The Development of a Four-Letter Language
DNA

The Griffith Experiment

- Chromosomes are comprised of two types of macromolecules, proteins and DNA, but which one is the stuff of genes?
  - the answer was discovered from a variety of different experiments, all of which shared the same basic design
    - *if you separate the DNA in an individual's chromosomes from the protein, which of the two materials is able to change another individual's genes?*

- Frederick Griffith in 1928 experimented with pathogenic (i.e., disease-causing) bacteria
  - he experimented with two strains of *Streptococcus pneumoniae*
    - the virulent strain, called the S form, was coated with a polysaccharide capsule and caused infected mice to die of blood poisoning
    - a mutant form, called the R form, which lacked the capsule and was non-virulent

- Griffith determined that when dead bacteria of the S form were injected into mice, the mice remained healthy
- But, when Griffith injected mice with mixture of dead S bacteria and live bacteria of the R form, the mice unexpectedly died
  - the R form bacteria now had transformed into the virulent S variety

How Griffith discovered transformation

The Avery and Hershey-Chase Experiments

- The agent responsible for transforming *Streptococcus* went undiscovered until a classic series of experiments by Oswald Avery and his coworkers Colin MacLeod and Maclyn McCarty
  - they also worked with *Streptococcus* strains, both dead S and live R, but were able to remove first nearly 99.98% of the dead S
  - they found that the transforming principle was not reduced by the removal of the protein

- The Avery team discovered that the transforming principle resembled DNA in several ways
  - same chemistry and behavior as DNA
  - not affected by lipid and protein extraction
  - not destroyed by protein- or RNA-digesting enzymes
  - destroyed by DNA-digesting enzymes
- Based on this overwhelming evidence, the Avery team concluded that the heredity material was DNA

- Alfred Hershey and Martha Chase provided the final experimental evidence that pointed to DNA as the hereditary material
  - the team studied viruses that infect bacteria
  - the structure of these viruses is very simple: a core of DNA surrounded by a coat of protein
  - the viruses attach themselves to the surface of bacteria cells and inject their genes into the interior
    - the infected bacterial cell is then forced to make hundreds of copies of new viruses, which then burst out of the cell to infect new cells

- Hershey and Chase used radioactive isotopes to "label" or tag the DNA and the protein of the viruses
  - some viruses were grown so that their DNA contained radioactive phosphorus (^{32}P)
  - other viruses were grown so that their protein coats contained radioactive sulfur (^{35}S)

- After the labeled viruses were allowed to infect bacteria, only the viruses with ^{32}P had labeled tracer in their interior

- The conclusion was that the genes that viruses use to specify new viruses are made of DNA and not protein

Discovering the Structure of DNA

- In order to understand how DNA functioned as the molecules that stored heredity, researchers needed to understand the structure of DNA
  - DNA is comprised of subunits called nucleotides
  - each DNA nucleotide has three parts
    - a central deoxyribose sugar
    - a phosphate group
    - an organic base

- Nucleotides differ with regards to their bases
  - large bases (**purines**) with double-ring structure
    - either adenine (**A**) or guanine (**G**)
  - small bases (**pyrimidines**) with single rings
    - either cytosine (**C**) or thymine (**T**)
  - Edwin Chargaff noted that DNA molecules always had equal amounts of purines and pyrimidines
    - **Chargaff's rule** suggested that DNA had a regular structure
      - the amount of A always equaled the amount of T
      - the amount of C always equaled the amount of G

The four nucleotide subunits that make up DNA

- Rosalind Franklin's work in 1953 using

X-ray diffraction revealed that DNA had a regular structure that was shaped like a corkscrew, or **helix**

- Francis Crick and James Watson elaborated on the discoveries of Franklin and Chargaff and deduced that the structure of DNA was a **double helix**
  - two strands of DNA bind together by their bases
  - because a purine of one strand binds to a pyrimidine on the other strand to form a **base pair**, the molecule keeps a constant thickness

The DNA double helix

How the DNA Molecule Copies Itself

- The two strands of DNA that form the double helix DNA molecule are complementary to each other
  - each chain is essentially a mirror image of the other
  - this **complementarity** makes it possible for DNA to copy itself in preparation for cell division

- There are three possible alternatives as to how the DNA could serve as a template for the assembly of new DNA molecules
  - **conservation replication**
    - the two strands of DNA completely separate to act as templates for the assembly of two new strands
    - after replicating, the original strands rejoin, preserving the original DNA molecule and leaving a completely new one
  - **Semi-conservative replication**
    - the DNA unzips and new complementary strands are assembled using each strand as a template
    - one original strand is preserved in each duplex created
  - **dispersive replication**
    - replication results in both original and new DNA dispersed among the two daughter strands

Alternative Mechanisms for DNA replication

- Matthew Meselson and Franklin Stahl tested, in 1958, the three alternative hypotheses for the replication of DNA
  - they used radioactive isotopes of N to label DNA prior to and after replication
  - they found that DNA replication was semi-conservative

The Meselson-Stahl experiment

- The process of DNA replication involves several enzymes
  - **DNA polymerase**
    - adds the correct complementary nucleotide to the growing daughter strand

        - but can only add to an existing strand or primer
    - **helicase**
        - unzips the DNA to expose the templates
        - this creates a **replication fork**
    - **DNA ligase**
        - seals fragments of DNA together

How nucleotides are added in DNA replication

- At the replication fork, a primer must first be added to give a place for DNA polymerase to start
    - from one template, DNA polymerase adds nucleotides in a continuous fashion; this new daughter strand is called the **leading strand**
    - because the other template is a mirror image, directionality becomes a problem because DNA can build a new strand in one direction only
        - this second daughter strand is assembled is segments, each one beginning with a primer
        - the segments will be joined together to form the **lagging strand**

Building the leading and lagging strands

- Before the newly formed DNA molecules wind back into the double helix shape, the primers must be removed and the DNA fragments sealed together
    - DNA ligase joins the ends of the fragments of DNA to form continuous strands

How DNA replication works

- Because so much DNA is being replicated in the many cells of the body, there is a potential for errors to occur
    - **DNA repair** involves comparing the daughter strand to the parent DNA template to check for mistakes
        - the proofreading is not perfect because mutations are still possible, although rare

Mutation

- There are two general ways in which to alter the genetic message encoded in DNA
    - **mutation**
        - results from errors in replication
        - can involve changes, additions, or deletions to nucleotides
    - **recombination**
        - causes change in the position of all or part of a gene

- Mutations can alter the genetic message and affect protein synthesis
  - because most mutations occur randomly in a cell's DNA, most mutations are detrimental
  - the effect of a mutation depends on the identity of the cell where it occurs
    - mutations in germ-line cells
      - these mutations will be passed to future generations
      - they are important for evolutionary change
    - mutations in somatic cells
      - not passed to future generations but passed to all other somatic cells derived from it

- Some mutations alter the sequence of DNA nucleotides
  - **base substitution** changes the identity of a base or bases
  - **insertion** adds a base or bases
  - **deletion** removes a base of bases

- If the insertion or deletion throws the reading frame of the gene message out of register, a **frame-shift mutation results**
  - these are extremely detrimental because the final protein intended by the message may be altered or not made

Two types of mutations

- Some mutations affect how a genetic message is organized
  - **transposition** occurs when individual genes move from one place in the genome to another
  - sometimes entire regions of chromosomes may change their relative location or undergo duplication
    - this is called **chromosomal rearrangement**

Some Categories of Mutation

- All evolutionary change begins with mutation
  - mutation and recombination provide the raw materials for evolution

- Chemicals that causes mutation, called **mutagens**, appear to be linked to cancer
  - for example, chemicals in cigarette smoke cause cancer