Chapter 3.5
Protein Synthesis
Summary of Protein Synthesis

- Information transfer in process of protein synthesis
  - DNA ➔ mRNA ➔ protein

- transcription – the step from DNA to mRNA
  - occurs in the nucleus where DNA is located

- translation – the step from mRNA to protein
  - occurs in cytoplasm
  - 15-20% of proteins are synthesized in the nucleus
Step 1: Transcription

Step 2: Translation
Overview of Protein Synthesis

- all somatic cells contain identical chromosomes which contain many genes

- different genes are activated in different cells // some genes are “turned on” and other genes are “turned off”

- Note: Genes are receipts for making proteins

- any given cell uses 1/3 to 2/3rds of its genes
  - rest remain dormant

  - dormant genes in one cell type may be functional in another type of cell

  - Allows for different cells to make different products
Overview of Protein Synthesis

Activated genes / turned on / followed by transcription and translation // three type of ribonucleic acids required for protein synthesis

- **messenger RNA** (mRNA) is assembled
  - a mirror-image copy of the gene is made
  - migrates from the nucleus to cytoplasm
  - its code is read by the ribosomes

- **Ribosomal RNA** (rRNA)
  - cytoplasmic organelles composed of ribosomal RNA (rRNA) and enzymes
  - ribosomes assemble amino acids in the order directed by the codons of mRNA

- **transfer RNA** (tRNA) – delivers amino acids to the ribosome
Transcription

• DNA too large to leave nucleus and participate directly in protein synthesis which takes place in the cytoplasm

  – necessary to make a small mRNA copy of the DNA gene that can migrate through a nuclear pore into the cytoplasm

• Transcription = copying genetic instructions from DNA to RNA
RNA Polymerase – enzyme that binds to the DNA and assembles the mRNA

- base sequences TATATA or TATAAA inform the polymerase where to begin

- RNA polymerase opens up the DNA helix about 17 base pairs at a time

- reads base from one strand of DNA

- makes corresponding mRNA
  - where it finds C on the DNA, it adds G to the mRNA
  - where it finds A on the DNA, it adds U to the mRNA

- RNA polymerase rewinds the DNA helix behind it

- gene can be transcribed by several polymerase molecules at once

- terminator – base sequence at the end of a gene which signals polymerase to stop
In DNA replication, A binds to T, but in RNA replication (making mRNA), U substitutes for T, therefore the DNA template will bind to “U”.

```
Template DNA base sequence
```

```
Complementary RNA base sequence
```
Transcription

• **pre-mRNA** – immature RNA produced by transcription

• **exons** – “sense” portions of the immature RNA
  – will be translated to protein

• **introns** – “nonsense” portions of the immature RNA
  – must be removed before translation

• alternative splicing
  – removing the introns by enzymes
  – splicing and arranging the exons together into different “patterns” to form functional RNA molecule
  – one gene can code for more than one protein
Alternative Splicing of mRNA

- One gene can code for more than one protein
- Exons can be spliced together into a variety of different mRNAs.
Translation

• process that converts the language of nucleotides into the language of amino acids

• ribosomes (rRNA) - translate sequence of nucleotides into the sequence of amino acids
  – occur mainly in cytosol // but two different locations for different usage

  • on surface of rough ER and nuclear envelope (proteins for export)
  
  • free rRNA in cytoplasm (proteins for cell use)

  – consists of two granular subunits, large and small rRNA subunits // each made of several rRNA and enzyme molecules
Translation of mRNA

mRNA molecule begins with leader sequence

- acts as binding site for small ribosomal subunit
- large subunit attaches to small subunit
- ribosome pulls mRNA molecule through it like a ribbon, reading the bases as it goes
- when start **codon** (AUG) is reached, protein synthesis begins
- all proteins begin with **methionine** when first synthesized
Translation

- Requires the participation of transfer RNA (tRNA)
  - small RNA molecule
  - coils on itself to form an angular L shape
  - one end of the L includes three nucleotides called an anticodon
  - other end has binding site specific for one amino acid
  - each tRNA picks up specific amino acid from pool of free amino acids in cytosol
  - one ATP molecule is used to bind amino acid to site
  - provides energy for peptide bond formation
Translation

- some imprecision in codon-anticodon pairing
  - takes only 48 different tRNAs to pair with 61 different codons
- ribosome binds and holds tRNA with its specific amino acid
- large ribosomal subunit contains an enzyme that forms peptide bond that links amino acids together
- first tRNA released from ribosome
- second tRNA temporarily anchors growing peptide chain
- ribosome shifts and third tRNA brings its amino acid to the site
Polyribosomes

- polyribosome - one mRNA holding multiple ribosomes
  - One ribosome can assemble protein of 400 amino acids in 20 seconds
  - 300,000 identical mRNA molecules may be undergoing simultaneous translation
- cell can produce 150,000 protein molecules per second
(a) Components of a ribosome and their relationship to mRNA and protein during translation

(b) Interior view of tRNA binding sites
### Table 8.5 Translation

<table>
<thead>
<tr>
<th>Step</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>The mRNA molecule leaves the DNA transcription site and is transported to ribosomes in the cytoplasm. Ribosomal subunits come together and form sites to hold the mRNA and tRNAs. The ribosome begins to scan the mRNA by moving in the 5' to 3' direction along the mRNA. The first codon it encounters is called the <strong>START</strong> codon, which is almost always AUG (and, rarely, GUG). With the mRNA message in place on the assembled ribosome, the next step in translation involves entrance of tRNAs with their amino acids. The pool of cytoplasm contains a complete array of tRNAs, previously charged by having the correct amino acid attached. The step in which the complementary tRNA meets with the mRNA code is guided by the two sites on the large subunit of the ribosome called the P site (left) and the A site (right). The ribosome also has an exit or E site where used tRNAs are released. (P stands for peptide site; A stands for aminoacyl (amino acid) site; E stands for exit site.)</td>
</tr>
<tr>
<td>2</td>
<td>Rules of pairing dictate that the anticodon of this tRNA must be complementary to the mRNA codon AUG; thus, the tRNA with anticodon UAC will first occupy site P. It happens that the amino acid carried by the initiator tRNA in bacteria is <strong>formyl methionine</strong>. The formyl group provides a special signal that this amino acid is not part of the translated protein because usually fMet does not remain a permanent part of the finished protein but instead is cleaved from the finished peptide. The ribosome shifts its “reading frame” to the right along the mRNA from one codon to the next. This brings the next codon into place on the ribosome and makes a space for the next tRNA to enter the A position. A peptide bond is formed between the amino acids on the adjacent tRNAs, and the polypeptide grows in length. Elongation begins with the filling of the A site by a second tRNA. The identity of this tRNA and its amino acid is dictated by the second mRNA codon.</td>
</tr>
<tr>
<td>3</td>
<td>The entry of tRNA 2 into the A site brings the two adjacent tRNAs in favorable proximity for a peptide bond to form between the amino acids (aa) they carry. The fMet is transferred from the first tRNA to aa 2, resulting in two coupled amino acids called a dipeptide. For the next step to proceed, some room must be made on the ribosome, and the next codon in sequence must be brought into position for reading. This process is accomplished by translocation, the enzyme-directed shifting of the ribosome to the right along the mRNA strand, which causes the blank tRNA 1 to be discharged from the ribosome at the E site.</td>
</tr>
</tbody>
</table>

![Diagram of Translation Process](image-url)
**Table 8.5 Translation (continued)**

4. First translocation

- The tRNA holding the dipeptide moves to the P position. Site A is temporarily left empty. The tRNA that has been released is now free to drift off into the cytoplasm and become recharged with an amino acid for later additions to this or another protein.

5. Formation of peptide bond

- The stage is now set for the insertion of tRNA 3 at site A as directed by the third mRNA codon. This insertion is followed once again by peptide bond formation between the dipeptide and amino acid 3 (making a tripeptide), splitting of the peptide from tRNA 2, and translocation.

6. Discharge of tRNA 2; second translocation; enter tRNA 4

- This releases tRNA 2, shifts mRNA to the next position, moves tRNA 3 to position P, and opens position A for the next tRNA (which will be called tRNA 4).

7. Formation of peptide bond

- From this point on, peptide elongation proceeds repetitively by this same series of actions out to the end of the mRNA.

8. Peptide bond 3

- The termination of protein synthesis is not simply a matter of reaching the last codon on mRNA. It is brought about by the presence of at least one special codon occurring just after the codon for the last amino acid. Termination codons—UAA, UAG, and UGA—are codons for which there is no corresponding tRNA. Although they are often called nonsense codons, they carry a necessary and useful message: Stop here. When this codon is reached, a special enzyme breaks the bond between the final tRNA and the finished polypeptide chain, releasing it from the ribosome.
Review of “Steps” in Peptide Formation

1. DNA double helix

2. Seven base triplets on the template strand of DNA

3. The corresponding codons of mRNA transcribed from the DNA triplets

4. The anticodons of tRNA that bind to the mRNA codons

5. The amino acids carried by those six tRNA molecules

6. The amino acids linked into a peptide chain
### Possible “Codons” and Amino Acids Which Codons Code For

<table>
<thead>
<tr>
<th>First Base Position</th>
<th>Second Base Position</th>
<th>Third Base Position</th>
</tr>
</thead>
<tbody>
<tr>
<td>U</td>
<td>UU</td>
<td>UU</td>
</tr>
<tr>
<td></td>
<td>UUC</td>
<td>UUC</td>
</tr>
<tr>
<td></td>
<td>UUA</td>
<td>UUA</td>
</tr>
<tr>
<td></td>
<td>UUG</td>
<td>UUG</td>
</tr>
<tr>
<td></td>
<td>CUU</td>
<td>CUU</td>
</tr>
<tr>
<td></td>
<td>CUC</td>
<td>CUC</td>
</tr>
<tr>
<td></td>
<td>CUA</td>
<td>CUA</td>
</tr>
<tr>
<td></td>
<td>CUG</td>
<td>CUG</td>
</tr>
<tr>
<td></td>
<td>AUU</td>
<td>AUU</td>
</tr>
<tr>
<td></td>
<td>AUC</td>
<td>AUC</td>
</tr>
<tr>
<td></td>
<td>AUA</td>
<td>AUA</td>
</tr>
<tr>
<td></td>
<td>AUG (START)</td>
<td>AUG</td>
</tr>
<tr>
<td></td>
<td>GUU</td>
<td>GUU</td>
</tr>
<tr>
<td></td>
<td>GUC</td>
<td>GUC</td>
</tr>
<tr>
<td></td>
<td>GUA</td>
<td>GUA</td>
</tr>
<tr>
<td></td>
<td>GUG</td>
<td>GUG</td>
</tr>
</tbody>
</table>

**Possible Codons and Amino Acids**

- **Phenylalanine**: UUU, UUC
- **Leucine**: UUA, UUG, CUU, CUC
- **Valine**: GUU, GUC, GUA, GUG
- **Isoleucine**: AUU, AUC, AUA, AUG
- **Tryptophan**: UGU, UGC, UGA, UGG
- **Stop**: UAA, UAG
- **Start**: AUG

**Amino Acids**

- **Phenylalanine**, **Valine**, **Isoleucine**, **Leucine**, **Tryptophan**, **Stop**, **Start**

**Notes**

- This codon initiates translation.
- For these codons, which give the orders to stop translation, there are no corresponding tRNAs and no amino acids.
Details of ribosomal subunits

Large subunit + Small subunit → Complete functional ribosome
Protein Processing and Secretion

- protein synthesis is not finished when the amino acid sequence (primary structure) has been assembled.

- to be functional it must coil or fold into precise secondary and tertiary structure

- Chaperone proteins
  - older proteins that pick up new proteins and guides the new protein in folding into the proper shapes
  - helps to prevent improper association between different proteins
  - also called stress proteins or heat-shock proteins
    - chaperones produced in response to heat or stress
    - help damaged protein fold back into correct functional shapes
Protein Packaging and Secretion

1. Protein formed by ribosomes on rough ER.

2. Protein packaged into transport vesicle, which buds from ER.

3. Transport vesicles fuse into clusters that unload protein into Golgi complex.


5. Golgi vesicle containing finished protein is formed.


Rough ER

Ribosomes

Clathrin-coated transport vesicle

Nucleus

Lysosome

Golgi complex
Protein Processing and Secretion

• proteins to be used in the cytosol are likely to be made on free ribosomes in cytosol

• proteins destined for packaging into lysosomes or secretion from the cell are assembled on rough ER and sent to golgi complex for packaging
  – entire polyribosome migrated to the rough ER and docks on its surface
  – assembled amino acid chain completed on rough ER
  – then sent to Golgi for final modification
Protein Processing and Secretion

- proteins assembled on ER surface
- threads itself through a pore in the ER membrane into cisterna
- modifies protein by posttranslational modification
  - removing some amino acid segments
  - folding the protein
  - stabilizing protein with disulfide bridges
  - adding carbohydrates
Protein Processing and Secretion

• when rough ER finished with protein

  – pinches off bubblelike transport vesicle coated with clathrin

  – clathrin helps select the proteins to be transported in vesicles and helps mold forming vesicle

  – vesicles detach from ER and carry protein to the nearest cisterna of Golgi complex
Protein Processing and Secretion

- vesicles fuse and unloads proteins into Golgi cisterna

- Golgi complex further modifies the protein

- passes from cisterna closest to ER to cisterna farthest from ER

- buds off new coated Golgi vesicles containing finished protein

- some Golgi vesicles become lysosomes

- **others become secretory vesicles** migrate to plasma membrane, fuse to it, and release their cell product by **exocytosis**
(b) Transverse section
(b) Several lysosomes
Mitochondria Undergo Mitosis Independent of the Cell’s Genome

(What does this suggest? – endosymbiosis!)

All mitochondria are “maternal” meaning they come from the egg. (sperm do not contribute any mitochondria to the zygote)

Mitochondria have their “own” genetic information.

Mitochondria’s DNA is a “circular” chromosome // prokaryote architecture.

At one time in history mitochondria lived as an independent self sustaining organism. Much like the bacteria of today.
(b) Transverse section

- **Outer mitochondrial membrane**
- **Inner mitochondrial membrane**
- **Mitochondrial matrix**
- **Mitochondrial cristae**
How Genetic Mutations Lead to Disease

Gene mutations can disrupt biology at multiple levels (molecules, cells, tissues and organs) to cause disease. Certain mutations are particularly prevalent in Amish and Mennonite populations. For each patient the clinic sees, it applies advanced technologies to identify the individual’s genetic variants, understand their causal links to disease, and devise ways to alleviate or prevent the mutations’ harmful effects. In related work, the clinic and its collaborators recently identified a gene mutation linked to bipolar disorder among the Amish, and they are now constructing a picture of how it might impair emotional regulation (below). This knowledge could lead to a deeper understanding of bipolar disorder in the general population and to new strategies for prevention and treatment.

Gene
A gene consists of a sequence of DNA “letters” that spell out the amino acids needed to make a protein. Proteins are the main workhorses of cells. A mutation in a gene can alter the functioning of the encoded protein. The bipolar study pinpointed a mutation in a gene called KCNH7.

Protein
To function properly, proteins must have the right structure, location and abundance in each cell. KCNH7 encodes a protein that spans the cell membrane, forming a channel that regulates the flow of potassium ions. The mutant is altered at just a single amino acid, but this subtle change affects potassium movement across the membrane.

Cell
All cells contain the same genes, but many genes are expressed (that is, give rise to proteins) only in select cell types. The ion channel encoded by KCNH7 is used by neurons throughout the brain. Potassium currents critically shape each neuron’s electrical behavior, and the mutant alters the cells’ firing patterns.

Tissue
Tissues can contain a mixture of cell types. Brain tissue, for instance, includes neurons and supporting cells called glia. The mutant KCNH7 gene would be expected to disrupt the operation, not only of individual nerve cells, but of whole neuronal circuits, such as those regulating emotions and behavior.

Organ
Nerve cells throughout the brain make the ion channel encoded by the KCNH7 gene, but the channel is most abundant in brain regions underlying emotions and cognition. Consistent with that finding, mutation of the gene has been tied to mania observed in laboratory animals.

Behavior
Bipolar disorder is marked by a spectrum of behaviors that can include depression, mania and psychosis. New insight into how the KCNH7 mutation affects each level of biology—from misspelled protein to perturbed brain function—could lead to fresh ideas for interrupting the chain of events underlying the disorder.
Summary of Protein Synthesis
(Images Only)
(a) Components of a ribosome and their relationship to mRNA and protein during translation
1. Initiator tRNA attaches to a start codon.

2. Large and small ribosomal subunits join to form a functional ribosome and initiator tRNA fits into P site.

3. Anticodon of incoming tRNA pairs with next mRNA codon at A site.

4. Amino acid on tRNA at P site forms a peptide bond with amino acid at A site.

5. The two-peptide protein created from the formation of the peptide bond becomes attached to tRNA at A site.

6. Ribosome shifts by one codon; tRNA previously at P site enters E site and is released from ribosome; tRNA previously at A site is now at P site.

7. Protein synthesis stops when the ribosome reaches stop codon on mRNA.

Key:
- Green = Adenine
- Blue = Guanine
- Orange = Cytosine
- Purple = Uracil
1. Initiator tRNA attaches to a start codon.
Large and small ribosomal subunits join to form a functional ribosome and initiator tRNA fits into P site.
3 Anticodon of incoming tRNA pairs with next mRNA codon at A site.
Amino acid on tRNA at P site forms a peptide bond with amino acid at A site.
5 The two-peptide protein created from the formation of the peptide bond becomes attached to tRNA at A site.
6 Ribosome shifts by one codon: tRNA previously at P site enters E site and is released from ribosome; tRNA previously at A site is now at P site.
Summary of movement of ribosome along mRNA
Protein synthesis stops when the ribosome reaches stop codon on mRNA.
In DNA replication A binds to T but in RNA replication (making mRNA) U substitutes for T therefore the DNA template will bind to “U”
Flagellum
Proteasome
Intermediate filament
Centrosome
Lysosome
Smooth ER
Peroxisome
Microtubule
Cilium
NUCLEUS
CYTOPLASM
PLASMA MEMBRANE
Ribosome on rough ER
Golgi complex
Mitochondrion
Microfilament