Protein Synthesis

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"Protein Synthesis (Translation) Notes"

I. Overview

- Genetic information flow:

  DNA (replication) → RNA (transcription) → Protein (translation).

- Proteome: Complete set of proteins expressed in a cell.

- Translation:

  - "Language" of nucleotides → converted into "language" of amino acids.
  - Requires the genetic code.

- Errors:

  - Mutation in nucleotide sequence → wrong amino acid → defective protein → disease or death.

- Nascent proteins:
    - Must fold properly.
    - Misfolding → aggregation or degradation.
    - Many undergo covalent modifications (e.g., phosphorylation, glycosylation).
    - Targeted to final destinations via signal sequences.

II. The Genetic Code

Definition

- A "dictionary" that relates nucleotide triplets (codons) in mRNA to amino acids.

A. Codons

- Written in 5′ → 3′ direction.
- Total combinations: 4 bases (A, U, G, C) taken 3 at a time → 64 codons.
- Start codon: AUG → codes for Methionine (Met) → also the initiation codon.
- Sense codons: 61 codons specify the 20 standard amino acids.

- Stop codons (nonsense): UAA, UAG, UGA → terminate translation.

🔑 Flow (codon → amino acid):

mRNA codon → read by ribosome → corresponding amino acid added to growing polypeptide.

B. Characteristics of the Genetic Code

1. Specificity (Unambiguous):
    - A particular codon always codes for the same amino acid.
    - Example: AUG → always Met.
2. Universality:
    - Same codon meaning in almost all organisms.
    - ☆ Exception: In mitochondria, some codons differ.
        - e.g., UGA → codes for Tryptophan (Trp) instead of stop.
3. Degeneracy (Redundancy):

- Multiple codons can code for the same amino acid.
- Example: Arginine (Arg) → 6 codons.
- Only Met & Trp have a single codon.
- Usually, difference lies in the 3rd base (wobble position).

4. Nonoverlapping & Commaless:

- Codons read continuously from start point → no overlap, no "commas."
- Example: mRNA AGCUGGAUACAU → read as AGC | UGG | AUA | CAU.
- Reading frame: Correct grouping of codons → determines correct protein sequence.

☆ Exam Points

- AUG → universal start codon, codes for Met.
- Stop codons: UAA, UAG, UGA (mnemonic: "U Are Annoying, U Are Gone, U Go Away").
- Degeneracy protects against mutations (silent mutations).
- Universality → used in recombinant DNA tech (bacteria

can express human proteins).

- Mitochondrial genetic code is slightly different → high-yield viva question.

📊 Mini Table: Start vs Stop Codons

Codon	Function	Codes for
AUG	Start	Methionine (Met)
UAA	Stop	—
UAG	Stop	—
UGA	Stop (in cytoplasm) / Trp (in mitochondria)	— / Trp

C. Consequences of Altering the Nucleotide Sequence

Point mutations → change of a single nucleotide in coding region → outcomes:

1. Silent mutation

- Base change does not alter amino acid (due to degeneracy of code).
- Example: UCA → UCU (both code for Serine).

2. Missense mutation

- Base change → different amino acid.
- Example: UCA (Ser) → CCA (Proline).
- Can be conservative (similar AA) or non-conservative (different properties).

3. Nonsense mutation

- Base change → stop codon introduced.
- Example: UCA (Ser) → UAA (stop).
- Results in premature termination → truncated, usually nonfunctional protein.
- Nonsense-mediated decay may degrade such mRNAs.

4. Other important mutations

a. Trinucleotide repeat expansions

- Abnormal amplification of triplet sequences.
- In coding region → abnormal protein with multiple repeats.
    - Example: CAG repeat in *huntingtin* gene → extra glutamines → Huntington disease.
- In UTR → altered regulation, ↓ protein production.
    - Example: Fragile X syndrome, Myotonic dystrophy.
- ☆ Over 20 such disorders known.

b. Splice-site mutations

- Affect intron removal → aberrant proteins.
- Example: Myotonic dystrophy (triplet expansion + splicing defects).

c. Frameshift mutations

- Addition/deletion of 1 or 2 nucleotides → alters reading frame.
    - → abnormal protein or premature stop.
- Addition/deletion of 3 nucleotides → reading frame intact, but:
    - Addition → extra amino acid or premature stop.
    - Deletion → loss of one amino acid.
- Example: Cystic Fibrosis (ΔFS08 mutation)
    - Loss of phenylalanine at position 508 in CFTR.
    - Misfolded → destroyed by proteasome.
    - CFTR normally = chloride channel.
    - Defect → thick secretions in lungs/pancreas → lung damage, pancreatic insufficiency.
    - Common in Northern Europeans (1 in 3300).
        - 70% of CF cases due to ΔFS08.

☆ Exam Points on Mutations

- Silent vs Missense vs Nonsense: classic MCQ.
- Trinucleotide expansions → Huntington (CAG), Fragile X,

Myotonic dystrophy.

- Frameshift → Cystic fibrosis ΔF508 (loss of Phe).
- Nonsense codons = UAA, UAG, UGA.

📊 Mini Table: Mutation Types

Mutation type	Effect	Example
Silent	Same amino acid	UCA → UCU (Ser)
Missense	Different amino acid	UCA (Ser) → CCA (Pro)
Nonsense	Stop codon formed	UCA (Ser) → UAA (stop)
Trinucleotide repeat	Abnormal repeats	Huntington (CAG)
Frameshift	Altered reading frame	Cystic Fibrosis (ΔF508)

III. Components Required for Translation

A. Amino acids

- All 20 amino acids must be present.
- If even one is missing, translation halts at its codon.
- 🔑 Explains importance of dietary essential AAs.

B. Transfer RNA (tRNA)

- At least one tRNA per amino acid (≥50 in humans; ≥ 30 in bacteria).
- Some AAs → multiple tRNAs (isoacceptors).
- Key features:

1. Amino acid attachment site:
    - At 3′ end (CCA sequence).
    - Amino acid attached to ribose's 3′–OH (ester linkage).
    - Charged tRNA = with AA; uncharged tRNA = without AA.
2. Anticodon:

    - 3-base sequence complementary to mRNA codon.
    - Ensures correct AA insertion into growing chain.

C. Aminoacyl-tRNA synthetases

- Family of 20 enzymes (one per amino acid).
- Functions:
    - Recognize amino acid + corresponding tRNAs (isoacceptors).
    - Catalyze charging of tRNA (AA + tRNA binding at 3′ CCA).
    - Reaction requires ATP → AMP + PPi.
- Proofreading function: Removes incorrectly attached amino acids.
- ☆ Ensures fidelity of translation.

D. Messenger RNA (mRNA)

- Provides the template encoding protein sequence.
- Must be present to direct translation.

E. Functionally Competent Ribosomes

- Ribosomes = large complexes of rRNA + proteins (rRNA predominates).

- Composed of two subunits (large + small).

Sedimentation coefficients (Svedberg values):

- Prokaryotes: 50S + 30S = 70S ribosome

- Eukaryotes: 60S + 40S = 80S ribosome

  (Note: S values are not strictly additive — depend on shape + size.)

Functions of subunits:

- Small subunit: binds mRNA; ensures correct codon–anticodon pairing.

- Large subunit: catalyzes peptide bond formation.

1. Ribosomal RNA (rRNA)

- Prokaryotes: 3 species of rRNA.
- Eukaryotes: 4 species of rRNA.
- Derived from single pre-rRNA, processed by ribonucleases + base modifications.

2. Ribosomal proteins

- More numerous in eukaryotes.
- Stabilize structure + facilitate interactions during translation.

3. A, P, and E sites

- A site: (Aminoacyl-tRNA) binds incoming charged tRNA.
- P site: (Peptidyl-tRNA) holds growing polypeptide chain.
- E site: (Exit site) holds empty tRNA before leaving ribosome.

4. Cellular location

- Eukaryotes:

    - Free ribosomes (cytosol) → proteins for cytosol, nucleus, mitochondria, peroxisomes.
    - RER-bound ribosomes → proteins for secretion, membranes, lysosomes.

- Mitochondria: contain own SSS ribosomes + circular DNA (but most mitochondrial proteins are nuclear-encoded).

III-F. Protein Factors

- Required for initiation, elongation, termination.

- Some are catalytic, others stabilize translation machinery.

- Many are small GTP-binding proteins:

    - Active = GTP-bound
    - Inactive = GDP-bound

III-G. Energy Sources

- Translation = energy-intensive.

- Adding 1 amino acid requires cleavage of 4 high-energy

bonds:

1. 2 ATP equivalents: charging tRNA (aminoacyl-tRNA synthetase).
2. 1 GTP: binding aminoacyl-tRNA to A site.
3. 1 GTP: ribosomal translocation step.

- Extra ATP/GTP used in initiation + termination.

IV. Codon Recognition by tRNA

A. Antiparallel binding

- Codon (mRNA) read 5′ → 3′.
- Anticodon (tRNA) binds 3′ → 5′ (complementary + antiparallel).

B. Wobble Hypothesis

- Explains how fewer tRNAs recognize all codons.
- Pairing rules:
  - First 2 codon bases = strict Watson-Crick pairing.

    - 3rd base = "wobble" position → flexible pairing.

- Allows 1 tRNA to recognize multiple codons.
- Example: Inosine (I) in anticodon can pair with U, C, or A.
- Result: <61 tRNAs needed for 61 sense codons.

☆ Exam Points

- Prokaryotic ribosomes = 70S; eukaryotic = 80S.
- Ribosomal A, P, E sites = core to elongation mechanism.
- Translation uses 4 high-energy bonds per AA (big exam favorite).
- Wobble hypothesis = explains genetic code degeneracy (esp. 3rd codon base).

❖ Steps in Translation

Definition:

Translation = Process of protein synthesis where the nucleotide sequence of mRNA (3-letter codons) is decoded into the amino acid sequence of a polypeptide chain.

- Direction of reading mRNA: 5′ → 3′
- Direction of protein synthesis: N-terminal → C-terminal
- Prokaryotic mRNA: Polycistronic (multiple coding regions → multiple proteins)
- Eukaryotic mRNA: Monocistronic (one coding region → one protein)
- Prokaryotes: Translation + transcription occur simultaneously (no nuclear membrane).
- Eukaryotes: Transcription (nucleus) and translation (cytosol/RER) are separate.

I. Initiation

Goal: Assembly of complete ribosomal initiation complex before peptide bond formation.

Components required:

- Small + large ribosomal subunits
- mRNA
- Initiator aminoacyl-tRNA
- GTP
- Initiation factors (IFs in prokaryotes, eIFs in eukaryotes)
- ATP (extra requirement in eukaryotes)

Recognition of Start Codon (AUG):

1. Prokaryotes (Shine-Dalgarno sequence):
    - Purine-rich sequence ~6-10 bases upstream of AUG.
    - 16S rRNA of 30S subunit base-pairs with SD sequence → correct alignment.
2. Eukaryotes (5′ Cap scanning):
    - 40S subunit binds 5′ cap (with help of eIF-4 proteins).
    - Scans 5′ → 3′ until AUG encountered.
    - Requires ATP.
    - Cap-independent initiation: 40S binds IRES (internal

ribosome entry site).

3. Initiator tRNA:

    - Prokaryotes → tRNAi carrying N-formylmethionine (fMet) (formyl group added by transformylase).
    - Eukaryotes → tRNAi carrying Met (not formylated).
    - Only initiator tRNA goes directly to P site.

Final initiation event:

- Large ribosomal subunit joins.
- Initiator tRNA positioned in P site.
- A site remains empty, ready for next aminoacyl-tRNA.
- GTP on IF-2/eIF-2 hydrolyzed → complex stabilized.

II. Elongation

Goal: Add amino acids sequentially to the C-terminal end.

Steps:

1. Decoding (A site entry):

- Aminoacyl-tRNA enters A site.
- Requires elongation factors (EF-Tu-GTP in prokaryotes, EF-1α-GTP in eukaryotes).
- GTP hydrolyzed.

2. Peptide bond formation (Transpeptidation):

- Catalyzed by peptidyl transferase (rRNA of large subunit → ribozyme).
- Peptide chain transferred from P site tRNA → amino acid at A site.

3. Translocation:

- Ribosome shifts 3 nucleotides toward 3′ end of mRNA.
- Requires EF-G-GTP (prokaryotes) or EF-2-GTP (eukaryotes).
- Result:
  - Empty tRNA → E site (exits).
  - Peptidyl-tRNA → moves A → P site.
  - A site becomes free again.

Note: Multiple ribosomes can translate simultaneously = polyribosome (polysome).

III. Termination

Trigger: Stop codon (UAA, UAG, UGA) enters A site.

- Prokaryotes:
    - RF-1 → recognizes UAA, UAG
    - RF-2 → recognizes UAA, UGA
    - RF-3-GTP → releases RF-1/2 after peptide release
- Eukaryotes:
    - Single release factor (eRF) → recognizes all stop codons
    - eRF-3 (GTPase) → helps release

Event: Hydrolysis of bond between peptide + tRNA at P site → polypeptide released.

- Ribosomal subunits + mRNA + tRNA recycled (ribosome recycling factors in prokaryotes; ATP + eRF in eukaryotes).

IV. Regulation of Translation

- Most gene regulation occurs at transcription.

- Translation regulation also possible:

    - Eukaryotes: Phosphorylation of eIF-2 → inactivation → inhibits initiation.
    - mRNA-binding proteins → block translation of specific mRNAs.

Flowchart: Steps of Translation

mRNA + small subunit + initiator tRNA + IFs → Initiation complex

↓

Large subunit joins → tRNAi in P site → A site empty

↓

Elongation cycle:

A site entry (aminoacyl-tRNA + EF-GTP) → Peptide bond formation (peptidyl transferase) → Translocation (ribosome moves, EF-GTP hydrolyzed)

↓

Stop codon enters A site → Release factor binding → Peptide released

↓

Ribosome recycled

☆ Exam Points

- Start codon = AUG (Met/fMet).
- Prokaryotic initiation → Shine-Dalgarno sequence + 16S rRNA.
- Eukaryotic initiation → 5′ cap scanning.
- Initiator tRNA → only tRNA that goes to P site first.
- Peptidyl transferase → rRNA (ribozyme).
- Multiple ribosomes on one mRNA = polysome.
- Termination codons (UAA, UAG, UGA) → no tRNA corresponds.

Translation: Protein Folding, Targeting & Post-Translational

Modifications

E. Protein Folding

- Proteins must fold into their native 3D conformation to be functional.

- Folding can be:

    - Spontaneous → driven by amino acid sequence.
    - Chaperone-mediated → specialized proteins assist folding.
        - Example: Heat shock proteins (HSPs).
        - Prevent misfolding & aggregation.

F. Protein Targeting

- Although most proteins begin synthesis in the cytoplasm, many must be directed to other organelles or outside the cell.

- Signal sequences (short amino acid motifs) → direct proteins to final location.

Examples:

1. Secreted proteins → have N-terminal hydrophobic signal sequence.

    - Recognized by Signal Recognition Particle (SRP).
    - SRP halts elongation → brings ribosome-peptide complex to RER.
    - Delivered to translocon channel in RER membrane.
    - Translation resumes inside RER lumen → signal peptide cleaved.
    - Protein processed → Golgi → secreted.
    - (Process = cotranslational targeting).

2. Post-translational targeting:

    - Nucleus → short, basic Nuclear Localization Signal (NLS).
    - Mitochondria → N-terminal, amphipathic α-helix.
    - Peroxisome → C-terminal tripeptide (PTS1).

VI. Co- & Posttranslational Modifications

A. Trimming

- Many proteins made as inactive precursors (zymogens/pro-proteins).

- Cleavage by endoproteases → active protein.
- Sites: RER, Golgi, secretory vesicles, or extracellular.
    - Example: Insulin (from proinsulin).
    - Example: Collagen trimming after secretion.

B. Covalent Modifications

1. Phosphorylation
    - On Ser, Thr, Tyr residues.
    - Catalyzed by kinases; reversed by phosphatases.
    - Alters activity (↑ or ↓).
    - Example: Regulation of glycogen metabolism.
2. Glycosylation
    - N-linked (to Asn) → in RER.
    - O-linked (to Ser/Thr/Hyp) → in Golgi.
    - Special case: Mannose-6-P tagging → lysosomal enzymes.
3. Hydroxylation
    - Proline & lysine residues of collagen.

- Requires Vitamin C.
- Defect → Scurvy.

4. Other modifications

- γ-Carboxylation of Glu residues → Vitamin K dependent → clotting factors (II, VII, IX, X).
- Biotinylation → lysine residues of biotin-dependent enzymes (e.g., pyruvate carboxylase).
- Lipid anchoring (e.g., farnesylation) → membrane targeting.
- Acetylation (N-terminal) → stability; histone acetylation regulates gene expression.

C. Protein Degradation

- Ubiquitination → marks proteins for destruction.
  - Ubiquitin = small, conserved protein.
  - Proteins degraded in proteasome (ATP-dependent).
- Examples:
  - Misfolded CFTR → degraded → Cystic Fibrosis.
  - Misfolded/unfolded proteins → accumulate in RER → ER stress → Unfolded Protein Response (UPR).

    - ↑ Chaperones.
    - ↓ Global translation (via eIF-2 phosphorylation).
    - Misfolded proteins exported to cytosol → ubiquitinated → proteasome degradation (ERAD: ER-associated degradation).

☆ Exam Points

- Chaperones = assist folding, prevent aggregation.
- SRP halts translation until ribosome docks on RER.
- Nuclear proteins → NLS; Mitochondrial proteins → N-terminal amphipathic helix.
- Insulin & collagen → classic examples of trimming.
- Phosphorylation = reversible ON/OFF switch.
- Vitamin C → hydroxylation of collagen.
- Vitamin K → γ-carboxylation of clotting factors.
- Ubiquitin-proteasome pathway = main protein degradation route.

- ER stress → triggers Unfolded Protein Response.

<--->