

Protein Synthesis

1. The connection between genes and proteins.
 - a. It was believed since the early 1900s that genes determine the way an organism looks through enzymes that catalyze specific chemical reactions in the cell. In other words, some diseases are caused by missing or defective enzymes.
 - b. In the 1930s George Beadle and Edward Tatum were able to definitively establish the link between genes and enzymes in their exploration of the metabolism of a bread mold.
 - i. They bombarded the mold with X-rays and screened the survivors for mutants that differed in their nutritional needs.
 - ii. The normal mold could grow on agar containing very little nutrients. Beadle and Tatum identified mutants that could not survive on this minimal medium, because they were unable to synthesize certain essential molecules from the minimal ingredients. However, most of these nutritional mutants were able to survive on a complete growth medium that includes all 20 amino acids and a few other nutrients.
 - iii. They suggested that the mutants were unable to survive because they lacked an enzyme necessary to make the particular nutrient missing from the medium.
 - iv. By providing the missing nutrient, the mutants were able to survive.
 - v. Their results provided strong evidence for the **one gene–one polypeptide hypothesis**.
 - c. Proteins carry information in their amino acid sequence and nucleic acids carry information in their nucleotide sequence.
 - d. To get from DNA (in nucleic acid language) to protein (in amino acid language) requires two steps:
 - i. During **transcription**, a DNA strand provides a template for the synthesis of a complementary RNA strand. This RNA molecule is called **mRNA**.
 - (1) The use of mRNA provides protection for the genetic information contained in DNA.
 - (2) Also, more protein can be made simultaneously because many mRNA copies of a gene can be made.
 - (3) Lastly, each mRNA can be translated many times.
 - ii. During **translation**, the instructions are converted from nucleic acid language to amino acid language.
2. The genetic code
 - a. There are only 4 bases but 20 amino acids so it is not sufficient for one nucleotide to represent one amino acid.
 - i. In 1961, Crick (yep, him again) figured out how many bases have to be in each “code word” to create enough combinations to code for each amino acid. $4^1 = 4$; $4^2 = 16$; $4^3 = 64$
 - ii. That is, the code must be a series of triplets (three bases) which indicate a particular amino acid. The genetic instructions for a polypeptide chain are written in DNA as a series of nonoverlapping three-nucleotide words called **codons**.
 - (1) Sixty-one of the 64 triplets code for amino acids.
 - (2) The codon AUG not only codes for the amino acid methionine, but also indicates the “start” of translation.

- (3) Three codons do not indicate amino acids but are “stop” signals marking the termination of translation.
 - iii. Some amino acids are coded for by two or more codons but a given codon ALWAYS codes for only one amino acid. *i.e.* there is redundancy but no ambiguity. *e.g.*, GAA and GAG both mean glutamic acid, but **never** mean any other amino acid.
3. The process of transcription is much like DNA replication except that DNA is acting as a template for the construction of mRNA rather than new DNA
- a. Initiation
 - i. **RNA polymerase** separates the DNA strands at a particular sequence called the promoter and bonds the RNA nucleotides as they base-pair along the DNA template.
 - ii. Like DNA polymerase, RNA polymerase can only assemble a polynucleotide in its 5' to 3' direction.
 - iii. Unlike DNA polymerase, RNA polymerase is able to start a chain from scratch; it does not need a primer.
 - b. Elongation
 - i. As RNA polymerase moves along the DNA, it untwists the double helix, 10 to 20 bases at time, adding bases by base pairing rules to form mRNA.
 - ii. Remember that in RNA, U rather than T is paired with A.
 - iii. Behind the point of RNA synthesis, the double helix re-forms and the mRNA molecule peels away.
 - iv. If there is high demand for a protein, the cell can have several RNA polymerases transcribing the same gene simultaneously to produce several mRNAs.
 - c. Termination
 - i. Transcription proceeds until RNA polymerase transcribes a terminator sequence (AATAAA).
 - ii. The mRNA is then released from the RNA polymerase and sent to the cytosol.
 - iii. A transcription unit is the sequence between the start and stop sequences - generally speaking, one gene.
4. Translation
- a. In the process of translation, a cell interprets a series of codons along an mRNA molecule and builds a polypeptide. That is, mRNA is translated from nucleic acid language to amino acid language.
 - b. The interpreter is **transfer RNA (tRNA)**, which carries amino acids to a ribosome. The ribosome then adds each amino acid carried by tRNA to the growing end of the polypeptide chain.
 - i. Each tRNA carries a specific amino acid attached to the amino acid attachment site.
 - ii. At the other end of the tRNA is a group of three nucleotides called the **anticodon** that binds by complementary base pairing to the nucleotides of a codon.
 - (1) *E.g.*, if the codon on mRNA is UUU, a tRNA with an AAA anticodon and carrying phenylalanine will bind to it.
 - iii. The tRNA molecule is a translator, because it can read a nucleic acid word (the mRNA codon) and translate it to a protein word (the amino acid).

c. **Initiation**

- i. Each ribosome has a binding site for mRNA and three binding sites for tRNA molecules.
 - (1) The **P site** holds the tRNA carrying the growing polypeptide chain.
 - (2) The **A site** carries the tRNA with the next amino acid to be added to the chain.
 - (3) A tRNA that has dropped off its amino acid leaves the ribosome at the **E (exit) site**.
- ii. A ribosome binds to mRNA and begins looking for the start codon (AUG)
 - (1) To extract the message from the genetic code requires specifying the correct starting point.
 - (2) This establishes the **reading frame**; subsequent codons are read in groups of three nucleotides.
- iii. When it is found, it is displayed in the P site so tRNA molecules can attempt to recognize it by complementary base pairing with their anticodon. When this occurs, the two ribosomal subunits come together to form the functional ribosome. The tRNA with the anticodon complementary to AUG always carries methionine (met) so it is always the first amino acid in every protein. The tRNA carrying methionine enters the P site
- iv. The ribosome now displays the next codon in the A site and waits for the tRNA with the complementary anticodon to recognize it
- v. If the cellular demand for a protein is high, several ribosomes can translate the same mRNA simultaneously

d. **Elongation** (~ 60 ms per peptide bond)

- i. The tRNA with an anticodon complementary to the next codon enters the A site
- ii. The growing polypeptide chain on the tRNA at the P site, now one amino acid longer, is transferred to the tRNA at the A site. The ribosome forms a new peptide bond by transferring the amino acid from tRNA in the P site to the amino acid on the tRNA in the A site
- iii. The ribosome moves over one codon on the mRNA so that the next codon is now displayed in the A site.
- iv. The tRNA (now empty) that had been in the P site is moved to the E site and then leaves the ribosome.
- v. the appropriate tRNA moves in and the ribosome attaches the amino acids (now 2 of them) from the tRNA in the P site to the amino acid on the tRNA in the A site. The chain is now three amino acids in length
- vi. These steps of elongation continue to add amino acids codon by codon until the polypeptide chain is completed.

e. **Termination**

- i. The elongation process continues until one of the three stop codons is reached displayed in the A site.
 - ii. There is no tRNA which recognizes any of the stop codons but a release factor binds to the stop codon and hydrolyzes the bond between the polypeptide and its tRNA in the P site.
 - iii. This frees the polypeptide, so it is released from the ribosome.
- f. A ribosome requires less than a minute to translate an average-sized mRNA into a polypeptide.

- g. During and after synthesis, a polypeptide coils and folds to its three-dimensional shape spontaneously.

5. Mutations are changes in the genetic material of a cell

- a. If a mutation occurs in gametes or cells producing gametes, they may be transmitted to future generations.
- b. A change in just one base pair of a gene causes a **point mutation**.
- c. A point mutation that results in the switching of one nucleotide with another is called a **base-pair substitution**.
 - i. Some base-pair substitutions have little or no impact on protein function.
 - (1) In a **silent mutation**, the new nucleotide still codes for the same amino acid because of the redundancy in the genetic code.
 - (2) Some changes lead to switches from one amino acid to another with similar properties.
 - (3) The change could occur in a non-critical part of the protein.
 - ii. Sometimes, base-pair substitutions cause a detectable change in a protein.
 - (1) These are usually detrimental but can occasionally lead to an improved protein or one with new features.
 - iii. **Insertions and deletions** are additions or losses of nucleotide pairs in a sequence of DNA and nearly always have a quite serious effect on the protein.
 - (1) Unless the insertion or deletion is a multiple of three nucleotides, they cause a **frameshift mutation**.
 - (2) All the nucleotides after the deletion or insertion will be improperly grouped into codons.
- d. Mutations can be caused by several things
 - i. Errors can occur during DNA replication.
 - ii. A **mutagen** is anything that interacts with DNA to increase the natural frequency of mutations.
 - (1) Some mutagens are chemicals.
 - (a) Some chemicals are similar in shape to one of the nucleotides and may be substituted into DNA, but they pair incorrectly during DNA replication.
 - (b) Other mutagens interfere with DNA replication by inserting into DNA and distorting the double helix.
 - (c) Still others cause chemical changes in bases that change their pairing properties.
 - (2) X-rays and ultraviolet radiation are mutagens.
 - (a) They cause abnormal base pairing or irreversible bonding.
 - (b) Radiation can also break the ladder and repair enzymes sometimes put the pieces back together in the wrong order.
 - iii. Exposure to mutagens is especially dangerous for pregnant women because the embryo is growing rapidly and relatively few embryonic cells give rise to many adult cells.
 - iv. Note that only mutations in germ cells are passed on to offspring. Somatic cell mutations are not hereditary.

6. Gene therapy is the manipulation of genes for our benefit
 - a. In gene insertion, a normal copy of a gene is inserted into the correct position in DNA if a cell is missing it.
 - i. This is usually done by having a virus (called a vector) carry the gene into the cell.
 - (1) *e.g.*, nasal spray for cystic fibrosis.
 - (2) *e.g.*, insert gene for insulin into pancreatic cells and implant in patient. As new cells divide, pancreas would slowly become non-diabetic
 - ii. One problem is that genes are often controlled by their location on the chromosome so might not behave normally.
 - b. A gene transplant involves removing a gene and replacing it with another one.
 - i. This could be done to give an organism some trait that it does not naturally have.