Chapter 10: How Proteins are Made

Section 1: From Genes to Proteins
From DNA to Protein

DNA → RNA → Protein → Trait

- **Ribonucleic acid (RNA):**
  - Present in all living cells
  - A nucleic acid (like DNA) made up of linked nucleotides
  - Plays role in protein synthesis
DNA versus RNA

• DNA:
  – Double stranded
  – Contains deoxyribose as sugar
  – Contains thymine (T) as complementary base pair to adenine (A)

• RNA:
  – Single stranded
  – Contains ribose as sugar
  – Contains uracil (U) as complementary base pair to adenine (A)
Types of RNA

• There are **three** types of RNA that participate in the process of gene expression:
  – Messenger RNA
  – Transfer RNA
  – Ribosomal RNA
Messenger RNA

• **Messenger RNA (mRNA):**
  – Form of RNA that carries the instructions for making a protein from a gene
  – Delivers it to the site of translation
Transfer RNA

Transfer RNA (tRNA):
- Transfers amino acids to the growing end of a polypeptide chain during translation
- Folded into compact shape and contains anticodon

anticodon
Ribosomal RNA

• **Ribosomal RNA (rRNA):**
  – Part of structure of ribosomes
  – Each ribosome temporarily holds 1 mRNA and 2 tRNA molecules
Introduction to Protein Synthesis

DNA → Transcription → RNA → Translation → Protein

- Uracil
Transcription

- **Transcription:**
  - Process of forming a nucleic acid by using another molecule as a template
  - More specifically, the process of synthesizing RNA by using one strand of DNA as a template
  - Location:
    - Eukaryotes: nucleus
    - Prokaryotes: cytoplasm
  - Three-step process
Transcription: Step 1

• Step 1:
  – RNA polymerase binds to the promoter site on DNA

• RNA polymerase: enzyme that catalyzes the formation of RNA by using a strand of DNA as a template

• Promoter: specific sequence of DNA that acts as a “start” signal for transcription
Transcription: Step 2

- Step 2:
  - RNA polymerase unwinds and separates the two strands of the double helix of DNA, exposing DNA nucleotides on each strand
Transcription: Step 3

• Step 3:
  – RNA polymerase adds and then links, using covalent bonds, complementary RNA nucleotides as it “reads” the gene

• Recall:
  – A ↔ U
  – C ↔ G
Transcription: What’s Next?

- RNA polymerase moves along nucleotides of DNA strand, proceeding until it reaches a “stop” signal on the DNA.
- Behind RNA polymerase, the two strands of DNA close up by reforming hydrogen bonds between complementary base pairs.
Speed of Transcription

• During transcription, many identical RNA molecules are made simultaneously from a single gene
  – Eukaryotes:
    • 100 RNA polymerase molecules per gene
    • 60 nucleotides added per second by each RNA polymerase
DNA Replication versus Transcription

• DNA Replication:
  – DNA polymerase is used
  – DNA nucleotides are linked
  – Both strands of DNA serve as a template

• Transcription:
  – RNA polymerase is used
  – RNA nucleotides are linked
  – Only one part of one strand (the gene) is used as a template
From RNA to Protein...

- The information must now be translated from the language of RNA – nucleotides - to that of proteins – amino acids

- **Codon**: three nucleotide sequence
  - Encodes an amino acid
  - Signifies start or stop signal
  - Discovered in 1961 by American biochemist Marshall Nirenberg
The Genetic Code

• **Genetic code**: rule that describes how a sequence of nucleotides, read in groups of three consecutive nucleotides (triplets) that correspond to specific amino acids, specifies the amino acid sequence of proteins.
# Codes in mRNA

<table>
<thead>
<tr>
<th>First base</th>
<th>U</th>
<th>Second base</th>
<th>A</th>
<th>G</th>
<th>Third base</th>
</tr>
</thead>
<tbody>
<tr>
<td>U</td>
<td>UUU</td>
<td></td>
<td>UAU</td>
<td>UGU</td>
<td>U</td>
</tr>
<tr>
<td></td>
<td>UUC</td>
<td></td>
<td>UAC</td>
<td>UGC</td>
<td>C</td>
</tr>
<tr>
<td></td>
<td>UUA</td>
<td></td>
<td>UAA</td>
<td>UGA</td>
<td>A</td>
</tr>
<tr>
<td></td>
<td>UUG</td>
<td></td>
<td>UAG</td>
<td>UGG</td>
<td>G</td>
</tr>
<tr>
<td>C</td>
<td>CUU</td>
<td></td>
<td>CAU</td>
<td>CGU</td>
<td>U</td>
</tr>
<tr>
<td></td>
<td>CUC</td>
<td></td>
<td>CAC</td>
<td>CGC</td>
<td>C</td>
</tr>
<tr>
<td></td>
<td>CUA</td>
<td></td>
<td>CAA</td>
<td>CGA</td>
<td>A</td>
</tr>
<tr>
<td></td>
<td>CUG</td>
<td></td>
<td>CAG</td>
<td>CGG</td>
<td>G</td>
</tr>
<tr>
<td>A</td>
<td>AUU</td>
<td></td>
<td>AUA</td>
<td>AGU</td>
<td>U</td>
</tr>
<tr>
<td></td>
<td>AUC</td>
<td></td>
<td>AAC</td>
<td>AGC</td>
<td>C</td>
</tr>
<tr>
<td></td>
<td>AUA</td>
<td></td>
<td>ACA</td>
<td>AGA</td>
<td>A</td>
</tr>
<tr>
<td></td>
<td>AUG</td>
<td></td>
<td>AAG</td>
<td>AGG</td>
<td>G</td>
</tr>
<tr>
<td>G</td>
<td>GUU</td>
<td></td>
<td>GAU</td>
<td>GGU</td>
<td>U</td>
</tr>
<tr>
<td></td>
<td>GUC</td>
<td></td>
<td>GAC</td>
<td>GGU</td>
<td>C</td>
</tr>
<tr>
<td></td>
<td>GUA</td>
<td></td>
<td>GAA</td>
<td>GGC</td>
<td>A</td>
</tr>
<tr>
<td></td>
<td>GUG</td>
<td></td>
<td>GAG</td>
<td>GGA</td>
<td>G</td>
</tr>
</tbody>
</table>

- **Phenylalanine**
- **Leucine**
- **Serine**
- **Tyrosine**
- **Stop**
- **Cysteine**
- **Glycine**
Translation

- Portion of protein synthesis that takes place at ribosomes in the cytoplasm
- Uses codons of mRNA molecules to specify the sequence of amino acids in protein chains
- 7 step process
Ribosomes: The Site of Protein Synthesis

• Each ribosome can hold:
  • 1 mRNA molecule
  • 2 tRNA molecules, one at each of the two possible regions
  • A site
  • P site
What Happens After Transcription?

- After transcription, mRNA travels into cytoplasm and anchors to the ribosome, forming a ribosome-mRNA complex.
  - Recall: mRNA contains a universal “start” codon (AUG) signaling where a gene begins and, hence, where translation will begin.
  - AUG oriented in P site of ribosome
- Meanwhile, tRNA molecules in the cytoplasm are binding loosely with specific amino acids.
Translation: Step 1

- Once the mRNA is anchored on the ribosome, a tRNA molecule carrying the amino acid Methionine (Met) binds to the start codon on mRNA
  - Why Met?
    - AUG codes for Met/start
  - How does tRNA bind to mRNA?
    - Recall: each tRNA molecule contains an **anticodon**, a 3-nucleotide sequence on tRNA that is complementary to an mRNA codon
Translation: Step 2

• Let’s review:
  – P site is now holding 1 tRNA
  – A site is still open, with the next codon on mRNA ready to bind to its complementary tRNA anticodon

• tRNA carrying the amino acid specified by the codon on the A site arrives
Translation: Step 3

• Let’s Review:
  – Both the P site and A site are holding a tRNA molecule, each carrying a specific amino acid

• A peptide bond (linkage of amino acids) forms between the amino acids of the 2 tRNA molecules in the P site and A site
  – Enzymes help form these peptide bonds
Translation: Step 4

- tRNA in the P site detaches and leaves its amino acid behind
  - Peptide bond between amino acids holds them together, leading to the formation of a growing protein chain
Translation: Step 5

• Let’s Review:
  – P site is empty
  – A site still holding a tRNA molecule

• As a unit, the bound mRNA and tRNA in A site move to P site
  – A site is now empty, leaving the next codon on mRNA free to bind with complementary tRNA anticodon

• tRNA carrying the amino acid specified by the codon in the A site arrives and another peptide bond is formed
Translation: Step 6

- tRNA in P site detaches and leaves its amino acid behind and peptide bond is formed.
- A site is again empty, leaving the next mRNA codon ready for its complementary tRNA anticodon.
Translation: Step 7

- Process is repeated until a stop codon is reached
  - Ribosome complex falls part
  - Newly-synthesized protein is released
- Why stop codons?
  - No complementary anticodon on tRNA molecule
- Recall:
  - 3 mRNA stop codons
    - UAG
    - UAA
    - UGA
Translation: What’s Next?

• Many copies of the same protein can be made from a single mRNA molecule
  – As mRNA moves along ribosome, another ribosome can find the AUG codon on the same mRNA
The Genetic Code: Evolutionary Clues?

- Genetic code is nearly universal
  - Genetic code is the same in all organisms with few exceptions (see p. 214)
- Supports theory that all life-forms have common evolutionary ancestor
Chapter 10: How Proteins are Made

Section 2: Gene Regulation and Structure
Regulation of Protein Synthesis

• Organisms contain thousands of genes
  – Prokaryotes: ~ 2,000 genes
  – Eukaryotes: Variable
• Human genome (largest of all eukaryotes) contains 30,000 genes

• Why regulate?
  – Not all genes must be expressed consistently
  – Waste of cell’s time and energy
Gene Regulation in Prokaryotes

- Gene expression regulated using different components:
  - Operator
  - Operon
  - Repressor
Operator

- **Operator**: piece of DNA overlapping the promoter site (recall: “start” signal)
  - Serves as on-off switch
  - Controls RNA polymerase’s access to genes due to its position
Operon

- **Operon**: Unit of gene regulation comprised of three parts:
  - Promoter site
  - Operator
  - One or more structural genes
Repressor

- **Repressor**: Protein that binds to operator
  - Physically blocks RNA polymerase from binding to promoter site
  - Transcription ceases
Gene Regulation in Prokaryotes: The Metabolism of Lactose

- Dissacharide lactose found in dairy products is absorbed and broken down by E.coli in the intestinal tract
  - Lactose broken down into 2 components:
    - Glucose
    - Galactose
- Provides energy and materials for making other compounds
Genes Involved in Lactose Use

- 3 separate enzymes, each coded for by a different gene, are necessary for the processes involved in the metabolism of lactose:
  - Recognition
  - Consummation
  - Break down

- Genes coding for the enzymes located next to one another and are controlled by same promoter site
The *lac* Operon

- *lac operon*: gene system consisting of:
  - Operator gene
  - Three structural genes coding for enzymes
- Together control lactose metabolism in E.coli
The *lac* Operon: “Off” Mode

- If lactose is ABSENT:
  - Repressor is bound to operator
  - RNA polymerase cannot bind to promoter site
  - Transcription cannot begin
  - Bacterium saves energy, since enzymes are not produced until lactose is available
The *lac* Operon: “On” Mode

- If lactose is PRESENT:
  - Lactose binds to repressor
  - Repressor changes shape and falls off the operator
  - RNA polymerase can now bind to promoter site, allowing the process of transcription to begin
Gene Regulation in Eukaryotes

- Because nuclear envelope separates transcription from translation, more opportunities exist for regulating gene expression
  - Before, during, or after transcription
  - After translation
  - When protein is functional
  - Most regulation occurs at the onset of transcription, when RNA polymerase binds to a gene
Controlling the Onset of Transcription

• Rather than using operons, eukaryotic cells typically control protein synthesis using other units:
  – **Transcription factors**: regulatory proteins
    • Some help arrange RNA polymerase in the correct position on promoter
    • Others (**activators**) bind to enhancers
  – **Enhancers**: non-coding segments of DNA involved in regulation of protein synthesis
    • Typically located 1000’s of nucleotide bases away from promoter
Eukaryotic Regulation of Gene Expression, Illustrated

- Transcription begins when the activator bound to the enhancer comes into contact with the transcription factor and RNA polymerase at the promoter.
Intervening DNA in Eukaryotic Cells

• Unlike prokaryotic cells, that contain long uninterrupted stretches of nucleotides coding for a protein, genes of eukaryotic cells contain:
  – **Introns**: long segments of nucleotides that have NO coding information
  – **Exons**: portions of the gene that are translated (expressed) into proteins
  – **Spliceosomes**: complex assemblies of RNA and protein used to “cut out” introns from mRNA after transcription

• Once introns are removed from the mRNA, exons are “stitched” back together by the spliceosome, leaving a smaller mRNA molecule ready for translation
Removal of Introns After Transcription

1. DNA is transcribed into pre-mRNA.
2. Introns are removed.
3. The remaining exons are spliced together in mRNA.
4. The mRNA strand leaves the nucleus and enters the cytoplasm for translation into a protein.
Introns: Of Biological Significance?

- Evolutionary flexibility
  - Shuffling of exons between genes provides many options for producing different proteins
  - Source of genetic diversity essential for evolution
Gene Alterations

Gene alterations: mutation that changes a gene
- Point mutations
- Insertion mutations
- Deletion mutations

Usually disrupts protein’s function
Point Mutations

- **Point mutation**: substitution, addition, or removal of a single nucleotide
  - **Substitution**: one nucleotide in a codon is replaced with a different nucleotide, resulting in a new codon
Insertion and Deletion Mutations

- **Insertion mutation**: one or more nucleotides are added to a gene
- **Deletion mutation**: one or more nucleotides in a gene are lost
  - **Frameshift mutation**: a mutation that results in the misreading of the genetic code during translation because of a change in the reading frame
Gene Rearrangements

- Gene rearrangement: mutations that move an entire gene to a new location
  - Transposition
  - Chromosomal rearrangement
- Often disrupts gene function b/c gene is exposed to new regulatory controls
Transposition

- **Transposition**: occurs when mobile segments of DNA (transposons) carry a gene, moving randomly from one position to another on chromosomes.

- Transposons make up 45% of human genome.
Chromosomal Rearrangement

- **Chromosomal rearrangement**: portion of the chromosome containing a gene is rearranged during meiosis.