

## CHAPTER 6

### GRIFFITH/HERSHEY/CHASE: DNA IS THE GENETIC MATERIAL

*In 1928, Frederick Griffith was able to transform harmless bacteria into virulent pathogens with an extract that Oswald Avery proved, in 1944, to be DNA. In 1952, Martha Chase and Alfred Hershey used radioactively labeled virus DNA to infect bacteria, proving the same point. These important experiments established that DNA is the genetic material.*

#### IDENTIFICATION OF DNA

Deoxyribonucleic acid (DNA) was first described by Friedrich Miescher in 1869, only four years after Mendel's work was published. But it took over 80 years for its role as the genetic material of most organisms to become firmly established. DNA was first characterized as acid-precipitable material from the cell nuclei of pus and fish sperm. The proportion of nitrogen and phosphorus was very unusual compared to other known organic substances, convincing Miescher he had discovered a new biological substance. He called it *nuclein*, because it was associated exclusively with the nucleus. Further work demonstrated that nuclein is a complex of protein and DNA.

#### DNA AND HEREDITY

Although clear experiments linking DNA to heredity were not performed until the mid 1940s, there was a good deal of circumstantial evidence that this was the case. In higher organisms, DNA was found almost exclusively in the chromosomes. The histone proteins and RNA, which chromosomes also contain, did not seem likely candidates as genetic material; sperm contained almost no RNA, and the histones are replaced in sperm by a different protein, protamine.

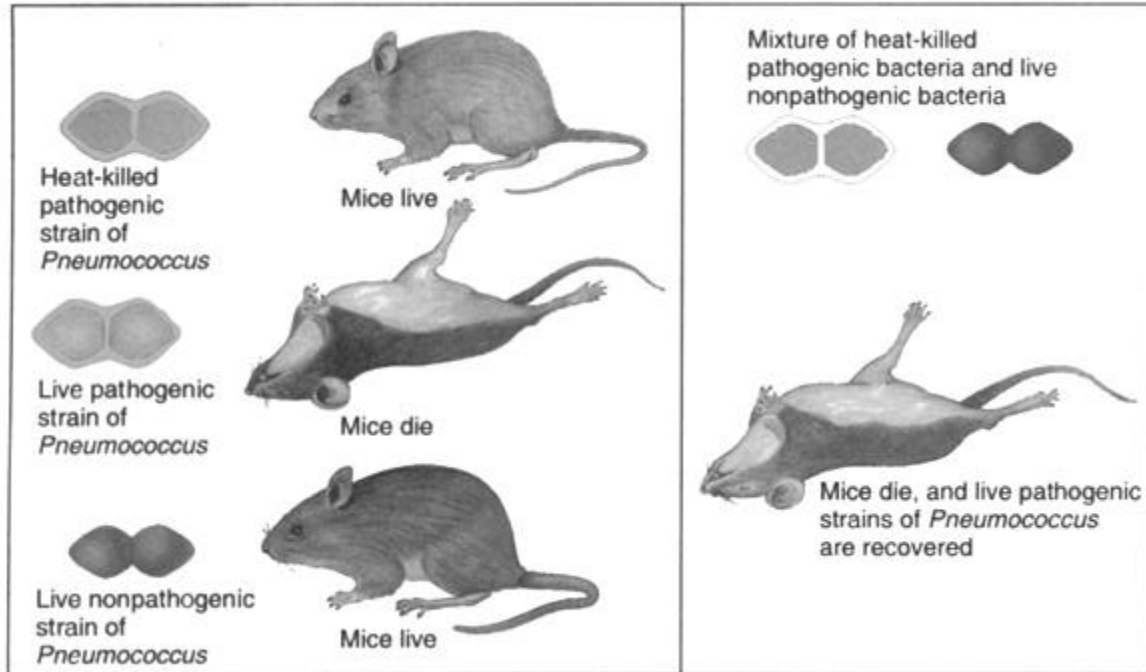
Unlike RNA and protein, every diploid cell of an organism has about the same amount of DNA. In the hen, for example, the red blood cells contain  $2.6 \times 10^{-12}$ g of DNA per cell, the kidney contains  $2.3 \times 10^{-12}$ g per cell, and the liver contains  $2.6 \times 10^{-12}$ g per cell. Furthermore, the amount of DNA seems correlated with chromosomal division; entering mitosis, the amount of cellular DNA doubles, while the haploid products of meiosis have only half the normal amount (thus rooster sperm contains  $1.3 \times 10^{-12}$ g of DNA). In polyploid plants, which contain multiples of the diploid number of chromosomes, the quantity of DNA is also a multiple of the diploid amount. Thus, the close association of DNA with chromosomes strongly implicates DNA as the genetic material.

#### DNA CAN GENETICALLY TRANSFORM CELLS

The first unambiguous evidence that DNA was the hereditary material came from Frederick Griffith's studies in 1928. Griffith used chemical mutagens to isolate a nonvirulent form of the bacterium that causes pneumonia, *Diplococcus pneumoniae*. Virulence required the presence of a polysaccharide capsule around the bacterium. The nonvirulent mutants lacked this capsule. Colonies of nonvirulent capsuleless bacteria appeared rough and were designated *R*. In contrast, the virulent form produced colonies that appeared smooth, so it was designated *S*. Several virulent forms were known, each with a characteristic polysaccharide capsule (called IS, IIS, IIIS, etc.), which is genetically inherited and is immunologically distinct from other forms.

A smooth bacterium of a particular capsule type (say IIS) can mutate to a nonencapsulated, nonvirulent form (IIR, because it derives from a type II cell). This happens at a very low frequency (in less than one in

a million cells), but it is inherited when it does occur. Similarly, the IIR cell can mutate back to the IIS virulent form at low frequency. However, the IIR cell line can *not* mutate to a IIIS virulent form. This property provides the key to the experiment.



**Figure 6.1**  
Griffith's discovery of the "transforming principle."

## **GRIFFITH'S EXPERIMENT**

Griffith mixed *Pneumococcus* type IIR with IIS cells that had been killed and rendered nonvirulent by heating them to 65°C, and he injected them into a host rabbit or, in other experiments, into a mouse. Neither strain injected alone produced disease, and no disease was expected from the mixed injections, as neither strain was virulent. However, many of the rabbits given mixed injections *did* come down with pneumonia and died. When analyzed, they all contained living virulent type IIIS cells! These cells could not have arisen from the type IIR cells by mutations (they would have produced type IIS cells), and the type IIIS cells were demonstrably dead (injected alone they caused no disease). Some factor must have passed from the dead IIIS cells to the live IIR ones, endowing them with the ability to make a capsule of the III type. Griffith called the factor "transforming principle" and the process genetic *transformation* (figure 6.1).

The transforming principle could be isolated as a cell-free extract and was fully active. The stability of the principle's transforming activity to heat treatment at 65°C suggested that it was not a protein (such high temperatures denature most proteins). In 1944, Oswald Avery, C. M. MacLeod, and M. J. McCarty succeeded in isolating a highly purified preparation of DNA from the type IIIS bacteria. The preparation of this type IIIS DNA was fully active as a transforming agent and could transform type IIR cells into type IIIS cells in a test tube. If the DNA was destroyed by deoxyribonuclease (an enzyme that specifically attacks DNA), all transforming activity was lost. It therefore seemed clear that DNA was "functionally active in determining the biochemical activities and specific characteristics of pneumococcal cells."

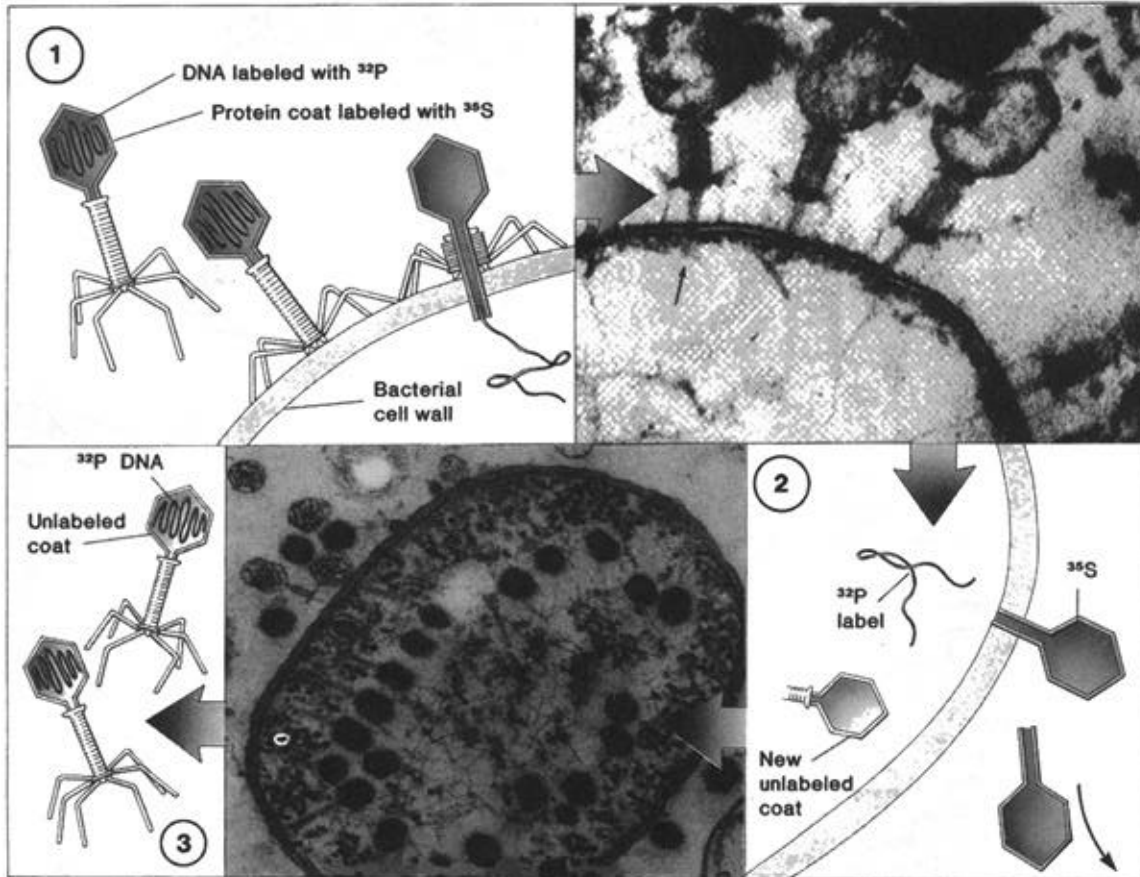
These experiments by themselves, however, do not establish that DNA is itself the genetic material. Perhaps DNA acts upon the genetic material of the recipient cell changing its genes to resemble the genes of the DNA donor? A clear demonstration was provided by experiments on bacterial viruses.

## **HERSHEY AND CHASE'S EXPERIMENT**

These experiments that clearly linked DNA and heredity were those performed by Alfred Hershey and Martha Chase in 1952 (figure 6.2). They chose to explore the genetic properties of DNA using bacterial viruses. Viruses are small, very simple aggregates of nucleic acid and protein. Several types of viruses attack bacteria and are known as bacteriophages (literally: "bacteria-eaters"). One of the viruses that attacks the bacterium *Escherichia coli* is the bacteriophage T2. It contains only protein and DNA; the DNA forms the central core of the virus, while the protein surrounds the core like a coat. Phages infect bacteria by adsorbing to the cell walls and injecting the genetic material into the bacteria. This material causes the production of many new viruses within the cell. Eventually the cell is ruptured (lysed), and the new viruses are released.

The chemical make-up of protein and of DNA is quite different. Hershey and Chase used these differences to distinguish between them. DNA contains phosphorus and proteins do not; proteins, on the other hand, usually contain sulfur, and DNA does not. By specifically labeling the phosphorus and sulfur atoms with radioisotopes, Hershey and Chase could distinguish unambiguously between the protein and the DNA of the phage and determine whether either or both were injected into the bacterial cell during the course of infection. When bacteriophage labeled with  $^{32}\text{P}$  DNA were allowed to infect a cell, almost all the label entered the cell. If such infected cells were allowed to lyse, the label was found among the progeny viruses.

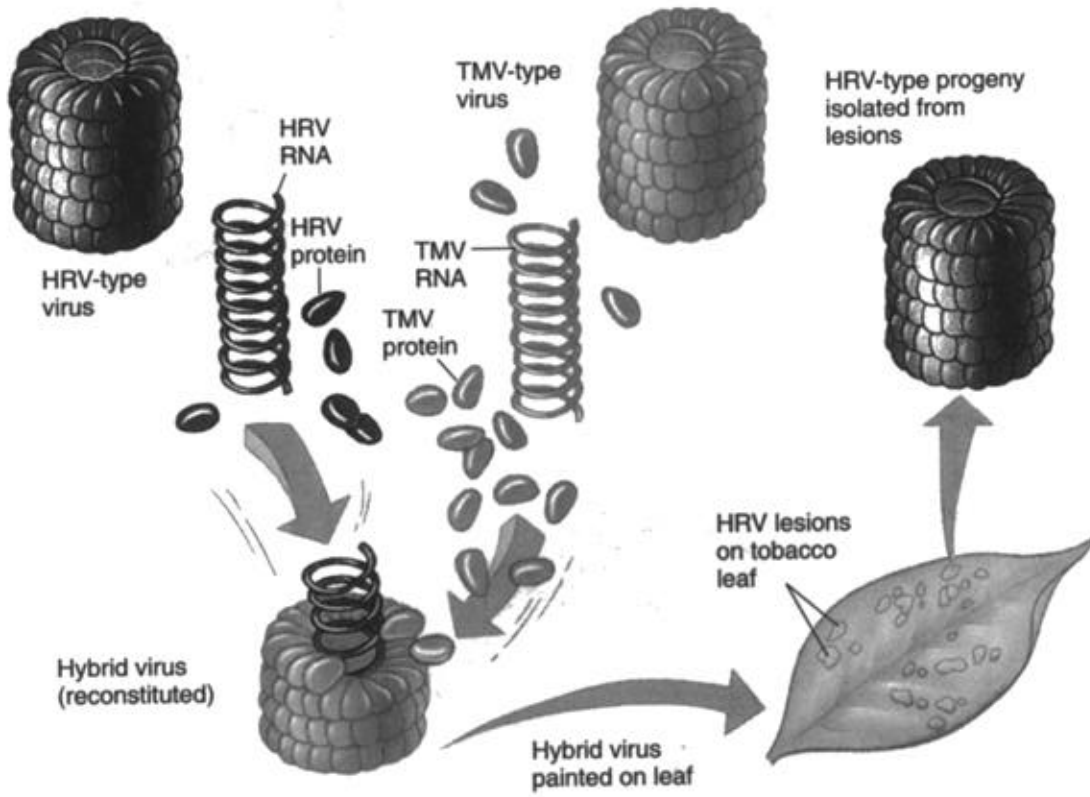
The opposite occurred when  $^{35}\text{S}$ -labeled phage infected a bacterial culture. Almost all label remains on the outside of the bacterium, bound to fragments of the cell wall. A small amount of protein did enter the bacterial cell in the course of infection. That this was not involved in the production of new bacteriophage could be demonstrated by repeating the experiment with bacteria stripped of their cell walls (*protoplasts*). If protoplasts were infected with  $^{32}\text{P}$  phage DNA free of protein, virulent phage were produced. If the purified  $^{32}\text{P}$  was first treated with DNAase, no progeny phage were produced. Clearly the labeled DNA contained all the information necessary to produce new virus particles.



*Figure 6.2*  
*The Hershey-Chase experiment with bacterial viruses.*

### **THE TOBACCO MOSAIC VIRUS (TMV)**

Some viruses do not contain DNA, being made up instead of protein and RNA (ribonucleic acid). The tobacco mosaic virus (TMV) is such an RNA virus. H. Fraenkel-Conrat and others were able to dissociate the TMV into its constituent protein and RNA parts (figure 6.3). When the parts were mixed, they reformed TMV particles that were normal in every respect. That the RNA contained the genetic information was demonstrated by isolating protein and RNA from several different types of TMV, with subsequent combinations of protein and RNA mixed together. These reconstituted viruses, containing protein from one type and RNA from another, were then allowed to infect tobacco cells. In every case the progeny TMVs proved to have the protein coats of the type that had contributed the RNA, and not of the type that had contributed the protein. Thus, in the tobacco mosaic virus, the RNA, rather than the protein, must be acting as the genetic material.



*Figure 6.3*  
*Fraenkel-Conrat's virus-reconstitution experiment.*