On 6 May 1952, at King’s College London in London, England, Rosalind Franklin photographed her fifty-first X-ray diffraction pattern of deoxyribonucleic acid, or DNA. Photograph 51, or Photo 51, revealed information about DNA’s three-dimensional structure by displaying the way a beam of X-rays scattered off a pure fiber of DNA. Franklin took Photo 51 after scientists confirmed that DNA contained genes. Maurice Wilkins, Franklin’s colleague showed James Watson and Francis Crick Photo 51 without Franklin’s knowledge. Watson and Crick used that image to develop their structural model of DNA. In 1962, after Franklin’s death, Watson, Crick, and Wilkins shared the Nobel Prize in Physiology or Medicine for their findings about DNA. Franklin’s Photo 51 helped scientists learn more about the three-dimensional structure of DNA and enabled scientists to understand DNA’s role in heredity.

X-ray crystallography, the technique Franklin used to produce Photo 51 of DNA, is a method scientists use to determine the three-dimensional structure of a crystal. Crystals are solids with regular, repeating units of atoms. Some biological macromolecules, such as DNA, can form fibers suitable for analysis using X-ray crystallography because their solid forms consist of atoms arranged in a regular pattern. Photo 51 used DNA fibers, DNA crystals first produced in the 1970s. To perform an X-ray crystallography, scientists mount a purified fiber or crystal in an X-ray tube. The X-ray tube generates X-rays that strike the purified material. X-rays are electromagnetic waves that have a shorter wavelength and higher energy than visible light. Because of their short wavelength, X-rays can pass through a crystal and interact with the electrons of the atoms within the crystal. When X-rays interact with electrons in a crystal the X-rays scatter, or diffract, at angles that indicate the arrangement of atoms in the crystal, or its structure. When the X-rays scatter, they strike a film mounted behind the crystal and leave a pattern of dark marks. The pattern of dark marks on the film gives scientists information about the structure of the crystal.

Scientists began collecting X-ray diffraction patterns of DNA in the 1930s before they confirmed that DNA contained genes. William Thomas Astbury, a crystallographer working at the University of Leeds in Leeds, England, gathered the first diffraction patterns of DNA in 1937. However, Astbury’s diffraction patterns were blurry and difficult to interpret. At the time of Astbury’s experiments, scientists had determined the chemical composition of DNA. However, at that time scientists generally agreed that DNA merely provided structural support for cells and that protein must be genetic material. In 1944 Oswald Avery, Colin MacLeod and Maclyn McCarty published an experiment that isolated DNA as the material that contained genes.

Maurice Wilkins, a scientist working at King’s College London, collected X-ray diffraction patterns of DNA in 1950. Wilkins and his graduate student, Raymond Gosling, later Franklin’s graduate student, collected X-ray diffraction patterns of DNA purified in a way that produced longer fibers than those accessible to Astbury. When mounting the DNA fibers for viewing, Wilkins and Gosling were able to bundle many of the thin fibers together and pull them tight to provide a larger sample to better diffract X-rays. Furthermore, the two researchers kept the DNA fibers wet with water by keeping them in a humid environment. The resulting X-ray diffraction pattern of DNA was of a higher quality than any patterns collected prior.

Franklin, a specialist in X-ray crystallography, continued previous X-ray crystallography experiments on DNA with Gosling when she joined the King’s College London lab in 1951. Before joining the lab, Franklin conducted X-ray diffraction experiments on carbon compounds at a government lab in Paris, France, and published several papers on X-ray crystallography of coal and coal compounds. Throughout Franklin’s early work at King’s College London, she found that DNA fibers with a higher water content produced a different diffraction pattern than DNA fibers with a lower water content, indicating that wet and dry DNA adopted different three-dimensional conformations. Franklin later defined the drier DNA conformation as the A-Form DNA and the wetter DNA conformation as B-Form DNA. As of 2018, scientists continue to use the A Form and B Form designations for the two conformations of DNA. In addition to identifying the two forms of DNA, Franklin determined that Astbury’s diffraction patterns of DNA came from a mixture of A and B-Forms of DNA.

By improving her methods of collecting DNA X-ray diffraction images, Franklin obtained Photo 51 from an X-ray crystallography experiment she conducted on 6 May 1952. First, she minimized how much the X-rays scattered off the air surrounding the crystal by pumping hydrogen gas around the crystal. Because hydrogen only has one electron, it does not scatter X-rays well. She pumped hydrogen gas through a salt solution to maintain the targeted hydration of the DNA fibers. Franklin tuned the salt concentration of the solution and the humidity surrounding the crystal to keep DNA entirely in the B-Form. After exposing the DNA fibers to X-rays for a total of sixty-two hours, Franklin collected the resulting diffraction pattern and labeled it Number 51 that
became Photo 51.

Photo 51 presents a clear diffraction pattern for B-Form DNA. The outermost edge of the diffraction pattern consists of a black diamond shape. The diamond has rounded corners with the darkest corners situated at the top and bottom of the film. The diamond shape of the DNA diffraction pattern is not made of fine, definite lines, but rather thick, fuzzy boarders that vary in darkness such that the boarders fade on the left and right hand sides of the film. Inside the diamond is a cross shape like the letter "X." The X shape is not made of continuous lines. Instead, along each line of the X are four horizontal dashes, called spots that become darker moving closer to the center of the film. There is a hole at the center of the film, with dark spots lining the outside of the center hole.

Researchers could interpret an X-ray diffraction pattern of DNA with knowledge about DNA’s composition, which scientists had at the time Franklin collected photo 51. Years prior to Franklin’s work, scientists determined that DNA consists of a chain of repeating units called nucleotides. Each nucleotide has three key features. Each nucleotide consists of a center sugar ring called deoxyribose. Attached to one end of the deoxyribose ring is a negatively charged phosphate group consisting of phosphorus and oxygen atoms. Attached to the other end of the deoxyribose ring is a molecule called a base consisting of either single or double rings of carbon and nitrogen. There are four types of bases in DNA.

Using the available knowledge about DNA’s composition and mathematical techniques, Franklin learned of some key features regarding the structure of B-Form DNA from Photo 51. The presence of the X shape in the diffraction pattern indicated to Franklin that DNA strands were helical. Each dash of the X shape marks the repetition of atoms, or atomic repeats, in DNA. Therefore, based on the distances between the dashes, Franklin determined the distance between nucleotides, the smallest repeating units in DNA. The angles of the X shape revealed to Franklin the radius of DNA, or half the horizontal distance from one side of the molecule to the other. From the distance between the top and bottom of the outer diamond shape, Franklin found that there are ten nucleotides between each turn of the DNA molecule. Lastly, the lighter nature of the diamond on the top and bottom of the film showed Franklin that the DNA bases face the inside of the helix whereas the phosphate groups face outside. With knowledge of the density, mass per unit volume, of her DNA samples, Franklin also concluded that DNA contained two strands. While Franklin obtained Photo 51 in May 1952, she did not complete her analysis of Photo 51 until early 1953.

In January 1953, Watson visited King’s College London. While visiting, Wilkins showed Watson one of Franklin’s X-ray diffraction images of DNA, which historians claim was one of the clearest image of DNA, Photo 51, without Franklin’s knowledge. From the image, Watson concluded that DNA was helical. During his meeting with Wilkins, Watson also obtained necessary dimensions of DNA derived from Photo 51 that he and Crick later used to develop their proposed structure of DNA. Later, Watson and Crick received an internal King’s College London research report written by Franklin about her DNA diffraction images. From that report, Crick determined that DNA contains two strands, with each strand running in opposite directions.

Watson and Crick, two scientists at the University of Cambridge in Cambridge, England, relied on Franklin’s Photo 51 to propose a three-dimensional structure of DNA and in April 1953, they suggested a three-dimensional structure of DNA partly based on Photo 51. The model they suggested consisted of two helical strands of repeating nucleotides wound around each other making a double helix. The double helix had ten nucleotides between each turn. The phosphate groups faced outside the double helix and the DNA bases faced horizontally inward of the helix. The two strands held together through interactions between the bases of each strand. The DNA strands ran in opposite directions. As of 2019, Watson and Crick’s proposed DNA structure has remained the verified structure with a few variations of B-Form DNA, the major form of DNA in living cells.

Later, in May 1953, Watson and Crick proposed a replication mechanism for DNA using their DNA structure. Their replication mechanism, later called semi-conservative replication, described how to copy the DNA molecule that contained the genes[5] and to pass the genes[5] from cell to cell and from parent to offspring. Many features of B-Form DNA present in Photo 51 are necessary for semi-conservative replication, such as the DNA bases facing horizontally inward in the double helix. In addition, some aspects of B-Form DNA as indicated in Photo 51 posed challenges for semi-conservative replication. Watson and Crick proposed that the DNA strands needed to unwind and separate in order to replicate. However, because of the helical nature of
DNA, as shown in the X-ray diffraction pattern of Photo 51, some scientists argued that the DNA strands would be too difficult to unwind and separate. Some years passed before scientists accepted semi-conservative replication due to the perceived difficulty of unwinding the helical strands.

For their findings related to DNA, Watson, Crick, and Wilkins received the 1962 Nobel Prize in Physiology or Medicine. Franklin also contributed to understanding DNA structure, especially through her collection of Photo 51. She also determined many important features about DNA’s structure independently using Photo 51. The award of the Nobel Prize is never posthumously and Franklin died in 1958 before the award of the 1962 Nobel Prize. Some controversy and speculation surrounds the 1962 Nobel Prize concerning Franklin and her contributions to Watson and Crick’s DNA model. Only after the publication of Watson’s, book The Double Helix: A Personal Account of the Discovery of the Structure of DNA in 1968 was the roll that Franklin played in the discovery of the structure of DNA realized.

Photo 51, a clear X-ray diffraction pattern of DNA, showed structural features of DNA necessary for scientific understanding of DNA’s three-dimensional structure. By understanding DNA structure, scientists could learn about how DNA functioned as genetic material. The DNA structure revealed in Photo 51 related the essential functions of a gene how its information is preserved and carried from cells to cell and from parent to offspring.

Sources


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