# Translation



- Objectives
  - Mutation
  - Steps of Protein synthesis
    - Prokaryotes
    - Eukaryotes
  - Post translational Modification
  - Clinical implications

# Case 1

- A 3-year-old Caucasian boy is brought to the clinic for a chronic productive cough not responding to antibiotics given recently. He has no fever or sick contacts. His medical history is significant for abdominal distention, failure to pass stool, and emesis as an infant. He continues to have bulky, foul-smelling stools. No diarrhea is present. He has several relatives with chronic lung and “stomach” problems, and some have even died at a young age. The examination reveals an ill appearing, slender male in moderate distress. The lung exam reveals poor air movement in the base of lungs bilateral and coarse rhonchi throughout both lung fields. A chloride sweat test was performed and was positive, indicating cystic fibrosis (CF).

- ◆ **What is the mechanism of the disease?**
- ◆ **How might gel electrophoresis assist in making the diagnosis**



# Case 2

- An 8-year-old boy is brought to his pediatrician by his mother because she was concerned that he was having language-speech problems, was hyperactive, and was told by teachers that he may have mental retardation. The mother reports a strong family history of mental retardation in males. The boy on exam is found to have a large jaw, prominent ears, and enlarged testes (macroorchidism). The mother was told her family had a genetic problem causing the mental retardation. The patient underwent a series of blood tests and was scheduled to see a genetic counselor, who expressed that the etiology of the genetic defect was likely transmitted from his mother. The genetic counselor states that his mother likely has a silent mutation.

- **What is the most likely diagnosis?**
- **◆ Which chromosome is likely to be affected?**
- **◆ What are some types of biochemical mutations?**
- **◆ What is the biochemical basis of the different types of mutations?**



# Case 3

- A 15-year-old African-American female presents to the emergency room with complaints of bilateral thigh and hip pain. The pain has been present for 1 day and is steadily increasing in severity. Acetaminophen and ibuprofen have not relieved her symptoms. She denies any recent trauma or excessive exercise. She does report feeling fatigued and has been having burning with urination along with urinating frequently. She reports having similar pain episodes in the past, sometimes requiring hospitalization. On examination, she is afebrile (without fever) and in no acute distress. No one in her family has similar episodes. Her conjunctiva and mucosal membranes are slightly pale in coloration. She has nonspecific bilateral anterior thigh pain with no abnormalities appreciated. The remainder of her examination is completely normal. Her white blood cell count is elevated at 17,000/mm3, and her hemoglobin (Hb) level is decreased at 7.1 g/dL. The urinalysis demonstrated an abnormal number of numerous bacteria.

- **What is the most likely diagnosis?**
- **◆ What is the molecular genetics behind this disorder?**
- **◆ What is the pathophysiologic mechanism of her symptoms?**



# Sickle cell disease (pain crisis).

- **CLINICAL CORRELATION**
- This 15-year-old female's description of her pain is typical of a sickle cell pain crisis.
- infection is a trigger, most commonly pneumonia or a urinary tract infection. This case is consistent with a urinary tract infection, indicated by her symptoms of urinary frequency, and burning with urination (dysuria).
- Her white blood cell count is elevated in response to the infection.
- The low hemoglobin level is consistent with sickle cell anemia.
- Since she is homozygous (both genes coding for sickle hemoglobin), both her parents have sickle cell trait (heterozygous) and thus do not have symptoms.
- The diagnosis can be established with hemoglobin electrophoresis.
- Treatment includes searching for an underlying cause of crisis (infection, hypoxia, fever, excessive exercise, and extreme changes in temperature), administration of **oxygen**, intravenous fluids for hydration, pain management, and consideration of a blood transfusion.

**Proteome:**

complete set of proteins expressed in a cell

**Genetic code:**

A dictionary that identifies the correspondence between a sequence of nucleotide bases and a sequence of amino acids.



| First Nucleotide | Second Nucleotide | | | | Third Nucleotide |
|---|---|---|---|---|---|
| | U | C | A | G | |
| U | Phe | Ser | Tyr | Cys | U |
| | Phe | Ser | Tyr | Cys | C |
| | Leu | Ser | Term | Term[b] | A |
| | Leu | Ser | Term | Trp | G |
| C | Leu | Pro | His | Arg | U |
| | Leu | Pro | His | Arg | C |
| | Leu | Pro | Gln | Arg | A |
| | Leu | Pro | Gln | Arg | G |
| A | Ile | Thr | Asn | Ser | U |
| | Ile | Thr | Asn | Ser | C |
| | Ile[a] | Thr | Lys | Arg[b] | A |
| | Met | Thr | Lys | Arg[b] | G |
| G | Val | Ala | Asp | Gly | U |
| | Val | Ala | Asp | Gly | C |
| | Val | Ala | Glu | Gly | A |
| | Val | Ala | Glu | Gly | G |

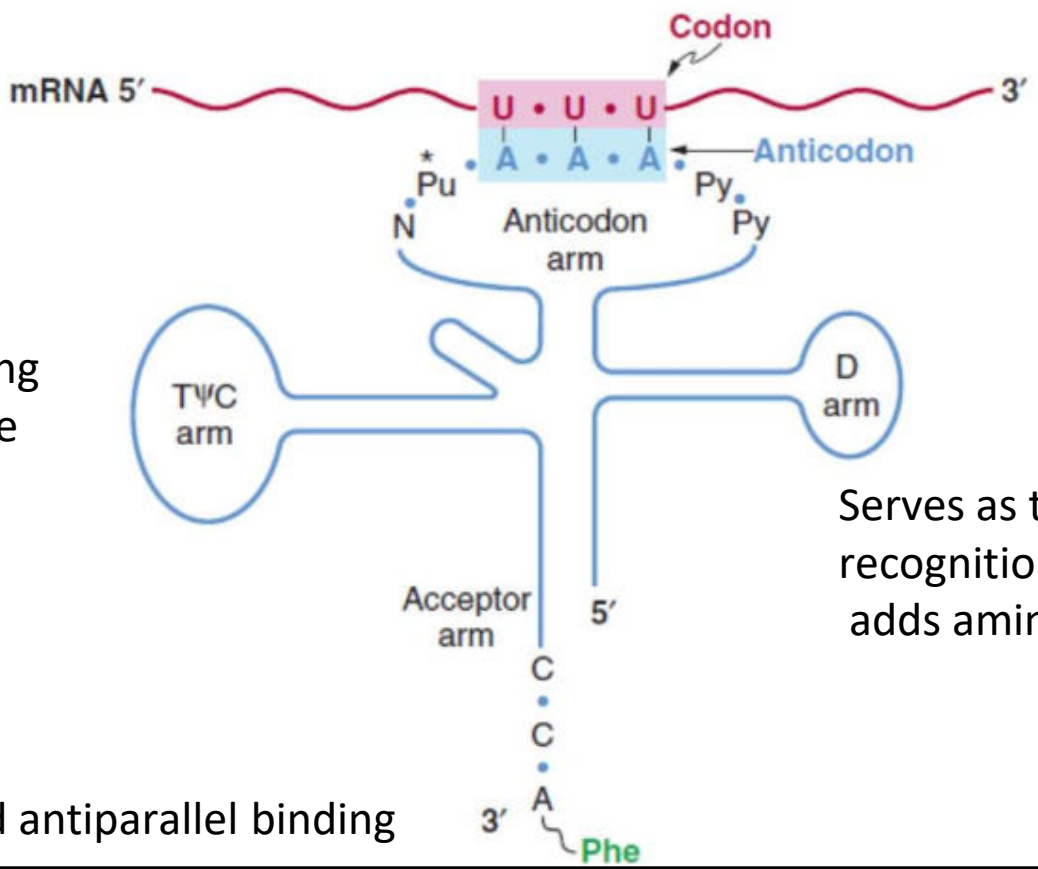
# Features of the Genetic Code

- Degenerate
- Unambiguous
- Nonoverlapping
- Not punctuated
- Universal

In mitochondria: AUA= Met, UGA= trp;
AGA&AGG= stop codon



# Recognition of the codon by the anticodon




Involved in binding tRNA to ribosome

Serves as the recognition site for enzyme: adds amino acid

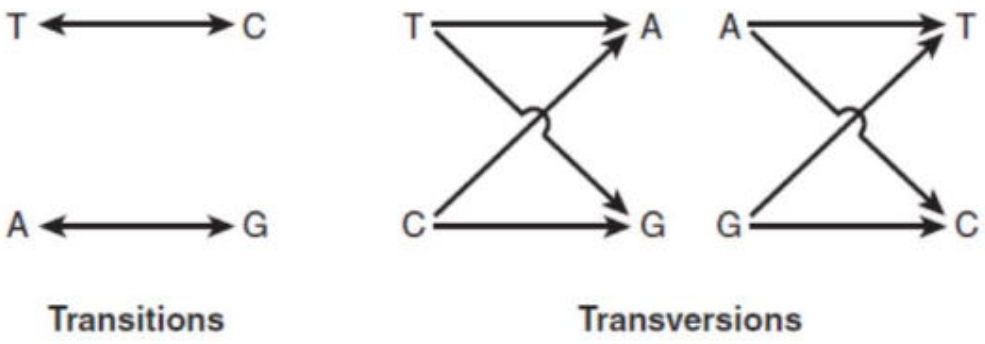
Complimentary and antiparallel binding



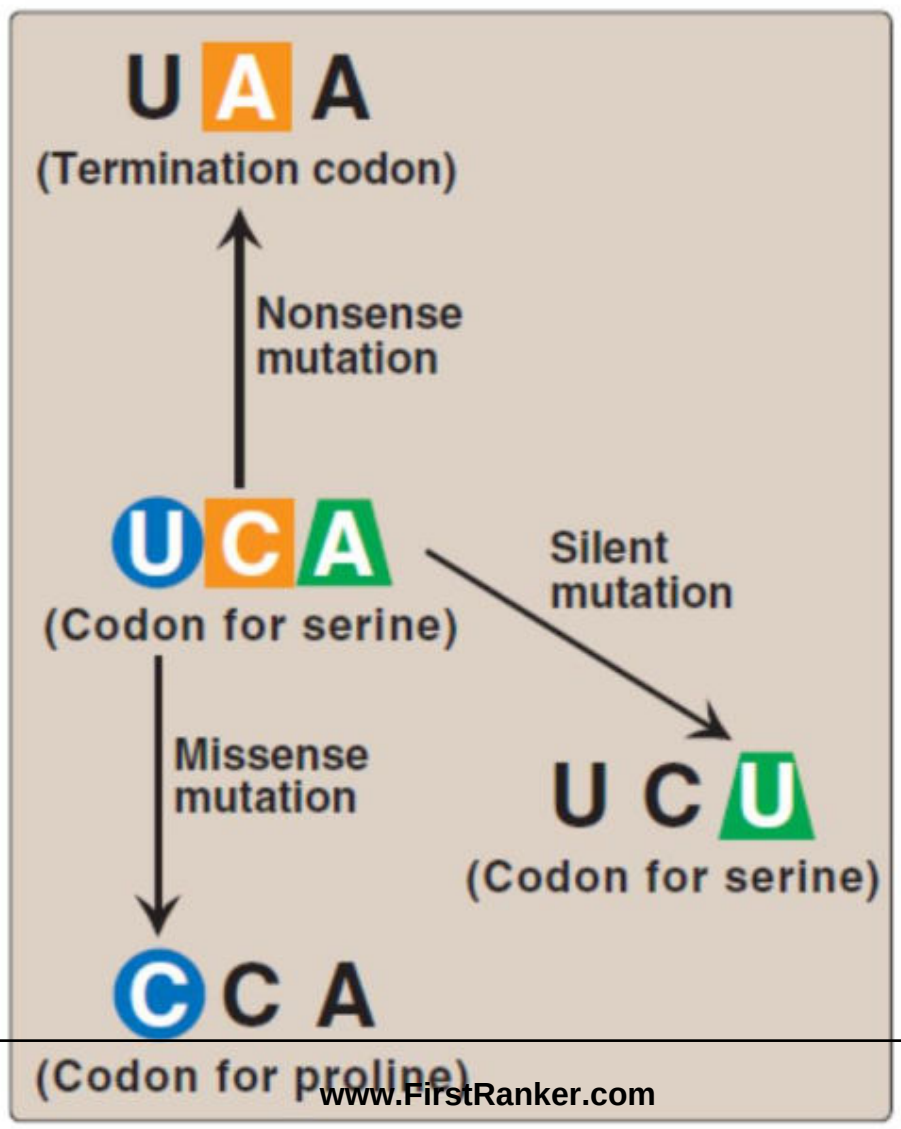


Adapted from Harper's Biochemistry

# Point mutation: Single nucleotide change in coding region




1. Silent mutation
2. Missense mutation
3. Nonsense mutation








Adapted from Lippincott,s Biochemistry

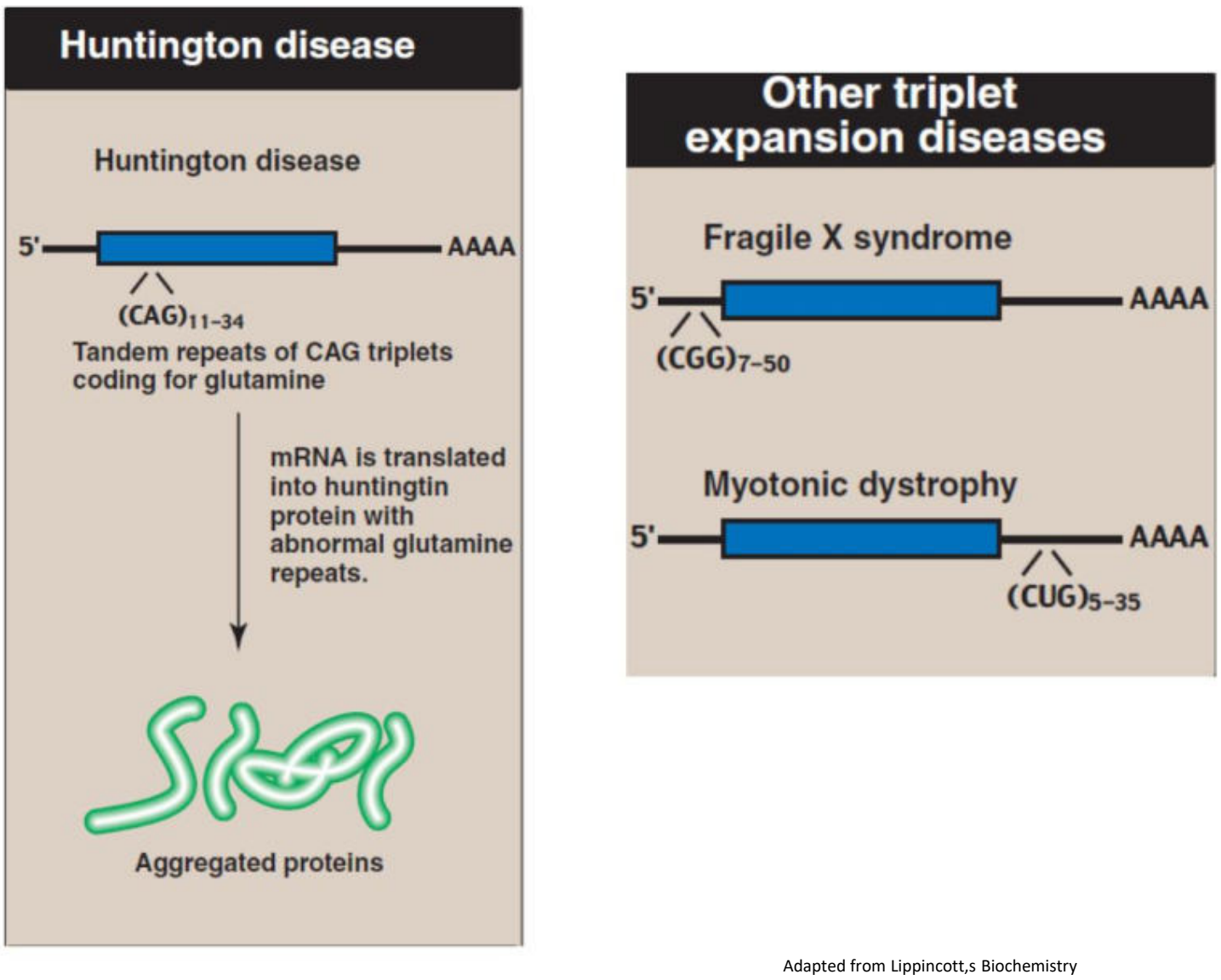
# Missense mutations resulting in abnormal hemoglobin chains

| | Protein molecule | Amino acid | Codons |
|---|---|---|---|
| Acceptable missense | Hb A, β chain → Hb Hikari, β chain | 61 Lysine → Asparagine | AAA or AAG → AAU or AAC |
| Partially acceptable missense | Hb A, β chain → Hb S, β chain | 6 Glutamate → Valine | GAA or GAG → GUA or GUG |
| Unacceptable missense | Hb A, α chain → Hb M (Boston), α chain | 58 Histidine → Tyrosine | CAU or CAC → UAU or UAC |



# Other mutations

# 1. Trinucleotide repeat expansion




Adapted from Lippincott,s Biochemistry

# Fragile X

- Fragile X is the most common inherited form of mental retardation,
- Affecting primarily males
- moderate to severe mental retardation, hyperactivity, typical facies such as large jaw and large ears. Pigmented skin lesions (cafe au lait) can also be seen.
- The fragile X mental retardation (FMR) gene product is affected and, through a little-understood mechanism, leads to mental retardation.

# Fragile X syndrome

- **Molecular basis of disease:**

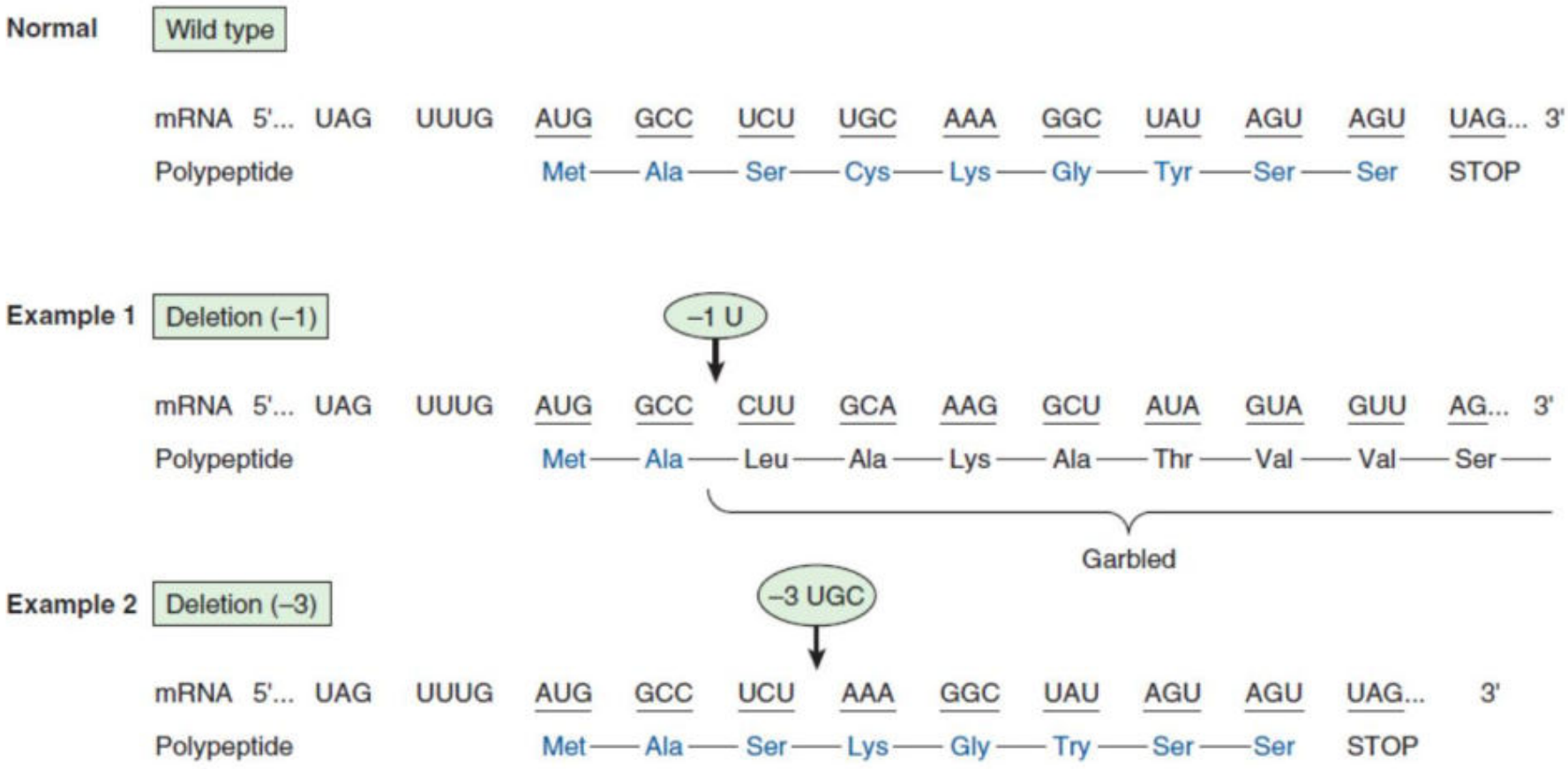
  **Mutation resulting in an increased number** of CGG repeats on the X chromosome. When the number of repeats reaches a critical size, it can be methylated and inactivated resulting in the disorder. Individuals who carry 50 to 199 repeats are phenotypically normal and carry a premutation. If repeats exceed 200, the patient has a full mutation; and if methylation occurs, he or she will be affected.



- 2. Splice site mutation

- 3. Frameshift mutation
  - Deletion
  - Addition

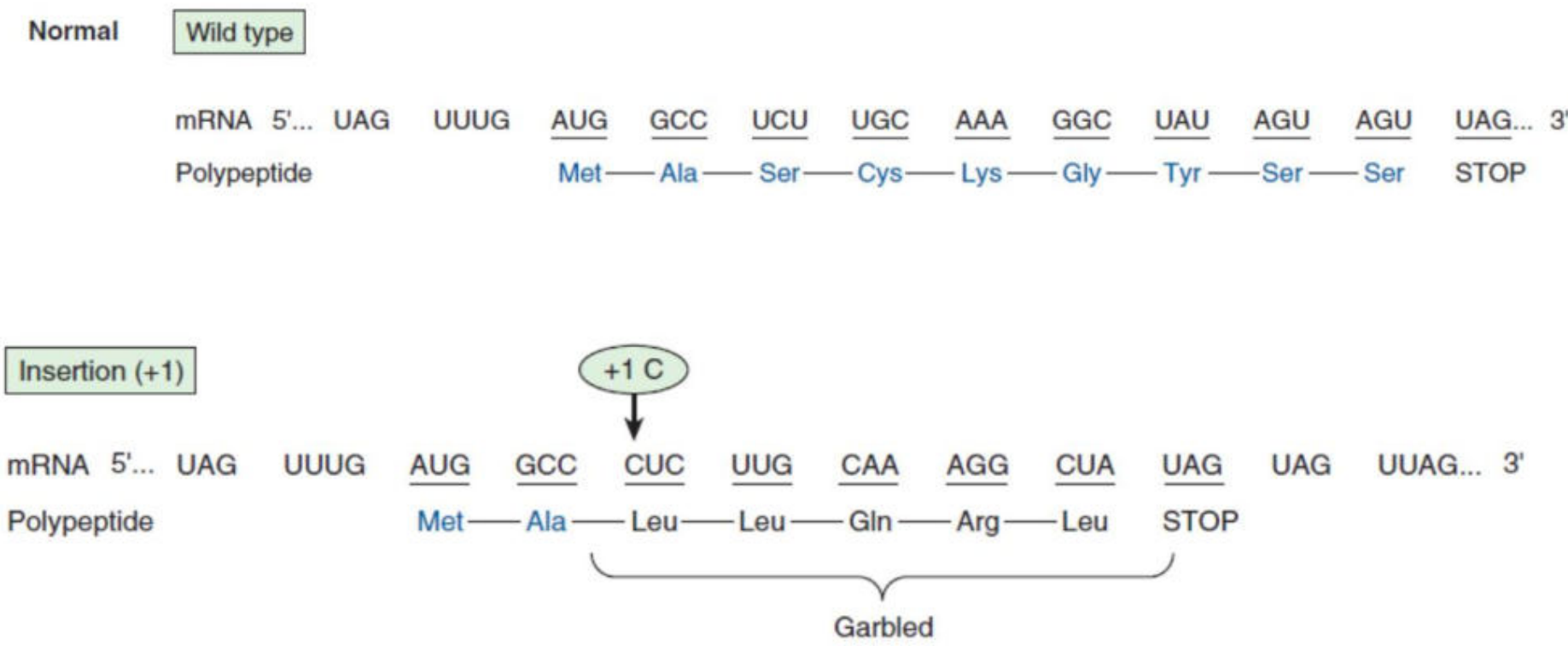
# Effects of deletions in a gene on the sequence of the mRNA transcript and of the polypeptide chain translated therefrom



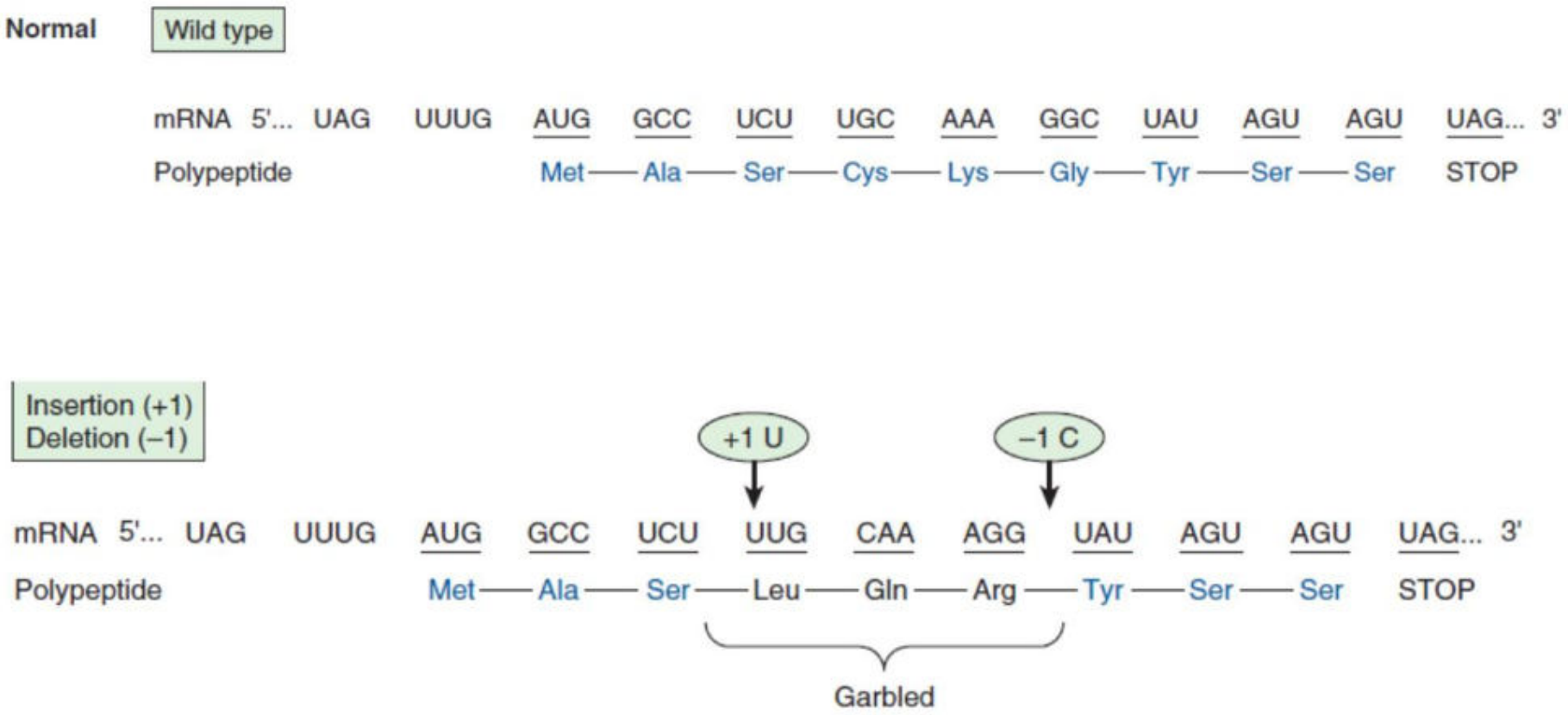



Adapted from Harper,s Biochemistry

# The effects of insertions in a gene on the sequence of the mRNA transcript and of the polypeptide chain translated therefrom



**The effects of deletions and insertions in a gene on the sequence of the mRNA transcript and of the polypeptide chain translated there from**






# Cystic fibrosis

- is an inherited condition affecting approximately 1 in 2500 white individuals.
- Affected patients usually have abnormal mucus secretion and eccrine sweat glands leading to respiratory infections, gastrointestinal obstruction, pancreatic enzyme dysfunction leading to malabsorption of nutrients, and excessive electrolyte secretion.
- The protein cystic fibrosis transmembrane conductance regulator (CFTR) is defective, leading to abnormal chloride transport.
- Approximately 70 percent of mutations are accounted for by **deletion** of three specific base pairs at the F 508 position of the CFTR.

# Case 4

- A 40-year-old male returned from a deer-hunting trip approximately 6 weeks ago, and presents to clinic with multiple complaints. He states that recently he has had worsening joint pain and “arthritis” in multiple joints that seems to move to different spots. Patient also complains of some numbness in his feet bilaterally.
- The patient denies any medical problems and he had a normal annual physical prior to hunting trip. On further questioning, he remembered having a rash on his body and the lesions were circular and appeared to be resolving in the center. He noted that he felt really bad once he got home with muscle ache (myalgias), joint ache (arthralgias), stiff neck, and severe headache. He also remembered that many of his hunting friends had experienced flea and tick bites and is quite sure he was bitten as well. The physical exam is essentially normal except some joint tenderness of left knee and right shoulder. After making your diagnosis you gave him a prescription for erythromycin.
- ◆ **What is the most likely diagnosis?**
- ◆ **What is the biochemical mechanism of action of erythromycin?**



## Steps of protein synthesis

Sequences of signal for initiation (Prokaryote and Eukaryote)

Peptide bond formation does not require energy– How it is possible

# DIFFERENCES IN PROKARYOTIC AND EUKARYOTIC PROTEIN SYNTHESIS

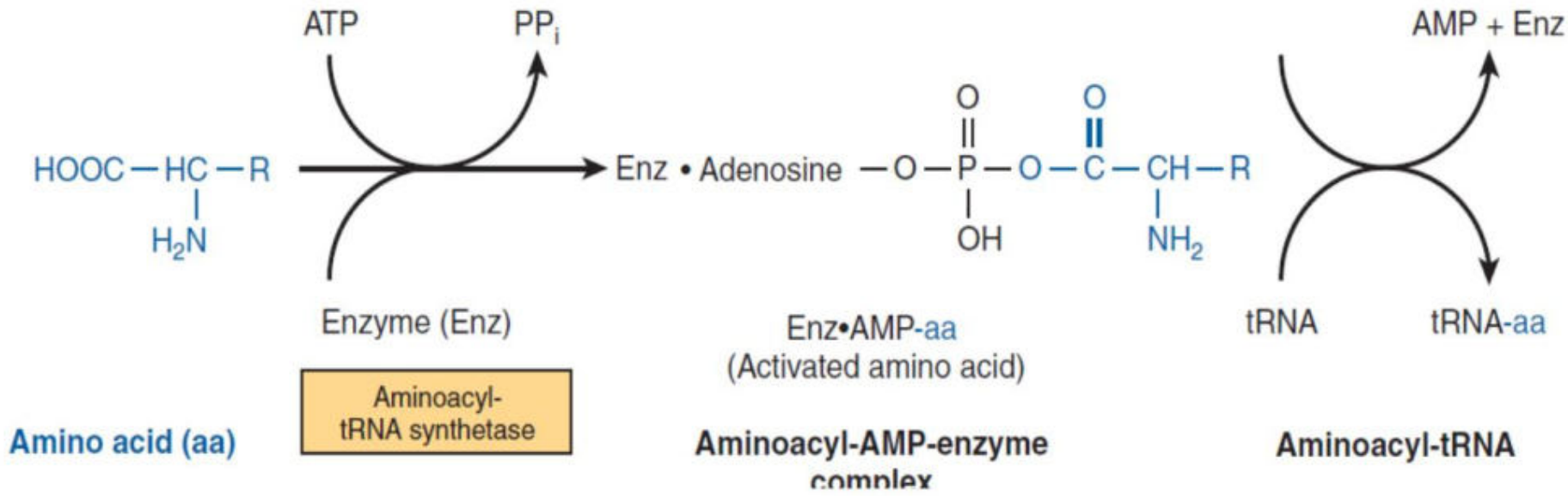
| | PROKARYOTE | EUKARYOTE |
|---|---|---|
| Start | fMet-tRNA | Met-tRNA |
| Recognition sequence | Shine-Dalgarno sequence | 5’ caps direct e-IFs |
| Initiation factors | IF-1, IF-2, IF-3 | multiple e-Ifs (>10) |
| Elongation factors | EF-Tu, EF-G, EF-Ts | Multi-subunit eEF-1, eEF-2, eEF-3 |
| | | |



# Components required for translation

- Amino acids
- tRNA
  - Aminoacid attachment site
  - Anticodon
  - DHU loop
  - Pseudouridine loop
  - Codon recognition by tRNA
    - Antiparallel binding between codon and anticodon
    - Wobble hypothesis
- Aminoacyl tRNA synthetase

# Activation of Amino acid





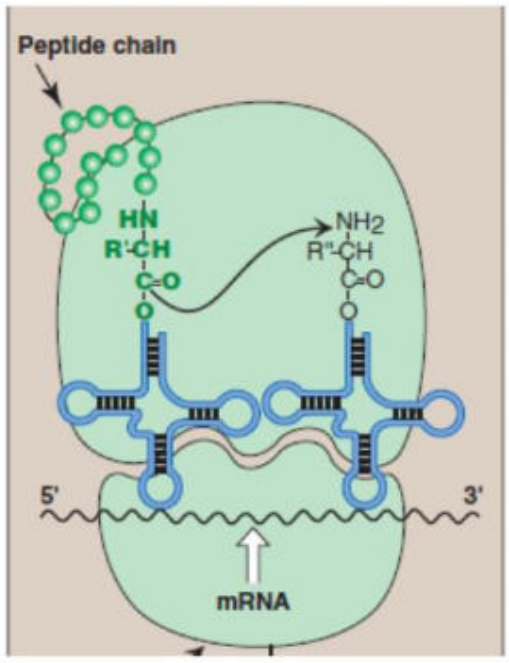

- Messenger RNA
- Functionally competent ribosome
  - rRNA
  - Ribosomal protein
  - A,P and E sites
  - Cellular location
    - Free in the cytosol
    - Associated with endoplasmic reticulum
- Protein factors for initiation, elongation and termination

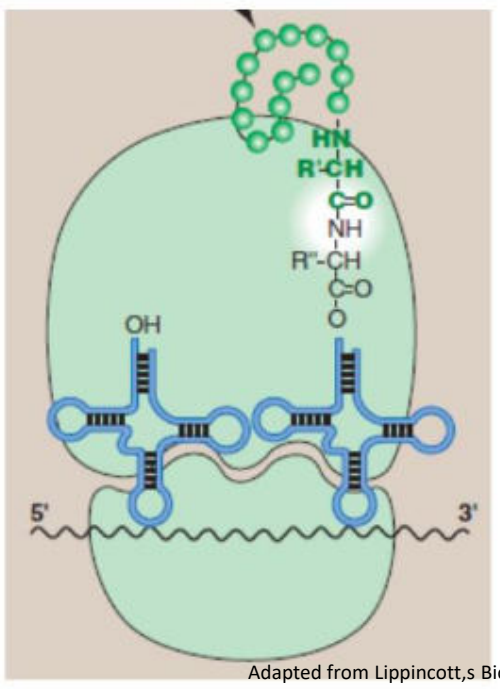
- Energy sources
  - Four high energy bonds cleavage for addition of one amino acid to the polypeptide chain
    - 2 from ATP- aminoacyl tRNA synthetase reaction
    - 2 from GTP
      - 1 for binding aminoacyl tRNA to the A site
      - 1 for translocation
  - ATP and GTP molecules for initiation in eukaryotes
  - GTP required for termination in both pro and Eu

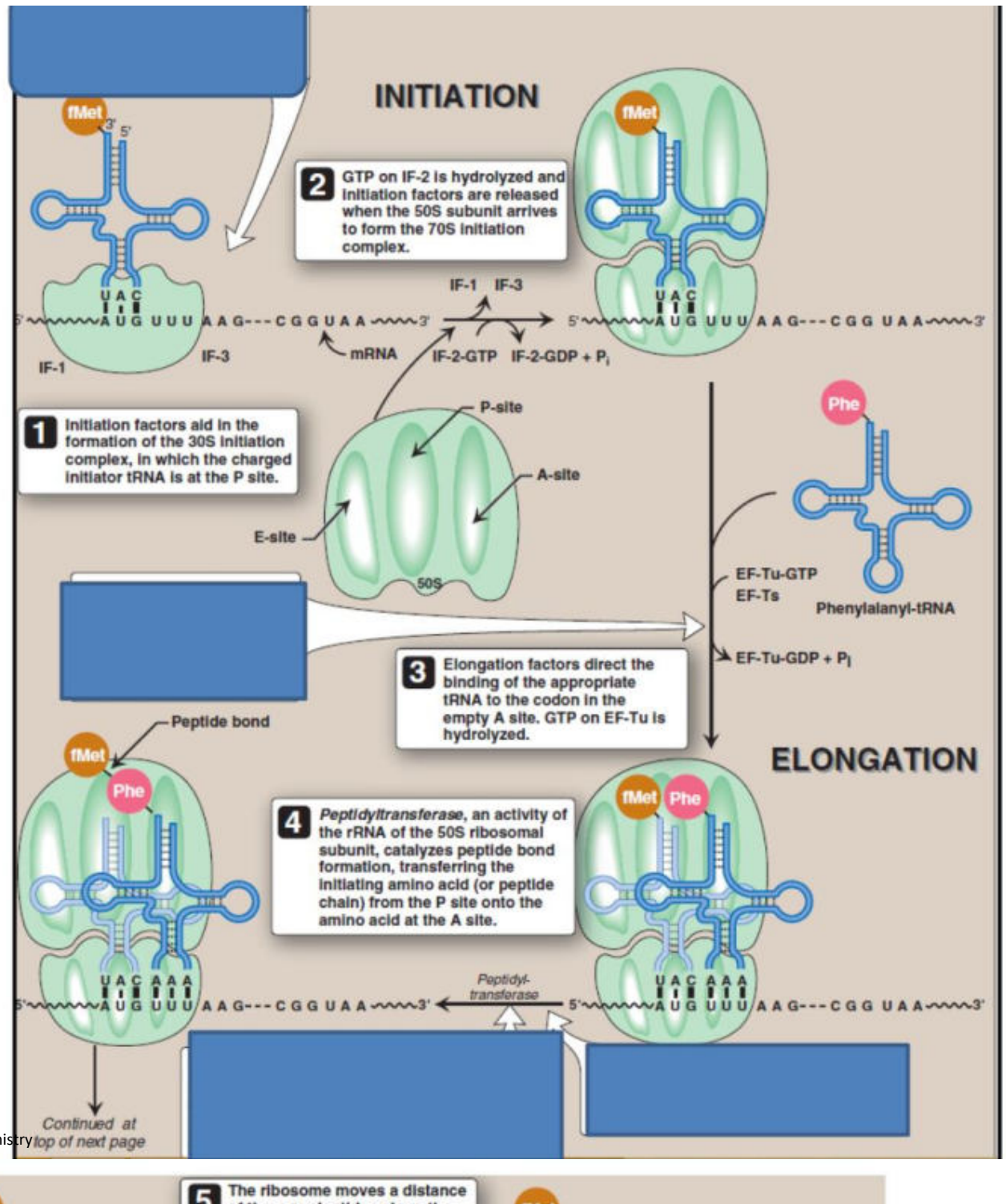


# Steps of protein synthesis

- Initiation
- Elongation
- Termination




Adapted from Lippincott,s Biochemistry





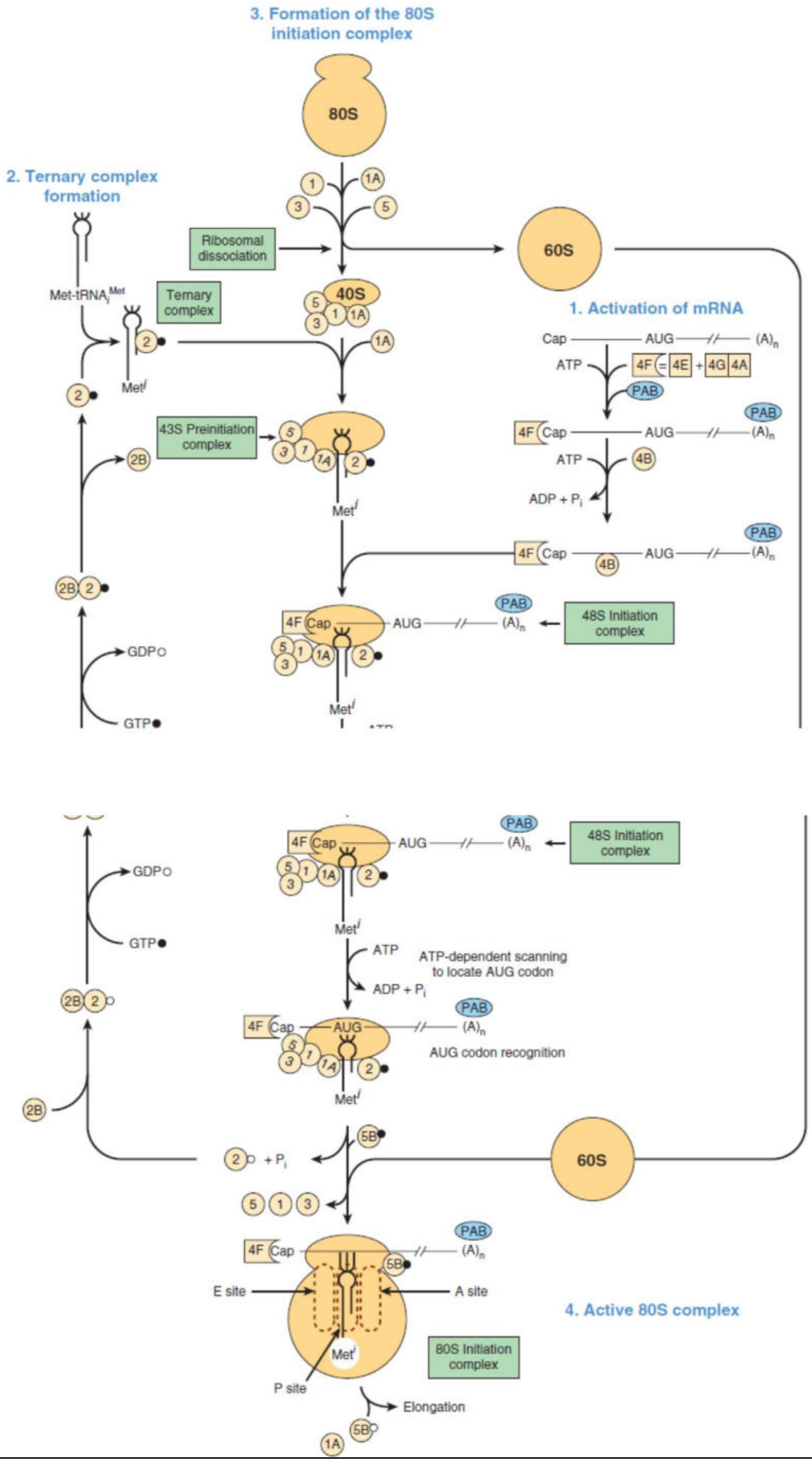
Adapted from Lippincott,s Biochemistry

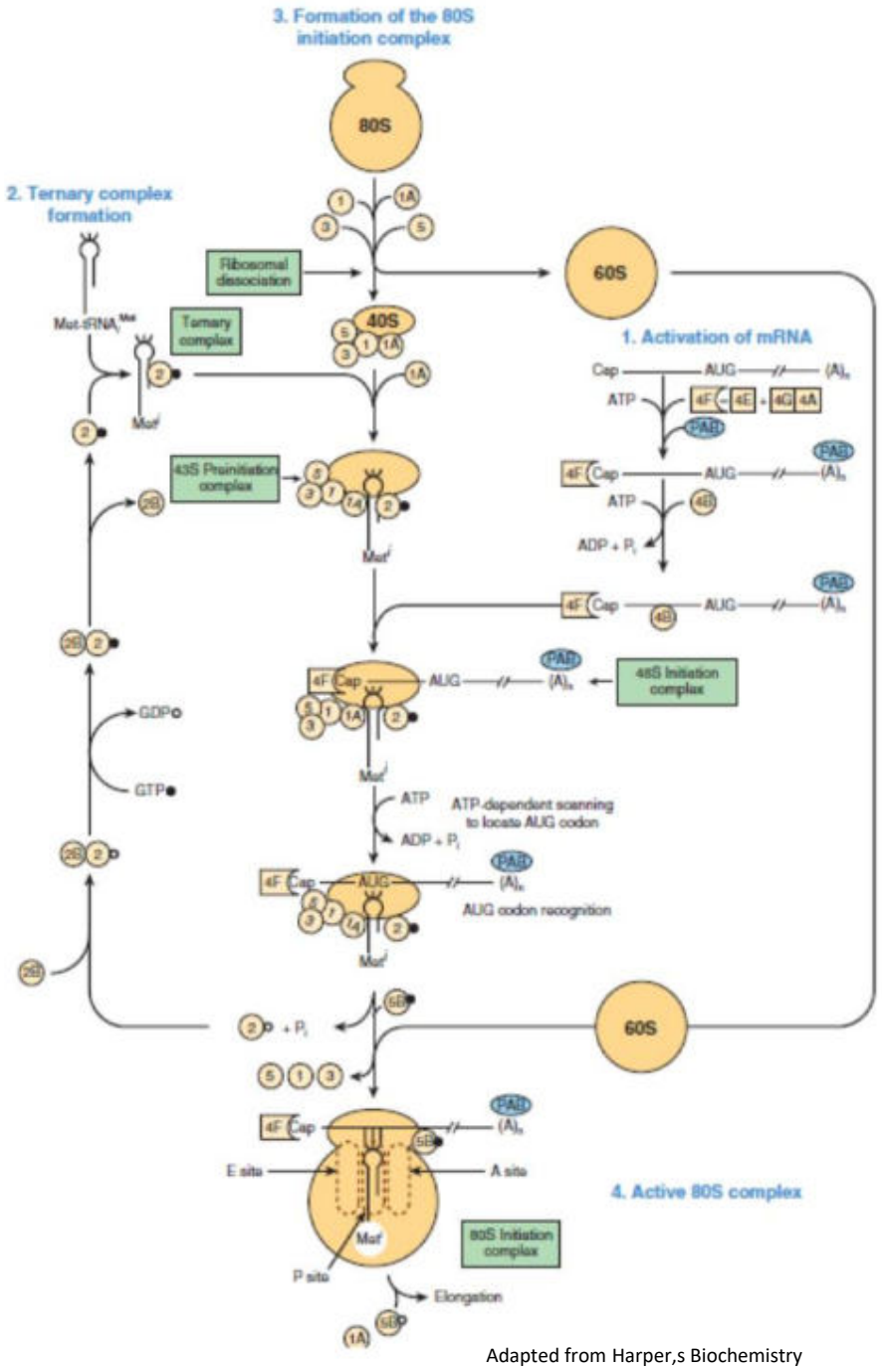
# Video on Prok translation



# Eukaryotic translation

- ~~**Initiation:**~~
- (1) dissociation of the ribosome into its 40S and 60S subunits;
- (2) binding of a ternary complex consisting of the **initiator methionyl-tRNA,** (**met-tRNAi), GTP, and eIF-2 to the 40S ribosome to form the 43S preinitiation complex;**
- **(3) binding of mRNA to the 40S** preinitiation complex to form the **48S initiation complex; and**
- (4) combination of the 48S initiation complex with the 60S ribosomal subunit to form the **80S initiation complex.**

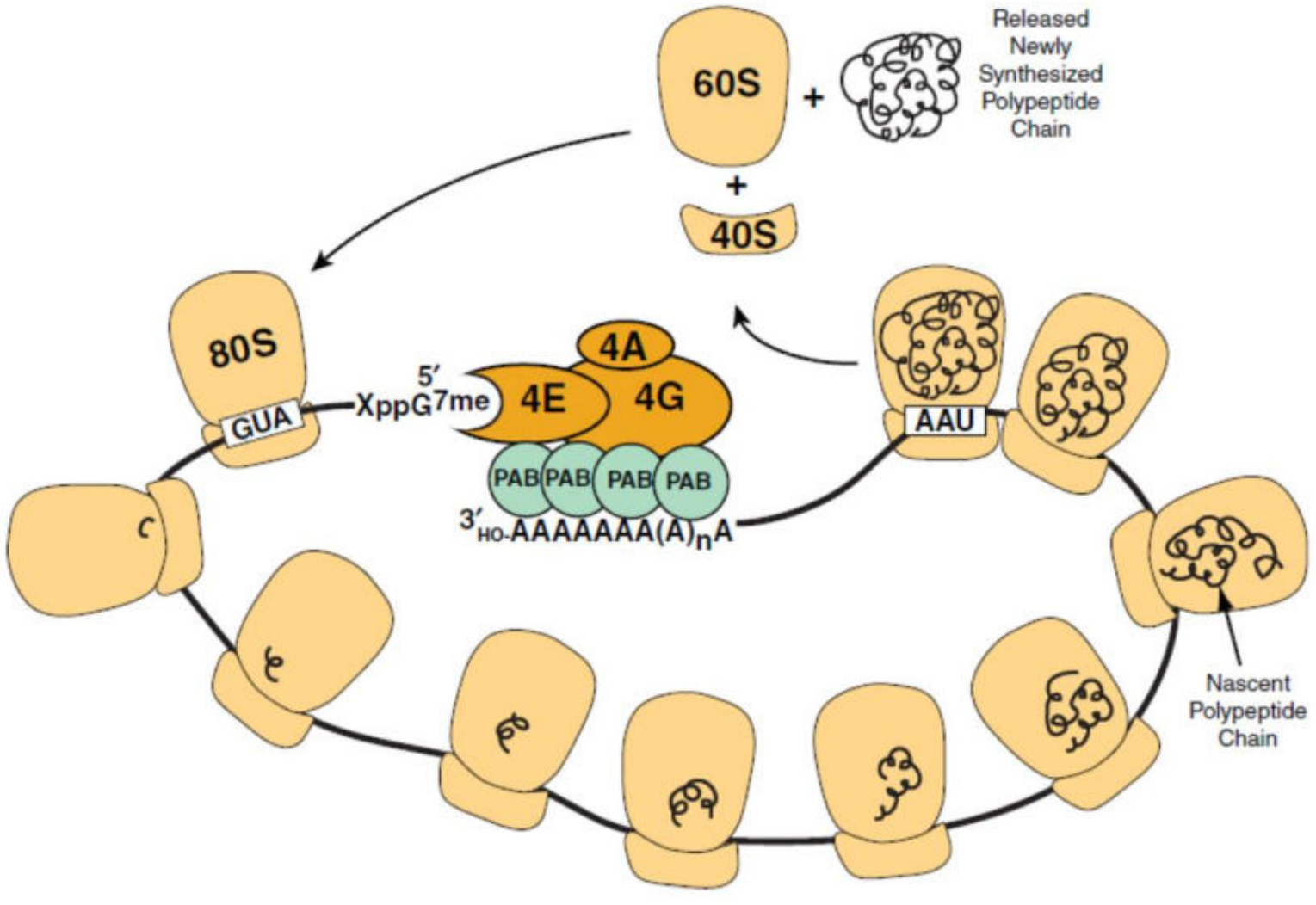





Adapted from Harper,s Biochemistry



## The circularization of mRNA through protein-protein interactions between 7meG cap-bound eIF4F and poly A tail-bound poly A binding protein

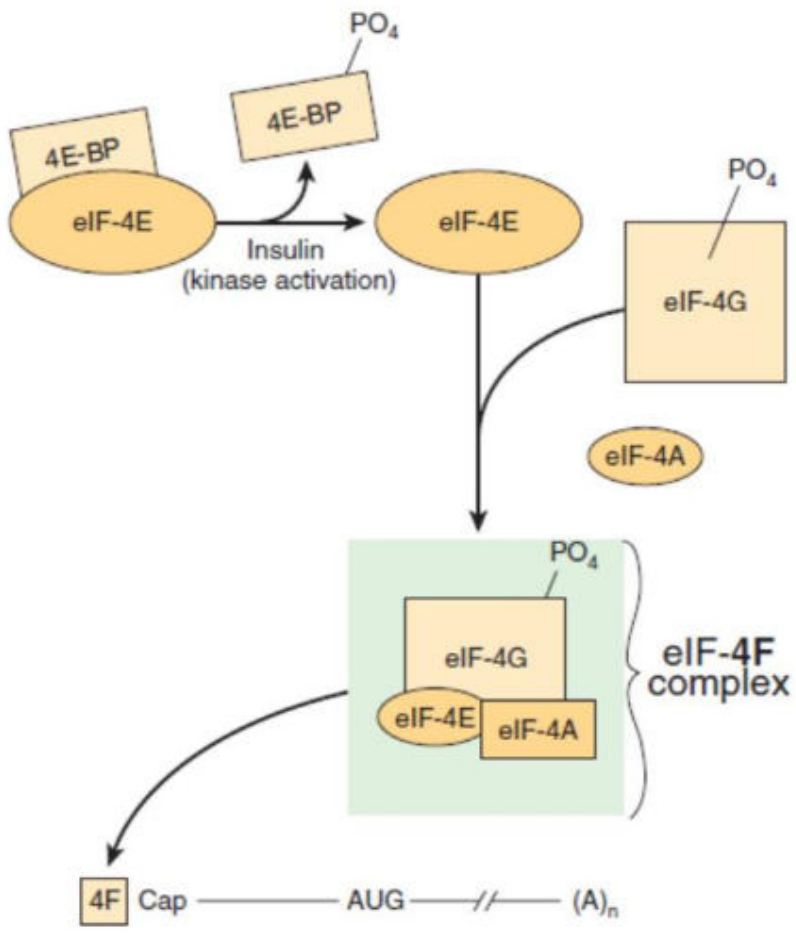







Adapted from Harper,s Biochemistry

# Activation of eIF-4E by insulin and formation of the cap binding eIF-4F complex

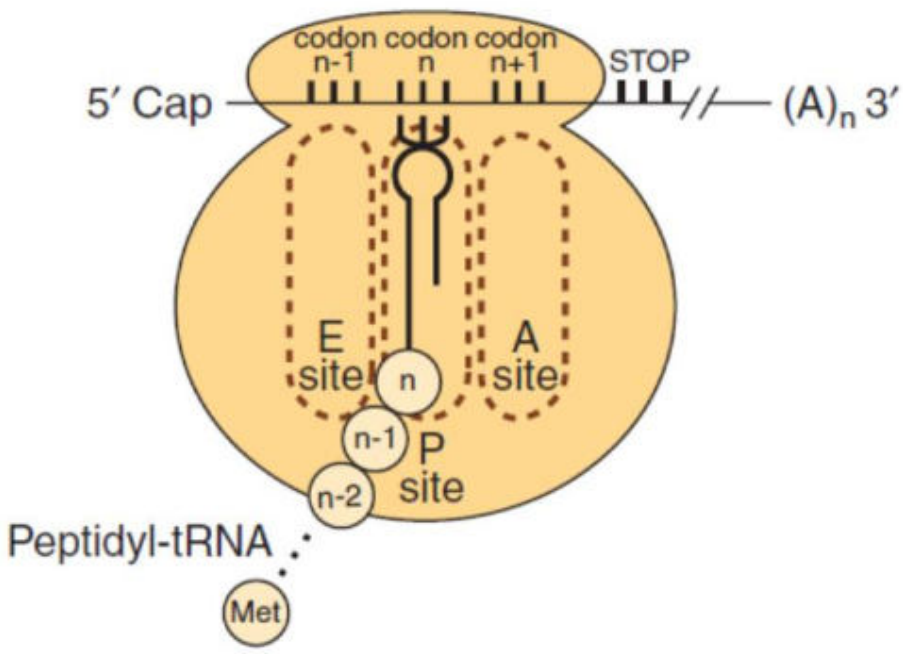



Adapted from Harper,s Biochemistry

# Elongation

- (1) binding of aminoacyl-tRNA to the A site,
- (2) peptide bond formation,
- (3) translocation of the ribosome on the mRNA, and
- (4) expulsion of the deacylated tRNA from the P- and E-sites.

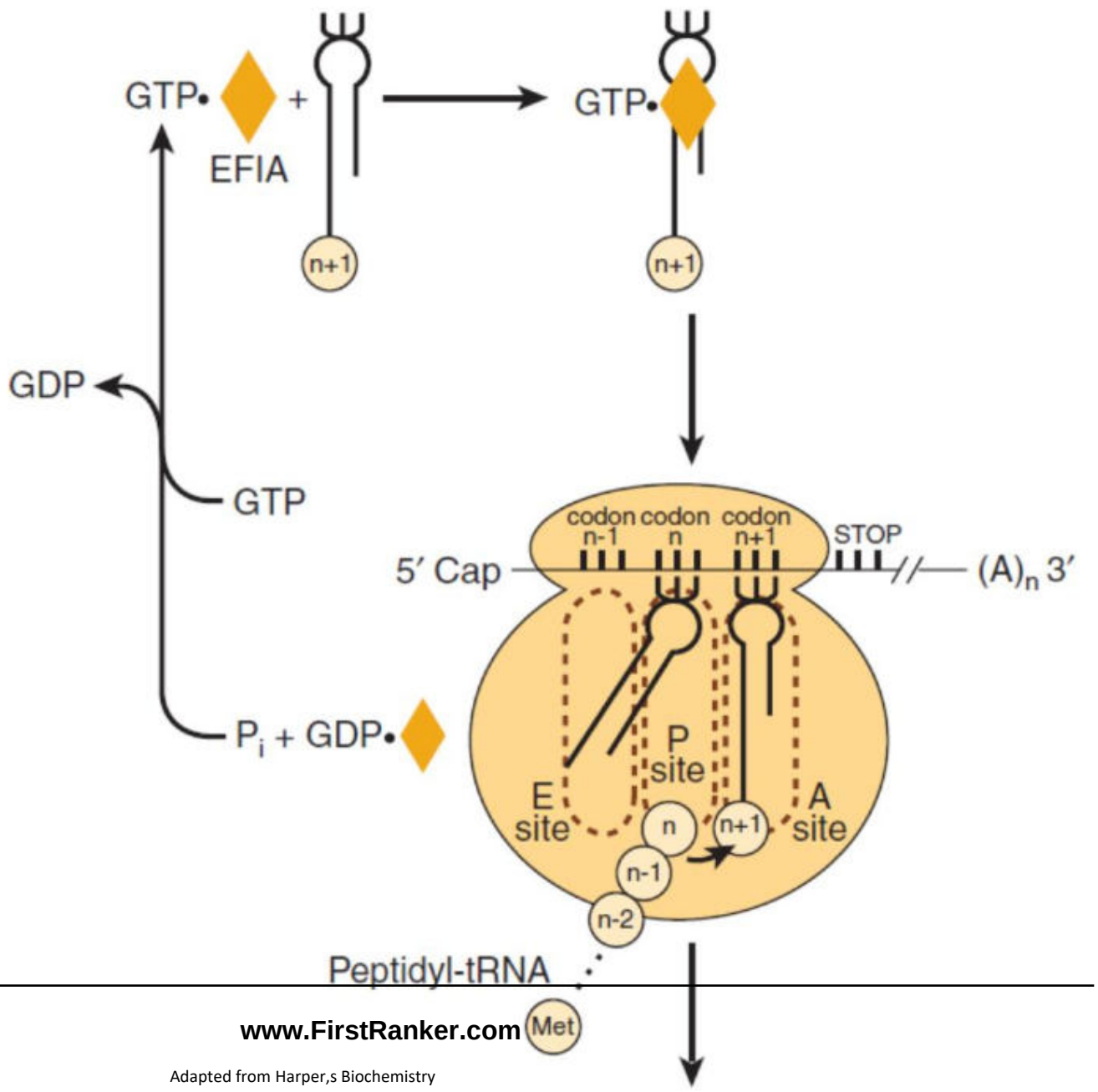
# Diagrammatic representation of the peptide elongation process of protein synthesis





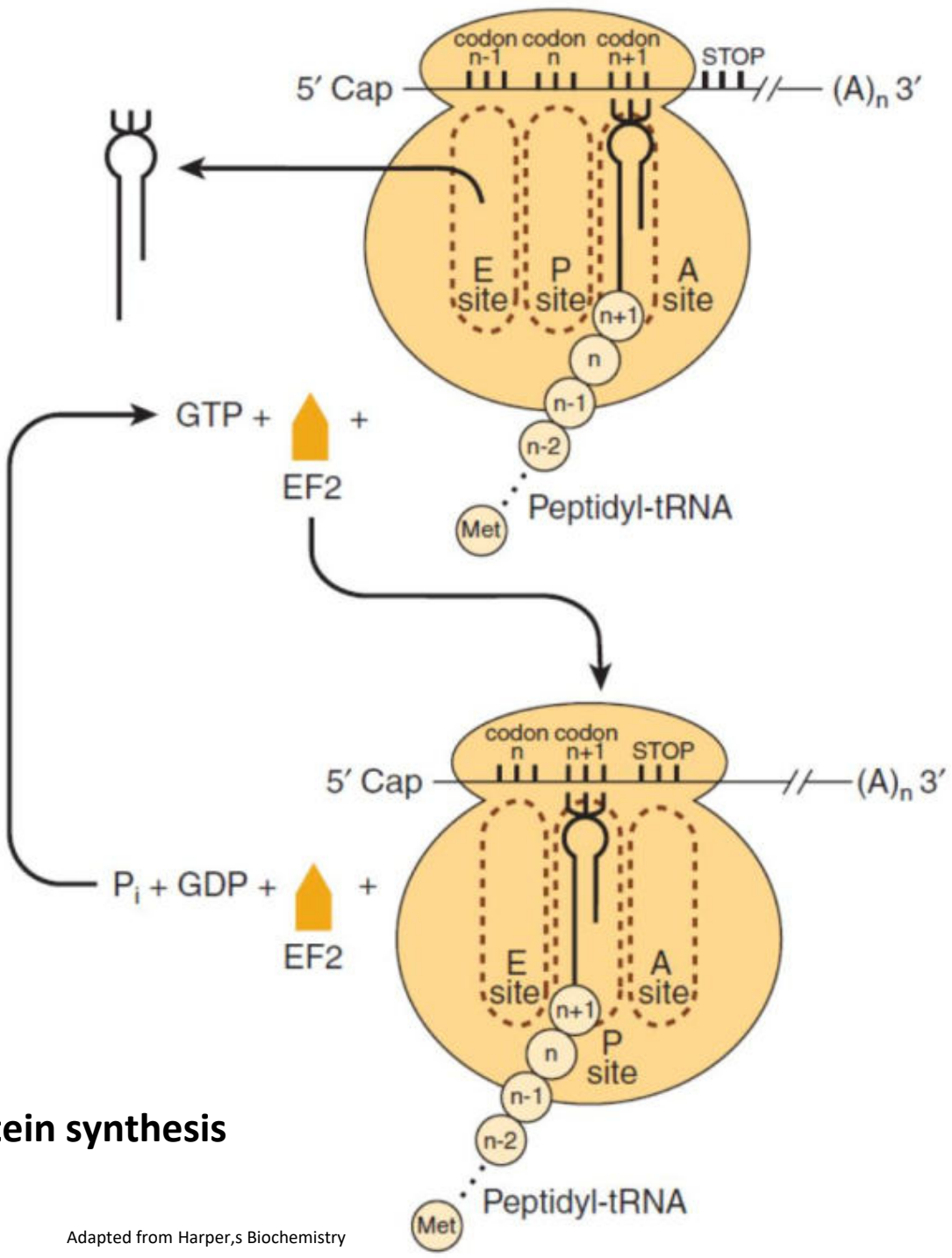

Adapted from Harper,s Biochemistry

# Peptide elongation process of protein synthesis





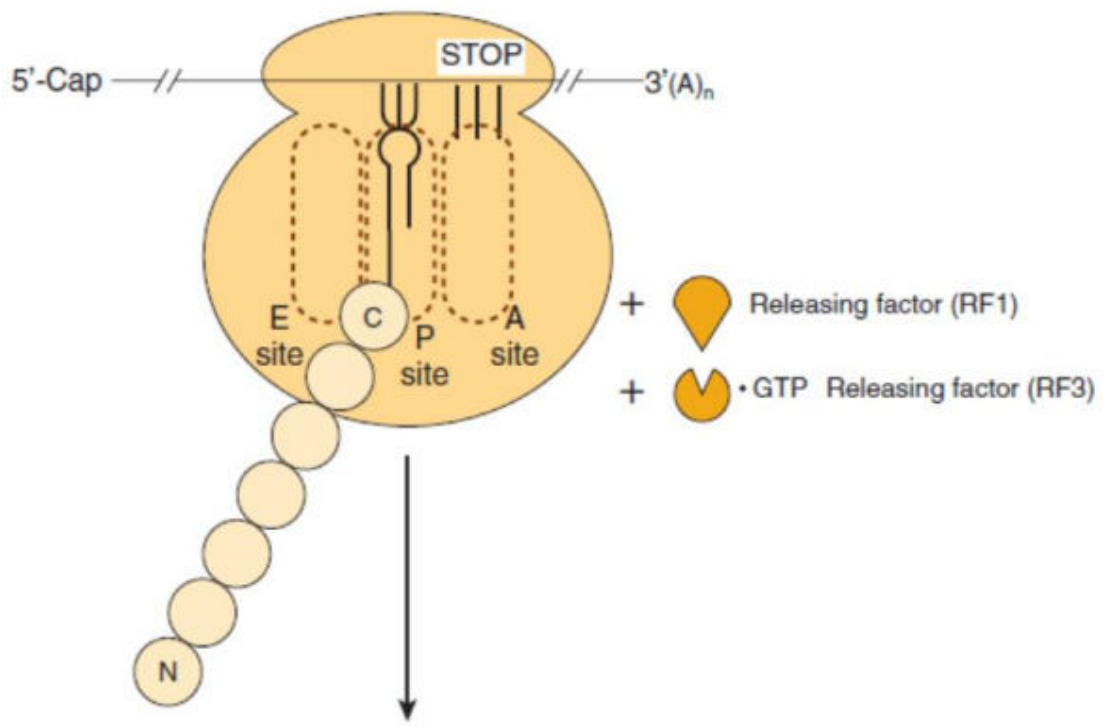

Adapted from Harper,s Biochemistry




**Peptide elongation process of protein synthesis**

Adapted from Harper,s Biochemistry

# Diagrammatic representation of the termination process of protein synthesis

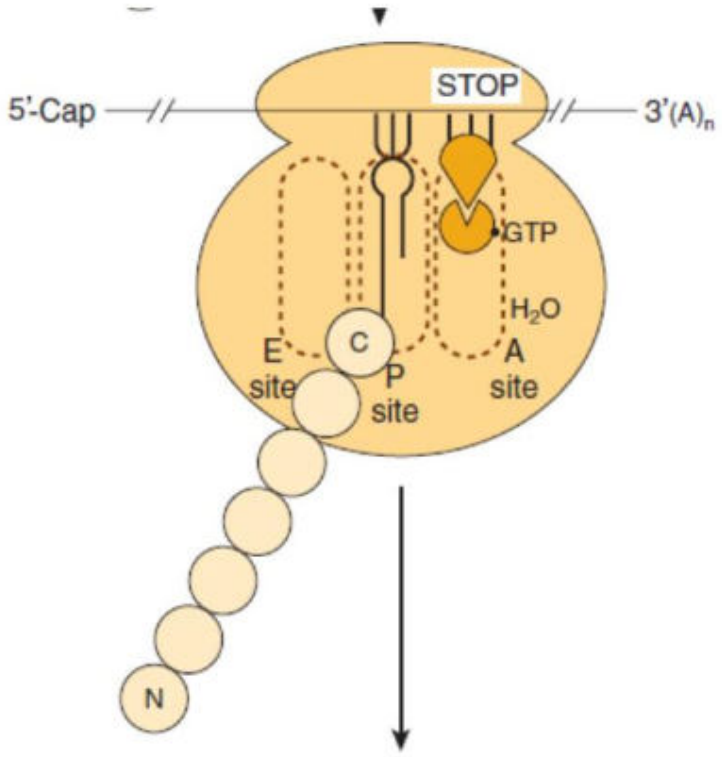






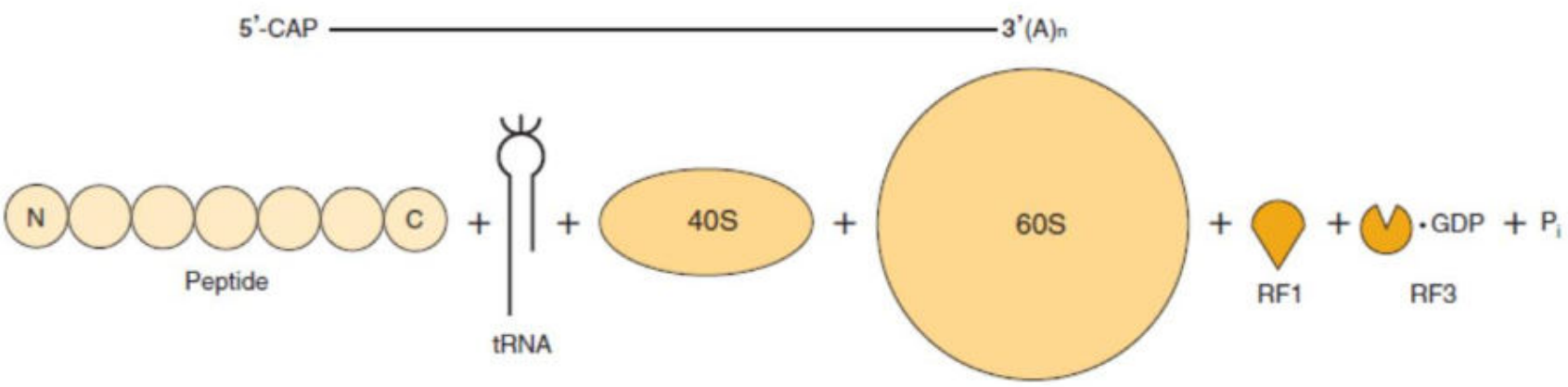

Adapted from Harper,s Biochemistry

# The termination process of protein synthesis




Adapted from Harper,s Biochemistry

# The termination process of protein synthesis








Adapted from Harper,s Biochemistry

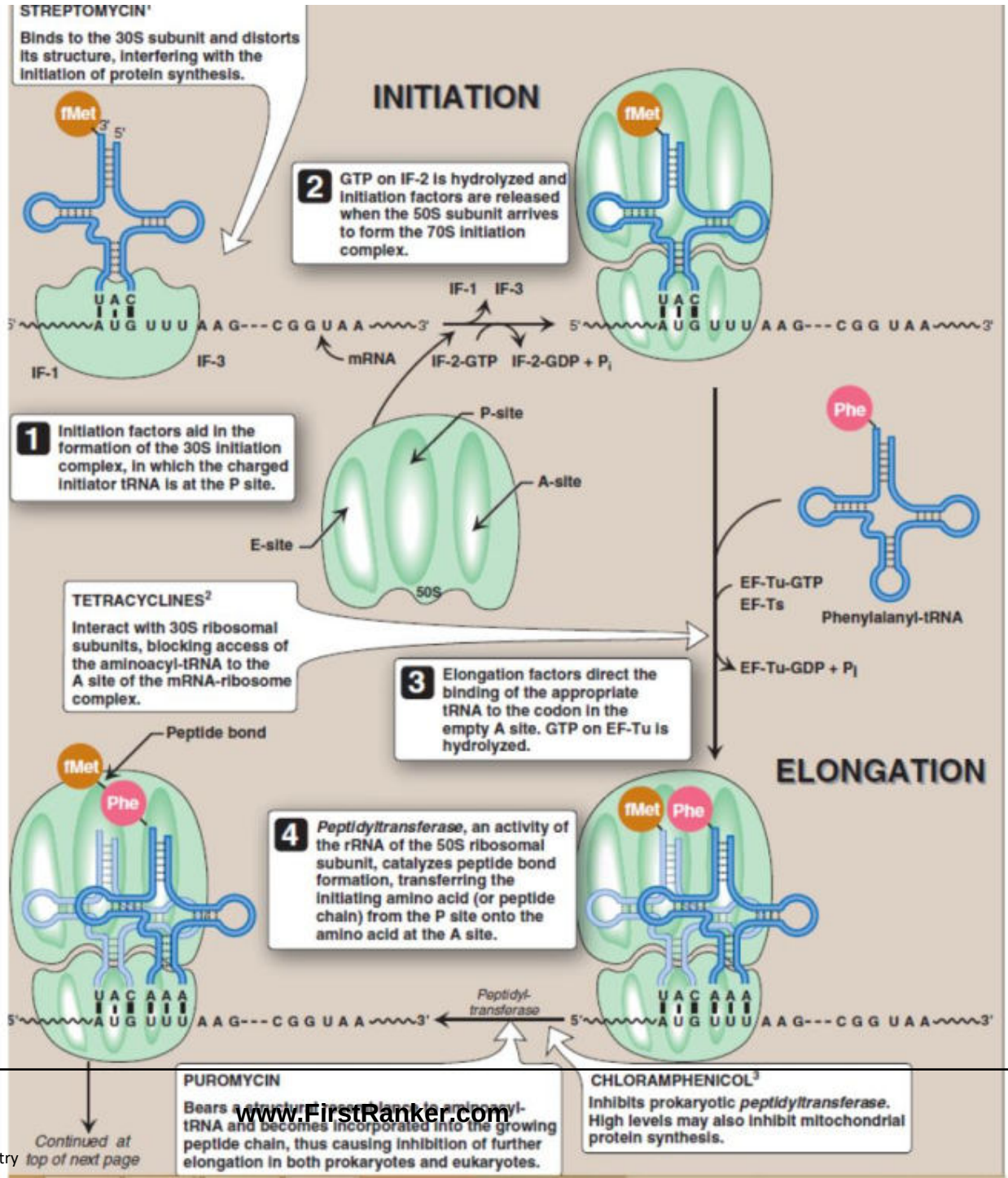
# Comparison between Prokaryotes and Eukaryotes

| Cell | Factor | Function |
|---|---|---|
| | **Initiation** | |
| P<br>E | IF2 GTP<br>eIF2-GTP | Bring charged initiating tRNA to P site |
| P<br>E | IF3<br>eIF3 | Prevent association of subunits |
| | **Elongation** | |
| P<br>E | EF-Tu-GTP<br>EF-1α-GTP | Bring all other charged tRNA to A site |
| P<br>E | EF-Ts<br>EF-1γβ | Guanine nucleotide exchange factor |
| P<br>E | EF-G-GTP<br>EF-2-GTP | Translocation |
| | **Termination** | |
| P<br>E | RF-1,2<br>eRF | Recognize stop codon |
| P<br>E | RF-3-GTP<br>eRF-3-GTP | Release of other RF |

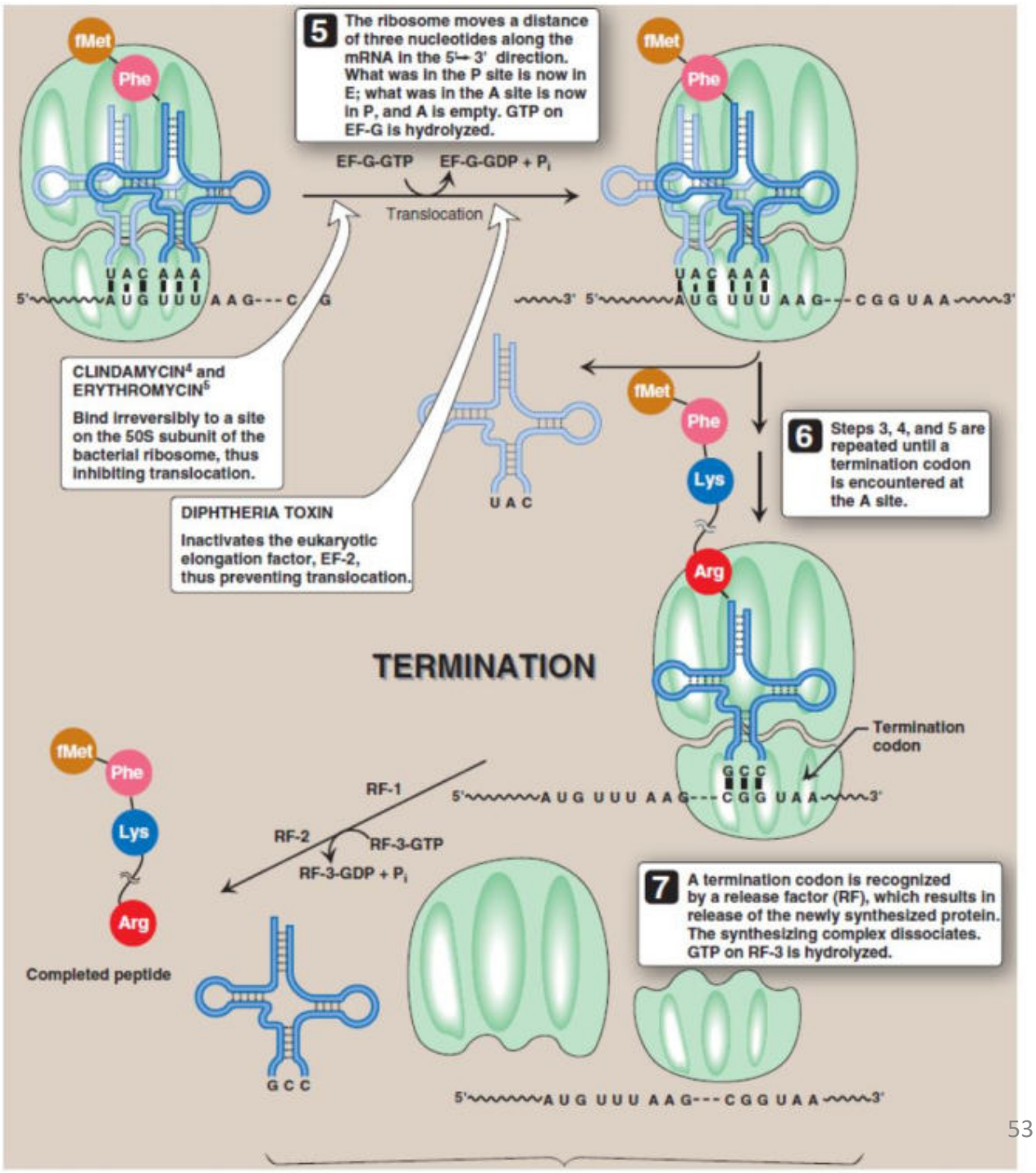


# Video on EUK translation

# Clinical implication




Adapted from Lippincott,s Biochemistry




Adapted from Lippincott,s Biochemistry

# Post translational modification

**A. Trimming**

**B. Covalent attachment**

Methylation

Acetylation

Glycosylation

N glycosidic linkage

O glycosidic linkage

Lipidation

GPI (glycosyl phosphatidyl inositol)

S palmitoylation

## Post translational modification

Phosphorylation
Formation of disulphide bonds

Gammacarboxylation----glutamic residue
Hydroxylation ----Proline and lysine

## C. Protein degradation

Ubiquitination
Proteolysis



# MCQ1

- The 6-year-old son of a migrant worker is brought to a clinic with chills, headache, nausea, vomiting, and sore throat. The examining physician notes a persistent grayish colored membrane near the tonsils. History reveals that the patient has not been immunized against diphtheria. Diphtheria toxin is potentially lethal in this unimmunized patient because it causes which of the following?

- A. Inactivates an elongation factor required for translocation in protein synthesis
- B. Binds to the ribosome and prevents peptide bond formation
- C. Prevents binding of mRNA to the 60S ribosomal subunit
- D. Inactivates an initiation factor
- E. Inhibits the synthesis of aminoacyl-charged tRNA

# MCQ2

- Many antimicrobials inhibit protein translation. Which of the following antimicrobials is correctly paired with its mechanism of action?

- A. Tetracyclines inhibit peptidyltransferase.
- B. Diphtheria toxin binds to the 30S ribosomal subunit.
- C. Puromycin inactivates EF-2.
- D. Clindamycin binds to the 30S ribosomal subunit.
- E. Erythromycin binds to the 50S ribosomal subunit.



# MCQ3

- Translation of a synthetic polyribonucleotide containing the repeating sequence CAA in a cell-free protein- synthesizing system produces three homopolypeptides: polyglutamine, polyasparagine, and polythreonine. If the codon for glutamine and asparagine are CAA and AAC, respectively, which of the following triplets is the codon for threonine?

- A. AAC.
- B. CAA.
- C. CAC.
- D. CCA.
- E. ACA.

# MCQ4

- A tRNA molecule that is supposed to carry cysteine (tRNAcys) is ischarged, so that it actually carries alanine (ala-tRNAcys). Assuming no correction occurs, what will be the fate of this alanine residue during protein synthesis?

- A. It will be incorporated into a protein in response to an alanine codon.
- B. It will remain attached to the tRNA, as it cannot be used for protein synthesis.
- C. It will be incorporated randomly at any codon.
- D. It will be chemically converted to cysteine by cellular enzymes.
- E. It will be incorporated into a protein in response to a cysteine codon