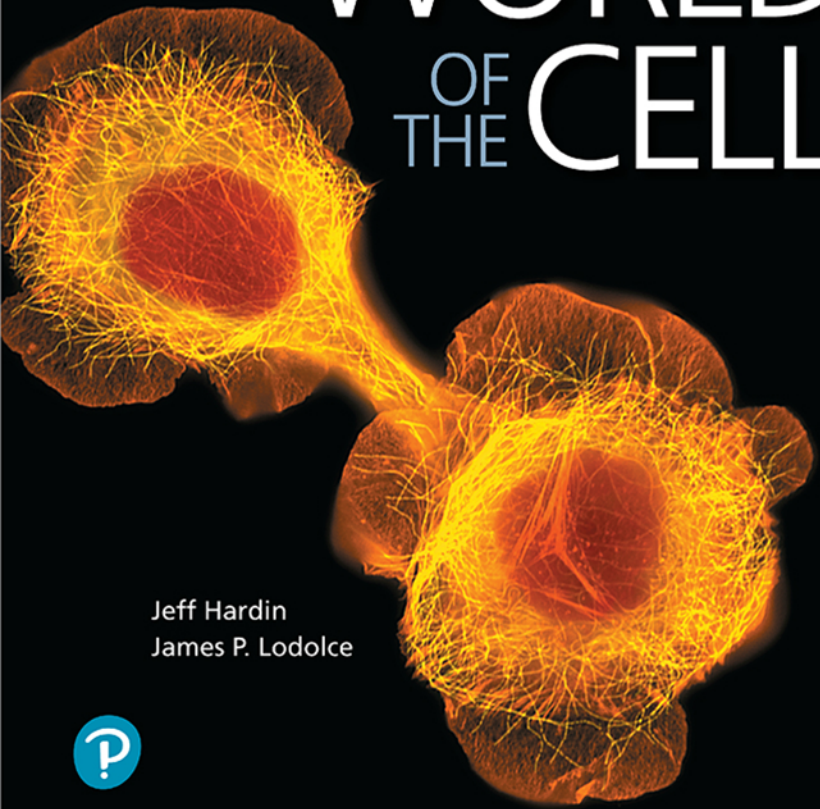
Becker's

10th Edition

WORLD OF THE CELL



Jeff Hardin
James P. Lodolce

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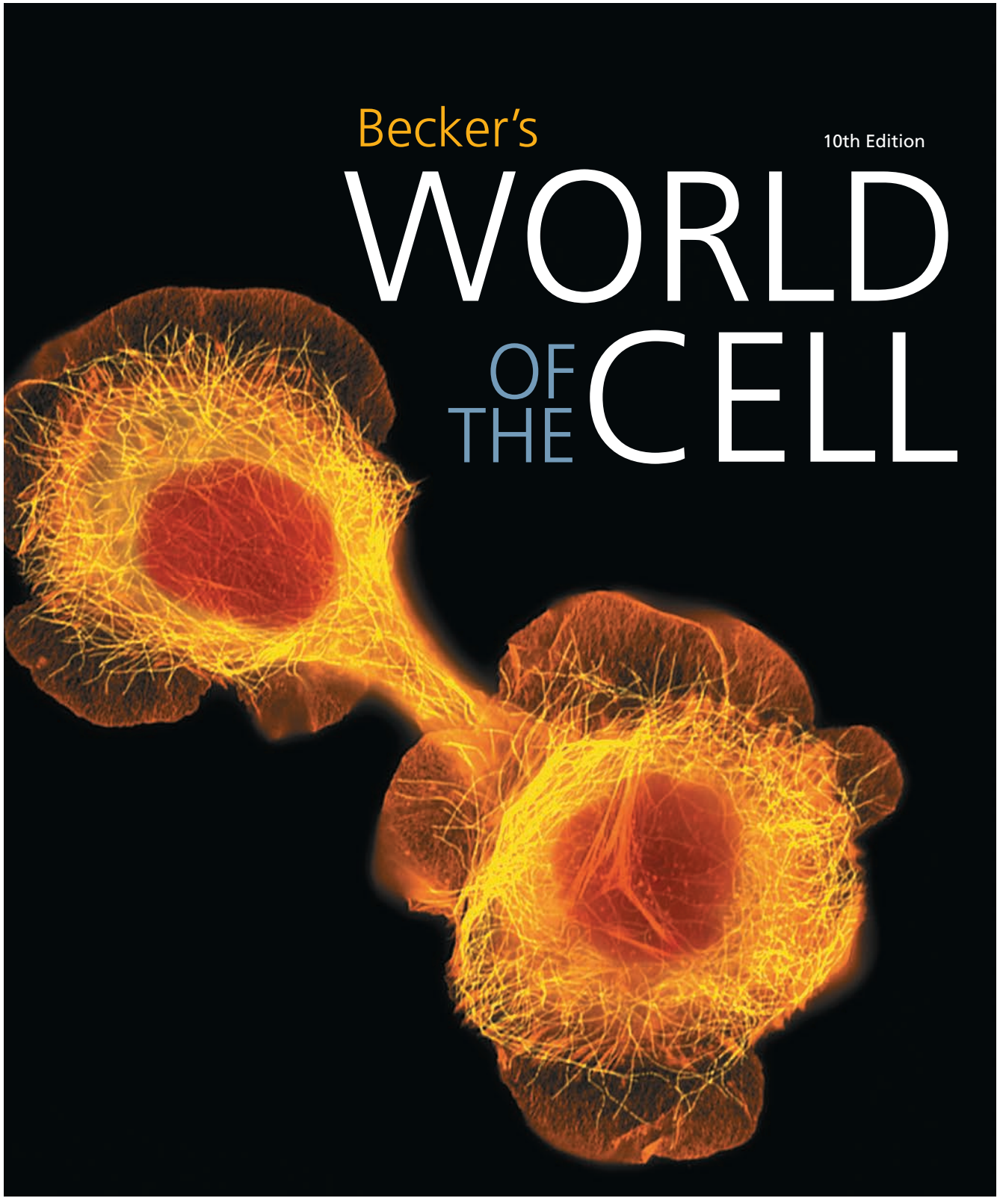

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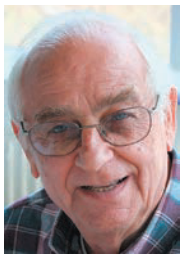
ABOUT THE AUTHORS

JEFF HARDIN received his Ph.D. in Biophysics from the University of California–Berkeley. He is the Raymond E. Keller Professor and Chair of the Department of Integrative Biology at the University of Wisconsin–Madison, where he has been since 1991. For 18 years he was Faculty Director of the Biology Core Curriculum, a four-semester honors biology sequence for undergraduates at Wisconsin known for its teaching innovations. Jeff's research focuses on how cells migrate and adhere to one another during early embryonic development. Jeff's teaching is enhanced by his extensive use of digital microscopy and his web-based teaching materials, which are used on many campuses in the United States and in other countries. Jeff was a founding member of the UW Teaching Academy, and has received several teaching awards, including a Lily Teaching Fellowship, a National Science Foundation Young Investigator Award, and a Chancellor's Distinguished Teaching Award.

JAMES P. LODOLCE earned his Ph.D. in Immunology from the University of Chicago in 2002. His thesis examined the signals that promote the survival of memory lymphocytes. As a postdoctoral fellow in the laboratory of Dr. David Boone, he studied the genetics and regulation of inflammation in autoimmunity. Cell biology was the first class that James taught when he arrived at Loyola University Chicago in 2010. He currently holds the title of Senior Lecturer and teaches a variety of courses ranging from molecular biology to virology. James is an active member of the Department of Biology and was appointed Co-Chairperson of Loyola's 2021 Pre-Health Professions Advisory Committee. In his career at Loyola, James has received several teaching honors, including a nomination for the 2014 Ignatius Loyola Award for Excellence in Teaching, the 2016 Master Teacher Award in the College of Arts and Sciences, and the 2020 Edwin T. and Vivijeanne F. Sujack Award for Teaching Excellence.



WAYNE M. BECKER taught cell biology at the University of Wisconsin–Madison for 30 years until his retirement. His interest in textbook writing grew out of notes, outlines, and problem sets that he assembled for his students, culminating in *Energy and the Living Cell,* a paperback text on bioenergetics published in 1977, and *The World of the Cell,* the first edition of which appeared in 1986. All his degrees are in biochemistry from the University of Wisconsin–Madison, an orientation that is readily discernible in his writing. His research interests were in plant molecular biology, focused on the expression of genes that encode enzymes of the photorespiratory pathway. Later in his career he focused on teaching, especially students from underrepresented groups. His honors include a Chancellor's Award for Distinguished Teaching, Guggenheim and Fulbright Fellowships, and a Visiting Scholar Award from the Royal Society of London. This text builds on his foundation and is inspired by his legacy.

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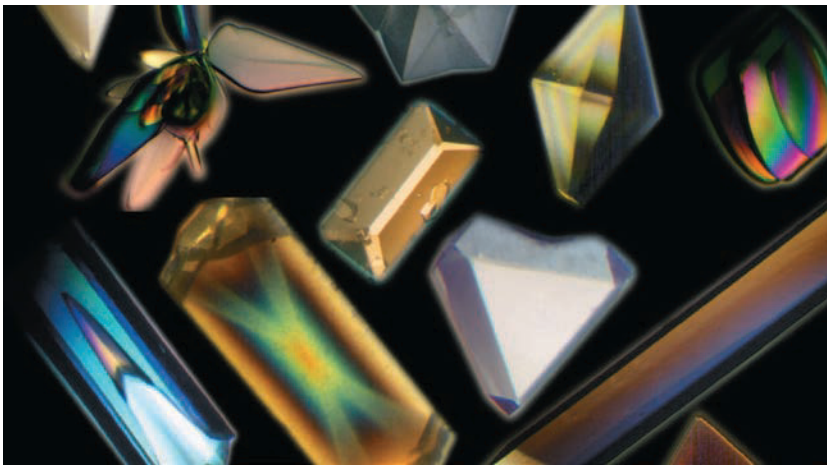
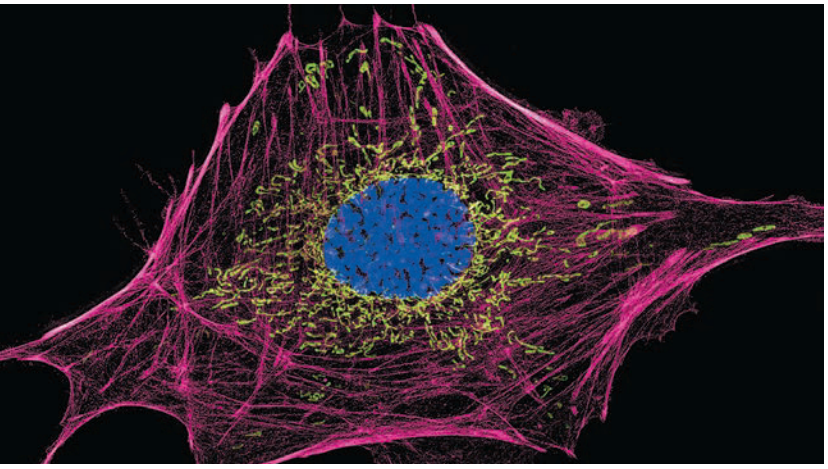
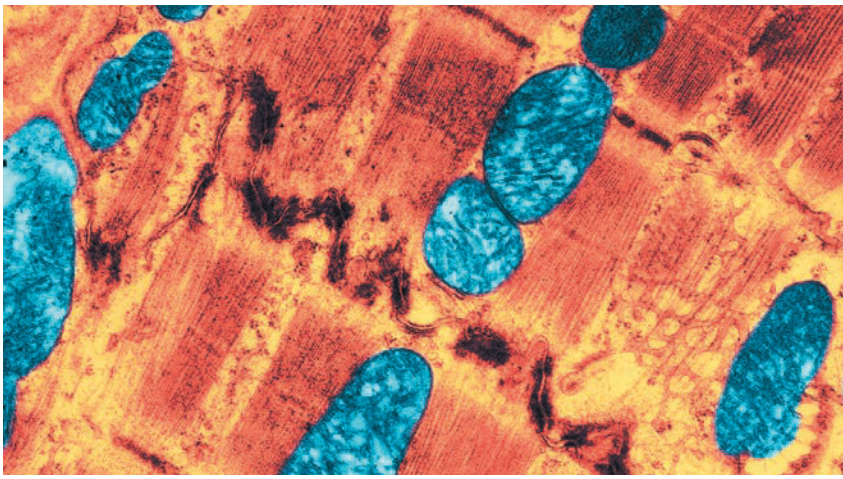
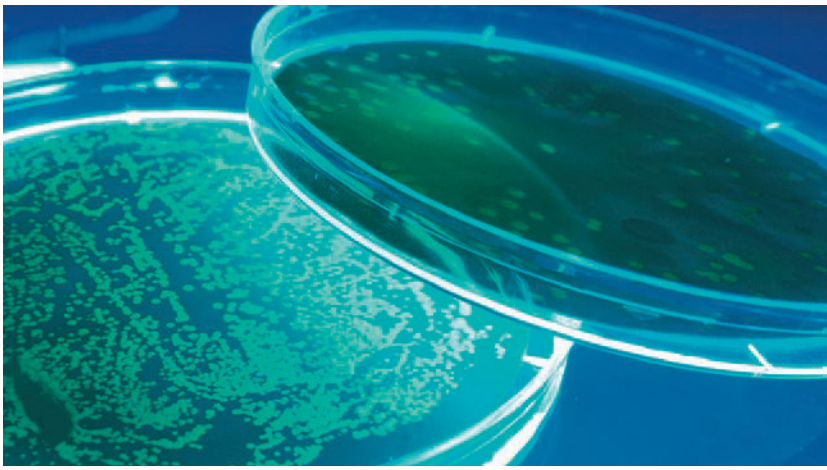
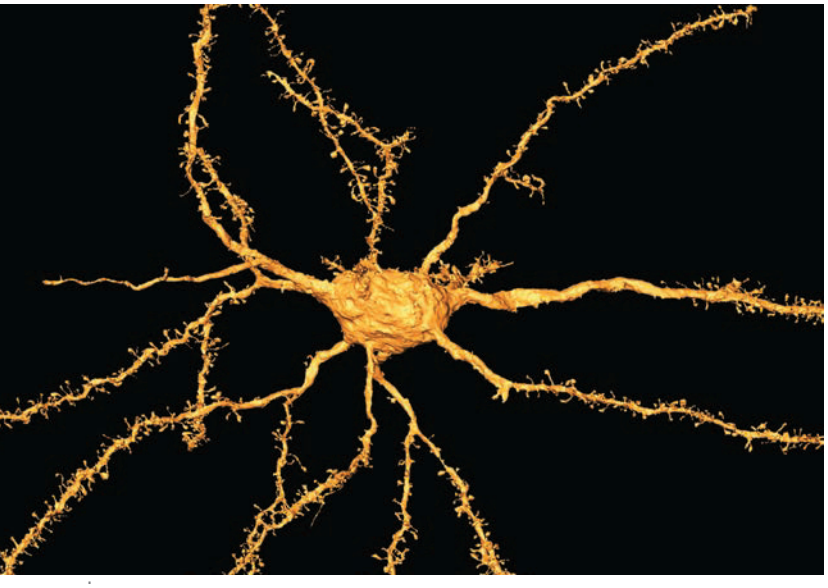
DETAILED CONTENTS





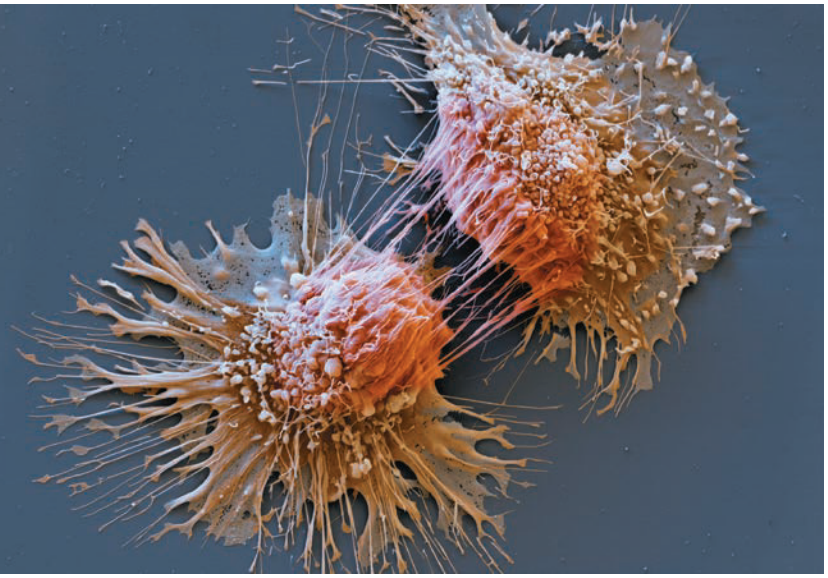






22 Signal Transduction Mechanisms: I. Electrical and Synaptic Signaling in Neurons *663*

23 Signal Transduction Mechanisms: II. Messengers and Receptors *689*



Cells are the fundamental building blocks of life on this planet. Despite their tiny size, they are wonders of intricacy. Moment by moment, the cells of our bodies are engaged in a dazzling repertoire of biochemical events, including signaling processes, transmission of genetic information, and delicately choreographed movements. Understanding the basic functions of cells also gives us insight when something goes wrong, as in the case of a disease, or when the cell is highjacked, as in the case of a viral infection. Helping our students to appreciate the complexities of this amazing cellular world lies at the heart of our goals as authors of *Becker's World of the Cell*. The motivations that drove our colleague, Wayne Becker, to write the first edition of this book continue to drive us today. We believe that our students should have biology textbooks that are clearly written, make the subject matter relevant, and help them to appreciate not only how much we already know about cell biology but also the exciting journey of continued discovery that lies ahead. We, as authors, have an extensive history of teaching undergraduate courses in cell biology and related areas, and we treasure our contact with students as one of the most rewarding aspects of being faculty members.

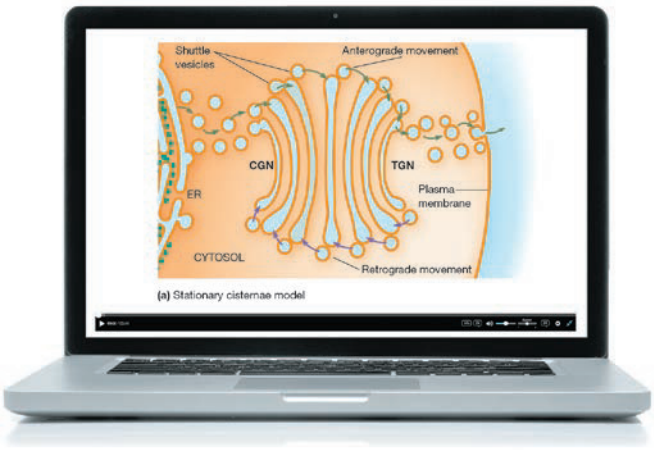
The amazing success of modern cell biology creates both exciting opportunities and central challenges in our teaching. How can we capture the core elements of modern cell biology in a way that draws our students in without overwhelming them? The enormous profusion of information challenges us to keep *Becker's World of the Cell* up to date while ensuring that it remains both manageable in length and readily comprehensible to students studying cell and molecular biology for the first time.

This tenth edition engages students with new innovative features in each chapter and an exciting, fresh look. In addition, a major goal of this edition has been to reorganize the presentation of several key topics. We hope that the often-requested consolidation of translation of secreted and plasma membrane-associated proteins with the larger discussion of the endomembrane system has led to an even more compelling presentation of these important topics. We also hope students and instructors will find that the continued emphasis on molecular biology throughout the tenth edition reinforces how indispensable these techniques are in the everyday work of modern cell biologists.

As with the previous editions, we remain committed to three central goals. First, our primary goal is to introduce students to the fundamental principles that guide cellular organization and function. Second, we want students to understand some of the key scientific evidence that has helped us formulate these central concepts. And third, we have sought to accomplish these goals in a book of manageable length that is easily read and understood by beginning cell biology students—and that still fits in their backpacks! We have therefore been necessarily selective both in the examples chosen to illustrate key concepts and in the quantity of scientific evidence included. The result is an update that we hope students and instructors will be as excited about as we are.

What's New in This Edition

- **Make Connection questions:** Two new questions in every chapter ask students to make connections across concepts and chapters in the text. By reinforcing fundamental conceptual connections throughout cell biology, these features help overcome students' tendencies to compartmentalize information. These questions are also assignable and automatically graded in **Mastering Biology.**
- **Data Analysis questions:** Every chapter of *World of the Cell* now has a Data Analysis question for students to practice their ability to interpret data. Students must be able to analyze data in order to make informed decisions, generate well-formed, testable hypotheses, design follow-up experiments, and provide compelling evidence for results. These questions are also assignable and automatically graded in **Mastering Biology.**
- **Figure Walkthroughs:** In the *World of the Cell* e-text, Figure Walkthroughs guide students through key figures with narrated explanations and figure mark-ups that reinforce important points. All walkthroughs are also assignable in **Mastering Biology** and paired with several auto-gradable questions for student assessment.



- **Reorganization of material on translation and intracellular trafficking:** Because the molecular genetics material comes earlier in the book, topics that relate to translation of secreted and plasma membrane-associated proteins are now more naturally integrated into the discussion of intracellular trafficking. These topics are now combined in Chapter 12, which focuses on the endomembrane system, including cotranslational import into the endoplasmic reticulum of proteins destined for secretion or insertion into the plasma membrane.

Hallmark Features

- **Key Technique boxes in every chapter:** Twenty-six Key Technique boxes are integrated throughout the text, demonstrating how cutting-edge technologies can be used to answer key questions in cell biology.

Key Technique Determining the Chemical Fingerprint of a Cell Using Mass Spectrometry

A Scientist Preparing an Injection for Mass Spectrometry.

PROBLEM: In cell biology, scientists typically study processes that involve changes in the chemistry of the cell, such as cell growth and division. Researchers often want to be able to identify small molecules in a cellular extract, or they may want to determine the chemical structure of a new compound. How is such analysis accomplished?

SOLUTION: *Mass spectrometry* (often called *mass spec*) is a method used to identify and measure the relative abundance of individual molecules in a sample, as well as to determine their chemical structure. Purified molecules are broken into fragments, and these fragments can be analyzed to determine their masses and the arrangement of covalent bonds that hold atoms of the molecule together.

Figure 2A-1 A Mass Spectrometer.

Key Tools: Mass spectrometer; an ionized sample; a computer to analyze the results.

Details: Mass spectrometry can identify chemical compounds within a sample with high resolution, differentiating between compounds that can vary by as little as 1 atomic mass unit (amu), the mass of a hydrogen atom. Analysis of a compound using a mass spectrometer (**Figure 2A-1**) involves three main steps: *ionization and fragmentation* of the sample, *deflection* of the ionized fragments by an electromagnet, and *detection* of the individual ions and measurement of their abundance.

Ionization and Fragmentation. Commonly, the sample is ionized by bombarding it with a stream of high-energy electrons from an electron gun. The stream has enough energy to knock an

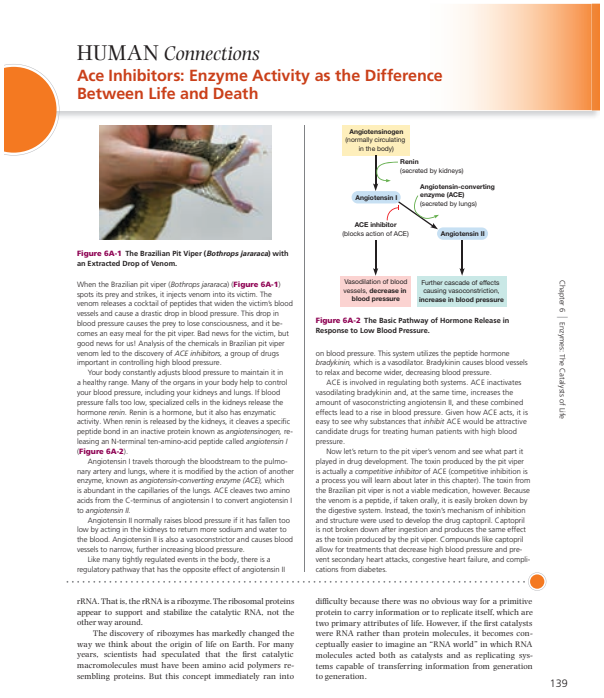
a single asymmetric carbon atom (in the center) and thus has two stereoisomers, called L-alanine and D-alanine (Figure 2-7a). Neither of the other two carbon atoms of alanine is an asymmetric carbon atom because one has three identical substituents (hydrogen atoms) and the other has two bonds to a single oxygen atom and thus is only bonded to three substituents. Both stereoisomers of alanine occur in nature, but only L-alanine is present as a component of proteins.

As an example of a compound with multiple asymmetric carbon atoms, consider the six-carbon sugar *glucose* shown in Figure 2-7b. Of the six carbon atoms of glucose, the four shown in boldface are asymmetric. (Can you figure out why the other two carbon atoms are not asymmetric?) With four asymmetric carbon atoms, the structure shown (D-glucose) is only one of 2^4, or 16, possible stereoisomers of the $C_6H_{12}O_6$ molecule.

CONCEPT CHECK 2.1
What properties of the carbon atom make it especially suitable as the structural basis for nearly all biomolecules?

26

- **Human Connections boxes in every chapter:** Twenty-six Human Connections boxes emphasize the relevance of cell biology to human health and society, from the story of Henrietta Lacks and the HeLa cell line to the relevance of biochemical pathways to our diet, to the many cases in which cell biology helps us diagnose and treat human disease.



HUMAN *Connections*

Ace Inhibitors: Enzyme Activity as the Difference Between Life and Death

Figure 6A-1 The Brazilian Pit Viper (*Bothrops jararaca*) with an Extracted Drop of Venom.

When the Brazilian pit viper (*Bothrops jararaca*) (**Figure 6A-1**) spots its prey and strikes, it injects venom into its victim. The venom releases a cocktail of peptides that widen the victim's blood vessels and cause a drastic drop in blood pressure. This drop in blood pressure causes the prey to lose consciousness, and it becomes an easy meal for the pit viper. Bad news for the victim, but good news for us! Analysis of the chemicals in Brazilian pit viper venom led to the discovery of *ACE inhibitors*, a group of drugs important in controlling high blood pressure.

Your body constantly adjusts blood pressure to maintain it in a healthy range. Many of the organs in your body help to control your blood pressure, including your kidneys and lungs. If blood pressure falls too low, specialized cells in the kidneys release the hormone *renin*. Renin is a hormone, but it also has enzymatic activity. When renin is released by the kidneys, it cleaves a specific peptide bond in an inactive protein known as *angiotensinogen*, releasing an N-terminal ten-amino-acid peptide called *angiotensin I* (**Figure 6A-2**).

Angiotensin I travels thorough the bloodstream to the pulmonary artery and lungs, where it is modified by the action of another enzyme, known as *angiotensin-converting enzyme (ACE)*, which is abundant in the capillaries of the lungs. ACE cleaves two amino acids from the C-terminus of angiotensin I to convert angiotensin I to *angiotensin II*.

Angiotensin II normally raises blood pressure if it has fallen too low by acting in the kidneys to return more sodium and water to the blood. Angiotensin II is also a vasoconstrictor and causes blood vessels to narrow, further increasing blood pressure.

Like many tightly regulated events in the body, there is a regulatory pathway that has the opposite effect of angiotensin II

Figure 6A-2 The Basic Pathway of Hormone Release in Response to Low Blood Pressure.

on blood pressure. This system utilizes the peptide hormone *bradykinin*, which is a vasodilator. Bradykinin causes blood vessels to relax and become wider, decreasing blood pressure.

ACE is involved in regulating both systems. ACE inactivates vasodilating bradykinin and, at the same time, increases the amount of vasoconstricting angiotensin II, and these combined effects lead to a rise in blood pressure. Given how ACE acts, it is easy to see why substances that *inhibit* ACE would be attractive candidate drugs for treating human patients with high blood pressure.

Now let's return to the pit viper's venom and see what part it played in drug development. The toxin produced by the pit viper is actually a *competitive inhibitor* of ACE (competitive inhibition is a process you will learn about later in this chapter). The toxin from the Brazilian pit viper is not a viable medication, however. Because the venom is a peptide, if taken orally, it is easily broken down by the digestive system. Instead, the toxin's mechanism of inhibition and structure were used to develop the drug captopril. Captopril is not broken down after ingestion and produces the same effect as the toxin produced by the pit viper. Compounds like captopril allow for treatments that decrease high blood pressure and prevent secondary heart attacks, congestive heart failure, and complications from diabetes.

rRNA. That is, the rRNA is a ribozyme. The ribosomal proteins appear to support and stabilize the catalytic RNA, not the other way around.

The discovery of ribozymes has markedly changed the way we think about the origin of life on Earth. For many years, scientists had speculated that the first catalytic macromolecules must have been amino acid polymers resembling proteins. But this concept immediately ran into difficulty because there was no obvious way for a primitive protein to carry information or to replicate itself, which are two primary attributes of life. However, if the first catalysts were RNA rather than protein molecules, it becomes conceptually easier to imagine an "RNA world" in which RNA molecules acted both as catalysts and as replicating systems capable of transferring information from generation to generation.

Chapter 6 | Enzymes: The Catalysts of Life

139

- **Concept Check questions:** Each main section of a chapter ends with a Concept Check question. These questions provide students with numerous opportunities to assess their understanding as they read. Answers to these questions are available at the back of the book.
- **Quantitative questions in every end-of-chapter Problem Set:** New and existing quantitative questions are flagged at the end of each chapter to encourage students to work on developing their ability to perform calculations or to interpret quantitative information. Most of these questions are assignable through **Mastering Biology.**
- **Content updates:** Updated information highlighting the most recent advances in cell and molecular biology has been added throughout the book (see Content Highlights of the Tenth Edition below).

Mastering Biology is an innovative online homework, tutorial, and assessment system that delivers self-paced tutorials with individualized coaching, hints, and feedback. The Mastering system helps instructors and students with customizable, easy-to-assign, and automatically graded assignments.

Integrated links in every chapter of the textbook point students to a variety of interactive online materials, including the following:

- 52 assignable Make Connection questions help students make connections across chapters and concepts
- 10 figure walkthrough tutorials walk students through key figures and then assess their understanding
- More than 100 tutorials and activities that teach complex cell processes
- More than 100 molecular and microscopy videos, which provide vivid images of cellular processes
- 240 Reading Quiz questions, which encourage students to read before class
- Many end-of-chapter questions and problems that are assignable and automatically gradable
- Test Bank questions for every chapter
- The e-text, also available through **Mastering Biology**, which provides both access to the complete textbook and powerful interactive and customizable functions
- A suite of Instructor Resources, including PowerPoint lecture outlines containing all the figures and photos and five to ten personal response system (PRS) clicker questions per chapter
- Learning Catalytics is a "bring your own device" assessment and active classroom system that expands the possibilities for student engagement beyond standard clickers where instructors can deliver a wide range of auto-gradable or open-ended questions that test content knowledge and build critical thinking skills

Content Highlights of the Tenth Edition

Updated material and new information have been added throughout the book in both the text and art. Major topics that have been altered, updated, or added include the following:

Chapter 1: Created new Figure 1-1 (Hooke's microscope and drawing of cork). Added CRISPR genome editing to Figure 1-3 and added a new subsection on CRISPR to Section 1.2. Condensed the three microscopy subsections (The Light Microscope, Specialized Light Microscopes, and The Electron Microscope) into one large subsection subtitled "Microscopy." Modified Figure 1-8 to better illustrate the central dogma in a cell. Added a new Data Analysis question.

Chapter 2: Added reference to organic carbon discoveries made by Mars rover to Section 2.1 and the importance of water transport to Section 2.2. Added new subsection on prion self-assembly to Section 2.5. Added a new Data Analysis question.

Chapter 3: Added reference to gecko pad and van der Waals interactions. Added information about the Folding @ Home initiative. Added subsection on chaperones in protein folding to Section 3.1. Added a new figure to the Human Connections box on Tau tangle formation. Added a new Data Analysis question.

Chapter 4: Significantly updated the discussion of the endosymbiont theory, including discussion of "inside-out" and "outside-in" proposals in a largely revised figure. Moved three domains of life discussion and figure from 9e Ch. 21 to Ch. 4.

Chapter 5: Added a new Data Analysis question; updated Figure 5-1 to add improved concentration work diagram.

Chapter 6: Majorly revised Figure 6-11 and relevant text to conform to the majority of advanced biochemistry texts regarding inhibitors. Removed sucrase discussion to comport with deletion of the relevant figure in the previous edition and generated a new figure showing the catalytic site of lysozyme accordingly. Shortened the discussion of ACE inhibitors in the Human Connections box. Replaced one Problem Set question on biological relevance with another graphical analysis problem on competitive inhibitors.

Chapter 7: Moved SDS-PAGE material to Ch. 21. Reduced treatment of lipid rafts to reflect ongoing controversy in the field. Added a new Key Technique box on fluorescence recovery after photobleaching (FRAP). Added a Human Connections box, adapted from 9e Ch. 12. Reinstated a more detailed structure diagram in Figure 7-6.

Chapter 8: Improved clarity of Figure 8-7. Added panel to Figure 8-10 to show frog oocytes. Added a new Data Analysis question.

Chapter 9: Shortened discussion of other uses of glycolytic enzymes. Improved several biochemical pathway diagrams for clarity.

Chapter 10: Revised the discussion of ATP yield in aerobic respiration while retaining the theoretical yield discussion as a *via media*. Substantially revised electron transport details in several figures. Substantially revised and improved Q cycle discussion and the relevant figure. Trimmed discussion and figure coverage of cristae and added a light micrograph showing mitochondria. Integrated TIM/TOM discussion into this chapter, moving it out of 9e Ch. 19 to join the discussion of the structure of mitochondria. Added figure on location of ATP synthesis in bacteria to compare to mitochondria. Replaced problem on thermogenin with Data Analysis question.

Chapter 11: Added information and figure about carboxysomes in cyanobacteria. Improved the molecular model presentation of light-harvesting complexes. Improved the treatment of electron flow in the chloroplast, including improving and shortening the discussion of the Q cycle. Updated information on protons per ATP. Improved depiction of the glycolate pathway and C_3/C_4 plant leaf anatomy. Added Quantitative and Data Analysis questions.

Chapter 12: Added an update on the types of models used to explain movement through the Golgi. Provided some rationale for grouping peroxisomes into the endomembrane system. Moved protein trafficking/sorting sections from 9E Ch. 19 to here. Added paragraph on how viruses can co-opt endosomes for infection. Combined 9e Sections 12.7 and 12.8 into one section (since the plant vacuole is a digestive compartment). Authored new Human Connections box on the role of autophagy in human disease.

Chapter 13: Updated *MreB* discussion to match current understanding of MreB function. Changed microtubule figures to show curved protofilaments at plus ends as per recent TEM work. Updated discussion of MT minus-end binding proteins; added information on augmin and branched MTs. Added info on CRWN proteins in higher plants to the IF section.

Chapter 14: Made minor changes to Figure 14A-2. Added a new Data Analysis question.

Chapter 15: Added brief mention of mechanotransduction via α-catenin. Added a new Data Analysis question.

Chapter 16: Added a purines/pyrimidine column in Table 16-1 on Chargaff's rules. Added detail on new studies on how histone H1 interacts with the nucleosome. Included an introduction to epigenetics in the section on chromatin remodeling. Mentioned how mRNA modifications are important in nuclear export of mRNA. Added possibility of NMCPs functioning as lamins in plant cells. Mentioned telomere dysfunction as a potential cause of premature aging in HGPS. Added detail about how charges in the histone tails affect DNA packaging. Moved section and figure on retroviruses from 9e Ch. 18 into this chapter.

Chapter 17: Added oxidation damage to Section 17.2. Authored a new Key Technique box on CRISPR genome modification. Updated the mutagenic mechanism of BrdU. Added a description of heteroduplex DNA to the homologous recombination section. Added a note on most likely mechanism of strand discrimination in eukaryotic mismatch repair. Updated nucleotide excision repair figure with more recent mechanism (Figure 17-27). Added quote from Francis Crick about the importance of DNA repair. Moved section and figure on retrotransposons from 9e Ch. 18 into this chapter. Added a new Data Analysis question.

Chapter 18: Improved the flow and organization of the chapter by moving discussion/figure about retroviruses to Ch. 16, moving retrotransposon discussion/figure to Ch. 17, and moving genetic code discussion/figures to Ch. 19. Authored a new Human Connections box on death cap mushrooms. Modified figure on the central dogma to include advances since Francis Crick's first proposal. Authored a new Concept Check question for Section 18.1. Added a note on the discovery of ribozymes, a subsection on mature mRNA nuclear export to Section 18.3, and a new Data Analysis question.

Chapter 19: Significantly reorganized the chapter flow by moving genetic code section from 9e Ch. 18 into new Section 19.1 and moving 9e Section 19.5 on protein targeting and sorting into Ch. 12. Added a new subsection on codon usage bias to Section 19.1.

Chapter 20: Added reference to temperature-sensitive riboswitches in Section 20.1, a paragraph on histone modifications to epigenetics in Section 20.2, and a new Data Analysis question.

Chapter 21: Moved 9e Figure 21-13 (tree of life) to Ch. 4 (new Figure 4-3) and the Key Technique box from 9e Ch. 17 (PCR) into this chapter. Worked the 9e Key Technique box from this chapter (DNA cloning) into the text in Section 21.1. Updated Southern blotting and Western blotting techniques for modern approach of not using film. Reorganized the techniques in Section 21.1 in a more logical way and moved all sequencing techniques into Section 21.2. Updated the description of next-generation and third-generation sequencing techniques to include state of the art in the field. Added a new subsection on quantitative PCR (qPCR). Expanded RNA-seq with details on single-cell RNAseq. Added description of conditional knockout mice engineering.

Chapter 22: Added a Make Connections question on *shibire* mutants in *Drosophila* that was needed in the synaptic transmission section.

Chapter 23: Changed title of Section 23.3 from Protein Kinase-Associated Receptors to Enzyme-Coupled Receptors. Added a subsection to the end of this section on other enzyme-coupled receptors (phosphatase receptor and guanylyl receptor families).

Chapter 24: Updated some sections with more modern treatment at the molecular level, including kinetochore (including revised Figure 24-4), chromosomal congression, FtsZ/divisome in bacteria, and spindle assembly checkpoint. Altered Figure 24-25 (9e 24-23) to improve clarity and moved to a later position.

Chapter 25: Added a paragraph on the potential role of the double-strand break repair model of homologous recombination in meiotic recombination in Section 25.6.

Chapter 26: Updated smoking statistics in Figure 26-7a through 2015 and added gender-specific data. Improved HPV figure (Figure 26-17). Changed emphasis to reflect replication/repair errors as a major cause of cancer, including discussion of the recent work by the Vogelstein group. Updated hallmarks of cancer discussion to correspond to the revised Weinberg paper from 2011. Added more detail in the immunotherapy section on Nobel Prize-winning work and CAR T cells, including a new small figure on CAR T cells. Added detail on Cdk4/6 therapy in the Key Technique box.

Appendix: Added explicit mention of GCaMP proteins in the calcium imaging section. Added a discussion of serial blockface SEM. Updated the cryoEM example image.

Building on the Strengths of Previous Editions

We have retained and built upon the strengths of prior editions in four key areas:

1. **The chapter organization focuses on main concepts.**
   - Each chapter is divided into sections that begin with a numbered *concept statement heading,* which summarizes the material and helps students focus on the main points to study and review.
   - Chapters are written and organized to allow instructors to assign chapters and chapter sections in different sequences, making the book adaptable to a wide variety of course plans.
   - Each chapter ends with a bulleted *Summary of Key Points* that briefly describes the main points covered in each section of the chapter.
2. **The illustrations teach concepts at an appropriate level of detail.**
   - Many of the more complex figures incorporate *mini-captions* to help students grasp concepts more quickly by drawing their focus to the body of an illustration rather than depending solely on a separate figure legend to describe what is taking place.
   - *Overview figures* outline complicated structures or processes in broad strokes and are followed by text and figures that present supporting details.
   - Carefully selected micrographs showing key cellular structures are accompanied by scale bars to indicate magnification.
3. **Important terminology is highlighted and defined in several ways.**
   - **Boldface type** is used to highlight the most important terms in each chapter, all of which are defined in the Glossary.

- *Italic type* is used to identify additional technical terms that are less important than boldfaced terms but significant in their own right. Occasionally, italics is also used to highlight important phrases or sentences.
- The Glossary includes definitions and page references for all boldfaced key terms and acronyms in every chapter—more than 1500 terms in all, a veritable dictionary of cell biology in its own right.

4. **Each chapter helps students learn the process of science, not just facts.**
   - Text discussions emphasize the experimental evidence that underlies our understanding of cell structure and function, to remind readers that advances in cell biology, as in all branches of science, come not from lecturers in their classrooms or textbook authors at their computers but from researchers in their laboratories.
   - The inclusion of a *Problem Set* at the end of each chapter reflects our conviction that we learn science not just by reading or hearing about it but by working with it. The problems are designed to emphasize understanding and application, rather than rote recall. These include highlighted questions that involve quantitative analysis and data analysis. Many are class-tested, having been selected from problem sets and exams we have used in our own courses. Detailed answers for all problems are available for students in the *Solutions Manual,* available on **Mastering Biology**.

Supplementary Learning Aids

Instructor Resources Area for *Becker's World of the Cell* (See Instructor Resource Area of Mastering Biology)

- PowerPoint lecture tools, including lecture outlines containing all of the figures, photos, and embedded animations, with five to ten personal response system clicker questions per chapter.
- JPEG images of all textbook figures and photos.
- Videos and animations of key concepts, organized by chapter for ease of use in the classroom.

Test Bank for *Becker's World of the Cell* (See Instructor Resource Area of Mastering Biology)

The test bank provides more than 1000 multiple-choice, short-answer, and inquiry/activity questions.

Solutions Manual for *Becker's World of the Cell* (See Instructor Resource Area of Mastering Biology)

Written by the authors, this manual includes complete, detailed answers for all of the Make Connections questions and end-of-chapter problems.

We Welcome Your Comments and Suggestions

The ultimate test of any textbook is how effectively it helps instructors teach and students learn. We welcome feedback and suggestions from readers and will try to acknowledge all correspondence. Please send your comments, criticisms, and suggestions to the appropriate authors listed here.

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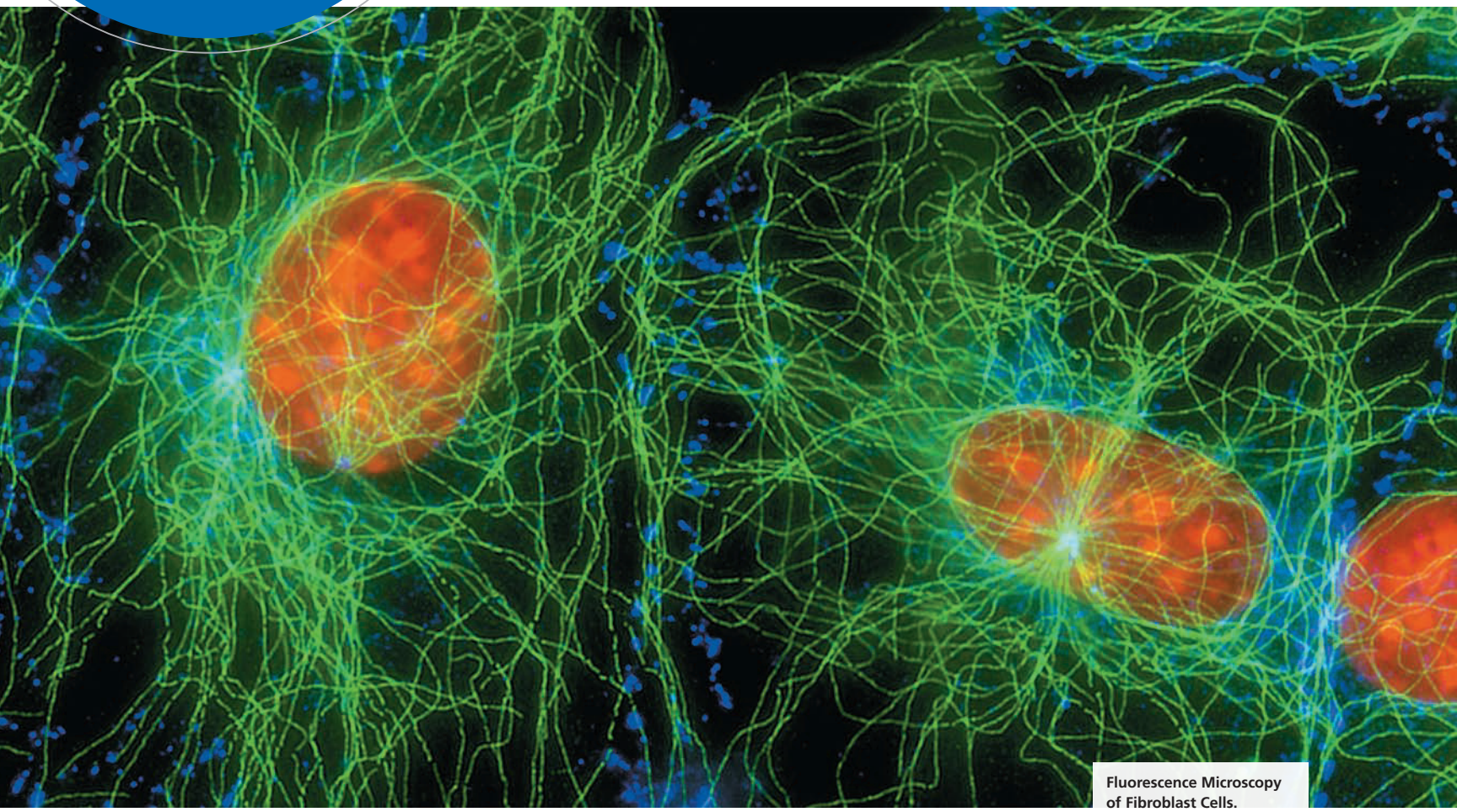
1 A Preview of Cell Biology



Fluorescence Microscopy of Fibroblast Cells. This image shows fluorescently labeled cell nuclei (red), microtubules (green), and cell-cell contacts (blue).

The **cell** is the basic unit of biology. Every organism either consists of cells or is itself a single cell. Therefore, it is only by understanding the structure and function of cells that we can appreciate both the capabilities and the limitations of living organisms, whether they are animals, plants, fungi, or microorganisms.

The field of cell biology is rapidly changing as scientists from a variety of related disciplines work together to gain a better understanding of how cells are constructed and how they carry out all the intricate functions necessary for life. Particularly significant is the dynamic nature of the cell. Cells are constantly changing; they have the capacity to grow, reproduce, and become specialized. In addition, once specialized, they have the ability to respond to stimuli and adapt to changes in the environment. The convergence of cytology, genetics, and biochemistry has made modern cell biology one of the most exciting and dynamic disciplines in all of biology. Nowhere is this excitement more evident than in the recent advances being made in our ability to modify genomes. If this text helps you appreciate the marvels and diversity of cellular

functions and allows you to experience the excitement of discovery, then one of our main goals in writing this book for you will have been met.

In this introductory chapter, we will look briefly at the origin of cell biology as a discipline. Then we will consider the three main historical strands of cytology, genetics, and biochemistry that have formed our current understanding of what cells are and how they work. The chapter concludes with a brief discussion of the nature of scientific knowledge itself by considering biological facts, the scientific method, experimental design, and the use of some common model organisms to answer important questions in modern cell biology.

1.1 The Cell Theory: A Brief History

The story of cell biology started to unfold more than 300 years ago, as European scientists began to focus their crude microscopes on a variety of biological material ranging from tree bark to bacteria to human sperm. One such scientist was Robert Hooke, Curator of Instruments for the Royal Society of London. In 1665, Hooke built a microscope and examined thin slices of cork (**Figure 1-1**). He observed and sketched a network of tiny boxlike compartments that reminded him of a honeycomb and called these little compartments *cells,* from the Latin word *cellula,* meaning "little room."

What Hooke observed were not cells at all. Those empty boxlike compartments were formed by the cell walls of dead plant tissue, which is what cork is. However, Hooke would not have thought of these cells as dead because he did not understand that they could be alive. Although he noticed that cells in other plant tissues were filled with what he called "juices," he concentrated instead on the more prominent cell walls of the dead cork cells that he had first encountered.

(a) Hooke's microscope

(b) Hooke's drawing of cork

Figure 1-1 The Birth of Microscopy. (a) Pictured is a reconstruction of Robert Hooke's original microscope, which he used to observe cork. **(b)** Hooke then sketched his observations.

Advances in Microscopy Allowed Detailed Studies of Cells

Hooke's observations were limited by the *magnification power* of his microscope, which enlarged objects to only 30 times (30×) their normal size. This made it difficult to learn much about the internal organization of cells. A few years later, Antonie van Leeuwenhoek, a Dutch textile merchant, produced small lenses that could magnify objects to almost 300 times (300×) their size. Using these superior lenses, van Leeuwenhoek became the first to observe living cells, including blood cells, sperm cells, bacteria, and single-celled organisms (algae and protozoa) found in pond water. He reported his observations to the Royal Society of London in a series of letters during the late 1600s. His detailed reports attest to both the high quality of his lenses and his keen powers of observation.

Two factors restricted further understanding of the nature of cells. First, the microscopes of the day had limited *resolution (resolving power)*—the ability to see fine details of structure. Even van Leeuwenhoek's superior instruments could push this limit only so far. The second factor was the descriptive nature of seventeenth-century biology. It was an age of observation, with little thought given to explaining the intriguing architectural details being discovered in biological materials.

More than a century passed before the combination of improved microscopes and more experimentally minded microscopists resulted in a series of developments that led to an understanding of the importance of cells in biological organization. By the 1830s, important optical improvements were made in lens quality and in the *compound microscope,* an instrument in which one lens (the eyepiece) magnifies the image created by a second lens (the objective). This allowed both higher magnification and better resolution. At that point, structures only 1 micrometer (μm) in size could be seen clearly.

The Cell Theory Applies to All Organisms

Aided by such improved lenses, the Scottish botanist Robert Brown found that every plant cell he looked at contained a rounded structure, which he called a *nucleus,* a term derived from the Latin word for "kernel." In 1838, his German colleague Matthias Schleiden came to the important conclusion that all plant tissues are composed of cells and that an embryonic plant always arises from a single cell. A year later, German cytologist Theodor Schwann reported similar conclusions concerning animal tissue, thereby discrediting earlier speculations that plants and animals do not resemble each other structurally. These speculations arose because plant cell walls form conspicuous boundaries between cells that are readily visible even with a crude microscope, whereas individual animal cells, which lack cell walls, are much harder to distinguish in a tissue sample. However, when Schwann examined animal cartilage cells, he saw that they were unlike most other animal cells because they have boundaries that are well defined by thick deposits of collagen fibers. Thus, he became convinced of the fundamental similarity between plant and animal tissue. Based on his astute observations, Schwann

developed a single unified theory of cellular organization. This theory has stood the test of time and continues to be the basis for our own understanding of the importance of cells and cell biology. (The recent discovery of certain giant viruses has led some to speculate that this definition may someday be expanded.)

As originally postulated by Schwann in 1839, the **cell theory** had two basic principles:

1. All organisms consist of one or more cells.
2. The cell is the basic unit of structure for all organisms.

Less than 20 years later, a third principle was added. This grew out of Brown's original description of nuclei, which Swiss botanist Karl Nägeli extended to include observations on the nature of cell division. By 1855 Rudolf Virchow, a German physiologist, concluded that cells arose only by the division of other, preexisting cells. Virchow encapsulated this conclusion in the now-famous Latin phrase *omnis cellula e cellula,* which in translation becomes the third principle of the modern cell theory:

3. All cells arise only from preexisting cells.

Thus, the cell is not only the basic unit of structure for all organisms but also the basic unit of reproduction. No wonder, then, that we must understand cells and their properties to appreciate all other aspects of biology. Because many of you have seen examples of "typical" cells in textbooks that may give the false impression that there are relatively few different types of cells, let's take a look at a few examples of the diversity of cells that exist in our world (**Figure 1-2**).

Cells exist in a wide variety of shapes and sizes, ranging from filamentous fungal cells to spiral-shaped *Treponema* bacteria to the differently shaped cells of the human blood system (Figure 1-2a–c). Other cells have much more exotic shapes,

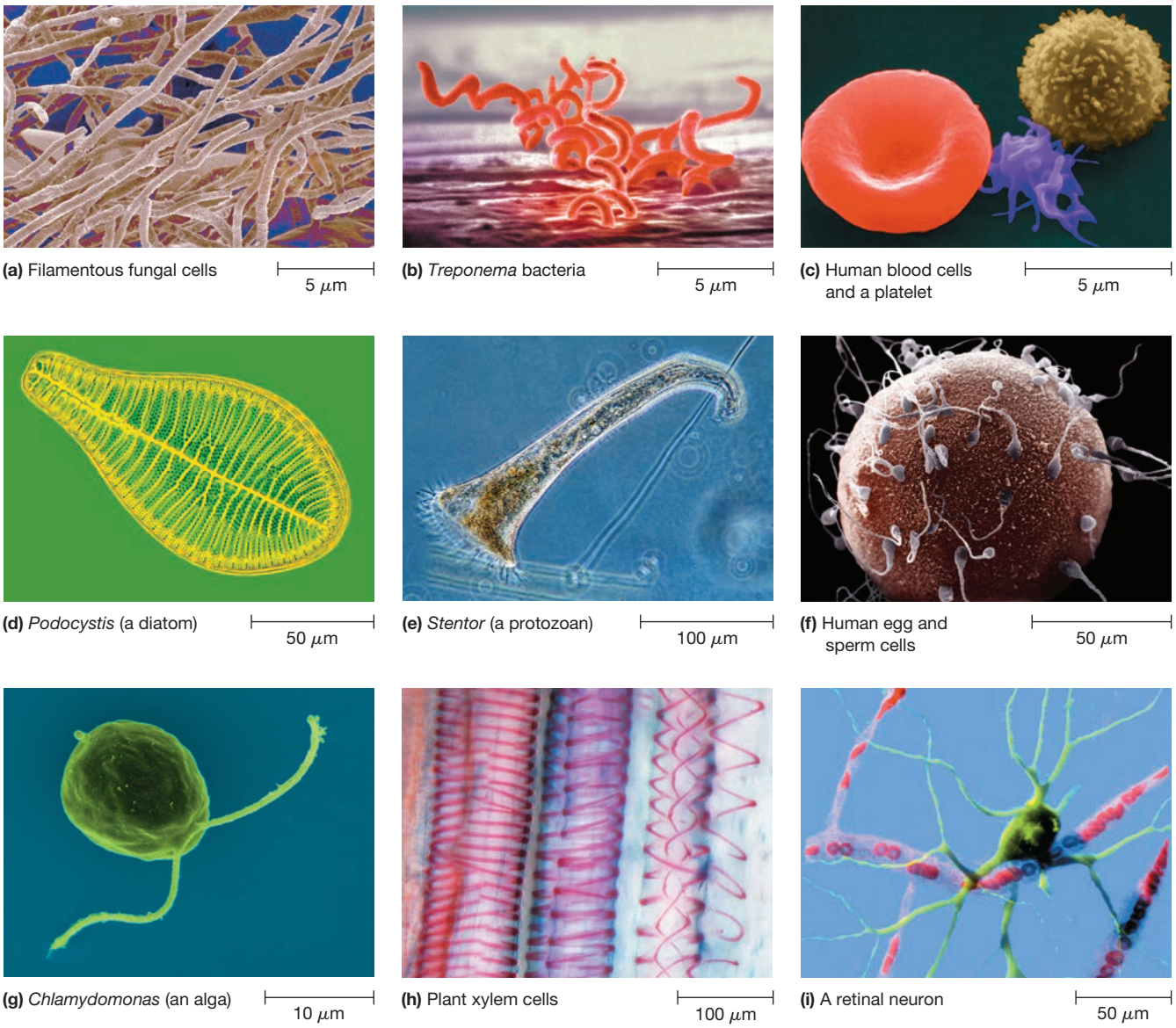


Figure 1-2 The Cells of the World. The diversity of cell types existing all around us includes the examples shown in this figure and thousands upon thousands more.

such as the diatom and the protozoan shown in Figure 1-2d and 1-2e. Note how the two human single-celled gametes, the egg and the sperm, differ greatly in size and shape (Figure 1-2f). As in leaves, the green chlorophyll in a *Chlamydomonas* cell shows that these algae carry out photosynthesis (Figure 1-2g). Often, a cell's shape and structure give clues about its function. For example, the spiral thickenings in the cell walls of plant xylem tissue give strength to these water-conducting vessels in wood (Figure 1-2h), and the highly branched cells of a human neuron allow it to interact with numerous other neurons (Figure 1-2i). In our studies throughout this textbook, we will see many other interesting examples of diversity in cell structure and function. First, though, let's examine the historical roots leading to the development of contemporary cell biology.

CONCEPT CHECK 1.1

What evidence led scientists to develop the basic principles of the cell theory? Note how technology played a role in its development.

1.2 The Emergence of Modern Cell Biology

Modern cell biology results from the weaving together of three different strands of biological inquiry—cytology, biochemistry, and genetics—into a single cord. As the timeline in **Figure 1-3** illustrates, each of the strands had its own historical origins, and each one makes unique and significant contributions to modern cell biology. Contemporary cell biologists must be adequately informed about all three strands, regardless of their own immediate interests.

Historically, the first of the strands to emerge was **cytology**, which is concerned primarily with cellular structure. In biological studies, you will often encounter words containing the Greek prefix *cyto–* or the suffix *–cyte,* both of which mean "hollow vessel" and refer to cells. Cytology had its origins more than three centuries ago and depended heavily on the light microscope for its initial impetus. The advent of electron microscopy and other advanced optical techniques has dramatically increased our understanding of cell structure and function.

The second strand represents the contributions of **biochemistry** to our understanding of cellular structure and function. Most of the developments in this field have occurred over the past 95 years, though the roots go back at least a century earlier. Especially important has been the development of laboratory techniques such as ultracentrifugation, chromatography, radioactive labeling, electrophoresis, and mass spectrometry for separating and identifying cellular components. You will encounter these and other techniques later in your studies as you learn how specific details of cellular structure and function were discovered using these techniques.

The third strand contributing to the development of modern cell biology is **genetics**. Although the timeline for genetics stretches back more than 150 years, most of our present understanding has been gained within the past 75 years. An especially important discovery was the demonstration that, in all organisms, DNA (deoxyribonucleic acid) is the bearer of genetic information. It encodes the tremendous variety of proteins and RNA (ribonucleic acid) molecules responsible for most of the functional and structural features of cells. Recent accomplishments on the genetic strand include the sequencing of the entire **genome** (all of the DNA) of humans and other species, the *cloning* (production of genetically identical organisms) of mammals, including livestock, pets, and primates, and the editing of genomes.

Therefore, an understanding of present-day cell biology requires an appreciation of its diverse roots and the important contributions made by each of its component strands to our current understanding of what a cell is and what it can do. Each of the three historical strands of cell biology is discussed briefly here; a deeper appreciation of these historical strands will come in later chapters as we explore cells in detail. Keep in mind also that in addition to developments in cytology, biochemistry, and genetics, the field of cell biology has benefited greatly from advancements in other fields of study such as chemistry, physics, computer science, and engineering.

The Cytological Strand Deals with Cellular Structure

Strictly speaking, cytology is the study of cells. Historically, however, cytology has dealt primarily with cellular structure, mainly through the use of optical techniques. Here we will describe briefly some of the microscopy that is important in cell biology. (For more detailed discussion of microscopic techniques, see the Appendix.) Microscopy has been invaluable in helping cell biologists overcome a fundamental problem—the problem of small size.

Cellular Dimensions. One challenge involved in understanding cellular structure and organization is the fact that most cells and their organelles are too small to be seen by the unaided eye. The cellular structures that microscopists routinely deal with are measured using units that may not be familiar to you.

The **micrometer** (μm) is the most useful unit for expressing the size of cells and organelles (**Figure 1-4**, on page 6). A micrometer (historically called a *micron*) is one-millionth of a meter (10^{-6} m). One inch equals approximately 25,000 μm. In general, bacterial cells are a few micrometers in diameter, and the cells of plants and animals are 10 to 20 times larger. Organelles such as mitochondria and chloroplasts tend to be a few micrometers in size and are thus comparable in size to whole bacterial cells. In general, if you can see it with a light microscope, you can express its dimensions conveniently in micrometers (Figure 1-4a).

The **nanometer** (nm) is the unit of choice for molecules and subcellular structures that are too small to be seen using the light microscope. A nanometer is one-billionth of a meter (10^{-9} m), so it takes 1000 nanometers to equal 1 micrometer. A ribosome has a diameter of about 25 to 30 nm. Other structures that can be measured conveniently in nanometers are cell membranes, microtubules, microfilaments, and DNA molecules (Figure 1-4b). A slightly smaller unit, the angstrom (Å), is used in cell biology when measuring dimensions within proteins and DNA molecules. An angstrom equals 0.1 nm, which is about the size of a hydrogen atom.

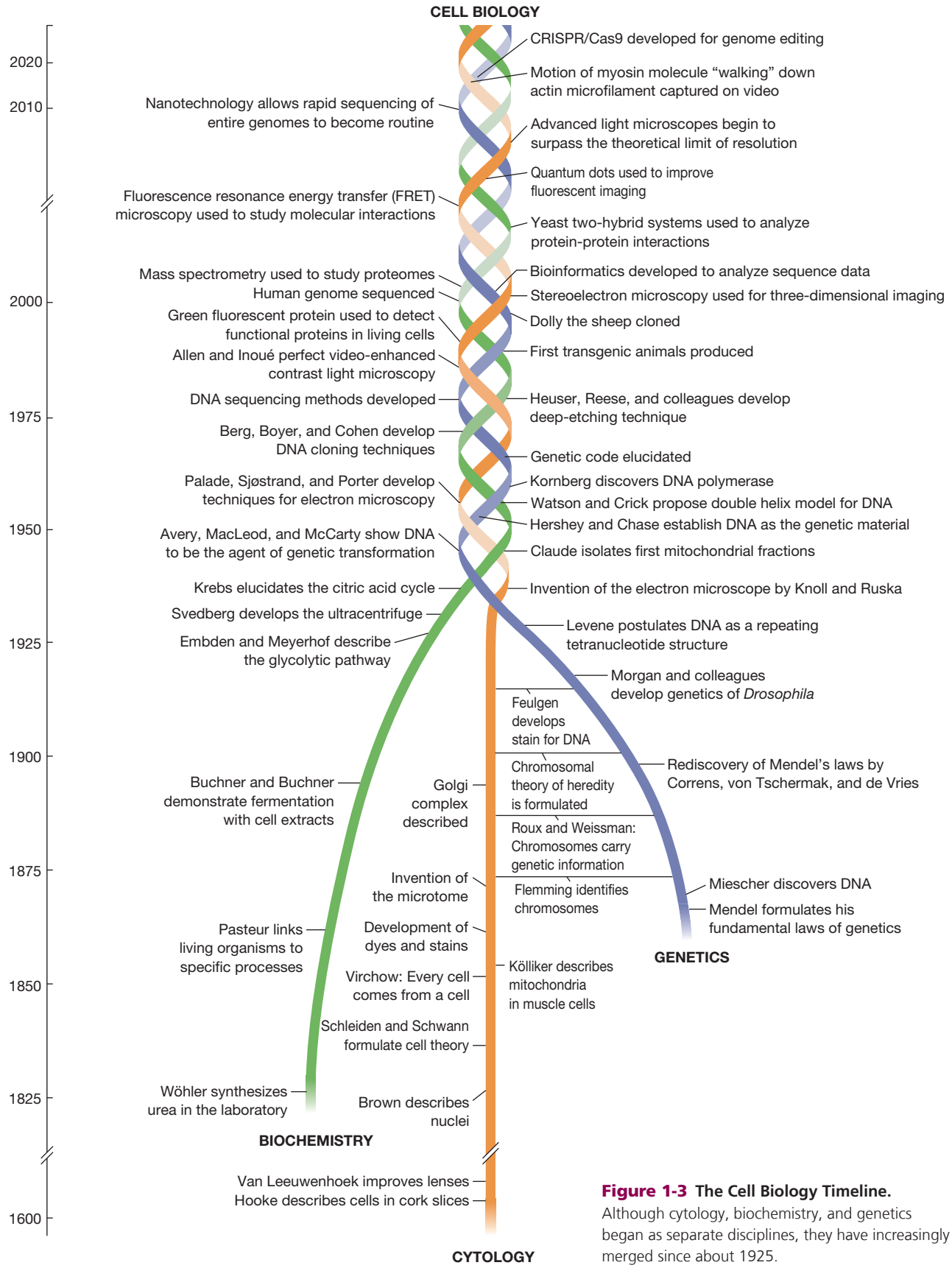


Figure 1-3 The Cell Biology Timeline.
Although cytology, biochemistry, and genetics began as separate disciplines, they have increasingly merged since about 1925.

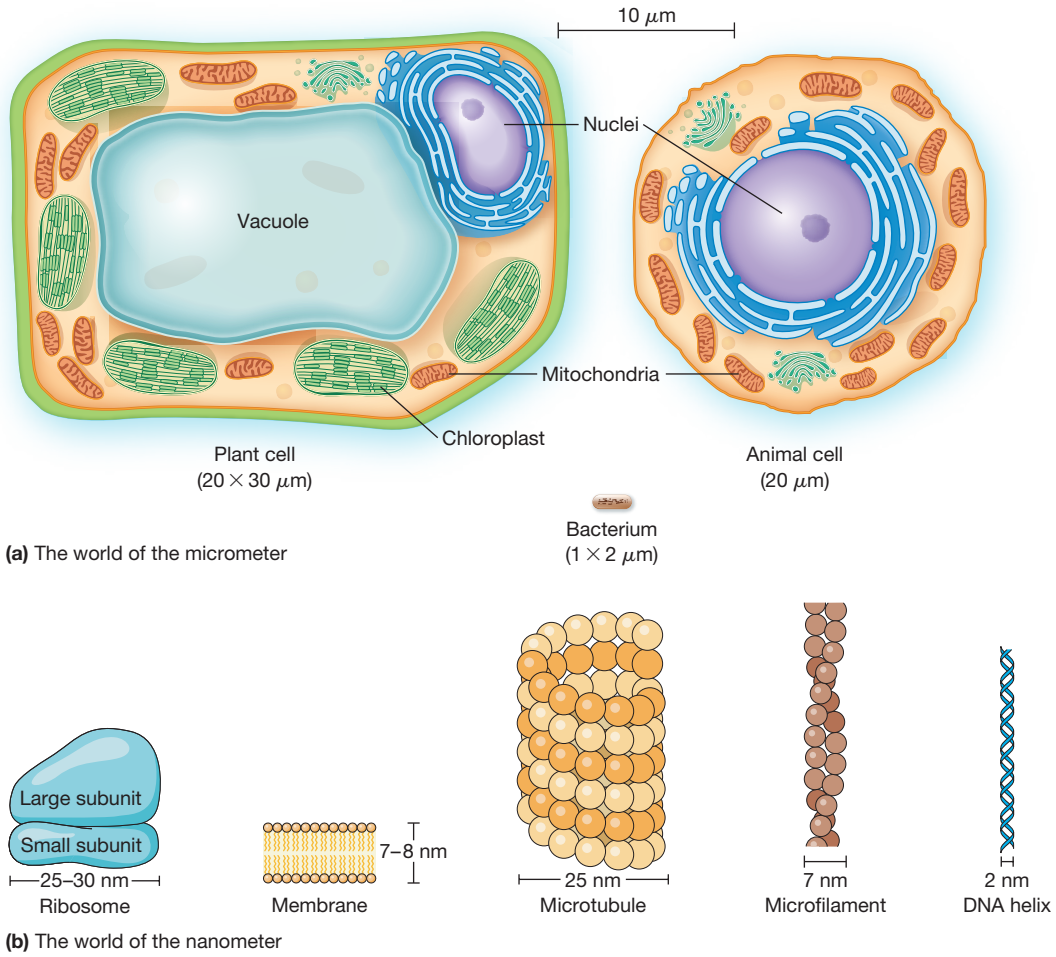


Figure 1-4 The Worlds of the Micrometer and Nanometer. Illustrations show **(a)** typical cells and **(b)** common cellular structures.

Microscopy. The most important technique within the cytological strand is microscopy. This technique allows scientists to visualize cells and cellular components at the previously mentioned cellular dimensions. Depending on the level of resolution required, the two major forms of microscopy used are light microscopy and electron microscopy.

The **light microscope** was the earliest tool of the cytologists and continues to play an important role in our elucidation of cellular structure. Light microscopy allowed cytologists to identify membrane-bounded structures such as *nuclei, mitochondria,* and *chloroplasts* within a variety of cell types. Such structures are called *organelles* ("little organs") and are prominent features of most plant and animal (but not bacterial) cells. (Chapter 4 presents an overview of organelle types, and later chapters investigate their structure and function in more detail.)

The basic type of light microscopy is called *brightfield microscopy* because white light is passed directly through a specimen that is either stained or unstained and the background (the field) is illuminated. A significant limitation of this approach is that specimens often must be chemically fixed (preserved), dehydrated, embedded in paraffin or plastic for slicing into thin sections, and stained to highlight otherwise transparent features. Fixed and stained specimens are no longer alive; therefore, features observed using this method could be distortions caused by slide preparation processes and might not be typical of living cells.

To overcome the limitations of a brightfield microscope, a variety of specialized light microscopes have been developed for observing living cells directly. These techniques include phase-contrast microscopy, differential interference contrast microscopy, fluorescence microscopy, and confocal microscopy. Each of these forms of microscopy is introduced below. (More detail on these techniques, including sample images using them, can be found in the Appendix.)

Phase-contrast and *differential interference contrast* microscopy make it possible to see living cells clearly. Like water waves, light waves have crests and troughs, and the precise positions of these maxima and minima as light travels are known as the *phase* of the light. Both techniques enhance and amplify slight changes in the phase of transmitted light as it passes through a structure having a different density than the surrounding medium.

Fluorescence microscopy is a powerful method that enables researchers to detect specific proteins, DNA sequences, or other molecules that are made fluorescent by coupling them to a fluorescent dye or a fluorescent protein or by binding them to a fluorescently labeled antibody. An **antibody** is a protein molecule produced by the immune system that binds one particular target molecule, known as its antigen.

By simultaneously using two or more such dyes or antibodies, each emitting light of a different color, researchers can follow the distributions of different kinds of molecules in the same cell. Antibody labeling is a powerful method to both visualize and identify specific molecules within cells and is described in more detail (**see Key Technique, pages 8–9**). In recent years, green fluorescent protein (GFP) from the bioluminescent jellyfish *Aequorea victoria* has become an invaluable tool for studying the temporal and spatial distribution of particular proteins in a cell. When a protein of interest is fused with GFP, its synthesis and movement can be followed in living cells using a fluorescence microscope.

An inherent limitation of fluorescence microscopy is that the viewer can focus on only a single plane of the specimen at a time, yet fluorescent light is emitted throughout the specimen, blurring the image. This problem is largely overcome by *confocal microscopy*, which uses a laser beam to illuminate just one plane of the specimen at a time. When used with thick specimens such as whole cells, this approach gives much better resolution.

Another recent development in light microscopy is *digital video microscopy*, which allows researchers to observe cells for extended periods of time using very low levels of light. This image intensification is particularly useful to visualize fluorescent molecules present at low levels in living cells and even to see and identify individual *macromolecules* such as DNA and protein molecules. In fact, extremely powerful *superresolution* light microscopy methods have been developed that use imaging and computational methods so advanced that they can visualize structures 50–100 nm in size, which, until the past few years, were believed impossible to see with any light microscope. However, despite recent significant advances, light microscopy is inevitably subject to the limit of resolution imposed by the wavelength of the light used to view the sample.

As used in microscopy, the **limit of resolution** refers to how far apart adjacent objects must be to appear as separate entities. For example, if the limit of resolution of a microscope is 400 nm, objects must be at least 400 nm apart to be recognizable as separate entities. The smaller the limit of resolution, the greater the **resolving power**, or ability to see fine details of structure, of the microscope. Therefore, a better microscope might have a resolution of 200 nm, meaning that objects only 200 nm apart can be distinguished from each other.

Because of the physical nature of light itself, the theoretical limit of resolution for the light microscope is approximately half the size of the wavelength of light used for illumination, allowing maximum magnifications of about 1000–1400×. For *visible light* (wavelengths of 400–700 nm), the limit of resolution is about 200–350 nm. **Figure 1-5** illustrates the useful range of the light microscope and compares its resolving power with that of the human eye and the electron microscope.

A major breakthrough in resolving power came with the development of the **electron microscope**, which was invented in Germany in 1931 by Max Knoll and Ernst Ruska. In place of visible light and optical lenses, the electron microscope uses a beam of electrons that is deflected and focused by an electromagnetic field. Because the wavelength of electrons is so much shorter than the wavelengths of visible light, the practical limit of resolution for the electron microscope is much better—generally about 100 times better than a light microscope, or 2 nm (see Figure 1-5). As a result, the useful magnification of the electron microscope is also much higher—up to 100,000×.

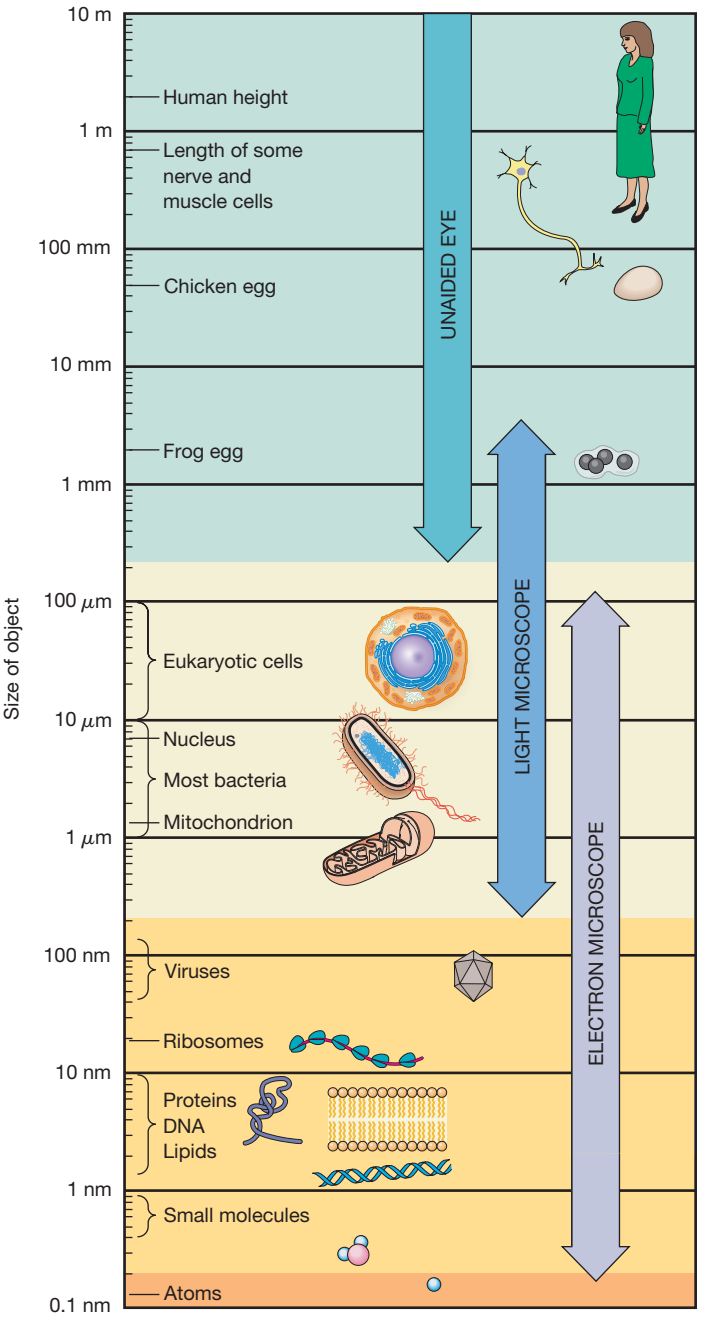


Figure 1-5 Relative Resolving Power of the Human Eye, the Light Microscope, and the Electron Microscope. Notice that the vertical axis is on a logarithmic scale to accommodate the wide range of sizes shown (based on powers of 10).

Electron microscopy continues to revolutionize our understanding of cellular architecture by making detailed ultrastructural investigations possible. Whereas organelles such as nuclei or mitochondria are large enough to be seen with a light microscope, they can be studied in much greater detail with an electron microscope. In addition, electron microscopy has revealed cellular structures that are too small to be seen with a light microscope. These include ribosomes, cell membranes,

Key Technique

Using Immunofluorescence to Identify Specific Cell Components

PROBLEM: Cells are made of thousands of different types of molecules that make up a wide variety of cellular structures. With so many different molecules present, how can researchers determine the presence and location of one specific type of molecule within a cell?

SOLUTION: *Immunofluorescence* is a technique in which a fluorescent molecule is attached to an *antibody,* which recognizes and binds to one specific complementary target molecule, known as its *antigen*. Using a fluorescence or confocal microscope, a researcher can then identify and locate the specific target molecule within the cell.

Key Tools: Fluorescence or confocal microscope; antibodies labeled with a fluorescent dye.

Details: One of the amazing features of animals is the ability of their immune systems to recognize and neutralize a wide variety of potential pathogens. In vertebrates, certain white blood cells, known as *B lymphocytes,* secrete antibodies into the bloodstream, and each different antibody recognizes one specific type of antigen, targeting it for destruction by other white blood cells. An antibody is a protein that has a constant region (C) that is the same for all antibodies of a particular type and variable regions (V) that are identical to each other but unique for each antibody (**Figure 1A-1**). The unique V regions at the tips of the Y contain a binding pocket into which only one specific antigen will fit.

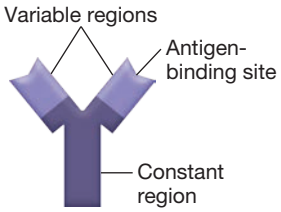


Figure 1A-1 Antibody Structure.

Immunofluorescence exploits the specificity of antibodies for their antigen targets. Rather than targeting antigens for destruction, however, immunofluorescence is used to detect where the antigen is located within a cell. Antibodies can be generated in the laboratory by injecting a foreign protein or other macromolecule into an animal host, such as a rabbit or mouse, producing antibodies that will bind selectively to virtually any protein that a scientist wishes to study. Using *primary* (or *direct*) *immunofluorescence,* antibody molecules are labeled with a fluorescent dye, known as a *fluorophore,* that is covalently linked to the C region of each antibody molecule (**Figure 1A-2**). The antibody recognizes and binds to the target molecule, which can then be detected using fluorescence or confocal microscopy.

More commonly, researchers use *secondary* (or *indirect*) *immunofluorescence.* In this case, a tissue or cell is treated with an antibody that is not labeled with dye (**Figure 1A-3**). This antibody, called the *primary antibody,* attaches to specific antigenic sites within the tissue or cell. A second type of antibody, called the *secondary antibody,* is labeled with a fluorescent dye and then added to the sample, where it attaches to the primary antibody. Because more than one primary antibody molecule can attach to an antigen and more than one secondary antibody molecule can

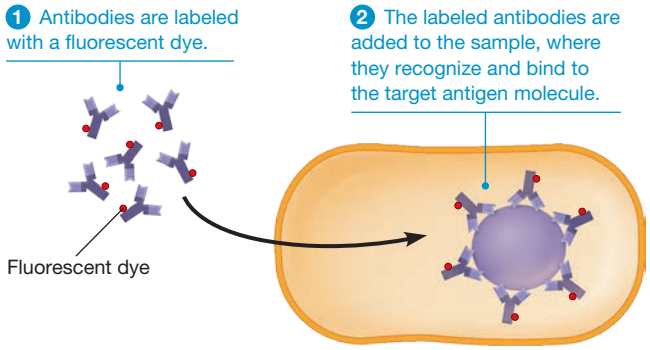


Figure 1A-2 Primary Immunofluorescence. In primary immunofluorescence, an antibody that binds to a specific antigen in a tissue or cell is labeled with a fluorescent dye. The labeled antibody is then added to the sample, where it binds to its target molecule. The pattern of fluorescence that results is visualized using fluorescence or confocal microscopy.

microtubules, and microfilaments (see Figure 1-4b), as well as some *macromolecules* such as DNA and protein molecules.

Most electron microscopes have one of two basic designs: the **transmission electron microscope (TEM)** and the **scanning electron microscope (SEM)**. Images from each are shown in **Figure 1-6** on page 10. Transmission and scanning electron microscopes are similar in that each employs a beam of electrons, but they use quite different mechanisms to form the image. As the name implies, a TEM forms an image from electrons that are transmitted through the specimen. An SEM, on the other hand, scans the surface of the specimen and forms an image by detecting electrons that are deflected from its outer surface. Scanning electron microscopy is an especially spectacular technique because of the sense of depth it gives to biological structures.

Electron microscopy is constantly evolving. Several specialized techniques for electron microscopy allow visualization of specimens in three dimensions and can determine structures of some macromolecules such as proteins. Still other techniques combine some of the principles of TEM and SEM and even allow visualization of cells in liquid without the need for a vacuum. (All of these microscopy techniques are described in detail in the Appendix.)

The Biochemical Strand Concerns the Chemistry of Biological Structure and Function

At about the same time that cytologists started exploring cellular structure with their microscopes, other scientists were making observations that began to explain and clarify cellular function. Using techniques derived from classical chemistry,

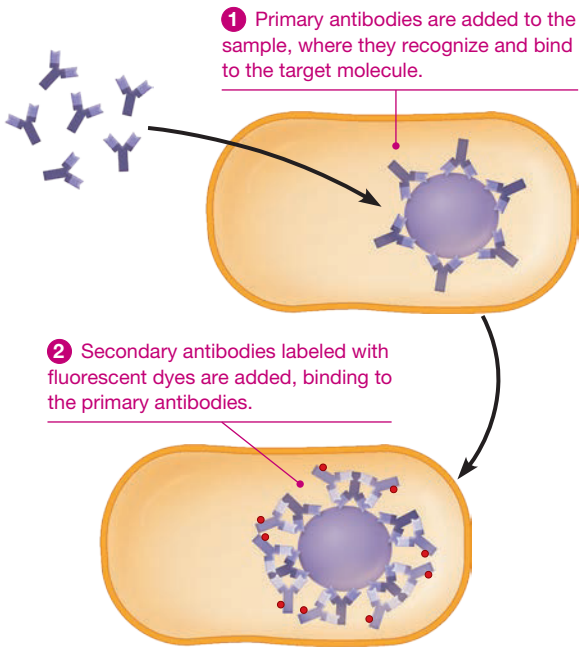


Figure 1A-3 Secondary Immunofluorescence. In secondary immunofluorescence, a primary antibody is added to a tissue or cell. A secondary antibody that is labeled with a fluorescent dye is then added to the sample, where it binds to the primary antibody, amplifying the fluorescence signal.

attach to each primary antibody, more fluorescent molecules are concentrated near the target molecule. As a result, indirect immunofluorescence amplifies the fluorescence signal and is much more sensitive than the use of a primary antibody alone.

Antibodies that recognize any one of thousands of specific antigens are commercially available. By using different combinations of antibodies and dyes, more than one molecule in a cell can be labeled at the same time. Different dyes can be imaged using

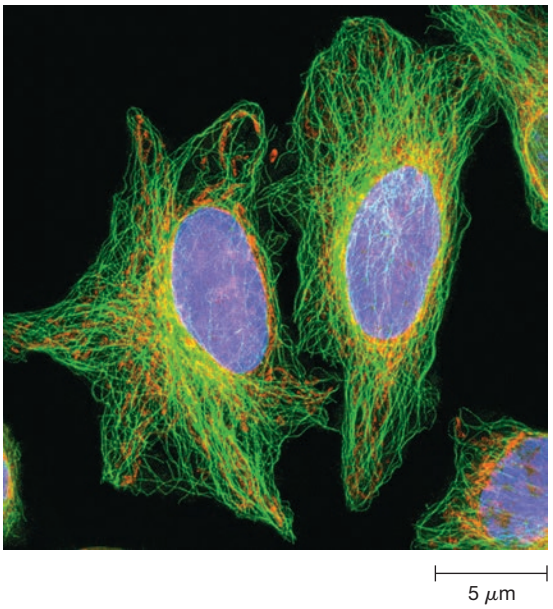


Figure 1A-4 Labeling Cells Using Two Different Colors. HeLa cells were stained using indirect immunofluorescence for microtubules (green) and a dye to label DNA (purple).

different combinations of fluorescent filters, and the different images can be combined to generate striking pictures of cellular structures (**Figure 1A-4**). In some cases, instead of a fluorescent dye, antibodies can be linked to an enzyme performing a chemical reaction, resulting in a colored precipitation product that can be seen using a standard microscope.

QUESTION: Why is a high degree of antibody-antigen specificity so important for identification of cellular components?

these scientists began to understand the structure and function of biological molecules, launching a field that came to be known as biochemistry.

Biochemical Reactions and Pathways. Much of what is now called biochemistry dates from a discovery reported in 1828 by German chemist Friedrich Wöhler, a contemporary and fellow countryman of Schleiden and Schwann. Wöhler revolutionized our thinking about biology and chemistry by demonstrating that urea, an organic compound of biological origin, could be synthesized in the laboratory from an inorganic starting material, ammonium cyanate.

Until Wöhler reported his results, it had been widely held that living organisms were unique, not governed by the laws of chemistry and physics that apply to the nonliving world. By showing that a compound made by living organisms—a "biochemical"—could be synthesized in a laboratory just like any other chemical, Wöhler helped dispel the notion that biochemical processes were somehow exempt from the laws of chemistry and physics.

Another major advance came about 30 years later, when French chemist and biologist Louis Pasteur showed that living yeast cells were responsible for the fermentation of sugar into alcohol. In 1897, German bacteriologists Eduard and Hans Buchner found that fermentation could take place with isolated extracts from yeast cells—that is, the intact cells themselves were not required. Gradually, it became clear that the active agents in the extracts were specific biological catalysts that have since come to be called **enzymes**—from *zyme*, a Greek word meaning "yeast."

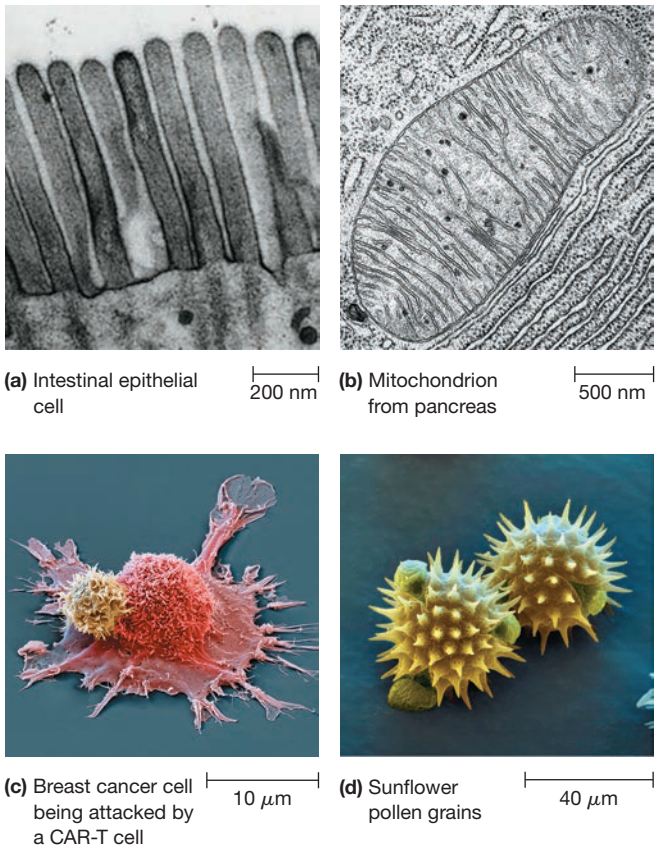


Figure 1-6 Electron Microscopy. A transmission electron microscope was used to produce the images in parts **(a)** and **(b)** (TEMs). A scanning electron microscope was used to make the images in parts **(c)** and **(d)** (colorized SEMs).

In the 1920s and 1930s, individual steps in the complex, multistep biochemical pathways for fermentation and related cellular processes were elucidated. German biochemists, such as Gustav Embden, Otto Meyerhof, Otto Warburg, and Hans Krebs, described the enzymatic steps in the Embden–Meyerhof pathway for *glycolysis* for glucose breakdown and the *Krebs cycle* for energy production. Both of these pathways are important because of their role in the process by which cells extract energy from glucose and other foodstuffs. (For a detailed examination of these biochemical pathways, see Chapters 9 and 10.) At about the same time, Fritz Lipmann, an American biochemist, showed that the high-energy compound *adenosine triphosphate (ATP)* is the principal energy storage compound in most cells.

An important advance in the study of biochemical reactions and pathways came as radioactive isotopes such as ^{3}H, ^{14}C, and ^{32}P were first used to trace the metabolic fate of specific atoms and molecules. American chemist Melvin Calvin and his colleagues at the University of California, Berkeley, were pioneers in this field as they traced the fate of ^{14}C-labeled carbon dioxide ($^{14}CO_2$) in illuminated algal cells that were actively photosynthesizing. Their work, carried out in the late 1940s and early 1950s, led to the elucidation of the *Calvin cycle*—the most common pathway for photosynthetic carbon metabolism. The Calvin cycle was the first metabolic pathway to be elucidated using a radioisotope.

Biochemistry Methods. Biochemistry took another major step forward with the development of techniques for isolating, purifying, and analyzing subcomponents of cells.

Centrifugation is a means of separating and isolating subcellular structures and macromolecules based on their size, shape, and/or density—a process called **subcellular fractionation**. This process helps us study specific parts of the cell, such as the nucleus or specific proteins.

Especially useful for resolving small organelles and macromolecules is the **ultracentrifuge**, developed by Swedish chemist Theodor Svedberg in the late 1920s. An ultracentrifuge is capable of very high speeds—more than 100,000 revolutions per minute—and can thereby subject samples to forces exceeding 500,000 times the force of gravity. In many ways, the ultracentrifuge is as significant to biochemistry as the electron microscope is to cytology. In fact, both instruments were developed at about the same time, so the ability to see organelles and other subcellular structures coincided with the capability to isolate and purify them.

Other biochemical techniques that have proven useful for separating and purifying subcellular components include chromatography and electrophoresis. **Chromatography** is a general term describing a variety of techniques by which a mixture of molecules in solution is separated into individual components. Chromatographic techniques separate molecules based on their size, charge, or affinity for specific molecules or functional groups (**Figure 1-7**). In fact, this technique gets its name from its early use in separating differently colored plant pigments, as shown in part a.

Electrophoresis refers to several related techniques that use an electrical field to separate macromolecules based on their mobility through a semisolid gel. Different molecules move at different rates depending on their size and charge.

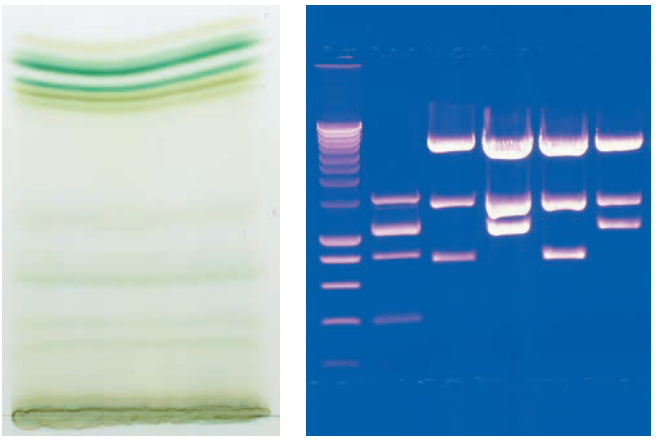


Figure 1-7 Separation of Molecules by Chromatography and Electrophoresis. (a) Filter paper is used as the stationary medium in this chromatogram of plant pigments. As the solvent containing the pigments moves across the filter, different pigments move at different rates and can be separated and then purified. **(b)** An agarose gel containing samples of mixed DNA molecules is separated by electrophoresis, stained with ethidium bromide, and illuminated with ultraviolet light to show each DNA fragment as a visible band.

Electrophoresis is used extensively to isolate and characterize DNA, RNA, and protein molecules. An example of separation of different DNA fragments using gel electrophoresis is shown in Figure 1-7b. After proteins have been separated by electrophoresis, **mass spectrometry** is commonly used to determine the size and composition of individual proteins. This technique allows researchers to determine the identity and characteristics of individual proteins.

To summarize, with the enhanced ability to see subcellular structures, to fractionate them, and to isolate them, cytologists and biochemists began to realize how well their respective observations on cellular structure and function could complement each other. These scientists were laying the foundations for modern cell biology.

The Genetic Strand Focuses on Information Flow

The third strand in the historical cord of cell biology is genetics, the study of the inheritance of characteristics from generation to generation. Although more than 2000 years ago the Greek philosopher Aristotle referred to a physical entity he called the "germ" and stated that it "springs forth from a definite parent and gives rise to a predictable progeny," it was not until the nineteenth century that scientists discovered the nature of these inherited physical entities, now known as genes.

Classical Genetics. The genetic strand begins with Gregor Mendel, whose studies with the pea plants he grew in a monastery garden must surely rank among the most famous experiments in all of biology. His findings were published in 1866, laying out the principles of segregation and independent assortment of the "hereditary factors" known today as **genes**. But Mendel was clearly a man ahead of his time. His work went almost unnoticed when it was first published and was not fully appreciated until its rediscovery nearly 35 years later.

In the decade following publication of Mendel's work, the role of the nucleus in the genetic continuity of cells came to be appreciated. In 1880, German biologist Walther Flemming identified **chromosomes**, threadlike bodies seen in dividing cells. Flemming called the division process *mitosis,* from the Greek word for "thread." Chromosome number soon came to be recognized as a distinctive characteristic of a species and was shown to remain constant from generation to generation. That the chromosomes themselves might be the actual bearers of genetic information was suggested by German anatomist Wilhelm Roux as early as 1883 and expressed more formally by his countryman, biologist August Weissman, shortly thereafter.

With the roles of the nucleus and chromosomes established and appreciated, the stage was set for the rediscovery of Mendel's initial observations. This came in 1900, when three plant geneticists working independently cited his studies almost simultaneously: Carl Correns in Germany, Ernst von Tschermak in Austria, and Hugo de Vries in Holland. Within three years, the **chromosome theory of heredity** was formulated, following work by American physician Walter Sutton and German biologist Theodor Boveri. The chromosome theory of heredity proposed that the hereditary factors responsible for Mendelian inheritance are located on the chromosomes within the nucleus. This hypothesis received its strongest confirmation from the work of American biologist Thomas Hunt Morgan and his students, Calvin Bridges and Alfred Sturtevant, at Columbia University during the first two decades of the twentieth century. Using *Drosophila melanogaster,* the common fruit fly, as their experimental model organism, they identified a variety of morphological mutants of *Drosophila* and were able to link specific traits to specific chromosomes.

Meanwhile, the foundation for our understanding of the chemical basis of inheritance was slowly being laid. An important milestone was the discovery of DNA by Swiss biologist Johann Friedrich Miescher in 1869. Using such unlikely sources as salmon sperm and human pus from surgical bandages, Miescher isolated and described what he called "nuclein." But, like Mendel, Miescher was ahead of his time. It would be about 75 years before the role of his nuclein as the genetic information of the cell came to be fully appreciated.

As early as 1914, DNA was implicated as an important component of chromosomes by the German chemist Robert Feulgen's staining technique, a method to identify DNA that is still in use today. But it was considered quite unlikely that DNA could be the bearer of genetic information due to its apparently monotonous structure. By 1930, DNA was known to be composed of only four different nucleotides—and this did not seem to be enough variety to account for all the diversity seen in living organisms. Proteins, on the other hand, were much more diverse, being composed of 20 different amino acids. In fact, until the middle of the twentieth century, it was widely thought that proteins were the carriers of genetic information from generation to generation because they seemed to be the only nuclear components with enough variety to account for the obvious diversity of genes.

A landmark experiment clearly pointing to DNA as the genetic material was reported in 1944 by Canadian scientists Oswald Avery and Colin MacLeod and American scientist Maclyn McCarty, working together at Rockefeller University. Their work (discussed in more detail in Chapter 16) showed that DNA could "transform" a nonpathogenic strain of bacteria into a pathogenic strain, causing a heritable genetic change. Eight years later, American biochemists Alfred Hershey and Martha Chase showed that DNA, and not protein, enters a bacterial cell when it is infected and genetically altered by a bacterial virus. Meanwhile, American biologists George Beadle and Edward Tatum, working in the 1940s with the bread mold *Neurospora crassa,* formulated the "one gene–one enzyme" concept, asserting that each gene controls the production of a single, specific protein.

Molecular Genetics. Shortly thereafter, in 1953, the unlikely team of former ornithology student James Watson and physicist Francis Crick, using images provided by X-ray crystallographer Rosalind Franklin, proposed their now-famous *double helix model* for the structure of DNA, which immediately suggested how replication during cell division could occur by precise base pairing between complementary strands. The 1960s brought more significant developments, including the discovery of the polymerase enzymes that synthesize DNA and RNA and the "cracking" of the genetic code, which

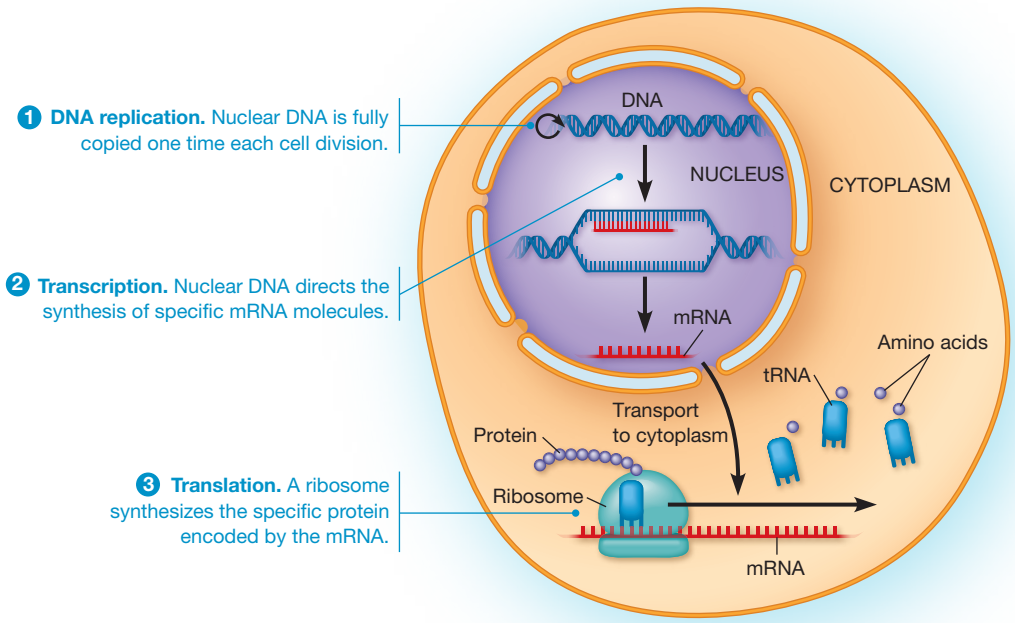


Figure 1-8 Central Dogma: The Flow of Genetic Information in the Cell. In eukaryotes, most of the DNA in a cell is located in the nucleus. 1 This DNA is copied each time the cell replicates. 2 DNA also contains instructions for the synthesis of a complementary messenger RNA (mRNA) in the process of transcription. 3 The mRNA then travels to the cytoplasm, where it is used by ribosomes to synthesize protein in the process of translation.

specifies the relationship between the order of nucleotides in a DNA or RNA molecule and the order of amino acids in a protein. At about the same time, biochemist Jacques Monod and geneticist François Jacob of France deduced the mechanism responsible for regulating bacterial gene expression.

Soon after the double-helical model of DNA was proposed in 1953, Francis Crick articulated a molecularly based model of genetic information flow, which he christened the *central dogma of molecular biology*. The steps shown in **Figure 1-8** summarize this model. Notice how the flow of genetic information involves *replication* of DNA to produce two identical copies, *transcription* of information carried by DNA into the form of RNA, and *translation* of this information from RNA into protein. The term *transcription* refers to RNA synthesis using DNA as a template to emphasize that this phase of gene expression is simply a transfer of information from one nucleic acid to another, so the basic "language" remains the same. In contrast, protein synthesis is called *translation* because it involves a language change—from the nucleotide sequence of an RNA molecule to the amino acid sequence of a polypeptide chain.

The discovery that three different kinds of RNA molecules serve as intermediates in protein synthesis completed our basic understanding of how the central dogma operates in cells (Figure 1-8). RNA that is translated into protein is called *messenger RNA (mRNA)* because it carries a genetic message from DNA to macromolecular complexes known as ribosomes, where protein synthesis actually takes place. *Ribosomal RNA (rRNA)* molecules are integral components of the ribosome itself. *Transfer RNA (tRNA)* molecules serve as intermediaries that recognize the coded base sequence of an mRNA and bring the appropriate amino acids to the ribosome for protein synthesis.

In the years since it was first formulated by Crick, the central dogma has been refined in various ways. For example, many viruses with RNA genomes have been found to synthesize mRNA molecules using RNA as a template. Other RNA viruses, such as HIV, carry out reverse transcription, whereby the viral RNA is used as a template for DNA synthesis—a "backward" flow of genetic information. But despite these variations on the original model, the principle that information flows from DNA to RNA to protein remains the main operating principle by which all cells express their genetic information. (The roles of DNA and RNA in the storage, transmission, and expression of genetic information will be considered in full detail in Chapters 16–20.)

Our current understanding of gene expression has relied heavily on the development of **recombinant DNA technology** since the 1970s. This technology was made possible by the discovery of *restriction enzymes*, which have the ability to cleave DNA molecules at specific sequences so that scientists can create *recombinant DNA molecules* containing DNA sequences from two different sources. This capability led quickly to the development of *DNA cloning*, a process used to generate many copies of specific DNA sequences for detailed study and further manipulation, and *DNA transformation*, the process of introducing DNA into cells. (These important techniques are explained and explored in detail in Chapter 21.)

At about the same time, **DNA sequencing** methods were devised for rapidly determining the base sequences of DNA molecules. This technology is now routinely applied not just to individual genes but also to entire genomes. Initially, genome sequencing was applied mainly to bacterial genomes because they are relatively small—a few million bases, typically. But DNA sequencing has long since been successfully applied to much larger genomes, including those from species of yeast, roundworm, plants, and animals that are of special interest to researchers. A major triumph was the sequencing of the entire human genome, which contains about 3.2 billion bases. This feat was accomplished by the *Human Genome Project*, a cooperative international effort that began in 1990, involved hundreds of scientists, cost billions of dollars, and established the complete sequence of the human genome by 2003.

Bioinformatics and "–Omics." The challenge of analyzing the vast amount of data generated by DNA sequencing has led to a new discipline, called **bioinformatics**, which merges computer science and biology as a means of making sense of sequence data. This approach has led to the recognition that the human genome contains approximately 20,000 protein-coding genes, about half of which were not characterized before genome sequencing. *Genomics,* the study of all the genes of an organism, is providing remarkable insights into cell biology and human health. Similarly, using modern techniques and bioinformatics, scientists can also study the *proteome,* the total protein content of a cell. In the emerging field of *proteomics,* researchers are attempting to understand the functions and interactions of all of the proteins present in a particular cell. Proteomic studies aim to understand the structure and properties of every protein produced by a genome and to learn how these proteins interact with each other in biological networks to regulate cellular functions.

Numerous bioinformatic tools are publicly available through the National Center for Biotechnology Information (NCBI), which is operated by the U.S. National Institutes of Health (NIH). In addition to housing PubMed, a searchable archive of more than 30 million citations from life science journals, NCBI maintains GenBank, a comprehensive database of all publicly available DNA sequences (over 217 million as of mid-2020). Similarly, the Protein Knowledgebase (UniProtKB) maintains a database of more than 560,000 protein sequences. Also available are numerous tools to compare gene and protein sequences from all organisms and to analyze their structure and function. For example, using a program known as BLAST, or Basic Local Alignment Search Tool, a researcher can compare the sequence of a newly discovered gene to all known gene sequences in a matter of minutes! In addition, NCBI provides a wealth of biological information, such as the OMIM (Online Mendelian Inheritance in Man) database, which is an encyclopedic collection of information regarding human genetic disorders and mutations involving almost 16,000 genes.

The past decade has seen major advances in technology that have miniaturized and automated molecular analyses, allowing them to be performed much faster. These *high-throughput* methods have resulted in dramatic increases in speed. The first human genome took 13 years to sequence, but now a genome can be sequenced in just a few hours and for a fraction of the cost. Likewise, the expression levels of hundreds or even thousands of genes can be monitored simultaneously, making it possible to study all the genes in a genome at the same time.

The ability to simultaneously analyze thousands of molecules on a global basis throughout the cell has led to a proliferation of "–omics" studies in addition to genomics and proteomics. For example, recent advanced methods of RNA sequencing allow us to determine the complete set of genes transcribed in a cell. This type of study is called *transcriptomics.* Scientists can also conduct *metabolomics,* the analysis of all metabolic reactions happening at a given time in a cell, *lipidomics,* the study of all the lipids in a cell, and even *ionomics,* the global study of all the ions in a cell. Keep your eyes open in the next few years, as this explosion of biological information will likely lead to a host of new fields of "–omics" studies.

CRISPR Genome Editing. Since the discovery of restriction enzymes, scientists have been trying to manipulate genomes. The key to these manipulations is having the right tools to modify DNA at specific sequences in specific ways. For example, precise sequence manipulation at the genome level is now being achieved through a new technique known as CRISPR/Cas9 genome editing (see Key Technique in Chapter 17 pages 500–501).

CRISPR (pronounced "crisper") is the abbreviation of **clustered regularly interspaced short palindromic repeats**. Although the CRISPR/Cas9 system is being used extensively for genome editing, it was first discovered as a prokaryotic defense mechanism against viral infection. Think of it as a bacterial immune system. (You will learn more details about this bacterial defense system in Chapter 20 [Figure 20-10].)

So how is a system designed to protect bacteria from invading viruses useful for editing genomes? The key is the generation of a double-stranded break at a precise location in the genome, which is targeted by a short nucleotide called a **guide RNA (gRNA)**. Double-stranded breaks in DNA are notoriously difficult for the cell to repair. Often, errors occur in this repair process, leading to the inactivation of the target gene. It is also possible to provide a piece of DNA with an alternative sequence that the cell can use to repair the break (called a *repair template*) using a process known as homology-directed repair. Both genome-editing strategies are depicted in **Figure 1-9**.

These and other techniques helped launch an era of molecular analysis that continues to revolutionize biology. In the process, the historical strand of genetics that dates back to Mendel became intimately entwined with the strands of cytology and biochemistry, and the discipline of cell biology came into being.

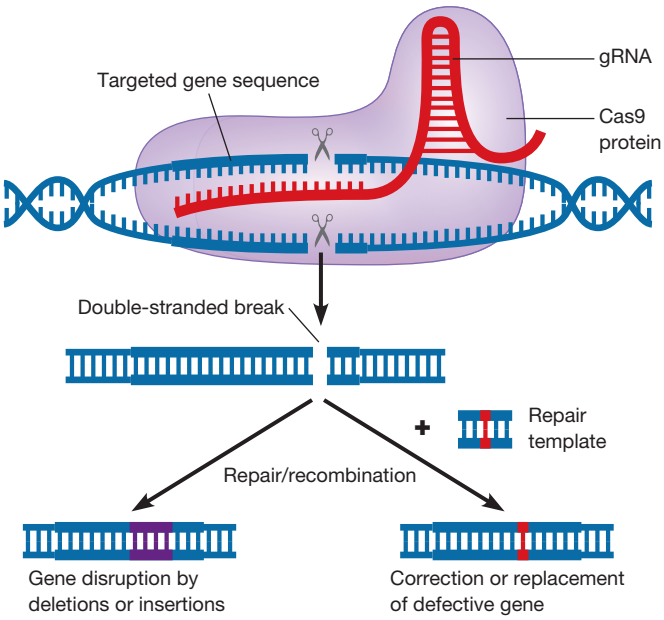


Figure 1-9 CRISPR Genome Editing. Guide RNA (gRNA) helps Cas9 target a specific place within the genome, where Cas9 causes a double-stranded break in the DNA. Repairs, which are often error prone, can lead to a gene disruption. If a repair template is added, homologous recombination will edit the genome using the DNA information from this alternative sequence.

CONCEPT CHECK 1.2

You have discovered a defective gene that allows cancer cells to use glucose more efficiently and therefore outgrow normal cells to form a tumor. Explain how your discovery likely involves contributions from all three historical strands of cell biology.

1.3 How Do We Know What We Know?

If asked what you expect to get out of this textbook, you might reply that you intend to learn the facts about cell biology. If pressed to explain what a "fact" is, most people would probably reply that a fact is "something that is known to be true." Saying, for example, that "DNA is the bearer of genetic information in cells" suggests that this statement is a fact of cell biology. But it must also be recognized that this statement actually replaced an earlier misconception that proteins carried the genetic information in cells.

Biological "Facts" May Turn Out to Be Incorrect

Cell biology is rich with examples of "facts" that were once widely held but have since been altered or even been discarded as cell biologists gained a better understanding of the phenomena those facts attempted to explain. As noted earlier, the early-nineteenth-century "fact" that living matter consisted of substances quite different from those in nonliving matter was discredited following work by Wöhler, who synthesized the biological compound urea from an inorganic compound, and by the Buchners, who showed that nonliving extracts from yeast cells could ferment sugar into alcohol. Thus, views held as fact by generations of scientists were eventually replaced by the new fact that living matter follows the same laws of chemistry and physics that inorganic materials do.

For a more contemporary example, until recently it was regarded as a fact that the sun is the ultimate source of all energy in the biosphere. Then came the discovery of *deep-sea thermal vents* and the thriving communities of organisms that live around them, none of which depend on solar energy. Instead, these organisms depend on energy derived from hydrogen sulfide (H_2S) by bacteria, which use this energy to synthesize organic compounds from carbon dioxide.

Thus, as you can see, sometimes biological "facts" are really much more provisional pieces of information than our everyday sense of the word might imply. Just like cells themselves, these "facts" are dynamic and subject to change, sometimes abruptly. Some results of biological research are, of course, not provisional in this sense. That most organisms are composed of cells is established beyond dispute. To a scientist, a "fact" is simply an attempt to state our best current understanding of the natural world around us, based on observations and experiments.

Experiments Test Specific Hypotheses

How does a new and deeper understanding of a cell biological process become available? First, a researcher will typically conduct a search of the scientific literature to determine what is known in the specific area of interest. Such information usually comes from a *peer-reviewed* scientific or medical journal, rather than from a nonreviewed site on the Internet. "Peer-reviewed" means that after scientists conducted their research and submitted a written article to a journal, the article was examined in detail by several experts in the particular field and found to be sound in methodology, experimental design, and analysis of results.

Once a cell biologist has assessed the current state of knowledge in her field, then she can formulate a **hypothesis**, a tentative explanation that can be tested experimentally or via further observation. Often, a hypothesis takes the form of a *model* that appears to provide a reasonable explanation of the phenomenon in question. Next, the investigator designs a controlled experiment to test the hypothesis by varying specific conditions while keeping other variables constant. The scientist then collects the data, interprets the results, and accepts or rejects the hypothesis, which must be consistent not only with the results of this particular experiment but also with prior knowledge.

Rather than try to prove a hypothesis, scientists typically try to rephrase the hypothesis as its opposite, known as the **null hypothesis**, and seek to prove the latter. Failure to confirm this null hypothesis in a sufficiently large number of attempts is indirect evidence that the hypothesis is correct. The certainty of its correctness increases with the number of experimental samples and the number of times the results are replicated.

For example, suppose you proposed the hypothesis that every person on Earth was under 20 feet tall. To prove this proposal rigorously, you would have to measure the height of everyone on Earth and determine that all were under 20 feet tall. The null hypothesis would be that there is indeed a person more than 20 feet tall. Failure to find such a person after years, perhaps centuries, of trying would provide reasonably solid evidence that the hypothesis is true—there is indeed no one on Earth more than 20 feet tall.

Experiments are often conducted in the laboratory using purified chemicals and cellular components. This type of experiment is described as in vitro, which literally means "in glass." But to fully understand how cells work, hypotheses may need to be tested in vivo ("in life"), meaning in live cells and organisms. Often, this is done using one of a variety of popular *model organisms,* which will be discussed in the next section. More recently, experiments using computers to test new hypotheses involving vast amounts of data have been described as in silico, referring to the silicon used to make computer chips. All of these approaches use the same basic steps for hypothesis formulation and testing as outlined above.

Model Organisms Play a Key Role in Modern Cell Biology Research

Although many studies of basic cellular functions can be carried out in the laboratory using isolated cellular components such as membranes, enzymes, and DNA molecules, the results obtained from such experiments may not directly reflect the corresponding process in an intact, living system. Scientists have developed a number of *model systems* to study cellular processes directly in living cells and organisms.

Cell and Tissue Cultures. Scientists make extensive use of *cell cultures* as model systems. Many types of cells can be grown in the laboratory outside their tissue of origin—such as skin cells, muscle cells, and cancer cells. Some of the first human cells ever grown in defined culture conditions in the laboratory were HeLa cells taken from cervical cancer tissue obtained from a woman named Henrietta Lacks in 1951. Descendants of her cells are still being grown today and are commonly used in cancer and virus research (**see Human Connections, page 16**).

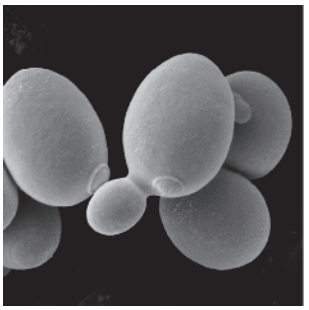
A variety of other cells are commonly used as model systems: egg cells from the frog *Xenopus* to study channel proteins; Chinese hamster ovary cells for cell signaling studies and commercial production of proteins; mouse 3T3 fibroblast cells to assess the carcinogenicity of new compounds; and undifferentiated embryonic stem cells to study cellular differentiation. In addition, cell cultures are indispensable for growing and studying viruses, which are tiny infectious noncellular particles that cannot multiply outside their host cells. But because what we learn by studying isolated cells in culture may not always reflect what happens in the intact organism, it is important to perform studies in the living organisms themselves.

Model Organisms. A **model organism** is a species that is widely studied, well characterized, and easy to manipulate and has particular advantages, making it useful for experimental studies. A few examples are the bacterium *Escherichia coli,* the yeast *Saccharomyces cerevisiae,* and the fruit fly *Drosophila melanogaster* (**Figure 1-10**).

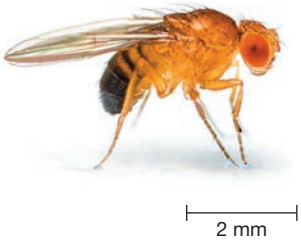
Much of what is known about basic cellular processes, such as DNA replication, membrane function, and protein synthesis, was learned using cells of the bacterium *E. coli* as a model system. *E. coli* is easy to grow in the lab, divides rapidly (it has a generation time of 20 minutes), and is easily mutagenized for studies of gene function. In 1997, it became the first bacterium to have its complete genome sequenced. Because it readily takes up DNA by transformation from virtually any organism, it has become the workhorse of cellular and molecular biology for isolating and cloning genes. It is routinely used for the analysis and production of genes and proteins for research, industrial, and medical uses.

To study processes unique to eukaryotic cells, such as chromosome pairing during cell division, organelle development, or cell signaling, cells of *S. cerevisiae* (bakers' and brewers' yeast) offer several similar experimental advantages. They are unicellular, as well as easy to grow and mutagenize, and well-characterized mutant lines are available. Using yeast cells as a model organism to isolate and characterize genetic mutants following *mutagenesis* by chemicals or radiation, scientists have learned much about how eukaryotic cells function. By studying mutant yeast strains that are deficient in particular genes and their protein products, researchers can determine the normal cellular function of those genes and proteins. For example, mutant yeast cells that display abnormal cell division because they are deficient in a particular gene necessary for proper cell division have been invaluable in helping us understand how normal cells divide. For example, much of what we know about human cell division originally came from the

(a) *E. coli* colonies on blood agar
- Unicellular bacterium with short generation time
- Easily mutagenized and transformed to study gene function
- Widely used for gene cloning and protein function



(b) *S. cerevisiae* cells (yeast; SEM)
- Unicellular eukaryotic cells that are easy to grow in the lab
- Thousands of mutant lines available to study gene function
- Widely used to study the cell cycle and protein-protein interactions



(c) Wild-type *D. melanogaster* (fruit fly)
- Multicellular eukaryotic organism with a short generation time
- Many mutant strains available with well characterized genetic defects
- Widely used for studies of embryogenesis and development

(d) *C. elegans* (roundworm)
- Multicellular eukaryotic organism with a short life cycle
- Nearly transparent with cell fates mapped out
- Widely used for cell differentiation and development studies

(e) *M. musculus* (house mouse)
- Mammalian organism with many similarities to humans
- Numerous strains with genetic "knockouts"
- Widely used as models for human disease

(f) *A. thaliana* (mustard plant)
- Rapid life cycle and one of the smallest plant genomes
- Easily mutagenized with thousands of mutant lines available
- Widely used for studies of plant-specific processes

Figure 1-10 Common Model Organisms. Examples of some model organisms frequently used in cell biology research are shown with descriptions of the advantages of each.

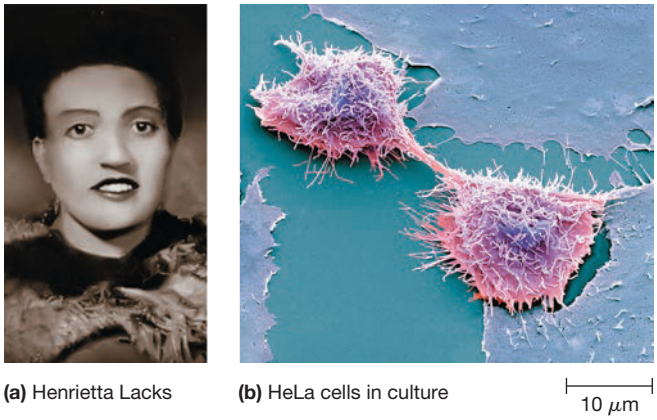
HUMAN *Connections*

The Immortal Cells of Henrietta Lacks

George Otto Gey was an anatomist at heart, who was fascinated by cancer and how cancer cells divided. He and his wife, Margaret, coordinated the cell culture lab at Johns Hopkins University and for decades attempted to produce a line of human cells that could live indefinitely if given the necessary environment and supplemental nutrition. Although the Geys were given cells from multiple human tissues, they had been unable to culture a line that would not age and eventually die. This would all change one day in 1951, when they were given a tissue sample biopsied from a cervical tumor growing in a woman reporting abnormal bleeding after the birth of her fifth child. After a few weeks the cells from this biopsy were obviously different. They grew well in culture and didn't seem to age and die. These cells, named *HeLa cells,* would become the first immortal human cell line. Almost 70 years later, they remain one of the most widely used human cell lines.

Creation of the HeLa line was a milestone in biomedical research. Using animals to study human diseases is time consuming and expensive, and animals do not always respond to disease in the same way that human cells do. HeLa cells have made an enormous contribution to science; they have been cited in more than 70,000 scientific papers, and they have been instrumental in research on viruses, cancer, and AIDS.

The name "HeLa" comes from the name of the woman from whom the cells were taken, Henrietta Lacks (**Figure 1B-1**). But who was the woman behind the cell line? Henrietta Lacks was born in 1920 to a poor African American family in rural Virginia. Henrietta moved to Baltimore in 1941 with her husband and started a family. In 1951, after the birth of her fifth child, Henrietta was diagnosed with cervical cancer. Tragically, Henrietta had a very aggressive form of cancer that did not respond to the treatment available at the time, and she died at the age of 31. At the time of her diagnosis and biopsy, there were no laws requiring informed consent before a doctor took tissue or blood samples, nor were there laws governing what the tissue could be used for after it was removed from the patient's body. After the HeLa cell culture line (shown in Figure 1B-1b) was created, it was freely disseminated to other scientists for use in their research. The Lacks family did not learn that Henrietta's cells were grown in laboratories around the world until two decades later.



(a) Henrietta Lacks **(b)** HeLa cells in culture

Figure 1B-1 Henrietta Lacks and Her Immortal Cells.
(a) Henrietta Lacks before her diagnosis. **(b)** HeLa cells in the process of dividing (colorized SEM).

From the beginning, Henrietta's cells were unique. Not only did they divide endlessly in culture, but they grew so vigorously that they would double in number every 24 hours! What made Henrietta's cells so different? Normal cells can divide only about 50 times before undergoing cell death because the ends of their chromosomes, known as *telomeres,* shorten (see Chapter 17). HeLa cells, however, are derived from cancer cells, which abnormally express the gene encoding *telomerase,* an enzyme that allows telomeres to be synthesized anew with each round of cell division. As a result, HeLa cells can divide indefinitely.

One cause of cervical cancer is the *human papilloma virus (HPV)*. HeLa cells contain a copy of HPV inserted into the chromosome near the *myc* gene, causing it to be overexpressed. The Myc protein normally controls cell growth, so overexpression of *myc* is associated with uncontrolled cell division and cancer (see Chapter 24). The presence of HPV near the *myc* locus may explain why Henrietta's cancer was so aggressive and why HeLa cells grow so vigorously in culture. In fact, HeLa cells grow so rapidly in culture that they frequently contaminate other cell lines. It is estimated that 10–20% of existing cell lines are actually HeLa contaminants that have overgrown and replaced the original cell culture.

HeLa cells played an important role in the development of a vaccine for polio. During the early 1900s, polio was the most dreaded disease in America. In 1952 the worst polio epidemic ever recorded swept across the United States. Of the 58,000 people who contracted polio, 21,000 were left with some form of paralysis and 3000 died. During the 1952 epidemic, HeLa cells were shipped to Jonas Salk and other polio researchers when it was discovered that HeLa cells could be infected with viruses and cellular responses to treatments could be easily monitored. Beginning with the introduction of the Salk vaccine in the mid-1950s and a later oral vaccine developed by Albert Sabin, polio, once a dreaded disease, was quickly eradicated in the United States and, eventually across most of the globe.

Once they were used to study human disease, the demand for HeLa cells exploded, and companies were established to grow HeLa cells and provide them to researchers. Ironically, the Lacks family never received any money from the sale of HeLa cells.

The Lacks family's plight raises several important ethical considerations. First, once a blood or tissue sample is removed from your body, is it still yours? Many people believe that donated tissue should be used in medical research for the common good. However, after the tissue has been donated, should the donor have control over the kinds of research his or her cells are used for? Second, if the donated tissue is used to develop a commercial product, should the donor be granted a share of the profits?

A third issue was raised in 2013, when a group of scientists published the DNA sequence of HeLa cells without the family's knowledge or permission. Requests for access to HeLa DNA sequence information are now reviewed by a committee consisting of scientists, physicians, and members of the Lacks family. Researchers who use HeLa cells or the genomic data are asked to acknowledge the contribution of Mrs. Lacks and her family in their publications. The use of HeLa cells, and HeLa sequence information, in biomedical research continues to benefit millions of people. But Henrietta's family finally has a say in how her genetic information will be used.

study of cell cycle mutants in yeast. Recently, use of the yeast *two-hybrid system* (see Chapter 21), which allows researchers to determine whether and how specific proteins interact within a living cell, has contributed greatly to our understanding of the complex molecular interactions involved in cellular function.

However, if you want to study processes such as communication between cells, differentiation of cells, or embryonic development, you may need to use a *multicellular* organism as a model system. You may have heard biologists talk about experiments using "flies and worms." They are referring to the tiny fruit fly *Drosophila melanogaster* and the roundworm *Caenorhabditis elegans,* both of which are extensively used for studies of the cell biology of multicellular, eukaryotic organisms.

Much of our basic understanding of genetics and gene function comes from using mutants of *Drosophila,* which has many experimental advantages: The flies are easy to grow and manipulate in the lab, have a short (2-week) generation time, produce numerous progeny, and have easily observable physical characteristics, such as eye color and wing shape. Thousands of mutant strains are available, each defective in a particular gene; this makes *Drosophila* quite valuable for studies of embryogenesis, developmental biology, and cell signaling.

Similarly, *C. elegans* is a widely used model organism for studies of cell differentiation and development in multicellular organisms. Its advantages include its ease of manipulation, relatively short life cycle, and small genome, the first of any multicellular organism to be sequenced. It is also one of the simplest animals to possess a nervous system. Its development from a fertilized egg is remarkably predictable, and the origin and fate of each of its approximately 1000 cells have been mapped out, as have the hundreds of connections among the roughly 200 nerve cells. In addition, the tiny worms are transparent, making it is easy to see individual cells under the microscope and to view fluorescently labeled molecules in the living organism.

For studies of cellular and physiological processes specific to mammals (including humans), the common laboratory mouse *(Mus musculus)* has become the primary model organism. It shares many cellular, anatomical, and physiological similarities with humans and is widely used for research in medicine, immunology, and aging. It is subject to, and therefore useful for the study of, a variety of diseases that also affect humans, such as cancer, diabetes, and osteoporosis. Numerous mouse strains have been bred or engineered in which particular genes have either been "knocked out" or introduced, making them extremely valuable in biomedical research.

For studies of processes in plants, such as photosynthesis and light perception, and some processes common to all organisms, *Chlamydomonas reinhardtii,* a unicellular green algae, is often used (see Figure 1-2g). Like *E. coli* and yeast, "*Chlamy*" is easily grown in the lab on Petri plates and has been used to study photosynthesis, light perception, mating type, cellular motility, and DNA methylation. For flowering plant studies, *Arabidopsis thaliana* is a powerful model organism. It has one of the smallest genomes of any plant and a rapid (6-week) life cycle, facilitating genetic studies. Its complete genome has been sequenced, and thousands of mutant strains have been created, enabling detailed studies of plant gene function.

Many other model organisms are currently being used in biology to study a wide variety of cellular, genetic, and biochemical processes. As you proceed through your studies in cell biology, keep in mind that much of our knowledge is based on research using relatively few of the millions of living organisms on Earth, and remember that it is always important to understand how this knowledge was obtained.

MAKE CONNECTIONS 1.1

What type of microscopy is *best* suited for taking advantage of the transparency of *C. elegans* in order to visualize a specific internal structure? (Ch. 1.2)

Well-Designed Experiments Alter Only One Variable at a Time

Modern cell biologists have an extensive array of tools to use as they consider how to perform experiments. How can they use model organisms, powerful microscopes, and genetic and biochemical techniques to meaningfully answer questions in cell biology? In a typical experiment in cell biology, many individual conditions can be varied, such as length of treatment or temperature, but it is best to vary, or perturb, only one condition, called the *independent variable,* and hold all others constant. The outcome of the change that is measured (which depends on the independent variable) is called the *dependent variable*. For example, if you wanted to test the rate of growth of cells at different temperatures, you would hold all culture conditions constant except temperature, which you would vary. Temperature is the independent variable that you set, and the growth you measure is the dependent variable whose value depends on the particular temperature.

The fact that there should be only one independent variable in an experiment is a key reason why genetic mutants are so valuable for studying gene function. For in vivo studies of gene function, for example, the classical genetic approach is to isolate a naturally occurring mutant form of an organism. It is now possible to artificially alter the DNA of an organism as well. In either case, an organism (*E. coli,* yeast, a mouse, or other model organism) with unaltered DNA is called the *wild type.* The mutant strain is identical to the wild type except that it lacks one particular gene's function.

Scientists can also perform in vitro experiments in which purified cellular components are added to a test tube to simulate a process that occurs in intact, living cells. Then the system can be perturbed systematically—adding, deleting, or modifying one ingredient of the mixture at a time—to test the hypothesis that a particular component is necessary. A powerful method (one you will see used throughout this text) is to introduce inhibitors or antibodies to block the function of a particular component in a reaction. Thus, by changing only one component or other variable at a time, scientists can determine the specific function of that component and the effect of that particular variable.

As you have now seen, the "facts" presented in biology textbooks such as this one are simply our best current attempts to describe and explain the biological world we live in. As you proceed through this text, notice how the process of science has been used to advance our knowledge of cell biology and pay attention to how this new knowledge was gained by experimentation. You will likely find that, regardless of the approach, the conclusions from an experiment add to our knowledge of how cells work but usually lead to more questions as well, continuing the cycle of scientific inquiry. We hope, as you gain greater understanding of cells and how they are studied

throughout this book, that you will learn to appreciate what experiments tell us and that you will learn how to design your own experiments to answer questions and test hypotheses.

MAKE CONNECTIONS 1.2

When generating knockout mice, scientists use inbred mouse stains. Based on what you know about well-designed experiments, why is this important? (Ch. 1.3)

CONCEPT CHECK 1.3

Suppose you often have heartburn on nights after you have eaten your favorite pepperoni, anchovy, and onion pizza. You wonder whether the pizza or one of its toppings might be causing the heartburn. Describe how you might determine whether the heartburn is due to the pizza and, if so, to which of the toppings.

Summary of Key Points

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1.1 The Cell Theory: A Brief History

- The biological world is a world of cells. The cell theory states that all organisms are made of cells, the basic units of biological structure, and that cells arise only from preexisting cells.
- The cell theory was developed through the work of many different scientists, including Hooke, van Leeuwenhoek, Brown, Schleiden, Schwann, Nägeli, and Virchow.
- Although the importance of cells in biological organization has been appreciated for about 150 years, the discipline of cell biology as we know it today is of much more recent origin.

1.2 The Emergence of Modern Cell Biology

- Modern cell biology has come about by the interweaving of three historically distinct strands—cytology, biochemistry, and genetics—which in their early development probably did not seem at all related.
- The contemporary cell biologist must understand all three strands because they complement one another in the quest to learn what cells are made of and how they function.
- The cytological strand deals with cellular structure.
- The cytological strand is best studied using microscopes, which include both light and electron microscopy. The light microscope has allowed us to visualize individual cells. Several types of light microscopes allow us to view preserved or living specimens, including brightfield, phase-contrast, differential interference contrast, fluorescence, confocal, and digital video microscopes. Historically, the limited resolving power of the light microscope did not allow us to see the finer details of cellular structure, but electron microscopes and modern light microscopes have solved this limitation. The electron microscope uses a beam of electrons, rather than visible light, for imaging specimens. It can magnify objects with a resolving power of less than 1 nm, enabling us to view subcellular structures such as membranes, ribosomes, organelles, and even individual DNA and protein molecules.
- The biochemical strand concerns the chemistry of biological structure and function.
- Discoveries in biochemistry have revealed how many of the chemical processes in cells are carried out, greatly expanding our knowledge of how cells function.
- Major discoveries in biochemistry were the identification of enzymes as biological catalysts, the discovery of adenosine triphosphate (ATP) as the main carrier of energy in living organisms, and the description of the major metabolic pathways cells use to harness energy and synthesize cellular components.
- Several important biochemical techniques that have allowed us to understand cell structure and function are subcellular fractionation, ultracentrifugation, chromatography, electrophoresis, and mass spectrometry.
- The genetic strand focuses on information flow.
- The chromosome theory of heredity states that the characteristics of organisms passed down from generation to generation result from the inheritance of chromosomes carrying discrete physical units known as genes.
- Each gene is a specific sequence of DNA that contains the information to direct the synthesis of one cellular protein.
- DNA itself is a double helix of complementary strands held together by precise base pairing. This structure allows the DNA to be accurately duplicated as it is passed down to successive generations.
- The flow of genetic information in cells is typically from DNA to RNA to protein, although exceptions such as reverse transcription exist. Expression of this genetic information to produce a protein requires several important types of RNA: mRNA, tRNA, and rRNA.
- Bioinformatics allows us to compare and analyze thousands of genes or other molecules simultaneously, causing a revolution in genomic, proteomic, and numerous other fields of "–omics" research.
- CRISPR genome editing is an exciting new technique that allows precise changes to genomic sequences.

1.3 How Do We Know What We Know?

- Science is not a collection of facts but a process of discovering answers to questions about our natural world. Scientists gain knowledge by using the scientific method, which involves creating a hypothesis that can be tested for validity by collecting data through well-designed, controlled experiments.
- A well-designed experiment will vary and test only one condition at a time to test a hypothesis. This can involve the use of mutants, experiments in which one component at a time is changed, and the use of inhibitors of specific cellular processes.
- Progress in science is based on the consistency and reproducibility of experimental results. These results are often presented in the form of peer-reviewed journal articles.
- Scientists use a variety of well-studied cell cultures and model organisms to test new hypotheses, develop new theories, and advance our knowledge of cell biology.

Problem Set

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1-1 The Historical Strands of Cell Biology. For each of the following events, deduce whether it belongs mainly to the cytological (C), biochemical (B), or genetic (G) strand in the historical development of cell biology.

(a) Köllicker describes "sarcosomes" (now called mitochondria) in muscle cells (1857).

(b) Hoppe-Seyler isolates the protein hemoglobin in crystalline form (1864).

(c) Haeckel postulates that the nucleus is responsible for heredity (1868).

(d) Ostwald proves that enzymes are catalysts (1893).

(e) Muller discovers that X-rays induce mutations (1927).

(f) Davson and Danielli postulate a model for the structure of cell membranes (1935).

(g) Beadle and Tatum formulate the one gene–one enzyme hypothesis (1940).

(h) Claude isolates the first mitochondrial fractions from rat liver (1940).

(i) Lipmann postulates the central importance of ATP in cellular energy transactions (1940).

(j) Avery, MacLeod, and McCarty demonstrate that bacterial transformation is attributable to DNA, not protein (1944).

(k) Palade, Porter, and Sjøstrand each develop techniques for fixing and sectioning biological tissue for electron microscopy (1952–1953).

(l) Lehninger demonstrates that oxidative phosphorylation depends for its immediate energy source on the transport of electrons in the mitochondrion (1957).

1-2 QUANTITATIVE Cell Sizes. To appreciate the differences in cell size illustrated in Figure 1-4a, consider these specific examples. *Escherichia coli,* a typical bacterial cell, is cylindrical in shape, with a diameter of about 1 μm and a length of about 2 μm. As a typical animal cell, consider a human liver cell, which is roughly spherical and has a diameter of about 20 μm. For a typical plant cell, consider the columnar palisade cells located just beneath the upper surface of many plant leaves. These cells are cylindrical, with a diameter of about 20 μm and a length of about 35 μm.

(a) Calculate the approximate volume of each of these three cell types in cubic micrometers. (Recall that $V = \pi r^2 h$ for a cylinder and that $V = 4\pi r^3/3$ for a sphere.)

(b) Approximately how many bacterial cells would fit in the internal volume of a human liver cell?

(c) Approximately how many liver cells would fit inside a palisade cell?

1-3 QUANTITATIVE Sizing Things Up. To appreciate the sizes of the subcellular structures shown in Figure 1-4b, consider the following calculations.

(a) All cells and many subcellular structures are surrounded by a membrane. Assuming a typical membrane to be about 8 nm wide, how many such membranes would have to be aligned side by side before the structure could be seen with the light microscope? How many with the electron microscope?

(b) Ribosomes are the cell structures in which the process of protein synthesis takes place. A human ribosome is a roughly spherical structure with a diameter of about 30 nm. How many ribosomes would fit in the internal volume of the human liver cell described in Problem 1-2 if the entire volume of the cell were filled with ribosomes?

(c) The genetic material of the *Escherichia coli* cell described in Problem 1-2 consists of a circular DNA molecule with a strand diameter of 2 nm and a total length of 1.36 mm. To be accommodated in a cell that is only a few micrometers long, this large DNA molecule is tightly coiled and folded into a *nucleoid* that occupies a small proportion of the cell's internal volume. Approximating the DNA molecule as a very thin cylinder, calculate the smallest possible volume the DNA molecule could fit into, and express it as a percentage of the internal volume of the bacterial cell that you calculated in Problem 1-2a.

1-4 QUANTITATIVE Limits of Resolution Then and Now. Based on what you learned in this chapter about the limit of resolution of a light microscope, answer each of the following questions. Assume that the unaided human eye has a limit of resolution of about 0.25 mm and that a modern light microscope has a useful magnification of about 1000×.

(a) Define *limit of resolution* in your own words. What was the limit of resolution of Hooke's microscope? What about van Leeuwenhoek's microscope?

(b) What are the approximate dimensions of the smallest structure that Hooke would have been able to observe with his microscope? Would he have been able to see any of the structures shown in Figure 1-4a? If so, which ones? And if not, why not?

(c) What are the approximate dimensions of the smallest structure that van Leeuwenhoek would have been able to observe with his microscope? Would he have been able to see any of the structures shown in Figure 1-4a? If so, which ones? And if not, why not?

(d) What are the approximate dimensions of the smallest structure that a contemporary cell biologist should be able to observe with a modern light microscope?

(e) Consider the eight structures shown in Figure 1-4a and 1-4b. Which of these structures would both Hooke and van Leeuwenhoek have been able to see with their respective microscopes? Which, if any, would van Leeuwenhoek have been able to see that Hooke could not? Explain your reasoning. Which, if any, that neither Hooke nor van Leeuwenhoek could see would a contemporary cell biologist be able to see using a modern light microscope?

1-5 The Contemporary Strands of Cell Biology. For each pair of techniques listed, indicate whether its members belong to the cytological (C), biochemical (B), or genetic (G) strand of cell biology (see Figure 1-3). Suggest one advantage that the second technique has over the first technique.

(a) Light microscopy/electron microscopy

(b) Centrifugation/ultracentrifugation

(c) Cell cultures/model organisms

(d) Sequencing of a genome/bioinformatics

(e) Transmission electron microscopy/scanning electron microscopy

(f) Chromatography/electrophoresis

1-6 The "Facts" of Life. Each of the following statements was once regarded as a biological fact but is now understood to be untrue. In each case, indicate why the statement was once thought to be true and why it is no longer considered a fact.

(a) Plant and animal tissues are constructed quite differently because animal tissues do not have conspicuous boundaries that divide them into cells.

(b) Living organisms are not governed by the laws of chemistry and physics, as is nonliving matter, but are subject to different laws that are responsible for the formation of organic compounds.

(c) Genes most likely consist of proteins because the only other likely candidate, DNA, is a relatively uninteresting molecule consisting of only four kinds of monomers (nucleotides) arranged in a relatively repetitive sequence.

(d) The fermentation of sugar to alcohol can take place only if living yeast cells are present.

1-7 Wrong Again. Explain why each of the following statements is false.

(a) Because of the wavelength of light, resolution of cellular structures smaller than 200 nm can never be achieved.

(b) Fluorescence microscopy can allow us to visualize cells but cannot help identify them.

(c) Because all DNA molecules have similar chemical composition, it is not possible to separate and characterize individual DNA molecules.

(d) The best way to carry out a scientific experiment is to try to prove a hypothesis by varying all the relevant conditions.

(e) The flow of genetic information is always from DNA to RNA to protein.

1-8 A New Biofuel. As a recent cell biology graduate, you have just been hired by a biotechnology company to develop a biofuel using algal cells that produce an oil very similar to diesel fuel. What model system(s) might you use for each of the following aspects of this project? What tools and techniques would you use?

(a) Determining which genes and enzymes are required to produce the oil

(b) Producing large amounts of a certain enzyme for further research

(c) Studying whether any of the cellular enzymes interact with each other

(d) Examining the involvement of any cellular organelles in storage or secretion of the oil

1-9 DATA ANALYSIS Worm Microscopy. With the use of microscopy, the localization of two proteins (A and B) using different fluorescent dyes can be examined in the developing *C. elegans* embryo images shown in **Figure 1-11**. The images of A and B are overlaid in the third image. Which type of microscopy is being used—light microscopy or electron microscopy? Are the two proteins likely to be interacting with each other? Provide evidence for your answers.

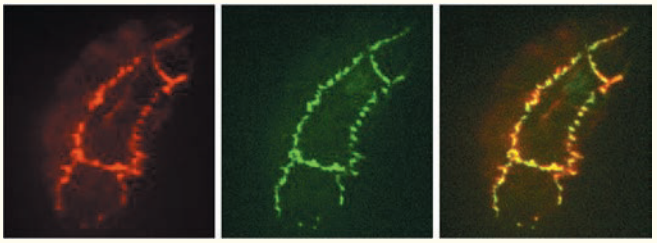
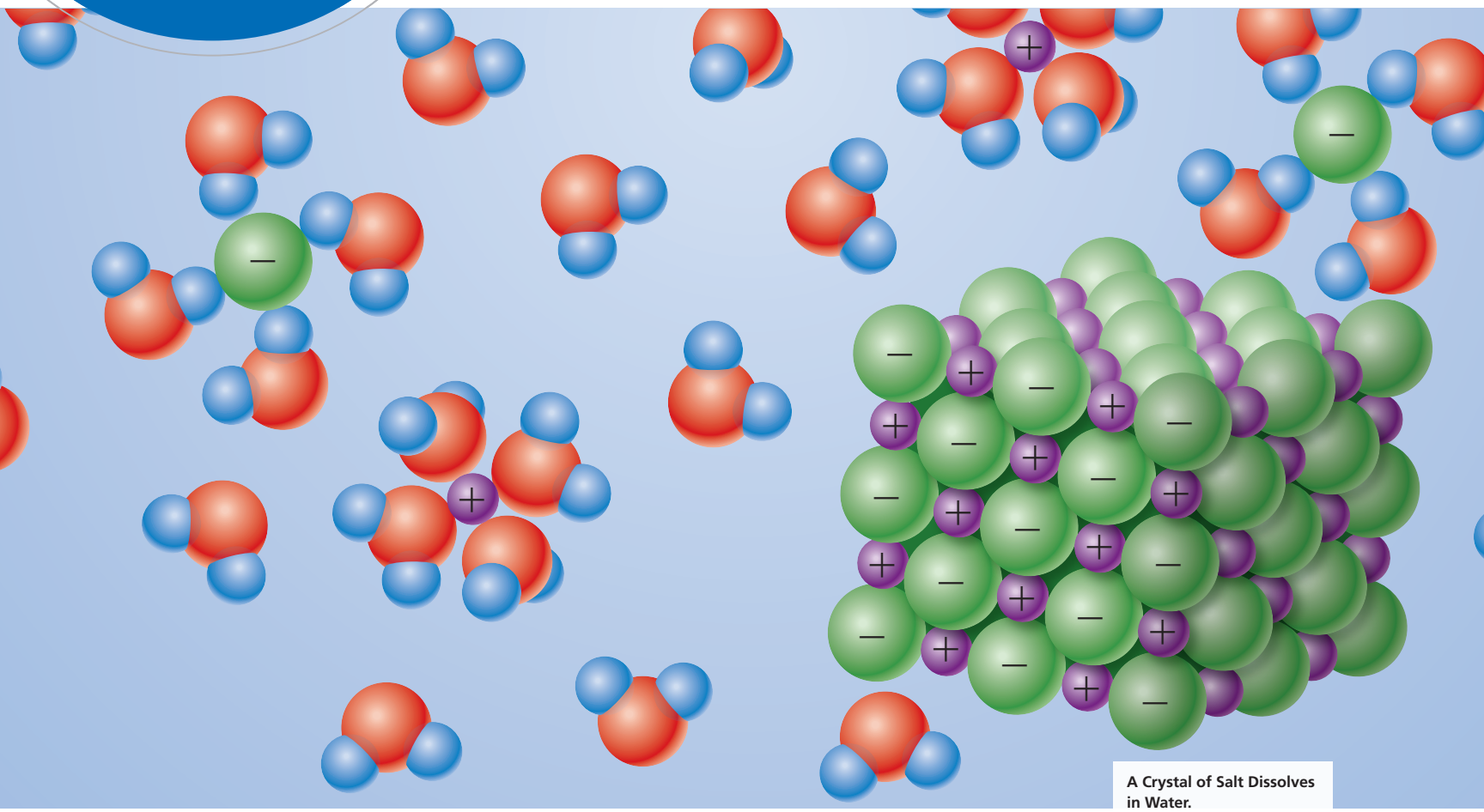


Figure 1-11 Localization of *C. elegans* Proteins Using Microscopy. See Problem 1-9.

2 The Chemistry of the Cell



A Crystal of Salt Dissolves in Water.
As a crystal of salt (green and purple structure) dissolves in water, the oxygen atoms of water (red) surround the positive ions, and its hydrogen atoms (blue) surround the negative ions.

Students just beginning in cell biology are sometimes surprised—and perhaps even dismayed—to find that courses and textbooks dealing with cell biology involve a substantial amount of chemistry. Yet biology in general and cell biology in particular depend heavily on both chemistry and physics. After all, cells and organisms follow all the laws of the physical universe, so biology is really just the study of chemistry and physics in systems that are alive. In fact, everything cells are and do has a molecular and chemical basis. Therefore, we can truly understand and appreciate cellular structure and function only when we can describe cellular structure in molecular terms and express cellular function in terms of chemical reactions and events.

Trying to appreciate cellular biology without a knowledge of chemistry would be like trying to appreciate a translation of Chekhov without a knowledge of Russian. Most of the meaning would probably get through, but much of the beauty and depth of appreciation would be lost in the translation. For this reason, we will consider the chemical background necessary for the cell biologist. Specifically, this chapter will provide an overview of

several chemical principles critical for understanding cellular biology and will introduce the major classes of macromolecules in cells: proteins, nucleic acids, carbohydrates, and lipids.

The main points of this chapter can conveniently be structured around five principles:

1. *The importance of carbon.* The carbon atom has several unique properties that make it especially suitable as the backbone of biologically important molecules.
2. *The importance of water.* The water molecule has several unique properties that make it especially suitable as the universal solvent of living systems.
3. *The importance of selectively permeable membranes.* Membranes define cellular compartments and control the movements of molecules and ions into and out of cells and organelles.
4. *The importance of synthesis by polymerization of small molecules.* Biological macromolecules are polymers formed by linking many similar or identical small molecules known as monomers.
5. *The importance of self-assembly.* Biological macromolecules are often capable of self-assembly into higher levels of structural organization because the information needed to specify the spatial configuration of the molecule is contained in the polymer.

Understanding these five principles will help you to appreciate the cellular chemistry necessary before venturing further into our exploration of what it means to be a cell.

2.1 The Importance of Carbon

To study cellular molecules really means to study compounds containing carbon. Almost without exception, molecules of importance to the cell biologist have a backbone, or skeleton, of carbon atoms linked together covalently in chains or rings. Actually, the study of carbon-containing compounds is the domain of **organic chemistry.** In its early days, organic chemistry was synonymous with *biological chemistry* because the carbon-containing compounds that chemists first investigated were obtained from biological sources (hence the word *organic*, acknowledging the organismal origins of the compounds).

The terms *organic chemistry* and *biological chemistry* have long since gone their separate ways, however, because organic chemists have now synthesized an incredible variety of carbon-containing compounds that do not occur naturally in the biological world. Organic chemistry therefore is the study of all classes of naturally occurring and synthetic carbon-containing compounds. **Biological chemistry** (*biochemistry* for short) deals specifically with the chemistry of living systems and is, as we have already seen, one of the several historical strands that form an integral part of modern cell biology (see Figure 1-3).

The **carbon atom (C)** is the most important atom in biological molecules. Carbon-containing compounds owe their diversity and stability to specific bonding properties of the carbon atom. Especially important are the ways that carbon atoms interact with each other and with other chemical elements of biological importance (**Figure 2-1**).

An extremely important property of the carbon atom is its **valence** of four, meaning it can form up to four chemical bonds with other atoms before filling its outer electron shell. Atoms can bond to each other via their outer electrons, and atoms are usually the most stable when they are surrounded by a total of eight electrons, satisfying what is known as the *octet rule*. The outermost electron orbital of a carbon atom has four electrons and therefore lacks four of the eight electrons needed to fill it completely and make it the most stable. Therefore, carbon atoms associate with each other or with other electron-deficient atoms, allowing adjacent atoms to share a pair of electrons, one from each atom, so that each

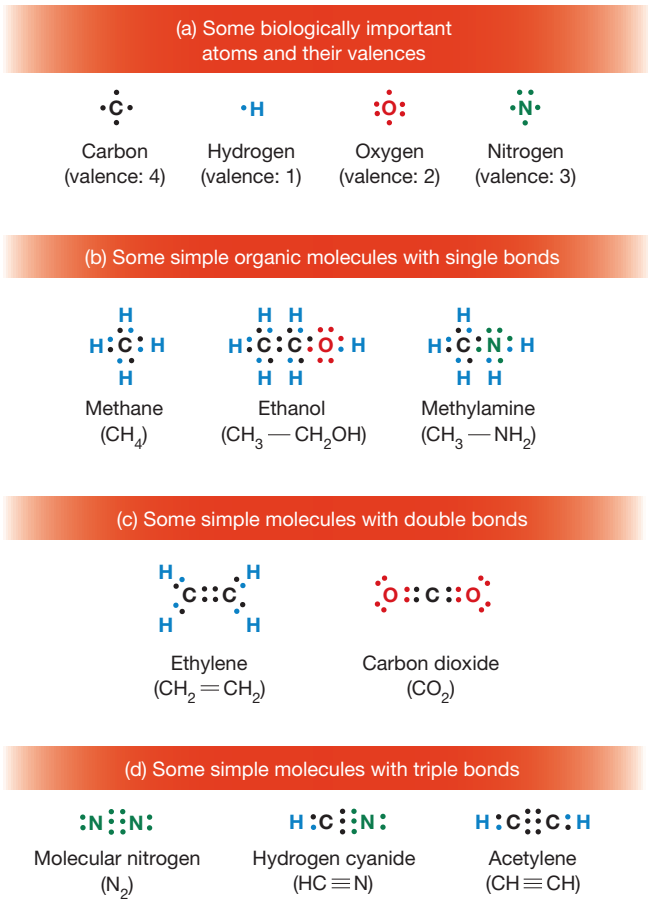


Figure 2-1 Electron Configurations of Some Biologically Important Atoms and Molecules. Electronic configurations are shown for **(a)** individual atoms and **(b–d)** some simple molecules. Only electrons in the outermost electron orbital are shown.

atom's outer orbital has a full set of eight electrons including shared electrons. (For hydrogen alone, a full set is only two electrons.) Atoms that share electrons in this way are held together and are said to be joined by a **covalent bond.** *Because four additional electrons are required to fill the outer orbital of carbon, stable organic compounds have four covalent bonds for every carbon atom.* This gives carbon-containing molecules great diversity in molecular structure and function.

Carbon atoms are most likely to form covalent bonds with other carbon atoms and with atoms of oxygen (O), hydrogen (H), nitrogen (N), and sulfur (S). The electronic configurations of several of these atoms are shown in Figure 2-1a. Sulfur, like oxygen, has six outer electrons and a valence of two. Notice that, in each case, one or more electrons are required to complete the outer orbital to a total of eight electrons. The number of "missing" electrons corresponds in each case to the valence of the atom, which is the number of covalent bonds the atom can form. Because hydrogen's outermost electron orbital can hold only two electrons, it has a valence of one and forms only one covalent bond.

The sharing of one pair of electrons between atoms results in a **single bond.** Methane, ethanol, and methylamine are simple examples of carbon-containing compounds containing only single bonds between atoms (Figure 2-1b), as represented by the pair of electrons between any two chemical symbols. Sometimes two or even three pairs of electrons can be shared by two atoms, giving rise to **double bonds** or **triple bonds.** Ethylene and carbon dioxide are examples of double-bonded compounds (Figure 2-1c). Notice that, in these compounds, each carbon atom still forms a total of four covalent bonds, either one double bond and two single bonds or two double bonds. Triple bonds are rare but can be found in molecular nitrogen, hydrogen cyanide, and acetylene (Figure 2-1d). Thus, both the valence and the low atomic weight of carbon give it unique properties that account for the diversity and stability of carbon-containing compounds, giving carbon a preeminent role in biological molecules.

Carbon-Containing Molecules Are Stable

The stability of organic molecules is a property of the favorable electronic configuration of each carbon atom in the molecule. This stability is expressed as **bond energy**—the amount of energy required to break 1 *mole* (about 6×10^{23}) of such bonds. (The term *bond energy* is a frequent source of confusion. Be careful not to think of it as energy that is somehow "stored" in the bond but rather as the amount of energy needed to *break* the bond.) Bond energies are usually expressed in *calories per mole (cal/mol),* where a **calorie** is the amount of energy needed to raise the temperature of 1 gram of water by 1°C and a *kilocalorie (kcal)* is equal to 1000 calories.

It takes a large amount of energy to break a covalent bond. For example, the carbon-carbon (C – C) bond has a bond energy of 83 kilocalories per mole (kcal/mol). The bond energies for carbon-nitrogen (C – N), carbon-oxygen (C – O), and carbon-hydrogen (C – H) bonds are all in the same range: 70, 84, and 99 kcal/mol, respectively. Even more energy is required to break a carbon-carbon double bond (C = C; 146 kcal/mol) or a carbon-carbon triple bond (C ≡ C; 212 kcal/mol), so these compounds are even more stable.

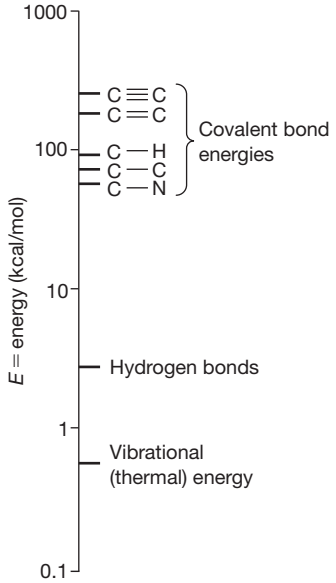


Figure 2-2 Energies of Biologically Important Bonds. Notice that energy is plotted on a logarithmic scale to accommodate the wide range of values shown.

We can appreciate the significance of these bond energies by comparing them with other relevant energy values, as shown in **Figure 2-2**. Most noncovalent bonds in biologically important molecules, such as the hydrogen bonds we will see later in this chapter, have energies of only a few kilocalories per mole. The energy of thermal vibration is even lower—only about 0.6 kcal/mol. Covalent bonds are much higher in energy than noncovalent bonds and are therefore much more stable.

The fitness of the carbon-carbon bond for biological chemistry on Earth is especially clear when we compare its energy with that of solar radiation. As shown in **Figure 2-3** on page 24, an inverse relationship exists between the wavelength of electromagnetic radiation and its energy content. This figure shows that the visible portion of sunlight (wavelengths of 380–750 nm) is lower in energy than the carbon-carbon bond is. If this were not the case, visible light would break covalent bonds spontaneously, and life as we know it would not exist.

Figure 2-3 illustrates another important point: the hazard that ultraviolet radiation poses to biological molecules due to its high energy. At a wavelength of 300 nm, for example, ultraviolet light has an energy content of about 95 kcal/mol. This is enough to break carbon-carbon bonds spontaneously. This threat underlies the current concern about pollutants that destroy the ozone layer in the upper atmosphere because the ozone layer filters out much of the ultraviolet radiation that would otherwise reach Earth's surface and disrupt the covalent bonds that hold biological molecules together.

The stability of carbon is evident not only through our study of organic chemistry here on Earth but also through exciting discoveries on other planets in our solar system. Recent work made possible by the Mars rover Curiosity has demonstrated the presence of carbon-containing compounds near the planet surface. The organic matter was detected despite the high levels of ionizing radiation bombarding the Martian planet's surface, highlighting carbon's stable nature and suggesting

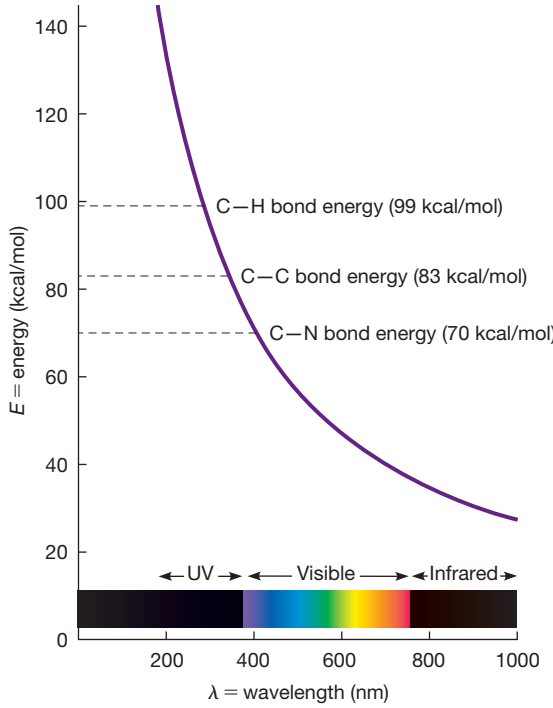


Figure 2-3 The Relationship Between Energy (*E*) and Wavelength (λ) for Electromagnetic Radiation. The dashed lines mark the bond energies of the C—H, the C—C, and the C—N single bonds. The bottom of the graph shows the approximate range of wavelengths for ultraviolet (UV), visible, and infrared radiation.

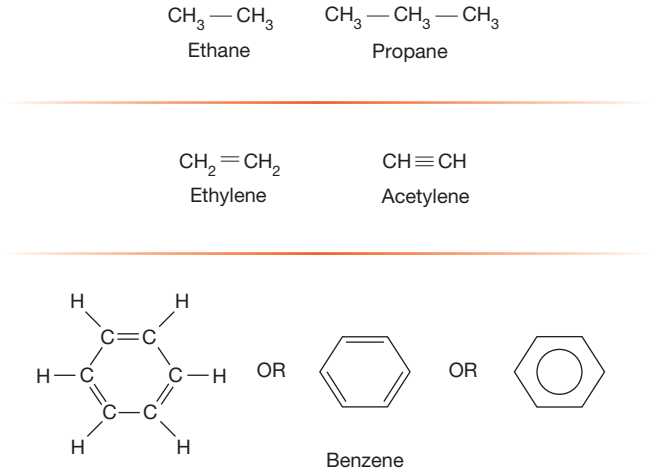


Figure 2-4 Some Simple Hydrocarbon Compounds. Compounds in the top row have single bonds only, whereas those in the second and third rows have double or triple bonds.

that more exciting organic compounds could be discovered farther below the surface of Mars where less radiation exists.

Carbon-Containing Molecules Are Diverse

In addition to their inherent stability, carbon-containing compounds are characterized by the great diversity of molecules that can be generated from relatively few different kinds of atoms. Again, this diversity is due to the tetravalent nature of the carbon atom and the resulting ability of each carbon atom to form covalent bonds to four other atoms. Because one or more of these bonds can be bonds to other carbon atoms, molecules consisting of long chains of carbon atoms can be built up. Ring compounds are also common. Further variety is possible by the introduction of branching and the presence of double bonds in the carbon-carbon chains.

When only hydrogen atoms are bonded to carbon atoms in linear or branched chains or in rings, the resulting compounds are called **hydrocarbons** (**Figure 2-4**). Economically important hydrocarbons such as hexane (C_6H_{14}), octane (C_8H_{18}), and decane ($C_{10}H_{22}$) are found in gasoline and other petroleum products. The natural gas that many of us use for fuel is a mixture of methane, ethane, propane, and butane, which are hydrocarbons with one to four carbon atoms, respectively. Benzene (C_6H_6) is a common industrial solvent.

In biology, on the other hand, hydrocarbons play only a limited role because they are essentially insoluble in water, the universal solvent in biological systems. One exception is ethylene (C_2H_4), a gas that acts as a plant hormone and is used commercially to promote fruit ripening. However, hydrocarbons do play an important role in the structure of biological membranes. The interior of every biological membrane is a nonaqueous environment consisting of the long hydrocarbon "tails" of phospholipid molecules that project into the interior of the membrane from either surface. This feature of membranes has important implications for their role as permeability barriers, as we will see shortly.

Most biological compounds contain, in addition to carbon and hydrogen, one or more atoms of oxygen and often nitrogen, phosphorus, or sulfur as well. These atoms are usually part of various **functional groups,** which are specific arrangements of atoms that confer characteristic chemical properties on the molecules to which they are attached. Some of the more common functional groups present in biological molecules are shown in **Figure 2-5**. At the near-neutral pH of most cells, several of these groups form **ions,** which are atoms or molecules that are charged because they have gained or lost an electron or a *proton* (a hydrogen atom without its electron).

For example, the *carboxyl* and *phosphate* groups, which are considered acidic because they have given up a proton, are negatively charged. By contrast, the *amino* group, which is considered basic because it has gained a proton, is positively charged. Other groups, such as the *hydroxyl, sulfhydryl, carbonyl,* and *aldehyde* groups, are uncharged at pH values near neutrality.

However, the presence of any oxygen or sulfur atoms bound to carbon or hydrogen results in a *polar bond* due to unequal sharing of electrons. This is because oxygen and sulfur have higher *electronegativity,* or affinity for electrons, than carbon and hydrogen. Therefore, when "sharing" electrons with carbon or hydrogen, an oxygen (or nitrogen) atom will have the electron more than half the time, giving it a slightly negative charge and giving the hydrogen (or carbon) a slightly positive charge. The resulting polar bonds have higher water solubility and chemical reactivity than nonpolar C – C or C – H bonds, in which electrons are equally shared.

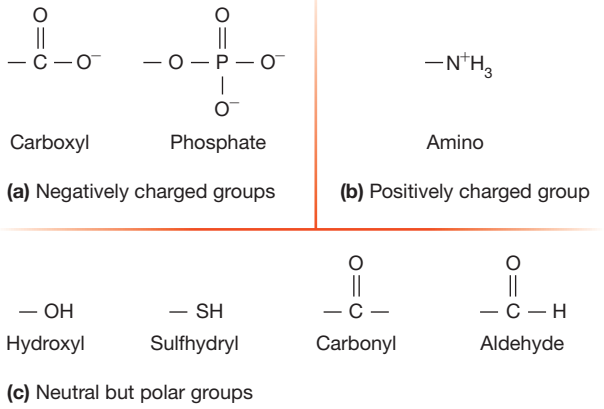


Figure 2-5 Some Common Functional Groups Found in Biological Molecules. Each functional group is shown in the form that predominates at the near-neutral pH of most cells. They are separated into **(a)** negatively charged, **(b)** positively charged, and **(c)** neutral but polar groups.

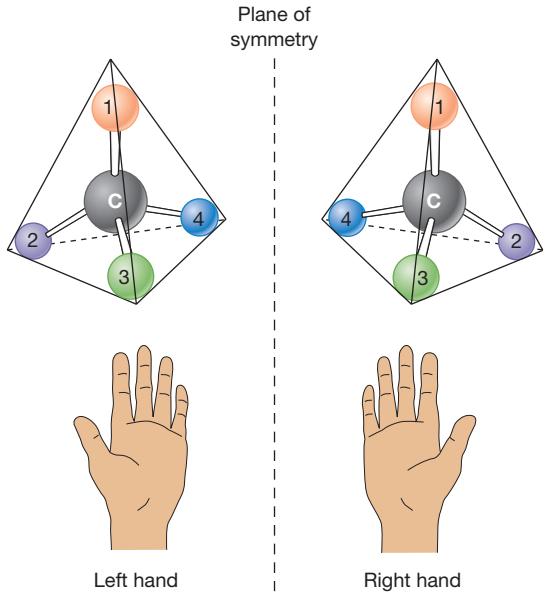


Figure 2-6 Stereoisomers. Stereoisomers of organic compounds occur when four different groups are attached to a tetrahedral carbon atom. Stereoisomers, like left and right hands, are mirror images of each other and cannot be superimposed on one another.

Often, carbon-containing compounds will lose electrons to other molecules such as molecular oxygen. This process is called *oxidation* and typically involves degradation and releases energy, as in the oxidation of glucose to carbon dioxide and water. The reverse process, by which carbon-containing compounds gain electrons, is known as *reduction* and typically is biosynthetic and requires energy, as in the photosynthetic reduction of carbon dioxide to glucose. (Oxidation and reduction will be discussed in more detail in Chapter 9; see Equations 9-7 to 9-10.)

Given the incredible diversity of chemical compounds in cells, you may wonder how it is possible to study individual compounds to determine their structure. **Key Technique, pages 26–27**, describes the use of mass spectrometry to determine the chemical structure and identity of individual chemical compounds in cells.

MAKE CONNECTIONS 2.1

Which strand of cell biology does mass spectrometry align best with? (Fig. 1-3)

Carbon-Containing Molecules Can Form Stereoisomers

Carbon-containing molecules are capable of still greater diversity because the carbon atom is a **tetrahedral** structure. When four different atoms or groups of atoms are bonded to the four corners of such a tetrahedral structure, two different spatial configurations are possible. Although both forms have the same structural formula, they are not superimposable but are, in fact, mirror images of each other as shown by the plane of symmetry, which represents the mirror. Such mirror-image forms of the same compound are called **stereoisomers** (**Figure 2-6**).

A carbon atom that has four different substituents (atoms or groups attached) is called an **asymmetric carbon atom (Figure 2-7).** Because two stereoisomers are possible for each asymmetric carbon atom, a compound with n asymmetric carbon atoms will have 2^n possible stereoisomers. As shown in Figure 2-7, the three-carbon amino acid *alanine* has

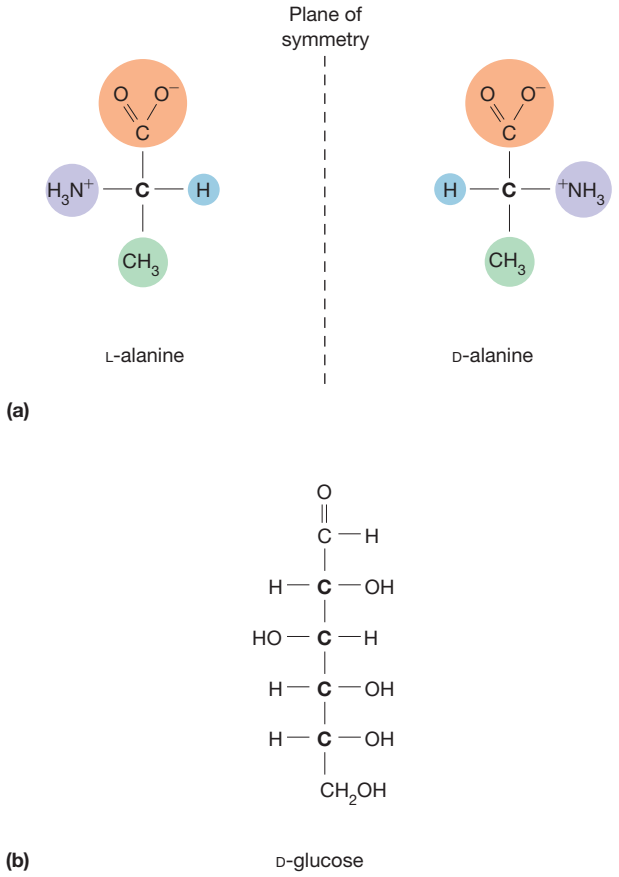


Figure 2-7 Stereoisomers of Biological Molecules. (a) The amino acid alanine has a single asymmetric carbon atom (in boldface) and can therefore exist in two spatially different forms, designated as L- and D-alanine. **(b)** The six-carbon sugar glucose has four asymmetric carbon atoms (in boldface).

Key Technique

Determining the Chemical Fingerprint of a Cell Using Mass Spectrometry

A Scientist Preparing an Injection for Mass Spectrometry.

PROBLEM: In cell biology, scientists typically study processes that involve changes in the chemistry of the cell, such as cell growth and division. Researchers often want to be able to identify small molecules in a cellular extract, or they may want to determine the chemical structure of a new compound. How is such analysis accomplished?

SOLUTION: *Mass spectrometry* (often called *mass spec*) is a method used to identify and measure the relative abundance of individual molecules in a sample, as well as to determine their chemical structure. Purified molecules are broken into fragments, and these fragments can be analyzed to determine their masses and the arrangement of covalent bonds that hold atoms of the molecule together.

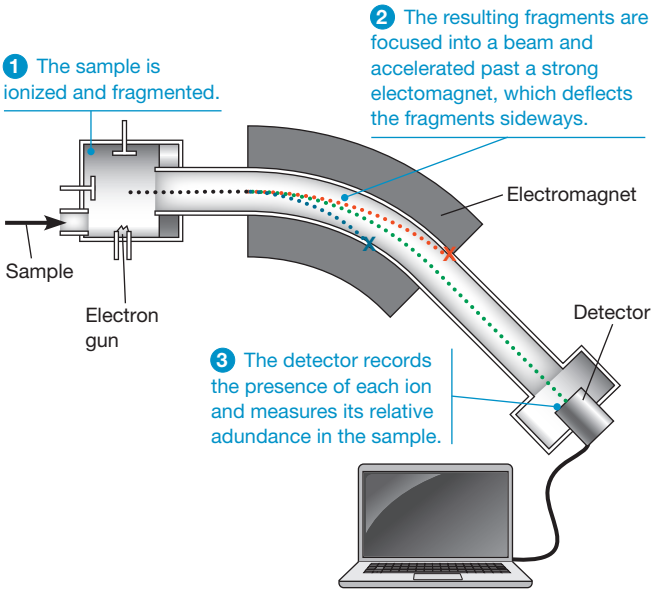


Figure 2A-1 A Mass Spectrometer.

Key Tools: Mass spectrometer; an ionized sample; a computer to analyze the results.

Details: Mass spectrometry can identify chemical compounds within a sample with high resolution, differentiating between compounds that can vary by as little as 1 atomic mass unit (amu), the mass of a hydrogen atom. Analysis of a compound using a mass spectrometer (**Figure 2A-1**) involves three main steps: *ionization and fragmentation* of the sample, *deflection* of the ionized fragments by an electromagnet, and *detection* of the individual ions and measurement of their abundance.

Ionization and Fragmentation. Commonly, the sample is ionized by bombarding it with a stream of high-energy electrons from an *electron gun*. The stream has enough energy to knock an

a single asymmetric carbon atom (in the center) and thus has two stereoisomers, called L-alanine and D-alanine (Figure 2-7a). Neither of the other two carbon atoms of alanine is an asymmetric carbon atom because one has three identical substituents (hydrogen atoms) and the other has two bonds to a single oxygen atom and thus is only bonded to three substituents. Both stereoisomers of alanine occur in nature, but only L-alanine is present as a component of proteins.

As an example of a compound with multiple asymmetric carbon atoms, consider the six-carbon sugar *glucose* shown in Figure 2-7b. Of the six carbon atoms of glucose, the four shown in boldface are asymmetric. (Can you figure out why the other two carbon atoms are not asymmetric?) With four asymmetric carbon atoms, the structure shown (D-glucose) is only one of 2^4, or 16, possible stereoisomers of the $C_6H_{12}O_6$ molecule.

CONCEPT CHECK 2.1

What properties of the carbon atom make it especially suitable as the structural basis for nearly all biomolecules?

electron off the sample to form a positively charged *molecular ion* (M^+). Molecular ions are generally unstable and will break up into smaller fragments, some of which will be positively charged and others neutral.

Deflection. The resulting fragments are focused into a fine beam and accelerated past a powerful electromagnet that separates them by mass. The mass of each fragment will depend on which particular covalent bond in the parent ion (M^+) was broken. Because each molecule fragments in a predictable pattern, the specific pattern of fragment masses can be used to identify a compound.

As the beam passes by the electromagnet, individual ions are pulled sideways, deflected from a straight path. The amount of deflection depends on the mass of the ion, with lighter ions being deflected more. The effect is like dropping a large cannonball and a small steel ball (such as can be found in a pinball machine) near a large magnet. The heavy cannonball will be pulled sideways during its flight much less than the smaller ball. The strength of the electromagnet can be increased (to deflect heavier ions) or decreased (to deflect lighter ions) so that ions of different masses are focused on a detector at the end of the spectrometer. (Only positively charged particles reach the detector.)

Detection and Analysis. The detector records the presence of each ion and, based on the strength of the magnetic field, can determine the ion's mass. It also records the number of ions having each different mass and calculates their abundance in the sample. A computer then converts this information into a graph showing vertical lines representing a *spectrum* of mass-to-charge ratios (m/z) across the *x*-axis, with the heights of the lines (also called peaks) showing each ion's relative abundance (*y*-axis). **Figure 2A-2** shows mass spectral results for a simple amino acid, glycine.

Data Interpretation. The heaviest ion in a mass spectrum (the one with the highest m/z value) is often the molecular ion. In Figure 2A-2, this is the peak at m/z = 75. (To confirm that a molecular weight of 75 corresponds to glycine, start with its elemental formula, $C_2H_5NO_2$, and sum the atomic weights of its carbons, 12 amu × 2; nitrogen, 14 amu × 1; oxygens, 16 amu × 2; and hydrogens, 1 amu × 5.)

The tallest peak in a mass spectrum, called the base peak, is assigned a *y*-axis value of 100%. The heights of all other lines are plotted relative to the base peak. The base peak typically corresponds to the most stable, hence most abundant, fragment ion. In this example, the base peak is at m/z = 30, which, for glycine, is a fragment with a single carbon, a nitrogen, and four hydrogens (CH_2NH_2). By comparing the pattern of the lines to the patterns of known compounds, a compound can often be identified. For novel compounds, the pattern of lines helps identify the fragments. By determining how the fragments would fit together to form an intact molecule, a researcher can predict the types and arrangement of the covalent bonds and determine the overall chemical structure of the novel compound.

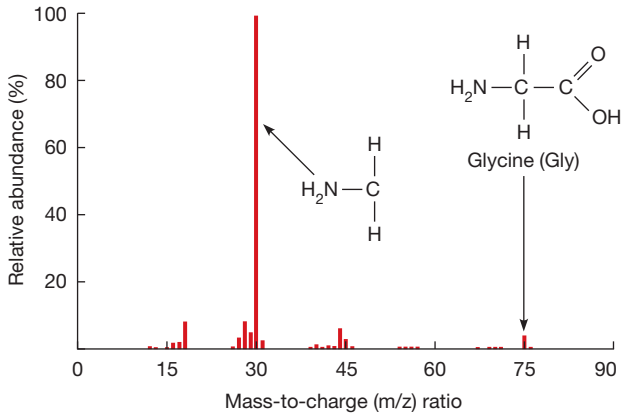


Figure 2A-2 The Mass Spectrum of Glycine. The non-ionized form of glycine is shown.

QUESTION: Compare the flights of the ionized molecular fragments whose paths are shown in blue and red in Figure 2A-1. Which fragment has the higher m/z ratio? What factors might explain why the deflection of that fragment differs from the other fragment?

2.2 The Importance of Water

Just as the carbon atom is uniquely significant because of its role as the universal backbone of biologically important molecules, the water molecule commands special attention because of its indispensable role as the universal solvent in biological systems. Water is, in fact, the single most abundant component of cells and organisms. Typically, about 75–85% of a cell by weight is water, and most cellular processes (like protein folding) take place in this aqueous environment. In addition, many cells depend on an extracellular environment that is essentially aqueous as well. In some cases, this is a body of water—whether an ocean, a lake, or a river—where the cell or organism lives, and in other cases, it may be the body fluids with which the cell is bathed. Therefore, we must always take into account the presence of water when considering the ways that a cell functions.

Water is indispensable for life. True, there are life forms that can become dormant and survive periods of severe water scarcity. Seeds of plants and spores of bacteria and fungi are

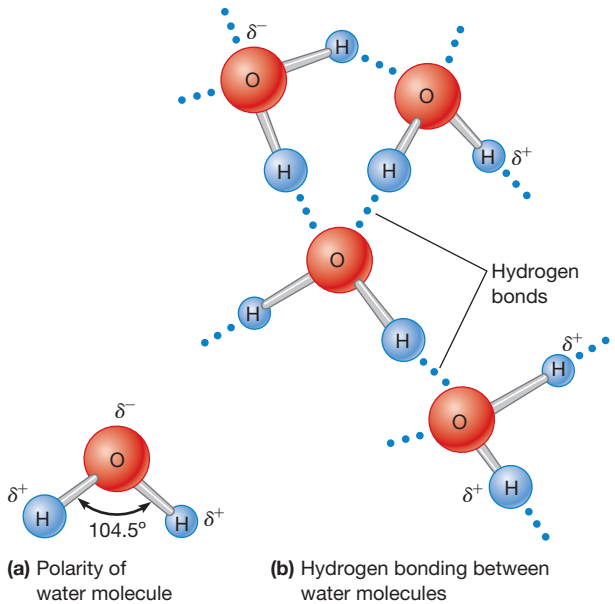
clearly in this category. Some plants and animals—notably certain mosses, lichens, nematodes, and rotifers—can also undergo physiological adaptations that allow them to dry out and survive in a highly dehydrated form, sometimes for surprisingly long periods of time. Such adaptations are clearly an advantage in environments characterized by periods of drought. Yet all of these are, at best, temporary survival mechanisms. Resumption of normal activity always requires rehydration.

The successful transport of water into and out of cells, as well between cells is also critical. Water can move across cellular membranes based on the concentration of solutes present in a process called **osmosis.** Whereas osmosis can be a slow process, water is able to move much more quickly through a specialized channel protein known as an **aquaporin (AQP).** For example, aquaporins allow water to flow rapidly between cells within certain organs like the kidneys. (You will learn more detail about the movement of water using both these mechanisms in Chapter 8.)

To understand why water is so uniquely suitable for its role, we need to look at its chemical properties. The most critical attribute of water is its *polarity* because this property accounts for its *cohesiveness,* its *temperature-stabilizing capacity,* and its *solvent properties,* all of which have important consequences for biological chemistry.

Water Molecules Are Polar

An unequal distribution of electrons gives the water molecule its **polarity,** which we can define as an uneven distribution of charge within a molecule. To understand the polar nature of water, we need to consider the shape of the molecule (**Figure 2-8**). As shown in Figure 2-8a, the water molecule is bent rather than linear in shape, with the two hydrogen atoms bonded to the oxygen at an angle of 104.5° rather than 180°. It is no overstatement to say that life depends critically on this angle because of the distinctive properties that the resulting asymmetry produces in the water molecule.



(a) Polarity of water molecule

(b) Hydrogen bonding between water molecules

Figure 2-8 Hydrogen Bonding Among Water Molecules. (a) The water molecule is polar because it has an asymmetric charge distribution, partly due to the high electronegativity of the oxygen atom. It has a partial negative charge (δ^-), and each of the two hydrogen atoms has a partial positive charge (δ^+). **(b)** The extensive association of water molecules with one another in either the liquid or the solid state is due to hydrogen bonds (blue dots).

Although the water molecule as a whole is uncharged, its electrons are unevenly distributed. The oxygen atom is highly **electronegative**—it tends to draw electrons toward it. Therefore, the oxygen atom has a partial negative charge (denoted as δ^-, with the Greek letter delta standing for "partial"), and each of the two hydrogen atoms has a partial positive charge (δ^+). This unequal distribution of charge makes any O – H bond polar and, along with the presence of two lone electron pairs, helps explain why water is such a highly polar molecule.

Water Molecules Are Cohesive

Because of their polarity, water molecules are attracted to each other so that the electronegative oxygen atom of one molecule is associated with the electropositive hydrogen atoms of adjacent molecules. This forms a **hydrogen bond** (dotted lines in Figure 2-8b), which is a type of noncovalent interaction that is about one-tenth as strong as a covalent bond.

Each oxygen atom can bond to two hydrogens, and both of the hydrogen atoms can associate in this way with the oxygen atoms of adjacent molecules. As a result, water is characterized by an extensive three-dimensional network of hydrogen-bonded molecules. Although individual hydrogen bonds are weak, the combined effect of large numbers of them can be quite significant. In liquid water, the hydrogen bonds between adjacent molecules are constantly being broken and re-formed, with a typical bond having a half-life of a few microseconds. On average, however, each molecule of water in the liquid state is hydrogen-bonded to at least three neighbor molecules at any given time. In ice, the hydrogen bonding is still more extensive, giving rise to a rigid, hexagonal crystalline lattice with every oxygen hydrogen-bonded to hydrogens of two adjacent molecules and every water molecule therefore hydrogen-bonded to four neighboring molecules.

It is this tendency to form hydrogen bonds between adjacent molecules that makes water so highly *cohesive*. This cohesiveness accounts for the high *surface tension* of water, as well as for its high *boiling point,* high *specific heat,* and high *heat of vaporization.* The high surface tension of water allows some insects to move across the surface of a pond without breaking the surface (**Figure 2-9**). High surface tension is also important in allowing water to move upward through the conducting tissues of plants.

Water Has a High Temperature-Stabilizing Capacity

An important property of water that stems directly from the hydrogen bonding between adjacent molecules is the high specific heat that gives water its *temperature-stabilizing capacity. Specific heat* is the amount of heat a substance must absorb per gram to increase its temperature 1°C. The specific heat of water is 1.0 calorie per gram.

Because of its extensive hydrogen bonding, the specific heat of water is much higher than that of most other liquids.

Figure 2-9 Walking on Water. The high surface tension of water results from the collective strength of vast numbers of hydrogen bonds. It enables insects such as this water strider to walk on the surface of a pond without breaking the surface.

In other liquids, much of the energy would cause an increase in the motion of solvent molecules and therefore an increase in temperature. In water, the energy is used instead to break hydrogen bonds between neighboring water molecules, buffering aqueous solutions against large changes in temperature. This capability is an important consideration for the cell biologist because cells release large amounts of energy as heat during metabolic reactions. If not for the extensive hydrogen bonding and the resulting high specific heat of water molecules, this release of energy would pose a serious overheating problem for cells, and life would not be possible.

Water also has a high *heat of vaporization,* which is defined as the amount of energy required to convert 1 gram of a liquid into vapor. This value is high for water because of the hydrogen bonds that must be disrupted in the process. This property makes water an excellent coolant and explains why people perspire, why dogs pant, and why plants lose water through transpiration. In each case, the heat required to evaporate water is drawn from the organism, which is therefore cooled in the process.

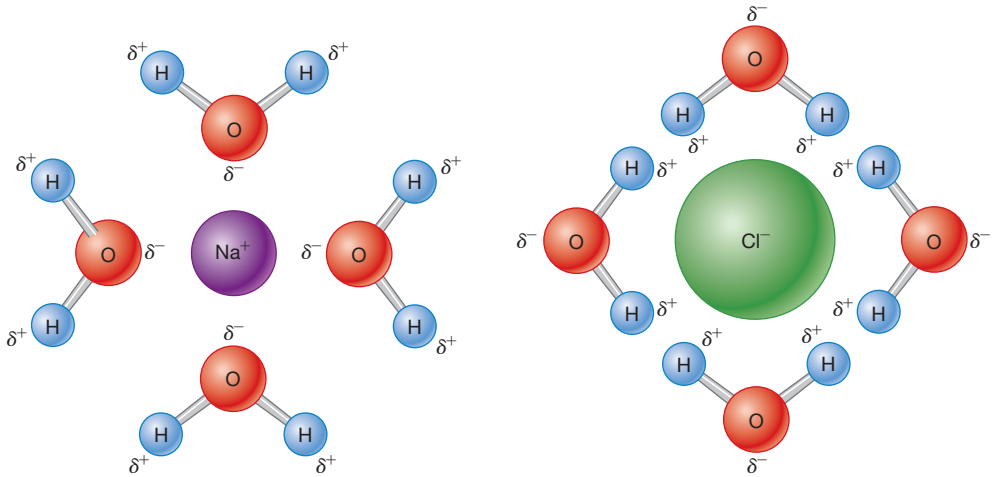
Water Is an Excellent Solvent

From a biological perspective, one of the most important properties of water is its excellence as a general solvent. A **solvent** is a fluid in which another substance, called the **solute,** can be dissolved. Water is an especially good solvent for biological purposes because of its remarkable capacity to dissolve a great variety of solutes.

It is the polarity of water that makes it so useful as a solvent. Many of the molecules in cells are also polar and therefore form hydrogen bonds with water molecules. Solutes that have an affinity for water and therefore dissolve readily in water are called **hydrophilic** ("water-loving"). Most small organic molecules found in cells are hydrophilic. Examples are sugars, organic acids, and some of the amino acids. Molecules that are not very soluble in water are termed **hydrophobic** ("water-fearing"). Among the more important hydrophobic compounds found in cells are the lipids and proteins found in biological membranes. In general, polar molecules and ions are hydrophilic, and nonpolar molecules are hydrophobic. Some biological macromolecules, notably proteins, have both hydrophobic and hydrophilic regions, so some parts of the molecule have an affinity for water whereas other parts of the molecule do not.

To understand why polar substances and ions dissolve so readily in water, let's consider a salt such as sodium chloride (NaCl) (**Figure 2-10**). Because it is a salt, NaCl exists in crystalline form as a lattice of positively charged sodium *cations* (Na^+) and negatively charged chloride *anions* (Cl^-). For NaCl to dissolve in a liquid, solvent molecules must overcome the attraction of the Na^+ cations and Cl^- anions for each other. When NaCl is placed in water, both the sodium and chloride ions become involved in *electrostatic interactions* with the water molecules instead of with each other, and the Na^+ and Cl^- ions separate and become dissolved. Because of their polarity, water molecules can form *spheres of hydration* around both Na^+ and Cl^-, thus neutralizing their attraction for each other and decreasing their likelihood of reassociation.

As Figure 2-10a shows, the sphere of hydration around a cation such as Na^+ involves water molecules clustered around the ion with their negative (oxygen) ends pointing toward it. For an anion such as Cl^-, the orientation of the



(a) Hydration of sodium ion

(b) Hydration of chloride ion

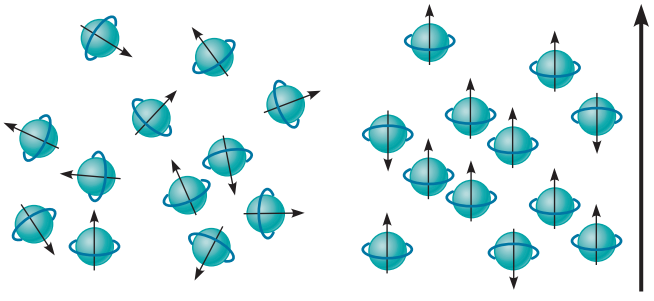
Figure 2-10 The Solubilization of Sodium Chloride. Sodium chloride (NaCl) dissolves in water because spheres of hydration are formed around both **(a)** the sodium ions and **(b)** the chloride ions. The oxygen atom and the sodium and chloride ions are drawn to scale.

Taking a Deeper Look: Magnetic Resonance Imaging (MRI)

We often take the water content of our bodies for granted. On average, the human body is 55–65% water, depending on age and weight. But the chemistry of water also provides an opportunity: each water molecule possesses two hydrogen atoms, which are constantly breaking and re-forming hydrogen bonds with surrounding molecules. In addition, most biological molecules contain hydrogen, and the products of many biological reactions release hydrogen ions into surrounding tissues. It is these hydrogen ions, so plentiful in water and tissue, especially the circulatory system, that make one of the safest imaging technologies possible: *magnetic resonance imaging,* or *MRI.*

It is very likely that you or someone you know has had an MRI to diagnose a medical condition, but how does an MRI device exploit water in tissues to produce images of the body? MRI is a noninvasive method that uses basic principles of chemistry and physics to visualize internal structures of the body. An MRI machine capitalizes on the ability of protons to align with an externally applied magnetic field by placing the patient inside the field of a powerful electromagnet. In the absence of an applied magnetic field, the magnetic fields of individual hydrogens are randomly oriented. When a magnetic field is applied, however, most of the hydrogen atoms in the patient's body will align in one of two directions: either "up" or "down" with respect to the applied field (**Figure 2B-1**). Then a second, oscillating magnetic field is applied, which causes the aligned hydrogen atoms to absorb energy, which they subsequently release in the form of radio waves as the magnetic field changes. By rapidly varying the main magnetic field using a special set of magnetic coils, the release of these radio waves is detected by a receiver and provides relevant spatial information. The rapid switching of these coils on and off gives rise to the familiar repetitive clicking sounds made by an MRI machine. Once the radio waves are detected, a computer creates a spatial map of energy differences to produce an image of the tissue.

All tissues in the body vary in density and water content. Because an MRI requires a change in orientation of hydrogen ions in a magnetic field, these differences throughout the body



(a) Before magnetic field applied: random orientation of proton spins

(b) After magnetic field (large arrow) applied: proton spins oriented

Figure 2B-1 Hydrogen Atom Spin Patterns. (a) The magnetic field orientation of hydrogen atoms in the absence of a magnetic field is random. **(b)** The magnetic fields of hydrogen atoms align in the presence of an applied magnetic field.

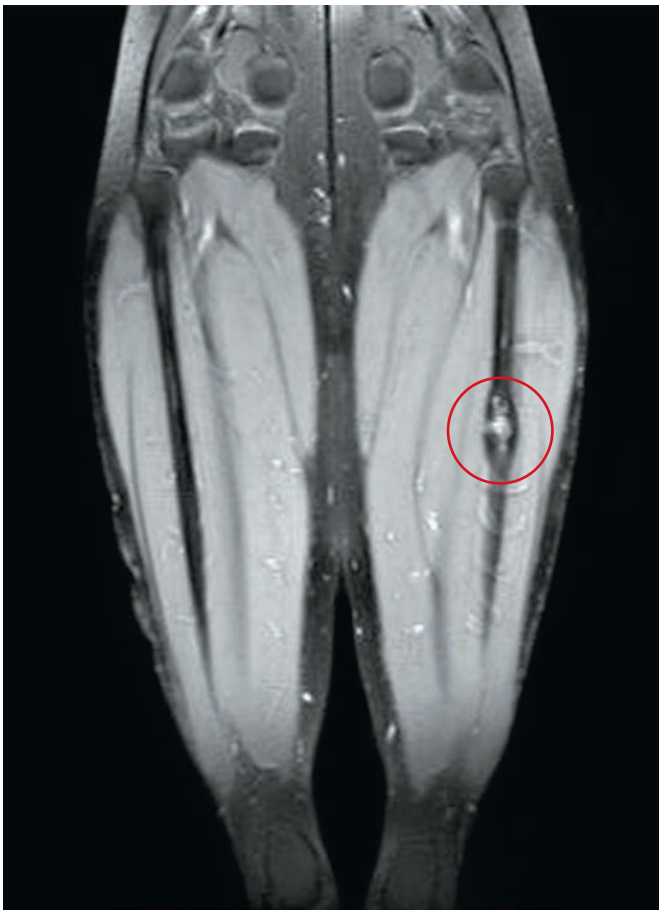


Figure 2B-2 MRI of Tumor on the Femur. The small tumor is easily visible (red circle).

actually provide the perfect contrast for visualization. By changing magnetic field strength or the frequency of the radio waves used during an MRI, a specific organ or tissue can be highlighted. For example, water and fluid-containing tissues are bright, whereas fatty tissues with little water are darker. These differences produce striking images of individual organs that can be examined for tumors or other abnormalities (**Figure 2B-2**). For many purposes, the images produced in an MRI scan are far better than those produced by taking multiple X-ray images and reconstructing them (a technique known as *computer-aided tomography,* or a *CAT scan*). With the addition of contrast agents injected into a patient's bloodstream or other tissues, specific structures such as blood vessels can be imaged with extraordinary detail using MRI.

The cost of an MRI procedure in the United States can range from $1000 to $2000. While more expensive than an X-ray, this procedure may be a better choice for many patients due to its potential for more accurate diagnoses. Unlike traditional X-rays, which can image only dense structures like bone and can cause damage to DNA, an MRI can give a detailed image of soft tissue such as internal organs, blood vessels, and nerves without posing a significant health risk.

water molecules is reversed, with the positive (hydrogen) ends of the solvent molecules pointing in toward the ion (Figure 2-10b). Similar spheres of hydration develop around charged functional groups (see Figure 2-5a, b), increasing their solubility. Even uncharged polar functional groups, such as aldehyde or sulfhydryl groups (see Figure 2-5c), will have a sphere of hydration, as the polar oxygen or sulfur atoms attract the positively charged ends of the polar water molecule and increase solubility.

Some biological compounds are soluble in water because they exist as ions at the near neutral pH of the cell and are therefore solubilized and hydrated like the ions of Figure 2-10. Compounds containing carboxyl, phosphate, or amino groups are in this category (see Figure 2-5). Most organic acids, for example, are almost completely ionized by deprotonation at a pH near 7 and therefore exist as anions that are kept in solution by spheres of hydration, as we have just seen with the chloride ion in Figure 2-10b. Amines, on the other hand, are usually protonated at cellular pH and thus exist as hydrated cations, and behave like the sodium ion in Figure 2-10a.

Often, organic molecules have no net charge but are nonetheless hydrophilic because they have some regions that are positively charged and other regions that are negatively charged and thus are soluble in water. Also, compounds containing the polar hydroxyl, sulfhydryl, carbonyl, or aldehyde groups shown in Figure 2-5 are also water soluble due to hydrogen bonding of these polar groups with water molecules.

Hydrophobic molecules such as hydrocarbons, on the other hand, have no such polar regions and therefore show no tendency to interact electrostatically with water molecules. In fact, because they disrupt the hydrogen-bonded structure of water, they tend to be excluded by the water molecules. Therefore, hydrophobic molecules tend to coalesce as they associate with one another rather than with the water. As we will see later in the chapter, such associations of hydrophobic molecules (or parts of molecules) are a major driving force in the folding of proteins, the assembly of cellular structures, and the organization of membranes.

Because typical cells are primarily water and most other biological molecules in cells contain hydrogen, the chemical and physical properties of the hydrogen atom can be exploited for medical imaging purposes. The common procedure known as magnetic resonance imaging (MRI) takes advantage of this abundance of water to image internal body tissues in a noninvasive manner (**see Human Connections, page 30**).

CONCEPT CHECK 2.2

How would the properties of water change if the water molecule were linear rather than bent? Why would it be less satisfactory as the basis for living cells?

2.3 The Importance of Selectively Permeable Membranes

Every cell and organelle needs some sort of physical barrier to keep its contents in and external materials out. A cell also needs some means of controlling exchange between its internal environment and the extracellular environment. Ideally, such a barrier should be impermeable to most of the molecules and ions found in cells and their surroundings. Otherwise, substances could diffuse freely in and out, and the cell would not really have a defined content at all. On the other hand, the barrier cannot be completely impermeable. If it were, necessary exchanges of material between the cell and its environment could not take place. The barrier must be insoluble in water so that it will not be dissolved by the aqueous medium of the cell. At the same time, it must be readily permeable to water because water is the basic solvent system of the cell and must be able to flow into and out of the cell as needed.

As you might expect, the membranes that surround cells and organelles satisfy these criteria admirably. A cellular **membrane** is essentially a hydrophobic permeability barrier that consists of *phospholipids, glycolipids,* and *membrane proteins*. In most organisms other than bacteria, the membranes also contain *sterols—cholesterol* in the case of animal cells, *ergosterols* in fungi, and *phytosterols* in the membranes of plant cells. (Don't be concerned if you haven't encountered these kinds of molecules before; we'll meet them all again in Chapter 3.)

Most membrane lipids and proteins are not simply hydrophobic or hydrophilic. They typically have both hydrophilic and hydrophobic regions and are therefore referred to as **amphipathic molecules** (the Greek prefix *amphi–* means "of both kinds" and pathic means "to feel"). The amphipathic nature of membrane phospholipids is illustrated in **Figure 2-11** on page 32, which shows the structure of *phosphatidylethanolamine,* a prominent phospholipid in many kinds of membranes. The distinguishing feature of amphipathic phospholipids is that each molecule consists of a polar *head* and two nonpolar hydrocarbon *tails.* The polarity of the hydrophilic head is due to the presence of a negatively charged phosphate group that is often linked to a positively charged group—an amino group, in the case of phosphatidylethanolamine and most other phosphoglycerides. (We will learn more about phospholipids in Chapter 3.)

Soap is a familiar amphipathic molecule that you all have likely used to dissolve grease, oil, and other nonpolar substances. The nonpolar hydrocarbon tails of the soap molecules interact with and surround the oil or grease, and the polar heads interact with water, enabling the oil or grease to be washed away. In the lab, we often use the amphipathic detergent sodium dodecyl sulfate (SDS) to isolate insoluble proteins and lipids. SDS has a negatively charged sulfate group attached to a single hydrocarbon chain of 12 carbons and acts much like the soap described above to solubilize nonpolar and amphipathic molecules.

A Membrane Is a Lipid Bilayer with Proteins Embedded in It

When exposed to an aqueous environment, amphipathic molecules undergo hydrophobic interactions. In a membrane, for example, phospholipids are organized into two layers: their polar heads face outward toward the aqueous environment on both sides, and their hydrophobic tails are hidden from the water by interacting with the tails of other molecules oriented in the opposite direction. The resulting structure is the

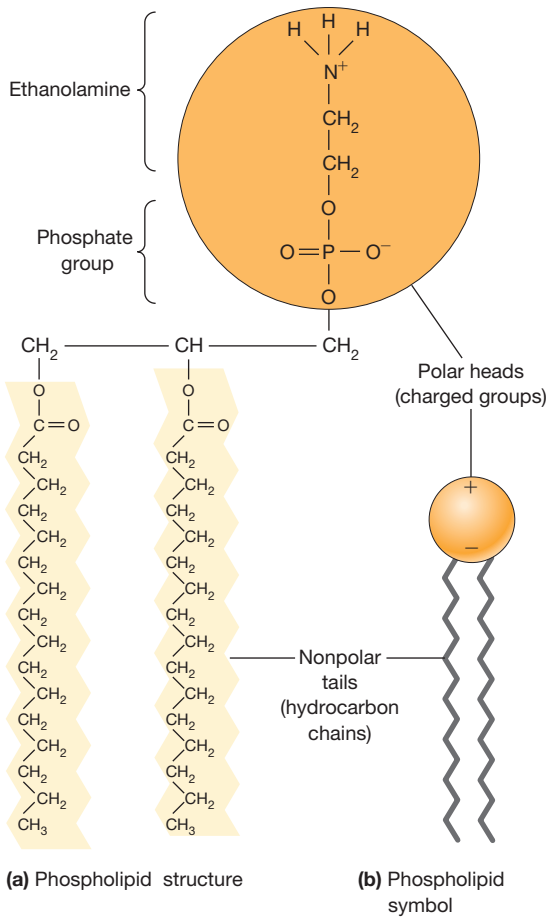


Figure 2-11 The Amphipathic Nature of Membrane Phospholipids. (a) A phospholipid molecule consists of two long nonpolar tails (yellow) and a polar head (orange). The polarity of the head of a phospholipid molecule results from a negatively charged phosphate group linked to a positively charged group—an amino group, in the case of the phosphatidylethanolamine shown here. **(b)** A phospholipid molecule is often represented schematically by a circle for the charged polar head and two zigzag lines for the nonpolar hydrocarbon chains.

lipid bilayer, shown in **Figure 2-12**. The heads of both layers face outward, and the hydrocarbon tails extend inward, forming the continuous hydrophobic interior of the membrane.

Every known biological membrane has such a lipid bilayer as its basic structure. Each of the lipid layers is typically 3–4 nm thick, so the bilayer has a width of ~7–8 nm.

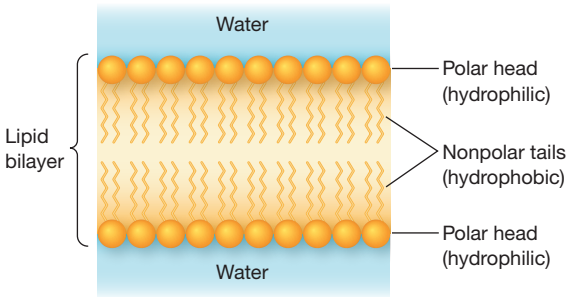


Figure 2-12 The Lipid Bilayer as the Basis of Membrane Structure. Phospholipids in an aqueous environment orient themselves in a double layer, with the hydrophobic tails buried on the inside and the hydrophilic heads interacting with the aqueous environment on either side of the membrane.

Lipid Bilayers Are Selectively Permeable

Because of its hydrophobic interior, a lipid bilayer is readily permeable to nonpolar molecules. However, it is quite impermeable to most polar molecules and is highly impermeable to all ions (**Figure 2-13**). Because most cellular constituents are either polar or charged, they have little or no affinity for the membrane interior and are effectively prevented from entering or escaping from the cell. Small, uncharged molecules are an exception, however. Compounds with molecular weights below about 100 readily diffuse across membranes, meaning they can freely and spontaneously pass through membranes, regardless of whether they are nonpolar (O_2 and CO_2) or polar (water and ethanol). Water is an especially important example of a very small molecule that, though polar, diffuses rapidly across membranes and can readily enter or leave cells.

Large, uncharged polar molecules, such as glucose and sucrose, can diffuse across the membrane, but to a lesser extent than small molecules. In contrast, even the smallest ions are effectively excluded from the hydrophobic interior of the membrane. For example, a lipid bilayer is at least 10^8 times less permeable to small cations such as Na^+ or K^+ than to water. This striking difference is due to both the charge on an ion and the sphere of hydration surrounding the ion.

Of course, it is essential that cells have ways of transferring not only ions such as $Na^+$and K^+ but also a wide variety of polar molecules across membranes that are not otherwise permeable to these substances. To transport these substances into and out of the cell, biological membranes are equipped with a wide variety of transport proteins (which we will discuss in detail in Chapter 8). A transport protein is a specialized transmembrane protein that serves either as a *hydrophilic channel* through an otherwise hydrophobic membrane or as a *carrier* that binds a specific solute on one side of the membrane and then undergoes a change to its **conformation,** or three-dimensional shape, in order to move the solute across the membrane.

Whether a channel or a carrier, each transport protein is specific for a particular molecule or ion (or, in some cases, for a class of closely related molecules or ions). Moreover, the

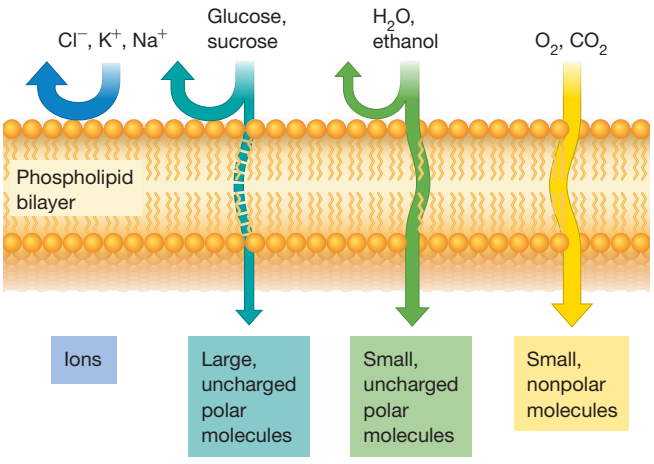


Figure 2-13 Permeability of Membranes to Various Classes of Solutes. The relative thicknesses of the arrows show the proportion of each substance that can freely cross the membrane (wavy arrows) or is repelled by the membrane (curved arrows.)