BCMB 3100 - Chapters 39 & 40
Translation (protein synthesis)

- Translation
- Genetic code
- tRNA
- Amino acyl tRNA
- Ribosomes
- Initiation
- Elongation
- Termination

How is the nucleotide code translated into a protein code?

transduction
DNA → RNA → protein

transcription

5’ UCA 3’ → NH₂ Ser COO⁻...

Adapter Molecule Hypothesis (Crick, 1958)
mRNA → protein
    (codon)       adapter molecule = tRNA

Adapter molecule = ___________ (transfer RNA)
                (anticodon)

___________: relation between the sequence of bases in RNA (DNA) and the sequence of amino acids in protein. It is a three base code that is sequential, non-overlapping, and degenerate.

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3 letter code 1st proposed by George Gamow

2 letter RNA code:  $4^2 = 16$
3 letter RNA code:  $4^3 = 64$
4 letter RNA code:  $4^4 = 256$
Overlapping vs nonoverlapping reading of the three-letter code

mRNA \[
\vdots \vdots \vdots \quad A \ U \ G \ C \ A \ U \ G \ C \ A \ U \ G \ C \ \vdots \vdots \vdots
\]

(a) Message read in overlapping triplet code
\[
A \ U \ G \\
U \ G \ C \\
G \ C \ A \\
C \ A \ U \\
\vdots \vdots \vdots
\]

(b) Message read in *** nonoverlapping **** triplet code
\[
A \ U \ G \\
C \ A \ U \\
G \ C \ A \\
U \ G \ C
\]

Three reading frames of mRNA

• Translation of the correct message requires selection of the correct reading frame

mRNA
\[
\vdots \vdots \vdots \quad A \ U \ G \ C \ A \ U \ G \ C \ A \ U \ G \ C \ \vdots \vdots \vdots
\]

Message read in reading frame 1
\[
\vdots \vdots \vdots \quad A \ U \ G \ C \ A \ U \ G \ C \ A \ U \ G \ C \ \vdots \vdots \vdots
\]

Message read in reading frame 2
\[
\vdots \vdots \vdots \quad A \ U \ G \ C \ A \ U \ G \ C \ A \ U \ G \ C \ \vdots \vdots \vdots
\]

Message read in reading frame 3
\[
\vdots \vdots \vdots \quad A \ U \ G \ C \ A \ U \ G \ C \ A \ U \ G \ C \ \vdots \vdots \vdots
\]
### Standard genetic code  (see Tables 39.1 & 39.2)

<table>
<thead>
<tr>
<th>First position (5' end)</th>
<th>Second position</th>
<th>Third position (3' end)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>U</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Phe Ser</td>
<td>Tyr Cys</td>
<td><strong>U</strong></td>
</tr>
<tr>
<td>Phe Ser</td>
<td>Tyr Cys</td>
<td><strong>C</strong></td>
</tr>
<tr>
<td>Leu Ser</td>
<td><em>STOP</em></td>
<td><strong>A</strong></td>
</tr>
<tr>
<td>Leu Ser</td>
<td><em>STOP</em></td>
<td><strong>G</strong></td>
</tr>
<tr>
<td><strong>C</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leu Pro</td>
<td>His Arg</td>
<td><strong>U</strong></td>
</tr>
<tr>
<td>Leu Pro</td>
<td>His Arg</td>
<td><strong>C</strong></td>
</tr>
<tr>
<td>Leu Pro</td>
<td>Gln Arg</td>
<td><strong>A</strong></td>
</tr>
<tr>
<td>Leu Pro</td>
<td>Gln Arg</td>
<td><strong>G</strong></td>
</tr>
<tr>
<td><strong>A</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ile Thr</td>
<td>Asn Ser</td>
<td><strong>U</strong></td>
</tr>
<tr>
<td>lle Thr</td>
<td>Asn Ser</td>
<td><strong>C</strong></td>
</tr>
<tr>
<td>lle Thr</td>
<td>Lys Arg</td>
<td><strong>A</strong></td>
</tr>
<tr>
<td>*Met Thr</td>
<td>Lys Arg</td>
<td><strong>G</strong></td>
</tr>
<tr>
<td><strong>G</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Val Ala</td>
<td>Asp Gly</td>
<td><strong>U</strong></td>
</tr>
<tr>
<td>Val Ala</td>
<td>Asp Gly</td>
<td><strong>C</strong></td>
</tr>
<tr>
<td>Val Ala</td>
<td>Glu Gly</td>
<td><strong>A</strong></td>
</tr>
<tr>
<td>Val Ala</td>
<td>Glu Gly</td>
<td><strong>G</strong></td>
</tr>
</tbody>
</table>

---

**BCMB 3100 - Chapters 39 & 40**

**Translation (protein synthesis)**

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Brief overview of synthesis of tRNA

1. Primary transcript may contain several tRNAs (prokaryotes)

2. **Endonuclease RNAs P cleaves 5’ end** side of tRNA
   - RNAs P = ribonucleoprotein
   - *E. coli* Rnase P = 377 nucleotide RNA (130 kD) + 18 kD protein
     - RNA is catalytic part of the complex!

3. Another **endonuclease cleaves 3’ side** of tRNA

4. **RNase D further cleaves 3’ end** to yield “final” 3’ end

5. **tRNA nucleotidyl transferase adds CCA to the 3’ end of tRNA**

6. ~ 30% of **nucleotides in tRNA are modified**

---

**Cloverleaf structure of tRNA**

- tRNAs 73-95 nucleotides long.
- Anticodon base pairs with codon in mRNA.
- 3’ end always ends in 3’ ACC……5’
- Amino acid is added to A at 3’ end

<table>
<thead>
<tr>
<th>D = dihydrouridylate</th>
<th>(\Psi) = pseudouridylate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Places where amino acid will be added</td>
<td></td>
</tr>
</tbody>
</table>

Place where amino acid will be added
Tertiary structure of tRNA

Fig. 39.1

Fig. 39.2
Some tRNAs recognize more than one codon because of **Wobble** in base-pairing

The anticodon forms base pairs with the codon:

By convention, sequences are written in the 5' to 3' direction. Thus the anticodon that pairs with AUG is written CAU.

Some tRNA molecules can recognize more than one codon. The **recognition of the third base in the codon** by the anticodon is sometimes less discriminating, a phenomenon called wobble.

---

**Generalizations of the codon–anticodon interactions are:**

1. Codons that differ in either of the first two nucleotides must be recognized by different tRNA.

2. The first base of the anticodon determines the degree of wobble. If the first base is inosine, the anticodon can recognize three codons.
Aminoacyl-tRNA Synthetases

- synthesize ________________ (specific amino acid covalently attached to 3’ end of specific tRNA (named as: alanyl-tRNA_{Ala}))
- At least 20 different aminoacyl-tRNA synthetases (1 per amino acid)
- Each synthetase specific for a particular amino acid, but may recognize isoacceptor tRNAs
- Synonymous codons may be recognized by isoacceptor tRNAs (different tRNAs that attach the same amino acid) (bacteria have 30-60 different tRNAs)
Multiple nucleotides in tRNAs recognized by the tRNA synthetases

Fig. 39.6.

NOTE: correct recognition is essential for fidelity of translation!

Aminoacyl-tRNA Synthetase Reaction

• Aminoacyl-tRNAs: high-energy molecules in which the amino acid has been “activated”

• Activation of amino acid by aminoacyl-tRNA synthetase requires ATP

\[
\text{Amino acid} + \text{tRNA} + \text{ATP} \rightarrow \text{Aminoacyl-tRNA} + \text{AMP} + \text{PP}_i
\]

Summary of overall reaction, note however, the reaction actually takes place in two steps.
Note: 2 P bond equivalents!
Step 1: ATP + amino acid $\rightarrow$ aminoacyl-adenylate intermediate + PPi

Step 2:
aminoacyl-adenylate + tRNA $\rightarrow$
aminoacyl-tRNA + AMP

3’ terminal end of t-RNA activated with an amino acid
Aminoacyl-tRNA Synthetases have highly discriminating amino acid activation sites

Each aminoacyl-tRNA synthetase is specific for particular amino acid.

Specificity attained by various means in different enzymes.

Example:

Threonyl-tRNA synthetase contains a zinc ion at the active site that interacts with the hydroxyl group of threonine. Valine is similar in overall structure to threonine but lacks hydroxyl group and thus is not joined to the tRNA\textsuperscript{Thr}.

Serine, although smaller than threonine, is occasionally linked to tRNA\textsuperscript{Thr} because of the presence of the hydroxyl group.
**Active site of threonyl-tRNA synthetase**

Fig. 39.4

Threonyl-tRNA synthetase has editing site, in addition to active site, to remove a serine inappropriately joined to tRNA\textsuperscript{Thr}.

CCA arm of tRNA\textsuperscript{Thr} can swing into editing site where the serine is removed.

Because threonine is larger than serine, it cannot fit into the editing site. (Note: editing sites select against smaller potential substrates)

The double sieve of an acylation site and an editing site increases the fidelity of many synthetases.

---

**Proofreading by Aminoacyl-tRNA Synthetases increases fidelity of protein synthesis**

Threonyl-tRNA synthetase has **editing site**, in addition to active site, to remove a serine inappropriately joined to tRNA\textsuperscript{Thr}.

CCA arm of tRNA\textsuperscript{Thr} can swing into editing site where the serine is removed.

Because threonine is larger than serine, it **cannot fit into the editing site**. (Note: editing sites select against smaller potential substrates)

The double sieve of an acylation site and an editing site increases the fidelity of many synthetases.
Editing site of aminoacyl-tRNA synthetases.
Flexible CCA arem of an aminoacyl-tRNA can move the newly attached amino acid from the activation site to the editing site. If the newly added amino acid fits well into the editing site, it is removed by hydrolysis, thus reducing errors in protein synthesis later.

Fig. 39.5
Ribosomes

- **Ribosome**: RNA-protein complex that interacts with accessory protein factors, mRNA and charged tRNA to synthesize proteins.

SEE Figure 39.7 to understand how much of the ribosome structure is RNA!

- **Initiation complex**: assembles at first mRNA codon; disassembles at termination step.
- **Ribosome moves 5’ → 3’ along mRNA**
- **Polypeptide** synthesized in N → C direction.

### Comparison of prokaryotic and eukaryotic ribosomes

![Comparison of prokaryotic and eukaryotic ribosomes](image-url)
**E. coli ribosome:** 2700 kd, 250 angstroms, 70S

*Fig. 39.7* 23S RNA (yellow); 5S RNA (orange); 16S RNA (green); proteins red and blue.

**rRNA is the actual catalyst for protein synthesis, with the ribosomal proteins making only a minor contribution!!!!**

The 3D structure of the ribosome depends on the secondary structure of RNA.

*Fig. 39.8* depicts the 2D and 3D structure of 16S rRNA.
**Polysomes:** a group of ribosomes bound to an mRNA and simultaneously carrying out translation (also called polyribosomes)

**Fig. 39.9.** In *E.coli* transcription is coupled to translation since there is no nucleus to separate the two events. Good view of polysomes in this figure.

**Sites for tRNA binding in ribosomes**

- Aminoacyl site
- Peptidyl site
- P site
- A site
- 5’
- 3’
- mRNA
- tRNA with amino acid
- Tunnel
- Growing peptide chain
There are 3 tRNA-binding sites, each with a different function, in a fully assembled ribosome: A, P and E site.

1. The **A (aminoacyl) site** binds the incoming tRNA.
2. The **P (peptidyl) site** binds the tRNA with the growing peptide chain.
3. The **E (exit) site** binds the uncharged tRNA before it leaves the ribosome.
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**Initiation: Structure of fMet-tRNA\textsubscript{Met}**

*First codon in mRNA is usually AUG
*recognized by **initiator** tRNA
*Bacteria: N-formylmethionyl-tRNA\textsubscript{fMet}

**Eukaryotes:** methionyl-tRNA\textsubscript{iMet}
In Bacteria

Initiator tRNA (tRNA_f) is charged with methionine and then a formyl group is transfered to the methionyl- tRNA_f from N10-formyltetrahydrofolate.

![Fig. 40.4](image-url)

Initiation Complexes Assemble at Initiation Codons

In prokaryotes 30S ribosome binds to a region of the mRNA (Shine-Dalgarno sequence; purine-rich sequence) upstream of the initiation sequence

- Ribosome-binding sites at 5' end of mRNA for E. coli proteins
- S-D sequences (red) occur immediately upstream of initiation codons (blue)
Fig. 40.3 Initiation sites in E. coli RNA.

- Complementary base pairing of S-D sequence

![Diagram of initiation sites in E. coli RNA]

- E. coli trpA
- E. coli araB
- E. coli thrA
- E. coli lacI
- φX174 phage A protein
- Qβ phage replicase
- R17 phage A protein
- λ phage cro

Pairs with 16S rRNA
Pairs with initiator tRNA
Comparison of prokaryotic and eukaryotic ribosomes

Initiation: formation of the prokaryotic 70S ribosome

Initiation factors are required to form a complex
(1F-1, IF-2, IF 3 in prokaryotes)

IF-1: binds to 30S and facilitates IF-2 & IF-3

IF-3: prevent premature association with 50S subunit; helps position fMET-tRNA & initiation codon at P site
The initiator tRNA binds to the P site of the ribosome!

Fig. 40.5
Overview of Translation initiation in prokaryotes.
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Elongation phase

Insertion of aa-tRNA by EF-Tu during chain elongation
Formation of the correct complex triggers hydrolysis of GTP, which alters the conformation of EF-Tu. EF-Tu dissociates, leaving behind a correctly inserted aminoacyl-tRNA.

Note: cost of one ATP

**Fig. 40.6**
Structure of Elongation factor Tu bonding to an aminoacyl-tRNA.

Note: EF-Tu does NOT interact with fMet-tRNA_{f}.
Cycling of EF-Tu-GTP

1. Aminoacyl-tRNA is delivered to the ribosome, and GTP is hydrolyzed, causing the EF-Tu-GDP complex to dissociate.

2. The inactive EF-Tu-GDP complex is recognized by elongation factor EF-Ts, which promotes dissociation of GDP.

3. The EF-Tu-EF-Ts complex binds GTP, which causes EF-Ts to dissociate.

4. Regenerated EF-Tu-GTP binds another aminoacyl-tRNA molecule.

EF-Tu-GTP-aminoacyl-tRNA complex
Formation of a peptide bond catalyzed by Peptidyl transferase (activity in large ribosomal subunit)

Catalytic activity from 23S rRNA (an RNA-catalyzed reaction!)

RNA has the catalytic activity of the ribosome large subunit

Atomic resolution crystal structures of the large subunit published since the middle of August 2000 prove that the peptidyl transferase center of the ribosome, which is the site of peptide-bond formation, is composed entirely of RNA; the ribosome is a ribozyme. They also demonstrate that alignment of the CCA ends of ribosome-bound peptidyl tRNA and aminoacyl tRNA in the peptidyl transferase center contributes significantly to its catalytic power.

Translocation step: new peptidyl-tRNA moved from A site to P site; mRNA shifts by one codon

(1) Deaminoacylated tRNA shifts from the P site to E site (exit site)

(2) Elongation factor G (EF-G) (translocase) bound to GTP competes for partially open A site

Note: cost of one ATP
Binding of EF-G-GTP to ribosome completes translocation of peptidyl-tRNA

Translocation shifts the peptidyl-tRNA completely into the P site, leaving the A site empty and ejecting the deaminoacylated tRNA from the E site.

Deaminoacylated tRNA is ejected.

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Termination of Translation

• One of three termination codons binds to A site: UGA, UAG, UAA

• No tRNA molecules recognize these codons; protein synthesis stalls

• One of release factors (RF-1, RF-2, RF-3 in *E. coli*) binds and causes hydrolysis of the peptidyl-tRNA to release the polypeptide chain

Protein Synthesis is Energetically Expensive

Four phosphoanhydride bonds cleaved for each amino acid added to polypeptide chain

**Amino acid activation:** Two ~P bonds

\[
\text{ATP} \longrightarrow \text{AMP} + 2 \text{P}_i
\]

**Chain elongation:** Two ~P bonds

\[
2 \text{GTP} \longrightarrow 2 \text{GDP} + 2 \text{P}_i \quad (EF-Tu \text{ and } EF-G)
\]
Bacteria and Eukaryotes differ in the initiation of Protein Synthesis.

Basic protein synthesis mechanisms same for all organisms, but eukaryotic protein synthesis more complex in number of ways:

1. Ribosomes larger, consist of 40S and 60S subunits and form 80S ribosome.

2. Protein synthesis begins with a methionine rather than formylmethionine. Special initiator tRNA called Met-tRNAi required.

3. Initiator codon always first AUG from the 5’ end of the mRNA. More protein initiation factors are required.

Bacteria and Eukaryotes differ in the initiation of Protein Synthesis. (continued)

4. mRNA is circular because of interactions between proteins that bind the 5’ cap and those that bind the poly A tail.

5. Elongation and termination similar in eukaryotes and bacteria except bacteria have multiple release factors while eukaryotes have only one.

6. Protein synthesis occurs in nucleus in eukaryote; protein synthesis machinery organized into large complexes associated with the cytoskeleton.
Comparison of rates of translation, transcription and DNA replication in *E. coli*.

Rate of protein synthesis: 18-40 aa/sec

Rate of transcription: 30-85 nucleotides/sec

Rate of DNA synthesis: 1000 nucleotides/sec

**Fig. 40.14** Protein interactions circularize eukaryote mRNA. Initiation factors interact with poly(A) tail binding protein (PABP1).
In eukaryotes, **protein sorting** or **protein targeting** is the process of directing proteins to distinct organelles such as the nucleus, mitochondria, and endoplasmic reticulum, or directing them out of the cell.

Two pathways are used to sort proteins. In one, completed proteins are synthesized in the cytosol and then delivered to the target.

The other pathway is called the **secretory pathway**, in which proteins are inserted into the ER membrane co-translationally.

Protein synthesis in the secretory pathway occurs on ribosomes bound to the ER. ER with ribosomes bound is called the **rough ER** or RER.

**Rough ER** is that ER which binds ribosomes and functions in co-translocation of proteins across the ER and into the secretory pathway or to other organelle membrane locations. **Fig. 40.19**
Synthesis of proteins bound for secretory pathway begins on ribosomes that are free in the cytoplasm.

Once a portion of the nascent protein that contains a specific signal emerges from the ribosome, synthesis is halted and the ribosome complex is directed to ER.

Once bound to ER, protein synthesis is reactivated, with the nascent protein now directed through the membrane of the ER.

Several components are required for cotranslational insertion of proteins into the ER.

1. **Signal sequence:**
   * sequence of 9 to 12 hydrophobic amino acids, sometimes with positively charge amino acids, often located at N-terminal region of the primary structure
   * identifies nascent protein as one that must cross ER membrane.
   * signal peptidase in lumen of ER may remove signal sequence.

2. **Signal-recognition particle (SRP):**
   * GTP-binding ribonucleoprotein with GTPase activity
   * binds signal sequence as it exists ribosome and directs complex to ER.
   * Binding of SRP to ribosome halts protein synthesis.
3. **SRP receptor**: a dimer integral membrane protein with GTPase activity, binds to SRP-ribosome complex.

4. **Translocon**:
   * protein-conducting channel
   * accepts ribosome from SRP-SRP receptor complex and protein synthesis begins again with protein now passing through the membrane in the translocon.

Upon GTP hydrolysis, the SRP and SRP-receptor dissociate and begin another cycle.

---

**Fig. 40.20**
Signal Recognition Particle (SRP) targeting cycle

---

*Figure 4.0.19* 
Iron is a key component of many important proteins, e.g., hemoglobin and cytochromes.

However, iron can generate destructive reaction oxygen species. Thus, iron transport and storage MUST be carefully regulated.

Proteins involved in iron metabolism:
* **transferrin**: blood protein that transports iron
* **transferrin receptor**: membrane protein that binds iron-rich transferrin and facilitates its entry into the cell
* **ferritin**: iron storage protein in the cell.

Translation can be regulated at several levels.

**Example:** regulation of proteins involved in iron uptake

**I: Post-transcriptional regulation of transcript expression**

Iron is a key component of many important proteins, e.g., hemoglobin and cytochromes.

However, iron can generate destructive reaction oxygen species. Thus, iron transport and storage MUST be carefully regulated.

**Proteins involved in iron metabolism:**
* **transferrin**: blood protein that transports iron
* **transferrin receptor**: membrane protein that binds iron-rich transferrin and facilitates its entry into the cell
* **ferritin**: iron storage protein in the cell.

Ferritin mRNA contains a stem-loop structure in the 5’ untranslated region called the iron response element (IRE).

In absence of iron, the protein IRE-binding protein (IRE-BP) binds to IRE and prevents translation.

When iron is present, it binds IRE-BP causing it to dissociate from the IRE thereby allowing translation to occur.

Thus in absence of iron, the protein that transports iron (i.e., transferrin) is not unnecessarily made.
Translation can be regulated at several levels.

Another Example: regulation of proteins involved in iron uptake

II. Regulation of stability of mRNA

Transferrin-receptor also has several IRE located in 3’ untranslated region.

When little iron is present, IRE-BP binds to IRE, thereby allowing the transferrin-receptor mRNA to be translated.

When present, iron binds to IRE-BP causing it to dissociate from transferrin-receptor mRNA. Devoid of the IRE-BP, the receptor mRNA is degraded.

Fig. 40.22
Transferrin-receptor mRNA and its IREs

Thus in the presence of high amounts of iron, IRE-PB dissociates from the mRNA and the RNA is degraded. Thus excess iron is not stored in the cell.
**THUS: IRE-BPs serve as iron sensors.**

When enough iron is present to bind IRE-BP, ferritin is synthesized to store the iron.

If enough iron is present to be stored, the uptake receptor is no longer needed, so its mRNA is degraded.

*Iron balance is maintained!*

---

**Small RNAs can regulated mRNA stability and use.**

**RNA interference (RNAi)** leads to mRNA degradation induced by presence of foreign double-stranded RNA, which may be present during certain viral infection.

* **Dicer**: a ribonuclease, cleaves double-stranded RNA into 21-nucleotide fragments. Single-stranded components of these are called small interfering RNA (siRNA).

* **siRNA** are bound by class of proteins called Argonaute family to form RNA induced silencing complex (RISC).

* **RNA induced silencing complex (RISC)**: locates mRNA complementary to the siRNA and degrades the mRNA.
Small RNAs, called microRNAs (miRNA) are generated from large precursor RNAs encoded in the genome. The association of these miRNAs with Argonaute to form a complex regulates translation in one of two ways.

1) If siRNA binds to mRNA by precise Watson-Crick base-pairing, mRNA is degraded.
2) If base-pairing is not precise, translation of the mRNA is inhibited but mRNA not destroyed.

60% of human genes are regulated by miRNA.