DNA Structure and Function: A History

6.2

About 150 years ago, Mendel hypothesized the existence of a hereditary molecule that passes genetic information through the generations. Less than 70 years ago, scientists determined that this hereditary material is, in fact, DNA. Scientists have now sequenced the genome of many species, including humans, important crop plants, disease-causing micro-organisms, commonly used research animals and plants, and close genetic relatives of humans, such as the chimpanzee. This information has enhanced our understanding of the role that genetic mutations play in diseases such as breast cancer, prostate cancer, muscular dystrophy, and cystic fibrosis. Currently, scientists are using this information to develop gene therapy technologies and trial vaccines that directly target the genetic mechanisms in viruses. CAREER LINK

Outside of medicine, DNA analysis has been used to determine the lineage of King Tutankhamun, an Egyptian pharaoh. Police investigators use DNA technology to help them solve crimes and convict criminals. Every day, scientists around the world discover more about how DNA guides the functioning and evolution of life on Earth.

These discoveries have been possible due to the accumulated findings of many scientists. A single researcher rarely makes a major discovery without input from other scientists. The nature of science is collaborative and cumulative. Many hard-working and dedicated people, over many years, have added their contributions to the pool of scientific knowledge. While some of these people receive praise, even awards such as the Nobel Prize, others remain virtually unknown. The story of the discovery of the structure and function of DNA—like any other scientific discovery—is filled with many interesting people and their research developments.

Establishing DNA as the Hereditary Molecule

In 1868, Swiss physician Frederick Meischer was interested in the composition of the cell nucleus (**Figure 1**). At the time, proteins were thought to be the hereditary material because they were known to be complex and carry out numerous biological functions. Meischer collected pus (mainly white blood cells) from the bandages of his patients. From these cells, he extracted large quantities of an unknown substance that was acidic and had a large amount of phosphorus. Not knowing the true nature of this substance, he named it "nuclein" because he found it in the nucleus of the white blood cells.

Figure 1 Frederick Meischer discovered "nuclein," later identified as DNA.

At the time of Meischer's discovery, scientists knew little about the molecules and processes that underlie heredity. Although Mendel was crossbreeding his peas at about the same time (1865), his work was generally unnoticed until the early 1900s. Many scientists believed that heredity involved a mixing of characteristics from the two parents. However, they could not explain why a short woman and a tall man produced children with a variety of heights, not a height that was an average of the two parents' heights. The chemical composition and function of Meischer's newly discovered molecule, nuclein, were not determined for more than 50 years, and its structure was not determined until some decades after that.

Frederick Griffith: The Transforming Principle

The role of Meischer's nuclein as the hereditary material was still unknown when, in the last days of World War I, a pneumonia epidemic struck Europe. Frederick Griffith, a medical officer for the British military, decided to research this disease. In 1928, he carried out an experiment that accidentally shed light on the function of DNA in inheritance.

Griffith used two different strains of the pneumonia bacterium. One strain had a capsule that surrounded each cell and caused the bacterial colonies to look smooth and glossy when grown on agar. Griffith called this the smooth strain, or S-strain, of pneumonia. The second strain of pneumonia lacked this smooth capsule and, as a result, formed rough and irregular colonies when cultured. He called this strain the rough strain, or R-strain. When Griffith injected mice with the S-strain, the mice contracted

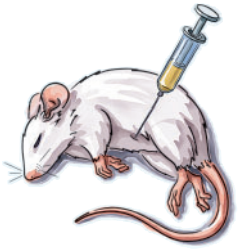
pneumonia and died in a matter of days. The S-strain bacteria were highly virulent (pathogenic) (**Figure 2(a)**). However, when Griffith injected mice with the R-strain of bacteria, the mice showed no signs of pneumonia and survived (**Figure 2(b)**). Griffith concluded that the capsule surrounding the S-strain was responsible for the virulence.

Griffith's next step was the important one. He heated the S-strain cells, destroying the capsule that surrounded the cells and killing the bacteria. The dead S-strain no longer caused an infection in the mice (**Figure 2(c)**). However, when he mixed the heat-killed S-strain with the live non-virulent R-strain, many of the mice contracted pneumonia and died (**Figure 2(d)**). Griffith then isolated living bacteria that appeared to be S-strain bacteria from the dead mice. Somehow, the living R-strain bacteria acquired some factor from the heat-killed S-strain that made them virulent. The newly virulent R-strain bacteria even formed smooth colonies when cultured, just like the living S-strain bacteria. We now know that bacteria can take up genetic material from nearby bacteria and use this DNA as their own.

(a) Mice injected with live S-strain cells died.

(b) Mice injected with live R-strain cells lived.

(c) Mice injected with heat-killed S-strain cells lived, showing that S-strain cells must be alive to be virulent to mice.



(d) Mice injected with heat-killed S-strain cells plus live R-strain cells died. Live R-strain cells were converted to virulent S-strain cells with some factor from dead S-strain cells.

Figure 2 Mixing heat-killed S-strain cells with live R-strain cells made the R-strain cells virulent.

Although Griffith could not identify the exact material involved in inheritance, he understood that some hereditary substance had passed from the dead S-strain cells to the live R-strain cells. When the R-strain bacteria acquired this material, they were effectively transformed into infectious S-strain bacteria. He called this process **transformation**, and he called the factor that was responsible the transforming principle. At the time, the most likely candidates for the transforming principle were proteins and DNA, but further experiments were required to determine its identity.

transformation a change in a genotype or phenotype caused by the direct uptake of genetic material by a cell

Avery, McLeod, and McCarty: DNA Transformation Confirmed

Building on Griffith's findings, physician Oswald Avery and his co-workers Colin McLeod and Maclyn McCarty carried out a series of similar experiments in 1944. The researchers grew different strains of *Streptococcus* bacteria in culture tubes that contained a growth medium. They kept the different strains isolated from one another. Like the pneumonia bacteria, *Streptococcus* have S- and R-strains, which differ in their disease-causing ability. Avery and his colleagues wanted to determine which part of the S-strain bacteria cell was responsible for making R-strain bacteria virulent. The possible transforming substances were DNA, RNA, and proteins. Like Griffiths, they heat-killed the S-strain. Then they treated molecules extracted from the dead bacteria with one of three enzymes to destroy

one of the three possible transforming substances. They mixed each extract with R-strain bacteria and recorded how the new cultures looked when grown in culture tubes.

Although their results clearly showed that DNA was the transforming substance, Avery and his colleagues were hesitant to report this directly. At the time, most scientists believed that proteins were the genetic material. If some protein had not been destroyed by the enzymes, their results would be incorrect. Furthermore, precise experiments were required to establish the role of DNA as the carrier of genetic information.

Hershey and Chase: DNA Is the Hereditary Material

In 1952, bacteriologists Alfred D. Hershey and Martha Chase attempted to determine whether proteins or DNA functioned as the genetic material. They used a virus and the bacteria *E. coli*. The virus they used was a **bacteriophage**: a virus that specifically infects bacteria. The particular bacteriophage they used had both DNA and a protein coat. When a bacteriophage infects a bacterium, it inserts its genetic material into the bacterium and uses the bacterium's cellular processes to produce new bacteriophages. Keep in mind that, at the time, no one knew what part or parts of a virus entered cells during an infection or even what a virus looked like. Viruses were much too small to be seen with the most powerful microscopes available. WEB LINK

bacteriophage a virus that infects bacteria

Hershey and Chase knew that bacteria could be transformed by viruses, but they did not know which part of the virus—the protein coat or the DNA (or RNA)—did the transforming. They used radioisotopes to label the different molecules. An isotope is an atom of an element that has a different number of neutrons than usual, and a radioisotope is an isotope that emits radiation.

Hershey and Chase used the radioisotopes of phosphorus and sulfur, ^{32}P and ^{35}S. Sulfur was used to label proteins because proteins contain sulfur but DNA molecules do not. Phosphorus was used to label DNA because DNA molecules contain phosphorus while proteins contain only a tiny amount. Hershey and Chase labelled some bacteriophages with the sulfur isotope and other bacteriophages with the phosphorus isotope, and then infected different colonies of the bacteria (**Figure 3**).

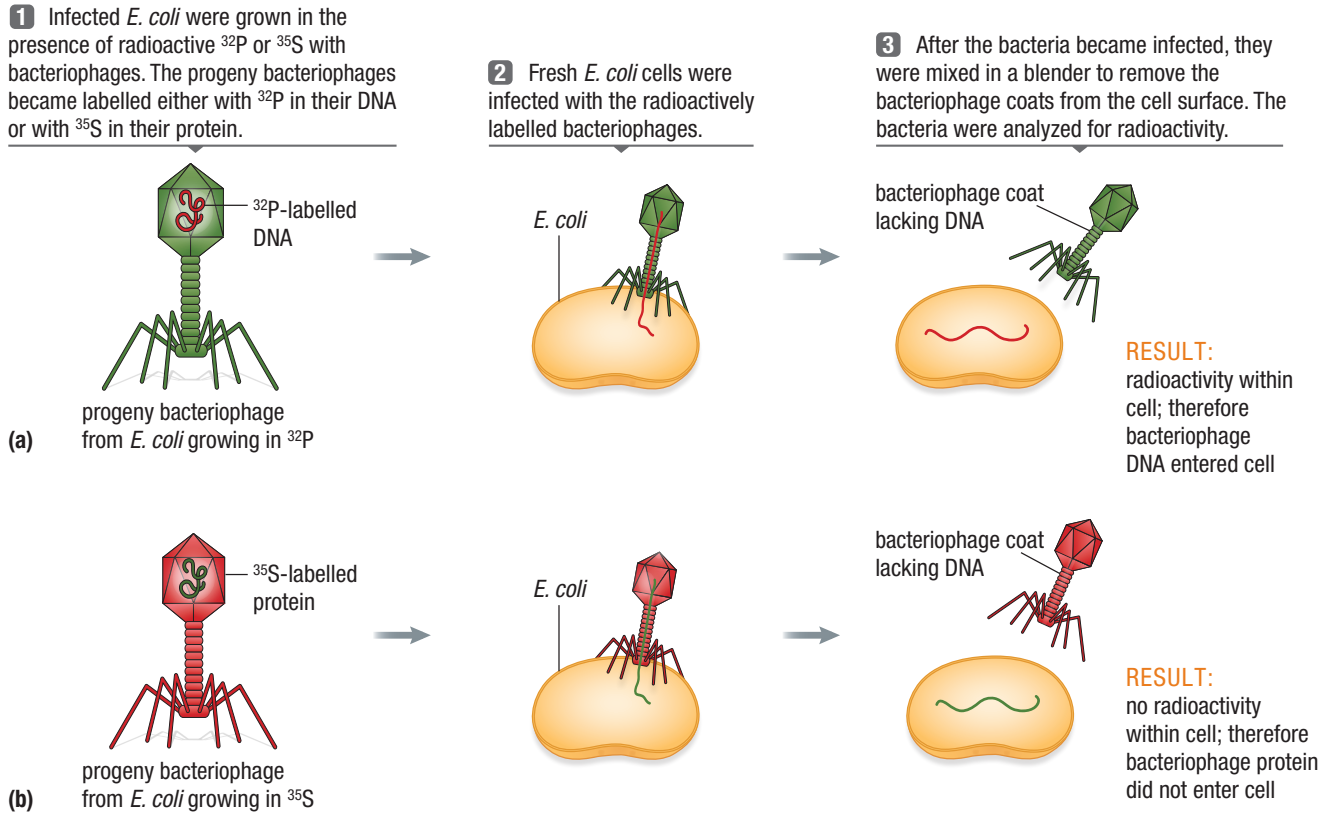


Figure 3 Hershey and Chase used progeny bacteriophages labelled with radioisotopes of either (a) phosphorus, ^{32}P, or (b) sulfur, ^{35}S. Their experiments indicated that DNA is the material transmitted from virus to bacteria.

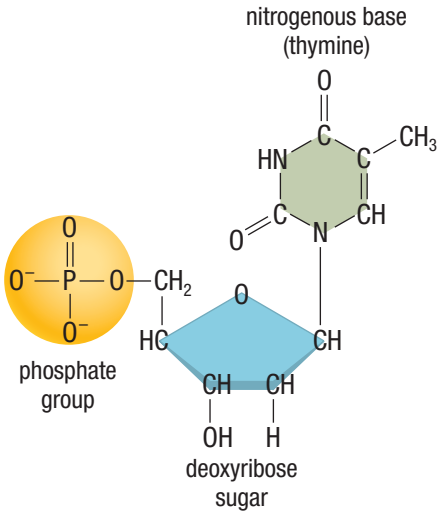


Figure 4 A nucleotide consists of a deoxyribose sugar, a phosphate group, and a nitrogenous base.

purine a class of nitrogenous bases with a double-ring structure; adenine and guanine are purines

pyrimidine a class of nitrogenous bases with a single-ring structure; thymine and cytosine are pyrimidines

After providing time for the bacteria to become infected, they separated the bacterial cells from any viral particles remaining outside the cell. Depending on which radioisotope they found within the bacterial cells, the true hereditary material would be found to be either DNA or protein. Their results were conclusive. Radioactivity was only detected within bacterial cells that had been infected by viruses containing DNA labelled with ^{32}P. The radioactive protein coats had remained outside the bacterial cells, while the radioactive DNA had entered the cells. Hershey and Chase concluded that DNA must be responsible for carrying genetic information. CAREER LINK

The Chemical Composition of DNA

Meanwhile, other scientists were researching the molecular composition of Meischer's nuclein. In the 1920s, Phoebus Levene reported that each DNA molecule contained three major components: deoxyribose sugars, phosphate groups, and nitrogenous bases. A DNA molecule is a polymer made of nucleotide subunits. Each nucleotide subunit consists of a nitrogenous base attached to one deoxyribose sugar, which is connected to a phosphate group (**Figure 4**). By 1949, the four nitrogenous bases had been identified (**Figure 5**). Adenine (A) and guanine (G) are double-ring structures known as **purines**, while thymine (T) and cytosine (C) are single-ring structures called **pyrimidines**.

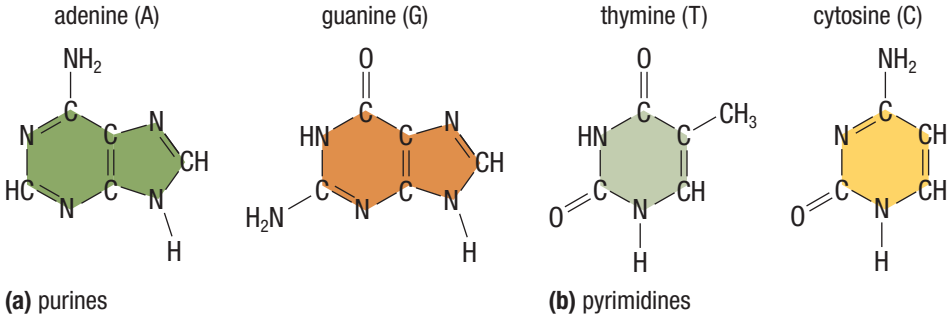


Figure 5 The four nitrogenous bases of DNA are classified as either (a) purines or (b) pyrimidines.

Edward Chargaff, an organic chemist, did not agree with researchers who suggested that DNA contained equal amounts of the four nitrogenous bases. In 1950, Chargaff found that these bases always occur in definite ratios. He also found that the quantities of thymine and adenine always matched, as did the quantities of guanine and cytosine. Human DNA, for example, was estimated to contain 30.9 % A, 29.4 % T, 19.9 % G, and 19.8 % C. This information was to prove vital to future research.

Scientists now knew DNA's chemical composition and its role as the molecule of inheritance. However, there were still many unknowns. For example, scientists did not know the structure of the DNA molecule. Nor did they know how the cell made the DNA or how it was passed from parent to offspring.

Wilkins and Franklin: Another Piece of the Puzzle

New discoveries in science are often facilitated by new research technologies. This is certainly true of the research into the chemical composition, structure, and function of DNA. One new technology at that time was X-ray crystallography. This technology involves X-rays bombarding a sample of a compound, which is usually in the form of a solid crystal. The atoms in the compound deflect the X-rays in a specific way, creating a pattern on a photographic plate. The pattern is then analyzed to help determine the molecular structure of the original sample.

Rosalind Franklin and Maurice Wilkins used X-ray crystallography to study the shape of the DNA molecule. They worked somewhat independently on two crystal forms. Although they were colleagues, working in the same laboratory at King's College in London, their relationship was less than congenial. Wilkins

had produced some preliminary crystallographs of DNA that suggested its helical structure, but Franklin was unconvinced. Wilkins's DNA samples were poorly prepared, so his crystallographs were difficult to interpret. Franklin prepared much purer crystallized DNA samples and was able to produce some excellent crystallographs. She noticed that the pattern produced by the X-rays was in the shape of an X (**Figure 6**).

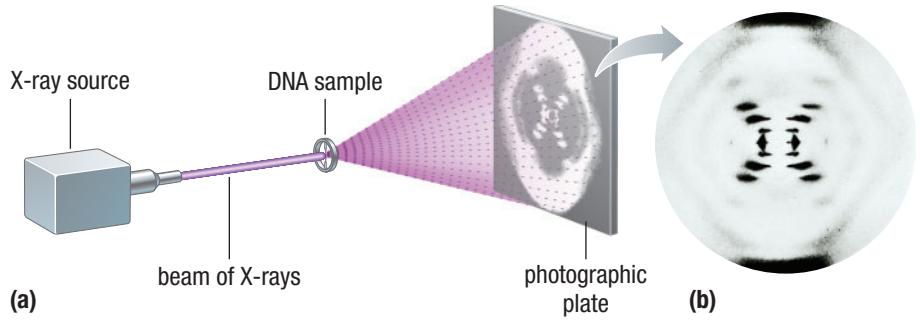


Figure 6 (a) X-ray diffraction was used to study DNA. (b) The X-shaped pattern of spots (dashed lines) that Rosalind Franklin obtained was correctly interpreted by Franklin to indicate that DNA has a helical structure, similar to a spiral staircase.

After analyzing the pattern in her crystallographs, Franklin suggested that the sugar-phosphate backbones of DNA faced the outside of the molecule, not the inside, as was generally believed at the time. She also suggested that DNA was a double helix, which rotated in a clockwise direction. She determined that the DNA molecule had a diameter of 2 nm and that one turn of the helix was 3.4 nm in length. However, she could not explain how the nitrogenous bases were associated in the centre of the helix, even though she was aware of Chargaff's findings. Not wishing to appear incomplete in her analysis, she was hesitant to publish her results.

Watson and Crick: Building a Model of DNA

In science, the synthesis of existing ideas is often just as important as the discovery of new ideas. How a series of discoveries fits together can be the key that unlocks their full meaning. In 1952, the team of James Watson and Francis Crick were building models of the DNA molecule, incorporating everything they knew about DNA. They had a wealth of information available to them:

- four different nitrogenous bases (A, T, C, and G)
- Chargaff's ratios of the nitrogenous bases
- the phosphate and sugar backbone

Without Franklin's knowledge, Maurice Wilkins revealed details of her work to James Watson. The information available to Watson and Crick now included Franklin's ideas about a double helix and inward-facing bases, and her calculations of the size of the DNA molecule. Watson and Crick realized that the double helix could incorporate all of the facts. In their model, each strand of the helix consisted of a phosphate and sugar backbone. The nitrogenous bases were attached to the backbone and directed toward the centre of the molecule. The strands twisted around each other in a clockwise direction. Down the centre of the molecule, each nitrogenous base on one strand was hydrogen bonded with a nitrogenous base on the other strand (**Figure 7**, next page).

Watson and Crick's model showed that the DNA molecule could only be stable if the strands ran antiparallel, that is, if they ran in opposite directions. In other words, one DNA strand must have the hydroxyl of the 3′ carbon attached to the deoxyribose sugar at one end and the phosphate attached to the 5′ carbon of the last sugar at the other end. The other strand must wind around the first with its 5′ end opposite the 3′ end of the first strand (Figure 7). Watson and Crick's model also showed that the nitrogenous bases are connected by hydrogen bonds, keeping the two strands

Investigation 6.2.1

Extracting DNA from Plants (p. 299)
All life uses DNA for its genetic material, but some cells contain more DNA than others. The number of chromosomes and the number of copies of each chromosome within a cell are directly related to how much DNA the cell contains. In this investigation, you will compare the amount of DNA extracted from different plant species.

Investigation 6.2.2

Extracting DNA: Design Your Own Experiment (p. 300)
In this investigation, you will examine how changing the experimental protocol affects the yield of DNA from strawberries.

complementary base pairing the chemical tendency of adenine to form hydrogen bonds with thymine, and cytosine to form hydrogen bonds with guanine

together. The purine and pyrimidine base pairs are linked to each other, according to Chargaff's rules. Therefore, thymine always bonds with adenine by two hydrogen bonds, and guanine always bonds with cytosine by three hydrogen bonds. This is known as **complementary base pairing**. The symmetry is key to the structure of DNA and its ability to divide itself accurately and convey genetic information.

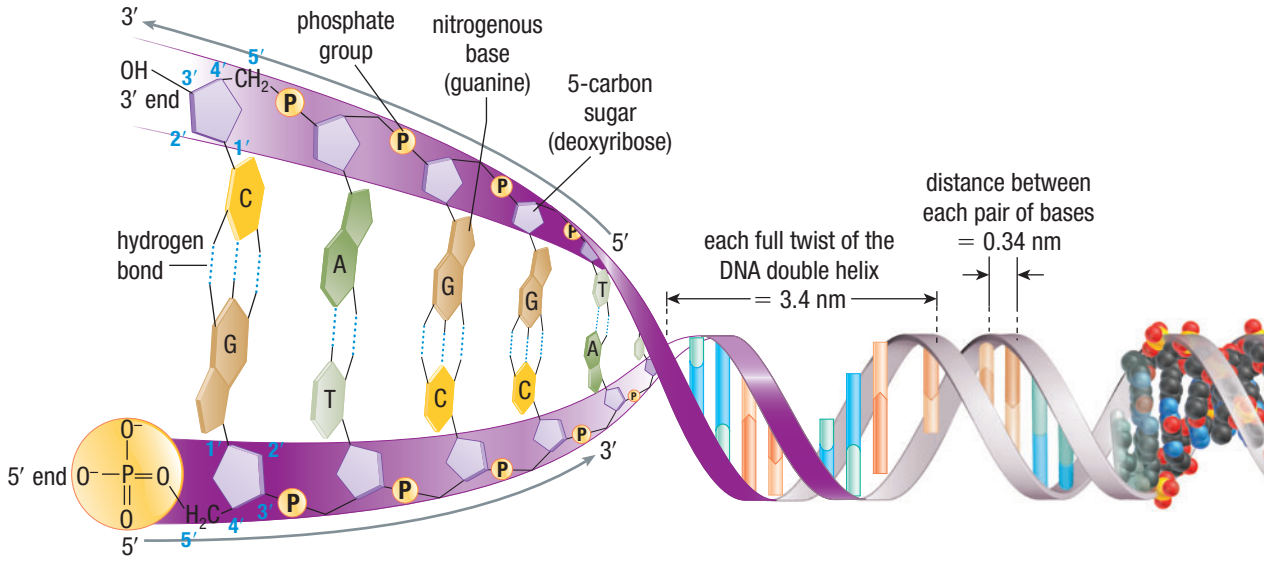


Figure 7 DNA is in the shape of a double helix.

Numerous future experiments supported Watson and Crick's model of the DNA molecule. Their work revolutionized our understanding of life and led to many scientific breakthroughs.

Research This

The Collaborative Nature of Science

Skills: Researching, Analyzing, Evaluating, Communicating

SKILLS HANDBOOK A4.1

Many scientists contributed to the information that Watson and Crick used to put together their famous model of DNA.

1. Choose one of the following scientists: Frederick Griffith, Oswald Avery, Erwin Chargaff, Alfred Hershey, Martha Chase, Rosalind Franklin, or Maurice Wilkins.
2. Use the Internet and other sources to research the following information about the scientist you chose:
   - the date of the scientist's discovery
   - the experiments that the scientist conducted and where the experiments were conducted
   - the scientist's findings and conclusions
   - the journal in which the scientist's findings and conclusions were published, and when they were published

A. In a one-page report, discuss the contributions of the scientist you researched. In addition to the information in Step 2, include a diagram and answer questions B to E. T/I C

B. How did Watson and Crick use the information discovered by this scientist to help them build their molecular model? T/I

C. Is science collaborative? Use examples from your research and Section 6.2 to support your answer. T/I

D. How does the use of journals enhance the collaboration between scientists? T/I

E. Why is it important that scientists collaborate? T/I C

WEB LINK

6.2 Review

Summary

- Griffith discovered a substance derived from infectious bacteria that could turn non-infectious bacteria into infectious bacteria.
- Avery, McLeod, and McCarty showed that DNA, rather than RNA or protein, was the transforming substance.
- Hershey and Chase confirmed that the DNA of a bacteriophage transformed the DNA of an infected bacterium.
- Chargaff discovered that the nitrogenous bases in DNA always occur in exact ratios, with the number of adenine matching the number of thymine, and the number of cytosine matching the number of guanine.
- Using X-ray crystallography, Franklin and Wilkins provided vital information about the size and shape of the DNA molecule.
- The discovery of the chemical composition, function, and structure of DNA involved the work of numerous scientists over many decades. Watson and Crick combined all of the available information into their double helix model of DNA.
- Each nucleotide in the DNA molecule consists of a deoxyribose sugar, a phosphate group, and one of the four nitrogenous bases. The phosphates and sugars are joined together to form the backbone of each strand. The molecule is double stranded and forms a helix. The two strands of the molecule are antiparallel.

Questions

1. What would Hershey and Chase have concluded if they had found radioactive sulfur instead of phosphorus in infected bacteria cells? K/U T/I
2. The nitrogenous base content of a sample of DNA was found to be 32 % adenine. Determine the amounts of the other three bases in this sample. K/U T/I
3. Describe and sketch the structure of DNA. K/U C
4. Write the complementary strand for the following sequence: GTGACTAACAGTGGCCAT K/U
5. In point-form notes or a timeline, summarize the findings of Frederick Griffith, Oswald Avery, Alfred Hershey and Martha Chase, Erwin Chargaff, Rosalind Franklin, Maurice Wilkins, and James Watson and Francis Crick. K/U C
6. Distinguish between the following substances:
   (a) nitrogenous bases and nucleotides
   (b) purines and pyrimidines
   (c) bacteria and bacteriophages K/U
7. How did the reproductive behaviour of bacteriophages allow Hershey and Chase to conduct their experiment? K/U
8. (a) Why were Avery, McLeod, and McCarty hesitant to report their findings?
   (b) Why is it important for scientists to report their findings? K/U T/I
9. Watson and Crick did not actually conduct any experiments with DNA. Do you think they can be considered scientists? Explain your reasoning. T/I A