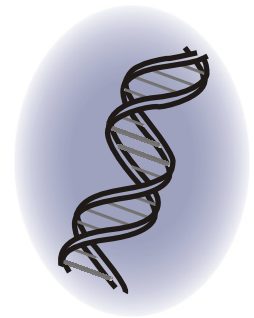


Model Building

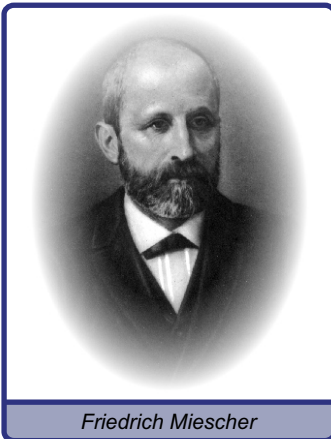
Piecing Together the Structure of DNA



Today we know that the blueprint for life lies in the nucleus of every cell in the human body. Often referred to simply as DNA, its full name is deoxyribonucleic acid. It looks like a twisted ladder, called a double helix. The steps are made of four nitrogen bases – adenine, thymine, cytosine, and guanine. They are more commonly referred to by their abbreviations A, T, C, and G respectively. Each of the bases has a complementary partner. T pairs with A, and C pairs with G. Every step in the DNA ladder is made of these pairs, stacked in different orders to build the genes that are the genetic code for an organism.

We now know a lot about DNA, but it's been a long journey to develop that understanding. James Watson and Francis Crick are the two names usually associated with determining the structure of DNA in 1953. They and Maurice Wilkins received the Nobel Prize in 1962 for that work. But efforts to understand the genetic material and the structure of DNA involved many more people over a long period of time.

The story of nucleic acids began in Germany in 1869. Friedrich Miescher had just finished medical school. Instead of becoming a physician, he opted to go into cell chemistry. His object of study was quite peculiar to us. He collected pus, the oozing stuff that comes out wounds. He



Friedrich Miescher

thought it might be useful in understanding proteins. Antiseptic didn't exist at that point, so most wounds had plenty of the whitish-yellow stuff. Miescher thought the pus cell nuclei would have a certain protein, but after investigation realized a different substance was also in the nucleus. Moreover, he found it in cells throughout the body. It was definitely not a protein. Since it came from nuclei in cells, he called it nuclein. He

thought it just stored phosphorous in the body.

The hereditary aspects of the cell proved difficult for scientists to master in the late 1800s. The problem was not entirely due to its very small size. In 1866, after a decade

of work studying pea plants, Gregor Mendel proposed how traits were passed from one generation to another. However, his work was largely ignored and forgotten until the turn of the next century. In 1884, fifteen years after Miescher's work, Oskar Hertwig observed cell fertilization under a microscope and announced nuclein to be responsible for the transfer of traits. The larger scientific community felt, however, that nuclein simply served an accessory or structural function inside the nucleus. Not until the next century would scientists get a better grasp on the processes behind heredity.

By 1900, scientists had learned a lot about nuclein. Nuclein had a sugar called ribose. It also contained phosphate. The phosphate groups connected ribose groups up and down the longer molecule. Deoxyribose was isolated in 1920. As its name suggests, it was ribose with one less oxygen atom. Scientists also knew about the four nitrogen bases, but little else regarding DNA's structure.

The Russian-born Phoebus Levene came to work in America in the early 1900s. He proposed his *tetranucleotide hypothesis* – that the four nitrogen bases were present in *exactly* equal amounts. The basic unit of DNA was thought to be simply a repeating tetranucleotide made up of one of each of the four different nucleotides. This meant that DNA would have little variance and could not be the agent of heredity. Many in the scientific community accepted this conclusion and continued their investigations of proteins. Their reasoning was that nucleic acids were too simple to account for the variability noted in organisms. Thus, they could not be the genetic material. Proteins, however, are made up of twenty-three possible amino acids and did appear to possess the variability expected in genetic material.

In 1914, Robert Feulgen, a German chemist, developed a staining procedure that was specific for DNA. Thus, the presence or absence of DNA in cells could be determined by viewing stained cells through a high-powered microscope. Moreover, the observed stain intensity was determined to be related to the amount of DNA present. Further staining work was interpreted as indicating that all cells (except egg and sperm cells) in a particular animal or plant contained the same amount of DNA. You might think that this would sway scientists toward considering DNA as

the genetic material, but that was not the case. DNA just didn't seem to have the necessary complexity that could produce the immense variations of life. Moreover, proteins were also determined to be in nuclei, and they possess the complexity that the genetic material was expected to have.

Fourteen years later in 1928, bacteriologist Fred Griffith was studying the disease-causing capability of two strains of *Streptococcus pneumoniae*, a bacteria associated with pneumonia. One strain had a smooth coat (S-strain) on its surface. When the S strain was injected in mice, the mice developed pneumonia and died (Figure 1). The other strain, called 'rough' (R-strain), did not have a smooth surface. When the R-strain was injected in mice, the mice did not develop pneumonia. Griffith then used heat to kill the disease causing S-strain and injected them into mice. The mice did not develop pneumonia. But when he mixed heat-killed S-strain bacteria with live R-strain bacteria (both harmless by themselves) and injected the mixture into mice, the mice developed pneumonia and died. Autopsy of the mice showed they were full of S-strain bacteria. What sense would you make of these results? Griffith reasoned that material in the heat-killed S-strain that caused the smooth coat was transferred to the live R-strain. But he did not know what this material was.

More than a decade of work was required to isolate the material responsible for the transformation first observed by Griffith. Techniques to destroy various compounds

found in bacteria were developed and Oswald Avery, Colin MacLeod and Maclyn McCarty applied these to solve the puzzle. One-by-one different components of the S-strain bacteria were destroyed prior to mixing them with live R-strain bacteria. Transformation always occurred except when the S-strain bacteria were treated with an enzyme that destroyed DNA. In 1944 Avery, MacLeod and McCarty announced that DNA carried the genetic information responsible for transforming the R-strain bacteria to the disease-causing S-strain bacteria.

That same year, a call to determine the physical structure of genes came from a physicist, Erwin Schrödinger. In his book, *What Is Life?*, he argued that physics and chemistry should be applied in solving the mystery of life. However, while more and more scientists began to accept that DNA played at least some role in heredity, other scientists remained skeptical. Watson was not one of these, and he later wrote:

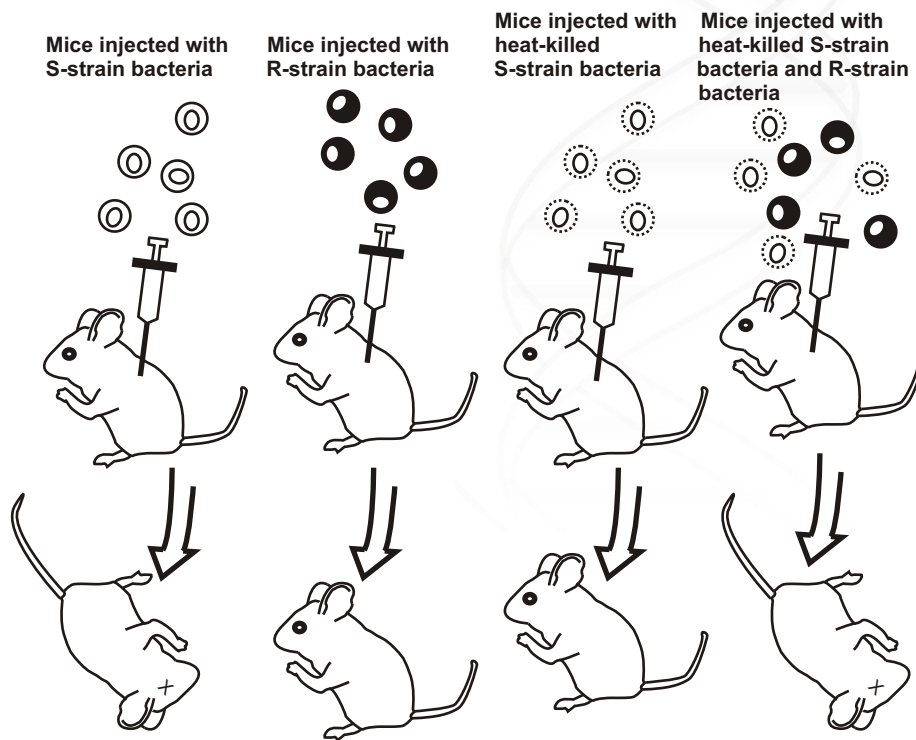
Of course there were scientists who thought the evidence favoring DNA was inconclusive and preferred to believe that genes were protein molecules. Francis [Crick] however, did not worry about these skeptics. Many were cantankerous fools who unflinchingly backed the wrong horses.

Watson admitted, however, that further experimental work was needed to show that all genes are composed of DNA. In 1952 Alfred Hershey and Martha Chase of Cold Spring Harbor Laboratory in Long Island, New York published further evidence in favor of DNA being the genetic material. They permitted bacteriophages (viruses that attack bacteria) to infect *E. coli* bacteria. Bacteriophages had first been seen with an electron microscope in 1940. The bacteriophage they used in their work was known to be essentially DNA with a protein coat. These bacteriophages land on bacteria and bore a hole through the cell surface. The virus injects something into the bacterium that instructs the bacterium to produce more viruses. But scientists did not know what that something was.

The key to Hershey and Chase's experimental work was that proteins have sulfur in their structure, but no phosphorous. DNA contains phosphorous, but no sulfur. Before infecting the *E. coli*, they went through a process that ensured the bacteriophages would be labeled with radioactive phosphorous (³²P)

FIGURE 1

Results of S-strain and R-strain injections in mice.



and radioactive sulfur (^{35}S). This would permit them to track whether the virus inserted protein, DNA, or both inside the bacterium. After permitting the bacteriophages to infect the bacteria, Hershey and Chase placed the culture containing the two in a blender and gently agitated it. This would knock loose the phages from the bacteria. They then placed the blended mix in a centrifuge to separate the cells from the phages. The sample of *E. coli* was determined to contain ^{32}P and an amount of ^{35}S that was deemed insignificant. This was interpreted by Hershey and Chase as indicating that DNA, and not protein, plays a role in heredity. While this work convinced many scientists that DNA was the genetic material, still not all agreed.

1. Hershey and Chase reported their work in 1952. However, Watson, Crick and many other scientists were already engaged in efforts to determine the structure of DNA, confident it was the genetic material. But not all scientists agreed that DNA was the genetic material. What does this disagreement among scientists imply about interpreting experimental data? What does this illustrate about how science works?

Meanwhile, Erwin Chargaff had always been skeptical of the *tetranucleotide hypothesis* put forward by Levene. Chargaff had been born in Austria, but was working in the United States at Columbia University. He struggled throughout the 1940s to determine the base ratios of a variety of organisms. By 1948, he had proposed the idea that DNA from different organisms had different nucleotide ratios. That is, they had different percentages of the four nitrogen bases. However, the percentage of A in any organism equals the percentage of T, and the percentage of G in any organism equals the percentage of C (Table 1).

TABLE 1
Demonstration of Chargaff's rule for several organisms.

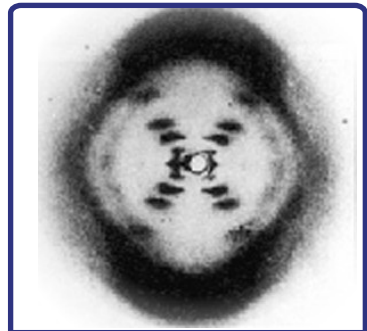
	A	T	C	G
Human	30.9%	29.4%	19.8%	19.9%
Salmon	29.7%	29.1%	20.4%	20.8%
Sheep	29.3%	28.3%	21.0%	21.4%
Turtle	29.7%	27.9%	21.3%	22.0%
Yeast	31.3%	32.9%	17.1%	18.7%
<i>E. coli</i>	24.7%	23.6%	25.7%	26.0%

Adapted from Wallace, R.A., King, J.L., & Sanders G.P. (1981). *Biology: The Science of Life*. Goodyear Publishing, Santa Monica.

The idea that the amount of A=T and C=G became known as Chargaff's rule. In a published paper he wrote:

The results serve to disprove the tetranucleotide hypothesis. It is, however, noteworthy—whether this is more than accidental cannot yet be said—that in all deoxyribose nucleic acids examined thus far the molar ratios... of adenine to thymine and of guanine to cytosine, were not far from one.

But Chargaff's work did not reveal anything about the structure of DNA, or how it passed traits from parents to progeny. That work began in earnest after 1950. At the time, the most modern technique available to scientists to collect information on the three-dimensional structure of molecules was called X-ray diffraction. Molecules, like DNA, were exposed to X-rays for up to 100 hours to produce an image hinting at the physical structure. X-ray diffraction gives patterns of light and dark marks that must be interpreted. Much skill was required to acquire good X-ray diffraction pictures and interpret them. Maurice Wilkins and Rosalind Franklin of the University College in London had these skills and had already been collecting such data when James Watson and Francis Crick began their quest to determine DNA's structure.



X-ray diffraction of DNA molecule.

The young James Watson graduated college at age 19, finished his doctorate at Indiana University at age 22, and then went to Europe to do post-doctorate work. Shy and quiet with a huge smile across his thin frame, Watson eventually attended a conference in Naples where he watched a presentation by Maurice Wilkins. Although Watson found Wilkins dry and unenthusiastic, the pictures of X-ray diffraction Watson saw at the conference inspired him to work on DNA.

Watson then moved to work at the University of Cambridge, England. There he shared an office with Francis Crick, who "talked louder and faster than anyone else," and could understand the most complex concepts almost instantly. Although Crick was fifteen years older than Watson, he had yet to finish his doctoral thesis. Crick's laughter echoing through the halls was vastly different than Watson's calmness. The two got along well despite their differences of personality.

Watson and Crick feared they might tread on the work of other DNA researchers, although they freely asked for help. Rosalind Franklin specialized in X-ray diffraction and gathered the data most valuable to Watson and Crick. Watson, however, did not get along with Franklin. This reflected difference in personalities, but also the sexism toward women in and out of science during the period. Despite this animosity, she was a brilliant scientist who lived a vibrant life and sought her just recognition.

As early as 1946, graduate student Sven Furberg had proposed DNA might be helical like polypeptides. In the late 1940s, Linus Pauling from Caltech in the United

States proposed amino acids were shaped in an alpha helix, and thought the same shape might apply to DNA. Wilkins and Franklin had X-ray diffraction evidence for a helical structure, but they didn't know whether it could be a single, double, or triple helix. Watson and Crick entered this debate and struggled for two years because of their lack of familiarity with the field. While paying attention to the X-ray diffraction work being done, they took a different approach. They would attack the DNA structure problem through model building. In Watson's words, the alpha helix had not been determined by Pauling by only looking at X-ray diffraction pictures:

...the essential trick, instead, was to ask which atoms like to sit next to each other. In place of pencil and paper, the main working tools were a set of molecular models superficially resembling the toys of preschool children.

Using this approach, they first proposed a triple helix with three sugar-phosphate backbones in the middle and the nitrogen bases sticking out. Confident in their model, they called Wilkins and asked him to come down to give his view. He, Franklin, and two others arrived the next day to see Watson and Crick's model. After observing the model and listening for a short while, Franklin and Wilkins made clear why the model could not work. Watson and Crick were embarrassed and their work stalled.

A major advance came when Rosalind Franklin developed a new way to image DNA. Prior X-ray diffraction was done on a "crystalline" form of DNA, called its "A form." Franklin determined that if she put the DNA in an environment of 70-90% humidity, the DNA opened up a bit. She called this the "B form." When the B form was subjected to X-ray diffraction, the resulting image was interpreted as clearly indicating a helical structure. Knowing the value of her discovery, she wanted to keep it quiet. One day, however, Watson visited Franklin to chat about helical structures. They got into an argument and she ran him out of the room. Watson took this to mean she detested the helix, although Franklin's personal notes indicate she was in favor of a helix. According to Watson, he feared that in the heat of their discussion, Franklin might strike him. But the tense situation was interrupted by Wilkins appearing at the doorway. All too familiar with his own tensions with Franklin, Wilkins began opening up to Watson and briefly showed him the new B form data, called photograph 51. Watson instantly interpreted the photo as clear evidence for a helix and raced back to Cambridge. Franklin, however, did not know that Wilkins had shown Watson her photo.

At this same time Linus Pauling had also been working on determining the DNA structure. He proposed a model that had several features similar to Watson and Crick's failed triple helix. When Watson and Crick learned that Pauling was on the hunt for the DNA structure, they devoted all

their efforts to beating him to the goal. By this time they had decided that the DNA sugar-phosphate backbone belonged on the outside, but they were uncertain whether it should be a double or triple helix. On the hunch that in biology, things tend to come in pairs, Watson began playing with two backbone models.

Watson spent considerable time trying to make a 'like-with-like' (i.e. C paired with C, G with G, T with T, and A with A) double stranded DNA structure work. However, he acknowledged that the difference in size between the pyrimidines and purines meant the sugar phosphate backbone would be quite irregular in width. Crick also noted that Watson's 'like-with-like' idea did not account for Chargaff's rule. Interestingly, Watson professed to have a "lukewarm" attitude towards Chargaff's experimental data. Although Watson continued to work with his 'like-with-like' idea, he eventually began entertaining other possibilities.

2. Note that Watson did not give up easily on his earlier idea despite the evidence against it. Why might this be the case with him or any other scientist? What does this illustrate about individual scientist's objectivity?

On February 21, 1953, Watson showed up early to his office. He began trying different arrangements of nitrogen base pairs beginning with his 'like-with-like' idea. Admitting that was fruitless, he began trying other possibilities. Watson suddenly became aware that an adenine-thymine pair was identical in shape to a guanine-cytosine pair. He later wrote:

...my morale skyrocketed, for I suspected that we now had the answer to the riddle of why the number of purine residues exactly equaled the number of pyrimidine residues. Chargaff's rule then suddenly stood out as a consequence of a double-helical structure for DNA.

3. Earlier Watson spoke poorly of scientists who did not accept the evidence for DNA being the genetic material. Yet Watson was resistant to accept Chargaff's experimental evidence. Why do you think Watson changed his mind about Chargaff's work? How does this story illustrate that scientific data does not tell scientists what to think?

When Crick showed up to the office later that morning, Watson excitedly shared his insight. Crick and other colleagues approved of the new configurations. In the days ahead, Watson and Crick began building a detailed model of their proposed structure to ensure its details would account for the available data. They wanted to let Wilkins and Franklin know of their model, but delayed in

calling. They remembered the disaster sixteen months earlier when they had prematurely asked Wilkins to come see their failed triple helix. Watson and Crick painstakingly completed the demonstration model, ensuring the bond angles and distances accurately accounted for the available data. When finished, they had a colleague call Wilkins and ask him to come see the DNA model that Watson and Crick had devised.

Wilkins quickly noted key features of the structure and liked the model. Before leaving, he said that he would compare the diffraction pattern predicted by the model to the X-ray diffraction data he and Franklin had collected. Two days later he called saying his and Franklin's data strongly supported a double helix. Shortly afterwards they all submitted articles to the journal *Nature* to announce to the world their structure.

! Watson and Crick's successful approach to solving this important scientific puzzle was to build DNA models that would account for the available data. This entailed a great deal of trial and error.

Major scientific insights often take much time to be accepted by the scientific community. However, the significance of the double helix was accepted relatively quickly. Of course, further work had to be done to provide overwhelming support for the model. Important supporting evidence came four years later in 1957, when Matthew Meselson and Franklin Stahl worked with the bacteria *E. coli*. Meselson and Stahl fed the *E. coli* a diet rich in heavy nitrogen-15 for one generation and then switched to normal nitrogen-14 for the subsequent generations. The first generation would have the traces of heavy nitrogen in its DNA, and if it really was a double helix, it would reproduce by splitting the helix in half. One half of the helix would go to each of the new cells and replicate a new strand of DNA using the normal nitrogen-14. So the original strand of heavy nitrogen-15 DNA would be present in each of its two daughter cells, then in two out of four of those cells, then two out of eight, and so on. Meselson and

Stahl provided compelling evidence in favor of the double helical structure of DNA and its implications for heredity.

In 1962, the Nobel Prize in Biology was awarded to James Watson, Francis Crick, and Maurice Wilkins. Rosalind Franklin would have assuredly also received a Nobel, but she tragically died of ovarian cancer in 1958. This was likely due to the extensive exposure to X-rays she received while performing her X-ray diffraction work. The Nobel Prize is not awarded posthumously. Unfortunately, Franklin's contributions are often overlooked. Watson characterized Franklin poorly in his popular 1968 book *The Double Helix*. Even Wilkins and Crick protested its publication. Other research and published books have more accurately depicted Franklin and her outstanding scientific work. But when the determination of the DNA structure is mentioned, still too often all credit appears to go to Watson, Crick, and sometimes Wilkins.

The story of the blueprint of life has now spanned over 140 years, and research in this area is accelerating. As we learn more, new questions arise. But the early history of DNA shows us that this is nothing new. Scientists worked for almost a hundred years to determine the genetic material, and the structure and replication of DNA. Much has been learned since the awarding of the Nobel Prize for this work in 1962, and yet many challenges remain. Watson and Crick may be the most well remembered scientists who worked on the structure of DNA, but they couldn't have succeeded without a large supporting cast.

4. Scientific journal articles that announced the structure of DNA to the scientific community provided evidence in support of the final structure. The missteps, personalities of participants, and significant interactions between individuals are left out of scientific journal articles. Why do you think that information is left out of science journals and science textbooks? How does leaving out that information result in science students having misconceptions regarding how science and scientists work?

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