DNA

1. Evidence for DNA as the genetic material.
   a. Until the 1940s, proteins were believed to be the genetic material.
   b. In 1944, Oswald Avery, Maclyn McCarty, and Colin MacLeod announced that the transforming substance was DNA. They followed up on work done by Frederick Griffith in 1928 on the ability of one strain of bacteria which caused pneumonia in mice to transform a strain that could not cause pneumonia.
   c. More evidence that DNA was the genetic material came from studies of the infection of bacteria by viruses.
      i. In 1952, Alfred Hershey and Martha Chase showed that DNA was the genetic material of a bacteriophage. They used radioactive S to label the protein of the bacteriophage and allowed it to infect the host bacterium. After centrifugation, the radioactive protein was found in the supernatant. This showed that protein is not injected into host. Then, radioactive P was used to label the nucleic acid of the virus. After infection and centrifugation, the radiation was in the pellet with the bacterial cells. This showed that nucleic acid is injected into host bacterium during infection.
   d. The fact that cells double the amount of DNA in a cell just before they divide and then distribute the DNA equally to each daughter cell provided some circumstantial evidence that DNA was the genetic material.
   e. In 1947, Erwin Chargaff had made some observations regarding the composition of DNA. He noted that:
      i. the DNA composition varies from species to species.
      ii. in any one species, the four bases are found in characteristic, but not necessarily equal, ratios. If the bases were chosen randomly, species would be expected to have the same relative amounts of each nucleotide.
      iii. in all organisms, the amount of adenine was approximately equal to the amount of thymine (%T = %A) and that the amount of guanine was approximately equal to the amount of cytosine (%G = %C).

2. The structure of DNA.
   a. Maurice Wilkins and Rosalind Franklin used X-ray crystallography to study the structure of DNA.
   b. James Watson and Francis Crick worked out the 3D structure of DNA using molecular models made of wire
      i. The molecule consists of 2 chains wound together in a spiral (*i.e.*, a double helix).
      ii. The sides of the chains are made of alternating sugars and phosphates, like the sides of a rope ladder.
      iii. The ladder forms a twist every ten bases.
      iv. Pairs of nitrogenous bases, one from each strand, form the rungs of the ladder. In order for the ladder to have a uniform width, a small base must be paired with a large base. A pairs with T and C pairs with G. This is called complementary base pairing.
      v. The two strands are held together by hydrogen bonding between bases.
      vi. Note that the chains have direction. Each strand has a 3' end with a free OH group attached to deoxyribose and a 5' end with a free phosphate (P) group attached to deoxyribose. This arrangement is called **antiparallel**.

3. Replication of DNA
   a. When a cell divides, the DNA must be doubled so that each daughter cell gets a complete copy. It is important for this process to be high fidelity because any errors made would be inherited by the offspring and these errors would tend to accumulate with each generation.
   b. Because each strand is complementary to the other, each can form a template when separated. When a cell copies a DNA molecule, each strand serves as a template for ordering nucleotides into a new complementary strand. One at a time, nucleotides line up along the template strand according to the base-pairing rules. (paper fan model)
   c. An experiment in the late 1950s by Matthew Meselson and Franklin Stahl demonstrated that replication was **semiconservative**.
      (1) Bacteria that had been growing on a heavy isotope of N were allowed to grow for one generation (*i.e.*, DNA replicated once) on a light isotope of N.
      (2) The cells had DNA of one weight. This meant that it was constructed from half heavy (old or parent) and half light (new or daughter) N.
      (3) After a second generation (*i.e.*, another replication) the cells had DNA of two distinct weights. Some cells had DNA that was all light and some cells had DNA that was a mixture of light and heavy. This showed that replication is semi-conservative.
   d. Origin of Replication
      i. The replication of a DNA molecule begins at special sites, **origins of replication.** A specific sequence of nucleotides marks the origin. (a sequence of about 150 nucleotides rich in GATC)
      ii. Humans have hundreds of origins from which replication proceeds on both strands in both directions.
      iii. At the origins, the DNA strands are separated, forming a replication "bubble" with **replication forks** at each end. An enzyme called **helicase** separates the strands.
   e. Elongating a new strand
      i. After the two strands are separated, DNA polymerase reads the bases on the template strand and attaches complementary bases to form a new strand. (DNA polymerase works at a rate of about 50 nucleotides per second)
      ii. DNA polymerase can only attach the 5' phosphate (P) of one nucleotide to the 3' hydroxyl (OH) of the previous nucleotide that is already part of a strand. The enzyme can only work by building a new strand in the 5' → 3' direction.
      iii. The new nucleotides (*e.g.*, ATP) that are attached lose two of their three phosphates which provides the energy to form the bond.
   f. Problem of antiparallel strands
      i. Remember that the DNA molecule is arranged with the strands going in opposite directions so the 3' end of one strand is aligned with the 5' end of the other.
      ii. DNA polymerase adds nucleotides only to the 3' end but can only do this on one strand, the **leading strand**.
      iii. The other strand has a 5' P at the end rather than a 3' OH like DNA polymerase needs. This strand, the **lagging strand**, must be made in short fragments (**Okazaki fragments**) going in the direction opposite to the leading strand. Another enzyme, **DNA ligase**, then fills in the gaps by joining the fragments together. (fragments are 100-200 nucleotides in eukaryotes; 1000-2000 in prokaryotes)

g. Priming DNA synthesis
    i. DNA polymerases cannot *initiate* the synthesis of a new strand of DNA.
    ii. A short stretch of RNA (5-10 nucleotides) with an available 3' end is built. This short piece is called a **primer** and is built by **primase**, a RNA polymerase.
    iii. After formation of the primer, DNA polymerase can add new nucleotides to the 3' end of the RNA primer.
    iv. The leading strand requires the formation of only a single primer as the replication fork continues to separate. For synthesis of the lagging strand, each Okazaki fragment must have its own primer.
    v. Another DNA polymerase then replaces the RNA nucleotides of the primers with DNA nucleotides.

h. Replication error rate, DNA damage and repair.
    i. The active site of DNA polymerase must recognize all four nucleotides. This means that it is difficult to determine if a nucleotide is mistakenly in the active site. Mistakes during the initial pairing of template nucleotides and complementary nucleotides occur at a rate of one error per 100,000 base pairs.
    ii. DNA polymerase checks for these errors by checking the width of the helix. The final error rate is only one per ten billion nucleotides.
    iii. Constant exposure to chemicals, viruses, and radiation also cause damage to DNA so human cells have about 130 enzymes which constantly check DNA for errors.

DNA Technology

1. DNA profiling
    a. Although 99.9% of human DNA sequences are the same in every person, some sequences are unique to each individual - like a fingerprint - so that they can be used to distinguish one individual from another.
        i. The process begins with a sample of an individual's DNA called a "**reference sample**." To make a DNA fingerprint DNA can be collected from cheek cells, saliva, blood, semen, hair follicles, or any other tissue sample.
        ii. The reference sample is then analyzed to create the individual's DNA profile which can then be compared to another sample to determine whether they are a match.
    b. A **restriction enzyme** is an enzyme that cuts DNA at or near specific nucleotide sequences known as **restriction sites**. These enzymes are found in bacteria and serve to defend against viral infection. Inside the bacterium, the restriction enzymes destroy foreign DNA by cutting it at restriction sites. Over 3000 restriction enzymes are known and more than 600 of these are available commercially.
    c. DNA is cut up into pieces by restriction enzymes, making strands of varying lengths. Because each person has slight differences in their DNA, restriction enzymes will cut at different places.
    d. The strands are separated based on length using the technique of gel electrophoresis. These fragments are then stained so that we can see them.

e. The unique DNA sequences of each person produce a unique collection of fragments of various lengths, which results in a unique pattern of bands on the gel. By looking at the pattern of bands, we can distinguish the DNA from different people. These differences are called **restriction fragment length polymorphisms**.
    i. Some of the uses of this technique include forensics, paternity determination, and screening for genetic disorders.

2. **Recombinant DNA** molecules are DNA molecules formed by bringing together genetic material from different sources, creating sequences that would not otherwise be found naturally in organisms. DNA can be taken from living organisms or sequences that do not occur anywhere in nature may be created chemically synthesizing DNA with the desired sequence. Examples of uses include:
    a. Bacterial plasmids
        i. A gene from one organism is spliced (inserted) into the DNA of a bacterial plasmid. A plasmid is a small, circular piece of DNA separate from the main chromosomal DNA.
        ii. The gene is duplicated along with the bacterial DNA when the cell divides and the protein is then synthesized by the bacterial cell.
        iii. This is often used as a means of cheaply, mass-producing protein (*e.g.,* human growth hormone, insulin, *etc.*)
    b. Plant DNA
        i. Bacterial DNA coding for an antibiotic is inserted in plant DNA.
        ii. The plant is grown and produces the antibiotic.
        iii. This confers resistance to various pests without having to spray chemicals on the plant.
        iv. Plants can be given other traits using this technique, such as improving shelf life or frost resistance.
    c. Producing proteins in other organisms.
        i. The gene for a particular protein (*e.g.*, antibiotics) can be placed inside yeast cells.
        ii. The yeast cells can be added to feed for some domestic animals and provide a dietary source of the protein.
        iii. The animal then has a source of the protein without having to use expensive supplements.
    d. Research
        i. A disease-causing gene can be inserted into animals to study the effects of the disease.
        ii. This can help in researching treatments or prevention.
    e. Gene therapy
        i. Some diseases are caused by malfunctioning or damaged genes.
        ii. A functional copy of the gene can be inserted into cells to alleviate (or eliminate) the symptoms. *e.g.*, cystic fibrosis, insulin-dependant diabetes.