Frederick Griffith – 1928

- Studied *Streptococcus pneumoniae*, a pathogenic bacterium causing pneumonia
- 2 strains of *Streptococcus*
  - S strain is virulent
  - R strain is nonvirulent
- Griffith infected mice with these strains

Griffith’s Experiments

- a. Live virulent strain of *S. pneumoniae* injection, mice die
- b. Live nonvirulent strain of *S. pneumoniae* injection, mice live
- c. Heat-killed virulent strain of *S. pneumoniae* injection, mice live
- d. Mixture of heat-killed virulent and live nonvirulent strains of *S. pneumoniae* injection, mice die
  - Their lungs contain live pathogenic strain of *S. pneumoniae*
Griffith’s Results

• **Transformation**
  – Information specifying virulence passed from the dead S strain cells into the live R strain cells
  
• Our modern interpretation is that genetic material was actually transferred between the bacterial cells

Avery, MacLeod, & McCarty – 1944

• Repeated Griffith’s experiment using purified cell extracts as transforming material
  
• Removal of all protein did not destroy ability to transform R strain cells
  
• DNA-digesting enzymes destroyed all transforming ability
  
• Supported DNA as the genetic material

Hershey & Chase – 1952

• Investigated bacteriophages
  – Viruses that infect bacteria
  
• Bacteriophage was composed of only DNA and protein
  
• Wanted to determine which of these molecules is the genetic material
  
• Only the DNA entered the bacteria and was used to produce more bacteriophage
  
• Conclusion: DNA is the genetic material
DNA Structure

- DNA is a nucleic acid
- Composed of **nucleotides**
  - 5-carbon sugar called **deoxyribose**
  - **Phosphate group** (PO₄)
    - Attached to 5' carbon of sugar
- **Nitrogenous base**
  - Adenine, thymine, cytosine, guanine
  - Free hydroxyl group (—OH)
    - Attached at the 3’ carbon of sugar

- **Phosphodiester bond**
  - Bond between adjacent nucleotides
  - Formed between the phosphate group of one nucleotide and the 3’—OH of the next nucleotide
- Each DNA strand has a 5’ end and a 3’ end

Chargaff’s Rules

- Erwin Chargaff determined that
  - Amount of adenine = amount of thymine
  - Amount of cytosine = amount of guanine
  - Always an equal proportion of purines (A and G) and pyrimidines (C and T)
Rosalind Franklin

- Performed X-ray diffraction studies to identify the 3-D structure
  - Discovered that DNA is helical
  - Using Maurice Wilkins’ DNA fibers, discovered that the molecule has a diameter of 2 nm and makes a complete turn of the helix every 3.4 nm

James Watson and Francis Crick – 1953

- Deduced the double helix structure of DNA using evidence from Chargaff, Franklin, and others

Double helix

- 2 strands are polymers of nucleotides
- Phosphodiester backbone – repeating sugar and phosphate units joined by phosphodiester bonds
- Antiparallel strands
- Complementarity of bases
  - A forms 2 hydrogen bonds with T
  - G forms 3 hydrogen bonds with C
- Gives consistent diameter
DNA Replication

- 3 possible models
  1. Conservative model
  2. Semiconservative model
  3. Dispersive model

1957 Meselson and Stahl - 3 Replication Models

Meselson-Stahl Experiment
Meselson-Stahl Experiment (cont.)

CsCl forms a density gradient during centrifugation, with the highest density at the bottom of the tube. DNA molecules move to positions where their density equals that of the CsCl solution and form bands. Shown are the positions of differently labeled DNA molecules. Experimentally the bonds are detected by absorbance of UV light.

<table>
<thead>
<tr>
<th>Label</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>14N–14N (light) DNA</td>
<td>DNA from 15N medium</td>
</tr>
<tr>
<td>15N–14N hybrid DNA</td>
<td>DNA after one replication in 14N</td>
</tr>
<tr>
<td>15N–15N (heavy) DNA</td>
<td>DNA after two replications in 14N</td>
</tr>
</tbody>
</table>

The results support the semiconservative model.

Complementary base pairing in the DNA double helix: A pairs with T, G pairs with C.

The two chains unwind and separate. Each “old” strand is a template for the addition of bases according to the base-pairing rules.

The result is two DNA helices that are exact copies of the parental DNA molecule with one “old” strand and one “new” strand.
DNA Replication

• Requires 3 things
  – Something to copy
    • Parental DNA molecule
  – Something to do the copying
    • Enzymes
  – Building blocks to make copy
    • Nucleotide triphosphates

Enzymes of DNA Replication

• **DNA polymerases** assemble nucleotides into a chain
  • Require 3’ end (e.g., RNA primer)
  • Synthesize in 5’-to-3’ direction only
• **Helicase** unwinds the DNA
• **Primase** synthesizes RNA primer (starting point for nucleotide assembly by DNA polymerases)
• **Nuclease** removes primers
• **DNA ligase** closes remaining single-chain nicks

Semidiscontinuous

• **DNA polymerase** can synthesize only in 1 direction
• **Leading strand** synthesized continuously from an initial primer
• **Lagging strand** synthesized discontinuously with multiple priming events
  – **Okazaki fragments**
Assembling Antiparallel Strands

Enzyme Activities

1. Helicase unwinds the DNA, and primases synthesize short RNA primers.

2. RNA primers are used as starting points for the addition of DNA nucleotides by DNA polymerases.

3. DNA unwinds further, and leading strand synthesis proceeds continuously, while a new primer is synthesized on the lagging strand template and extended by DNA polymerase III.

4. Another type of DNA polymerase (I) removes the RNA primer, replacing it with DNA, leaving a nick between the newly synthesized segments.

5. Nick is closed by DNA ligase.

6. DNA continues to unwind, and the synthesis cycle repeats as before: continuous synthesis of leading strand and synthesis of a new segment to be added to the lagging strand.
Prokaryotic Replication

- *E. coli* model
- Single circular molecule of DNA
- Replication begins at one **origin of replication**
- Proceeds in both directions around the chromosome

*E. coli* has 3 DNA polymerases
- DNA polymerase I (pol I)
  - Acts on lagging strand to remove primers and replace them with DNA
- DNA polymerase II (pol II)
  - Involved in DNA repair processes
- DNA polymerase III (pol III)
  - Main replication enzyme
- All 3 have 3’-to-5’ exonuclease activity – proofreading
- DNA pol I has 5’-to-3’ exonuclease activity
Eukaryotic Replication

• Complicated by
  – Larger amount of DNA in multiple chromosomes
  – Linear structure
• Basic enzymology is similar
  – Requires new enzymatic activity for dealing with ends only

Telomeres

• Specialized structures found on the ends of eukaryotic chromosomes
• Protect ends of chromosomes from nucleases and maintain the integrity of linear chromosomes
• Gradual shortening of chromosomes with each round of cell division
  – Unable to replicate last section of lagging strand

• Telomeres composed of short repeated sequences of DNA
• Telomerase – enzyme makes telomere of lagging strand using an internal RNA template (not the DNA itself)
  – Leading strand can be replicated to the end
• Telomerase developmentally regulated
  – Relationship between senescence and telomere length
• Cancer cells generally show activation of telomerase
DNA Repair

- Errors due to replication
  - DNA polymerases have proofreading ability
- Mutagens – any agent that increases the number of mutations above background level
  - Radiation and chemicals
- Importance of DNA repair is indicated by the multiplicity of repair systems that have been discovered

Excision repair

- Targets multiple types of lesions
- Damaged region is removed and replaced by DNA synthesis
- 3 steps
  1. Recognition of damage
  2. Removal of the damaged region
  3. Resynthesis using the information on the undamaged strand as a template