All This For Four Letters!?!
DNA and Its Role in Heredity

What Is the Evidence that the Gene Is DNA?

- By the 1920s, it was known that chromosomes consisted of DNA and proteins.
- A new dye stained DNA and provided circumstantial evidence that DNA was the genetic material:
  - It was in the right place
  - It varied among species
  - It was present in the right amount

- Frederick Griffith, working with two strains of *Streptococcus pneumoniae* determined that a "transforming principle" from dead cells of one strain produced a heritable change in the other strain.
- Identifying the transforming principle, Oswald Avery:
  - Treated samples to destroy different molecules; if DNA was destroyed, the transforming principle was lost.

- Hershey-Chase experiment:
  - Determined whether DNA or protein is the genetic material using bacteriophage T2 virus.
  - Bacteriophage proteins were labeled with 35S; the DNA was labeled with 32P.

- Next, genetic transformation of eukaryotic cells was demonstrated—called transfection.
- Use a genetic marker—a gene that confers an observable phenotype.
- Any cell can be transfected, even an egg cell—results in a transgenic organism.

What Is the Structure of DNA?

- The structure of DNA was determined using many lines of evidence.
- One crucial piece came from X-ray crystallography.
- A purified substance can be made to form crystals; position of atoms is inferred by the pattern of diffraction of X-rays passed through it.

- Chemical composition also provided clues:
- DNA is a polymer of nucleotides: deoxyribose, a phosphate group, and a nitrogen-containing base.
- The bases:
  - Purines: adenine (A), guanine (G)
  - Pyrimidines: cytosine (C), thymine (T)

- 1950: Erwin Chargaff found in the DNA from many different species:
  - amount of A = amount of T
  - amount of C = amount of G
  - Or, the abundance of purines = the abundance of pyrimidines—Chargaff's rule.

- Model building started by Linus Pauling—building 3-D models of possible molecular

structures.

- Francis Crick and James Watson used model building and combined all the knowledge of DNA to determine its structure.

- X-ray crystallography convinced them the molecule was helical.
- Other evidence suggested there were two polynucleotide chains that ran in opposite directions—antiparallel.
- 1953—Watson and Crick established the general structure of DNA.

- Key features of DNA:
  - A double-stranded helix, uniform diameter
  - It is right-handed
  - It is antiparallel
  - Outer edges of nitrogenous bases are exposed in the major and minor grooves

- Complementary base pairing:
  - Adenine pairs with thymine by two hydrogen bonds.
  - Cytosine pairs with guanine by three hydrogen bonds.
  - Every base pair consists of one purine and one pyrimidine.

- Antiparallel strands: direction of strand is determined by the sugar–phosphate bonds.
- Phosphate groups connect to the 3′ C of one sugar, and the 5′ C of the next sugar.
- At one end of the chain—a free 5′ phosphate group; at the other end a free 3′ hydroxyl.
- The flat base pairs are exposed in the major and minor grooves—accessible for hydrogen bonding.
- The C═O group in thymine, the N group in adenine, and others offer hydrogen bonding sites.
- Key to DNA–protein interactions in replication and gene expression.
- Functions of DNA:
  - Store genetic material—millions of nucleotides; base sequence stores and encodes huge amounts of information
  - Susceptible to mutation—change in information

- Genetic material is precisely replicated in cell division—by complementary base pairing.
- Genetic material is expressed as the phenotype—nucleotide sequence determines sequence of amino acids in proteins.

How Is DNA Replicated?

- Kornberg showed that DNA contains information for its own replication.
- In a test tube: DNA, the four deoxyribonucleoside triphosphates, and DNA polymerase enzyme.
- The DNA is a template for synthesis of new DNA.

- Three possible replication patterns:
  - Semiconservative replication
  - Conservative replication
  - Dispersive replication

- Meselson and Stahl showed that semiconservative replication was the correct model.
- They used density labeling to distinguish parent DNA strands from new DNA strands.
- DNA was labeled with 15N, making it more dense.

- Results of their experiment can only be explained by the semiconservative model.
- If it was conservative, the first generation of individuals would have all been high or low density, but not intermediate.
- If dispersive, density in the first generation would be half, but this density would not appear in subsequent generations.

- Two steps in DNA replication:
  - The double helix is unwound, making two template strands.
  - New nucleotides are added to the new strand at the 3′ end; joined by phosphodiester linkages. Sequence is determined by complementary base pairing.

- A large protein complex—the replication complex—catalyzes the reactions of replication.
- All chromosomes have a base sequence called origin of replication (ori).
- Replication complex binds to ori at start.
- DNA replicates in both directions, forming two replication forks.

- DNA helicase uses energy from ATP hydrolysis to unwind the DNA.
- Single-strand binding proteins keep the strands from getting back together.
- Small, circular chromosomes have a single origin of replication.
- As DNA moves through the replication complex, two interlocking circular chromosomes are formed.
- DNA topoisomerase separates the two chromosomes.

- Large linear chromosomes have many origins of replication.
- DNA is replicated simultaneously at the origins.

- DNA polymerases are much larger than their substrates.
- Shape is like a hand; the "finger" regions have precise shapes that recognize the shapes of the nucleotide bases.

- A primer is required to start DNA replication—a short single strand of RNA.
- Primer is synthesized by primase.
- Then DNA polymerase begins adding nucleotides to the 3' end of the primer.

- Cells have several DNA polymerases.
- One is for DNA replication; others are involved in primer removal and DNA repair.
- Other proteins are involved in the replication process.

- At the replication fork:
  - The leading strand is pointing in the "right" direction for replication.
  - The lagging strand is in the "wrong" direction.

  - Synthesis of the lagging strand occurs in small, discontinuous stretches—Okazaki fragments.
- Each Okazaki fragment requires a primer.
- The final phosphodiester linkage between fragments is catalyzed by DNA ligase

- DNA polymerases work very fast:
  - They are processive: catalyze many polymerizations each time they bind to DNA
  - Newly replicated strand is stabilized by a sliding DNA clamp (a protein)
- The new chromosome has a bit of single stranded DNA at each end (on the lagging strand)—this region is cut off.
- Eukaryote chromosomes have repetitive sequences at the ends called telomeres.
- Human chromosome telomeres (TTAGGG) are repeated about 2500 times.
- Chromosomes can lose 50–200 base pairs with each replication. After 20–30 divisions, the cell dies.
- Some cells—bone marrow stem cells, gamete-producing cells—have telomerase that catalyzes the addition of telomeres.
- 90% of human cancer cells have telomerase; normal cells do not. Some anticancer drugs target telomerase.

How Are Errors in DNA Repaired?

- DNA polymerases make mistakes in replication, and DNA can be damaged in living cells.
- Repair mechanisms:
  - Proofreading
  - Mismatch repair
  - Excision repair
- As DNA polymerase adds a nucleotide to a growing strand, it has a proofreading function—if bases are paired incorrectly, the nucleotide is removed.
- The newly replicated DNA is scanned for mistakes by other proteins.
- Mismatch repair mechanism detects mismatched bases—the new strand has not yet been modified (e.g., methylated in prokaryotes) so it can be recognized.
- If mismatch repair fails, the DNA is altered.
- DNA can be damaged by radiation, toxic chemicals, and random spontaneous chemical reactions.
- Excision repair: enzymes constantly scan DNA for mispaired bases, chemically modified bases, and extra bases—unpaired loops.

What Are Some Applications of Our Knowledge of DNA Structure and Replication?

- Copies of DNA sequences can be made by the polymerase chain reaction (PCR) technique.
- PCR is a cyclical process:
  - DNA fragments are denatured by heating.
  - A primer, plus nucleosides and DNA polymerase are added.
  - New DNA strands are synthesized.

- PCR results in many copies of the DNA fragment—referred to as amplifying the sequence.
- Primers are 15–20 bases, made in the laboratory. The base sequence at the 3’ end of the DNA fragment must be known.

- DNA polymerase that does not denature at high temperatures (90°C) was taken from a hot springs bacterium, *Thermus aquaticus*.

- DNA sequencing determines the base sequence of DNA molecules.
- Relies on altered nucleosides with fluorescent tags that emit different colors of light.
- DNA fragments are then denatured and separated by electrophoresis.