

BIOLOGY
THE UNITY AND DIVERSITY OF LIFE
ELEVENTH EDITION

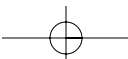
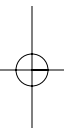
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13 DNA STRUCTURE AND FUNCTION

Goodbye, Dolly

In 1997 in Scotland, geneticist Ian Wilmut made headlines when he did not bother with the union of sperm and eggs to produce a new lamb. He wanted to make a genetic copy—a **clone**—of a fully grown sheep. He thought he could do so by slipping the nucleus of an adult's body cell into an unfertilized egg that had its own nucleus gently sucked out beforehand. He succeeded. One egg that his team modified developed into a cloned lamb, which they named Dolly.

Dolly grew up and later gave birth to six lambs of her own (Figure 13.1). Since then, researchers all over the world have been using adult DNA to make identical copies of other adult mammals. Mice, rabbits, pigs, cattle, goats, mules, deer, horses, and cats have all been cloned.

Sheep normally do not show symptoms of old age until they are about ten years old. By age five, Dolly had become arthritic and overweight. Less than a year later, an infection in her lungs proved irreversible and she was put to sleep.

Did Dolly develop health problems simply because she was a clone? Earlier studies of her telomeres had raised suspicions. Telomeres are short segments that cap the ends of chromosomes and stabilize them. They become shorter and shorter as an animal ages. When Dolly was only two years old, telomeres in some of her cells were as short as those of a six-year-old sheep—the exact age of the adult animal that was her genetic donor.

Using adult DNA to clone mammals is challenging. Most clones die before birth or shortly afterward. It took almost seven hundred attempts to get a clone of a guar, a wild ox on the endangered species list. Less than two days after his birth, he died of complications following an infection.

The clones that do survive often have health problems. Like Dolly, many become unusually overweight as they age. Other clones are exceptionally large from birth

or have some enlarged organs. Cloned mice develop lung and liver problems, and almost all die prematurely. Cloned pigs have heart problems, they limp, and one never did develop a tail or, worse still, an anus.

Physically moving the DNA from an adult's cell into an egg stripped of its own nucleus is only part of the challenge. Most genes in a mature cell are inactive. To guide the reproductive process, they have to be reprogrammed or switched on in controlled ways. Apparently, not all of the genes in all clones are being properly activated.

Some people want to put a stop to adult cloning because of the risk of bringing defective mammals into the world troubles them deeply. Other people want research into reprogramming DNA to continue. For instance, they point to patients who are desperate for organ transplants. People are already cloning pigs that were genetically modified to produce organs that human donors are less likely to reject. A few people on the fringes of bioethical common sense are toying with the idea of reprogramming DNA to clone an adult human.

Is DNA amazing? It certainly is. Are we amazing in our capacity to use and abuse its potential? You bet.



Figure 13.1 Dolly and one of her lambs. Dolly was the first mammal to be formed by way of adult DNA cloning. She awakened society to the goings-on of the molecular revolution by jarring our notions of what it takes to reproduce a complex animal.

Watch the video online!

IMPACTS, ISSUES



Figure 13.2 Watson, Crick, and the model for DNA that brought about a revolution in molecular biology.

With this chapter, we move past the chromosomal basis of inheritance and turn to the investigations and models that led to our current understanding of DNA (Figure 13.2). The story is more than a march through the details of how its molecular structure encodes hereditary information. *It also is revealing of how ideas are generated in science.*

On the one hand, having a shot at fame and fortune quickens the pulse of men and women in any profession, and scientists are no exception. On the other hand, science proceeds as a community effort, with individuals sharing not only what they can explain but also what they do not understand. Even when an experiment fails to produce the anticipated results, it may turn up information that others can use or lead to questions that others can answer. Unexpected results, too, might be clues to something important about the natural world.



How Would You Vote?

Abnormal animals often form during animal cloning experiments, but cloning research may also result in new drugs and organ replacements for human patients. Should animal cloning be banned? See BiologyNow for details, then vote online.



Key Concepts

DISCOVERY OF DNA'S FUNCTION

In all living cells, DNA molecules are storehouses of information that governs heritable traits. [Section 13.1](#)

THE DNA DOUBLE HELIX

DNA is a double-stranded molecule consisting of four kinds of nucleotides: adenine, thymine, guanine, and cytosine. The two strands coil together helically, like a spiral stairway.

Each nucleotide base of one strand is hydrogen-bonded to a base of the other strand. As a rule, adenine pairs with thymine, and guanine with cytosine.

The order in which one kind of base follows the next in a strand encodes heritable information. The DNA of each species has at least some unique base sequences that are not found in the DNA of any other species. [Section 13.2](#)

HOW CELLS DUPLICATE THEIR DNA

Before a cell divides, enzymes and other proteins replicate the DNA; they make a copy of it. Different kinds unwind the double helix and construct a new, complementary strand on the exposed bases of each parent strand. They do so according to the base-pairing rule for DNA.

Repair enzymes monitor mismatched base pairings and other changes in the DNA strands. [Section 13.3](#)

DNA AND THE CLONING CONTROVERSIES

What does it mean when we say that DNA holds heritable information? The answer hits home when the messages encoded in its base sequences are tapped to make an exact copy of an adult animal. [Section 13.4](#)

THE FRANKLIN FOOTNOTE

As in any profession, some were winners and some losers in the DNA chase. Some players might have taken less-than-noble shortcuts. [Section 13.5](#)



Links to Earlier Concepts

This chapter builds on your understanding of hydrogen bonding (Section 2.4), condensation reactions (3.2), and the earlier overview of DNA structure and function (3.7). Your knowledge of chromosomes, mitosis, and meiosis will help you understand the nuclear transfers that are part of cloning procedures (9.3, 10.3). Keep the image of the eight-cell stage of human development in mind when you read about embryo cloning, because the cells used are no more developed than this (12.11).

13.1 The Hunt for Fame, Fortune, and DNA

*Why, in the spring of 1868, was Johann Miescher collecting cells from the pus of open wounds and, later, from sperm of a fish? This physician wanted to identify the chemical composition of the nucleus. Such cells have little cytoplasm, which makes it easier to isolate the nuclear material. In time he isolated an acidic compound that contains nitrogen and phosphorus. He had discovered what came to be known many years later as **deoxyribonucleic acid**, or **DNA**.*

EARLY AND PUZZLING CLUES

At the time Miescher made his discovery, no one knew much about the physical basis of inheritance. That is, *which substance encodes the information about reproducing parental traits in offspring?* Few researchers thought that DNA might hold the answer. For a long time, most were thinking **PROTEINS!** Because heritable traits are so diverse, they assumed that molecules of inheritance had to be structurally diverse, too. Proteins, they said, consist of unlimited combinations of twenty kinds of amino acids. Other molecules just seemed too simple.

Now fast-forward to 1928. An army medical officer, Frederick Griffith, wanted to develop a vaccine against the bacterium *Streptococcus pneumoniae*, a major cause of pneumonia. He did not succeed, but he isolated and cultured two strains that unexpectedly shed light on inheritance. The colonies of one strain had a rough surface appearance; colonies of the other appeared smooth. Griffith designated the strains *R* and *S*, and he used them in a series of experiments (Figure 13.3).

First, he injected mice with live *R* cells. The mice did not develop pneumonia. *The R strain was harmless.*

Second, he injected other mice with live *S* cells. The mice died. Blood samples from them teemed with live *S* cells. *The S strain was pathogenic; it caused the disease.*

Third, he killed *S* cells by exposing them to high temperature. *Mice injected with dead S cells did not die.*

Fourth, he mixed live *R* cells with heat-killed *S* cells. He injected them into mice. The mice died—and *blood samples drawn from them teemed with live S cells!*

What went on in the fourth experiment? Maybe heat-killed *S* cells in the mix were not really dead. But if that were so, then the mice injected with heat-killed *S* cells in experiment 3 would have died. Or maybe the harmless *R* cells had mutated into a killer strain. But if that were so, then the mice injected with the *R* cells only in experiment 1 would have died.

The simplest explanation was this: *Heat had killed the S cells but did not destroy their hereditary material—including the part that specified “how to cause infection.”* Somehow, that material had been transferred from the dead *S* cells into living *R* cells, which put it to use.

Further tests made it clear that the transformation was permanently heritable. Even after a few hundred generations, *S* cell descendants were still infectious.

What was the hereditary material that caused the transformation? Scientists started looking in earnest, but most were still thinking **PROTEINS!**

Still, Griffith's results intrigued Oswald Avery, who began to transform harmless bacteria by mixing them with extracts of killed pathogenic cells. Avery asked: What part of the extracts caused the transformation? He found that adding protein-digesting enzymes to the extracts had no effect; cells were still transformed. However, adding a DNA-digesting enzyme to extracts prevented transformation. DNA was looking good.

CONFIRMATION OF DNA FUNCTION

By the 1950s, Max Delbrück, Alfred Hershey, Martha Chase, Salvador Luria, and other molecular sleuths were using viruses for experiments. These infectious particles hold information on substances required to make new virus particles. After viruses infect a host cell, their enzymes trick its metabolic machinery into synthesizing those substances. **Bacteriophages**, which only infect certain bacteria, were the viruses of choice for the early experiments.

As researchers knew, some bacteriophages consist only of DNA and a coat, probably of protein. Also, as

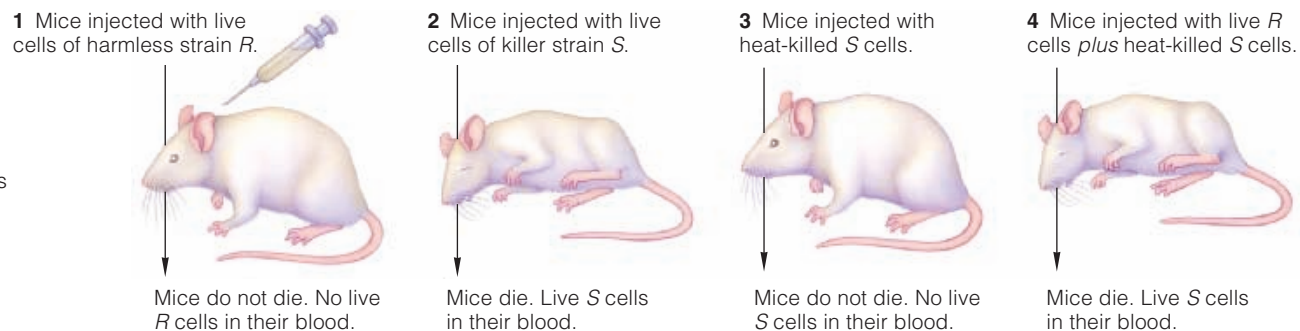


Figure 13.3
Animated!

Summary of results from Fred Griffith's experiments with *Streptococcus pneumoniae* and laboratory mice.

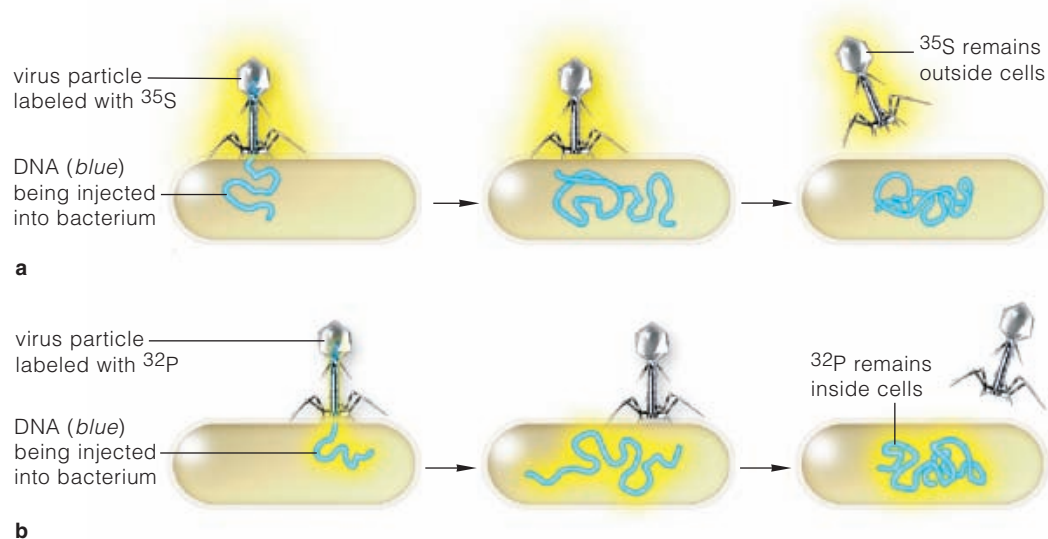
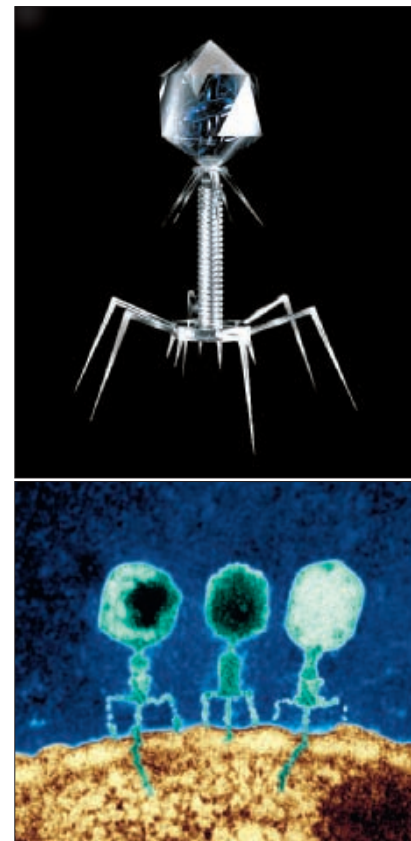


Figure 13.4 Animated! Example of the landmark experiments that tested whether genetic material resides in bacteriophage DNA, proteins, or both. Alfred Hershey and Martha Chase knew that sulfur (S) but not phosphorus (P) is a component of bacteriophage proteins. They also knew that phosphorus but not sulfur is a component of DNA.

(a) In one experiment, bacteria were grown in a culture medium with a tracer, the radioisotope ^{35}S . The cells used the ^{35}S when they built proteins. Bacteriophages infected the labeled cells, which started to make viral proteins. So the proteins of new virus particles became labeled with the ^{35}S . The labeled virus particles infected a new batch of unlabeled cells. The mixture was whirred in a kitchen blender. Whirring dislodged the viral coats from infected cells. Chemical analysis revealed the presence of labeled protein in the solution but only traces of it inside the cells.

(b) In another experiment, bacteriophages infected cells that had taken up the radioisotope ^{32}P . Later, the cells used ^{32}P when they built viral DNA. This labeled the DNA and new virus particles. The labeled viruses were used to infect bacteria in solution, then were dislodged from them. Most labeled viral DNA stayed in the cells—evidence that DNA is the genetic material of this virus.



c Above, model for a bacteriophage. Below, micrograph of virus particles injecting their DNA into an *E. coli* cell.

micrographs revealed, the coat remains on the *outer surface* of infected cells. Did viruses inject hereditary material only into cells? If so, then was the material protein, DNA, or both? Figure 13.4 outlines just two of many experiments that pointed to DNA.

Then Linus Pauling did something no one had done before. With his training in biochemistry, a talent for model building, and a dose of intuition, he deduced the structure of a protein—collagen. His discovery was electrifying. If someone could pry open the secrets of proteins, then why not DNA? And if DNA's structural details were deduced, would those details hold clues to how DNA functions in inheritance? *Someone could go down in history as having discovered the secret of life!*

ENTER WATSON AND CRICK

Scientists started to scramble after the prize. Among them were Francis H. Crick, a Cambridge University researcher, and James Watson, a postdoctoral fellow recently arrived from Indiana University. They spent

hours arguing over everything they had read about DNA's size, shape, and bonding requirements. They fiddled with cardboard cutouts, and they badgered chemists to help them identify possible bonds they might have overlooked. They built models from thin bits of metal connected with wire "bonds."

In 1953, Watson and Crick built a model that fit all the pertinent biochemical rules and all the clues they had gleaned from other sources. They had discovered the structure of DNA. The molecule has breathtaking simplicity, and it helped Crick answer another riddle—*how life can show unity at the molecular level and still give rise to such spectacular diversity at the level of whole organisms*. Turn now to high points in the community effort that gave us these insights.

DNA functions as the cell's treasurehouse of inheritance. The cumulative efforts of many scientists, building on one another's work, resulted in the discovery of that function.

13.2 The Discovery of DNA's Structure

LINKS TO
SECTIONS
3.2, 3.7



Long before the bacteriophage studies were under way, biochemists knew that DNA contains only four kinds of nucleotides that are the building blocks of nucleic acids. But how were the nucleotides arranged in DNA?

DNA'S BUILDING BLOCKS

Recall, from Section 3.7, that a **nucleotide** in DNA has a five-carbon sugar (deoxyribose), a phosphate group, and one of the following nitrogen-containing bases:

adenine	guanine	thymine	cytosine
A	G	T	C

T and **C** are pyrimidines, with a backbone of carbon and nitrogen that forms a single ring. **A** and **G** are purines—larger, bulkier molecules having two rings. Overall, the four types of nucleotides have the same bonding pattern, as Figure 13.5 indicates.

By 1949, the biochemist Erwin Chargaff had shared with the scientific community two insights about the proportions of nucleotides in DNA. First, the amount of adenine relative to guanine differs among species. Second, the amounts of thymine and adenine in DNA are identical, and so are the amounts of cytosine and guanine. We may show this as **A=T** and **G=C**.

These symmetrical proportions had to mean something. As biochemists already knew, the nucleotides in DNA are joined

to one another by way of condensation reactions that form long chains (Section 3.2). But how were the four kinds arranged in a chain, and in what order?

The first convincing clue to the actual arrangement emerged from Maurice Wilkins's research laboratory at Cambridge, England. Researcher Rosalind Franklin made exceptional **x-ray diffraction images** of DNA. Such images form after a beam of x-rays is directed at a molecule, which scatters the x-rays in a pattern that can be captured on film. The pattern consists only of dots and streaks; in itself, it is not the structure of the molecule. However, researchers can use it to calculate the positions of the molecule's atoms.

Before Franklin, researchers had been working with dehydrated DNA molecules. Franklin was the first to put DNA into a "wet" form—which is the form that occurs in cells—and make an exceptionally clear image of it. With that image, she painstakingly calculated that the DNA molecule is long and thin, and that it has a 2-nanometer diameter. She also found repeats of some molecular configuration every 0.34 nanometer along its length, and another repeat every 3.4 nanometers. These were crucial clues, but her part in the discovery process was downplayed until recently (Section 13.5).

What did the repeating variation in DNA mean? Could DNA be coiled along its length, like a circular stairway? Certainly Pauling thought so. After all, he had calculated that collagen is helically coiled. Like

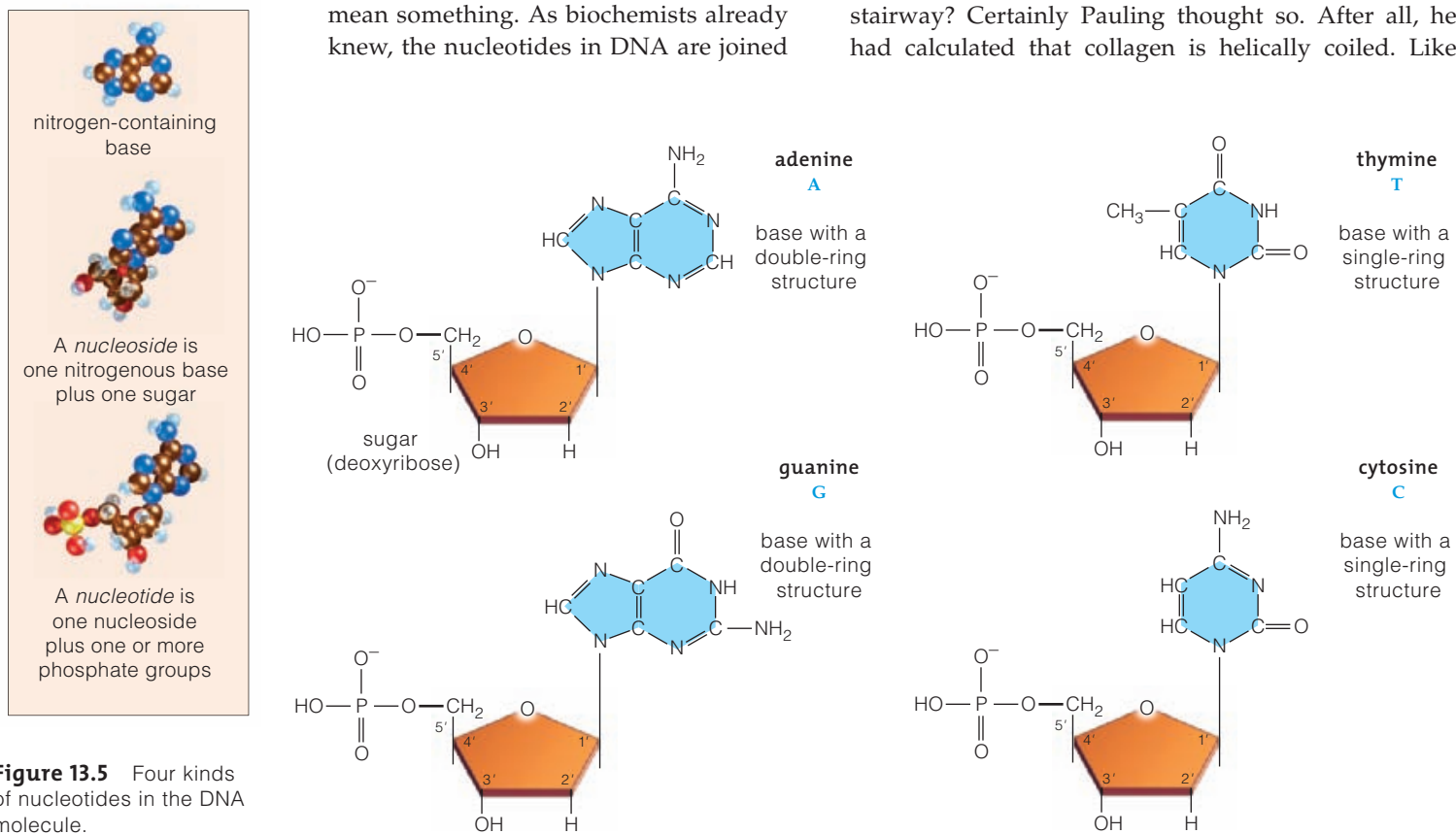


Figure 13.5 Four kinds of nucleotides in the DNA molecule.

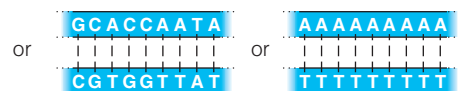
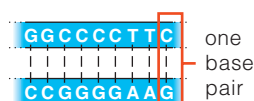
many others—including Wilkins, Watson, and Crick—he was thinking “helix.” As Watson later wrote, “We thought, why not try it on DNA? We were worried that Pauling would say, why not try it on DNA? Certainly he was a clever man. He was a hero of mine. But we beat him at his own game. I still can’t figure out why.”

Pauling, it turned out, made a big chemical mistake. His model had all the negatively charged phosphate groups facing the interior of the DNA helix instead of facing outward. If they were that close together, they would repel each other too much to remain stable.

PATTERNS OF BASE PAIRING

Franklin filed away her image of wet DNA, but it still came to the attention of Watson and Crick. From all the clues that had accumulated, they perceived that DNA must consist of two strands of nucleotides, held together at their bases by hydrogen bonds (Figure 13.6). Such bonds form when the two strands run in opposing directions and twist to form a double helix. Only two kinds of base pairings typically form along the molecule’s length: **A–T** and **G–C**.

This bonding pattern accommodates variation in the order of bases. For instance, a stretch of DNA from a rose, a human, or any other organism might be:



All DNA molecules show the same bonding pattern. Many stretches of base sequences are the same in all of them. But some are unique for each species and even vary among individuals of a species! *The constancy in DNA’s bonding pattern is the basis for life’s unity—and variation in base sequences is the basis for life’s diversity.*

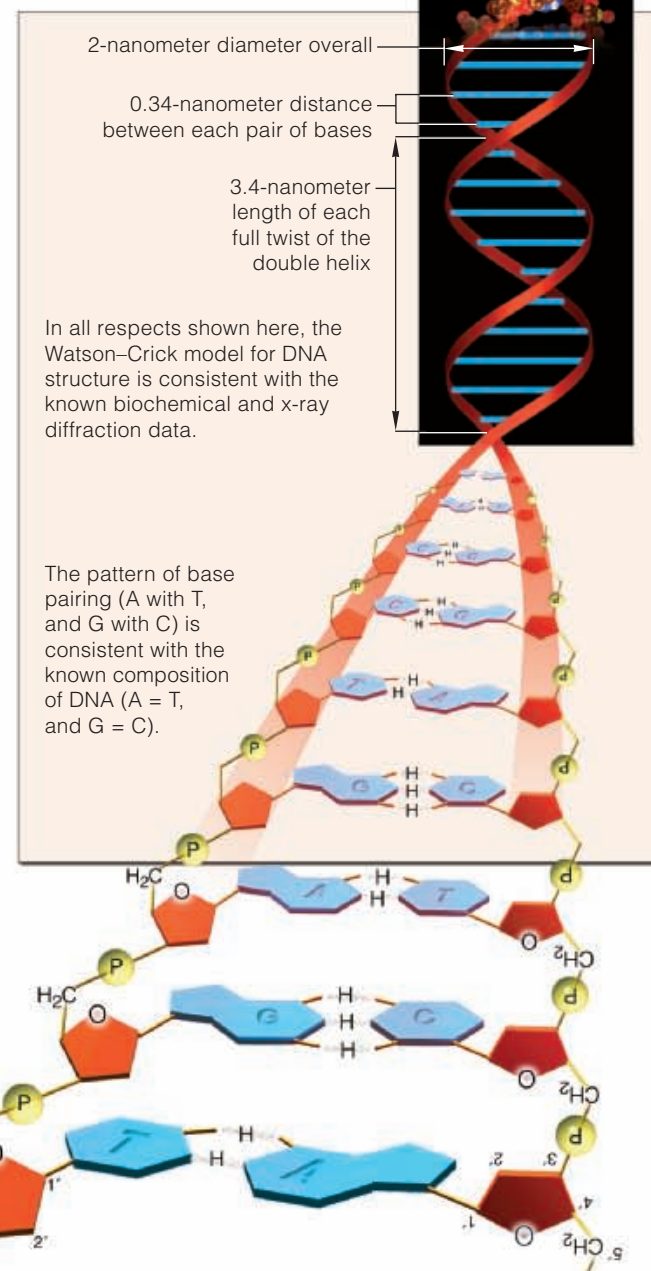
Intriguingly, computer simulations show that if you want to pack a string into the least space, coil it into a helix. Was this space-saving advantage a factor in the molecular origin of the DNA double helix? Maybe.

The pattern of base pairing between the two strands in DNA is constant for all species—A with T, and G with C. However, each species has a number of unique sequences of base pairs along the length of its DNA molecules.

Figure 13.6 Animated!

Composite of different ways to represent the DNA double helix. Two sugar–phosphate backbones run in *opposing* directions. Think of the sugar units (deoxyribose) of one strand as being upside down.

By comparing the numerals used to identify each carbon atom of DNA’s backbone (1’, 2’, 3’, and so on), you see that one strand runs in the 5’→3’ direction and the other runs in the 3’→5’ direction.



13.3 DNA Replication and Repair

LINKS TO
SECTIONS
9.2, 9.5, 12.8



The discovery of DNA structure was a turning point in the studies of inheritance. Crick saw at once how a parent cell could make copies of that molecule for its daughter cells.

HOW DNA IS DUPLICATED

Semiconservative Replication Until Watson and Crick presented their model, no one could explain **DNA replication**, or how the molecule of inheritance is duplicated before a cell divides. Such replication takes place in interphase of the cell cycle.

Enzymes easily break the hydrogen bonds between the two nucleotide strands of a DNA molecule. When enzymes and other proteins act on the molecule, one strand unwinds from the other and exposes stretches of its nucleotide bases. Cells contain stockpiles of free nucleotides that can pair with the exposed bases.

Each parent strand stays intact, and a companion strand is assembled on each one according to the base-pairing rules **A to T**, and **G to C**. As soon as a stretch of a new, partner strand forms on a stretch of a parent strand, the two twist together in a double helix. Each parent strand is conserved during replication, so that half of every double-stranded DNA molecule is “old” and half is “new.” Figures 13.7 and 13.8 offer a look into this process, called *semiconservative* replication.

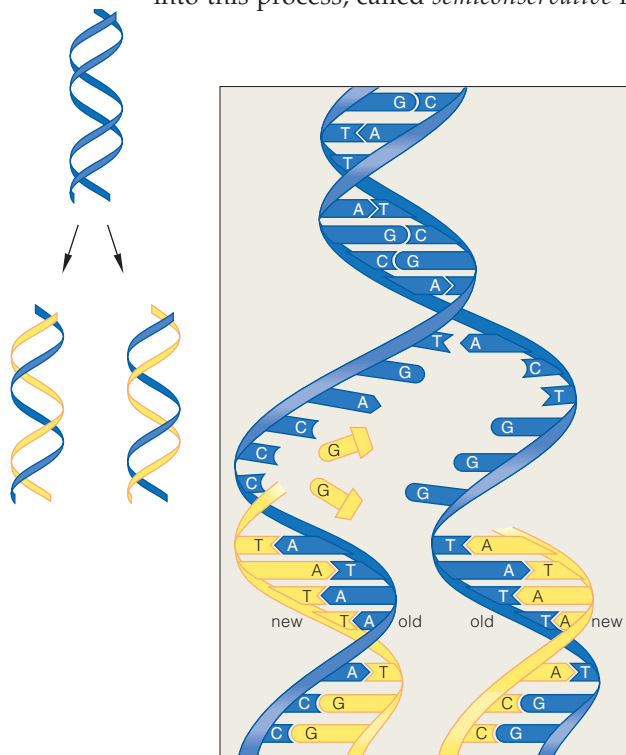


Figure 13.7 The semiconservative nature of DNA replication. The original two-stranded DNA molecule is coded *blue*. Each parent strand remains intact. One new strand (*gold*) is assembled on each of the parent strands.

Replication Enzymes DNA replication uses a team of molecular workers; Table 13.1 lists important ones. Signaling molecules that are part of the controls over the cell cycle activate replication enzymes. Starting at certain regions along the length of the DNA double helix, enzymes called **helicases** unzip the hydrogen bonds, which are individually weak and easy to break (Section 2.4). The two strands of the double helix now unwind from each other in both directions from the unzipped sites. The two parent strands are prevented from winding back together because small proteins temporarily bind with them.

Next, **DNA polymerases** catalyze the formation of two brand-new strands of DNA from free nucleotides. These enzymes also catalyze the hydrogen bonding of each new strand to the unwound region of one of the two parent DNA strands. But they can assemble new strands only in the 5' → 3' direction.

Check out Figure 13.5 to see what this directional strand assembly means. A free nucleotide has a tail of three phosphate groups dangling from the 5' carbon of their sugar component. DNA polymerase splits off two. The energy released drives the attachment of the last phosphate to an —OH group dangling from the 3' carbon of a sugar—which belongs to the most recent nucleotide addition to the growing strand.

What about the parent strand that runs the other way? Nucleotides are assembled in short stretches on the parent strand, then **DNA ligases** seal the stretches

Table 13.1 Three of the Enzymes With Roles in DNA Replication and Repair

Helicases

Catalyze the breaking of hydrogen bonds between base pairs in the DNA molecule, which unzips in two directions from double-stranded to single-stranded form. Protein factors work with helicases to keep the two parent strands unwound. The helicases are ATP-driven motors, similar to ATP synthases.

DNA polymerases

Catalyze the additions of free nucleotides to each new strand of deoxyribonucleotides on a parent DNA template. Also proofread; some DNA polymerases can reverse direction by one base pair and correct mismatches, which occur once in every thousand or so additions.

DNA ligases

Catalyze the sealing-together of short stretches of new nucleotides, which are assembled discontinuously on one of the parent DNA strands. Also can seal strand breaks.

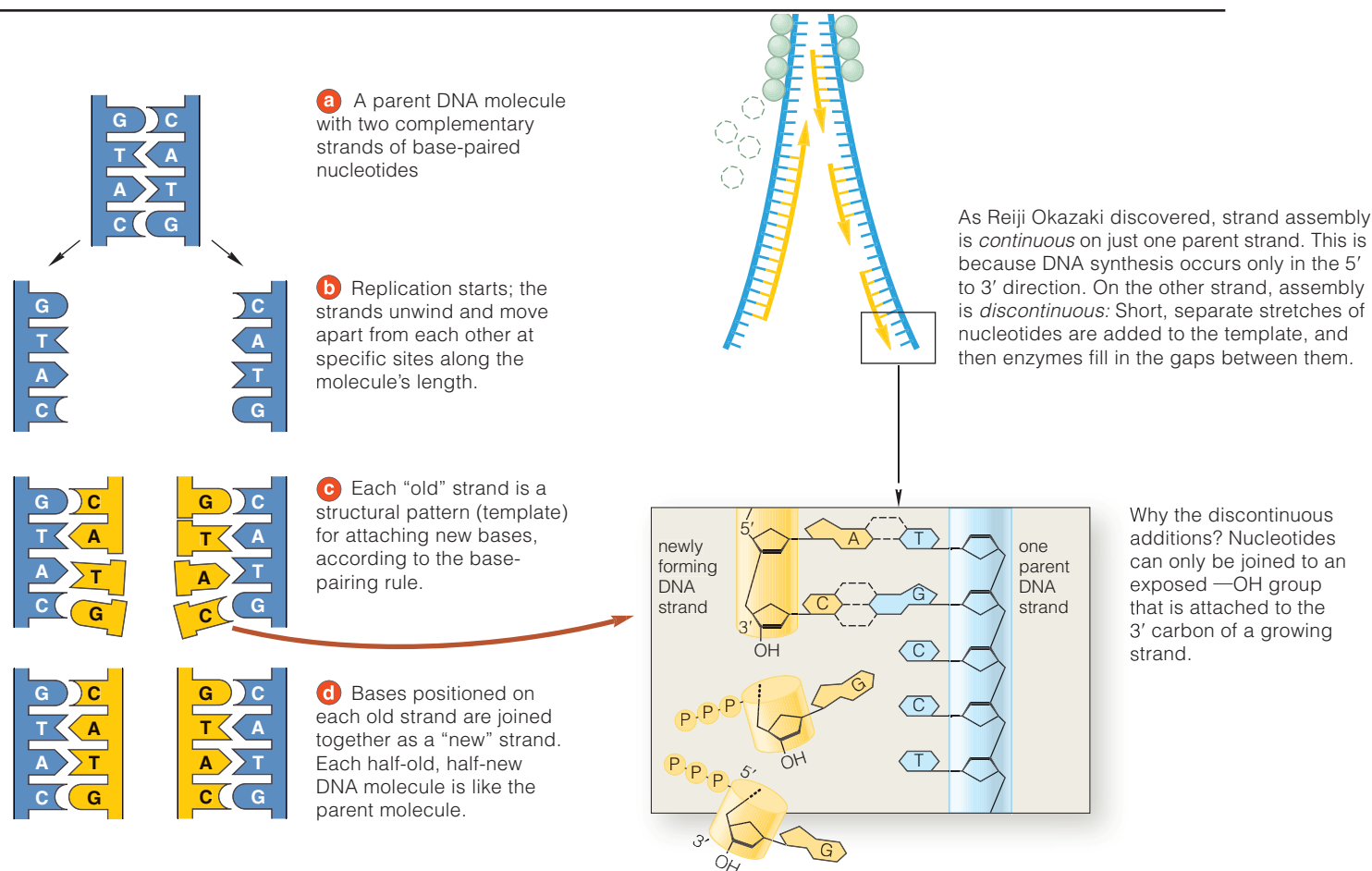


Figure 13.8 Animated! A closer look at DNA replication.

together into a continuous strand. A complementary strand now winds up with the parent strand that was its template. Two DNA double helixes are the result.

As you will read in Chapter 16, DNA polymerases and DNA ligases also have been put to good use as tools in recombinant DNA technology.

FIXING MISMATCHES AND BREAKS

Over the long term, changes in chromosomal DNA can give rise to variations in traits that help define a species (Section 12.8). However, in terms of a lifetime, the individual may not survive if something changes its DNA. For instance, on rare occasions, the wrong nucleotide is base-paired to a parent template (Table 13.1). Unless such mistakes are reversed, they might alter or weaken the functions of genes or the protein products. Chapter 12 has a number of examples of the kinds of genetic disorders that can follow changes.

DNA proofreading mechanisms swiftly fix most errors in replication and most of the strand breaks. For example, some DNA polymerases proofread new base pairings. They can reverse catalytic additions by one base and correct a mismatch. When they cannot,

replication is arrested, and controls over the cell cycle come into play (Sections 9.2 and 9.5).

Mismatches that slip past the proofreaders are only one type of DNA damage. One or both backbones of the double helix may break, as by ionizing radiation and some chemicals (Section 14.5). Also, a base in one strand may become covalently bonded to a base in the same strand or the partner strand.

Specialized sets of **repair enzymes** can repair some changes; they recognize and snip out a damaged site or mismatches. For instance, some glycosylases excise a mismatched base and replace it with a suitable one in a tiny burst of DNA synthesis.

DNA is replicated prior to cell division. At certain sites, helicases unzip hydrogen bonds between its two strands, so the double helix unwinds. Each strand remains intact—it is conserved—and DNA polymerases assemble a new, complementary strand on each parent strand.

DNA proofreading mechanisms and special sets of repair enzymes fix nearly all mismatched base pairs. Different kinds also seal or bypass most breaks.

13.4 Using DNA To Duplicate Existing Mammals

LINKS TO SECTIONS 9.3, 10.2, 12.11



Here we return to a topic introduced at the start of this chapter. Researchers can now isolate DNA from an adult mammal and use it to bypass meiosis, gamete formation, and fertilization. Unlike sexual reproduction, which gives rise to mixes of traits from two parents, adult DNA cloning produces an exact genetic copy of a single adult.

“Cloning” can be a confusing word. It can apply to a method in recombinant DNA technology that makes multiple copies of DNA fragments. It also applies to natural and manipulated interventions in the steps of reproduction and development. These interventions are called embryo cloning, adult cloning, and therapeutic cloning (Table 13.2).

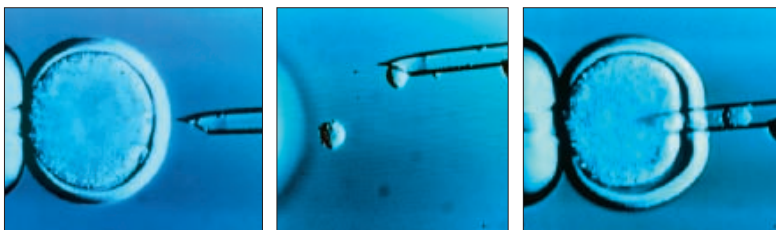
Embryo Cloning Embryo cloning occurs all the time in nature. For instance, soon after fertilized human eggs start dividing, a tiny ball of cells has formed. You saw one of these early developmental stages in Section 12.11. Once in every seventy-five or so pregnancies, the ball splits, and the two parts grow and develop into identical twins. A laboratory

procedure, “artificial twinning,” simply simulates what goes on in nature. For instance, the balls of cells grown from fertilized cattle eggs in petri dishes are encouraged to split into identical-twin embryos. Then they are implanted in surrogate mothers, which give birth to cloned calves.

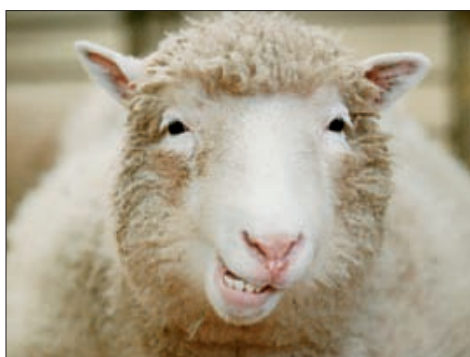
Embryo cloning has been practiced for decades. However, embryo clones inherit DNA from two parents, not one. If breeders are looking for a particularly valued trait, such as better milk production or mating vigor, they have to wait for clones to grow up to see if they display the trait.

Adult Cloning Because it takes so long to observe the outcome, some researchers were looking for an alternative to embryo cloning of cattle and other complex animals. It seemed that cloning a differentiated cell would be far more efficient, because the desired phenotype was already there, right in front of them.

You may wonder what “differentiated” means. As you already know after reading about mitosis and meiosis, all cells descended from a fertilized egg inherit the same chromosomal DNA (Sections 9.3 and 10.2). However, as the new embryo develops, different cells inside it start to select and use DNA’s information in different ways. Their selections commit them to becoming liver cells,



- 1 A microneedle is about to remove the nucleus from an unfertilized sheep egg (center).
- 2 The microneedle has now emptied the sheep egg of its own nucleus, which held the DNA.
- 3 A nucleus from a donor cell (in the microneedle) is about to be deposited in the egg that was stripped of its nucleus.
- 4 An electric current will stimulate the egg to enter mitotic cell division. After a few rounds of divisions, the ball of cells will be implanted in the womb of a female sheep (ewe).



the first sheep cloned from adult DNA

Figure 13.9 Animated! Transfer of an adult nucleus that led to Dolly.

Table 13.2 How Cloning Procedures Compare With Sexual Reproduction			
Sexual Reproduction			
Maternal DNA	Paternal DNA	fertilized egg	early embryo
			Individual with mix of parental traits
Embryo Cloning			
Maternal DNA	Paternal DNA	fertilized egg	artificial splitting of early embryo
			Identical twins with same mix of parental traits
Adult Cloning (reproductive cloning)			
Nucleus of cell from an adult	ball of cytoplasm (unfertilized egg stripped of nucleus)	early embryo	Genetically identical individual (clone of parent)
Therapeutic Cloning			
Cell nucleus from adult organ (e.g., nerve, heart, muscle)	ball of cytoplasm (stem cell stripped of nucleus)		New, replacement tissue for nerves, heart, muscles, other organs



Figure 13.10 *Left*, DNA donor Tahini, a Bengal cat. *Right*, Tabouli and Baba Ganoush, two of her clones. Eye color changes as a Bengal cat matures; in both clones, it will in time be the same as Tahini's. All three are household pets of the founder of a pet cloning company that, precisely because of its success, is at the center of a cloning controversy.

blood cells, or other specialists in structure, composition, and function in the adult. The mechanisms by which this happens are topics of later chapters.

With respect to adult cloning, a “grown-up” cell—one already differentiated—must be tricked into rewinding the developmental clock. Each of its descendants will have to start using a different subset of its DNA all over again to form a clone of its original owner.

Nuclear transfer is one way to trick a differentiated cell. A researcher with a good microscope and a very steady hand replaces the nucleus of an unfertilized egg with one from a differentiated cell of an adult animal, as in Figure 13.9. Small doses of chemicals or electric shocks may induce the cell to divide. If all goes well, then a cluster of embryonic cells forms and can be implanted inside a surrogate mother. In Dolly's case, the donor nucleus came from a cell from the lining of a sheep's udder.

A recipient egg is not always tricked into using the DNA as it is supposed to. For example, researchers studied many genes in the first cloned mice and found that 4 percent or so were being abnormally expressed. The cloning procedure had disrupted when and how mouse cells were supposed to use their genes. Even so, researchers around the world are becoming better at rewinding the developmental clock.

For instance, Genetic Savings and Clone is a company that uses adult DNA to clone beloved pet cats that are old and dying. So far, the pet owners who have requested the procedure report that their clones are healthy, lively, and uncannily like the DNA donor, not only in appearance but also in behavior. Figure 13.10 shows an example. Compare the markings on the top of the head, the face, the legs, and the tail of the donor and of the two clones.

Some people are deeply offended by the idea of spending tens of thousands of dollars to clone a cat when so many lost or orphaned cats are awaiting adoption in animal

shelters. Some owners say that they have bonded deeply with their particular pet, that they would grieve mightily over its loss, and that it is, after all, their money.

The real issue, of course, is that *humans*—like cats, mice, and pigs—are mammals. Not very long ago, mammalian cloning was fraught with technical problems. It still is. As researchers get better at what they do, however, the use of adult DNA cloning to make a genetic copy of a human no longer seems in the realm of science fiction. That is why most countries recently banned the use of federal funding for any research into adult human cloning.

Therapeutic Cloning *Therapeutic* cloning also uses nuclear transfers. In this case, the idea is to transplant DNA of a somatic cell from the heart, liver, muscles, or nerves into a stem cell. A *stem cell*, recall, is one that has not yet differentiated and retains the capacity to divide (Section 12.2). The descendant cells go on to differentiate into cell types of specific tissues and organs. The process is known as *somatic cell nuclear transfer* (SCNT).

SCNT already has produced stem cells that are an exact genetic match to an individual. This is not reproduction; no sperm are used. However, descendants of the modified cells may be able to regenerate a tissue for transplant back into a patient affected by an incurable disease or a spinal cord injury. Potentially, SCNT could regenerate organs. There are long waiting lists for organ transplants, and those who receive them have to take drugs to suppress the immune system for the rest of their lives.

We will return to some of these issues later on, after you learn more about the molecular basis of life. Make your own informed decisions about them, and remember this: For better or worse, our capacity to manipulate DNA started with Watson, Crick, and so many others who shared knowledge that became the underpinnings of a brave new world.

13.5 Rosalind's Story

FOCUS ON
BIOETHICS

There is a saying among researchers in any discipline—publish or perish. As soon as Watson and Crick's structural model of DNA fell into place, they immediately published a one-page paper that dazzled the world. All others who had helped fill in crucial pieces of the puzzle, including Franklin, received little or no recognition. Franklin's contribution is now receiving more attention.

Rosalind Franklin arrived at King's Laboratory in London with impressive credentials (Figure 13.11). She developed a refined x-ray diffraction method while studying the structure of coal in Paris. She took a new mathematical approach to interpreting x-ray diffraction images and, like Pauling, had built three-dimensional molecular models. Now she was asked to create and run a state-of-the-art x-ray crystallography laboratory. Her assignment was to investigate the structure of DNA.

No one bothered to tell Franklin that, just down the hall, Maurice Wilkins was already working on the puzzle. Even a graduate student assigned to assist her failed to mention it. No one bothered to tell Wilkins about Franklin's assignment; he assumed that she was a technician hired to do his x-ray crystallography work because he didn't know how to do it himself. And so a clash began. To Franklin, Wilkins seemed inexplicably prickly. To Wilkins, Franklin was appalling in her lack of deference to him.

Wilkins had a prized cache of DNA, which he gave to his "technician." Five months later, Franklin gave a talk on what she had learned so far. DNA, she said, may have two, three, or four parallel chains twisted into a helix, with phosphate groups projecting outward.

With his crystallography background, Crick would have recognized the significance of her report—if he had been there. (A pair of chains oriented in opposing directions would be the same even if flipped 180 degrees. Two pairs of chains? No. DNA's density ruled that out. But one pair of chains? Yes!) Watson was in the audience but did not know what Franklin was talking about.

Later on, Franklin produced her superb x-ray diffraction image of wet DNA fibers (Figure 13.12). The image fairly screamed *HELIX!* Franklin also worked out the length and the diameter of DNA. However, she had been working with dry fibers for a long time, and she chose not to dwell on the meaning of her new data. Wilkins did.

In 1953, without Franklin's knowledge, he let Watson see that image and reminded him of what she had reported more than a year before. When Watson and Crick did focus on her data, they had the final bit of information that they needed to build a plausible model of DNA—one with two helically twisted chains running in opposing directions.



Figure 13.11 Portrait of Rosalind Franklin arriving at Cambridge in style, from Paris.



Figure 13.12 Franklin's best x-ray diffraction image of DNA fibers.

<http://biology.brookscole.com/starr11>

Summary

Section 13.1 Experimental tests that used bacteria and bacteriophages offered the first solid evidence that DNA is the hereditary material in living organisms.

Biology Now

Learn about experiments that revealed the function of DNA with the animation on BiologyNow.

Section 13.2 The nucleotide monomers of DNA have a five-carbon sugar (deoxyribose), a phosphate group, and one of four kinds of nitrogen-containing bases: adenine, thymine, guanine, or cytosine.

A DNA molecule consists of two nucleotide strands coiled together into a double helix. The bases of one strand hydrogen-bond with bases of the other.

Bases of the two DNA strands pair in a constant way. Adenine pairs with thymine (**A-T**), and guanine with cytosine (**G-C**). Which base follows another along a strand varies among species. The DNA of each species incorporates some number of unique sequences of base pairs that set it apart from the DNA of all other species.

Biology Now

Investigate the structure of DNA with the animation on BiologyNow.

Section 13.3 In DNA replication, enzymes called helicases unwind the DNA double helix, and small proteins hold the two strands apart. DNA polymerases covalently bond free nucleotides into chains in a base sequence complementary to the parent strand that serves as its template. Two double-stranded DNA molecules result. One strand of each is old (is conserved); the other strand is new.

Strand assembly occurs only at an exposed —OH group at the 3' end of a growing nucleotide strand. DNA ligases seal tiny gaps between short stretches of nucleotides in one of the growing strands.

Proofreading mechanisms fix most base-pairing mistakes and strand breaks. Special repair enzymes recognize and snip out damaged sites in the DNA as well as mismatches.

Biology Now

See how a DNA molecule is replicated with the animation on BiologyNow.

Section 13.4 "Cloning" means copying fragments of DNA in recombinant DNA work. It also refers to three other procedures: Embryo cloning results in genetically identical twins. Adult cloning results in a genetically identical copy of an existing adult. Therapeutic cloning is a proposed method of producing stem cells that are an exact genetic match of a patient, the idea being to regenerate tissues and possibly organs.

Biology Now

Observe the procedure used to create Dolly and other clones with animation on BiologyNow.

Section 13.5 Science advances as a community effort that is both cooperative and competitive. Ideally,

individuals share their work and recognition for honors that come their way. As in all human endeavors, some fail to receive suitable recognition for their contribution.

Self-Quiz

Answers in Appendix II

- Which is *not* a nucleotide base in DNA?
 - adenine
 - guanine
 - uracil
 - thymine
 - cytosine
 - All are in DNA.
- What are the base-pairing rules for DNA?
 - A-G, T-C
 - A-C, T-G
 - A-U, C-G
 - A-T, G-C
- One species' DNA differs from others in its _____.
 - sugars
 - phosphates
 - base sequence
 - all of the above
- When DNA replication begins, _____.
 - the two DNA strands unwind from each other
 - the two DNA strands condense for base transfers
 - two DNA molecules bond
 - old strands move to find new strands
- DNA replication requires _____.
 - free nucleotides
 - new hydrogen bonds
 - many enzymes
 - all of the above
- _____ is the basis for the life's diversity.
 - constancy in DNA's bonding pattern
 - variation in the base sequences in DNA
- Adult cloning starts with _____.
 - an early embryo
 - nuclear transfers
 - artificial twinning
 - both b and c
- Match the terms appropriately.

_____ bacteriophage	a. nitrogen-containing base bonded to a sugar and one or more phosphate groups
_____ clone	b. breaks hydrogen bonds, starts unwinding of DNA during replication
_____ nucleotide	c. only DNA and protein
_____ helicase	d. fills in gaps, seals breaks in a DNA strand
_____ DNA ligase	e. carbon copy of dad or mom
_____ DNA polymerase	f. adds nucleotides to a growing DNA strand

Additional questions are available on **BiologyNow™**

Critical Thinking

- A pathogenic strain of *E. coli* has acquired an ability to produce a dangerous toxin that causes medical problems and fatalities. It is especially dangerous to young children who eat undercooked or raw contaminated beef. Develop hypotheses to explain how a normally harmless bacterium such as *E. coli* can become a pathogen.
- Matthew Meselson and Franklin Stahl's experiments supported a semiconservative model of DNA replication. These researchers obtained "heavy" DNA by growing *Escherichia coli* in a medium enriched with ^{15}N , a nitrogen radioisotope. They prepared "light" DNA by growing *E. coli* in the presence of ^{14}N , the more common isotope. An available technique helped them identify which replicated

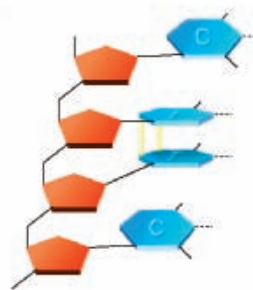
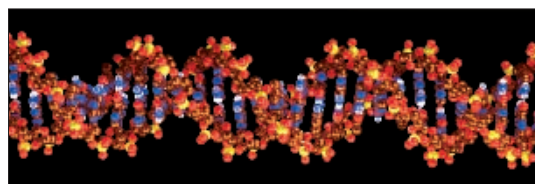


Figure 13.13 Above, a thymine dimer in a DNA strand. It can lead to xeroderma pigmentosum (right), a genetic disorder.



molecules were heavy, light, or hybrid (one heavy strand and one light). Use pencils of two colors, one for heavy strands and one for light. Assuming a DNA molecule has two heavy strands, arrange pencils to show how daughter molecules form after replication in a medium with ^{14}N . Represent four DNA molecules that form if the daughter molecules are replicated in the ^{14}N medium.

- If you are part of a biology class, split into groups. See which one writes up the clearest, most concise description of the component parts and organization of this molecule:



- Mutations, remember, are permanent changes in DNA base sequences—the original source of genetic variation and the raw material of evolution. Yet how can mutations accumulate, given that cells have repair systems that fix changes or breaks in DNA strands?
- In 1999, scientists discovered a woolly mammoth that had been frozen in glacial ice for the past 20,000 years. They thawed it very carefully so they could use its DNA to clone a woolly mammoth. It turns out there was not enough preserved material to work with. They plan to try again the next time a frozen woolly mammoth comes along. Reflect on Section 13.4, then speculate on the pros and cons of cloning an extinct animal.

6. *Xeroderma pigmentosum* (XP) is an autosomal recessive disorder that is characterized by the rapid formation of skin sores that can develop into cancers (Figure 13.13). Affected individuals have no mechanism for dealing with the damage that ultraviolet (UV) light can inflict on skin cells. They must avoid all forms of radiation—including sunlight and fluorescent lights.

Affected individuals do not have functioning DNA repair mechanisms. James Cleaver discovered this when he studied what happens in cells when DNA's nitrogen-containing bases absorb UV light. A covalent bond can form between two thymine bases in the same DNA strand. The resulting thymine dimer puts a kink in the strand. Propose what some of the consequences might be during interphase, when most proteins are synthesized, and then during DNA replication.