DNA as the genetic material

Introduction

Our modern understanding of DNA's role in heredity has led to a variety of practical applications, including forensic analysis, paternity testing, and genetic screening. Thanks to these wide-ranging uses, today many people have at least a basic awareness of DNA.

It may be surprising, then, to realize that less than a century ago, even the best-educated members of the scientific community did not know that DNA was the hereditary material!

In this article, we'll look at some of the classic experiments that led to the identification of DNA as the carrier of genetic information.

Protein vs. DNA

The work of Gregor Mendel showed that traits (such as flower colors in pea plants) were not inherited directly, but rather, were specified by genes passed on from parents to offspring. The work of additional scientists around the turn of the 20th century, including Theodor Boveri, Walter Sutton, and Thomas Hunt Morgan, established that Mendel's heritable factors were most likely carried on chromosomes.

Scientists first thought that proteins, which are found in chromosomes along with DNA, would turn out to be the sought-after genetic material. Proteins were known to have diverse amino acid sequences, while DNA was thought to be a boring, repetitive polymer, due in part to an incorrect (but popular) model of its structure and composition^1start superscript, 1, end superscript.

Today, we know that DNA is not actually repetitive and can carry large amounts of information, as discussed further in the article on discovery of DNA structure. But how did scientists first come to realize that "boring" DNA might actually be the genetic material?

Frederick Griffith: Bacterial transformation

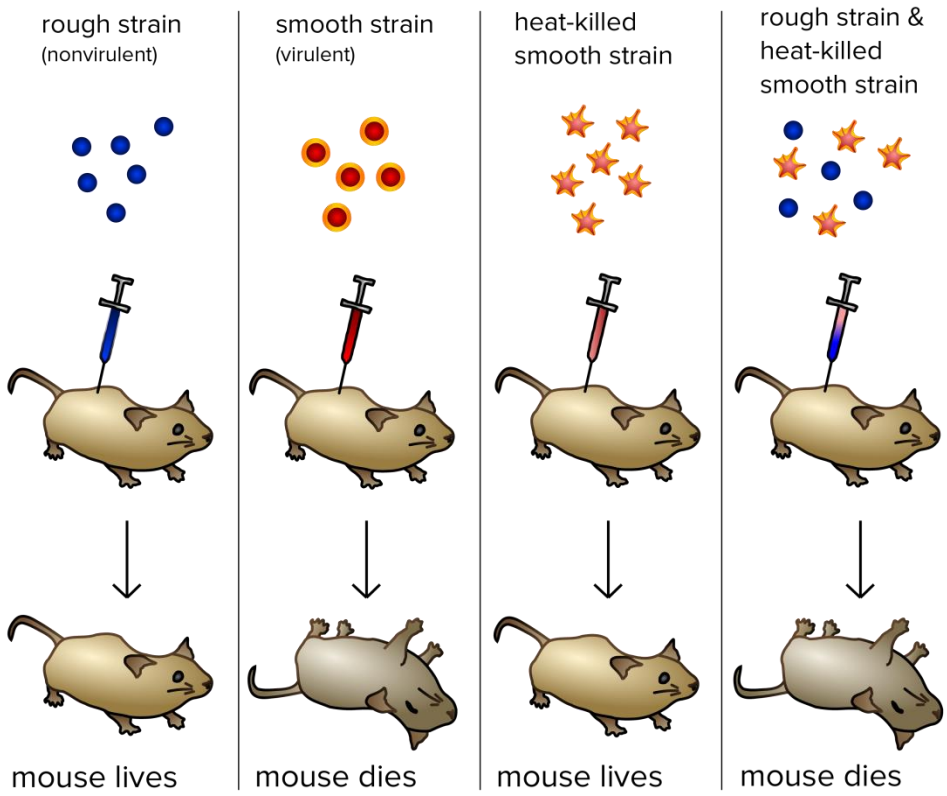
In 1928, British bacteriologist Frederick Griffith conducted a series of experiments using *Streptococcus pneumoniae* bacteria and mice. Griffith wasn't trying to identify the genetic material, but rather, trying to develop a vaccine against pneumonia. In his experiments, Griffith used two related strains of bacteria, known as R and S.

- **R strain.** When grown in a petri dish, the R bacteria formed colonies, or clumps of related bacteria, that had well-defined edges and a rough appearance (hence the abbreviation "R"). The R bacteria were nonvirulent, meaning that they did not cause sickness when injected into a mouse.
- **S strain.** S bacteria formed colonies that were rounded and smooth (hence the abbreviation "S"). The smooth appearance was due to a polysaccharide, or sugar-based, coat produced by the bacteria. This coat protected the S bacteria from the mouse immune system, making them virulent (capable of causing disease). Mice injected with live S bacteria developed pneumonia and died.

  As part of his experiments, Griffith tried injecting mice with heat-killed S bacteria (that is, S bacteria that had been heated to high temperatures, causing the cells to die). Unsurprisingly, the heat-killed S bacteria did not cause disease in mice.

The experiments took an unexpected turn, however, when harmless R bacteria were combined with harmless heat-killed S bacteria and injected into a mouse. Not only did the mouse develop pnenumonia and die, but when Griffith took a blood sample from the dead mouse, he found that it contained living S bacteria!

Griffith concluded that the R-strain bacteria must have taken up what he called a "transforming principle" from the heat-killed S bacteria, which allowed them to "transform" into smooth-coated bacteria and become virulent.



Avery, McCarty, and MacLeod: Identifying the transforming principle

In 1944, three Canadian and American researchers, Oswald Avery, Maclyn McCarty, and Colin MacLeod, set out to identify Griffith's "transforming principle."

To do so, they began with large cultures of heat-killed S cells and, through a long series of biochemical steps (determined by careful experimentation), progressively purified the transforming principle by washing away, separating out, or enzymatically destroying the other cellular components. By this method, they were able to obtain small amounts of highly purified transforming principle, which they could then analyze through other tests to determine its identity^2squared.

Several lines of evidence suggested to Avery and his colleagues that the transforming principle might be DNA^2squared:

- The purified substance gave a negative result in chemical tests known to detect proteins, but a strongly positive result in a chemical test known to detect DNA.
- The elemental composition of the purified transforming principle closely resembled DNA in its ratio of nitrogen and phosphorous.
- Protein- and RNA-degrading enzymes had little effect on the transforming principle, but enzymes able to degrade DNA eliminated the transforming activity.

These results all pointed to DNA as the likely transforming principle. However, Avery was cautious in interpreting his results. He realized that it was still possible that some contaminating substance present in small amounts, not DNA, was the actual transforming principle^3cubed.

Because of this possibility, debate over DNA's role continued until 1952, when Alfred Hershey and Martha Chase used a different approach to conclusively identify DNA as the genetic material.

The Hershey-Chase experiments

In their now-legendary experiments, Hershey and Chase studied **bacteriophage**, or viruses that attack bacteria. The phages they used were simple particles composed of protein and DNA, with the outer structures made of protein and the inner core consisting of DNA.

Hershey and Chase knew that the phages attached to the surface of a host bacterial cell and injected some substance (either DNA or protein) into the host. This substance gave "instructions" that caused the host bacterium to start making lots and lots of phages—in other words, it was the phage's genetic material. Before the experiment, Hershey thought that the genetic material would prove to be protein^4start superscript, 4, end superscript.

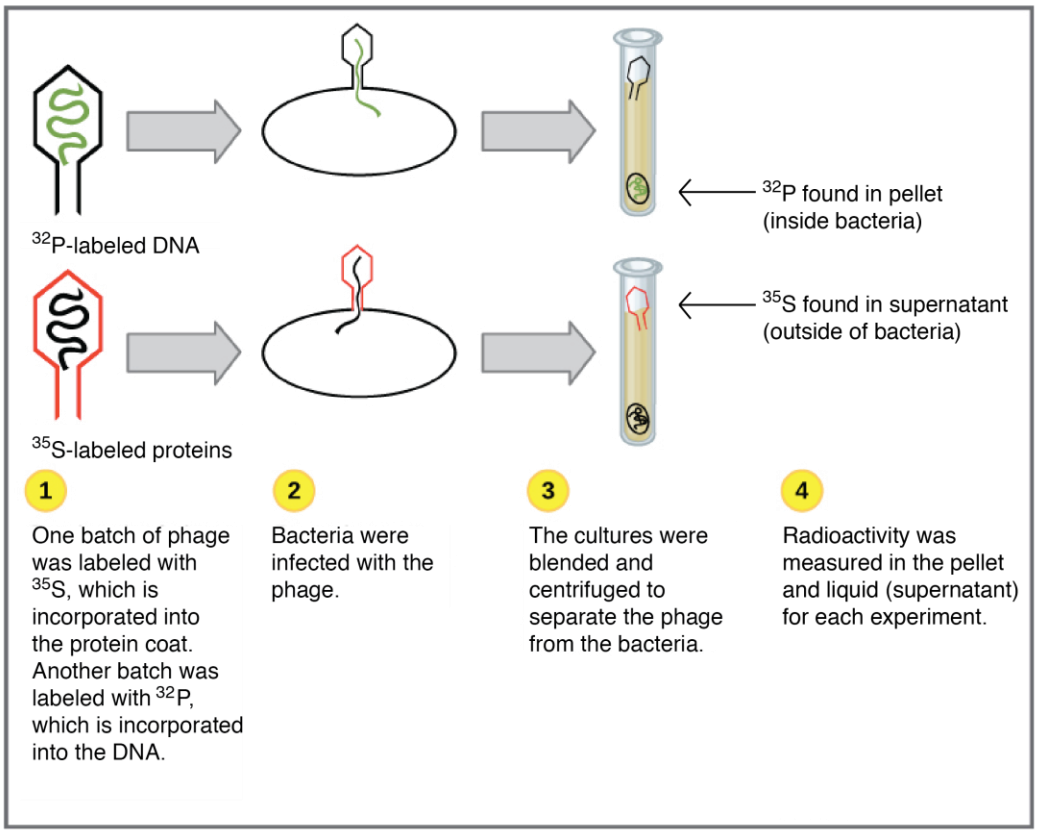
To establish whether the phage injected DNA or protein into host bacteria, Hershey and Chase prepared two different batches of phage. In each batch, the phage were produced in the presence of a specific radioactive element, which was incorporated into the macromolecules (DNA and protein) that made up the phage.

- One sample was produced in the presence of ^{35}\text S35Sstart superscript, 35, end superscript, start text, S, end text, a radioactive isotope of sulfur. Sulfur is found in many proteins and is absent from DNA, so only phage proteins were radioactively labeled by this treatment.
- The other sample was produced in the presence of ^{32}\text P32Pstart superscript, 32, end superscript, start text, P, end text, a radioactive isotope of

phosphorous. Phosphorous is found in DNA and not in proteins, so only phage DNA (and not phage proteins) was radioactively labeled by this treatment.

Each batch of phage was used to infect a different culture of bacteria. After infection had taken place, each culture was whirled in a blender, removing any remaining phage and phage parts from the outside of the bacterial cells. Finally, the cultures were centrifuged, or spun at high speeds, to separate the bacteria from the phage debris.

Centrifugation causes heavier material, such as bacteria, to move to the bottom of the tube and form a lump called a **pellet**. Lighter material, such as the medium (broth) used to grow the cultures, along with phage and phage parts, remains near the top of the tube and forms a liquid layer called the **supernatant**.



When Hershey and Chase measured radioactivity in the pellet and supernatant from both of their experiments, they found that a large amount of ^{32}\text P32Pstart superscript, 32, end superscript, start text, P, end text appeared in the pellet, whereas almost all of the ^{35}\text S35Sstart superscript, 35, end superscript, start text, S, end text appeared in the supernatant. Based on this and similar experiments, Hershey and Chase concluded that DNA, not protein, was injected into host cells and made up the genetic material of the phage.

Introduction

Today, the DNA double helix is probably the most iconic of all biological molecules. It's inspired staircases, decorations, pedestrian bridges (like the one in Singapore, shown below), and more.

I have to agree with the architects and designers: the double helix is a beautiful structure, one whose form fits its function in a remarkable way. But the double helix was not always part of our cultural lexicon. In fact, until the 1950s, the structure of DNA remained a mystery.

In this article, we'll briefly explore how the double-helical structure of DNA was discovered through the work of James Watson, Francis Crick, Rosalind Franklin, and other researchers. Then, we'll take a look at the properties of the double helix itself.

The components of DNA

From the work of biochemist Phoebus Levene and others, scientists in Watson and Crick's time knew that DNA was composed of subunits called **nucleotides^1**1start superscript, 1, end superscript. A nucleotide is

made up of a sugar (deoxyribose), a phosphate group, and one of four nitrogenous bases: adenine (A), thymine (T), guanine (G) or cytosine (C).

C and T bases, which have just one ring, are called **pyrimidines**, while A and G bases, which have two rings, are called **purines**.

DNA nucleotides assemble in chains linked by covalent bonds, which form between the deoxyribose sugar of one nucleotide and the phosphate group of the next. This arrangement makes an alternating chain of deoxyribose sugar and phosphate groups in the DNA polymer, a structure known as the **sugar-phosphate backbone**

Chargaff's rules

One other key piece of information related to the structure of DNA came from Austrian biochemist Erwin Chargaff. Chargaff analyzed the DNA of different species, determining its composition of A, T, C, and G bases. He made several key observations:

- A, T, C, and G were not found in equal quantities (as some models at the time would have predicted)
- The amounts of the bases varied among species, but not between individuals of the same species
- The amount of A always equalled the amount of T, and the amount of C always equalled the amount of G (A = T and G = C)

These findings, called **Chargaff's rules**, turned out to be crucial to Watson and Crick's model of the DNA double helix.

Watson, Crick, and Rosalind Franklin

In the early 1950s, American biologist James Watson and British physicist Francis Crick came up with their famous model of the DNA double helix. They were the first to cross the finish line in this scientific "race," with others such as Linus Pauling (who discovered protein secondary structure) also trying to find the correct model.

Rather than carrying out new experiments in the lab, Watson and Crick mostly collected and analyzed existing pieces of data, putting them together in new and insightful ways^2squared. Some of their most crucial clues to DNA's structure came from Rosalind Franklin, a chemist working in the lab of physicist Maurice Wilkins.

Franklin was an expert in a powerful technique for determining the structure of molecules, known as **X-ray crystallography**. When the crystallized form of a molecule such as DNA is exposed to X-rays, some of the rays are deflected by the atoms in the crystal, forming a **diffraction pattern** that gives clues about the molecule's structure.

Franklin’s crystallography gave Watson and Crick important clues to the structure of DNA. Some of these came from the famous “image 51,” a remarkably clear and striking X-ray diffraction image of DNA produced by Franklin and her graduate student. (A modern example of the diffraction pattern produced by DNA is shown above.) To Watson, the X-shaped diffraction pattern of Franklin's image immediately suggested a helical, two-stranded structure for DNA^3cubed.

RNA as Genetic Material:

The genome of viruses may be DNA or RNA. Most of the plant viruses have RNA as their hereditary material. Fraenkel-Conrat (1957)

conducted experiments on tobacco mosaic virus (TMV) to demonstrate that in some viruses RNA acts as genetic material.

TMV is a small virus composed of a single molecule of spring-like RNA encapsulated in a cylindrical protein coat. Different strains of TMV can be identified on the basis of differences in the chemical composition of their protein coats. By using the appropriate chemical treatments, proteins and RNA of RNV can be separated.

Moreover, these processes are reversible by missing the protein and RNA under appropriate conditions—reconstitution will occur yielding complete infective TMV particles. Fraenkel-Conrat and Singer took two different strains of TMV and separated the RNAs from protein coats, reconstituted hybrid viruses by mixing the proteins of one strain with the RNA of the second strain, and vice versa.

When the hybrid or reconstituted viruses were rubbed into live tobacco leaves, the progeny viruses produced were always found to be phenotypically and genotypically identical to the parental type from where the RNA had been isolated (Fig. 12.7). Thus the genetic information of TMV is stored in the RNA and not in the protein.