Chapter for today: Chap. XIII

Major points for the day:

1. Early experiments showed DNA as the genetic material
2. Structure of DNA

Molecular Biology explores the nature of heredity

During the last fifty years the new discipline of molecular biology has generated a revolution in the biological sciences

- At the beginning of the 1940s we didn't know what the chemical basis of heredity was
- We didn't know how hereditary instructions were propagated to control the way cells behaved or developed

Molecular biology is a discipline which attempts to trace the molecular causes of a variety of biological phenomena, though the problem of heredity has been central to molecular biology since its beginnings

As a direct result of the work of molecular biologists over the last several decades we now understand the molecular basis of heredity to a large extent

We also understand the basics of how hereditary instructions are given to cells

In addition, we have come to understand how those instructions can be controlled so that they are given only in the correct circumstances

During the next four lectures we will consider

- How molecular biologists came to understand the structural basis of heredity—that is, the structure of the DNA molecule
- How the instructions in DNA are transfered to an unstable messenger molecule called RNA
- How the RNA code is translated into proteins, the chemical products of the hereditary instructions
- And finally, the ways in which scientists are using the tools of molecular biology to alter genetic instructions in order to understand better how cells work

DNA as the "genetic material"

The book describes how in 1868 Friedrich Miescher, a Swiss physician, first isolated a molecule he called "nuclein"

Miescher knew that the genetic material resided in nuclei, and was probably carried on the bodies which microscopists had called chromosomes

However, as you can imagine, seeing chromosomes does not <u>explain how they work</u>

Miescher wanted to understand them chemically

Nuclein we would call deoxyribonucleic acid (literally, an acid from nuclei which contains the sugar deoxyribose)

I'm sure that most of you take it for granted that DNA is the genetic material

I know that you have heard that DNA is the genetic material because it is inherited from one generation to the next

But what is the **evidence** that it is so?

The early history of molecular biology is punctuated by a series of experiments so simple, and yet so elegant, that they have become known by the authors names which constitute almost a pantheon of biology—Avery, MacLeod and McCarty; Watson & Crick; Hershey–Chase; Meselson & Stahl; Jacob & Monod

Most of these experiments date from the first decade of modern molecular biology—the 1950s

- Though they are over 40 years old, nearly every biology student learns about these experiments
- They form the intellectual basis for much of modern biology, or at least of modern genetics

Avery, MacLeod & McCarty provide evidence for DNA as the genetic material

Much of the first half of the century microbiologists attempted to identify organisms responsible for disease

Fred Griffith was an Army doctor who in 1928 wanted to make a vaccine against *Streptococcus pneumoniae*, which caused a type of pneumonia

Since the time of Pasteur, about 50 years before, vaccines had been made using killed microorganisms which could be injected into patients to elicit the immune response of live cells without risk of disease

Though he failed in making the vaccine he stumbled on a demonstration of the transmission of genetic instructions by a process we now call **transformation**

- He found that the bacterium had two forms when grown on agar plates, a smooth (S) and a rough (R) form
- The R bacteria were harmless, but the S bacteria were lethal when injected into mice
- Heat–killed S cells were also harmless—the same effect seen by Pasteur
- However, surprisingly when live R cells were mixed with killed S cells and injected into mice the mice died, and the bacteria rescued from the mice had been “transformed” into S type

This experiment strongly implied that genetic material had been transferred from the dead to the live cell

It was hard to be certain of this, or to know what the material was in this crude experiment

Sixteen years later in 1944 the team of Avery, MacLeod and McCarty revisited this experiment and attempted a more definitive experiment

- A favorite hypothesis of the 1930s was that the genetic instructions in chromosomes was protein
- Chromosomes, as we shall see, are largely made of proteins, and biologists thought that DNA performed a structural role as a scaffold to hold these informational proteins
- However, chemical analysis of DNA had begun to suggest that it might be a more interesting molecule than had been supposed

Avery, MacLeod and McCarty repeated Griffith’s experiment using purified DNA molecules and got the same “transformation” of R cells into S cells

- Since it is often the case that favored hypotheses are given up only very grudgingly, they did some critical control experiments
- Knowing that many would claim that their DNA was contaminated with protein which was the true active principle, they treated the “DNA” with two enzymes:
  - Protease: this degrades proteins to its monomers (amino acids)

  - DNAse: this degrades DNA to its monomers (nucleotides)
- Pre–treatment with protease had no effect on transformation, while pre–treatment with DNAse eliminated transformation

Through another example of bloody–mindedness in scientific history many refused to accept these results, and suggested that it might apply only to bacteria!

The Hershey–Chase experiment

The second classical demonstration of DNA as the genetic material was an outgrowth of a group of molecular biologists who in the late 1940s and early 1950s started to use viruses that attack bacteria (bacteriophages—or literally “bacteria eaters”) to study the nature of heredity

Alfred Hershey was one of the founders of this group. One of his students, Martha Chase, performed an experiment which clearly distinguished between DNA and protein as the genetic material

The phage group had identified a series of bacteriophage and shown that they consisted of a combination of only DNA and protein

They had also shown that these viruses attacked cells from the outside, but that most of the virus never entered the cell

  - Later work showed that the viruses are like microscopic hypodermic needles, injected their genome into the inside of the bacteria

Hershey & Chase used a simple radiolabling protocol to distinguish between the DNA and protein portions of the viruses being the genetic material

- They knew that protein includes the atom sulfur (in two of the amino acids) but that DNA does not
- And that DNA contains phosphorus while the proteins of the viruses did not
- They labeled viruses with either ^{35}S or ^{32}P and used them to infect cells
- After allowing the viruses to infect the cells they put them through a blender—this knocked the viruses off of the cells without affecting the cells
- They found that the cells were still infected after this treatment
- They looked for the radioactivity of the ^{35}S or the ^{32}P and found that some of the ^{32}P went with the cells, while all of the ^{35}S did not

This simple experiment clearly suggested that the nucleic acid, but not the protein, was the genetic material

Chargaff’s rules and the structure of DNA

One of the facts about DNA that had begun in the 1940s to suggest that DNA might be more interesting a molecule was “Chargaff’s rules”

Erwin Chargaff was a biochemist interested in the structure of DNA

He found that DNA contained four nitrogen–containing, ring–shaped, basic molecules called adenine (A), guanine (G), thymine (T) and cytosine (C)

After studying the composition of DNA from many species he found that though the absolute amounts of these four molecules could vary widely, the amount of A always equaled the amount of T, and the amount of G equaled C

$$A = T \qquad G = C$$

X–ray crystallographers Maurice Wilkens and Rosalind Franklin had shown that DNA was a fiber about 2 nanometers in width repetitive steps of some sort every 0.34 nm, and longer steps every 3.4 nm

Watson & Crick used this information to propose a double–helical structure in which nucleotides repeated every 0.34 nm and the helix made one turn every 3.4 nm or 10 nucleotides

- The critical leap was to recognize that Chargaff's rules might have a structural basis
- They proposed that a pairing interaction between A and T, and G and C held together the two chains of the double helix
  - The two strands of the DNA run in opposite directions (antiparallel)
  - That is, one strand runs from 5' to 3' while the other runs from 3' to 5'
  - This means that when the helix is turned upside down it looks the same
  - If the strands were parallel, turning the structure could not be turned upside down and still look the same
- The association of A–T and G–C they called a base pair

- Because of this simple rule knowing the sequence of one strand of the DNA allows you to predict the sequence of the other