TRANSLATION OF MRNA TO PROTEIN

MOLECULAR MECHANISMS, REGULATION, AND BIOMEDICAL IMPLICATIONS

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Molecular Biology

Semester I

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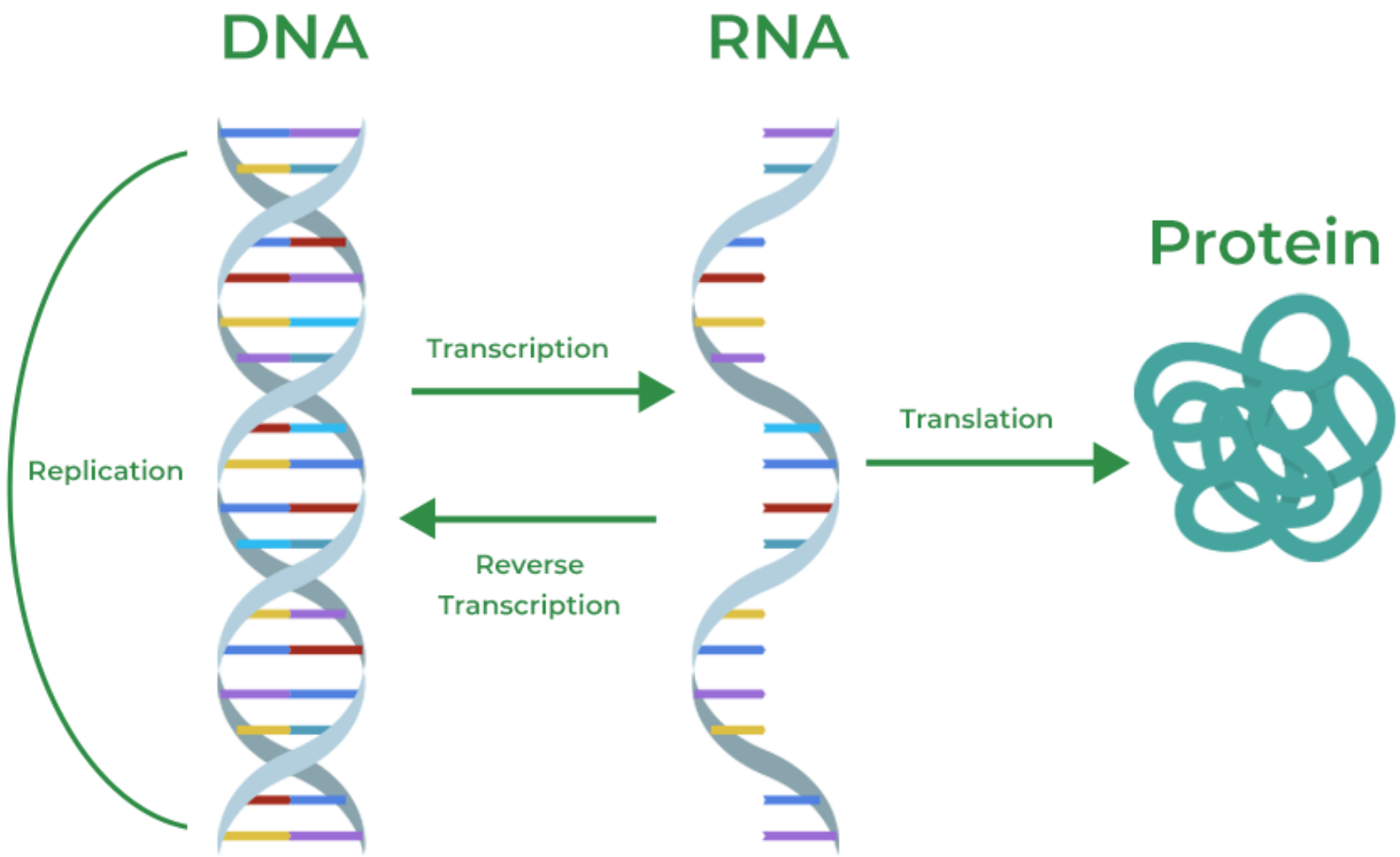
Learning Objectives

- This lecture provides an overview of how cells decode messenger RNA (mRNA) to synthesize proteins.
- Explore the molecular machinery involved, highlight key mechanistic steps, and compare translation in prokaryotes and eukaryotes.

Review: Central Dogma Overview

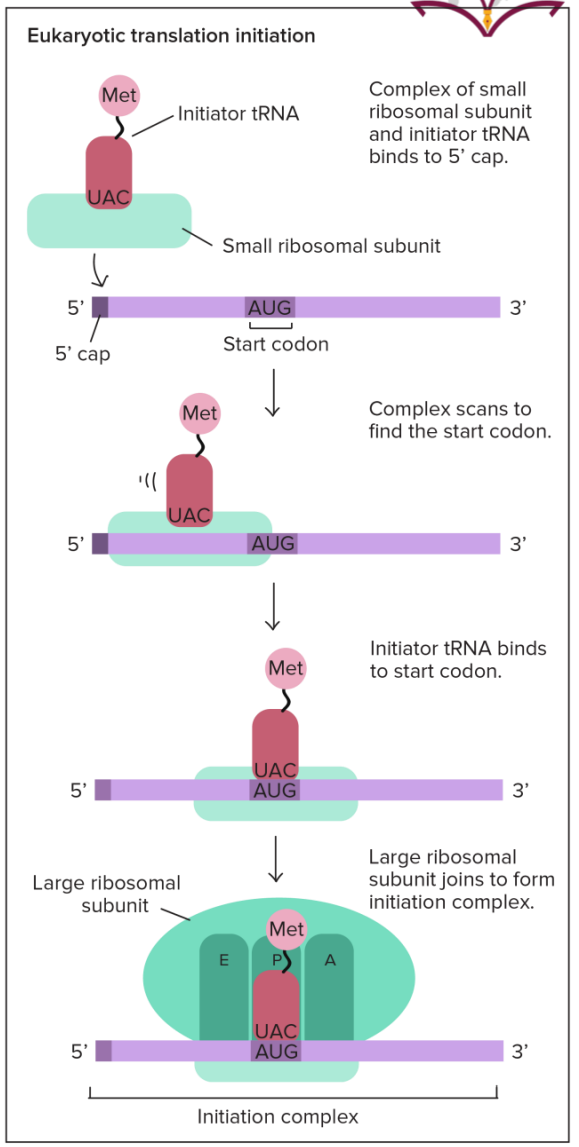


Overview of Translation

•Translation is the cellular process in which ribosomes read the nucleotide sequence of mRNA and convert it into the amino-acid sequence of a protein. It is one of the most highly conserved biological processes across all domains of life.

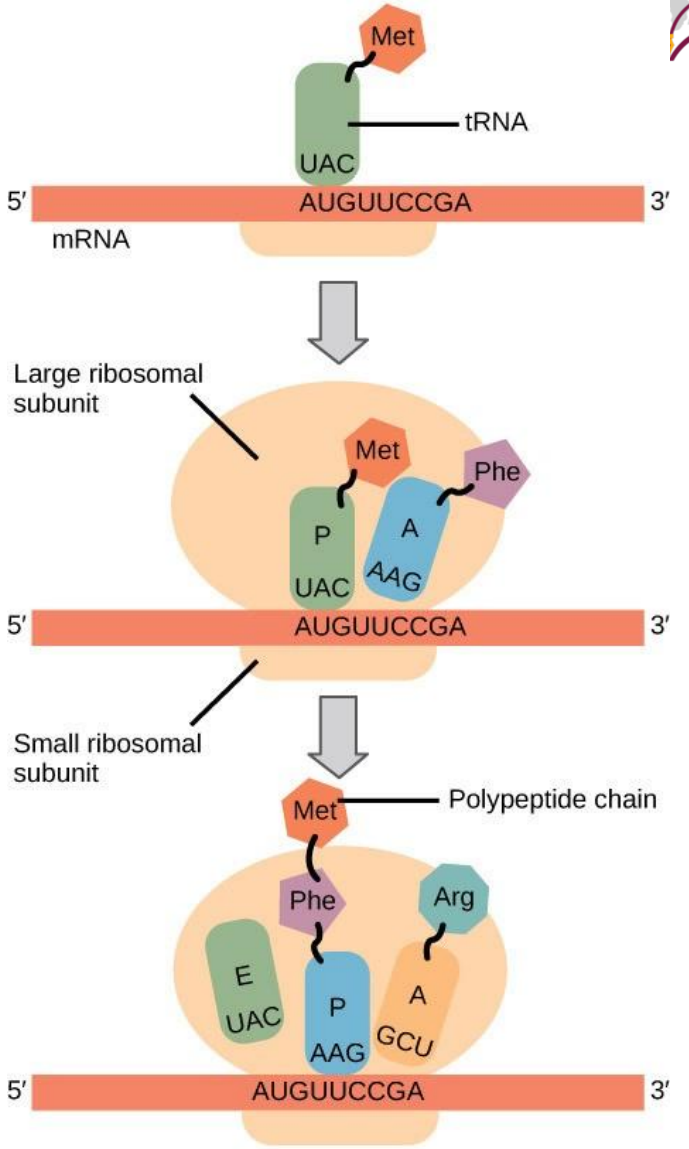
- How ribosomes, tRNAs, and mRNA interact
- Differences between eukaryotic and prokaryotic translation
- The functional significance of the genetic code

• Requires integration of **mRNA, tRNAs, rRNA, ribosomal proteins, initiation factors (eIFs), elongation factors, and energy (ATP/GTP)**.



Overview of Protein Synthesis

- Protein synthesis depends on the linear sequence of codons within mRNA. Because nucleic acids and proteins use different chemical languages, a translation system is required.
- This system relies on the **genetic code**, which establishes a precise correspondence between triplet nucleotides and amino acids.
- Discovered by Marshall Nirenberg and colleagues in the 1960s, the code links **three nucleotides (a codon)** to **one amino acid**.
- Translation begins at an AUG (Met) codon and terminates at a stop codon.



The Genetic Code

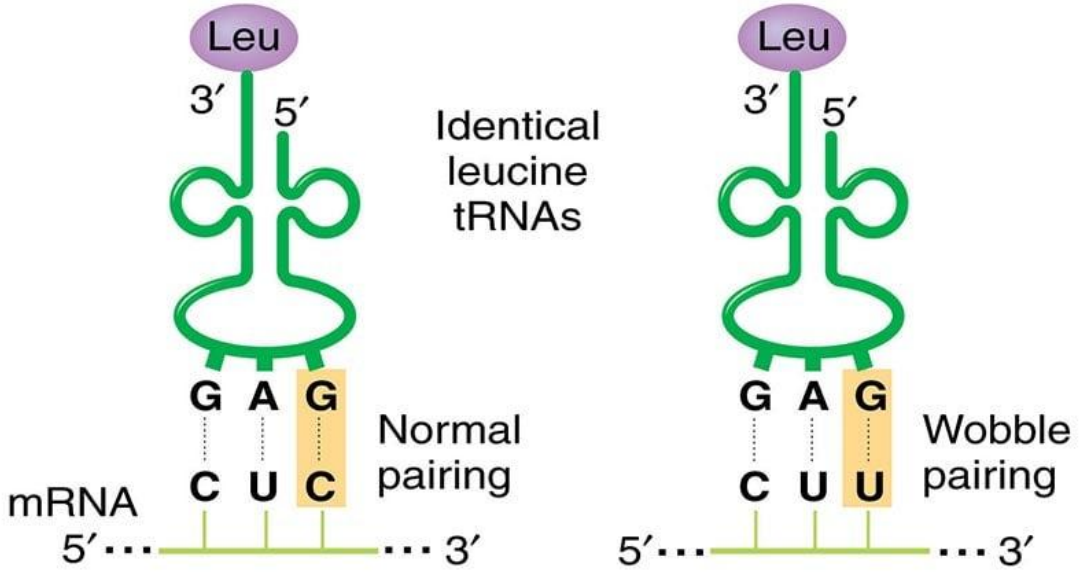
The standard genetic code assigns 61 codons to 20 amino acids and 3 codons as termination signals (UAA, UAG, UGA).
Key features:

- Translation always begins with **methionine (AUG)**
- Stop codons signal ribosome release
- The same code is used for mRNA; for DNA, T replaces U
- A reading frame between ATG → stop codon defines an **open reading frame (ORF)**
  An ORF is typically, but not always, a protein-coding region.

First letter	Second letter: U	Second letter: C	Second letter: A	Second letter: G	Third letter
U	UUU Phe UUC Phe UUA Leu UUG Leu	UCU Ser UCC Ser UCA Ser UCG Ser	UAU Tyr UAC Tyr **UAA Stop** **UAG Stop**	UGU Cys UGC Cys **UGA Stop** UGG Trp	U C A G
C	CUU Leu CUC Leu CUA Leu CUG Leu	CCU Pro CCC Pro CCA Pro CCG Pro	CAU His CAC His CAA Gln CAG Gln	CGU Arg CGC Arg CGA Arg CGG Arg	U C A G
A	AUU Ile AUC Ile AUA Ile **AUG Met**	ACU Thr ACC Thr ACA Thr ACG Thr	AAU Asn AAC Asn AAA Lys AAG Lys	AGU Ser AGC Ser AGA Arg AGG Arg	U C A G
G	GUU Val GUC Val GUA Val GUG Val	GCU Ala GCC Ala GCA Ala GCG Ala	GAU Asp GAC Asp GAA Glu GAG Glu	GGU Gly GGC Gly GGA Gly GGG Gly	U C A G

Order and Logic in the Genetic Code

- The genetic code is organized, not random.
- Amino acids with multiple codons usually share identical first two bases, differing in the third — the **wobble base**.
- This arrangement minimizes the impact of mutations and enhances translation flexibility.



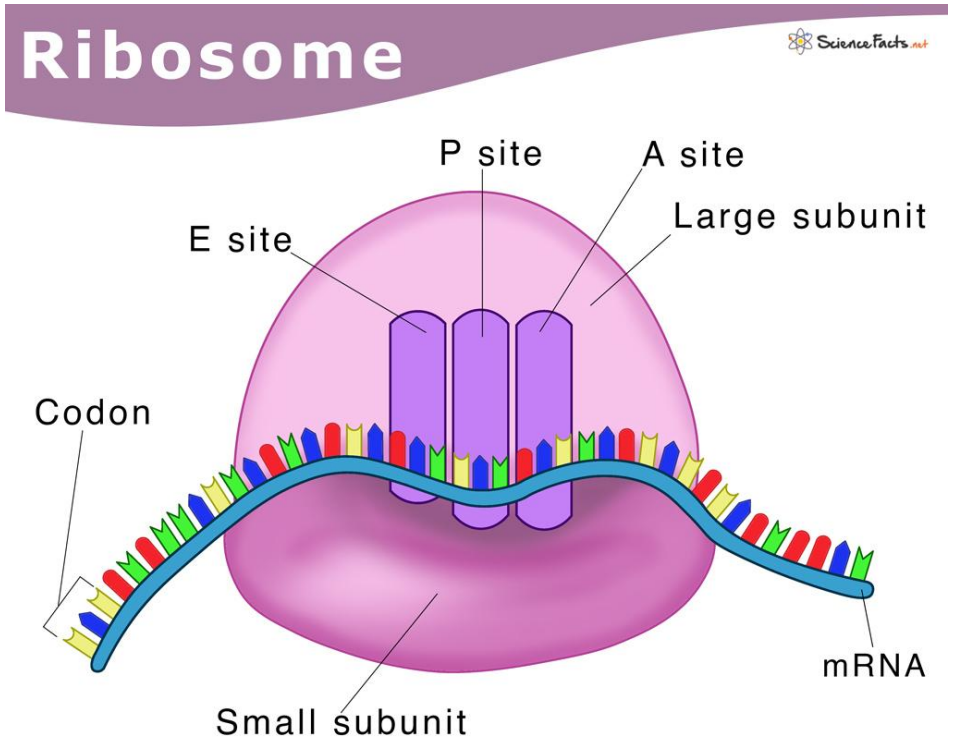
Ribosome Structure and Function

Ribosomes are ribonucleoprotein complexes that catalyze peptide-bond formation.

- **Prokaryotes:** 70S ribosomes (30S + 50S)
- **Eukaryotes:** 80S ribosomes (40S + 60S)

Despite differences in size, both systems follow the same fundamental mechanism:

- mRNA is read **5' → 3'**
- Polypeptides grow **N-terminus → C-terminus**
- rRNA performs catalytic functions (peptidyl transferase)

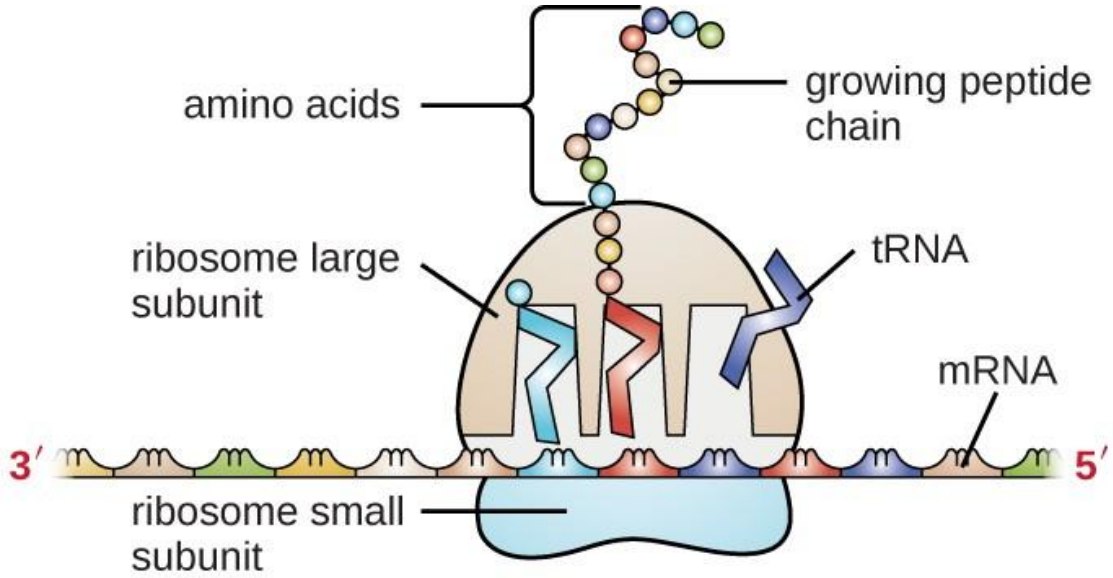


Molecular Components of Translation

Protein synthesis requires coordinated interactions among:

- **mRNA** — the genetic blueprint
- **tRNAs** — adaptor molecules that decode codons
- **rRNAs** — catalytic and structural ribosomal components
- **Translation factors** — proteins that regulate initiation, elongation, and termination

This ensures accuracy, speed, and fidelity.



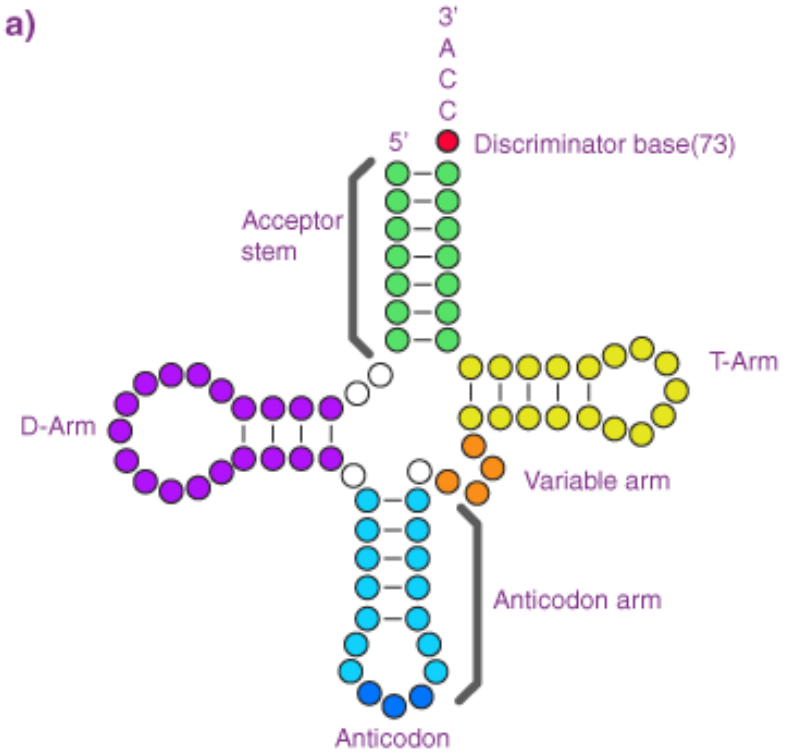
tRNA: Structure and Function

tRNAs are 70–80 nucleotide molecules with a classic cloverleaf secondary structure.

Features:

- All end with **CCA at the 3′ terminus**, where amino acids attach
- The **anticodon loop** base-pairs with the corresponding mRNA codon

  This structural duality—amino acid attachment on one end, codon recognition on the other—allows tRNAs to act as adaptors between nucleotides and amino acids.



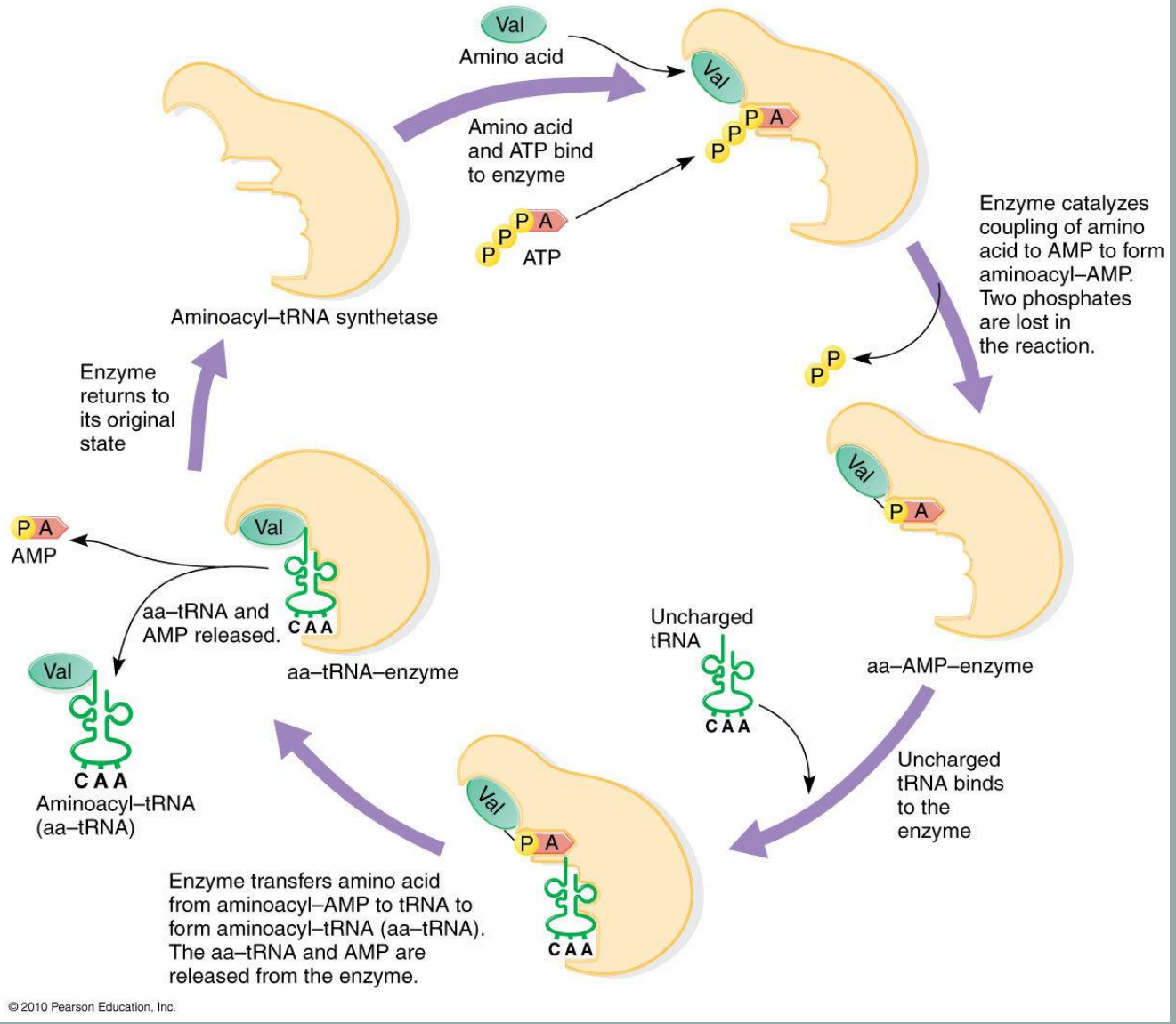
Aminoacyl-tRNA Synthetases

Accuracy in translation depends on the correct pairing of amino acids with their corresponding tRNAs, mediated by **aminoacyl-tRNA synthetases**.

Two-step charging reaction:

1. **Amino acid activation** → aminoacyl-AMP
2. **Transfer to tRNA** → aminoacyl-tRNA

   Each synthetase is highly specific, ensuring fidelity during protein synthesis.

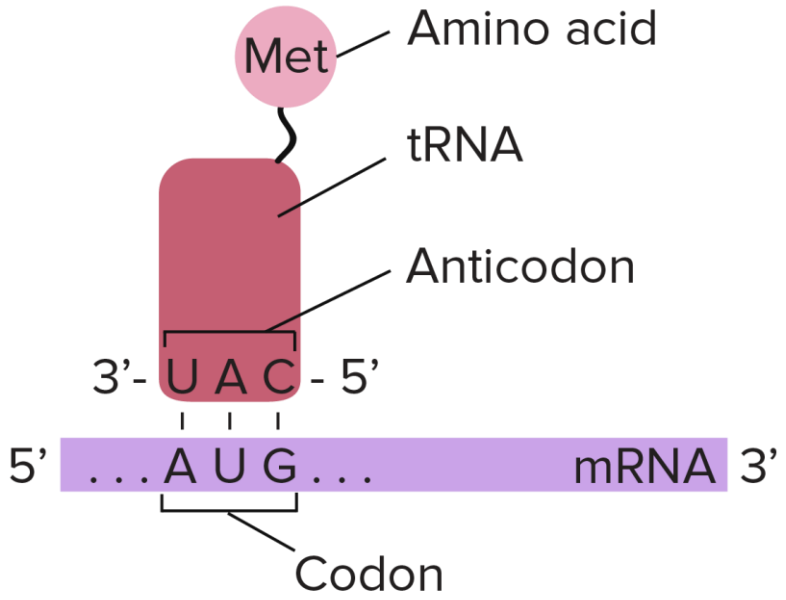


Codon–Anticodon Pairing & Wobble

Codon–anticodon interaction follows standard Watson–Crick pairing except at the wobble position (3rd codon base).

This flexibility allows:

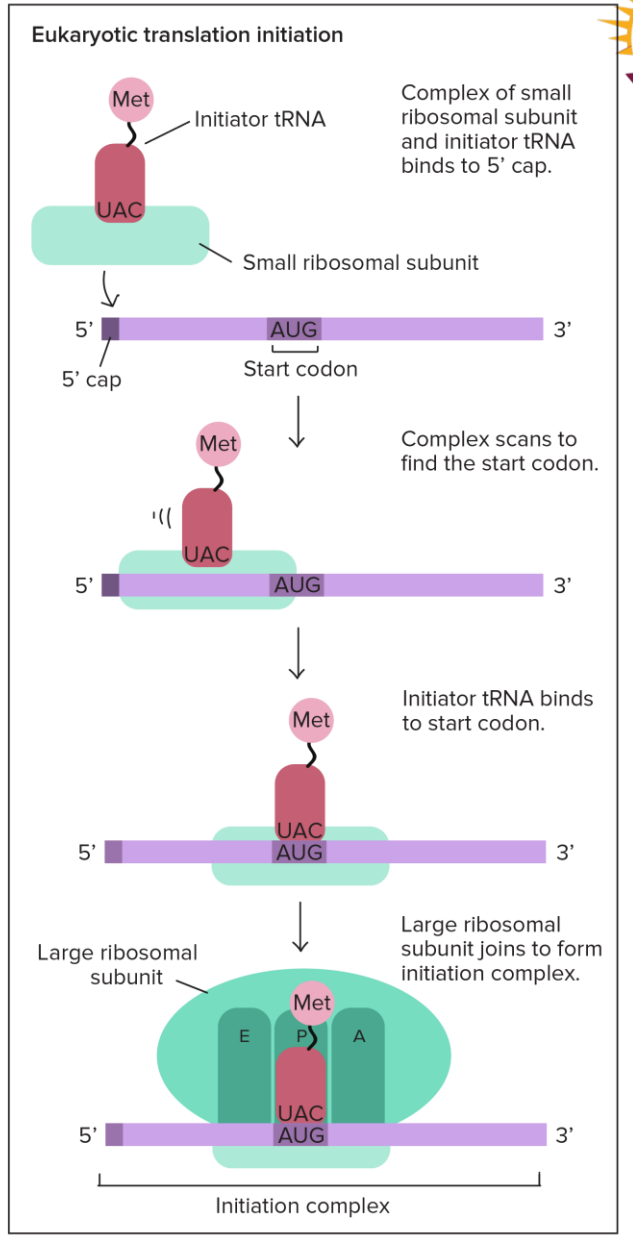
- One tRNA to recognize multiple codons
- Genetic code redundancy
  Inosine (I) in tRNA anticodons can base-pair with C, U, or A, greatly expanding pairing possibilities.



Stages of Translation

The translation cycle consists of three coordinated phases:

1) **Initiation** — assembly of ribosome on mRNA
2) **Elongation** — sequential addition of amino acids
3) **Termination** — release of the completed polypeptide



Initiation in Eukaryotes

Eukaryotic initiation is more complex and involves >10 eIFs.

Steps:

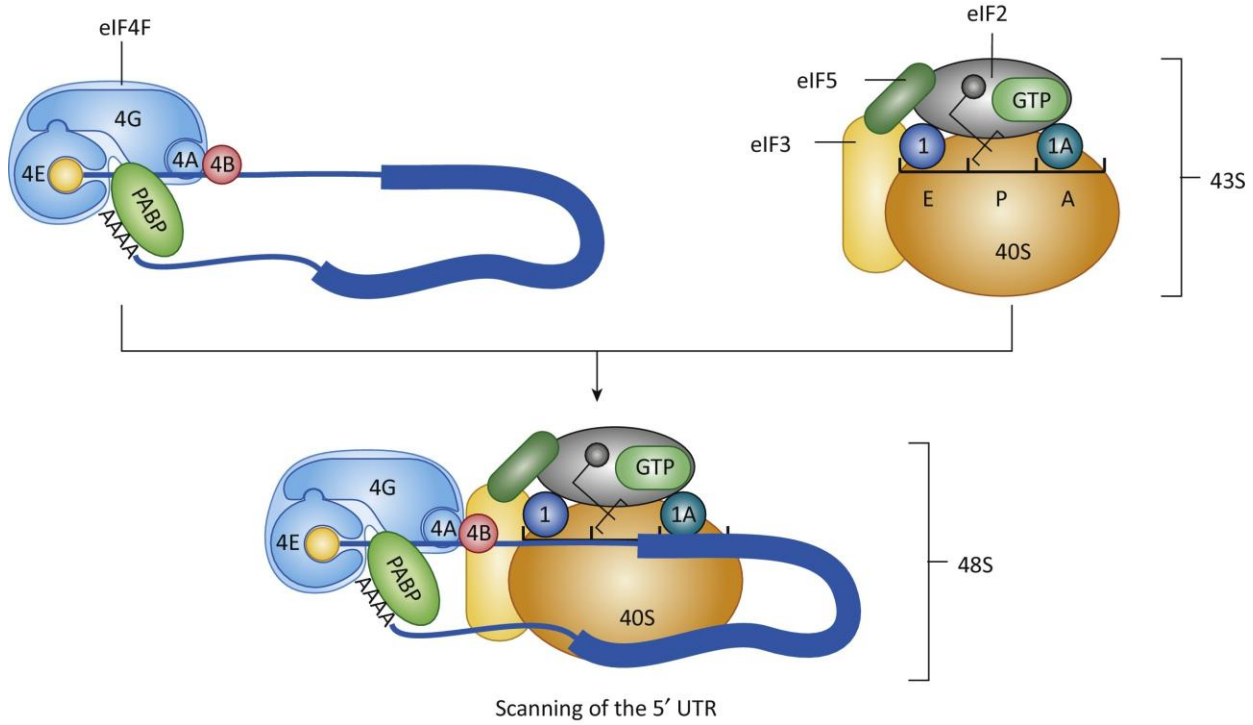
1.eIFs bind 40S subunit and initiator Met-tRNA

2.mRNA is recruited via the 5′ cap and poly-A-binding proteins

3.40S complex scans for AUG

4.60S joins to form the complete **80S initiation complex**

This stage is a major point of translational regulation.



Elongation Mechanism

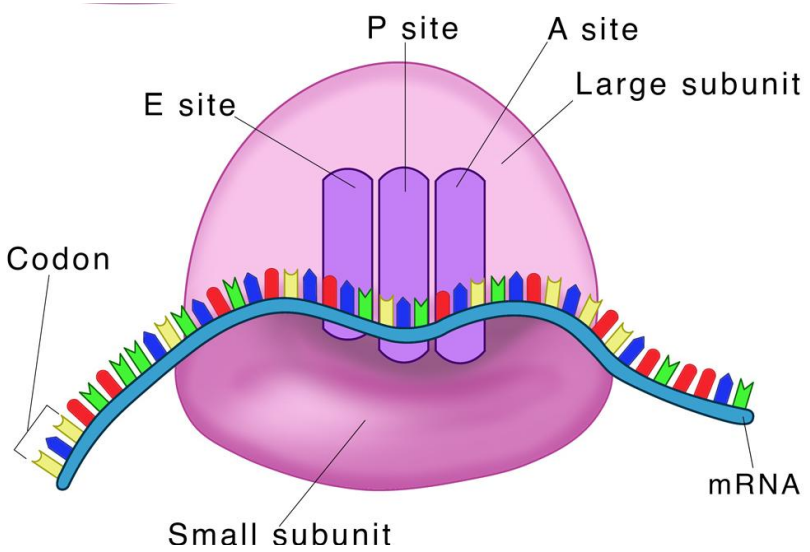
Ribosomal subunits contain three sites:

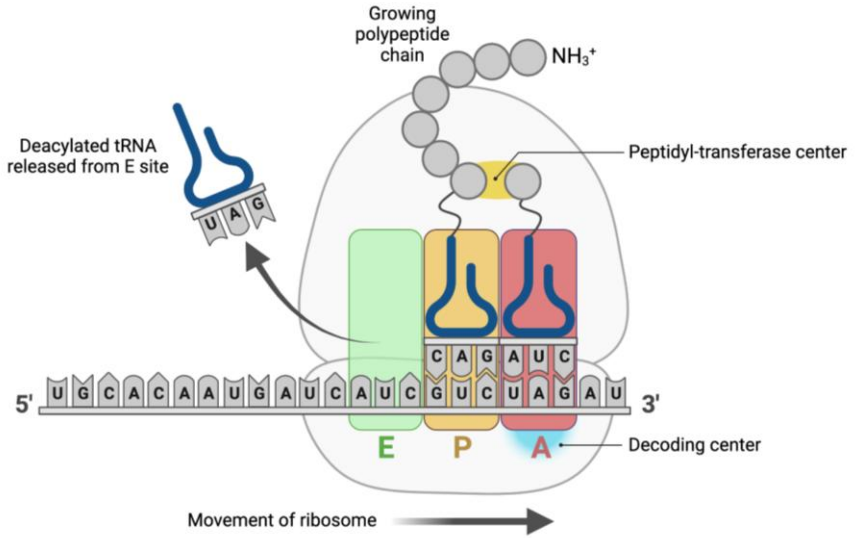
- **A site** — entry of aminoacyl-tRNA
- **P site** — holds peptidyl-tRNA
- **E site** — exit of uncharged tRNA

Elongation cycle:

1. Aminoacyl-tRNA delivered to A site
2. Peptide bond formation (catalyzed by rRNA)
3. Translocation moves ribosome one codon
4. tRNAs shift A→P→E
5. New aminoacyl-tRNA enters A site

   This cycle repeats until a stop codon appears.



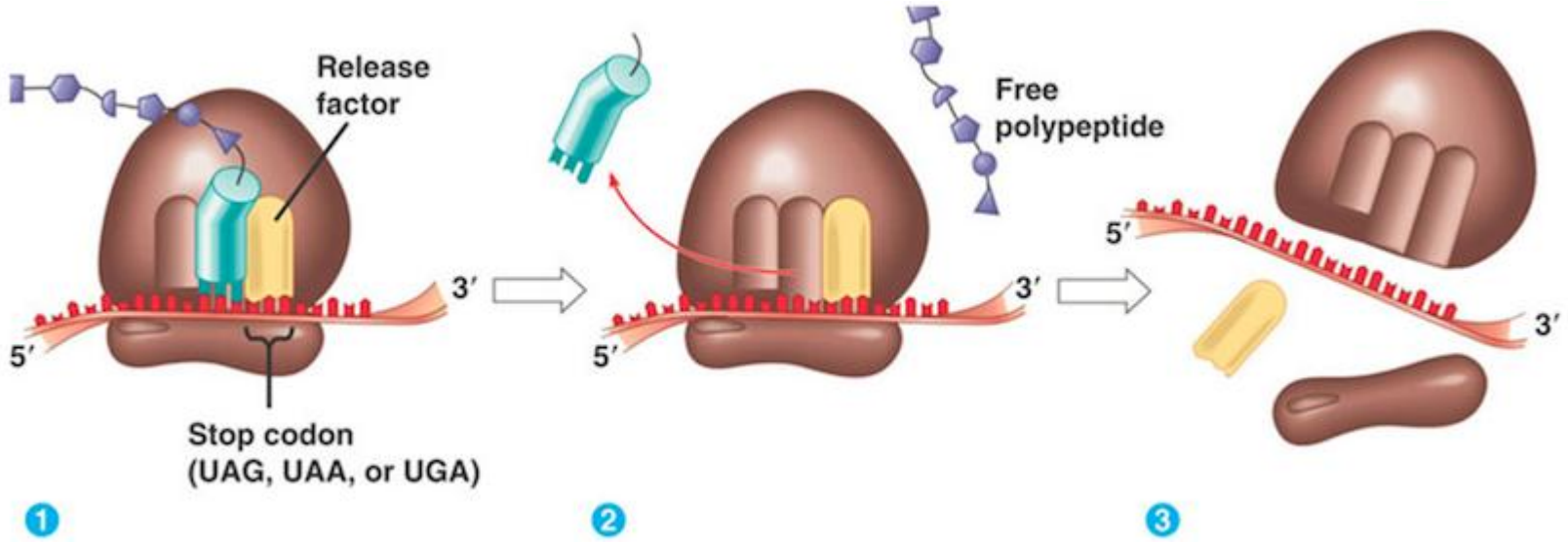


Termination

When a stop codon (UAA, UAG, UGA) enters the A site:

- No tRNA can recognize these codons
- **Release factors** bind instead
- Peptide-tRNA bond in the P site is hydrolyzed
- Polypeptide is released
- Ribosomal subunits disassemble and recycle

Termination ensures that the polypeptide is released with the correct length and sequence.



Translational Regulation in Eukaryotes

Global regulators

- **mTORC1** → phosphorylates 4EBP1 → releases eIF4E → increases translation.
- Stress (ER stress, hypoxia, viral infection) activates **eIF2α phosphorylation**, globally reducing translation except for stress-responsive mRNAs.
- **mRNA-**specific regulation
- **miRNAs:** inhibit translation or induce degradation.
- **RNA-binding proteins (RBPs):** HuR, TTP, IGF2BP1 regulate stability and translation.
- **uORFs, G-quadruplexes, structured 5'UTRs** decrease scanning efficiency.

Translation in Disease & Cancer Biology

- Oncogenes (MYC, RAS) upregulate translation machinery.
- Many cancers overexpress eIF4E, eIF3 subunits, ribosomal proteins.
- Deregulated translation enhances oncogenic signaling, metastasis, immune evasion.
- Therapeutic targets: **mTOR inhibitors, eIF4A inhibitors (silvestrol), eEF2 inhibitors**.

Translation in Immunology

- Rapid translation needed for T cell activation, cytokine production, and metabolic reprogramming.
- Codon usage shifts during effector T cell differentiation.
- Translational control modulates immune exhaustion, memory formation, and tolerance.
- Viral immune evasion: host translation shut-off vs. IRES-driven viral protein synthesis.

Antibiotics Targeting Translation (Advanced)

Inhibitors (bacteria-specific unless noted):

- **Tetracycline:** A-site entry blocker.
- **Macrolides (erythromycin):** block exit tunnel.
- **Aminoglycosides:** misreading of codons.
- **Cycloheximide (eukaryotes):** inhibits translocation.
- **Puromycin:** causes premature chain termination.

Mechanistic understanding of inhibitors informs therapeutic design and experimental applications.

Experimental Methods to Study Translation

Classical:

- Polysome profiling.
- Ribosome run-off assays.

Modern / high-resolution:

- **Ribosome profiling (Ribo-seq):** maps ribosome footprints genome-wide.
- **TRAP-seq:** cell-type–specific translatome profiling.
- **SunTag translation imaging:** real-time visualization of translation in single cells.
- **Mass spectrometry of nascent chains (NC-MS).**

Summary

- Translation is a highly regulated, energy-intensive, and error-sensitive process.
- Controlled at initiation, elongation, and termination stages.
- Integrates signals from metabolism, stress pathways, immunity, and disease states.
- Targetable in cancer, viral infections, and genetic diseases.
- Advances in ribosome profiling and single-molecule imaging are transforming our understanding of protein synthesis.

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