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Biotechnology

David P. Clark
Nanette J. Pazdernik

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Authors

David P. Clark
Department of Microbiology
Southern Illinois University
Carbondale, Illinois

Nanette J. Pazdernik
School of Medicine
Washington University
St. Louis, Missouri

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Academic Cell Update

This book is dedicated to Donna. —DPC

This book is dedicated to my children and husband. Their patience and understanding have given me the time and inspiration to research and write this text. —NJP

HOW WE GOT HERE

In speaking with professors across the biological sciences and going to conferences, we, the editors at Academic Press and Cell Press, saw how often journal content was being incorporated in the classroom. We understood the benefits students were receiving by being exposed to journal articles early: to add perspective, improve analytical skills, and bring the most current content into the classroom. We also learned how much additional preparation time was required on the part of instructors finding the articles, then obtaining the images for presentations and providing additional assessment.

So we collaborated to offer instructors and students a solution, and *Academic Cell* was born. We offer the benefits of a traditional textbook (to serve as a reference to students and a framework to instructors), but we also offer much more. With the purchase of every copy of an *Academic Cell* book, students can access an online study guide containing relevant, recent Cell Press articles and providing bridge material to help ease them into the articles. In addition, the images from the articles are available in PowerPoint and we have optional test bank questions.

We plan to expand this initiative, as future editions will be further integrated with unique pedagogical features incorporating current research from the pages of Cell Press journals into the textbook itself.

PREFACE

From the simple acts of brewing beer and baking bread has emerged a field now known as biotechnology. Over the ages the meaning of the word biotechnology has evolved along with our growing technical knowledge. Biotechnology began as the ability to culture microorganisms to create a variety of food and drinks. Today, biotechnology is defined as any application of biology that uses living organisms or bioprocesses to create new bioproducts. The idea is basically unchanged; biotechnology is using an organism to create a new or improved product.

The fields of genetics, molecular biology, microbiology, and biochemistry are merging their respective discoveries into the expanding field of biotechnology, and advances are occurring at a record pace. Two or three years of research can dramatically alter the approaches that are of practical use. For example, the simple discovery that double-stranded RNA can block expression of any gene with a matching sequence has revolutionized how we study and apply genetic interactions in less than a ten-year period.

This rapid increase in knowledge is very hard to incorporate into a textbook format, and often instructors who teach advanced molecular biology classes rely on the primary research to teach students novel concepts and applications. This type of teaching is difficult and requires lots of hours to plan and organize.

The new partnership between Academic Press and Cell Press has adopted a solution to teaching advanced molecular biology and biotechnology courses. The partnership combines years of textbook publishing experience with the most relevant and high impact research. What has emerged is a new teaching paradigm. In *Biotechnology, Academic Cell Update Edition,* the basic ideas and methodologies are explained using very clear and concise language. These techniques are supplemented with a wide variety of diagrams and illustrations to simplify the complex biotechnology processes.

These basics are then supported with a *Biotechnology* online study guide that not only tests the student's knowledge of the textbook chapter, but also contains primary research articles. The articles are chosen from the Cell Press family of journals, which includes such high-impact journals as *Cell, Molecular Cell,* and *Current Biology*. The articles expand upon a topic presented in each chapter or provide an exemplary research paper for that particular chapter. The entire full-color research article is included online.

In addition to the article, the *Biotechnology* study guide includes a synopsis of the research paper. The synopsis includes a thorough discussion of the relevant background information that is often assumed knowledge in most primary research articles. Then each synopsis breaks the paper into sections, explaining each individual experiment separately. Each experiment is explained by defining the underlying hypothesis or question, the methods used to study the question, and the results. The final section of the synopsis provides the overall conclusions for the paper. This approach reinforces the basic scientific method. The instructor does not have to find an article, create a presentation on the background, and then work with the student to explain each of the methods and results. The study guide synopsis provides all of this information already.

The online format ensures that only the most recent papers are associated with the chapter. The combination of the online study guide with the newest relevant research and a solid basic textbook provides the instructor with the best of both worlds. You can teach students the basic concepts using the textbook, and then use the relevant research paper to stretch the student's knowledge of current research in the field of biotechnology.

ACKNOWLEDGMENTS

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MODERN BIOTECHNOLOGY RELIES ON ADVANCES IN MOLECULAR BIOLOGY AND COMPUTER TECHNOLOGY

Traditional biotechnology goes back thousands of years. It includes the selective breeding of livestock and crop plants as well as the invention of alcoholic beverages, dairy products, paper, silk, and other natural products. Only in the past couple of centuries has genetics emerged as a field of scientific study. Recent rapid advances in this area have in turn allowed the breeding of crops and livestock by deliberate genetic manipulation rather than trial and error. The so-called green revolution of the period from 1960 to 1980 applied genetic knowledge to natural breeding and had a massive impact on crop productivity in particular. Today, plants and animals are being directly altered by genetic engineering.

New varieties of several plants and animals have already been made, and some are in agricultural use. Animals and plants used as human food sources are being engineered to adapt them to conditions that were previously unfavorable. Farm animals that are resistant to disease and crop plants that are resistant to pests are being developed in order to increase yields and reduce costs. The impact of these genetically modified organisms on other species and on the environment is presently a controversial issue.

Modern biotechnology applies not only modern genetics but also advances in other sciences. For example, dealing with vast amounts of genetic information depends on advances in computing power. Indeed, the sequencing of the human genome would have been impossible without the development of ever more sophisticated computers and software. It is sometimes claimed that we are in the middle of two scientific revolutions, one in information technology and the other in molecular biology. Both involve handling large amounts of encoded information. In one case the information is human made, or at any rate man-encoded, and the mechanisms are artificial; the other case deals with the genetic information that underlies life.

However, there is a third revolution that is just emerging—nanotechnology. The development of techniques to visualize and manipulate atoms individually or in small clusters is opening the way to an ever-finer analysis of living systems. Nanoscale techniques are now beginning to play significant roles in many areas of biotechnology.

This raises the question of what exactly defines biotechnology. To this there is no real answer. A generation ago, brewing and baking would have been viewed as biotechnology. Today, the application of modern genetics or other equivalent modern technology is usually seen as necessary for a process to count as "biotechnology." Thus, the definition of *biotechnology* has become partly a matter of fashion. In this book, we regard (modern) biotechnology as resulting in a broad manner from the merger of classical biotechnology with modern genetics, molecular biology, computer technology, and nanotechnology.

The resulting field is of necessity large and poorly defined. It includes more than just agriculture: it also affects many aspects of human health and medicine, such as vaccine development and gene therapy. We have attempted to provide a unified approach that is based on genetic information, while at the same time indicate how biotechnology has begun to sprawl, often rather erratically, into many related fields of human endeavor.

CONTENTS

CHAPTER 1

Basics of Biotechnology

ADVENT OF THE BIOTECHNOLOGY REVOLUTION

Biotechnology involves the use of living organisms in industrial processes—particularly in agriculture, food processing, and medicine. Biotechnology has been around since the dawn of time, ever since humans began manipulating the natural environment to improve their food supply, housing, and health. Biotechnology is not limited to humankind. Beavers cut up trees to build homes. Elephants deliberately drink fermented fruit to get an alcohol buzz. People have been making wine, beer, cheese, and bread for centuries. All these processes rely on microorganisms to modify the original ingredients (Fig. 1.1). Over the ages, farmers have chosen higher yielding crops by trial and error, so that many modern crop plants have much larger fruit or seeds than their ancestors (Fig. 1.2).

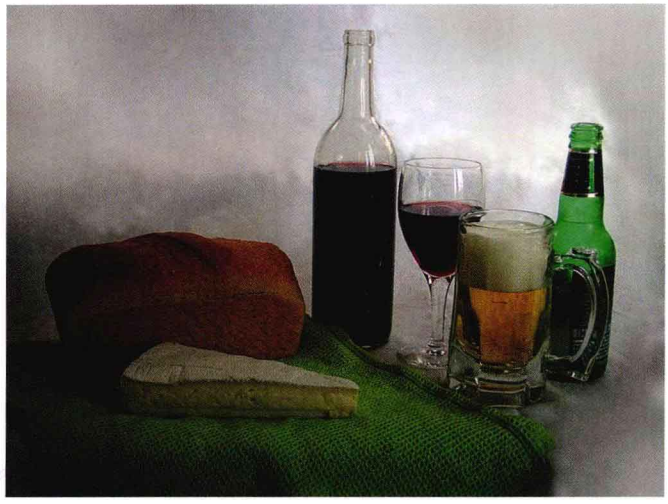


FIGURE 1.1 Traditional Biotechnology Products
Bread, cheese, wine, and beer have been made worldwide for many centuries using microorganisms, such as yeast.

FIGURE 1.2 Teosinte versus Modern Corn
Throughout history, people have improved many plants for higher yields. Teosinte (smaller cob and green seeds) is considered the ancestor of commercial corn (larger cob; a blue-seeded variety is shown). Courtesy of Wayne Campbell, Hila Science Camp.

The reason we think of biotechnology as modern is because of recent advances in molecular biology and genetic engineering. Huge strides have been made in our understanding of microorganisms, plants, livestock, as well as the human body and the natural environment. This has caused an explosion in the number and variety of biotechnology products. Face creams contain antioxidants—supposedly to fight the aging process. Genetically modified plants have genes inserted to protect them from insects, thus increasing the crop yield while decreasing the amount of insecticides used. Medicines are becoming more specific and compatible with our physiology. For example, insulin for diabetics is now genuine human insulin, although produced by genetically modified bacteria. Almost everyone has been affected by the recent advances in genetics and biochemistry.

Mendel's early work that described how genetic characteristics are inherited from one generation to the next was the beginning of modern genetics (see Box 1.1). Next came the discovery of the chemical material of which genes are made—**DNA (deoxyribonucleic acid)**. This in turn led to the central dogma of genetics; the concept that genes made of DNA are expressed as an **RNA (ribonucleic acid)** intermediary that is then decoded to make **proteins**. These three steps are universal, applying to every type of organism on earth. Yet these three steps are so malleable that life is found in almost every available niche on our planet.

Biotechnology affects all of our lives and has altered everything we encounter in life.

Box 1.1 Gregor Johann Mendel (1822–1884): Founder of Modern Genetics

As a young man, Mendel spent his time doing genetics research and teaching math, physics, and Greek to high school children in Brno (now in the Czech Republic). Mendel studied the inheritance of various traits of the common garden pea, *Pisum sativum*, because he was able to raise two generations a year. He studied many different physical traits of the pea, such as flower color, flower position, seed color and shape, and pod color and shape. Mendel grew different plants next to each other, looking for traits that mixed together. Luckily, the traits he studied were each due to a single gene that was either dominant or recessive, although he did not know this at the time. Consequently, he never saw them "mix." For example, when he grew yellow peas next to green peas, the offspring looked exactly like their parents. This showed that traits do not blend in the offspring, which was a common theory at the time.

Next Mendel moved pollen from one plant to another with different traits. He counted the number of offspring that inherited each trait and found that they were inherited in specific ratios. For example, when he cross-pollinated the yellow and green pea plants, their offspring, the F_1 generation, was all yellow. Thus the yellow trait must dominate or mask the green trait. He then let the F_1 plants produce offspring, and grew all of the seeds. These, the F_2 generation, segregated into 3/4 yellow and 1/4 green. When green seeds reappeared after skipping a generation, Mendel concluded that a "factor" for the trait—what we call a gene nowadays—must have been present in the parent, even though the trait was not actually displayed.

Mendel demonstrated many principles that form the basis of modern genetics. First, units or factors (now called genes) for each trait are passed on to successive generations. Each parent has two copies of each gene but contributes only one copy of the gene to each offspring. This is called the **principle of segregation**. Second, the **principle of independent assortment** states that different offspring from the same parents can get separate sets of genes. The same phenotype (the observable physical traits) can be represented by different genotypes (combinations of genes). In other words, although a gene is present, the corresponding trait may not be seen in each generation. When Mendel began these experiments, he used purebred pea plants—that is, each trait always appeared the same in each generation. So when he first crossed a yellow pea with a green pea, each parent had two identical copies or **alleles** of each gene. The green pea had two green alleles, and the yellow pea had two yellow alleles. Consequently, each F_1 offspring received one yellow allele and one green allele. Despite this, the F_1 plants all had yellow peas. Thus yellow is dominant to green. Finally when the F_1 generation was self-pollinated, the F_2 plants included some that inherited two recessive green alleles and had a green phenotype (Fig. A).

Mendel published these results, but no one recognized the significance of his research until after his death. Later in life he became the abbot of a monastery and did not pursue his genetics research.

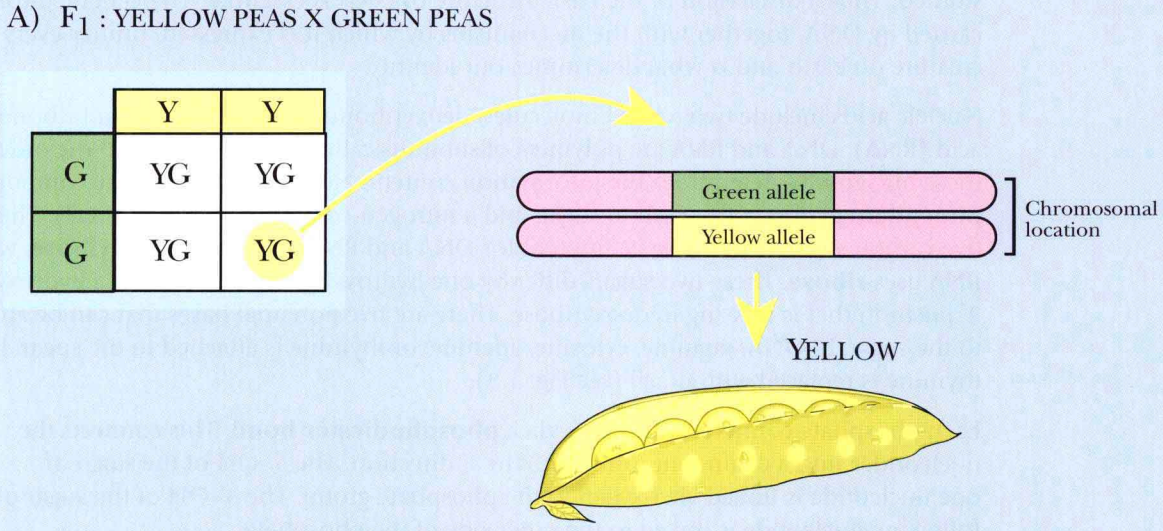


FIGURE A Relationship of Genotype and Phenotype
(A) Each parent has two alleles, either two yellow or two green. Any offspring will be heterozygous, each having a yellow and a green allele. Since the yellow allele is dominant, the peas look yellow.

(*Continued*)

Box 1.1 Gregor Johann Mendel (1822–1884): Founder of Modern Genetics—cont'd

B) F_2 : HETEROZYGOUS SELF-FERTILIZATION

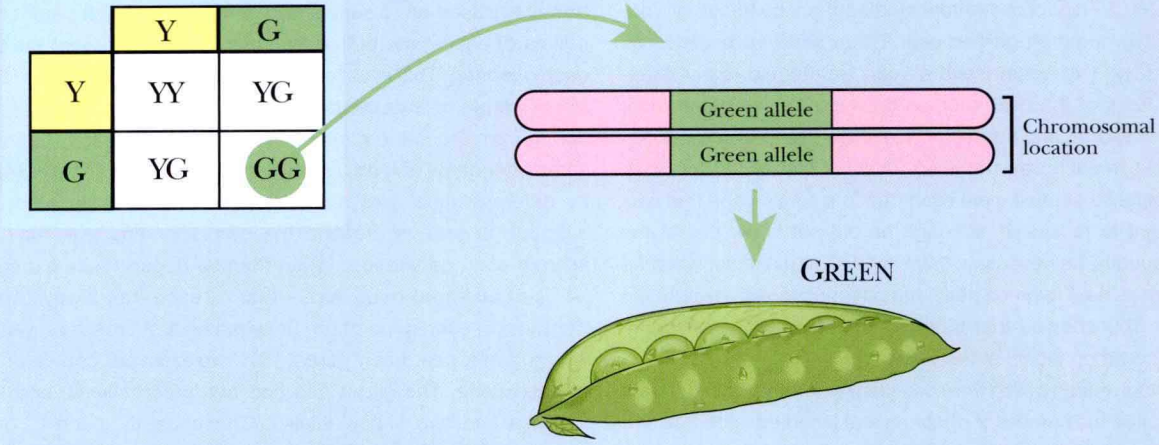


FIGURE A Relationship of Genotype and Phenotype, cont'd
(B) When the heterozygous F_1 offspring self-fertilize, the green phenotype re-emerges in one-fourth of the F_2 generation.

CHEMICAL STRUCTURE OF NUCLEIC ACIDS

The upcoming discussions introduce the organisms used extensively in molecular biology and genetics research. Each of these has genes made of DNA that can be manipulated and studied. Thus a discussion of the basic structure of DNA is essential. The genetic information carried by DNA, together with the mechanisms by which it is expressed, unifies every creature on earth and is what determines our identity.

Nucleic acids include two related molecules, deoxyribonucleic acid (DNA) and ribonucleic acid (RNA). DNA and RNA are polymers of subunits called **nucleotides**, and the order of these nucleotides determines the information content. Nucleotides have three components: a **phosphate group**, a five-carbon sugar, and a nitrogen-containing **base** (Fig. 1.3). The five-carbon sugar or **pentose** is different for DNA and RNA. DNA has **deoxyribose**, whereas RNA uses **ribose**. These two sugars differ by one hydroxyl group. Ribose has a hydroxyl at the 2′ position that is missing in deoxyribose. There are five potential bases that can be attached to the sugar. In DNA, guanine, cytosine, adenine, or thymine is attached to the sugar. In RNA, thymine is replaced with uracil (see Fig. 1.3).

Each phosphate connects two sugars via a **phosphodiester bond**. This connects the nucleotides into a chain that runs in a 5′ to 3′ direction. The 5′-OH of the sugar of one nucleotide is linked via oxygen to the phosphate group. The 3′-OH of the sugar of the following nucleotide is linked to the other side of the phosphate.

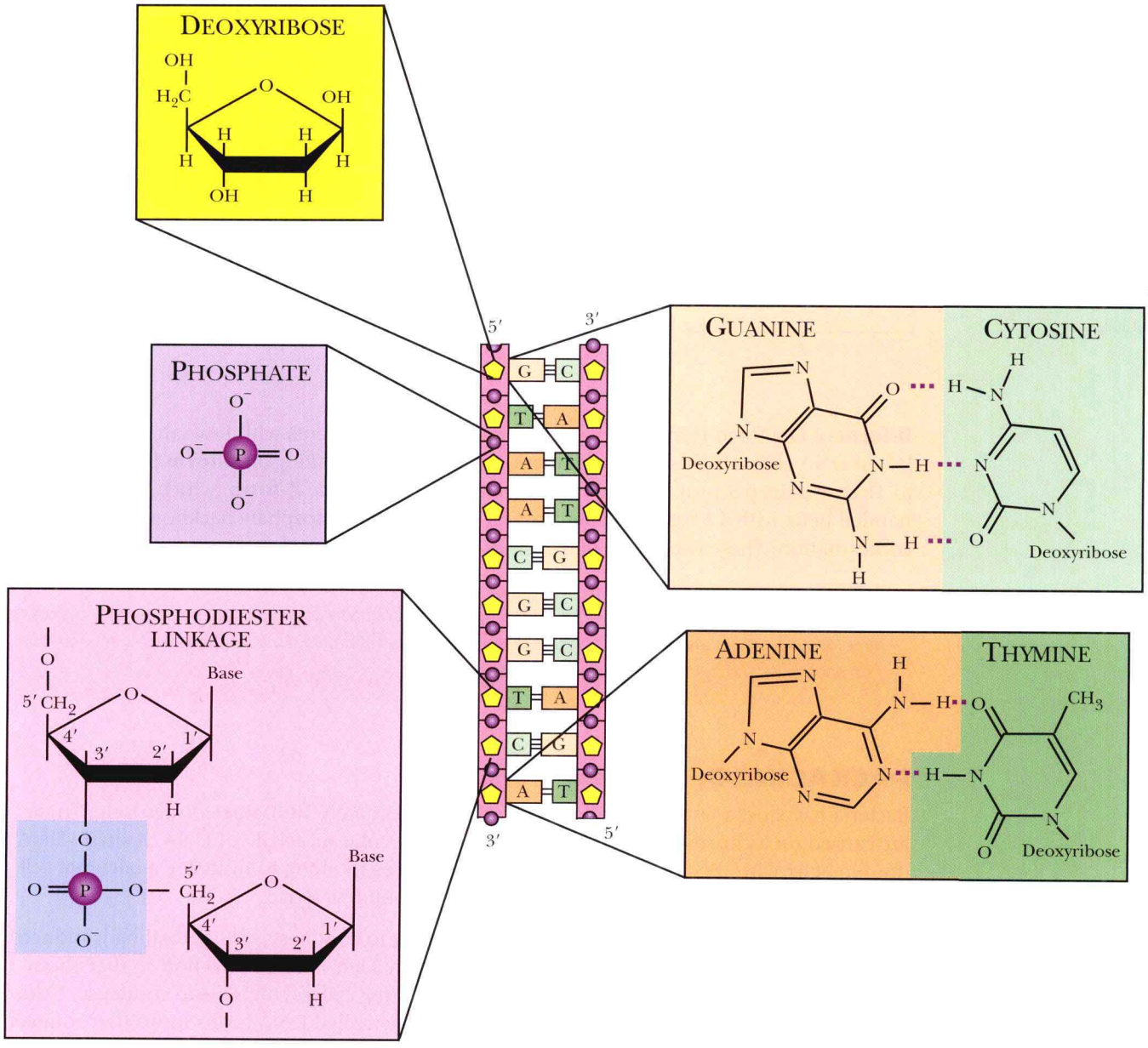
The nucleic acid bases jut out from the sugar phosphate backbone and are free to form connections with other molecules. The most stable structure occurs when another single strand of nucleotides aligns with the first to form a double-stranded molecule, as seen in the DNA **double helix**. Each base forms hydrogen bonds to a base in the other strand. The two strands are **antiparallel**, that is, they run in opposite directions with the 5′end of the first strand opposite the 3′end of its partner and vice versa.

The bases are of two types, **purines** (guanine and adenine) and **pyrimidines** (cytosine and thymine). Each base pair consists of one purine connected to a pyrimidine via hydrogen bonds. Guanine pairs only with cytosine (G-C) via three hydrogen bonds. Adenine pairs only with thymine (A-T) in DNA or uracil (A-U) in RNA. Because an adenine-thymine (A-T) or adenine-uracil (A-U) base pair is held together with only two hydrogen bonds, it requires less energy to break the connection between the bases than in a G-C pair.

The double-stranded DNA takes the three-dimensional shape that has the lowest energy constraints. The most stable shape is a double-stranded helix. The helix turns around a central axis in a clockwise manner and is considered a **right-handed helix**. One complete turn is 34 Å in length and has about 10 base pairs. DNA is not static, but can alter its conformation in response to various environmental changes. The typical conformation just described is the

FIGURE 1.3 Nucleic Acid Structure
(A) DNA has two strands antiparallel to each other. The structure of the subcomponents is shown to the sides.

A) Structure of DNA



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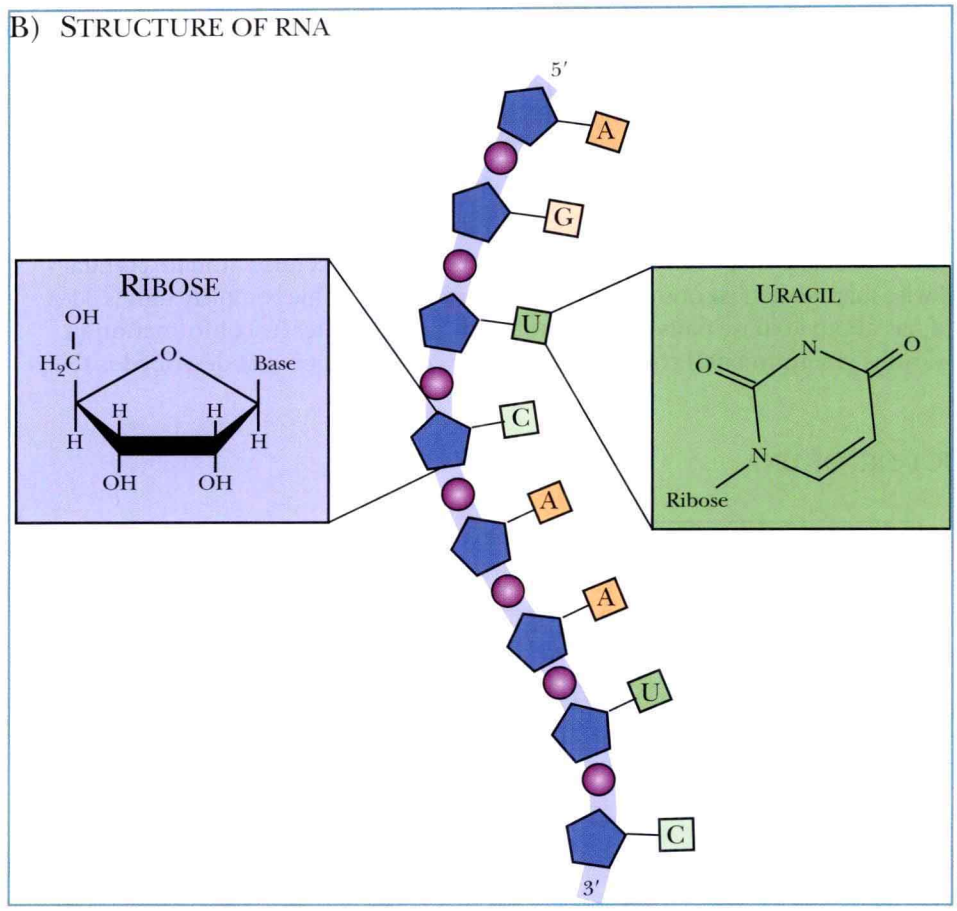

FIGURE 1.3, cont'd
(B) RNA is usually single-stranded and has two chemical differences from DNA. First, an extra hydroxyl group (-OH) is found at the 2′ position of ribose, and second, thymine is replaced by uracil.

B-form of DNA and is most prevalent in aqueous environments with low salt concentrations. When DNA is in a high-salt environment, the helix alters, making an **A-form** that has closer to 11 base pairs per turn. Another conformation of DNA is the **Z-form**, which has a left-handed helix with 12 base pairs per turn. In this form, the phosphate backbone has a zigzag conformation. These two forms may be biologically relevant under certain conditions.

DNA and RNA are both structures with alternating phosphate and sugar residues linked to form a backbone. Base residues attach to the sugar and project out from the backbone. These bases can base-pair with another strand to form double-stranded helices.

PACKAGING OF NUCLEIC ACIDS

Bacteria have just a few thousand genes, each approximately 1000 nucleotides long. These are carried on a chromosome that is a single giant circular molecule of DNA. A single DNA double helix with this many genes is about 1000 times too long to fit inside a bacterial cell without being condensed somehow in order to take up less space.

In bacteria, the double helix undergoes **supercoiling** to condense it. Supercoiling is induced by the enzyme **DNA gyrase**, which twists the DNA in a left-handed direction so that about 200 nucleotides are found in one supercoil. The twisting causes the DNA to condense. Extra supercoils are removed by **topoisomerase I**. The supercoiled DNA forms loops that connect to a protein scaffold (see Fig. 1.4).