6.2: Protein Synthesis

**Proteins:**
- Proteins are **linear polymers** of amino acids linked together by **peptide bonds** joining the amino and carboxyl groups of successive amino acids.
- Proteins are formed by one or several polypeptide chains. **Polypeptides** usually refer to long peptides whereas **oligopeptides** are short peptides (< 10 amino acids). Proteins are made up of one or more polypeptides with more than 50 amino acids.
- The sequence of the polypeptide chain is defined by a gene with genetic code.
- While all proteins are polypeptides, not all polypeptides are proteins.
- There are only 20 standard amino acids are exists in living organism, consisting of carbon, hydrogen, nitrogen, oxygen, and two that contain sulfur.
- Ten of these amino acids have side groups that are attracted to water, while the other ten do not. Therefore, when a protein is in a water-based environment, the hydrophobic amino acids fold inwards while the hydrophilic remain on the outside. The backbone of amino acids form strong covalent bonds and the actual amino acids form temporary weak bonds. These weak bonds allow the amino acids to change shape, remain mobile, and attain flexibility.

**Protein synthesis:** Protein synthesis occurs in the cytoplasm and involves ribosomes. All the three non-genetic RNAs i.e. mRNA, tRNA and rRNA involved in protein synthesis are synthesized directly on DNA which acts as a template for RNA synthesis. Proteins are molecules that have a variety of functions in cells such as providing structure, storing energy, providing movement, transporting other substances, catalyzing biological reactions, and protecting against disease. Proteins make up more than 50% of a cell's dry weight. Amount of RNA in each cell is directly proportional to the amount of protein synthesis.

In eukaryotes, **three different RNA polymerases (I, II, III) catalyse the synthesis of ribosomal RNA (rRNA), mRNA and tRNA** respectively. In prokaryotes **a single RNA polymerase** performs this function.
Two major steps are involved in protein synthesis. 1. **Transcription** - Francis Crick proposed that information flows from DNA to RNA in a process called transcription, and is then used to synthesize polypeptides by a process called 2. **Translation.**

### Transcription

- There are differences in transcription in prokaryotes and eukaryotes, but most of the basic process is the same.
- The base sequence of a DNA strand is converted into the complementary base sequence of mRNA.
- Transcription has similarities to DNA replication but only involves a small portion of the DNA molecule.
- Only one strand is used as a template for making the mRNA.
- A single gene may be transcribed thousands of times.
- After transcription, the DNA strands rejoin.

#### Steps of transcription

I. **DNA unwinds:** During the process of transcription, an enzyme known as RNA polymerase separates the relatively weak hydrogen bonds between the bases of complementary strands so as to expose an area of the double strands of DNA. A/T pairs joined by two hydrogen bond are easier to separate than G/C pair joined by three bond.

II. One of these strands is selected as a template for the formation of a complementary single strand of mRNA. The mRNA will then serve as a template from which the resulting polypeptide will be produced via translation.

III. RNA polymerase first binds to a region of DNA known as a promoter region on the DNA and proceeding until it reaches a termination signal. Promoter is the region immediately in front of transcription start site. Bacterial RNA polymerase binds to a specific region of about 60 base pairs of DNA. This is therefore said to be upstream of or “5” to start site. The promoter identifies the start of a gene, which strand is to be copied, and the direction that it is to be copied. It then begins to transcribe, or copy, the DNA into RNA at an initiation site.

IV. The DNA double helix is partially unwound by the polymerase and transcription always proceeds in a 3’ to 5’ direction on the DNA template so that the RNA produced is extended in a 5’ to 3’ direction.

V. Any region of a DNA molecule could be transcribed into two RNA molecules (one form each strand). However only one strand is copied at any given time, although it is not always the same strand for different genes.

VI. Typical mRNA is 70 – 10,000 nucleotides in length and is codeified by RNA splicing before becoming functional. The process is rapid and proceeds at a rate of about 30 nucleotides per second.

VII. Within transcription bubble the nascent RNA forms base pairs with the template DNA strand. The RNA polymerase determine the fidelity of transcription, which ensures that each new ribonucleotide must pair with a base in the template, as per rule G pairs with C, C pairs with G, A pairs with U and T pairs with A. Ribonucleotides which
are not complementary to the template base are discarded from the active site, allowing others to enter. The new ribonucleotide (joining one ribonucleotide after another) is attached covalently to the growing RNA chain, in a condensation reaction, which is catalysed by enzyme RNA polymerase. The polymerase then moves along the DNA and elongates the RNA molecule. As the polymerase travels along the DNA it both unwinds, catalyzes copying and rewinds the DNA. When the polymerase comes upon a certain sequence of DNA, called the termination sequence, it stops transcription and releases the RNA. The resulting RNA contains an exact copy of the gene.

**Processing the mRNA Transcript:**

- In eukaryotic cells, the newly-formed mRNA transcript (also called heterogenous nuclear RNA or hnRNA) must be further modified before it can be used.
- A modified guanine cap is added to the 5’ end and a poly-A tail (150 to 200 Adenines) is added to the 3’ end of the molecule by some enzymes.
- The newly-formed mRNA has regions that do not contain a genetic message. These regions are called introns and must be removed. Their function is unknown.
- The remaining portions of mRNA are called exons. They are spliced together to form a mature mRNA transcript.
- Transcription and mRNA processing occur in the nucleus.
- After the mature mRNA transcript is produced, it moves out of the nucleus and into the cytoplasm through pores in the nuclear membrane where it can be translated into a protein.

### Subsection 2

**Translation (mRNA to Protein in cytoplasm)** Translation is the mechanism by which the *triplet base sequences of mRNA molecules are converted into a specific sequence of amino acids in a polypeptide chain*. Several ribosomes may get attached to a molecule of mRNA like beads on a string know as polysome. Translation occurs at ribosomes located on the surface of rough endoplasmic reticulum and as groups of ribosomes (polysomes) found free-floating in the cytoplasm. Ribosomes contain two subunits. Both the large and the small subunit are composed of protein molecules and ribosomal RNA (rRNA) molecules forms a reversible attachment to the surface of the smaller sub unit in the presence of magnesium ions (Mg2+). On getting attached to the ribosome, two mRNA codons are exposed to the larger subunit. Ribosomes translate the nucleotide "language" of mRNA codons into the amino acid "language" of proteins. A mRNA codon consists of a sequence of three nitrogenous bases (eg. GAA or UUA) and these code for specific amino acids such as glutamic acid and leucine. Single amino acids are delivered to the ribosome one at a time by transfer RNA (tRNA) that have an anti-codon specific to the codons of mRNA. It is the mRNA codons that determine the order of the amino acids that are placed into the protein.
Translation has three steps; initiation, elongation and termination.

• **Initiation:** Ribosomes consist of a small (in E. coli, 30S) and larger (50S) subunits. The smaller subunit has a binding site for the mRNA. The larger subunit has two binding sites for tRNA. The **initiator codon (AUG) codes for the amino acid N-formylmethionine (f-Met).** f-Met is always the first amino acid in a polypeptide chain, although it is frequently removed after translation. The initiator tRNA/mRNA/subunit is called the initiation complex. The larger subunit attaches to the initiation complex. The initiator tRNA is found in the first site and the second site is ready for the next tRNA to deliver another amino acid.

![Initiation Diagram](image)

• **Elongation** In elongation the addition of amino acids to the growing polypeptide chain takes place. A second tRNA molecule with an anti-codon complementary to the second codon on mRNA moves to the second site on the ribosome. The initiator tRNA then transfers its amino acid to the amino acid on the second tRNA and a peptide bond is formed between the two amino acids by a ribozyme and ATP energy. The first tRNA then leaves the ribosome to pick up a new amino acid before returning to the ribosome. The first binding site is now empty and the ribosome moves forward shifting the tRNA and the attached amino acid chain from site two to site one on the ribosome (this is called translocation). The second site is now empty and a third tRNA with an amino acid can move into place at the second site on the ribosome. The polypeptide continues to be transferred and peptide bonded to the incoming amino acid on the second site tRNA and then the ribosome will move forward again. This process occurs over and over at an impressive rate (15 times each second) creating a polypeptide/protein that can be very long.

![Elongation Diagram](image)

• **Termination** Termination brings protein synthesis to an end. This event occurs when the ribosome reaches a stop codon such as UAA, UGA, or UAG. These three do not code for any amino acid and therefore do not recruit a tRNA. Instead cytoplasmic "release factors" bind to the A site on the ribosome and causes the peptidyl transferase to add an H2O instead of forming a peptide bond, thus freeing the carboxy terminus of the polypeptide chain. Since this is the attachment to the peptidyl tRNA and the ribosome the newly synthesized polypeptide is released. Lastly, the ribosome breaks apart into its two subunits (large and small subunit) and the mRNA and tRNA molecules are released.
The image contains a diagram related to protein synthesis. The diagram illustrates the process of translation, where the ribosome moves along the messenger RNA (mRNA) molecule, reading codons and pairing them with their corresponding amino acids to form a polypeptide chain. The ribosome translates the mRNA codons into amino acid sequences to assemble a protein. The diagram also shows the involvement of tRNA molecules, which shuttle the correct amino acids to the ribosome at the appropriate location based on the mRNA sequence.