SIII strain and living RII strain. He found that most of the mice died from pneumonia, and when he tested blood cultures from the dead mice, he detected living SIII bacteria. Knowing that this outcome could not have been the result of a simple mutational event, Griffith proposed that a molecular component he called the transformation factor was responsible for transforming RII into SIII.

Griffith concluded that the transforming factor was a molecule that carried hereditary information, but he was unable to suggest the class of molecule. Today biologists know that the process identified by Griffith is a naturally occurring process called transformation, which is used by bacteria to transfer DNA.

**DNA Is the Transformation Factor**

Shortly after Griffith published his report on the transformation factor, Martin Dawson, working with Oswald Avery, developed an in vitro transformation procedure to mix living R cells with a purified extract of cellular material derived from heat-killed SIII cells containing the transformation factor. Biochemical assays indicated that the SIII extract consisted mostly of DNA, along with a small amount of RNA and trace amounts of proteins, lipids, and polysaccharides.

The most direct evidence that DNA was the transformation factor came from an experiment performed by Avery and his colleagues MacLeod and McCarty (Figure 3).

**Figure 3** Avery, MacLeod, and McCarty’s use of in vitro transformation to identify DNA as the most likely hereditary molecule. A purified extract from heat-killed SIII bacteria successfully transforms RII cells in the control experiment 1. Destruction of lipids and polysaccharides 2, proteins 3, or RNA 4 does not affect transformation; however, destruction of DNA 5 prevents transformation.
This experiment identified the role of DNA in transformation by eliminating lipids, polysaccharides, protein, RNA, and DNA one at a time from the SIII extract. In each experimental trial, the SIII extract was treated to remove one component at a time, and the treated extract was mixed with RII cells. The in vitro transformation reaction was allowed to take place, and the resulting cells were injected into mice. Blood from the injected mice was then cultured to determine if SIII cells were present—an indication that transformation had occurred.

Figure 3 shows that in vitro transformation takes place and mice are killed by SIII infection in the control experiment 1, and when lipids and polysaccharides 2, proteins 3, or RNA 4 are removed from the extract. In contrast to the other results, experiment 5, which uses DNase to specifically degrade DNA, does not result in transformation. Blood drawn from mice injected with DNase-treated extract reveals no living SIII bacteria—a clear indication that transformation is blocked by the destruction of DNA. Based on these observations Avery, MacLeod, and McCarty correctly concluded that DNA is the transformation factor and the probable hereditary material.

DNA Is the Hereditary Molecule

Avery, MacLeod, and McCarty’s work convinced many biologists that DNA was the long-sought hereditary material, and a great deal of research in the late 1940s and early 1950s was devoted to deducing the physical structure of DNA. Biologists realized that once the structure of DNA was known, the chemical nature of genes would be identified, and biological research would move into the realm of genetic molecular biology. As clear and convincing as the work of Avery and his colleagues seems in retrospect, however, there were several unanswered questions about the role of DNA in heredity. There was also a need to demonstrate directly that the presence of a specific DNA molecule induces the appearance of a particular phenotype. That evidence came in a 1952 report by Alfred Hershey and Martha Chase, who showed that DNA, but not protein, is responsible for bacteriophage infection of bacterial cells.

**Bacteriophages**, also known as phages, are viruses that infect bacteria. Phages such as T2, for example, consist of a protein shell with a tail segment that attaches to a host bacterial cell and a head segment that contains DNA. T2 phages are among the many bacteriophage that do not carry any RNA. Like other viruses, T2 must infect host cells, bacterial cells in this case, in order to reproduce. Infection by a phage begins with the injection of phage DNA into a bacterial cell; the emptied phage shell remains attached to the outside of the bacterial cell. Inside the infected bacterial cell, phage DNA then replicates, and phage proteins are produced (Figure 4). These proteins are assembled into progeny phages that are each filled with a copy of the phage chromosome. Progeny phages are released by lysis of the host bacterial cell.

In their experiment, Hershey and Chase took advantage of an essential difference between the chemical composition of DNA and protein to confirm the hereditary role of DNA (Figure 5). Proteins contain large amounts of sulfur but almost no phosphorus; conversely, DNA
contains a large amount of phosphorus but no sulfur. To prepare phages for the experiment, Hershey and Chase initially grew phage cultures in different growth media. One growth medium contained $^{35}$S, the radioactive form of sulfur, to label protein 1; the other environment contained radioactive phosphorus, $^{32}$P, to label DNA 1. The researchers used radioactively labeled phages from each medium to infect unlabeled host bacterial cells in parallel experiments 2 2.

After a short time, each mixture was agitated in a blender to separate bacterial cells from the now empty phage shells. Such empty phage shells are called “ghosts” 3 3. The relatively large bacterial cells were easily separated from the ghosts by centrifugation. The heavier bacteria collect in a pellet at the bottom of the centrifuge tube, while the lighter ghosts remain suspended in the supernatant. Testing each fraction for radioactivity revealed that virtually all the $^{32}$P label was associated with newly infected bacterial cells and almost none with ghost particles 4. On the other hand, the $^{35}$S label was found in the ghost-particle fraction, and only trace amounts were found associated with the bacterial pellet 4. This result demonstrates that phage DNA, but not phage protein, is transferred to host bacterial cells and directs the synthesis of phage DNA and proteins, the assembly of progeny phage particles, and ultimately the lysis of infected cells. The experiment demonstrated that the transformation factor identified previously by Griffith was DNA; it also showed that Avery, MacLeod, and McCarty were correct in concluding that DNA is the hereditary material.
Genetic Insight. Griffith identified the transformation factor as a molecule carrying hereditary information. Avery, MacLeod, and McCarty showed that only the enzymatic degradation of DNA disrupts in vitro transformation. Hershey and Chase demonstrated that the physical transfer of DNA from phage to bacterial host initiates the lytic cycle.

2 The DNA Double Helix Consists of Two Complementary and Antiparallel Strands

Watson and Crick’s model of the secondary structure of DNA indicates that in some respects, the molecule is a simple one. It is composed of four kinds of nucleotides that are joined by covalent phosphodiester bonds into polynucleotide chains. Two polynucleotide chains come together along their lengths to form a double helix, also called a DNA duplex, by complementary pairing and hydrogen bonding between the nucleotide bases of each strand. Yet for all its simplicity—being composed of just four types of nucleotides—DNA is a complex informational molecule that serves as a permanent repository of genetic information in cells, and it directs the production of RNA molecules that carry out actions in cells or carry information for protein assembly. These essential functions of DNA derive from its molecular structure.

DNA Nucleotides

A nucleotide in DNA has three parts: (1) a sugar, (2) one of four nitrogenous bases, and (3) up to three phosphate groups (Figure 6). Deoxyribose, the sugar of DNA nucleotides, contains 5 carbons that are identified as 1’, 2’, 3’, 4’, and 5’. An oxygen atom connects the 1’ carbon to the 4’ to form a five-sided (pentose) ring, and the 5’ carbon projects outward from the 4’ carbon (and from the ring). A nucleotide base is attached to the 1’ carbon; a hydroxyl group (OH) is attached to the 3’ carbon; and a single phosphate molecule, or a chain of phosphates up to three molecules long, is attached at the 5’ carbon. Deoxyribose carries a hydrogen molecule at the 2’ carbon instead of a hydroxyl (OH) group. This is the basis for naming the sugar deoxyribose.

The nitrogenous bases in DNA are of two structural types—a single-ringed form called a pyrimidine, and a double-ringed form called a purine. Cytosine (C) and thymine (T) are pyrimidines, and adenine (A) and guanine (G) are purines. DNA nucleotides that are part of a polynucleotide chain have one phosphate group that forms the covalent phosphodiester bond with the adjacent nucleotide in the strand. Deoxyadenosine 5’-monophosphate (dAMP) and deoxyguanosine 5’-monophosphate (dGMP) carry the purine bases adenine and guanine, and deoxycytidine 5’-monophosphate (dCMP) and deoxythymidine 5’-monophosphate (dTMP) carry the pyrimidine bases cytosine and thymine. Collectively, these are identified as the deoxynucleotide monophosphates (dNMPs), where N can refer to any of the four nucleotide bases. In contrast, free (reactive) DNA nucleotides that are not part of a polynucleotide chain carry a string of three phosphate groups at the 5’ carbon and are identified as dATP, dGTP, dCTP, and dTTP. Collectively, these are the deoxynucleotide triphosphates (dNTPs).

Individual nucleotides are assembled into a polynucleotide chain by the enzyme DNA polymerase, which catalyzes the formation of a phosphodiester bond between the 3’ hydroxyl group of one nucleotide and the 5’ phosphate group of an adjacent nucleotide (Figure 7). Two of the three phosphates of a dNTP are removed (as a pyrophosphate group) during phosphodiester bond formation, leaving the nucleotides of a polynucleotide chain in their monophosphate form. Each polynucleotide chain has a sugar-phosphate
Figure 7 DNA strand elongation. (a) Nucleotides complementary to the template strand are added to the 3’ end of the new strand by DNA polymerase. (b) DNA nucleotide triphosphates are recruited by DNA polymerase, which uses catalytic action to remove two phosphates (the pyrophosphate group) and form a new phosphodiester bond.