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قسم التقنيات الاحيائية الطبية

Molecular Biology

Lec. 5

DNA Translation

by

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Lecture five : DNA Translation

1. Introduction to Translation

Translation is the **second major step** in gene expression, following transcription.
It is the **biological process** in which the nucleotide sequence of an **mRNA molecule** is converted into the **amino acid sequence** of a protein.

Central Dogma:
DNA → RNA → Protein

The sequence of bases in mRNA determines the order of amino acids in the protein.

2. Site of Translation

- **Prokaryotes:** Occurs in the **cytoplasm**, simultaneously with transcription (coupled).
- **Eukaryotes:** Occurs in the **cytoplasm** or on the **rough endoplasmic reticulum (RER)** after mRNA processing.
- The machinery of translation is located on **ribosomes** — complex particles composed of **rRNA and proteins**.

3. Components of the Translation Machinery

Component	Description	Function
mRNA	Transcribed copy of a gene	Carries codons that specify amino acid sequence
tRNA (transfer RNA)	Small cloverleaf RNA with anticodon	Brings the correct amino acid to ribosome

Component	Description	Function
rRNA (ribosomal RNA)	RNA component of ribosomes	Structural + catalytic role (forms peptide bonds)
Ribosomes	70S (prokaryotes) / 80S (eukaryotes)	Site of translation
Aminoacyl-tRNA synthetases	Enzymes	Attach amino acids to correct tRNA
Initiation/Elongation/Termination factors	Proteins (IFs, EFs, RFs)	Regulate and catalyze translation
Energy molecules (GTP, ATP)	Energy source	Drive bond formation and translocation

4. The Genetic Code

- The **genetic code** is a set of rules defining how codons (triplets of nucleotides) specify amino acids.
- There are **64 codons** total (4^3 combinations).

Key features:

1. **Triplet**: Each codon = 3 nucleotides (e.g., AUG).
2. **Non-overlapping**: Codons read one after another.
3. **Universal**: Shared across nearly all organisms.
4. **Degenerate**: Most amino acids have multiple codons.
5. **Start codon**: **AUG** (Methionine or formyl-Met in bacteria).
6. **Stop codons**: **UAA, UAG, UGA** (signal translation termination).

5. Stages of Translation

(A) Initiation

1. **Small ribosomal subunit** binds to mRNA near the 5′ end (Shine-Dalgarno sequence in prokaryotes or 5′ cap in eukaryotes).
2. **Initiator tRNA** carrying **Methionine (Met)** binds to the **start codon (AUG)** at the **P-site**.
3. **Large ribosomal subunit** joins → forming a **complete initiation complex**.
4. **Initiation factors (IF-1, IF-2, IF-3)** in bacteria or **eIFs** in eukaryotes assist in assembly.
5. **GTP** is hydrolyzed to provide energy.

Result: Ribosome is ready to elongate the polypeptide.

(B) Elongation

The growing polypeptide chain is built **one amino acid at a time**.

1. **tRNA Entry**: A new aminoacyl-tRNA enters the **A-site** (A = aminoacyl site).
2. **Peptide Bond Formation**: The **peptidyl transferase** activity of the **23S rRNA (50S subunit)** catalyzes bond formation between amino acids.
3. **Translocation**: The ribosome moves one codon forward along mRNA (requires **EF-G** and **GTP**).
4. The now-empty tRNA exits from the **E-site**, and the growing chain moves to the **P-site**.
5. The process repeats until a stop codon is reached.

(C) Termination

1. When the ribosome reaches a **stop codon (UAA, UAG, UGA)**, no tRNA matches it.
2. **Release factors (RF1, RF2, RF3)** bind to the A-site.
3. The finished **polypeptide** is released from the ribosome.
4. **Ribosome subunits** dissociate and can be reused.

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6. Energy Requirements

Translation is highly energy-consuming:

Step	Energy Source	Function
Charging tRNA	1 ATP	Aminoacyl-tRNA formation
Initiation	1 GTP	Ribosome assembly
Elongation	2 GTP per amino acid	tRNA entry & translocation
Termination	1 GTP	Release of polypeptide

Average: **~4 high-energy bonds per peptide bond**.

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7. Polysomes (Polyribosomes)

- Multiple ribosomes can translate the **same mRNA molecule simultaneously**, forming a **polysome**.
- Increases efficiency and speed of protein synthesis.

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8. Differences Between Prokaryotic and Eukaryotic Translation

Feature	Prokaryotes	Eukaryotes
Ribosome	70S (50S + 30S)	80S (60S + 40S)
Coupled with transcription	Yes	No

Feature	Prokaryotes	Eukaryotes
Initiator tRNA	fMet-tRNA	Met-tRNA
mRNA recognition	Shine-Dalgarno sequence	5′ cap scanning
Location	Cytoplasm	Cytoplasm / RER
Factors	IFs, EFs, RFs	eIFs, eEFs, eRFs

9. Post-Translational Modifications (PTMs)

Once synthesized, proteins often undergo:

- **Folding** (with chaperones).
- **Cleavage** of signal peptides.
- **Phosphorylation / Glycosylation / Methylation / Acetylation.**
- **Disulfide bond formation** for stability.
- **Targeting** to organelles or membranes.

These modifications make proteins functional and ready for their specific roles.

10. Inhibitors of Translation (Antibiotics)

Antibiotic	Target Mechanism
Streptomycin	Binds 30S; misreads codons
Tetracycline	Prevents tRNA binding to A-site
Chloramphenicol	Blocks peptidyl transferase

Antibiotic	Target Mechanism
Erythromycin	Prevents ribosome translocation
Puromycin	Causes premature chain termination
Cycloheximide	Inhibits eukaryotic 80S ribosomes

Used clinically to target **bacterial translation** without affecting human ribosomes.

11. Significance of Translation

- Final step in **gene expression** → produces functional proteins.
- Determines the **phenotype** of an organism.
- Essential for cell growth, metabolism, and reproduction.
- Understanding translation enables **drug design** and **biotechnological manipulation** (e.g., optimizing protein production in microbes).