Exam Questions 14 and 20

Question 14

4 pts

Hemoglobin consists of 4 subunits, each capable of binding one O_2 molecule. Carbon monoxide (CO) is released into the lungs as a result of smoking cigarettes. CO binds to the *same site* as O_2 in hemoglobin, this binding event is *irreversible*, and CO binding increases the affinity for O_2 to remain bound to the *other binding sites* within hemoglobin.

Which of the following is TRUE?

- ○ CO is only a competitive inhibitor of O_2 binding to hemoglobin as they are in direct competition for the same binding sites.
- ○ If 50% of a person's hemoglobin binding sites are occupied by CO, it is possible to overcome this inhibition by flooding the system with O_2 to outcompete the CO and return to maximal function.
- ○ CO is both a competitive inhibitor and an allosteric regulator of O_2 binding to hemoglobin.
- ○ CO is a non-competitive inhibitor of O_2 binding to hemoglobin as it regulates O_2 binding at sites other than the site where it binds and this effect cannot be overcome with an excess of O_2.

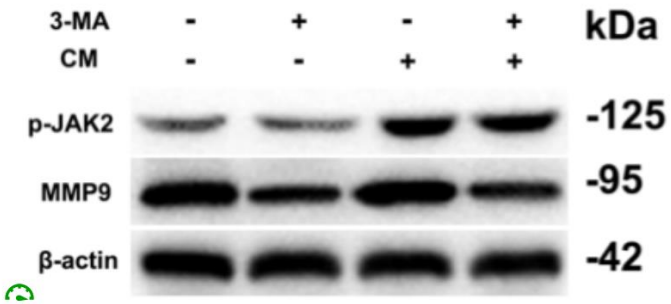
Question 20

4 pts

Consider the following western blot adapted from Chu et al.

3-MA and CM are different treatment conditions prior to analyzing protein expression, the plusses and minuses indicate whether or not that treatment was used. β-actin serves as the loading control.

Which of the following is a correct interpretation of the result?



- ○ The effect of 3-MA on MMP9 expression is more potent than the effect of CM on MMP9 expression.
- ○ 3-MA treatment increases the expression of MMP9.
- ○ Both 3-MA and CM have a notable effect on β-actin expression levels.
- ○ CM treatment reduces the expression level of p-JAK2.

Exam Questions 14 and 20

Question 14 4 pts

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- ○ CO is a non-competitive inhibitor of O_2 binding to hemoglobin as it regulates O_2 binding at sites other than the site where it binds and this effect cannot be overcome with an excess of O_2.

As CO binds to the *same site* as O_2 it is by definition a competitive inhibitor, not a non-competitive inhibitor. As this binding event is *irreversible*, it is not a traditional competitive inhibitor as once it has bound it cannot be unbound regardless of how much O_2 is added. As CO binding affects *other binding sites* within hemoglobin, it is also an allosteric regulator of O_2 binding to hemoglobin.

Exam Questions 14 and 20

Question 20 4 pts

Consider the following western blot adapted from Chu et al.

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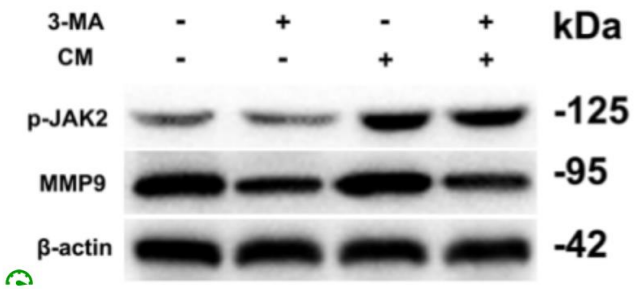

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- ○ 3-MA treatment increases the expression of MMP9.
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- ○ CM treatment reduces the expression level of p-JAK2.

A western blot is essentially showing the relative level of expression of a protein of interest under a specific condition. We use targeting antibodies to specifically recognize proteins of interest, if there is more of the protein present in the sample, more of the antibody will bind to it, which will be quantified as a darker, more prominent band on the blot.

Here we see that 3-MA treatment (the + columns) shows a less prominent band for MMP9, so this means it reduces the expression of this protein. Likewise, we see that CM treatment increases the expression of p-JAK2. β-actin is the loading control, which remains relatively constant across sample conditions a control measure to keep researchers honest by not simply adding different amounts of protein to different wells of the blot. Here we can see there is no notable change of expression in β-actin across the four conditions (i.e., the bands are roughly the same intensity).

However, when we compare the effect of the dual treatment of 3-MA and CM (the fourth column) on MMP9 we can see that the expression is reduced compared to the control condition (the first column). When we then compare the individual treatments, we see that 3-MA alone similarly reduces MMP9 expression (the second column) while CM alone does not really alter MMP9 expression (the third column). Therefore, we can conclude that the effect of 3-MA on MMP9 expression is more potent than the effect of CM on MMP9 expression.

Chapter 7 Part 2: How Cells Read the Genome Part 2: From RNA to Protein

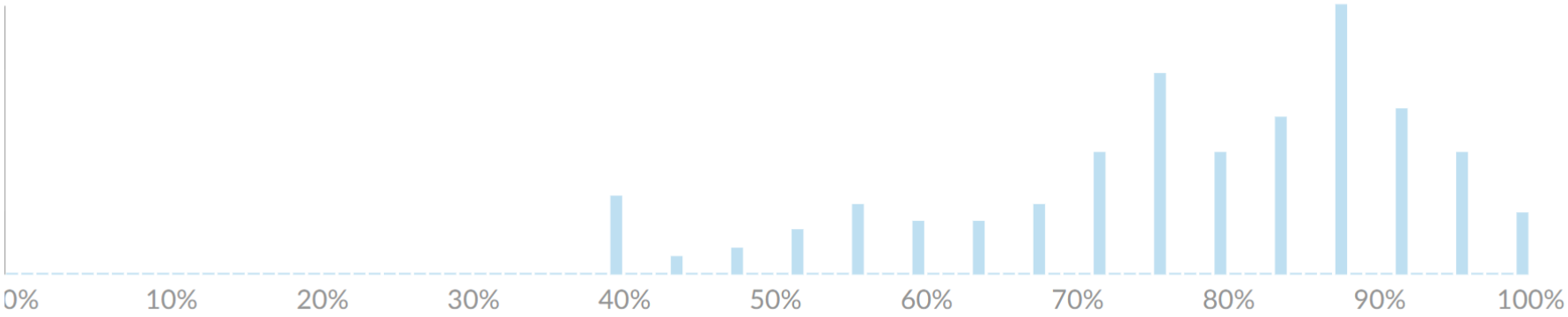
Dr. Matthew Ellis

Chapter 7 – Part 2

BIOL366

Exam 1 statistics

Average Score	High Score	Low Score	Standard Deviation	Average Time
78%	100%	40%	15.54	01:01:34



Spring 24

Average Score	High Score	Low Score	Standard Deviation	Average Time
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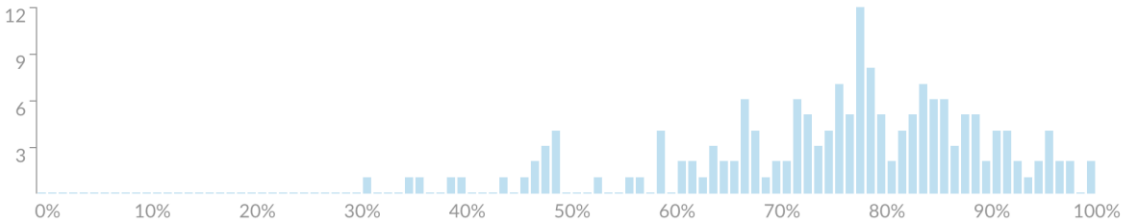

Fall 23

Average Score	High Score	Low Score	Standard Deviation	Average Time
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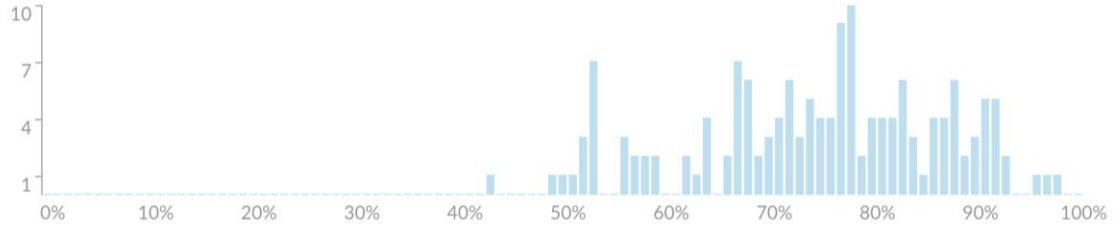

Learning Objectives for Chapter 7, Part 2:

Upon completing this module, **you should be able to**:

- Understand how the genetic code works and the role of tRNAs in translation.
- Explain the mechanism of protein synthesis (translation) via ribosomal initiation, elongation, and termination.
- Appreciate the main differences between translation features in prokaryotes vs. eukaryotes.
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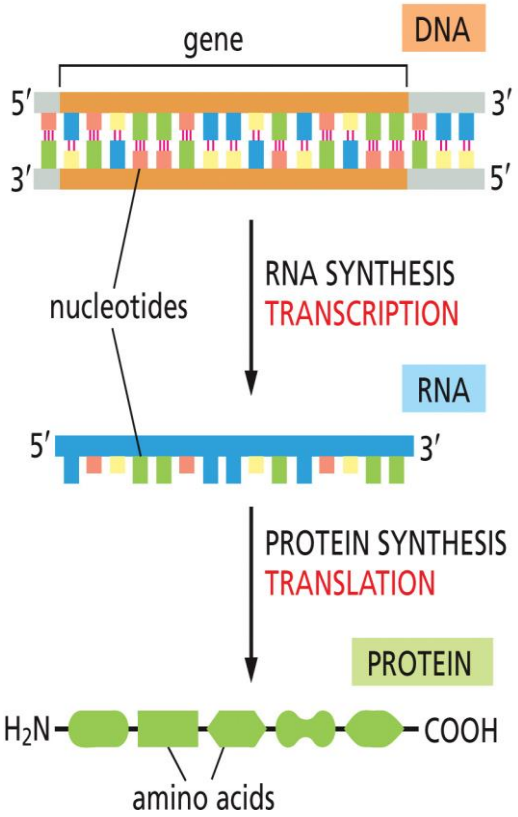
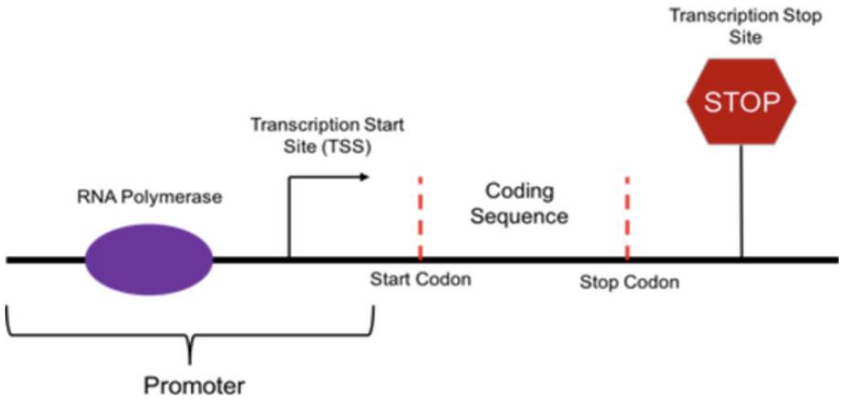
The Central Dogma

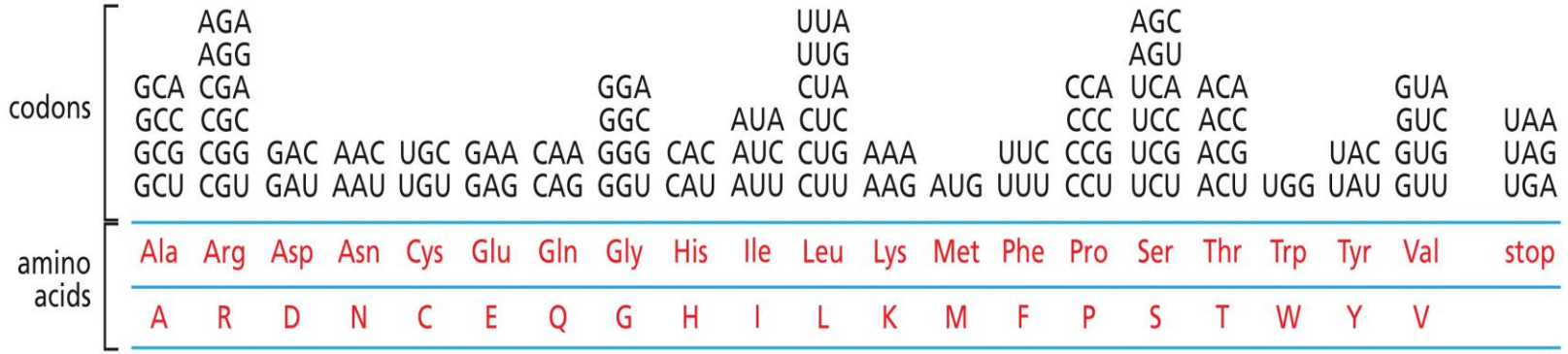
- *Gene Expression* is the process by which the information encoded in a DNA sequence is converted into a product.
- The synthesis of RNA molecules is called *transcription* (begins at promoter and ends at terminator sequence).
- The synthesis of proteins is called *translation* (begins at start codon and ends at stop codon sequence).





The Genetic Code tells us how to translate mRNA into protein

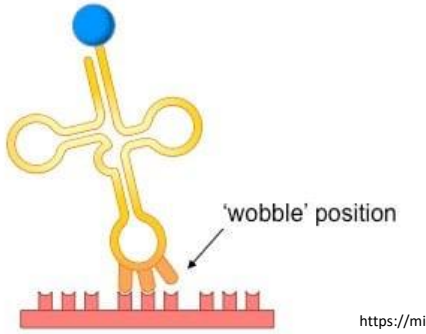
- Each group of 3 consecutive RNA nucleotides is called a **codon**
- The genetic code is *universal*, meaning all species use the same nucleotides (U,C,A,G) which code for the same amino acids
- The genetic code is *redundant*, meaning multiple codons can be used to translate the same amino acid
- The genetic code is *unambiguous*, meaning that a specific codon will always code for a specific amino acid (i.e., there are no repeat codons)



The Genetic Code tells us how to translate mRNA into protein

- Each codon specifies 1 amino acid
- 4 nucleotides in triplets: 4x4x4=64 possible combinations/amino acids
- 3rd nucleotide position is a "wobble" base, meaning base-pairing is more flexible at this position, so the same tRNA can be used across multiple codons

First letter	Second letter: U	Second letter: C	Second letter: A	Second letter: G	Third letter
U	UUU, UUC: Phenylalanine UUA, UUG: Leucine	UCU, UCC, UCA, UCG: Serine	UAU, UAC: Tyrosine UAA, UAG: Stop codon	UGU, UGC: Cysteine UGA: Stop codon UGG: Tryptophan	U C A G
C	CUU, CUC, CUA, CUG: Leucine	CCU, CCC, CCA, CCG: Proline	CAU, CAC: Histidine CAA, CAG: Glutamine	CGU, CGC, CGA, CGG: Arginine	U C A G
A	AUU, AUC, AUA: Isoleucine AUG: Methionine; start codon	ACU, ACC, ACA, ACG: Threonine	AAU, AAC: Asparagine AAA, AAG: Lysine	AGU, AGC: Serine AGA, AGG: Arginine	U C A G
G	GUU, GUC, GUA, GUG: Valine	GCU, GCC, GCA, GCG: Alanine	GAU, GAC: Aspartic acid GAA, GAG: Glutamic acid	GGU, GGC, GGA, GGG: Glycine	U C A G

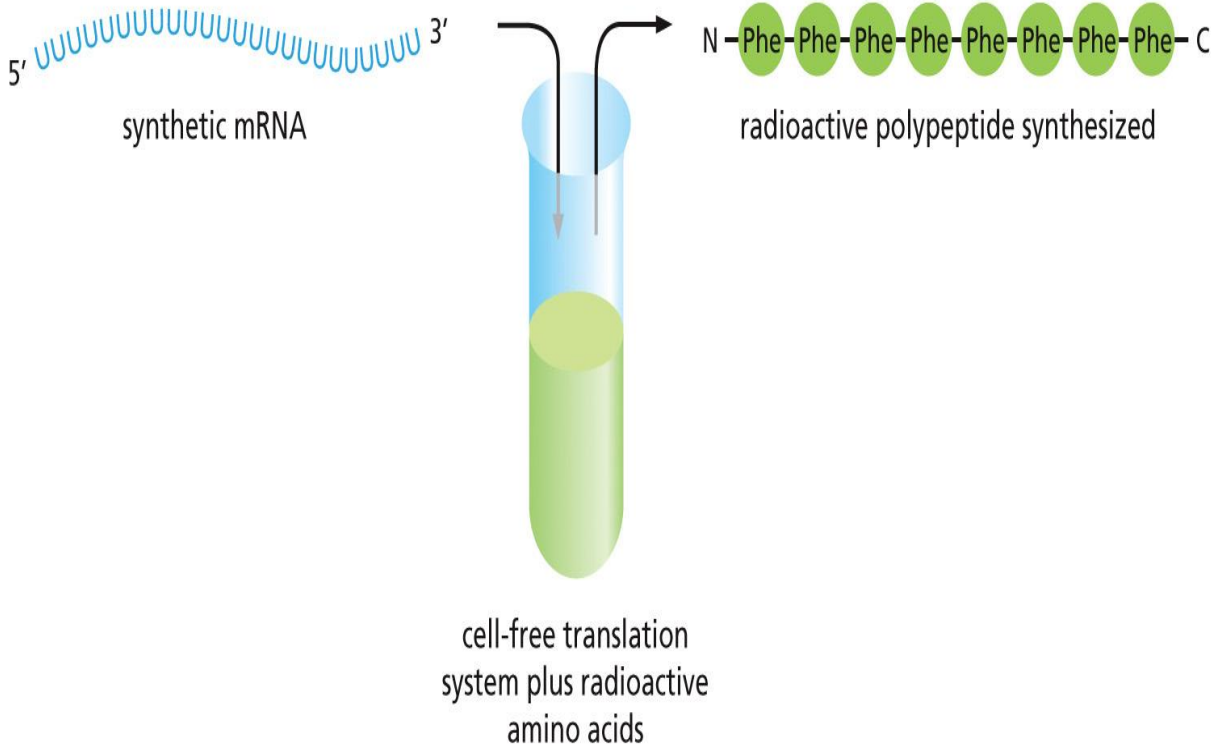


https://microbenotes.com/the-wobble-hypothesis/

How did we crack the genetic code?
In vitro reconstitution experiments

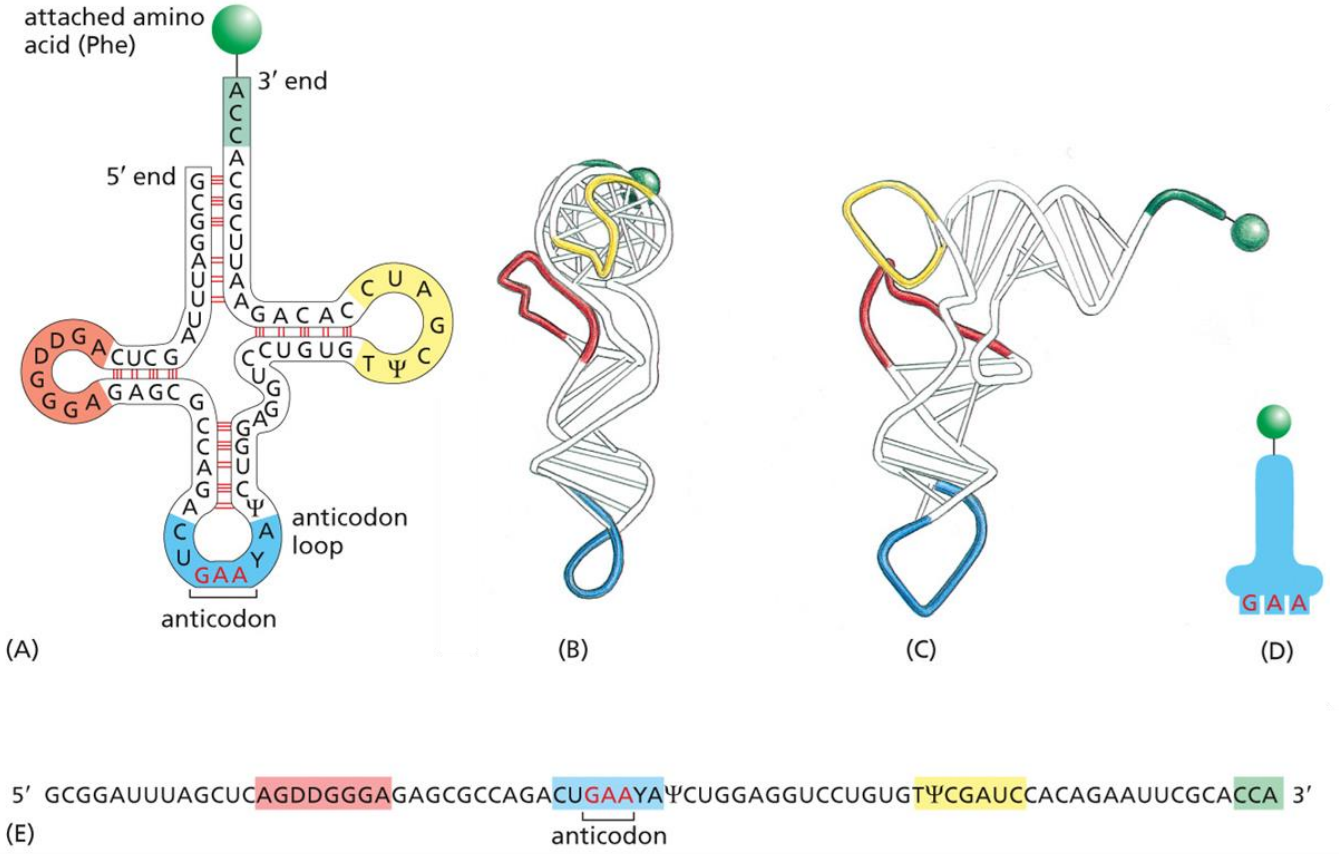
- **Experimental Setup:**
  - A cell-free translation system containing bacterial ribosomes, tRNAs, enzymes, and other small molecules was mixed with synthetic mRNAs.

- When the "correct" amino acid was added, a radioactive polypeptide was produced.
  - Ex. poly U encoded for a polypeptide containing only phenylalanine, poly C encoded only proline, etc.
  - Eventually fed in triplets of mRNA (all 64 possible codons) to determine the code



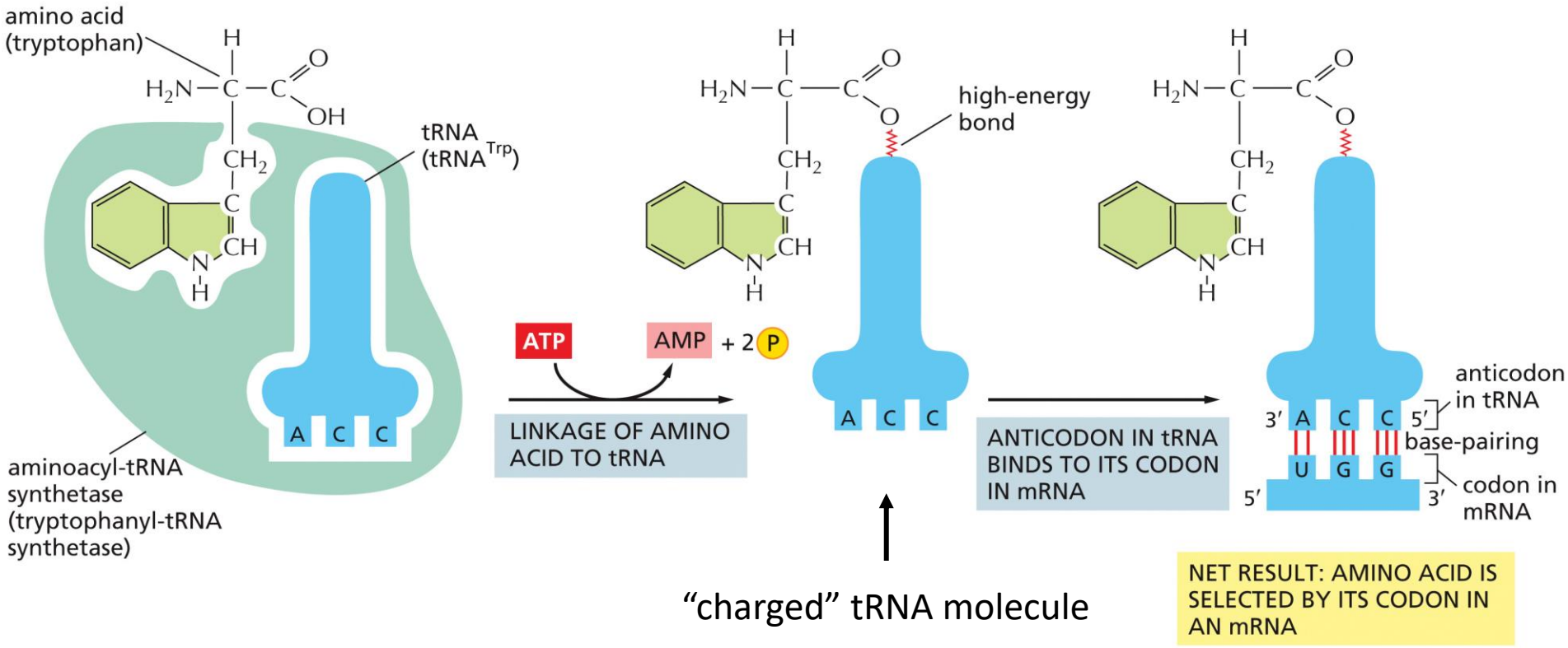
Transfer RNAs (tRNA) are molecular adaptors, linking amino acids to codons

- 3’ end of tRNA covalently linked to the amino acid corresponding to its *anticodon*
  - As with the anti-parallel nature of DNA replication and RNA transcription, the *anticodon* on the tRNA will be the complement to the *codon* on the mRNA



These 5 images are all representations of the same tRNA

Specific enzymes (aminoacyl-tRNA synthetases) couple the transfer of amino acids onto the correct tRNAs



Squarecap Q#1-2

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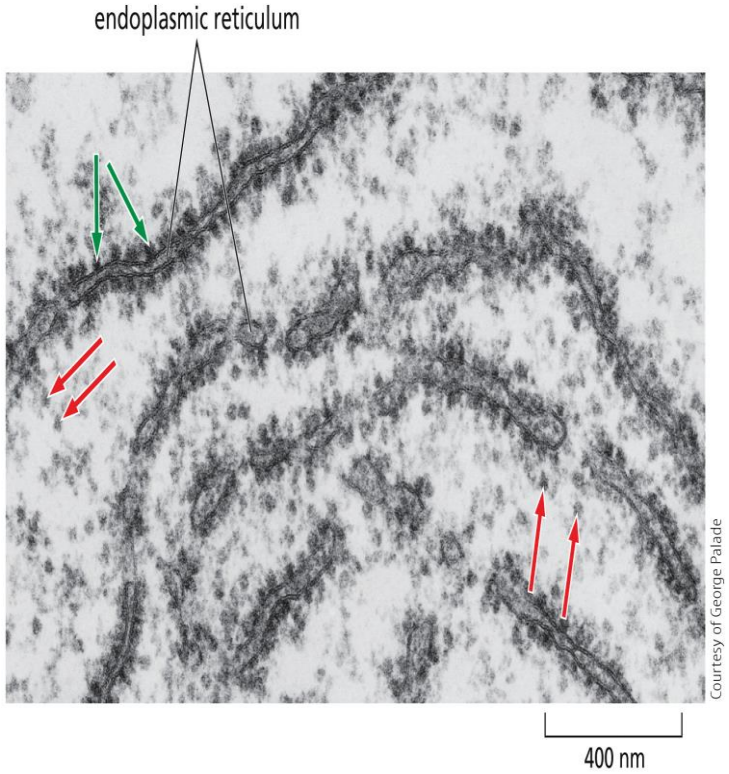
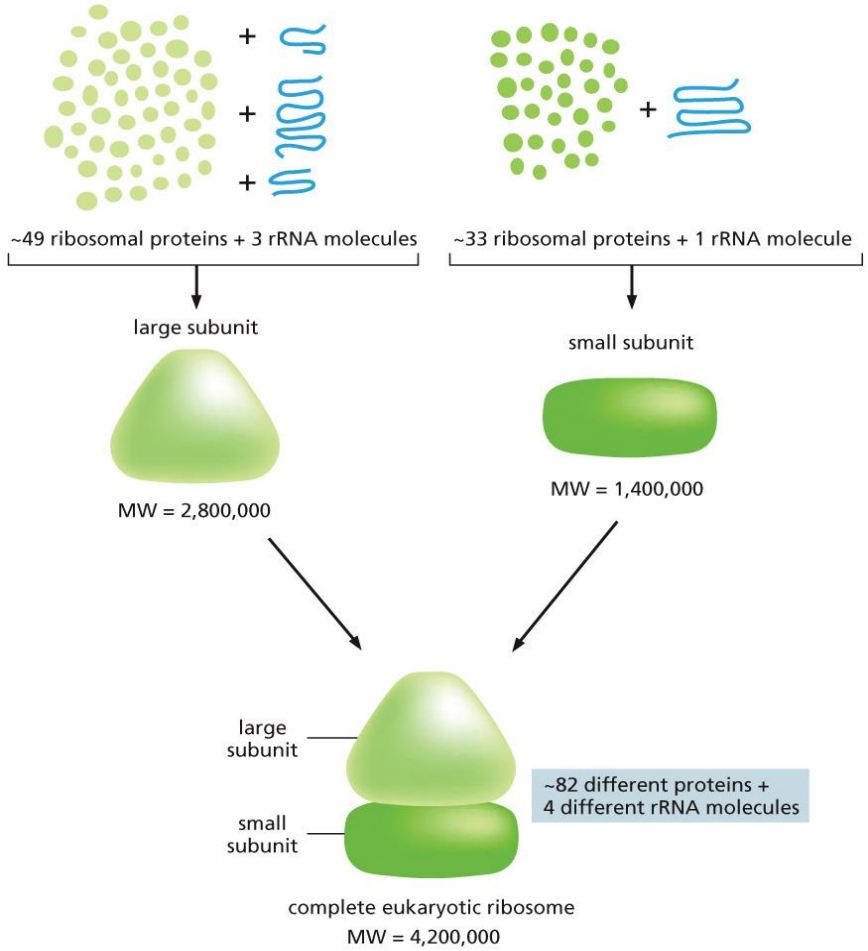
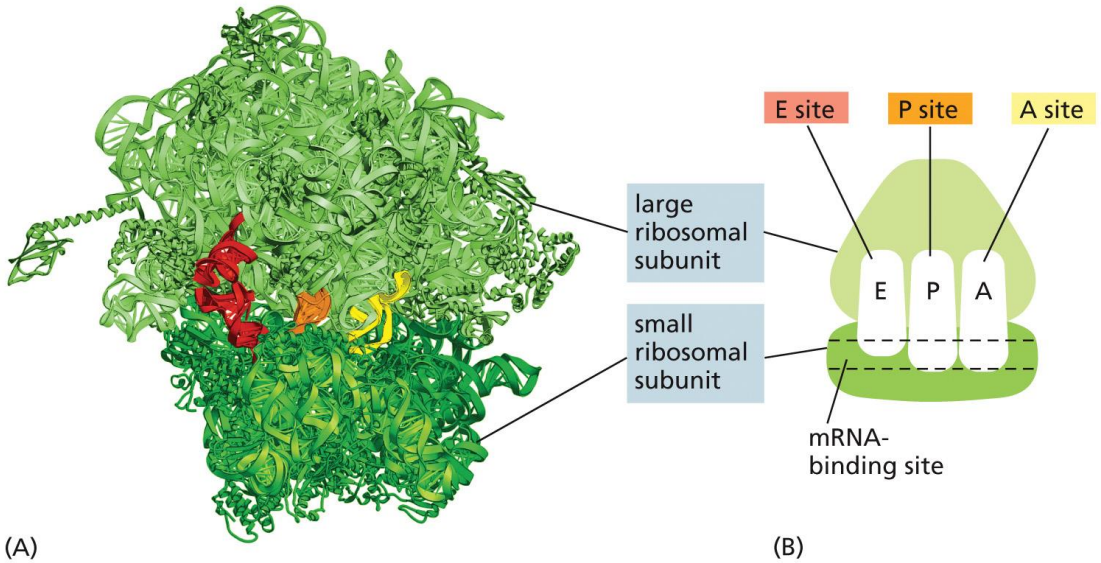
The mRNA message is decoded on ribosomes comprised of ribosomal RNAs (rRNAs) + proteins





Each ribosome has a binding site for one mRNA molecule and three binding sites for tRNAs



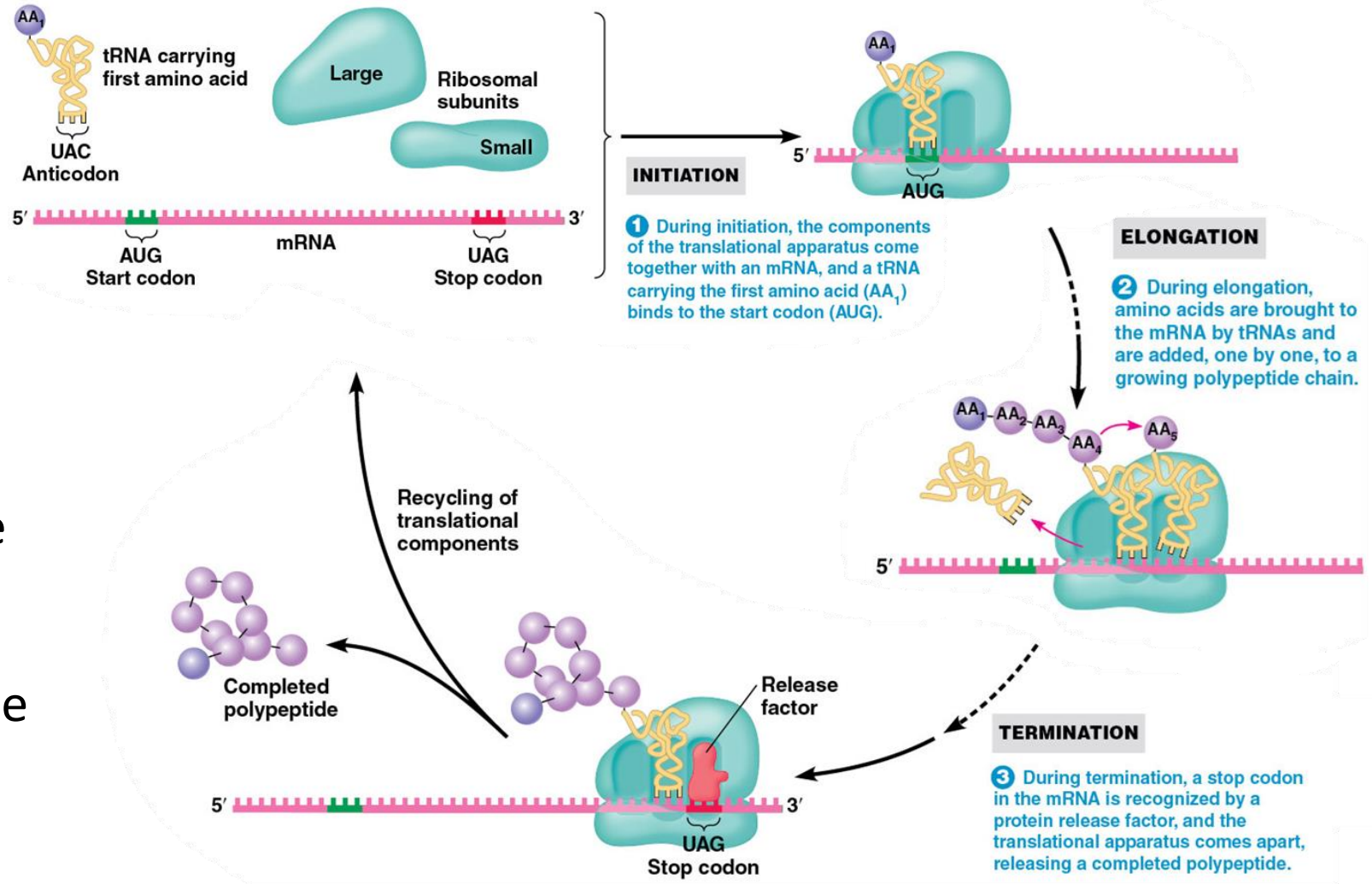
A adapted from M.M. Yusupov et al., *Science* 292:883–896, 2001. Courtesy of Albion A. Bausom and Harry Noller.

- A site (aminoacyl-tRNA): entry site for charged tRNA molecule
- P site (peptidyl-tRNA): connects growing peptide chain
- E site (exit): ejection site

Overview of Translation

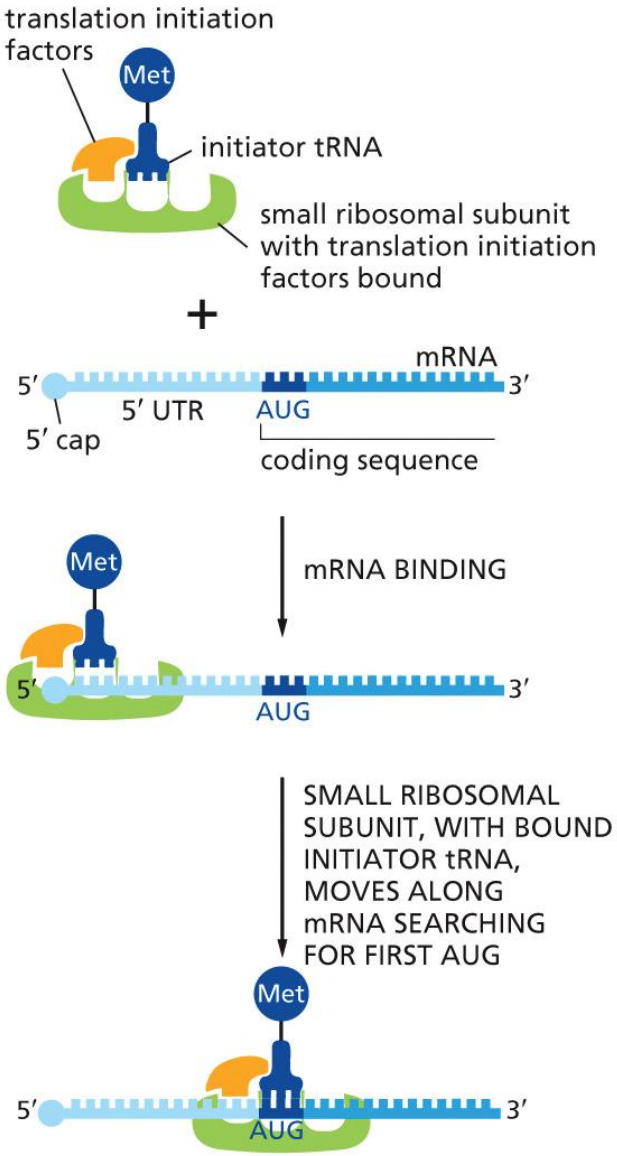
- Translation is divided into three stages:
  - Initiation
  - Elongation
  - Termination

- The mRNA is read in the 5’ to 3’ direction, so tRNAs enter the ribosome in the opposite way, from right to left.



Translation: **Initiation**

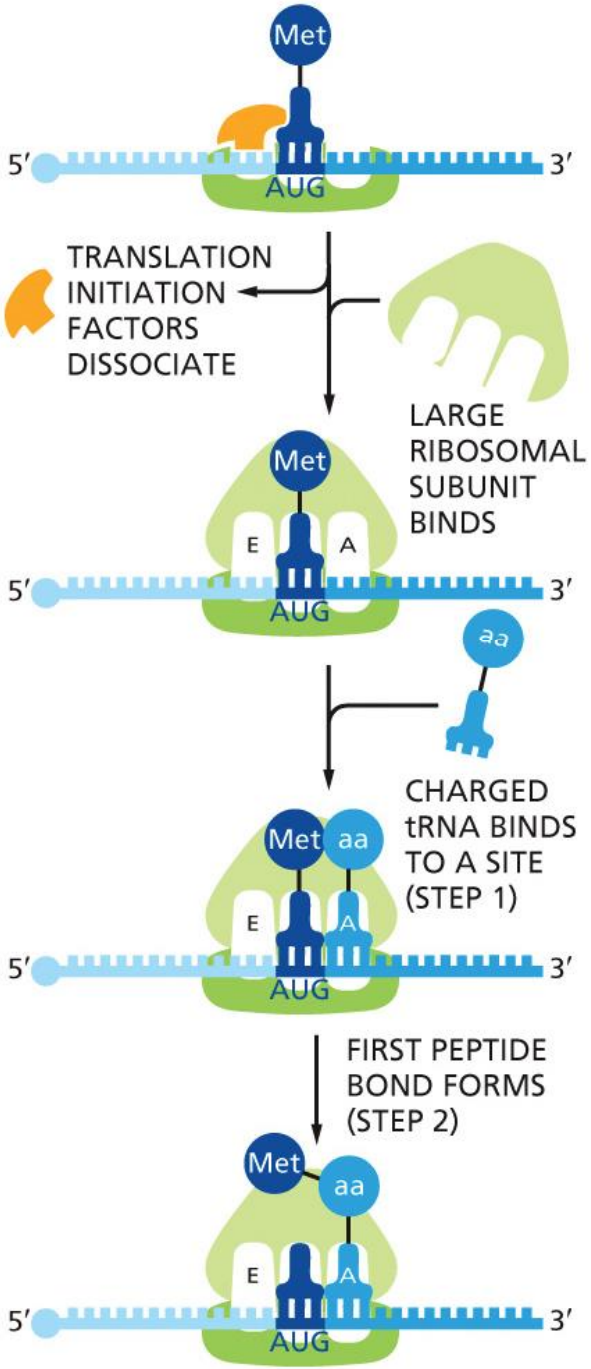
- A special charged initiator tRNA carrying methionine (Met) is loaded into the P site of small ribosomal subunit along with translation initiation factors
- Bind to 5’ end of mRNA molecule (marked by cap)
- Scans mRNA in 5’ to 3’ direction looking for **start codon (AUG)** which codes for methionine



Translation: **Initiation**

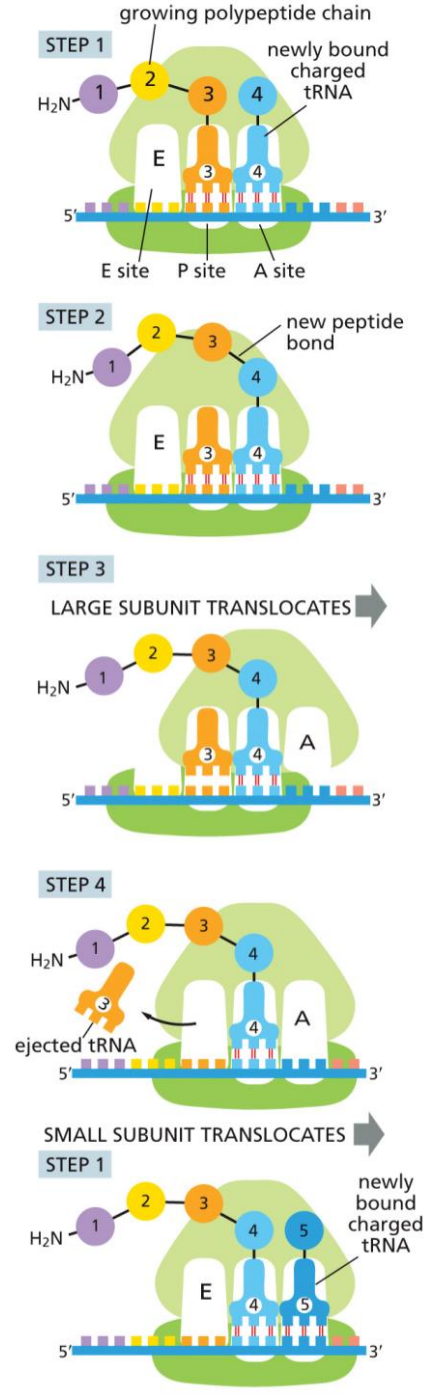
- Once bound to the start codon, translation initiation factors dissociate and allow the large ribosomal subunit to attach

- Protein synthesis is now ready to begin in the A site



Translation: **Elongation**

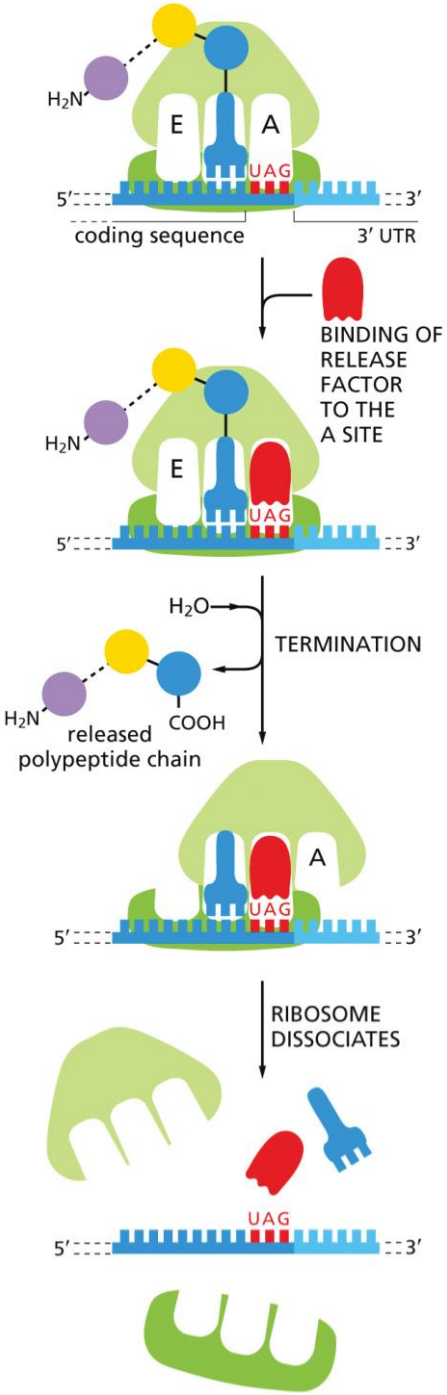
- Elongation occurs in 4 steps:
  - **Step 1:** A charged tRNA binds to vacant A site through base pairing (anticodon in tRNA to codon in mRNA)
  - **Step 2:** A catalytic site in large ribosomal subunit uncouples the polypeptide chain from the tRNA in the P site and catalyzes the formation of a peptide bond to the amino acid on the tRNA in the A site
  - **Step 3:** The large ribosomal subunit shifts to the right to move bound tRNAs into the E and P sites
  - **Step 4:** The small ribosomal subunit moves to the right to realign with large subunit. This movement ejects the spent tRNA from the E site and resets to accept another charged tRNA



Videos available in the Module on Canvas

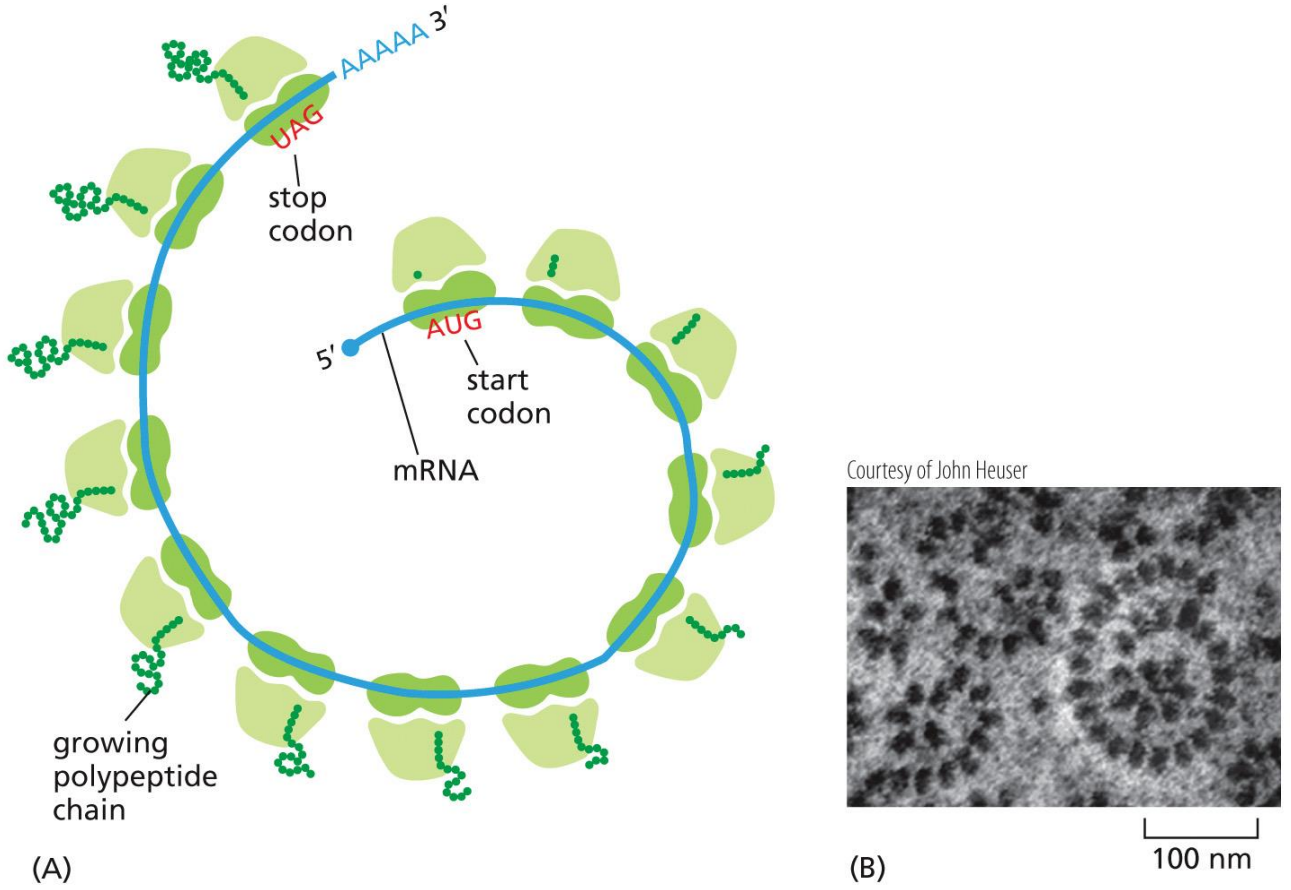
Translation: **Termination**

- End of translation is signaled by **STOP codons (UAA, UAG, and UGA)**
  - These are not recognized by any tRNA and do not specify an amino acid
- Instead, **release factors** bind to the stop codon that reaches the A site
  - Induces large ribosomal subunit to catalyze hydrolysis reaction to release the complete polypeptide chain
- Ribosome releases mRNA and dissociates into subunits to be reused for another round of translation



Many ribosomes can translate a single mRNA simultaneously

- Proteins are produced on **polyribosomes**: a group of ribosomes bound to an mRNA molecule



Squarecap Q#3-4

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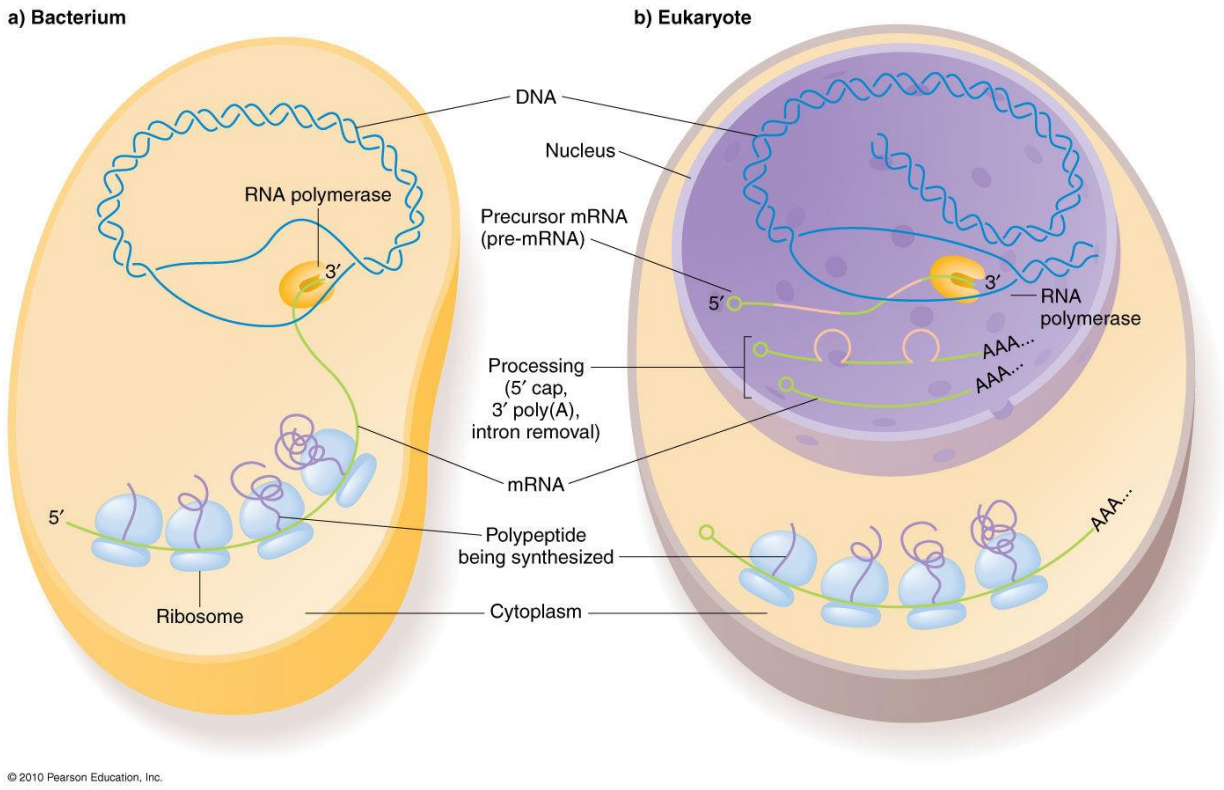
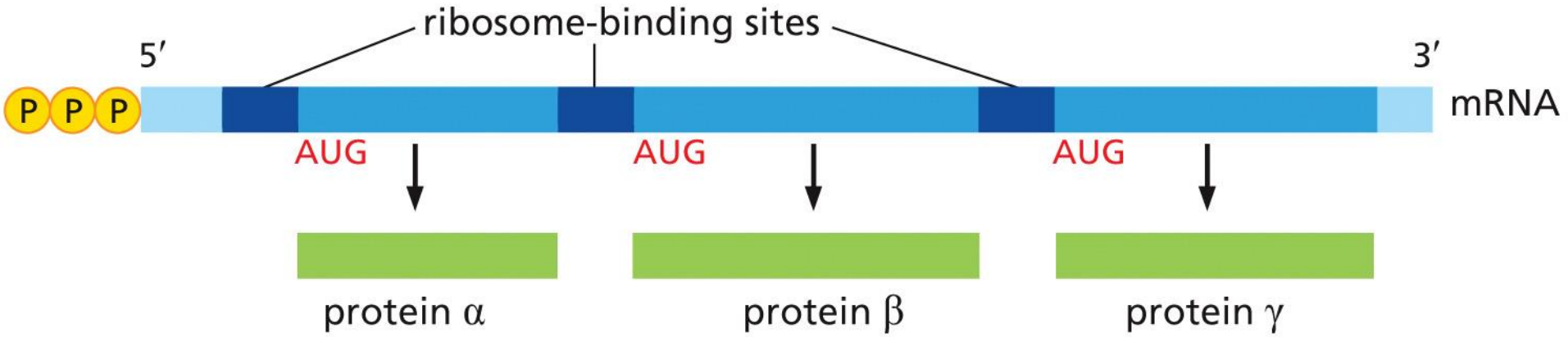
Where Transcription and Translation Occur Differs in Bacteria and Eukaryotes

- Bacteria do not have a nucleus; therefore, translation of mRNA can begin *before* its transcription is completed.
- The compartmentalization of eukaryotic cells leads to spatial separation of transcription and translation.



A single prokaryotic mRNA molecule can encode several different proteins

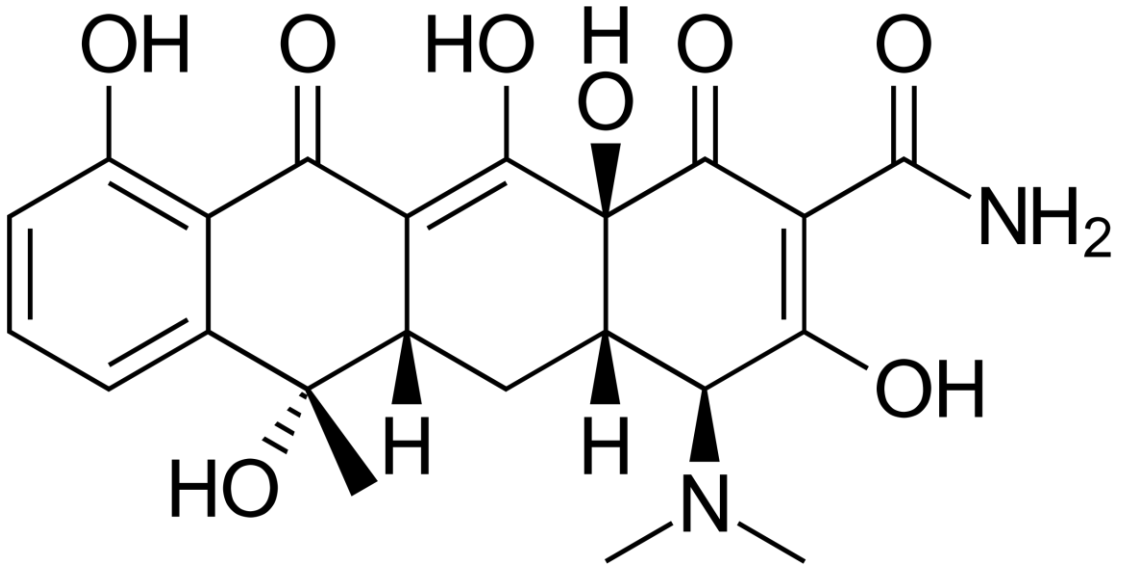


- Prokaryotes lack the 5’ cap and instead have a triphosphate at its 5’ end, so ribosomes instead bind to internal binding sequences and end at a stop codon

- Related genes are clustered together under a single promoter/ribosome binding site called an **operon**
  - More on operons next time!

Antibiotic application: Tetracycline

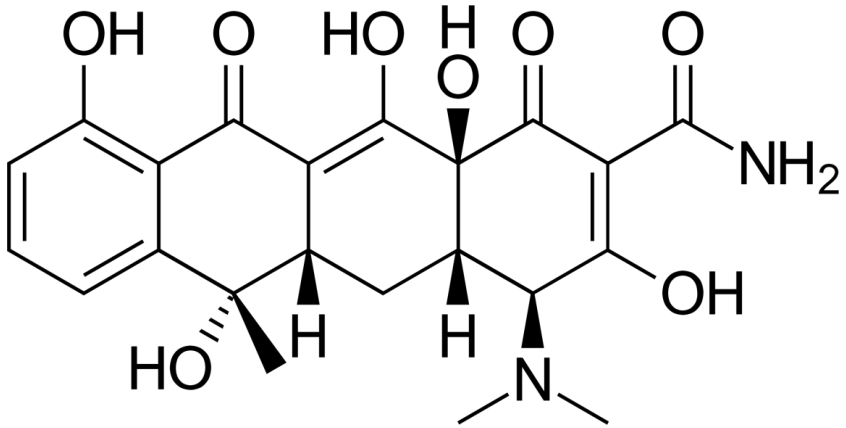
- Tetracycline binds to the A site of the bacterial ribosome.
- What effect do you think this has on bacterial protein synthesis?
- Why might tetracycline be better tolerated as an antibiotic in humans?

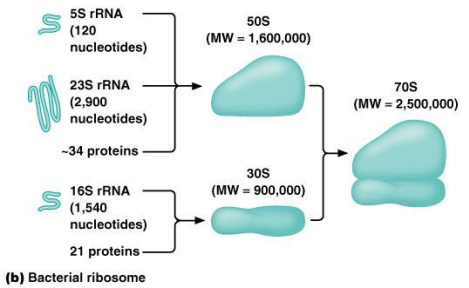


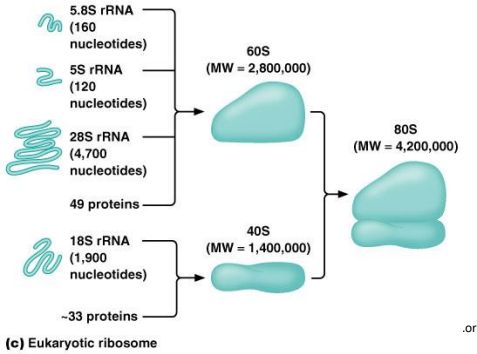
https://en.wikipedia.org/wiki/Tetracycline#/media/File:Tetracycline_skeletal.svg

Antibiotic application: Tetracycline

- Tetracycline binds to the A site of the bacterial ribosome.
- What effect do you think this has on bacterial protein synthesis?
  - With the A site blocked, a new charged aminoacyl-tRNA is no longer able to enter the ribosome and there will be no hydrogen bonding interactions between the existing amino acid in the growing peptide chain and the one attached to the next tRNA. And so, no protein chain elongation will occur.
- Why might tetracycline be better tolerated as an antibiotic in humans?
  - Tetracycline specifically binds to the 30S ribosomal subunit which is not present in humans, the specific proteins constituting the ribosome are not the same between bacteria and humans.



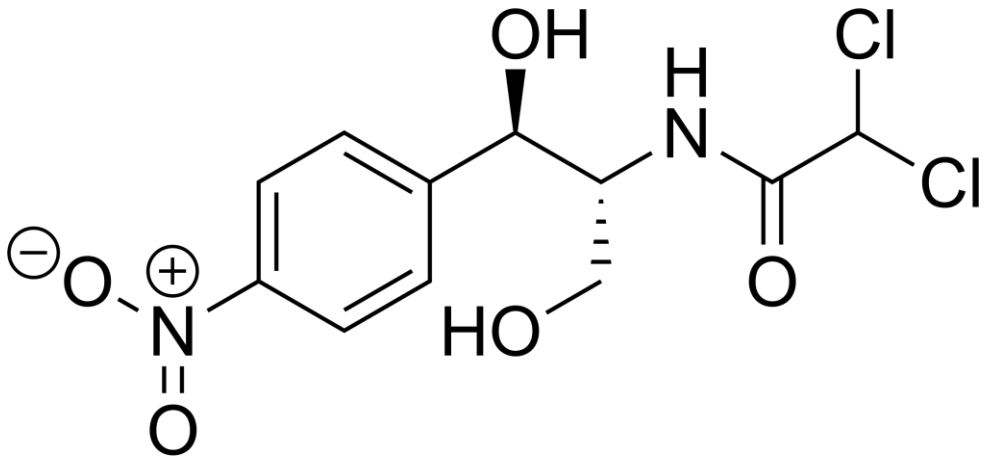


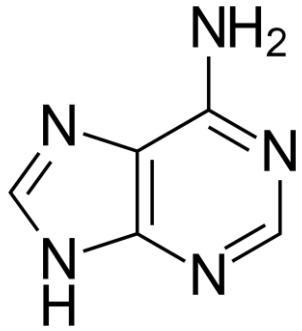


.org/wiki/Tetracycline#/media/File:Tetracycline_skeletal.svg

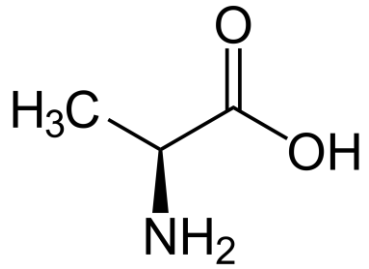
Antibiotic application: Chloramphenicol

- Chloramphenicol inhibits peptidyl transferase activity of the bacterial ribosome, preventing peptide bond formation.
- What effect do you think this has on bacterial protein synthesis?
- Chloramphenicol binds to residues A2451 and A2452 in the ribosome, based on the structures of adenine and alanine, which component of the ribosome is chloramphenicol binding to?





Adenine

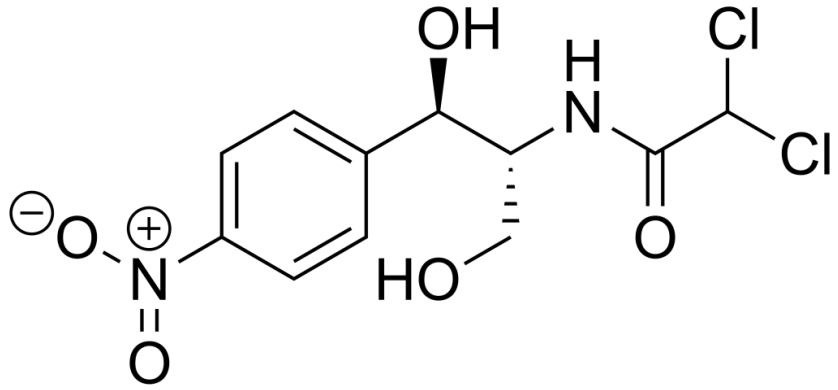


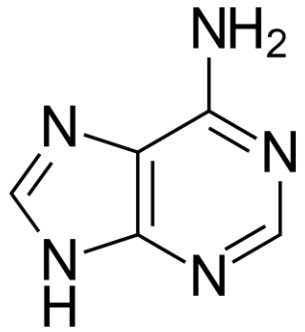
Alanine

https://en.wikipedia.org/wiki/Chloramphenicol#/media/File:Chloramphenicol.svg
https://en.wikipedia.org/wiki/Adenine#/media/File:Adenine.svg
https://en.wikipedia.org/wiki/Alanine#/media/File:L-Alanin_-_L-Alanine.svg

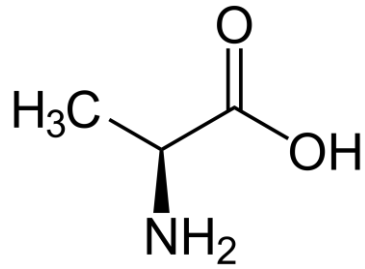
Antibiotic application: Chloramphenicol

- Chloramphenicol inhibits peptidyl transferase activity of the bacterial ribosome, preventing peptide bond formation.
- What effect do you think this has on bacterial protein synthesis?
  - Similarly, if the enzymatic reaction required to perform protein chain elongation is inhibited, it will not be possible for the ribosome to continue to build a growing polypeptide chain and translation will be halted.
- Chloramphenicol binds to residues A2451 and A2452 in the ribosome, based on the structures of adenine and alanine, which component of the ribosome is chloramphenicol binding to?
  - It will bind to the rRNA component (adenine) not to the protein component (alanine) of the bacterial ribosome. These neighboring residues create a hydrophobic core that the phenyl aromatic ring in chloramphenicol will be attracted to while having charged of highly electronegative groups (Cl) on either end to interact beneficially with the negatively charged phosphate groups in RNA as well as the aqueous environment.





Adenine



Alanine

Several antibiotics specifically target bacterial RNA or protein synthesis

TABLE 7–3 ANTIBIOTICS THAT INHIBIT BACTERIAL PROTEIN OR RNA SYNTHESIS	
Antibiotic	**Specific Effect**
Tetracycline	blocks binding of aminoacyl-tRNA to A site of ribosome (step 1 in Figure 7–37)
Streptomycin	prevents the transition from initiation complex to chain elongation (see Figure 7–39); also causes miscoding
Chloramphenicol	blocks the peptidyl transferase reaction on ribosomes (step 2 in Figure 7–37)
Cycloheximide	blocks the translocation step in translation (step 3 in Figure 7–37)
Rifamycin	blocks initiation of transcription by binding to and inhibiting RNA polymerase

Learning Objectives for Chapter 7, Part 2:

Upon completing this module, **you should be able to**:

- Understand how the genetic code works and the role of tRNAs in translation.
- Explain the mechanism of protein synthesis (translation) via ribosomal initiation, elongation, and termination.
- Appreciate the main differences between translation features in prokaryotes vs. eukaryotes.
- Apply your knowledge of the critical molecular interactions that occur during translation to experimental and disease scenarios.

Feedback/Reflection

https://forms.gle/vbdZXee89Dh9diY78