

Translation of mRNA to Protein

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Translation is the process where the sequence of codons in mRNA directs the synthesis of amino acids, forming polypeptides. Proteins, made of one or more polypeptides, are essential for cell structure and function.

Beadle and Tatum's One-Gene/One-Enzyme Hypothesis

- In the early 1940s, American geneticists George Beadle and Edward Tatum investigated the relationship between genes, enzymes, and traits.
- Beadle and Tatum concluded that one gene controls the synthesis of one enzyme, leading to the **one-gene/one-enzyme hypothesis**.

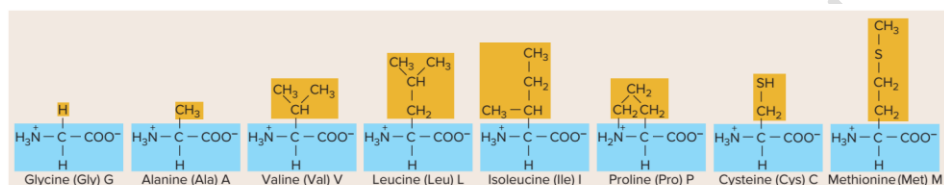
Modifications to the Hypothesis:

1. **Broader Protein Roles:** Enzymes are just one type of protein; all proteins are gene-encoded.
2. **Polypeptide Complexity:** Some proteins consist of multiple polypeptides, requiring multiple genes (e.g., hemoglobin).
3. **Non-Polypeptide Genes:** Some genes specify functional RNA, not polypeptides.
4. **Alternative Splicing:** One gene can produce multiple polypeptides due to processes like alternative splicing.

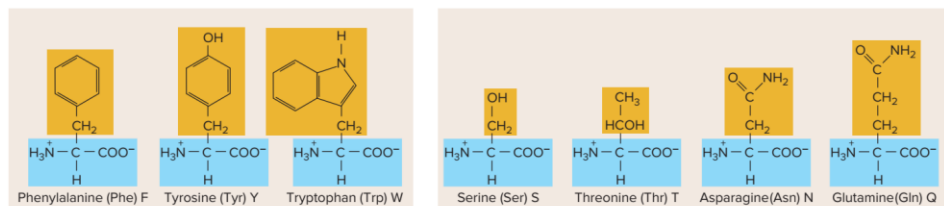
Central Question: *how the genetic information* in messenger RNA (mRNA) is *translated into the linear sequences of amino acids* in proteins—a process known as translation.

Amino Acids:

- There are 20 common amino acids, each with a unique side chain (R group) that influences its chemical properties.
 - Hydrophobic (water-fearing)** amino acids are typically buried within the protein's interior.
 - Hydrophilic (water-loving)** amino acids are more likely to be on the protein's surface, interacting favorably with surrounding fluids.

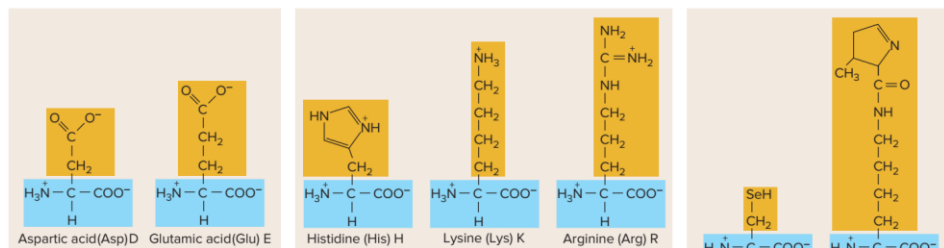


(a) Nonpolar, aliphatic amino acids



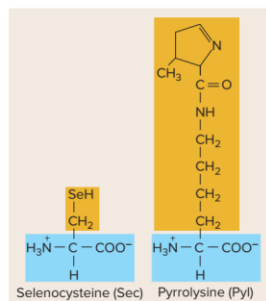
(b) Aromatic amino acids

(c) Polar, neutral amino acids



(d) Polar, acidic amino acids

(e) Polar, basic amino acids



(f) Nonstandard amino acids

Polypeptide Structure:

- Following transcription and translation, polypeptides acquire a defined amino acid sequence, known as the **primary structure**. This can range from hundreds to thousands of amino acids.

- Polypeptides do not remain linear; they quickly fold into a compact **three-dimensional structure** during or shortly after translation.

Folding Process:

- The folding is primarily dictated by the amino acid sequence, particularly the properties of the side chains. Some polypeptides require **chaperones** to assist in proper folding.
- The folding process occurs in multiple stages:
 - **Secondary Structure:** Involves regular shapes like α helices and β sheets, stabilized by hydrogen bonds in the polypeptide backbone.
 - **Tertiary Structure:** Formed by the folding of secondary structures, involving interactions such as hydrophobic avoidance, ionic interactions, hydrogen bonds, and van der Waals forces.

Functions of Proteins:

- Proteins perform a wide range of essential roles, including:
 - **Enzymatic Activity:** Catalyzing biochemical reactions.
 - **Structural Support:** Providing shape and support to cells and tissues.
 - **Transport:** Moving molecules across cell membranes and throughout the organism.
 - **Defense:** Acting as antibodies to protect against pathogens.
 - **Regulation:** Involving in signaling pathways that control cellular processes.

1. Significance of Translation:

- Translation is *highly conserved* across organisms and is energetically costly, *consuming up to 80% of energy* in rapidly growing bacterial cells.
- Synthesis of a single protein *requires* the coordinated action of *over 100 proteins and RNAs*.

2. Complexity of Translation:

- Translation is *more complex than transcription* due to the *lack of direct affinity between amino acids and nucleotide bases*.

- In **1955**, **Francis H. Crick** proposed that **amino acids must bind to an adaptor molecule** (later identified as transfer RNA, or tRNA) **to recognize mRNA codons**.

3. **Key Components of Translation:**

- **mRNA:** The template that **provides the sequence of codons**, each specifying an amino acid.
- **tRNA:** **Adaptor molecules** that transport specific amino acids to the ribosome, recognizing codons through base-pairing.
- **Aminoacyl-tRNA Synthetases:** Enzymes that **attach amino acids to their corresponding tRNAs**.
- **Ribosome:** A large molecular machine that coordinates mRNA and tRNA interactions, **facilitating peptide bond formation**.

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4. **Translation Process:**

- The **coding region of mRNA consists of open reading frames (ORFs)**, which are sequences of codons that specify proteins.
- **Translation begins at the start codon** (typically AUG) and **ends at stop codons** (UAG, UGA, UAA).
- In bacteria, the start codon is usually 5'-AUG-3', but 5'-GUG-3' and sometimes even 5'-UUG-3' are also used. Eukaryotic cells always use 5'-AUG-3' as the start codon.
- **Eukaryotic mRNAs** usually **contain a single ORF**, while **prokaryotic mRNAs can be polycistronic, encoding multiple proteins**.
- **Encodes two or more polypeptides within a single mRNA molecule**.

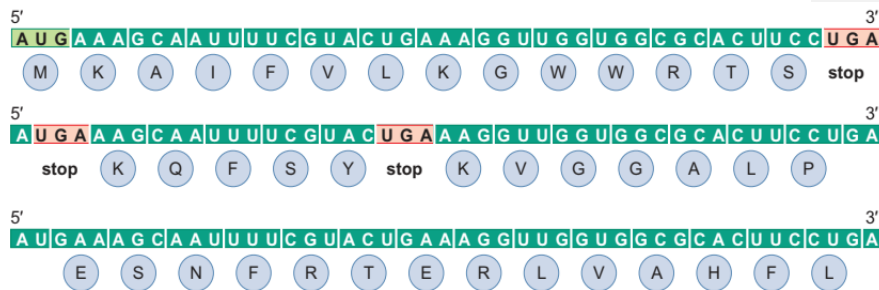


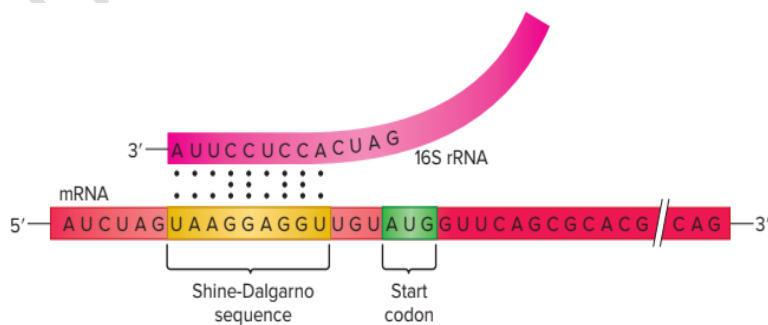
FIGURE 15-1 Three possible reading frames of the *Escherichia coli* *trp* leader sequence. Start codons are shaded in green, and stop codons are shaded in red. The amino acid sequence encoded by each reading frame is indicated in the single-letter code below each codon.

Ribosome Recruitment in Prokaryotic and Eukaryotic mRNAs

Prokaryotic mRNAs:

Ribosome-Binding Site (RBS):

- Located upstream (5' side) of the start codon
- Typically, 3-9 base pairs upstream of the start codon
- Complementary to a sequence in the 16S rRNA, aiding ribosome alignment with the ORF
- Known as the *Shine-Dalgarno sequence*
- Facilitates ribosome binding



- **Start Codon:**

- Indicates the *first amino acid* in a *polypeptide* sequence.
- Typically, *formylmethionine in bacteria or methionine in eukaryotes*.

- **Stop Codon:**

- Signals the *termination of polypeptide* synthesis.

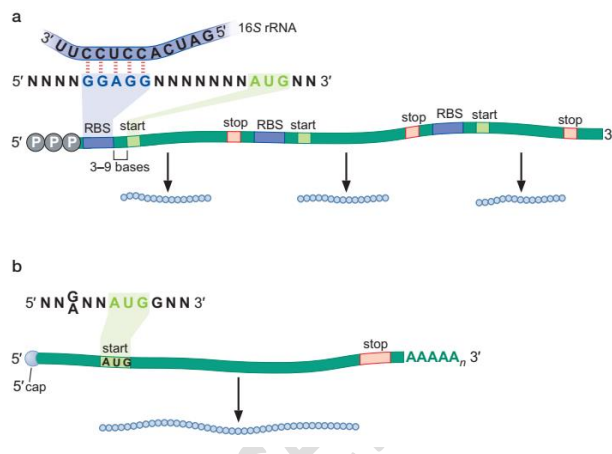


FIGURE 15-2 Structure of messenger RNA. (a) A polycistronic prokaryotic message with three ORFs. Each ribosome-binding site is indicated by a purple box labeled RBS. (b) A monocistronic eukaryotic message. The 5' cap is indicated by a "ball" at the end of the mRNA.

- **5' Cap:**

- A *methylated guanine nucleotide at the 5' end*, linked via a unique 5'-5' bond.
- *Essential for ribosome recruitment and scanning for the start codon*.

- **Poly-A Tail:**

- Added *enzymatically to the 3' end of the mRNA*
- *Increases translation* by enhancing the recruitment of translation initiation factors and *protects mRNA from degradation*

Poly A Polymerase :Commented [hn2]

- **Kozak Sequence:**

- *Aids in translation initiation*, typically *consisting of a purine three bases upstream and a guanine downstream of the start codon*

(5'-G/ANNAUGG-3')

- *Enhances translation efficiency* by interacting with the initiator tRNA

						Start Codon			
G	C	C	(A/G)	C	C	A	U	G	G
-6	-5	-4	-3	-2	-1	+1	+2	+3	+4

linked translation

- *Some ORFs lack strong RBS but are still translated* due to their position after another ORF in polycistronic mRNAs
- *Start codons of downstream ORFs often overlap with the stop codon of upstream ORFs (e.g., sequence 5'-AUGA-3'), allowing ribosome continuity*
- *Translation of the downstream ORF depends on the upstream ORF, demonstrating linked translation*

tRNAs as Adaptors in Protein Synthesis

tRNAs are *essential adaptors* that *bridge the genetic code and protein synthesis*, with their unique structures facilitating efficient translation

Function of tRNA:

- tRNA serves two primary functions during translation:
 - Recognizes a three-base codon sequence in mRNA.
 - Carries the corresponding amino acid specific to that codon.

Structure of tRNAs:

- tRNAs are typically *75-95 ribonucleotides long*
- *All tRNAs share common features*
 - *They end with the sequence 5'-CCA-3', which is where the amino acid attaches*
 - *They contain modified bases* (e.g., pseudouridine, dihydrouridine) created through enzymatic modifications, *enhancing tRNA function*

Secondary Structure:

Cloverleaf Model:

- *tRNAs exhibit a characteristic cloverleaf structure* formed by paired and unpaired regions:
 - **Acceptor Stem:** *Site for amino acid attachment, The 5'-CCA-3' sequence at the extreme 3' end of the molecule in a single-strand region*
 - **Pseudouridine Loop:** *Contains the unusual base*
 - **D Loop:** *Characterized by dihydrouridines*
 - **Anticodon Loop:** *Contains the anticodon, which pairs with the mRNA codon*
- **Codons:** Three-nucleotide sequences that specify particular amino acids.
 - **Variable Loop:** *Size varies from 3 to 21 bases, located between the anticodon and pseudouridine loops*

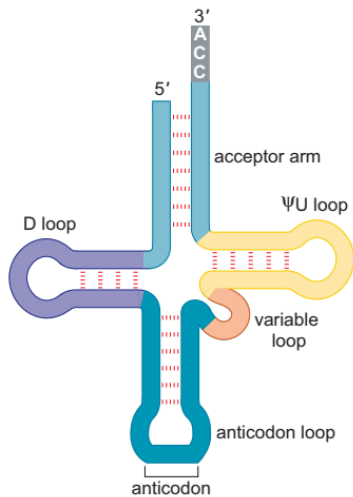


FIGURE 15-4 Cloverleaf representation of the secondary structure of tRNA. In this representation of a tRNA, the base pairings between different parts of the tRNA are indicated by the dotted red lines.

Tertiary Structure: L-Shaped Configuration:

- *X-ray crystallography reveals that tRNAs adopt an L-shaped tertiary structure*

Wobble Rules in Codon-Anticodon Pairing

- The genetic code is described as degenerate, meaning multiple codons can specify the same amino acid, particularly at the third position of the codon.

Degeneracy Example:

- For instance, valine is encoded by the codons GUU, GUC, GUA, and GUG. The first two bases are consistent (G and U), while the third can vary (U, C, A, or G).

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Genetic Code Characteristics:

- The genetic code comprises **64 codons** that specify **20 amino acids**, allowing for redundancy or **degeneracy**. This means multiple codons can specify the same amino acid (e.g., GGU, GGC, GGA, GGG all code for glycine).
- The genetic code consists of 64 codons, with **61 serving as sense codons** for the 20 amino acids.

		Second base					
		U	C	A	G		
First base	U	UUU Phenylalanine (Phe) UUC UUA Leucine (Leu) UUG	UCU Serine (Ser) UCC UCA UCG	UAU Tyrosine (Tyr) UAC UAA Stop codon UAG Stop codon	UGU Cysteine (Cys) UGC UGA Stop codon UGG Tryptophan (Trp)	U	Third base
	C	CUU Leucine (Leu) CUC CUA CUG	CCU Proline (Pro) CCC CCA CCG	CAU Histidine (His) CAC CAA Glutamine (Gln) CAG	CGU Arginine (Arg) CGC CGA CGG	C	
	A	AUU Isoleucine (Ile) AUC AUA AUG Methionine (Met); start codon	ACU Threonine (Thr) ACC ACA ACG	AAU Asparagine (Asn) AAC AAA Lysine (Lys) AAG	AGU Serine (Ser) AGC AGA Arginine (Arg) AGG	A	
	G	GUU Valine (Val) GUC GUA GUG	GCU Alanine (Ala) GCC GCA GCG	GAU Aspartic acid (Asp) GAC GAA Glutamic acid (Glu) GAG	GGU Glycine (Gly) GGC GGA GGG	G	

Wobble Hypothesis:

- Proposed by Francis Crick in 1966, the wobble rules explain the flexibility in codon-anticodon pairing.
- The first two positions adhere strictly to the AU/GC base pairing rules, while the third position allows for mismatches.

Isoacceptor tRNAs:

- When different tRNAs can recognize the same codon due to variations at the wobble position, they are termed isoacceptor tRNAs.
- For example, tRNAs with anticodons 3'-CCA-5' and 3'-CCG-5' both recognize the codon 5'-GGU-3', which encodes glycine.

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Aminoacyl-tRNA Synthetases

Overview of tRNA Charging

- **Charged vs. Uncharged tRNA:**
 - *Charged tRNAs have an amino acid attached; uncharged tRNAs do not*
- **Acyl Linkage:**
 - *Amino acids attach to the 3' terminal adenosine of tRNA via a high-energy acyl linkage, crucial for protein synthesis. Hydrolysis of this bond releases energy, facilitating peptide bond formation*

Mechanism of Charging by Aminoacyl-tRNA Synthetases

1. Two-Step Process:

- **Step 1: Adenylation:**
 - *The amino acid reacts with ATP, forming an adenylylated amino acid and releasing pyrophosphate. This step is driven by the hydrolysis of pyrophosphate.*
- **Step 2: tRNA Charging:**
 - *The adenylylated amino acid reacts with tRNA, transferring the amino acid to the tRNA and releasing AMP.*

2. Classes of Synthetases:

- **Class I:** *Attach amino acids to the 2'-OH of tRNA* (monomeric).
- **Class II:** *Attach to the 3'-OH of tRNA* (dimeric or tetrameric).

Synthetases face the daunting task of distinguishing between similar amino acids. Despite the small size and similarities of amino acids, *mischarging occurs at a remarkably low frequency—less than 1 in 1000 tRNAs are incorrectly charged.*

TABLE 15-1 Classes of Aminoacyl-tRNA Synthetases

Class II	Quarternary Structure	Class I	Quarternary Structure
Gly	($\alpha_2\beta_2$)	Glu	(α)
Ala	(α_4)	Gln	(α)
Pro	(α_2)	Arg	(α)
Ser	(α_2)	Cys	(α_2)
Thr	(α_2)	Met	(α_2)
His	(α_2)	Val	(α)
Asp	(α_2)	Ile	(α)
Asn	(α_2)	Leu	(α)
Lys	(α_2)	Tyr	(α)
Phe	($\alpha_2\beta_2$)	Trp	(α)

The Ribosome and Protein Synthesis

- The ribosome is a *complex macromolecular machine responsible for protein synthesis*.
- *It translates nucleic acid sequences (mRNA) into amino acid sequences (proteins).*
- *Located on Rough ER & Cytosol*

Complexity Compared to DNA/RNA Synthesis

- Ribosomes are *larger and more complex than* the machinery for DNA or RNA synthesis.
- *DNA replication* occurs at *200-1000 nucleotides per second*.
- *Translation* is slower, at *2-20 amino acids per second in prokaryotes*.
- *In eukaryotes, transcription occurs in the nucleus, while translation occurs in the cytoplasm, resulting in slower translation rate of 2-4 amino acids per second*.
- In *prokaryotic* cells, *translation and transcription* occur *simultaneously* in the *cytoplasm*

This coupling *allows ribosomes to begin translating mRNA even before transcription is complete*.

The *rate of translation*, around 20 codons (or 60 nucleotides) per second, *matches the speed of RNA polymerase during transcription*, facilitating rapid protein synthesis in response to cellular needs.

Structure of the Ribosome

- Ribosomes consist of *ribosomal RNA (rRNA)* and *ribosomal proteins*.
- *Comprised of two subunits*: large and small.

Svedberg Units

- Prokaryotic ribosome: 70S (50S + 30S)
- Eukaryotic ribosome: 80S (60S + 40S)

Composition

In prokaryotes

- *The large subunit (50S) contains the peptidyl transferase center for peptide bond formation*.
 - *includes 5S and 23S rRNA*
- *The small subunit (30S) contains the decoding center for reading mRNA codons*.
 - *contains 16S rRNA*

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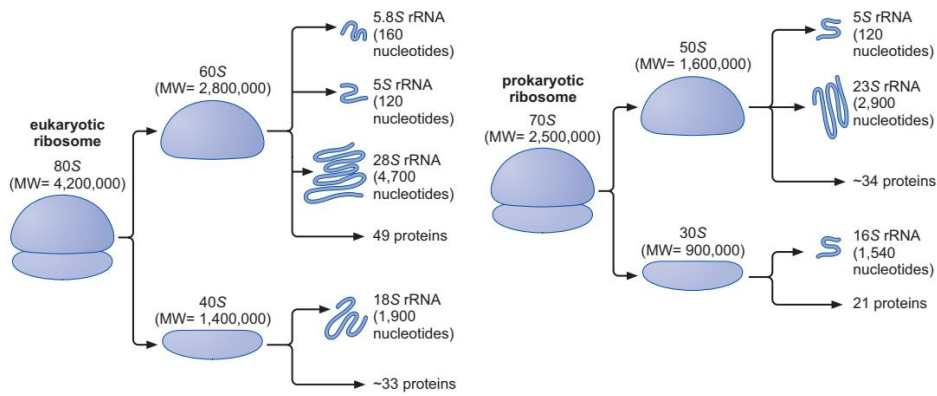
سرعت ته نشینی در واحدهای Svedberg (S) نامگذاری می‌شوند.

این واحدها به طور کلی به دو دسته تقسیم می‌شوند:

۱. زیر واحد ریبوزومی بزرگ

۲. زیر واحد ریبوزومی کوچک

این نامگذاری به دلیل تفاوت در ساختار و ترکیب پروتئینی و rRNA ریبوزومی است



Composition of Bacterial and Eukaryotic Ribosomes

	Small subunit	Large subunit	Assembled ribosome
Bacterial			
Sedimentation coefficient	30S	50S	70S
Number of proteins	21	34	55
rRNA molecule(s)	16S rRNA	5S rRNA, 23S rRNA	16S rRNA, 5S rRNA, 23S rRNA
Eukaryotic			
Sedimentation coefficient	40S	60S	80S
Number of proteins	33	49	82
rRNA molecule(s)	18S rRNA	5S rRNA, 5.8S rRNA, 28S rRNA	18S rRNA, 5S rRNA, 5.8S rRNA, 28S rRNA

tRNA Binding Sites:

The ribosome indeed has three key tRNA-binding sites that play crucial roles in protein synthesis:

1. **A-site (Aminoacyl-tRNA site):** This is *where the incoming aminoacyl-tRNA, which carries the next amino acid to be added to the growing polypeptide chain, binds.*

2. **P-site (Peptidyl-tRNA site):** This site holds the tRNA that carries the growing polypeptide chain. *It is where peptide bond formation occurs between the amino acid in the A-site and the polypeptide in the P-site.*

- ✓ The methionine tRNA (tRNA-fMet) first enters the **P-site (peptidyl-tRNA site)**
- ✓ Subsequently, other tRNAs enter the **A-site (aminoacyl-tRNA site)**

3. **E-site (Exiting tRNA site):** This site is *where the tRNA, which* has already *donated its amino acid* to the polypeptide chain, *exits the ribosome.*

- **A Site:** Binds aminoacyl-tRNA (charged tRNA).
- **P Site:** Contains peptidyl-tRNA (tRNA with attached peptide).
- **E Site:** Where uncharged tRNA exits.

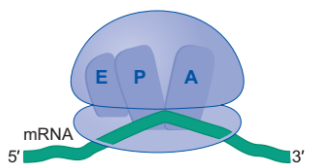


FIGURE 15-18 The ribosome has three tRNA-binding sites. The schematic illustration of the ribosome shows the three binding sites (E, P, and A) that each spans the two subunits.

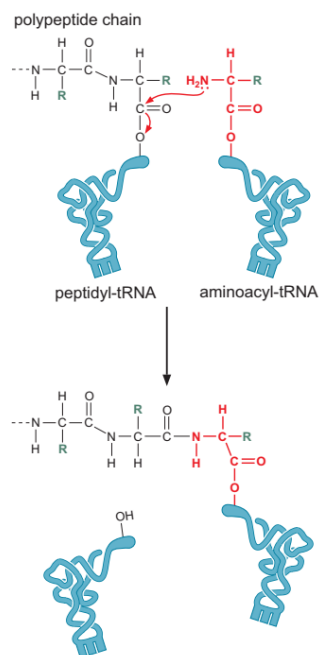
Additional notes

Peptide Bond Formation & Directionality in Protein Synthesis

- The *ribosome catalyzes peptide bond formation*
- *New amino acids* are *added to* the **carboxyl terminus** of the growing polypeptide chain, indicating *synthesis occurs in the amino-to-carboxy-terminal direction.*

N → C

- The first amino acid in a polypeptide is located at the **N-terminus**, characterized by a free amino group (NH_3^+), and is specified by a codon near the **5' end** of the mRNA.
 - The last amino acid is found at the **C-terminus**, marked by a free carboxyl group (COO^-), and is encoded by a codon closer to the **3' end** of the mRNA.
- This process involves:
- **Aminoacyl-tRNA** (*carries the incoming amino acid*).
 - **Peptidyl-tRNA** (*holds the growing polypeptide*).
- The reaction *does not require direct ATP hydrolysis*; instead, it *utilizes energy from the high-energy acyl bond formed during tRNA charging*.



Channels for mRNA and Polypeptide:

- The *ribosome contains channels that allow mRNA to enter* and the *growing polypeptide to exit*.
- *mRNA enters through narrow channels, ensuring it remains single-stranded* for decoding.
- *The peptide exit channel restricts the conformation of the polypeptide until it exits the ribosome, preventing premature folding*.

Polyribosomes

- *Multiple ribosomes can translate a single mRNA simultaneously, forming a polyribosome* (or polysome).
- This efficient use of ribosomes explains the relatively low abundance of mRNA in cells (*1%-5% of total RNA*).

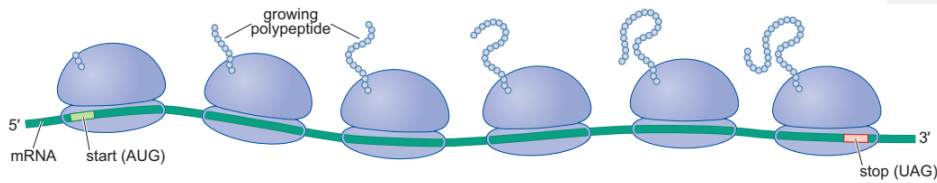


FIGURE 15-15 A polyribosome.

Ribosome Cycle

Involves a cycle where the ribosomal small and large subunits associate with mRNA to synthesize proteins and then dissociate.

- This process is known as the **ribosome cycle**.

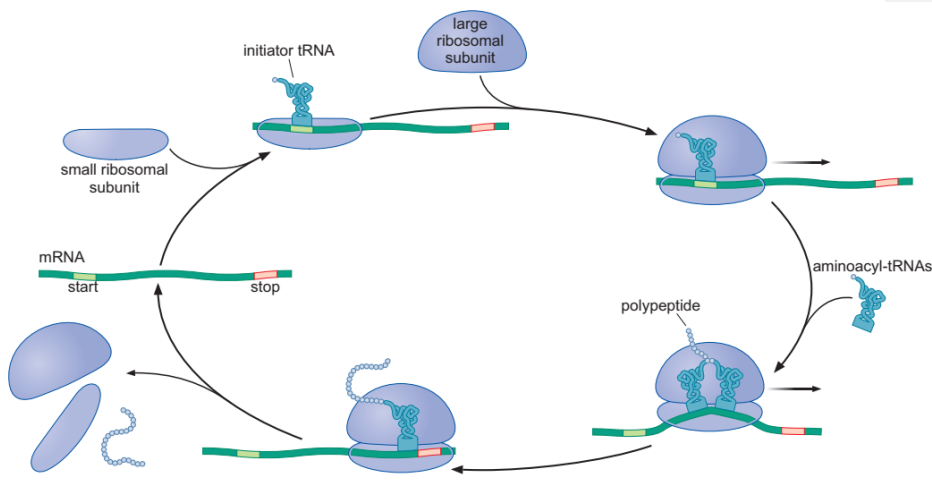


FIGURE 15-14 Overview of the events of translation: the ribosome cycle.

Key Steps in Translation: An Overview

1. Initiation:

- The *small ribosomal subunit binds to mRNA and an initiator tRNA*.
- The *large subunit joins to form a complete ribosome* with mRNA positioned between the subunits.

2. Elongation:

- *Charged tRNAs enter the ribosome, facilitating the addition of amino acids to the growing polypeptide chain*.

Once the initiation complex is formed, the ribosome moves along the mRNA in the 5' to 3' direction, progressing over the codons.

During this stage, tRNA molecules sequentially bind to the A site of the ribosome, each bringing the corresponding amino acid.

Amino acids are linked together in the order specified by the mRNA codon sequence.

3. Termination:

- Upon encountering a stop codon, the completed polypeptide is released.
- The ribosome dissociates into its subunits, ready to initiate a new cycle with a different mRNA.

Key Events for Prokaryotic Translation Initiation:

1. The ribosome must bind to the mRNA

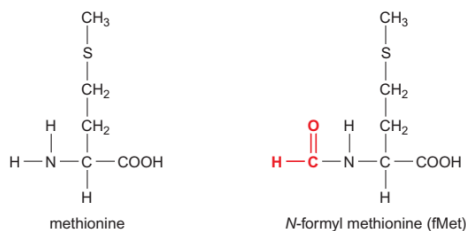
- The small ribosomal subunit first associates with mRNA via base-pairing between the ribosome binding site (RBS) and 16S rRNA
- The mRNA binds to the 30S subunit via the **Shine-Dalgarno sequence**, which is complementary to a region in 16S rRNA, facilitating hydrogen bonding.

From mRNA :Commented [hn6]

From Ribosome :Commented [hn7]

2. A charged tRNA is positioned in the P-site of the ribosome

- Initiator tRNA (~~fMet~~-tRNAⁱ fMet) binds directly to the P-site without occupying the A-site first
- This tRNA is charged with N-formyl methionine



3. The ribosome must align correctly over the start codon to establish the reading frame

Formation of Initiation complex

Prokaryotic translation begins with the assembly of an initiation complex

- Involving mRNA, initiator tRNA and small ribosomal subunit

- Facilitated by three key initiation factors: IF1, IF2, and IF3

Role of Initiation Factors:

- **IF1:** Prevents the binding of charged tRNAs to the *A-site* of the small subunit, ensuring that only the initiator tRNA can occupy the P-site.
- **IF2:** A GTPase that interacts with the small subunit, IF1, and the initiator tRNA (fMet-tRNAⁱ fMet).
- Facilitates the binding of fMet-tRNAⁱ fMet to the small subunit while blocking other charged tRNAs.
- **IF3:** Binds to the small subunit (prevent its reassociation with the large subunit) to keep it free for new translation cycles.
- IF1 and IF3 bind to the 30S ribosomal subunit, preventing the 50S subunit from associating prematurely.

Binding Dynamics:

- **IF1** binds to the *A-site*
- **IF2** interacts with both IF1 and the *P-site*
- **IF3** occupies the *E-site*

Bacterial Factors	Eukaryotic Factors*	Function
Initiation Factors		
	eIF4	Recognizes the 7-methylguanosine cap at the 5'-end of mRNA and facilitates the binding of the mRNA to the small ribosomal subunit
IF1, IF3	eIF1, eIF3, eIF6	Prevent the association between the small and large ribosomal subunits and favor their dissociation
IF2	eIF2	Promotes the binding of the initiator tRNA to the small ribosomal subunit
	eIF5	Helps to dissociate the initiation factors, which allows the two ribosomal subunits to assemble

Final Step:

Formation of the 70S Initiation Complex:

- Upon successful base pairing between the start codon (mRNA) and fMet-tRNA^{fMet}, the small subunit undergoes a conformational change, leading to the release of IF1 & IF3
- IF2 hydrolyzes GTP and released, allowing the 50S subunit to associate with the 30S subunit, forming the complete 70S initiation complex
- This process results in the ribosome being ready to accept a charged tRNA into the A-site and begin polypeptide synthesis

GTPase activity of IF2 :Commented [hn8]

Translation Initiation in Eukaryotes:

- Eukaryotic translation initiation *shares similarities with prokaryotic initiation* but employs *distinct mechanisms for mRNA recognition and start codon identification*.
- Eukaryotic initiation *shares similarities but requires additional factors* (designated eIF) *and uses methionine instead of N-formylmethionine*.
- *Eukaryotic mRNAs lack a Shine-Dalgarno sequence; instead, they are recognized by a 7-methylguanosine cap via the eIF4 complex.*
- The *ribosome scans the mRNA downstream from the cap to locate the AUG start codon, guided by the Kozak sequence* for optimal recognition.

Key Steps in Eukaryotic Translation Initiation:

1. Scanning for the Start Codon:

- *The small subunit scans the mRNA in the 5' to 3' direction to locate the first AUG start codon*
- *The mRNA's 5' cap is recognized by the cap-binding protein eIF4 which recruits additional initiation factors & facilitate mRNA binding*

2. Formation of the Preinitiation Complex:

- *The small ribosomal subunit binds to the initiator tRNA (Met-tRNAⁱ Met)*
- *This complex is referred to as the 43S preinitiation complex*

Eukaryotic Factors*	Function
eIF4	Recognizes the 7-methylguanosine cap at the 5'-end of mRNA and facilitates the binding of the mRNA to the small ribosomal subunit
eIF1, eIF3, eIF6	Prevent the association between the small and large ribosomal subunits and favor their dissociation
eIF2	Promotes the binding of the initiator tRNA to the small ribosomal subunit
eIF5	Helps to dissociate the initiation factors, which allows the two ribosomal subunits to assemble

3. Large Subunit Recruitment:

- Upon correct pairing, *eIF2 hydrolyzes GTP, leading to the release of initiation factors and the recruitment of the large ribosomal subunit (60S).*
- *This forms the 80S initiation complex, ready for peptide bond formation.*

Translation Elongation

Following the assembly of the ribosome with the charged initiator tRNA

- Elongation begins with a charged tRNA bringing a new amino acid to the the A-site of ribosome, polypeptide synthesis begins.
- Energy for this binding is provided by the hydrolysis of GTP mediated by the elongation factor **EF-Tu**.
 - The correct *aminoacyl-tRNA is loaded into the A-site* of the ribosome, guided by the codon present in the A-site.

2. Peptide Bond Formation:

The next step, called **peptidyl transfer**, involves transferring the polypeptide from the tRNA in the P site to the amino acid in the A site, forming a peptide bond.

This reaction is catalyzed by **peptidyl transferase**, a component of the 50S subunit, primarily composed of **23S rRNA**, highlighting the ribosome's role as a ribozyme.

- A **peptide bond is formed between** the **amino acid** of the aminoacyl-tRNA **in the A-site** and the **growing polypeptide chain attached to the peptidyl-tRNA in the P-site**.
- This reaction, **known as the peptidyltransferase reaction**

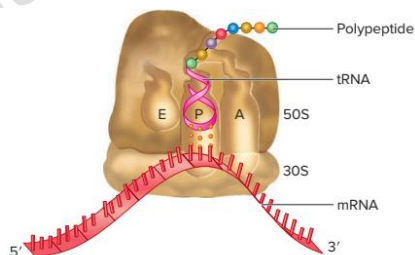
3. Translocation:

After the peptidyl transfer, the ribosome undergoes **translocation**, moving to the next codon on the mRNA.

This shifts the tRNAs: the tRNA in the P site moves to the E site, and the tRNA in the A site moves to the P site, allowing the uncharged tRNA to exit from the E site.

The A site is now vacant and ready for the next charged tRNA, continuing the cycle of elongation.

- This translocation is crucial for maintaining the correct reading frame of the mRNA, **allowing the ribosome to be ready for the next cycle of codon recognition and peptide bond formation**.



Termination of Translation

- The termination stage of translation occurs when a stop codon is reached in the mRNA, signaling the end of polypeptide synthesis.

1. Stop Codons:

- The three stop codons are **UAA**, **UAG**, and **UGA**.
- Unlike regular codons, stop codons are not recognized by tRNAs; instead, they are recognized by proteins called **release factors**.

2. Release Factors:

- In **bacteria**:
 - **RF1** recognizes UAA and UAG.
 - **RF2** recognizes UAA and UGA.
 - **RF3** is also required for termination.
- In **eukaryotes**:
 - A single release factor, **eRF1**, recognizes all three stop codons.
 - **eRF3** is also necessary for the termination process.

Release Factors

RF1, RF2	eRF1	Recognize a stop codon and trigger the cleavage of the polypeptide from the tRNA
RF3	eRF3	GTPases that are also involved in termination

Termination Process:

- Upon reaching a stop codon at the A site, RF1 or RF2 binds to the codon, while RF3 binds to another site on the ribosome.
- This binding triggers the hydrolysis of the bond between the completed polypeptide and the tRNA in the P site, resulting in the release of the polypeptide.
- The final step involves the disassembly of the ribosomal subunits, mRNA, and release factors, completing the translation process.

Bacterial Translation and Its Implications

1. Coupling of Transcription and Translation:

- In bacteria, translation begins before transcription is complete.
- As soon as an mRNA strand is sufficiently long, a ribosome attaches to the 5' end and starts translating, while RNA polymerase is still synthesizing the mRNA.
- This phenomenon is known as **coupling** between transcription and translation, which is not typical in eukaryotes due to their compartmentalized cellular structure (transcription occurs in the nucleus, translation in the cytosol).

Antibiotics and Bacterial Translation:

- Many diseases in humans and animals are caused by pathogenic bacteria.
- **Antibiotics** are substances produced by microorganisms that inhibit the growth of other microbes, particularly bacteria.
- Some antibiotics target bacterial translation specifically, exploiting the differences between bacterial and eukaryotic translation mechanisms.
- This selectivity allows for effective treatment of bacterial infections in humans, pets, and livestock without affecting eukaryotic cells.

Mechanisms of Inhibition of Bacterial Translation via Selected Antibiotics	
Antibiotic	Description
Chloramphenicol	Blocks elongation by acting as competitive inhibitor of peptidyl transferase.
Erythromycin	Binds to the 23S RNA and blocks elongation by interfering with the translocation step.
Puromycin	Binds to the A site and causes premature release of the polypeptide. This early termination of translation results in polypeptides that are shorter than normal.
Tetracycline	Blocks elongation by inhibiting the binding of aminoacyl-tRNAs to the ribosome.
Streptomycin	Interferes with normal pairing between aminoacyl-tRNAs and codons. This causes misreading, thereby producing abnormal proteins.

Overview of Energy Consumption in Translation:

- Each round of peptide-bond formation during translation elongation consumes a total of **three nucleoside triphosphates (NTPs): two GTP and one ATP**.
- This energy expenditure is crucial for the accurate and efficient synthesis of proteins.

1. ATP Consumption:

- One molecule of ATP is utilized by **aminoacyl-tRNA synthetase** to create a high-energy acyl bond that links the amino acid to its corresponding tRNA.
- The hydrolysis of this high-energy bond drives the **peptidyl transferase reaction**, which forms the peptide bond between amino acids.

2. GTP Consumption:

- **Two molecules of GTP** are consumed during the elongation process:
 - **First GTP:** Used by **EF-Tu** for the delivery of the charged tRNA to the A-site of the ribosome. This step also ensures correct codon-anticodon recognition.
 - **Second GTP:** Used by **EF-G** during the translocation phase, facilitating the movement of the ribosome along the mRNA.

Comparison of Prokaryotic and Eukaryotic Systems:

- While the elongation factors in prokaryotes (EF-Tu and EF-G) and eukaryotes (eEF1 and eEF2) have different names, their functions are remarkably similar.
- Both systems utilize GTP and ATP in comparable ways to ensure efficient translation elongation.

A Comparison of Bacterial and Eukaryotic Translation		
	Bacterial	Eukaryotic
Ribosome composition:	70S ribosomes: 30S subunit— 21 proteins + 1 rRNA 50S subunit— 34 proteins + 2 rRNAs	80S ribosomes: 40S subunit— 33 proteins + 1 rRNA 60S subunit— 49 proteins + 3 rRNAs
Initiator tRNA:	tRNA ^{Met}	tRNA ^{Met}
Formation of the initiation complex:	Requires IF1, IF2, and IF3	Requires more initiation factors compared to bacterial initiation
Initial binding of mRNA to the ribosome:	Requires a Shine-Dalgarno sequence	Requires a 7-methylguanosine cap
Selection of a start codon:	AUG, GUG, or UUG located just downstream from the Shine-Dalgarno sequence	According to Kozak's rules
Elongation rate:	Typically 15–20 amino acids per second	Typically 2–6 amino acids per second
Termination:	Requires RF1, RF2, and RF3	Requires eRF1 and eRF3
Location of translation:	Cytoplasm	Cytosol
Coupled to transcription:	Yes	No

Summary

Synthesis of Proteins on RNA Templates

Proteins are synthesized on messenger RNA (mRNA) templates through a process called translation. This process decodes the nucleotide sequence information in mRNA into the linear sequence of amino acids, forming a polypeptide chain. The machinery for protein synthesis consists of four main components:

1. **mRNA:** Contains the coding sequence for the protein and recognition elements for initiation and termination of translation.
2. **tRNAs (Transfer RNAs):** Adaptor RNAs that physically link the mRNA codons to the corresponding amino acids.
3. **Aminoacyl-tRNA Synthetases:** Enzymes that attach amino acids to their respective tRNAs.

4. **Ribosome:** A multi-subunit complex of proteins and RNA that catalyzes peptide bond formation.

Structure of mRNA

- The coding sequence within mRNA is referred to as the **open reading frame (ORF)**, which consists of a series of three-nucleotide-long units known as **codons**.
- Each ORF specifies a single polypeptide chain, starting with a **start codon** (usually AUG in eukaryotes) and ending with a **stop codon**.
- In prokaryotes, the start codon is preceded by a sequence that is complementary to the 16S rRNA of the ribosome, aiding in alignment. Eukaryotic mRNAs have a **5' cap** structure that recruits the ribosome, and they terminate with a **poly-A tail** to enhance translation efficiency.
- Prokaryotic mRNAs can be **polycistronic** (containing multiple ORFs), while eukaryotic mRNAs are typically **monocistronic** (containing a single ORF).

Role of tRNAs

- tRNAs serve as the link between mRNA codons and amino acids. They have an L-shaped structure with an **anticodon** loop at one end and a **3'-CCA** sequence at the other end.
- The anticodon is complementary to the codon on the mRNA, allowing for base pairing.
- Amino acids are attached to the 3'-CCA sequence via an acyl linkage.

Charging of tRNAs

- **Aminoacyl-tRNA synthetases** charge tRNAs by attaching the appropriate amino acid in a two-step process. Each synthetase is specific to one amino acid and recognizes the correct tRNAs through interactions with both ends of the tRNA molecule.
- These enzymes ensure high fidelity in charging tRNAs, and some have proofreading mechanisms to enhance accuracy.

Structure and Function of the Ribosome

- The ribosome is composed of a **large subunit** (containing the peptidyl transferase center) and a **small subunit** (containing the decoding center).
- Each subunit consists of one or more RNA molecules and multiple proteins, with the RNA playing crucial roles in structure and function.
- The ribosome has three tRNA-binding sites:
 - **A-site:** Where the charged tRNA enters.
 - **P-site:** Contains the peptidyl-tRNA.
 - **E-site:** Where deacylated tRNAs exit.

Translation Process

- The translation of a protein involves a cycle of association and dissociation of the ribosomal subunits.

- The small subunit assembles at the beginning of an ORF, followed by the large subunit, and they dissociate into free subunits once translation is complete.
- Translation starts at the 5' end of the ORF, synthesizing the polypeptide chain from the amino-terminal to the carboxy-terminal direction.

This intricate process ensures that proteins are accurately synthesized according to the genetic information encoded in mRNA, enabling the proper functioning of cells and organisms.

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