

The Genetic Code

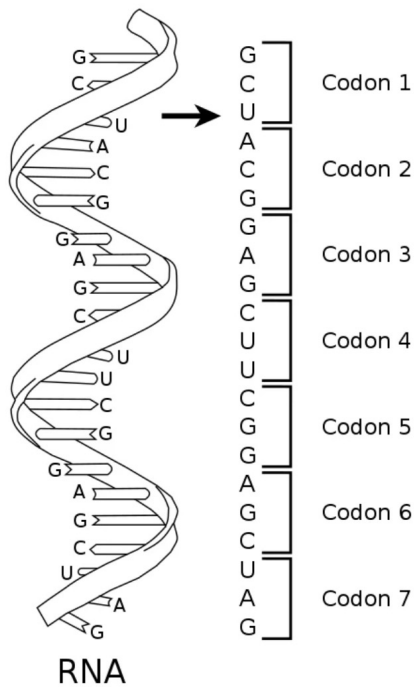
1. What are the main features of the genetic code?
2. How do aminoacyl tRNA synthetases help set up the genetic code, and how do they make sure they do it correctly?
3. What are the key parts of a bacterial ribosome?



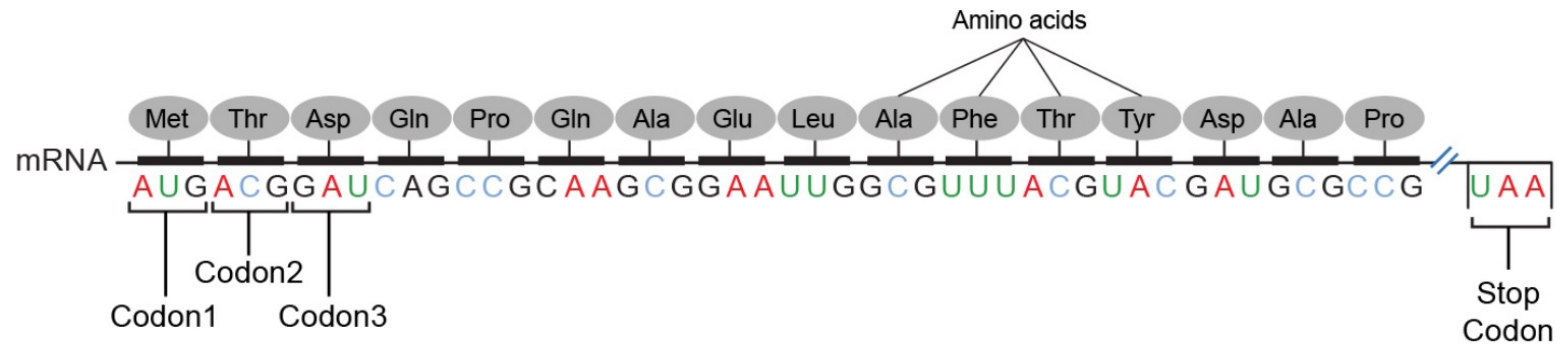
The Genetic Code Links Nucleic Acid and Protein Information

- Protein synthesis is a process of **translation**. Nucleic acid sequence information is translated into amino acid sequence information. The genetic code links these two types of information.
- Characteristics of the genetic code are:
 1. **Three nucleotides**, called a **codon**, encode an amino acid

What is a codon?



Ribonucleic acid



- A **codon** is a sequence of **three nucleotides** (trinucleotide) in DNA or RNA that codes for a specific **amino acid**.
- The **genetic code** defines how a sequence of DNA bases (**A, C, G, and T**) in a gene determines the **protein sequence** it encodes.
- The **cell reads** the gene sequence in **groups of three bases** (codons).
- There are **64 different codons**: **61 codons** code for **amino acids**.
 - **3 codons** act as **stop signals**, marking the end of protein synthesis.

Table 39.1 The genetic code

First position (5' end)	Second position				Third position (3' end)
	U	C	A	G	
U	Phe	Ser	Tyr	Cys	U
	Phe	Ser	Tyr	Cys	C
	Leu	Ser	Stop	Stop	A
	Leu	Ser	Stop	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	Thr	Lys	Arg	A
	Met	Thr	Lys	Arg	G
G	Val	Ala	Asp	Gly	U
	Val	Ala	Asp	Gly	C
	Val	Ala	Glu	Gly	A
	Val	Ala	Glu	Gly	G

Note: This table identifies the amino acid encoded by each triplet. For example, the codon 5'-AUG-3' on mRNA specifies methionine, whereas CAU specifies histidine. UAA, UAG, and UGA are termination signals. AUG is part of the initiation signal, in addition to coding for internal methionine residues.

The Genetic Code Links Nucleic Acid and Protein Information

➤ Protein synthesis is a process of **translation**. Nucleic acid sequence information is translated into amino acid sequence information. The **genetic code** links these two types of information.

➤ Characteristics of the genetic code are:

1. **Three nucleotides**, called a **codon**, encode an amino acid
2. The code is not overlapping
3. The code has no punctuation
4. The code is read in the 5' to 3' direction
5. The code is degenerate in that some amino acids are encoded by more than one codon.

Biological significance of degeneracy?

5'-ATTCCG-AGCCCG-AAT-3'

3'-TAA GGC TCG GGC TAA-5'

5'—GCGGCGACGCGCAGUAAUCCACAGCCGCCAGUUCGCUGGCGGCAU—3'

mRNA

3'—CGCCGCTGCGCGTCAATTAGGGTGTGGCGGTCAAGGCGACCGCCGTA—5'

Template (antisense) strand of DNA

5'—GCGGCGACGCGCAGTTAATCCACAGCCGCCAGTTCGCTGGCGGCAT—3'

Coding (sense) strand of DNA

5'-ATTCCGAGCCCGAAT-3'
 3'-TAA GGC TCG GGCTAA-5'
 5'-AUUCCGAGCCCGAAU-3' - mRNA

Codon	Standard code	Mitochondrial code
UGA	Stop	Trp
UGG	Trp	Trp
AUA	Ile	Met
AUG	Met	Met
AGA	Arg	Stop
AGG	Arg	Stop

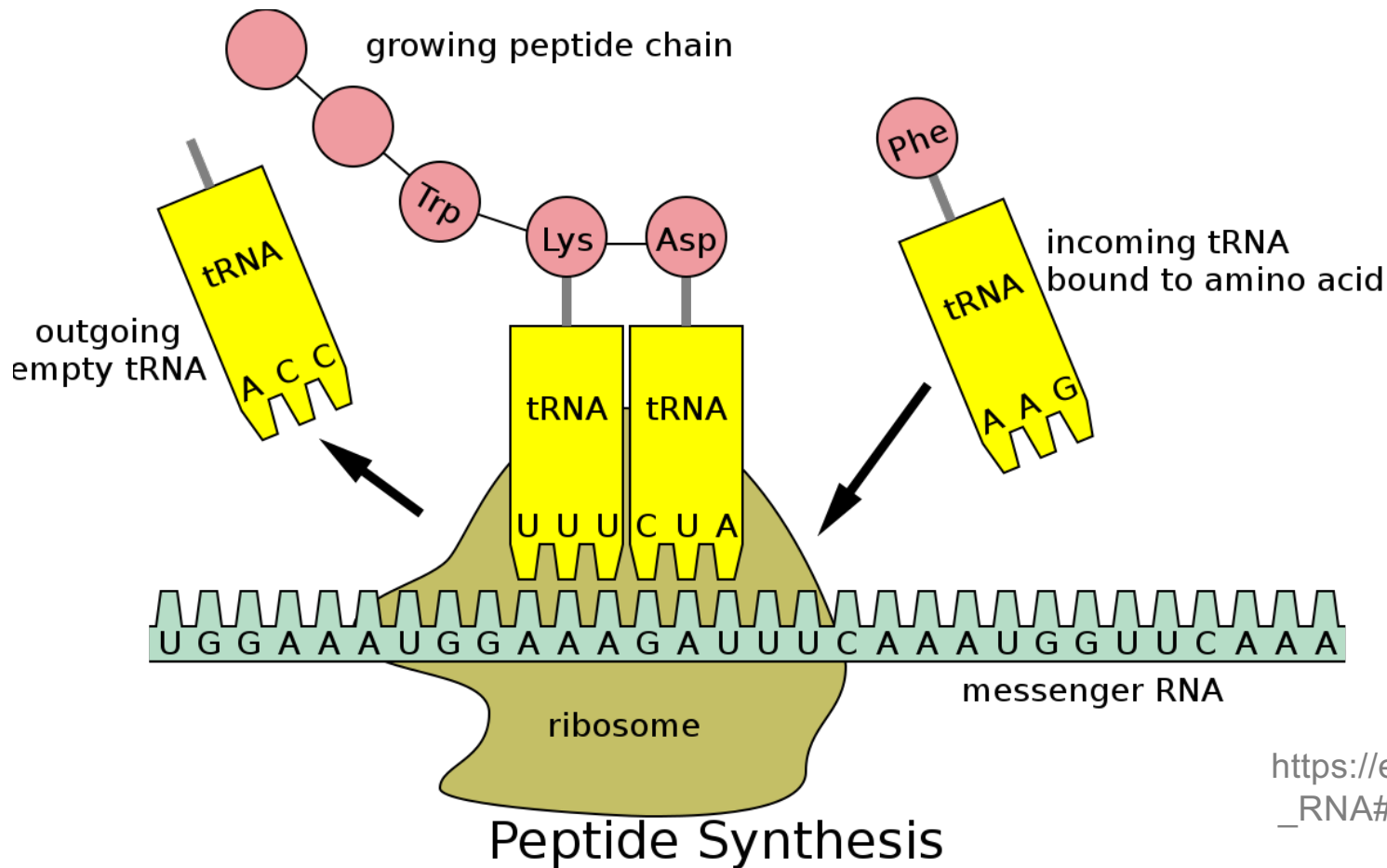
➤ Mitochondria also use variations of the genetic code.

Implications of having a nearly universal genetic code?

First letter of codon (5' end)

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How are proteins synthesized?

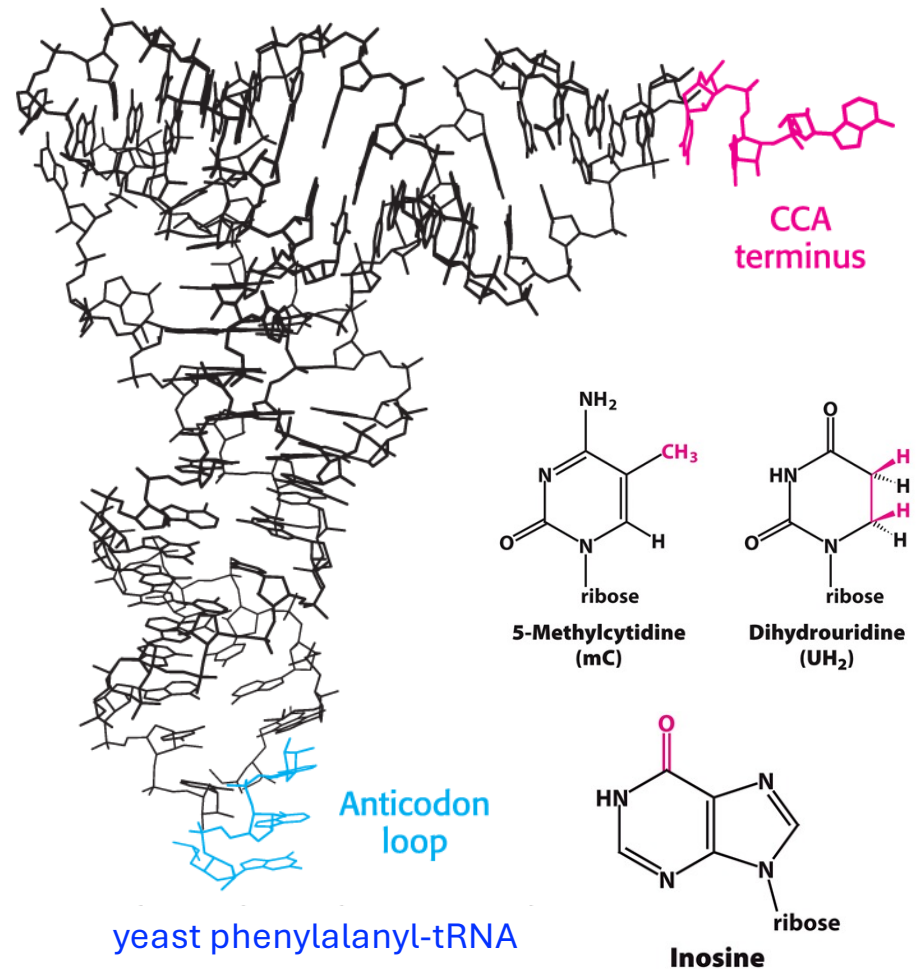


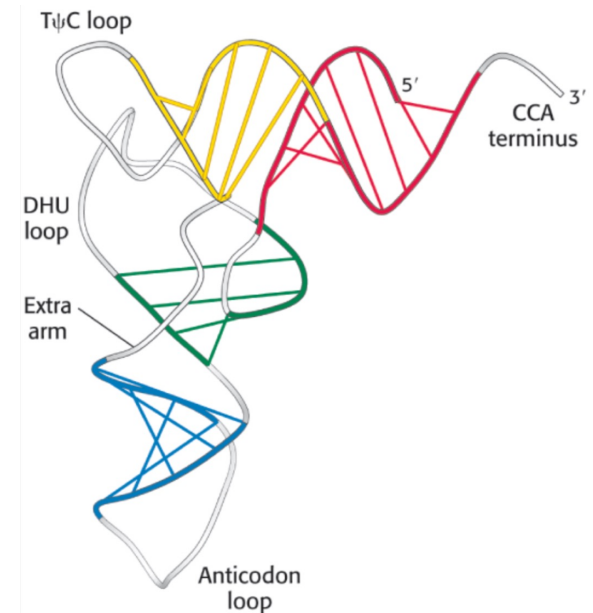
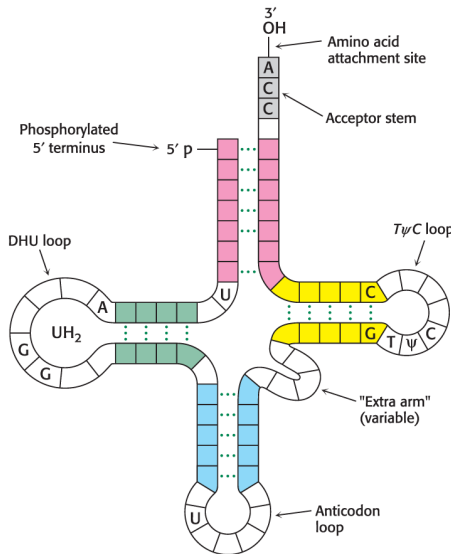
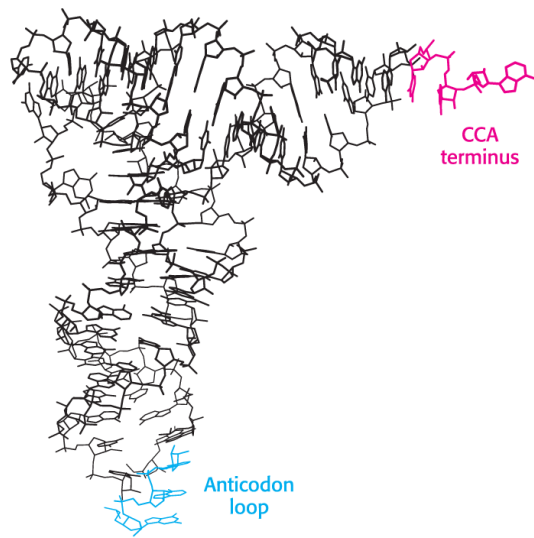
The interaction of tRNA and mRNA in protein synthesis.

https://en.wikipedia.org/wiki/Transfer_RNA#/media/File:Peptide_syn.svg

Codon recognition in mRNA

- **Transfer RNA (tRNA)** molecules function as an adapter molecule between a codon and an amino acid
- There is at least one tRNA molecule for each amino acid
- General characteristics of tRNA molecules include:
 1. Each is a single strand of RNA between 73 and 93 amino acids in length (~25 kDa)
 2. The three-dimensional structure of the molecule is L-shaped
 3. Transfer RNA molecules contain unusual bases, such as inosine, or bases that have been modified.

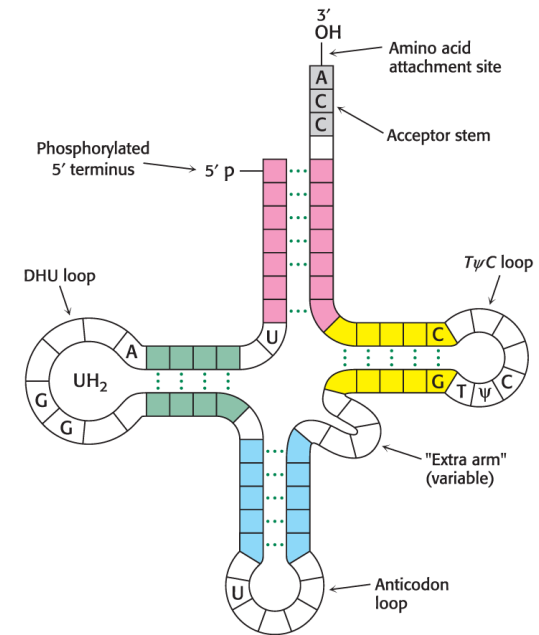




- tRNA folds into an L-shape by stacking its four double-stranded regions.
- Double-helical regions form the core of the tRNA structure.
- Secondary structure (cloverleaf) vs. tertiary structure (L-shape):
 - In 2D (secondary structure), tRNA looks like a cloverleaf.
 - In 3D (tertiary structure), the helices stack to create a compact L-shape.
- This shape is essential for proper function in translation.

All tRNAs take a cloverleaf shape

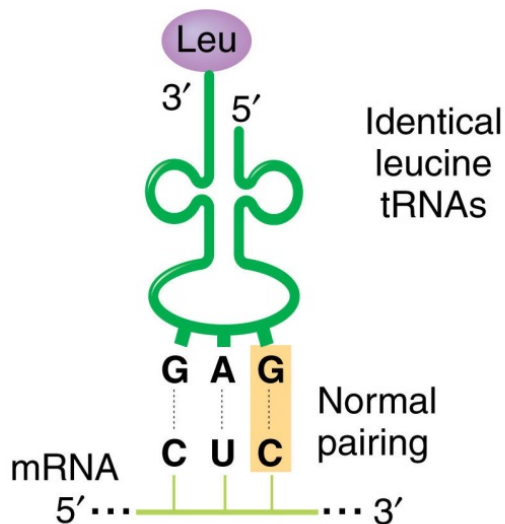
- ~**half** of the nucleotides in tRNAs form **double helices** through base pairing.
- **Five Non-Base-Paired Regions:**
 - **3' CCA terminal region** – Part of the acceptor stem, where amino acids attach.
 - **T ψ C loop** – Named after its ribothymine-pseudouracil-cytosine sequence.
 - **Extra arm** – A variable-length region that differs between tRNAs.
 - **Anticodon loop** – Contains the anticodon, which pairs with mRNA codons.
 - **DHU loop** – Contains dihydrouracil residues, involved in tRNA recognition.



- combination of helices and loops → tRNA's unique identity.
- Despite differences → all tRNAs fold into the same general tertiary structure → for function in translation

Codon recognition in mRNA

- The anticodon forms base pairs with the codon:
- By convention, sequences are written in the 5' to 3' direction. Thus the anticodon that pairs with AUG is written CAU.



Start **RNA** **Stop**

ACCA-**AUG**-AUA-GCC-GAU-GGG-**UGA**-GGAG

Met -Ile -Ala-Asp-Gly

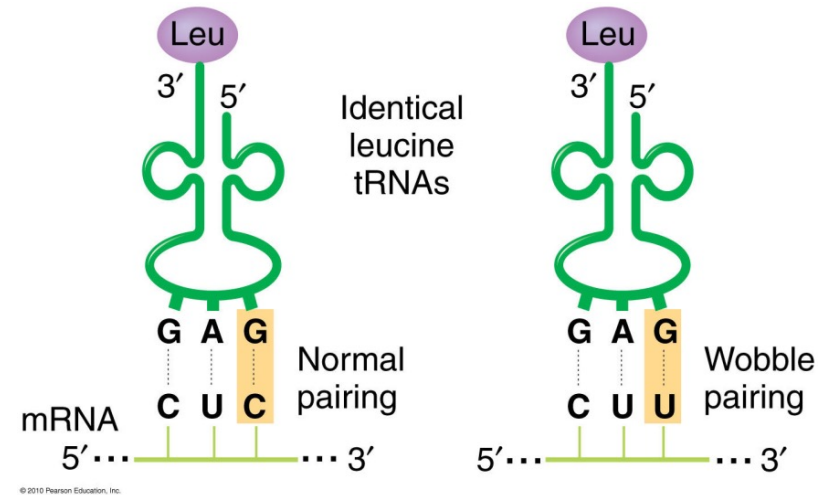
protein

The start codon is AUG and it also codes for Methionine

**There are three
stop codons
UGA, UAA, UAG**

Wobble in Base-Pairing

- Some tRNA molecules can recognize more than one codon. The recognition of the third base in the codon by the anticodon is sometimes less discriminating, a phenomenon called **wobble**.
- **Occurs at the third position** of the codon (5' end of the anticodon in tRNA, 3' end of the codon in mRNA).
- **Non-standard base pairing** happens at this position, allowing some **tRNAs to pair with more than one codon**.
- The first base of the anticodon determines the degree of wobble. If the first base is inosine, the anticodon can recognize three codons.
- **Wobble pairing** is a flexibility in the base-pairing rules of **codon-anticodon interactions** during translation. It allows a single **tRNA** to recognize **multiple codons**, helping cells translate mRNA efficiently with fewer tRNA molecules.



First base of anticodon	Third base of codon
C	G
A	U
U	A or G
G	U or C
I	U, C, or A

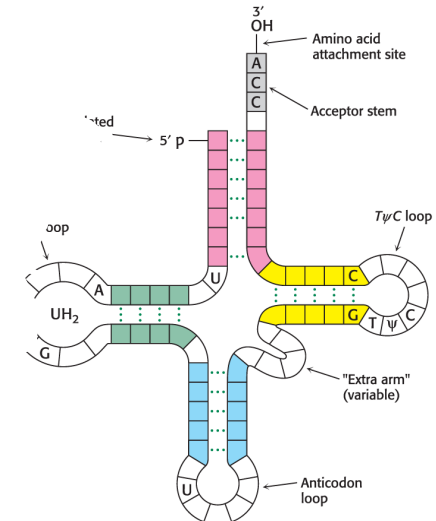
Quick Quiz 1

The genetic codon is degenerate. In this context which of the following statements is false?

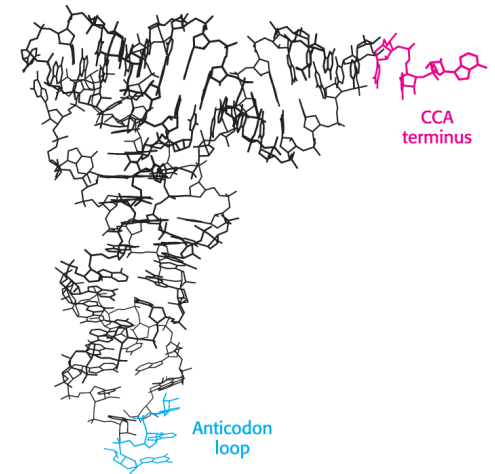
- A. There are 64 possible codons and only 20 amino acids
- B. Only three codons specify stop signals
- C. A total of 61 codons specify amino acids
- D. All amino acids have more than one codon specifying them
- E. Codons that specify the same amino acid are called synonyms.

CCA terminus

- Relevance of the CCA terminus at the 3' end of transfer RNA (tRNA) includes:
 - Amino Acid Attachment – The 3' end (adenosine) of the CCA sequence serves as the binding site for amino acids, which are attached by aminoacyl-tRNA synthetases.
 - Translation Efficiency – Ensures proper positioning of tRNA in the ribosome during translation.
 - Quality Control & tRNA Stability – Helps in recognition and quality control by enzymes that modify and charge tRNA, and contributes to tRNA stability.
 - Universality – Almost all tRNAs across organisms have a CCA sequence, either encoded in their genes or added by tRNA nucleotidyltransferase after transcription.
- Without a functional CCA terminus, tRNA cannot participate in translation, making it a critical feature for protein synthesis.

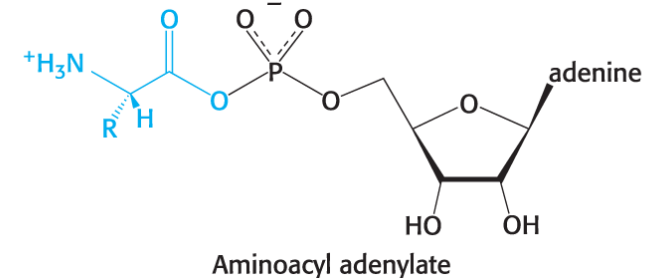
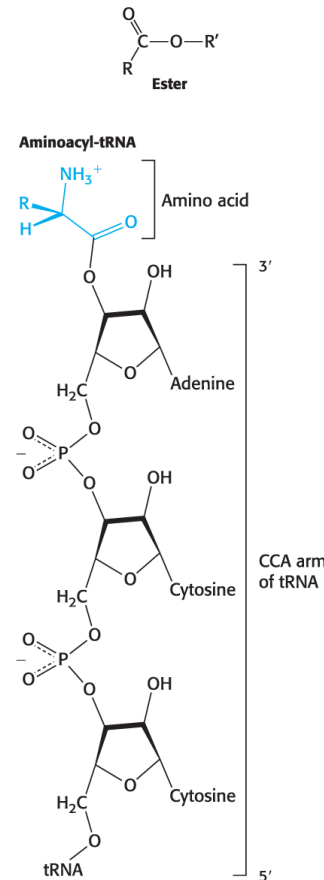


et al., *Biochemistry: A Short Course*, 4e, © 2019
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Amino Acids Are Activated by Attachment to transfer RNA

- In order to be incorporated into proteins, amino acids must be activated
- Amino acids are activated by formation of an ester linkage between the carboxyl group of the amino acid and either the 2' or 3' hydroxyl group of the terminal adenosine of the tRNA, forming an aminoacyl tRNA or charged tRNA
- **Aminoacyl tRNA synthetases** catalyze the activation of amino acids. The first step is the formation of aminoacyl adenylate or aminoacyl-AMP:



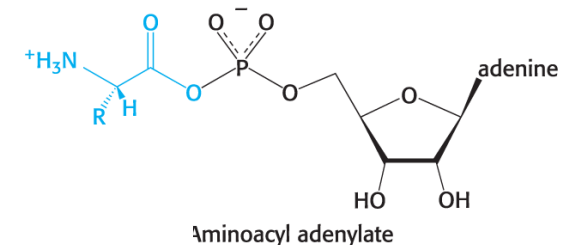
Amino Acids Are Activated by Attachment to transfer RNA

Amino acid + ATP \rightleftharpoons aminoacyl-AMP + PP_i

Aminoacyl-AMP + tRNA \rightleftharpoons aminoacyl-tRNA + AMP

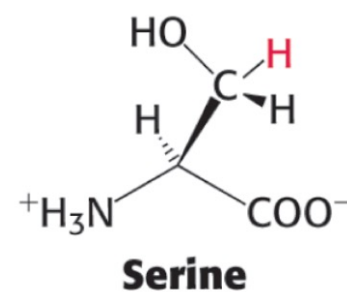
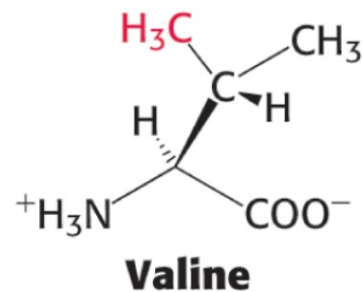
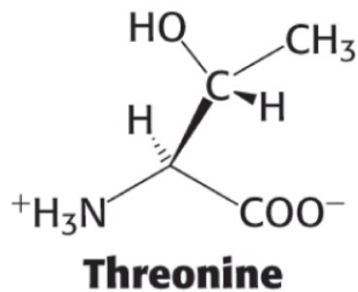
Amino acid + ATP + tRNA + H₂O \rightarrow aminoacyl-tRNA + AMP + PP_i
 (sum of activation and transfer steps)

Amino acid + ATP + tRNA + H₂O \rightarrow aminoacyl-tRNA + AMP + 2 P_i
 (the equivalent of two molecules of ATP is consumed in the synthesis of each aminoacyl-tRNA)



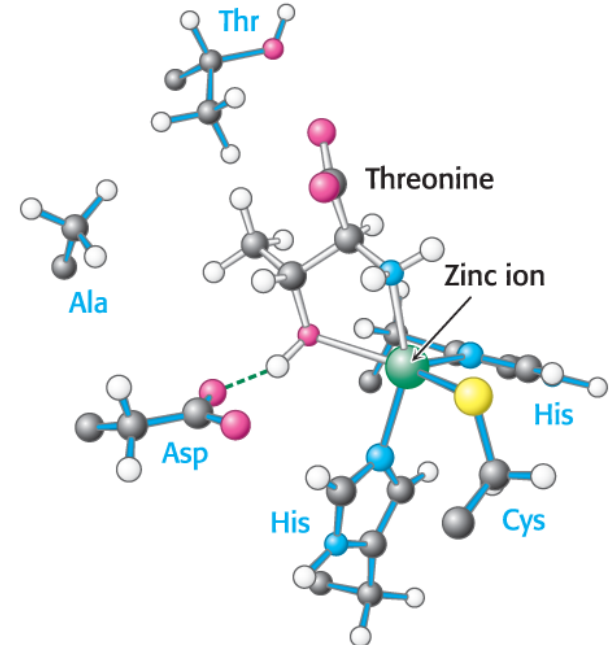
- Aminoacyl-tRNA synthetase catalyzes both the activation and transfer of an amino acid.
- The aminoacyl-AMP intermediate stays bound to the enzyme (does not dissociate).
- It is held in place by noncovalent interactions in the enzyme's active site.
- Translation happens when an ester bond forms between the amino acid and its specific tRNA.
- Aminoacyl-tRNA synthetases act as the true translators of the genetic code by ensuring the correct amino acid is attached to the right tRNA.

Aminoacyl-tRNA Synthetases have highly discriminating aa activation sites



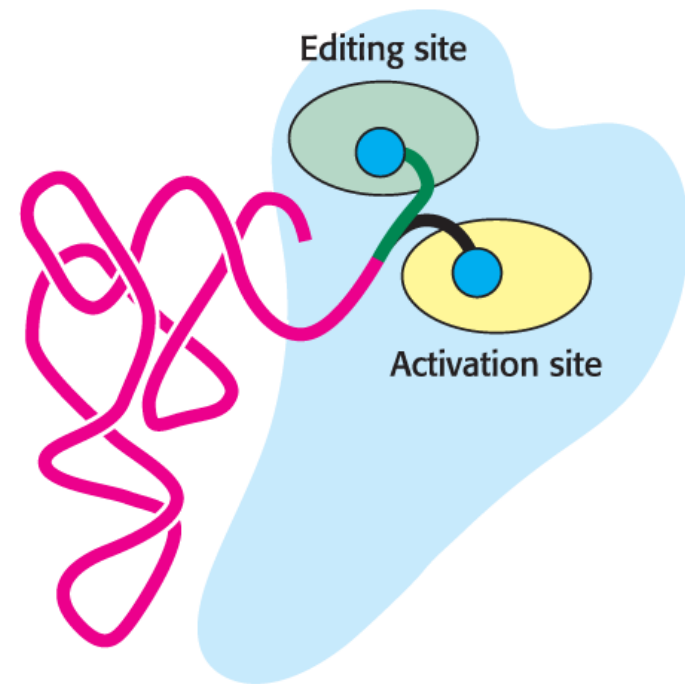
- The enzyme's active site contains a zinc ion. Threonine binds to the zinc ion using: (i) its amino group and (ii) its side-chain hydroxyl group.
- The hydroxyl group also forms a hydrogen bond with an aspartate residue for further recognition.
- Valine has a methyl group instead of a hydroxyl group, so:
 - It cannot bind to the active site.
 - It does not get adenylated or transferred to threonyl-tRNA.

How about Serine?



Proofreading increases the fidelity of protein synthesis

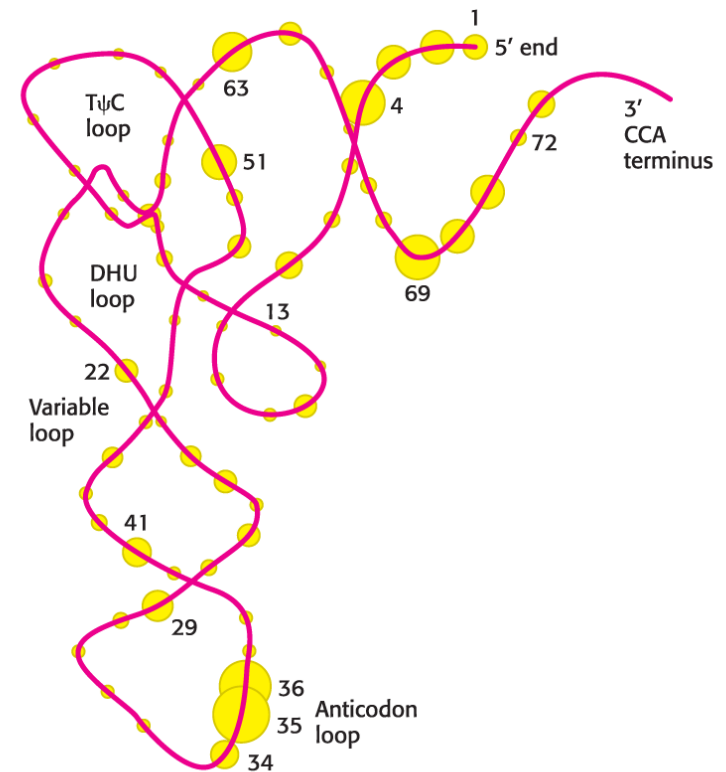
- Proofreading by aminoacyl-tRNA synthetases increases the fidelity of protein synthesis.
- Threonyl-tRNA synthetase has an editing site, in addition to an active site, to remove a serine inappropriately joined to tRNA^{Thr}.
- The CCA arm of tRNA^{Thr} can swing into the editing site where the serine is removed.
- Because threonine is larger than serine, it cannot fit into the editing site.
- The double sieve of an acylation site and an editing site increases the fidelity of many ***synthetases***.



If the amino acid fits well into the editing site, the amino acid is removed by hydrolysis. Only one amino acid is attached to the tRNA at a time, and this amino acid can only occupy either the editing site or the activation site.

How do synthetases choose their tRNA partners?

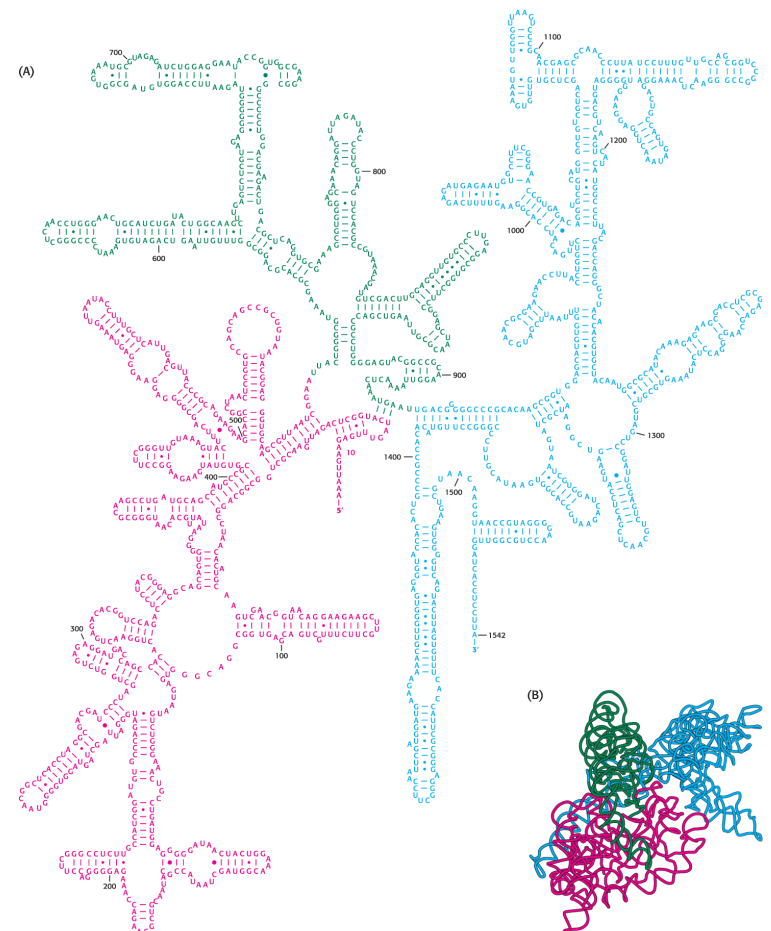
- **Aminoacyl-tRNA synthetases** match amino acids to their correct tRNAs, translating the genetic code into proteins.
- They are the only molecules in biology that directly "**read**" or "**know**" the genetic code.
- Their precise recognition of **correct tRNAs** is crucial for accurate protein synthesis.
- **Anticodons** on tRNAs are often the main recognition site, but not the only one.
- tRNA synthetases also recognize **other tRNA structural features**, such as **loops containing modified (unusual) bases**, that act as molecular identifiers.



tRNAs have multiple recognition sites for aminoacyl-tRNA synthetases

Ribosomal RNAs play the central role in protein synthesis

- Two-thirds of the mass of ribosomes is RNA, which is critical for the structure and function of the ribosome.
- The ribosomal RNAs fold into complex structures with many short duplex regions.
- Ribosomal RNA is the actual catalyst for protein synthesis, with the ribosomal proteins making only a minor contribution.
- Because transcription and translation both occur in the 5'-to-3' direction, bacterial protein synthesis begins before transcription is complete.
- Several ribosomes can translate an mRNA molecule at the same time, forming polyribosomes or polysomes.
- Having transcription and translation happen at the same time makes protein synthesis faster and more efficient.



Tymoczko et al., *Biochemistry: A Short Course*, 4e, © 2019 W. H. Freeman and Company
 (A) Courtesy of Dr. Bryn Weiser and Dr. Harry Noller; (B) drawn from 1FJG.pdb.

Quick Quiz 2

Why must tRNA molecules have both unique structural features and common structural features?

- ☐ tRNA molecules must interact nearly the same way with ribosomes, mRNA, and protein factors participating in translation.
- ☐ tRNA molecules must be distinguishable from other tRNA molecules to allow aminoacyl-tRNA synthetases to select the correct amino acid accurately.
- ☐ tRNA molecules must share common structural features to allow other species to utilize the same tRNA molecules.
- ☐ tRNA molecules must maintain all four double-stranded regions to prevent unwanted tRNA degradation.

Quick Quiz 3

The RNA transcript of a region of T₄ phage DNA contains the sequence

5'-AAAUGAGGA-3'

In theory, this sequence is capable of encoding three different polypeptides. What are they?

- ☐ Lys-Stop-Gly
- ☐ Asn-Glu
- ☐ Met-Arg
- ☐ Arg-Ser-Lys
- ☐ Gly-Val
- ☐ Glu-Stop

Quick Quiz 5

DNA polymerases can add nucleotides only to a free hydroxyl group. As a result, a primer made up of _____ is added by the enzyme _____.

- A. RNA; DNA polymerase II
- B. DNA; DNA polymerase I
- C. DNA; primase
- D. Okazaki fragments; primase
- E. RNA; primase

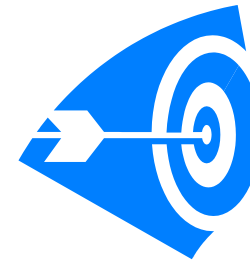
SELF-CHECK 5

Aminoacyl-tRNA synthetases are the only components of gene expression that know the genetic code. Explain.

Hide Answer

These enzymes convert nucleic acid information into protein information by binding the tRNA and linking it to the correct amino acid. Thus, through these enzymes the correct anticodon is paired with the correct amino acid to establish the code.

Assigned Problems



Chapter	Tymochko, Berg, Stryer, Biochemistry, 2 nd Edition,	Chapter	Tymochko, Berg, Stryer, Biochemistry, 2 nd Edition,
36	2, 6, 10, 11, 15, 18, 20, 23, 25, 27.	39	3, 5, 9, 11, 13, 14, 15, 19.
Chapter	Tymochko, Berg, Stryer, Biochemistry, 3 rd Edition, 4 th Edition (bottom line)	Chapter	Tymochko, Berg, Stryer, Biochemistry, 3 rd Edition, 4 th Edition (bottom line)
36	2, 6, 10, 11, 15, 18, 20, 23, 25, 27. 2, 6, 10, 11, 15, 18, 20, 23, 25, 27.	39	3, 5, 9, 11, 13, 15, 16, 20. 3, 5, 9, 11, 13, 14, 15, 19.