Unit 4: Molecular Genetics
Content Outline: DNA History, Structure and Replication (4.1) – Part 1

I. Alfred Hershey and Martha Chase (in 1952)
- A. They worked with the T2 **Bacteriophage** (a virus that infects bacteria) and E. Coli bacteria.
- B. Over time, this becomes known as the Hershey-Chase Experiment.
  1. They used *radioactive Sulfur 35* to label the virus's *protein* outer capsid in one container.
  2. They then used *radioactive Phosphorus 32* to label the *DNA* inside the virus in *another* container.
  3. The radioactive viruses where then exposed to bacteria. The viruses infected the bacteria.
  4. In the radioactive Sulfur container, the radioactive sulfur did <u>not</u> enter the bacteria. It *remained outside* the bacteria. When the viruses reproduced inside the bacteria, the reproduced viruses that came out of the dead bacteria were <u>not</u> radioactive.
  5. In the radioactive Phosphorus container, the radioactive phosphorus did enter the bacteria. When they reproduced inside the bacteria, the reproduced viruses that came out of the dead bacteria <u>were</u> radioactive from the phosphorus the possessed.
  6. This *proved with 100% accuracy*, that DNA was the *"transformation agent"* and that this carries the information "blueprint" from one generation to the next.

II. Erwin Chargaff (in 1947)
- A. He develops what becomes known as Chargaff's Rule.
- B. The rule states that, for <u>all</u> organisms**,** [A] = [T] and [C] = [G].
  1. For example: If you know a species has 32% Thymine; then there must ALSO be 32% Adenine. (32+32= 64%.) This means that there is 36% unaccounted for. (100- 64 = 36.) Since this 36% is BOTH Cytosine and Guanine, divide by 2 to find the percentage of each. (36÷ 2 = 18) There exists 18% Cytosine and 18% Guanine.

III. Rosalind Franklin (in the 1950's)
- A. She performed X-ray Crystallography on DNA. This picture was *extremely* important in helping James Watson and Francis Crick develop their model of DNA. (See below)
  1. The picture *indicates the Double Helix* (The picture would be from the view of looking down a strand of DNA. It would be similar to looking *down* a paper towel cardboard tube.
  2. The picture also indicates that the Nitrogen Bases (the X in the center) point inward and are *equal lengths* in binding, because it is <u>always</u> one pyrimidine (C and T) and one purine (A and G).
     a. Purines (A&G) are *larger* nitrogenous bases. Pyrimidines (C&T) are *smaller* nitrogenous bases. (Please discuss what the lengths would be if it were pyrimidine + pyrimidine or purine + purine and what the X ray might look like then.)
  3. The large areas *around* the "X" are the sugar phosphate backbone of DNA.

IV. James Watson and Francis Crick (in 1953)
- A. They construct the first accurate *model of DNA*.
- B. They used Chargaff's work and Franklin's work to fill in the gaps that they could not figure out. (Good point to discuss the collaborative nature of science and research scientists.)
- C. The Double Helix backbone is composed of Phosphorus and the 5-Carbon sugar Deoxyribose. It would be like the side supports on a ladder.
- D. The "rungs or steps of the ladder" would be the Purine base + Pyrimidine Base. (A=T and C=G)
- E. **Hydrogen Bonds** *hold the two sides together* and it is twisted into the Double Helix shape (It looks like a twisted ladder.) Remember, Hydrogen bonds are *weak* bonds. We will want to "open up" the DNA during DNA replication AND Protein Synthesis.

DNA History, Structure, and Replication – Part 2

I. DNA **Replica**tion
- A. The process of making of a *complete copy of an entire length of DNA.* (Applies to all Chromosomes.)
  1. This occurs *during the S-Phase* of the Cell Cycle for Mitosis or Meiosis.
  2. (Good place to *quickly* review those parts to the Cell Cycle.)
- B. It is easy to do for cells because the two sides are **Complimentary.** *(A with T and C with G always.)*
- C. The **Semi-conservative Model** *best* explains the process of DNA replication.
  1. Matthew Meselson and Franklin Stahl propose this model, in 1958.
  2. It shows one original DNA side serving as a *template (guide)* for making the *other* DNA side.
  3. Easy as A = T and C = G.
  4. The replication work is being done *in opposite directions*, but *on both sides at the same time.*
- D. In humans, it takes just a few hours to copy over 6 Billion nucleotides in our cells thanks to *enzymes*!

II. **Origins** Of Replication (**Starting points**)
- A. These are *specific nucleotide sequences* encoded in the DNA strands that act as "starting points".
- B. The enzyme **helicase** *unwinds the DNA double helix* to create a **Replication Bubble** (This provides *spaces* to do the actual building work of making the *new complimentary side* of the new DNA molecule by other enzymes.)

III. DNA Replication Elongation
- A. Elongation of the new DNA complimentary side will require the enzyme **DNA Polymerase III.** (This *enzyme* performs the *addition of new nucleotides* to the new DNA complimentary side and also acts as a *proofreader* to help prevent errors in construction from occurring. Look at the name and *see* the function. Remember, "polymers" means "many units" or "many monomers". In this case, the monomers are called nucleotides. The ending "ase" tells you it is an enzyme.) (Good place to remind students about proofreading their papers they write in English class.)
  1. The enzyme works at a rate of about 500 nucleotides being added *per second.*
- B. The two sides of the Double Helix are said to be **Anti-parallel.** (This means that the DNA information runs in *different directions.*)
  1. DNA is <u>always</u> *read and made 5'→3'.* (Remember this <u>important fact</u>!)
     a. The 5' Carbon of the sugar (Deoxyribose or Ribose) has a *phosphate* attached to it.
     b. The 1' Carbon of the sugar has the *Nitrogen Base* attached to it.
     c. The 3' Carbon of the sugar has an *open bond.* (This is the *connector site* for the *next* nucleoside.)
- C. **Helicase** enzyme causes the Double Helix to *unwind.*
- D. **Single-strand binding protein** keeps the two sides *apart and stable.* (Look at the name and *see* the function.)
- E. **Leading strand** of the *replication fork* (Remember, there are <u>two</u> forks going in <u>opposite</u> directions.)
  1. *This strand runs in a continuous 5'→3' direction as it opens.* (It is *leading* the way in the process.)
  2. To start adding nucleosides, we first need to attach an **RNA Primer**. (Remember, RNA) using **Primase** enzyme and go! (A "primer" is a *starting segment* of nucleotides.)
  3. (Please stress to the students that the names refer to the side of the DNA *being made* and <u>not</u> the template side.)

F. Lagging Strand
  1. This side of the replication fork has DNA *not running in a 5'→3' direction.* (Therefore it will always be lagging behind.) (Same as above.)
  2. This side of the fork has *to wait* for a long *segment* of DNA to become *exposed first* before we can start by adding a primer.
  3. When a long segment has been "opened" by Helicase, a RNA Primer (disposable) will attach and then DNA Polymerase III will *work backwards* making an **Okazaki fragment**.
  4. *The Okazaki fragments are "stitched" together using the enzyme* ***Ligase.***

IV. Correction of Errors (Proofreading)
- A. This function is performed by **DNA Polymerase III** as the *new DNA strand is being made.*
  1. **Mismatch Repair** is when the *wrong nucleotide is added* to the new sequence. DNA Polymerase will reverse a spot, remove the wrong nucleotide, and then replace with the correct nucleotide.
  2. (Please remind students of how the delete a misspelling when typing.)
- B. For errors that are created (what are called Mutations) <u>after</u> the DNA has been made – **Nucleotide Excision Repair** is used to correct these, if possible
  1. Step 1: **Nuclease** –*cuts around the faulty pairing* so they can be removed.
  2. Step 2: **DNA Polymerase III** – *replaces* the missing nucleotides.

3. Step 3: Ligase - *stitches back together* the fragments.

C. So What?

1. Errors in proofreading can result in some forms of cancer. For instance, some individuals are genetically predisposed to skin cancer because they have a mutation in the gene that codes for the excision repair enzyme. They can't fix damage caused by UV light on skin cells.