Chapter 16: DNA: The Genetic Material

1. **What must genetic material do?**

2. **Why did biologists used to think that proteins are the genetic material?**

3. **Describe Griffith's experiments with genetic transformation and how they (and follow-up experiments) helped determine the genetic material.**

4. **Describe the Hershey-Chase bacteriophage experiment, its results, and the conclusion.**

5. **Discuss how Watson and Crick determined the structure of DNA (including incorporation of Chargaff's rules and X-ray diffraction results from Franklin/Wilkins).**

6. **Draw the structure of DNA; indicate basepairs, 5' and 3' ends, antiparallel nature.**

7. **Compare and contrast conservative, semiconservative, and dispersive models of DNA replication.**

8. **Group activity on overhead: Meselson-Stahl experiment**

9. **Outline the process of DNA replication: what is required?**

10. **On a blank piece of paper, draw and label a replication fork (as completely as you can from memory).**

BIOL 1020 – CHAPTER 16 LECTURE NOTES

Chapter 16: DNA: The Genetic Material

I. Evidence that DNA is the genetic material

A. What must genetic material do?

1. the genetic material must be able to replicate itself
2. must be able to direct and control living processes

B. a model of genetic inheritance was in place in the early 1900s:

1. Mendel's "laws" of genetics – inherit one copy of each gene from each parent
2. chromosomes as locations/carriers of genes
3. distribution of chromosomes in making sex cells explains Mendel's laws

C. chromosomes are made of two things: protein and DNA

D. from the late 1800s until the mid-1900s, most biologists believed that the genetic material was made of proteins, and that nucleic acids were inconsequential

1. proteins are very complex
2. proteins have much variety

E. DNA is required for **genetic transformation** of bacteria

1. studies by Griffith in the 1920s of pneumococcus in mice
   - smooth (S) strain killed mice, rough (R) strain did not
   - heat-killed S strain did not kill mice, but heat-killed S + R strain killed mice
   - some "transforming principle" from the heat-killed S strain changed the R strain to make it deadly
2. studies by Avery and colleagues in the 1940s identified DNA as the "transforming principle" – but many were very skeptical of this result

F. viruses inject DNA into bacteria and take them over: the Hershey-Chase experiments

1. **viruses** that infect bacteria are called **bacteriophages** (shortened as **phages**)
2. viruses execute a "genetic takeover" of cells
3. using radioactive isotopes, phage were labeled with either ^{35}S to label proteins or ^{32}P to label DNA
4. phage were incubated with bacteria to allow infection, and then shaken off the bacteria
5. centrifugation then separated the bacteria into the pellet, with phage in the supernatant
6. found that ^{35}S stayed with the phage, while ^{32}P was with the bacteria
7. Hershey and Chase concluded that phage injected DNA into bacteria to infect them
8. this convinced many more biologists that DNA is the genetic material, and the race to find the structure of DNA began

9. evidence gathered since the mid-1900s that DNA is the generic material has been overwhelming (much of the rest of this unit will cover that evidence)

II. Structure of DNA

A. recall the DNA polymer structure from deoxyribonucleotide monomers

1. deoxyribonucleotide has 5-carbon deoxyribose sugar, phosphate, and nitrogenous base
2. bases are the purines **adenine (A)** and **guanine (G)**, and the pyrimidines **thymine (T)** and **cytosine (C)**
3. nucleotides are linked by a **3', 5' phosphodiester linkage**
4. resulting chain has a **5' end** and a **3' end**
5. the phosphates and sugars are collectively called the "backbone" of the strand
6. this structure had been fully worked out by the early 1950s

B. Chargaff and colleagues had found any one organism they tested had amounts of A ≈ T and C ≈ G

C. x-ray diffraction studies by Rosalind Franklin and Maurice Wilkins indicated a helical molecule

1. molecule has three repeating patterns that any model of its structure must account for
2. the data indicated a **helix**

D. the accepted model for the structure of the DNA double helix was published by James Watson and Francis Crick in 1953

1. DNA was envisioned as a twisted ladder, with the sugar-phosphate backbone forming the sides and basepairs forming the rungs
2. model explained all three repeating patterns seen in x-ray diffraction, as well Chargaff's data on base ratios
3. **double helix** with **antiparallel strands**
   - each strand a nucleotide chain held together by phosphodiester linkages
   - strands held together by hydrogen bonds between the bases (**basepairs**)
     - A paired with T, with 2 hydrogen bonds predicted
     - C paired with G, with 3 hydrogen bonds predicted
4. the strands were described as **complementary**: the sequence of one had to have an appropriate, complementary sequence on the other for the molecule to hold together
5. the double-helix model strongly suggested a way to store information in the sequence of bases, which indeed appears to be true

E. the determination of the DNA structure by Watson and Crick is considered the major landmark of modern biology

III. DNA replication is semiconservative

A. DNA structure suggests an obvious replication mechanism

1. Watson and Crick noted that "specific [base]pairing…immediately suggests a possible copying mechanism for the genetic material"
2. the model suggested that each strand could serve as a template for making a complementary strand, so-called **semiconservative replication**
   - one strand old, one new
3. competing, less-elegant models were conservative replication (both strands either old or new) and dispersive replication (each strand a mix of old and new)

B. experiments with *E. coli* supported the semiconservative replication model

1. Meselson and Stahl used nitrogen isotopes to mark old vs. newly synthesized DNA strands
2. bacteria grown in medium with ^{15}N were transferred to medium with ^{14}N; thus, old DNA strands had ^{15}N and new ones ^{14}N
3. isolated DNA after one generation: DNA molecules all had roughly equal amounts of ^{15}N and ^{14}N – disproved conservative replication
4. later generations: some ^{14}N only, some still with roughly equal amounts of ^{15}N and ^{14}N – disproved dispersive replication

IV. DNA replication: the process

A. overview

1. DNA replication requires the coordinated activity of many enzymes and other proteins
2. also requires the presence of nucleotide triphosphates

B. **origins of replication**

1. DNA replication begins at specific sites
   - synthesis generally proceeds in both directions from an origin, creating a "**replication bubble**"
   - there is usually only one origin of replication in the circular bacterial DNA
   - eukaryotic chromosomes usually have several origins of replication each
2. both strands are replicated at the same time on both sides of the replication bubble, producing Y-shaped **replication forks** on each side; the forks move as synthesis proceeds

C. unwinding and opening DNA

1. the twisted double helix must be unwound and the basepair bonds broken ("opening" the DNA molecule)
2. **DNA helicase** does the unwinding and opening
3. **single-strand DNA binding proteins** keep it open (also called helix-destabilizing proteins)
4. **topoisomerases** break and rejoin strands, resolving knots and strains that occur

D. direction of synthesis

1. **DNA polymerases** direct synthesis of new strands
2. synthesis proceeds by adding nucleotides onto the 3' end of a strand
3. thus, synthesis can only proceed in the **5' → 3'** direction
4. the nucleotide added is from a deoxynucleotide triphosphate; two phosphates are released in the process

E. priming new strands

1. DNA polymerase can only add onto an existing strand, so it can't start the strand
2. **primase** starts the strand by making an **RNA primer** that is a few (usually about 10) ribonucleotides long
3. DNA polymerase can then add nucleotides starting at the end of the RNA primer
4. the RNA primer is later degraded and (usually) replaced with DNA

F. leading and laggings strands

1. the 5' → 3' directionality of synthesis complicates the replication activity
2. one strand being synthesized, the **leading strand**, has its 3' end at the fork; thus, its synthesis can proceed continuously, in the direction that the fork moves
3. the other, **lagging strand** has its 5' end at the fork; it must be synthesized in the "opposite direction" from the leading strand
   - the lagging strand is thus made in short (100-1000 nucleotides) **Okazaki fragments**
   - fragments are later connected by **DNA ligase** (which also joins together DNA strands when replication forks meet)

G. **DNA proofreading** and **DNA repair**

1. DNA polymerase proofreads: initial error rate about 1 in 100,000; final rate about 1 in 100,000,000
2. cells have DNA repair mechanisms to fix most mistakes that get through as well as to fix most damaged DNA

H. the dead end: problem at the telomeres

1. the ends of chromosomes are called **telomeres**
2. they present special problems for DNA replication: the 5' end RNA primer cannot be replaced with DNA, creating 5' end gaps
3. this leads to shorting of chromosomes at the ends with each cell generation
4. in some cells, special **telomerase** enzymes can generate longer telomeres – telomerase is required in germ-line cells, and active in cancer cells as well

V. DNA packaging in chromosomes

A. the DNA molecule is too long if not folded

1. bacteria have much less DNA in their cells than eukaryotes do, but even so the length of their DNA molecule if stretched out would be 1000x the length of the cell itself
2. thus, even in the bacteria DNA must be "packaged", folded and coiled to make it fit in the cell
3. eukaryotes have even more DNA, and use somewhat elaborate means to package the DNA even when it is in "decondensed" chromatin

B. nucleosomes

1. **nucleosomes** are the main packaging mechanism for eukaryotic DNA
2. the nucleosome is made up of 8 protein subunits, acting like a "spool" for the DNA "thread"
3. the proteins are called **histones**
4. histones are positively charged, and thus able to associate with the negatively charged phosphates of the DNA backbone
5. the 8 proteins in a nucleosomes are 2 each of 4 different histones
6. nucleosomes are linked together with "**linker DNA**" regions, parts of the continuous DNA molecule that are not wound on histones
7. overall this gives an appearance of nucleosomes as "beads" on a DNA "string"
8. nucleosome packaging of DNA is found throughout the cell cycle, except when DNA is being replicated

C. further packaging: histone H1 and scaffolding proteins

1. even during interphase, most of the DNA is packed tighter than just being wound on nucleosomes
2. this next packing step uses another histone, **H1**, that associates with the linker DNA regions
3. H1 binding leads to packing of nucleosomes into a **30 nm chromatin fiber**
4. 30 nm fibers form **looped domains** that are ~300 nm wide and attached to non-histone **scaffolding proteins**
    - this level of packing is found only for some regions of DNA, except when chromosomes are condensed for cell division
5. the next step connects looped domains into an ~700 nm fiber that is considered fully **condensed chromatin**