

TRANSLATION

Mechanism, the Genetic Code, and a Detailed Comparison of Prokaryotic and Eukaryotic Translation

1. Introduction

Translation is the process by which the sequence of nucleotides in a messenger RNA (mRNA) molecule is decoded to build a specific sequence of amino acids, producing a polypeptide chain. It is the final step of gene expression, carried out by **ribosomes** with the help of **transfer RNAs (tRNAs)** that physically link each three-nucleotide codon to its corresponding amino acid.

- Ribosomes are ribonucleoprotein machines built from a **large subunit** and a **small subunit**, each composed of ribosomal RNA (rRNA) and proteins; the small subunit reads the mRNA while the large subunit catalyses peptide bond formation.
- **Transfer RNAs** are small, cloverleaf-shaped adaptor molecules, each charged with a specific amino acid at its 3' end and bearing an **anticodon** loop that base-pairs with a complementary mRNA codon.
- The ribosome has three tRNA-binding sites — the **A (aminoacyl) site** for the incoming charged tRNA, the **P (peptidyl) site** holding the tRNA attached to the growing chain, and the **E (exit) site**, through which the spent, deacylated tRNA leaves.
- Like transcription, translation proceeds in three stages: **initiation**, **elongation**, and **termination**.

The Ribosome During Translation Elongation

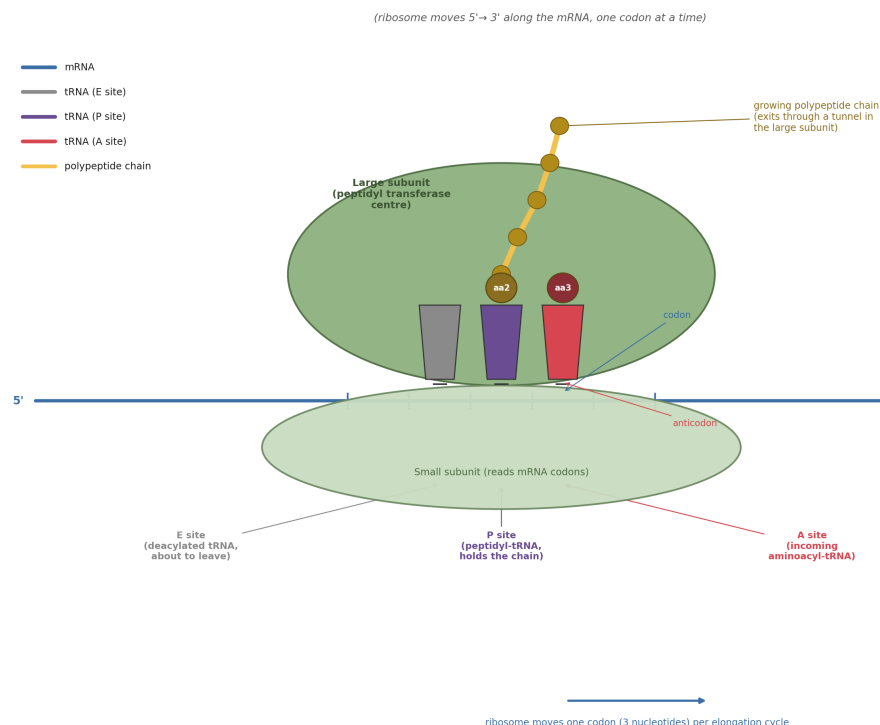


Fig. 1 — The ribosome during elongation: the A, P, and E sites, codon-anticodon pairing, and the growing polypeptide chain exiting through the large subunit.

(a) The Genetic Code

- Amino acids are specified by **codons** — triplets of mRNA nucleotides — giving $4^3 = 64$ possible codons for only 20 standard amino acids.
- The code is therefore **degenerate (redundant)**: most amino acids are specified by more than one codon (e.g. leucine has six), which buffers many mutations against changing the encoded protein.
- The code includes one **start codon (AUG)**, which also codes for methionine, and three **stop (nonsense) codons** — UAA, UAG, and UGA — which do not code for any amino acid and instead signal termination.
- The genetic code is very nearly **universal** across all forms of life (with a few minor exceptions, such as in mitochondria), strong evidence for the common ancestry of all living organisms.

2. Translation Initiation: Prokaryotes vs Eukaryotes

Initiation is the most heavily regulated and mechanistically distinct stage of translation between prokaryotes and eukaryotes, since each must solve the same problem — correctly finding the start codon on the mRNA — using very different strategies.

Translation Initiation: Prokaryotic vs Eukaryotic

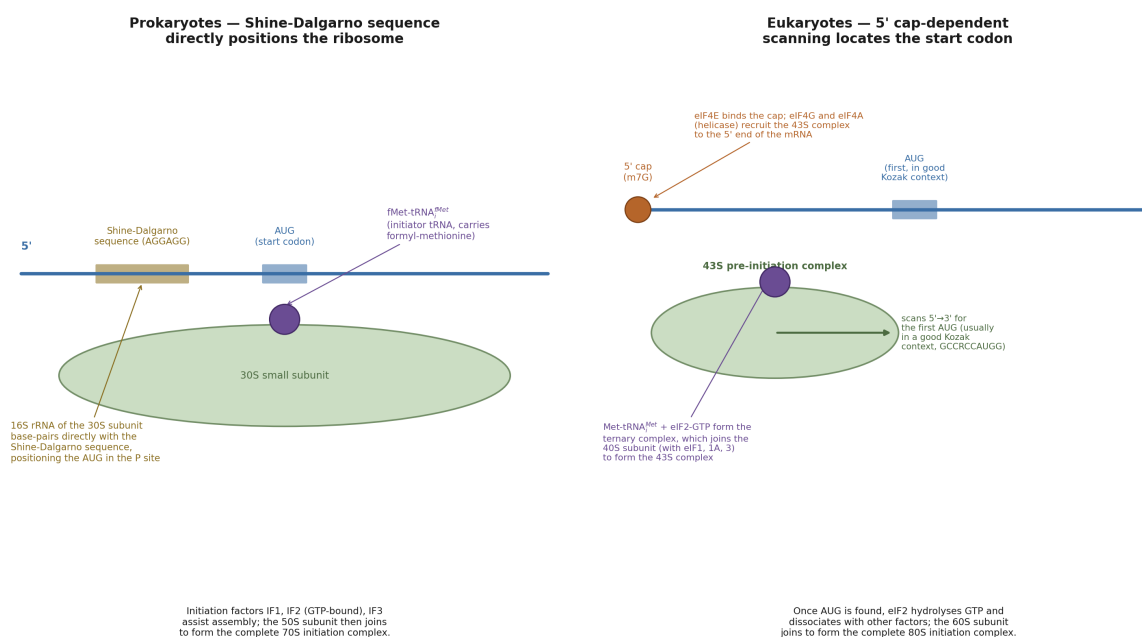


Fig. 2 — Prokaryotic initiation uses direct base-pairing (Shine-Dalgarno) to position the ribosome at AUG, while eukaryotic initiation recruits the small subunit to the 5' cap and scans downstream to find it.

(a) Prokaryotic Initiation

- The **30S small subunit**, aided by initiation factors **IF1**, **IF2 (GTP-bound)**, and **IF3**, binds the mRNA.
- A purine-rich sequence in the mRNA's 5' untranslated region, the **Shine-Dalgarno sequence** (consensus AGGAGG), base-pairs directly with a complementary sequence near the 3' end of the **16S rRNA** of the small subunit, positioning the nearby AUG start codon precisely in the P site.
- The special initiator tRNA, **fMet-tRNA^{fMet}** (charged with N-formylmethionine), enters the P site directly (not the A site) and pairs with the AUG codon.

- IF3 dissociates once the correct codon-anticodon pairing is confirmed, then IF2 hydrolyses GTP, releasing IF1 and IF2 and allowing the **50S large subunit** to join, forming the complete **70S initiation complex**.
- Because bacterial mRNAs are often **polycistronic** (encoding several proteins), each gene's own Shine-Dalgarno sequence allows internal initiation without needing to start scanning from the extreme 5' end.

(b) Eukaryotic Initiation

- A **ternary complex** of **Met-tRNA^{Met}** and **eIF2-GTP** joins the **40S small subunit** (together with eIF1, eIF1A, and eIF3) to form the **43S pre-initiation complex**.
- The cap-binding protein **eIF4E** binds the mRNA's 5' **m⁷G cap**; together with **eIF4G** (a scaffold) and the helicase **eIF4A**, it unwinds nearby secondary structure and recruits the 43S complex to the extreme 5' end of the mRNA.
- The 43S complex then **scans** along the mRNA in the 5'→3' direction until it encounters the first AUG codon, usually one embedded in a favourable **Kozak sequence** context (consensus **GCCACCAUGG**).
- Recognition of the start codon triggers hydrolysis of eIF2-bound GTP; the initiation factors are released, and the **60S large subunit** joins to form the complete **80S initiation complex**.
- Eukaryotic mRNAs are almost always **monocistronic** (one protein per mRNA), consistent with the linear scanning mechanism starting from a single capped 5' end.

Feature	Prokaryotic initiation	Eukaryotic initiation
Ribosomal subunits	30S + 50S → 70S	40S + 60S → 80S
Start-site recognition	Shine-Dalgarno sequence base-pairs with 16S rRNA	5' cap recruitment, then scanning to first AUG (Kozak context)
Initiator tRNA	fMet-tRNA (formylmethionine)	Met-tRNA (unformylated methionine)
mRNA organisation	Often polycistronic	Almost always monocistronic
Key initiation factors	IF1, IF2, IF3	eIF1, eIF1A, eIF2, eIF3, eIF4E, eIF4G, eIF4A, and others

3. Elongation

Elongation adds amino acids to the growing polypeptide chain one at a time, in a cyclical, three-step process that repeats for every codon in the reading frame. The core chemistry is essentially the same in prokaryotes and eukaryotes; the main differences are in the names of the elongation factors involved.

Elongation Cycle and Termination of Translation

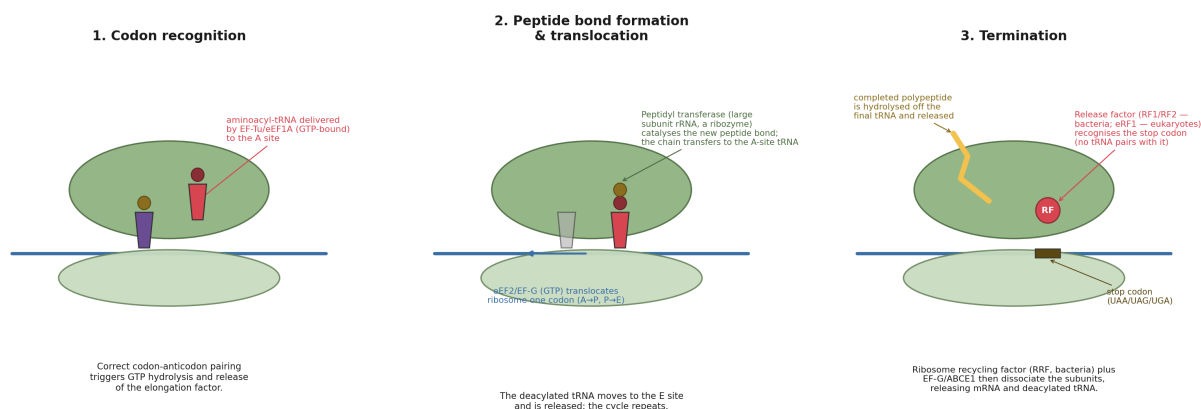


Fig. 3 — The elongation cycle (codon recognition, peptide bond formation, and translocation) followed by termination, when a release factor recognises a stop codon.

(a) Codon Recognition

- A charged aminoacyl-tRNA, escorted by an elongation factor bound to GTP — **EF-Tu** in bacteria, **eEF1A** in eukaryotes — enters the empty **A site**.
- If the tRNA's anticodon correctly base-pairs with the mRNA codon in the A site, the ribosome triggers GTP hydrolysis on the elongation factor, which then dissociates, leaving the aminoacyl-tRNA in place.

(b) Peptide Bond Formation

- The **peptidyl transferase centre**, formed by rRNA within the large subunit (making it a **ribozyme** — a catalytic RNA, not a protein enzyme), catalyses formation of a new peptide bond between the amino acid on the A-site tRNA and the growing chain on the P-site tRNA.
- The entire polypeptide chain is transferred onto the A-site tRNA, leaving the P-site tRNA deacylated (uncharged).

(c) Translocation

- Another GTP-dependent elongation factor — **EF-G** in bacteria, **eEF2** in eukaryotes — drives **translocation**: the ribosome moves exactly one codon (three nucleotides) along the mRNA.
- This shifts the tRNAs by one site each: the peptidyl-tRNA moves from the A site to the P site, and the deacylated tRNA moves from the P site to the E site, from which it is released; a new, empty A site is now positioned over the next codon, ready for the cycle to repeat.
- Elongation proceeds rapidly — around 20 amino acids per second in bacteria and 2–8 per second in eukaryotic cells.

Proofreading during elongation

The delay between initial codon-anticodon pairing and GTP hydrolysis by EF-Tu/eEF1A provides a kinetic proofreading step: incorrectly paired tRNAs dissociate more often during this delay than correctly paired ones, which is a major contributor to the overall accuracy of translation (roughly one error per 10,000 codons).

4. Termination

- Elongation continues until one of the three **stop codons** (UAA, UAG, UGA) enters the A site.

- No tRNA has an anticodon complementary to a stop codon; instead, a **release factor** — **RF1** (recognises UAA, UAG) or **RF2** (recognises UAA, UGA) in bacteria, or the single **eRF1** in eukaryotes — recognises the stop codon directly and enters the A site.
- The release factor triggers hydrolysis of the bond linking the completed polypeptide to the tRNA in the P site, releasing the finished protein.
- GTP-dependent factors (**RF3** in bacteria, **eRF3** in eukaryotes) help release the release factor itself, and finally the **ribosome recycling factor (RRF)** together with EF-G (bacteria), or **ABCE1** (eukaryotes), dissociates the ribosomal subunits from the mRNA, freeing them to be recycled for another round of translation.

5. Prokaryotic vs Eukaryotic Translation — Side by Side

Feature	Prokaryotic	Eukaryotic
Location	Cytoplasm, coupled to transcription	Cytoplasm, after mRNA export from the nucleus
Ribosome size	70S (30S + 50S)	80S (40S + 60S)
Start-codon selection	Shine-Dalgarno base-pairing	5' cap-dependent scanning
Initiator amino acid	N-formylmethionine	Methionine
mRNA structure	Polycistronic, no cap, short/no poly(A)	Monocistronic, 5' cap, poly(A) tail
Elongation factors	EF-Tu, EF-G	eEF1A, eEF2
Release factors	RF1, RF2, RF3	eRF1, eRF3
Rate of elongation	~20 amino acids/second	~2–8 amino acids/second
Ribosome recycling	RRF + EF-G	ABCE1

6. Additional Features of Translation

(a) Polyribosomes (Polysomes)

- Multiple ribosomes can translate the same mRNA molecule simultaneously, each at a different stage of progress along it, forming a **polyribosome (polysome)**.
- This allows a cell to produce many copies of a protein from a single mRNA transcript in a short time, and in bacteria — where there is no nuclear envelope — ribosomes can begin translating an mRNA while RNA polymerase is still transcribing it (coupled transcription-translation).

(b) Protein Targeting

- Proteins destined for secretion, the plasma membrane, or the endomembrane system carry an N-terminal **signal sequence** that is recognised by the **signal recognition particle (SRP)**, which halts translation and directs the ribosome to a receptor on the endoplasmic reticulum (eukaryotes) or plasma membrane (bacteria), where translation resumes and the growing chain is threaded across or into the membrane.
- Proteins without such a signal are synthesised on **free ribosomes** in the cytoplasm and typically remain there, or are subsequently imported into organelles such as mitochondria or the nucleus via other targeting signals.

(c) Antibiotics Targeting Translation

- Because prokaryotic (70S) and eukaryotic (80S) ribosomes differ structurally, many antibiotics selectively inhibit bacterial translation with minimal effect on human ribosomes.
- Examples include **streptomycin** and **tetracycline** (act on the 30S subunit), and **chloramphenicol** and **erythromycin** (act on the 50S subunit) — a key example of exploiting structural differences between prokaryotic and eukaryotic translation machinery for medicine.

7. Quick Revision Summary

Aspect	Key point
Genetic code	64 codons for 20 amino acids; degenerate; AUG = start, UAA/UAG/UGA = stop
Ribosome sites	A (incoming tRNA), P (peptidyl-tRNA), E (exiting tRNA)
Start-site recognition	Shine-Dalgarno/16S rRNA (prokaryotes) vs cap-dependent scanning for AUG (eukaryotes)
Initiator tRNA	fMet-tRNA (prokaryotes) vs Met-tRNA (eukaryotes)
Elongation factors	EF-Tu/EF-G (prokaryotes) vs eEF1A/eEF2 (eukaryotes)
Peptide bond formation	Catalysed by rRNA (a ribozyme) in the large subunit
Termination	Release factor recognises stop codon; no tRNA involved
Key size difference	70S ribosome (bacteria) vs 80S ribosome (eukaryotes)

Compiled study note — molecular biology reference. Figures are original schematic diagrams prepared for this note and are not to scale.