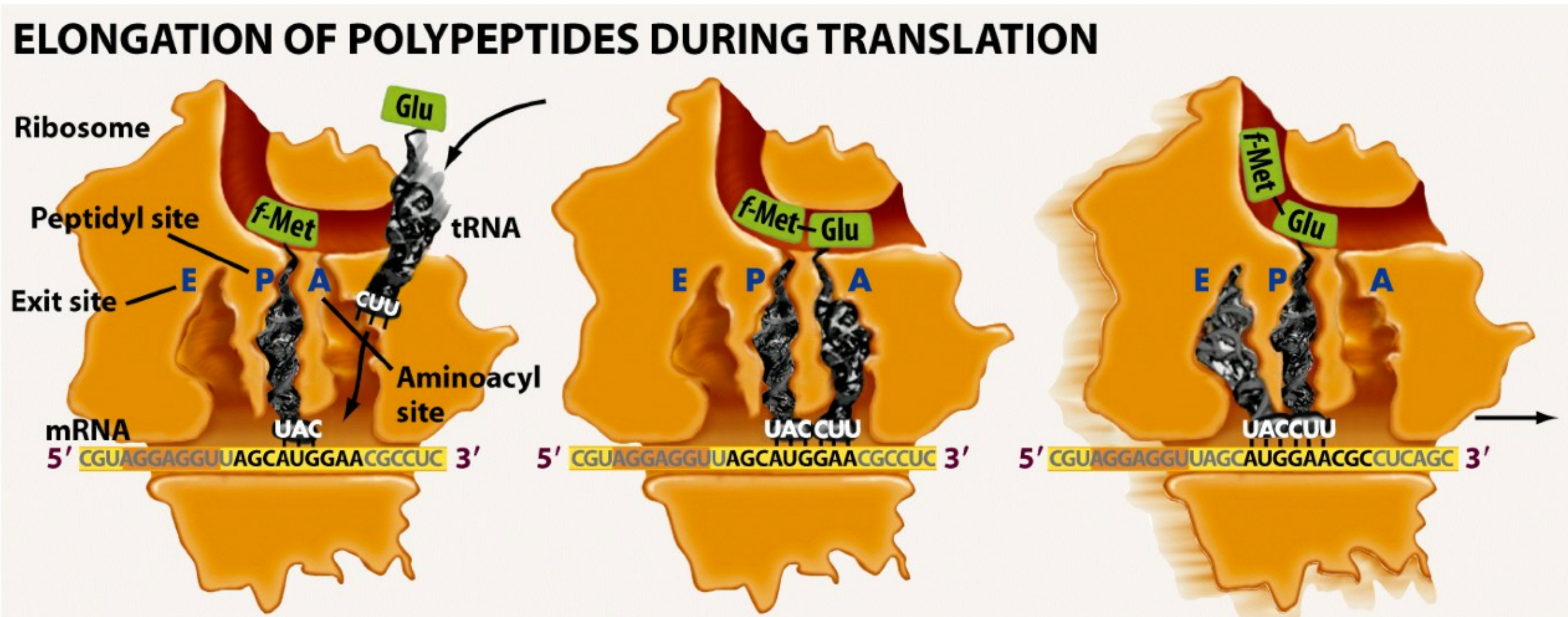
3. Translocation

Peptide bond formation



Translation elongation in bacteria

Summary 1



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

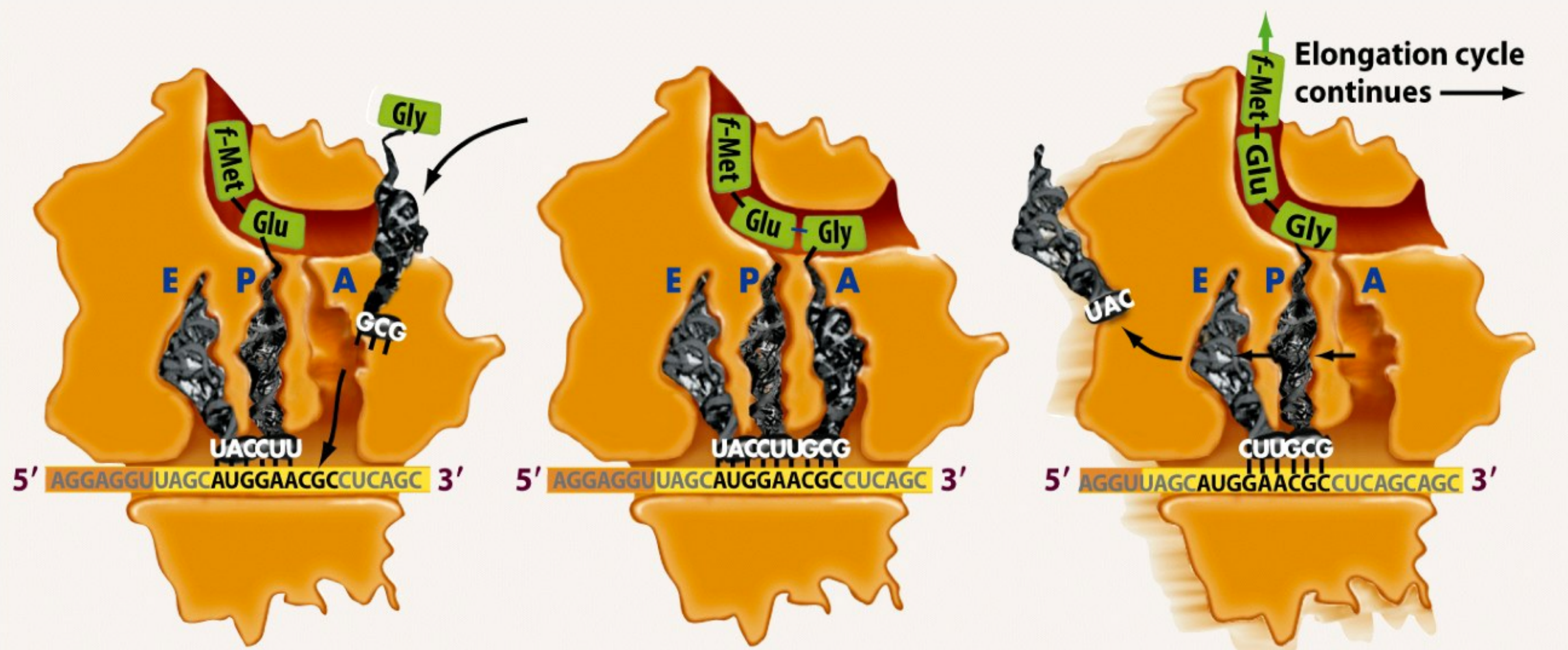
2. Peptide bond formation
The amino acid attached to the tRNA in the P site is transferred to the tRNA in the A site.

3. Translocation
Ribosome moves down mRNA. The tRNA attached to polypeptide chain moves into P site. The A site is empty.

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.

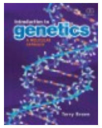
5. Peptide bond formation
The polypeptide chain attached to the tRNA in the P site is transferred to the tRNA in the A site.

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

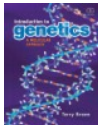
Elongation

- The stage during which the protein is synthesized step by step
- Individual amino acids being attached to the carboxyl terminus of the growing polypeptide
- Elongation is similar in bacteria and eukaryotes



- Bringing together the two ribosome subunits creates two sites at which aminoacyl-tRNAs can bind:
  - the **peptidyl** (or **P**) **site**
  - the **aminoacyl** (or **A**) **site**

- The **peptidyl** (or **P**) **site**,
  - Already occupied by the initiator $tRNA_f^{Met}$, charged with *N*-formylmethionine or methionine and base-paired with the initiation codon.
- The **aminoacyl** (or **A**) **site**
  - covers the second codon in the open reading frame



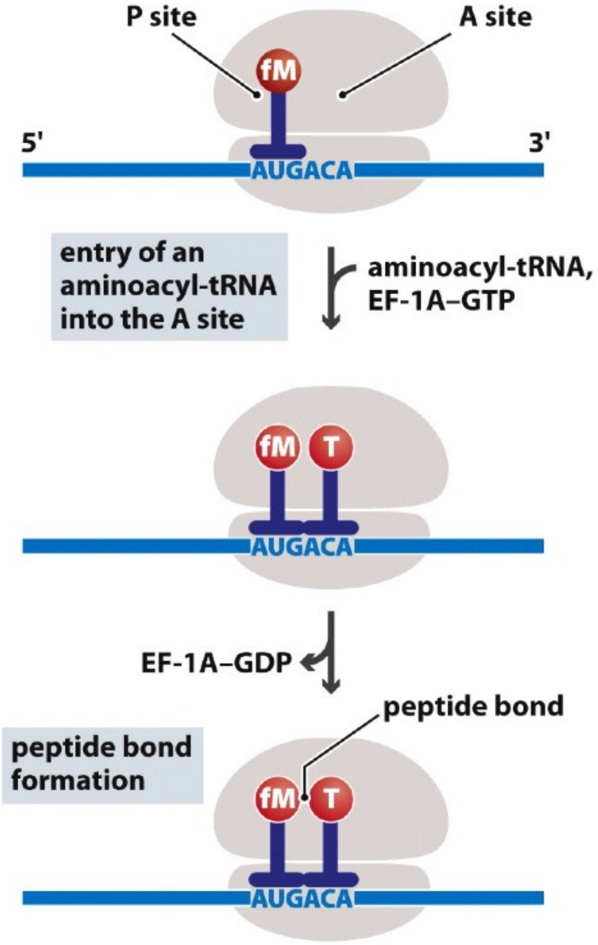
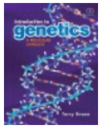
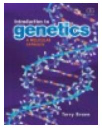


Figure. The first steps in the elongation phase of protein synthesis in *E. coli*.



- The ribosome structures revealed by X-ray crystallography show that these sites are located in the cavity between the large and small subunits of the ribosome
- The codon–anticodon interaction being associated with the small subunit and the aminoacyl end of the tRNA with the large subunit



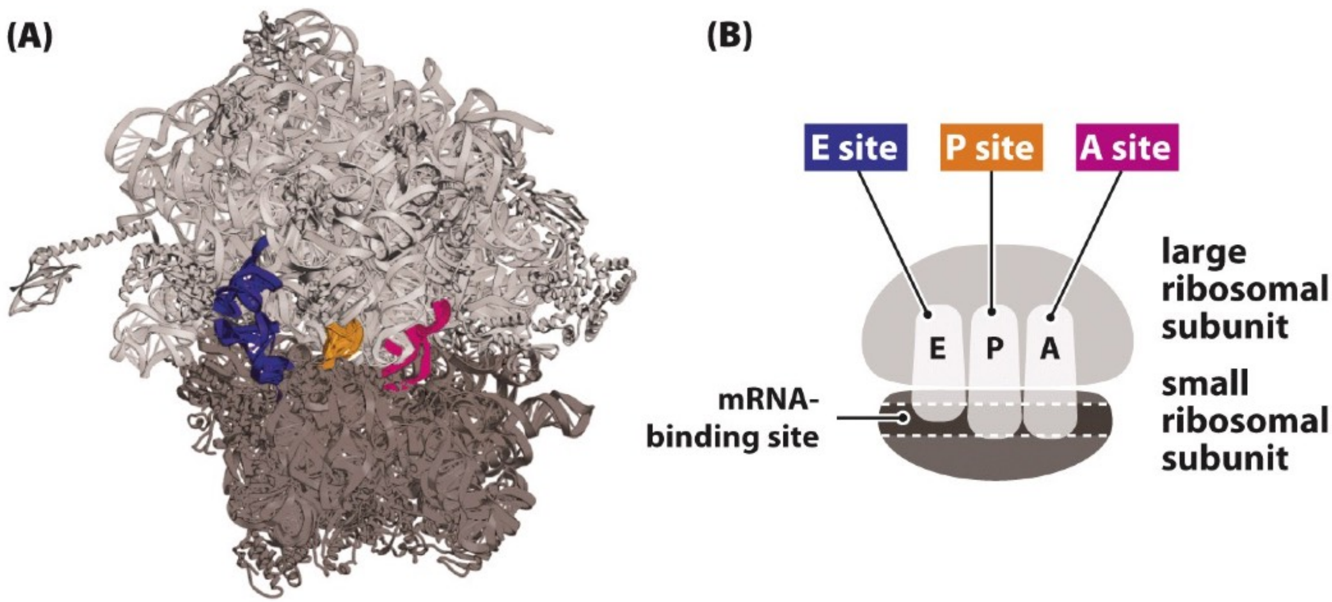
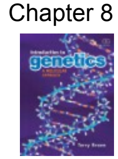


Figure. The structure of a bacterial ribosome during the elongation phase of translation.



To begin elongation

- The A site becomes filled with the appropriate aminoacyl- tRNA,
- which in *E. coli* is brought into position by the **elongation factor** EF-1A
- This factor helps to ensure that translation is accurate
- It allows only a tRNA that carries its correct amino acid to enter the ribosome
- EF-1A also binds a molecule of GTP, which will be hydrolyzed to release energy when the peptide bond is formed.
- In eukaryotes the equivalent factor is called eEF-1

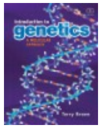
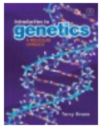


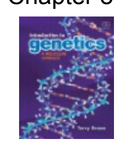
TABLE 8.4 FUNCTIONS OF ELONGATION FACTORS IN BACTERIA AND EUKARYOTES	
Initiation factor	**Function**
Bacteria	
EF-1A	Directs the next tRNA to its correct position in the ribosome
EF-1B	Regenerates EF-1A after the latter has yielded the energy contained in its attached GTP molecule
EF-2	Mediates translocation
Eukaryotes	
eEF-1	Complex of four subunits (eEF-1a, eEF-1b, eEF-1d, and eEF-1g); directs the next tRNA to its correct position in the ribosome
eEF-2	Mediates translocation
Note that the bacterial elongation factors have recently been renamed. The older designations were EF-Tu, EF-Ts, and EF-G for EF-1A, EF-1B, and EF-2, respectively.	

Table 8.4 Introduction to Genetics (© Garland Science 2012)

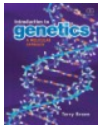
- Once in the A site, the anticodon of the tRNA must form base pairs with the next codon of the mRNA
- The tRNA and mRNA must fit exactly within the A site
- If there is a mispair at any position in the codon–anticodon interaction, then the tRNA is rejected



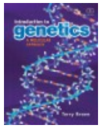
- When the correct aminoacyl-tRNA has entered the A site
- A peptide bond is formed between the two amino acids
- The bond is formed by the enzyme called **peptidyl transferase**
- In both bacteria and eukaryotes, peptidyl transferase is an example of a ribozyme—an RNA enzyme
- The catalytic activity for peptide bond formation is provided not by a protein
- But by the largest of the rRNAs present in the large subunit of the ribosome



- Peptide bond formation is energy-dependent
- It requires the hydrolysis of the GTP attached to EF-1A (eEF-1 in eukaryotes)
- This inactivates EF-1A, which is ejected from the ribosome and regenerated by EF-1B
- A eukaryotic equivalent of EF-1B has not been identified
    - It is possible that one of the subunits of eEF-1 provides the regenerative activity.

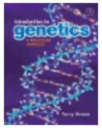


- Formation of the first peptide bond results in a dipeptide
- Which correspond to the first two codons of the open reading frame
- The attachment between the first amino acid and its tRNA is broken at this stage
- leaving the dipeptide attached to the tRNA located in the A site
- The next step is **translocation**, during which the ribosome moves three
- nucleotides along the mRNA, so a new codon enters the A site



The next step is translocation

- During which the ribosome moves three nucleotides along the mRNA
- Then a new codon enters the A site
- This moves the dipeptide-tRNA to the P site
- Which in turn displaces the deacylated tRNA
- In eukaryotes, the deacylated tRNA is simply ejected from the ribosome
- But in bacteria the deacylated tRNA departs via a third position the **exit** (or **E**) **site**



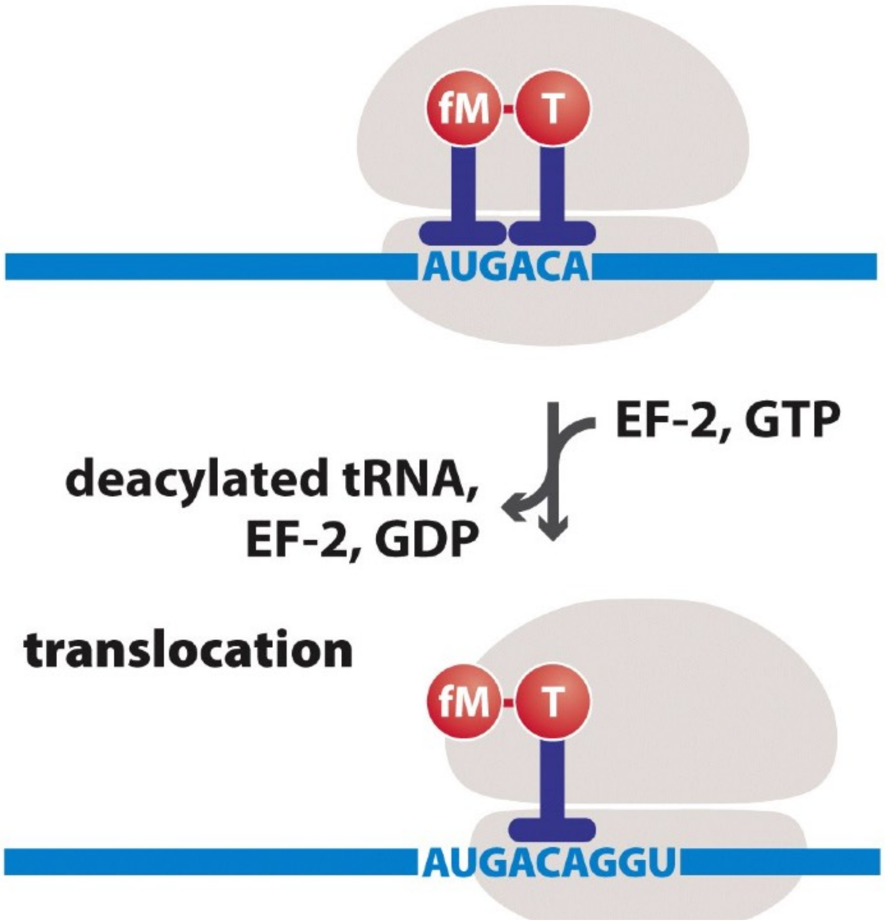
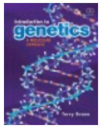
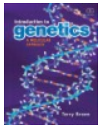


Figure. The first cycle of translocation during protein synthesis in *E. coli*.



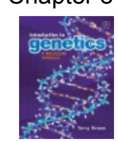
E-Site

- It was originally looked on as a simple exit point from the ribosome
- It is now known to have an important role in ensuring that translocation moves the ribosome along the mRNA by precisely three nucleotides
- To ensure that the ribosome keeps to the correct reading frame



- Translocation requires hydrolysis of a molecule of GTP and is mediated by EF-2 in bacteria and by eEF-2 in eukaryotes
- Electron microscopy of ribosomes at different intermediate stages in translocation shows that, in order to move along the mRNA, the ribosome adopts a less compact structure
- With the two subunits rotating slightly in opposite directions
- This opens up the space between them and enables the ribosome to slide along the mRNA
- Translocation results in the A site becoming vacant
- Allowing a new aminoacyl-tRNA to enter

- The elongation cycle is now repeated
- continues until a termination codon is reached
- After several cycles of elongation the start of the mRNA molecule is no longer associated with the ribosome
- A second ribosome can attach and begin to synthesize another copy of the protein
- The end result is a **polysome**
    - An mRNA that is being translated by several ribosomes at once
- Polysomes have been seen in electron microscopic images of both prokaryotic and eukaryotic cells.



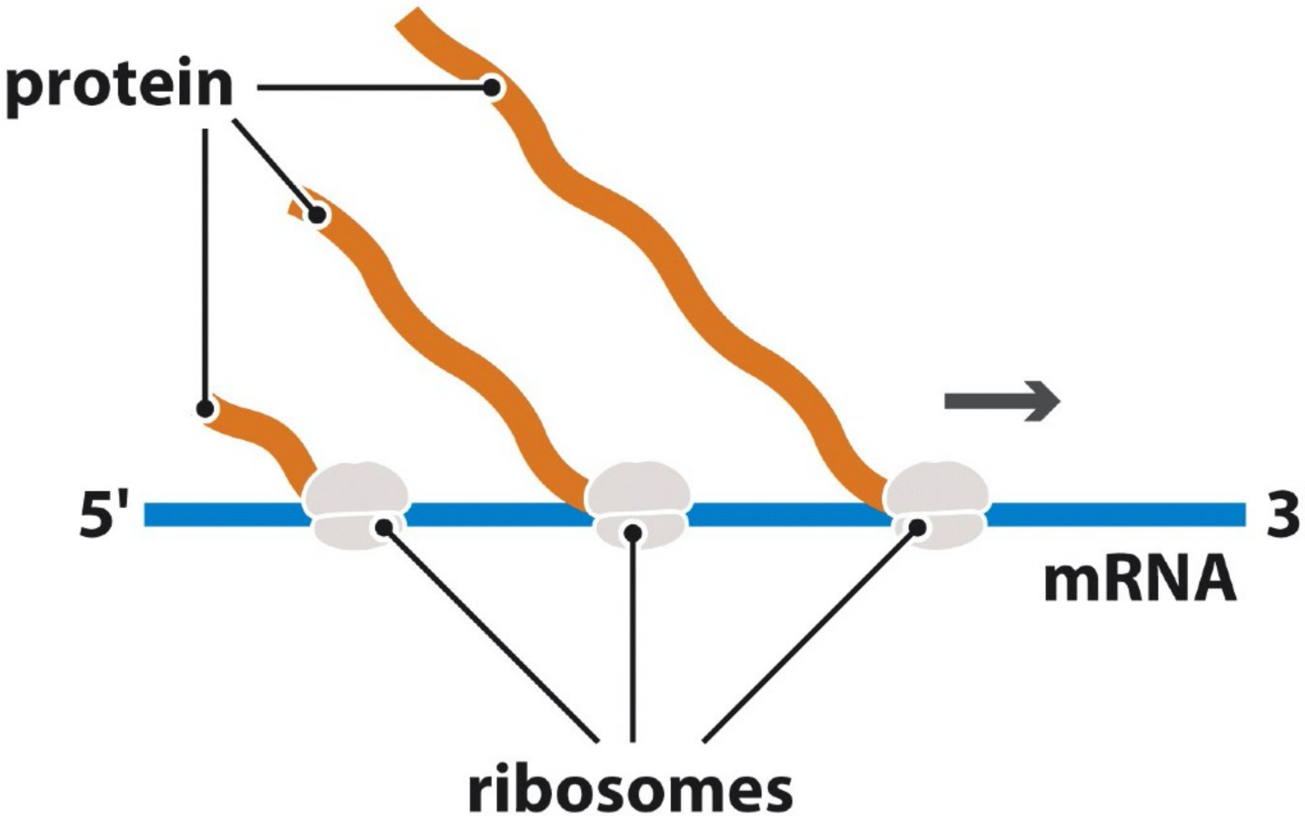
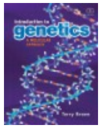


Figure. A polysome.



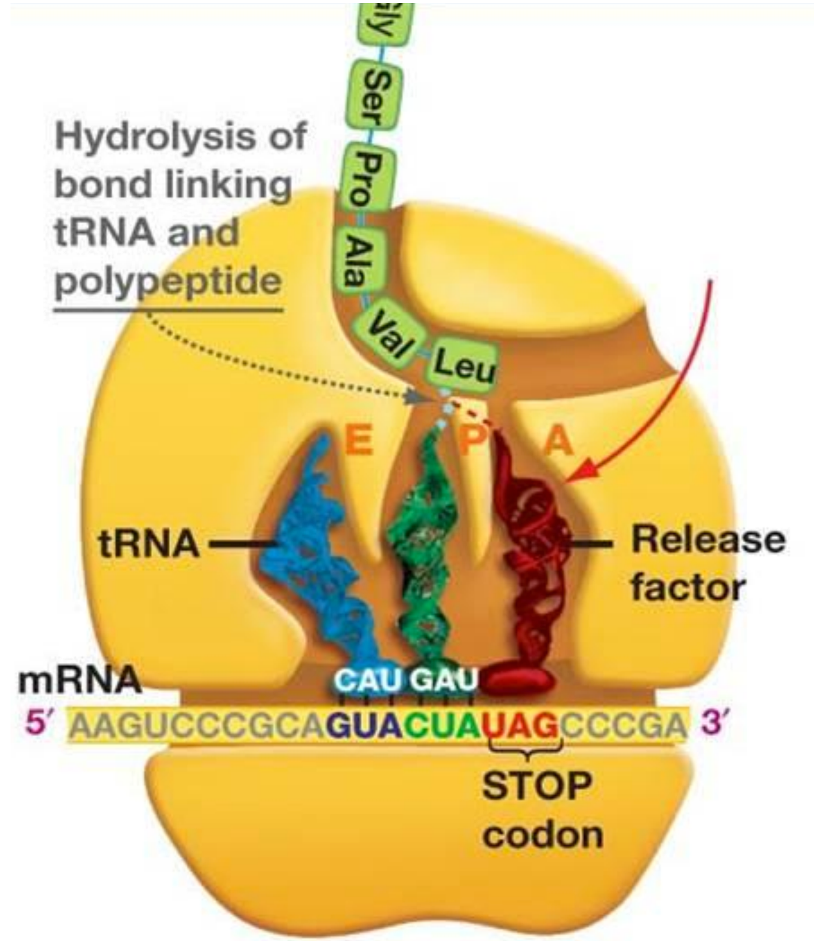
Translation termination in bacteria

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.

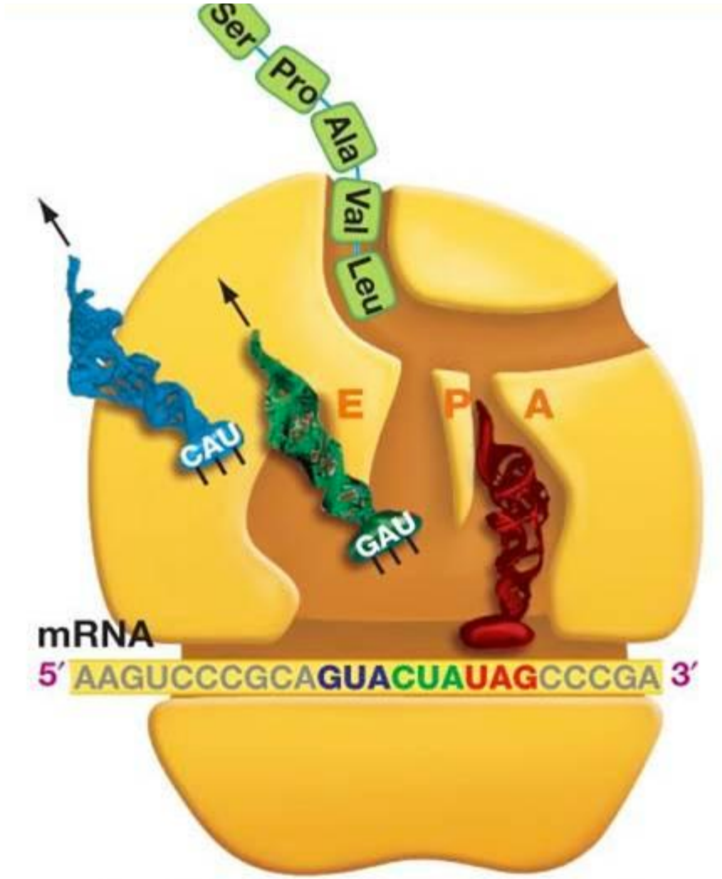


1. Release factor binds to stop codon.

Translation termination in bacteria

4. Amino acid chain is released.

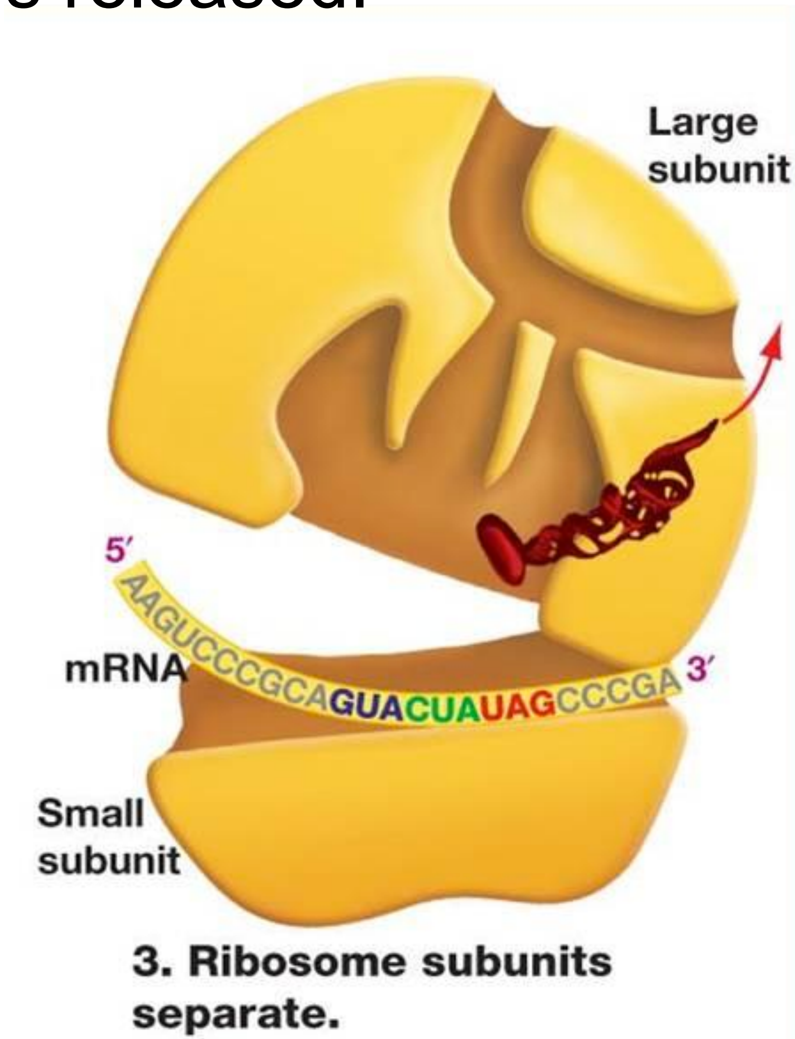
5. tRNA at E site and P site are released.



2. Polypeptide is released.

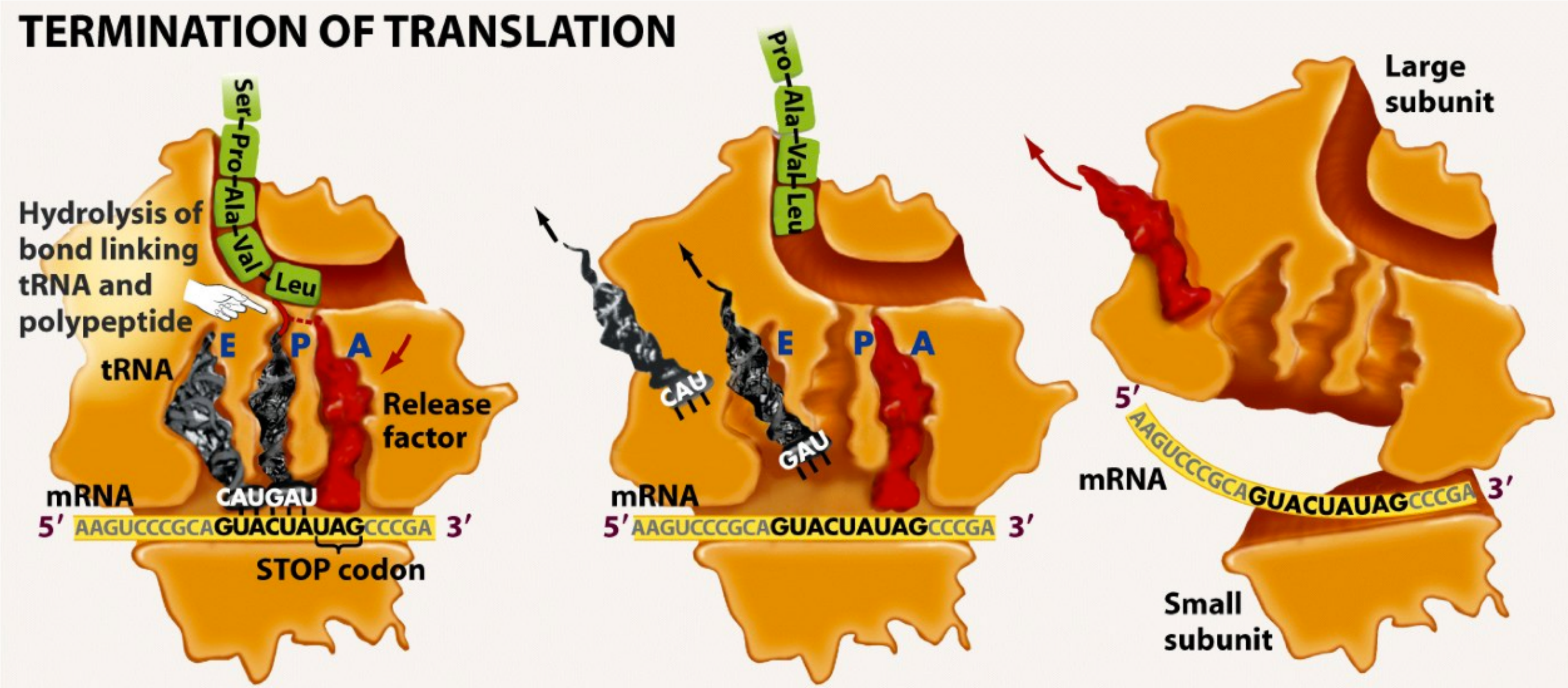
Translation termination in bacteria

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



Translation termination in bacteria

Summary



1. When translocation opens the A site and exposes one of the stop codons, a protein called a release factor fills the A site. The release factor catalyzes the hydrolysis of the bond linking the tRNA in the P site with the polypeptide chain.

2. The hydrolysis reaction frees the polypeptide, which is released from the ribosome. The empty tRNAs are released either along with the polypeptide or when the ribosome dissociates following release of the polypeptide.

3. The ribosome then separates from the mRNA, and the two ribosomal subunits dissociate. The subunits are ready to attach to the start codon of another message and start translation anew.

Termination

- Protein synthesis ends when one of the three termination codons enters the A site
- There are no tRNA molecules with anticodons able to basepair with any of the termination codons
- Aprotein **release factor** enters the A site in order to terminate translation

In bacteria

- In bacteria there are three release factors:
  - RF-1
  - RF-2
  - RF-3
- In eukaryotes:
  - eRF-1
  - eRF-3

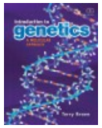
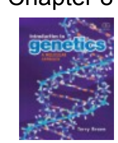


TABLE 8.5 RELEASE AND RIBOSOME RECYCLING FACTORS IN BACTERIA AND EUKARYOTES

Factor	Function
Bacteria	
RF-1	Recognizes the termination codons 5′-UAA-3′ and 5′-UAG-3′
RF-2	Recognizes 5′-UAA-3′ and 5′-UGA-3′
RF-3	Stimulates dissociation of RF-1 and RF-2 from the ribosome after termination
RRF	Ribosome release factor, responsible for separation of the ribosome subunits after translation has terminated
Eukaryotes	
eRF-1	Recognizes the termination codon
eRF-3	Possibly stimulates dissociation of eRF-1 from the ribosome after termination; possibly causes the ribosome subunits to disassociate after termination of translation

Table 8.5 Introduction to Genetics (© Garland Science 2012)



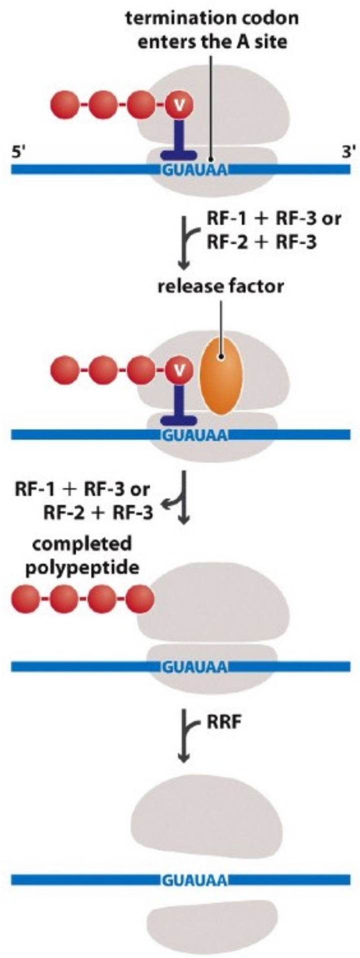
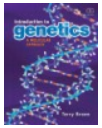


Figure Termination of translation in *E. coli*. In the top drawing, the ribosome has reached a termination codon



- The structure of eRF-1 has been solved by X-ray crystallography
- showing that the shape of this protein is very similar to that of a tRNA

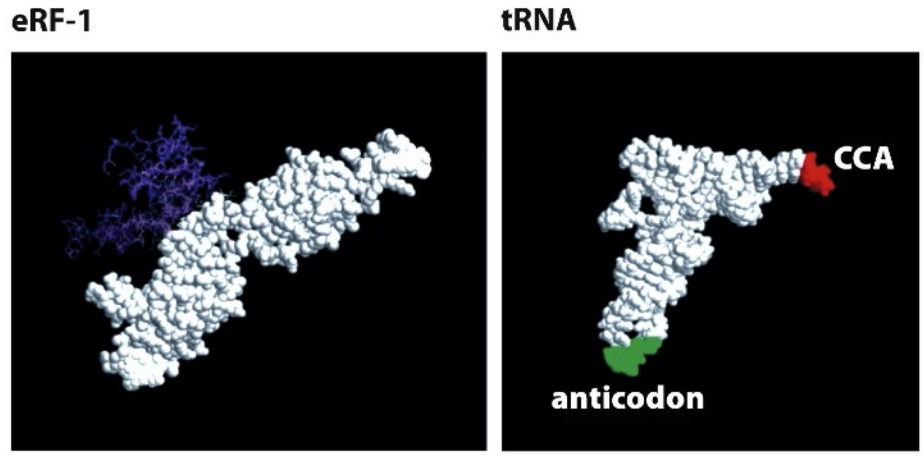
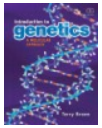
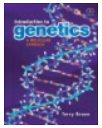


Figure 8.12 Introduction to Genetics (© Garland Science 2012)

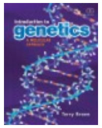


- The release factor is able to enter the A site by mimicking a tRNA
- But some researchers believe that the structural similarities are deceptive and that there are unresolved complexities about the way in which the release factors work

- The release factors terminate translation
- but they do not appear to be responsible for separation of the ribosomal subunits, at least not in bacteria.
- This is the function of an additional protein called **ribosome recycling factor** (**RRF**)
    - which also has a tRNA-like structure
- RRF probably enters the P or A site and “unlocks” the ribosome



- Separation of the subunits requires energy
- Which is released from GTP by EF-2, one of the elongation factors
- Also requires the initiation factor IF-3 to prevent the subunits from attaching together again
- A eukaryotic equivalent of RRF has not been identified
- and this may be one of the functions of eRF-3
- The ribosome subunits enter the cytoplasmic pool
- Where they remain until used again in another round of translation



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