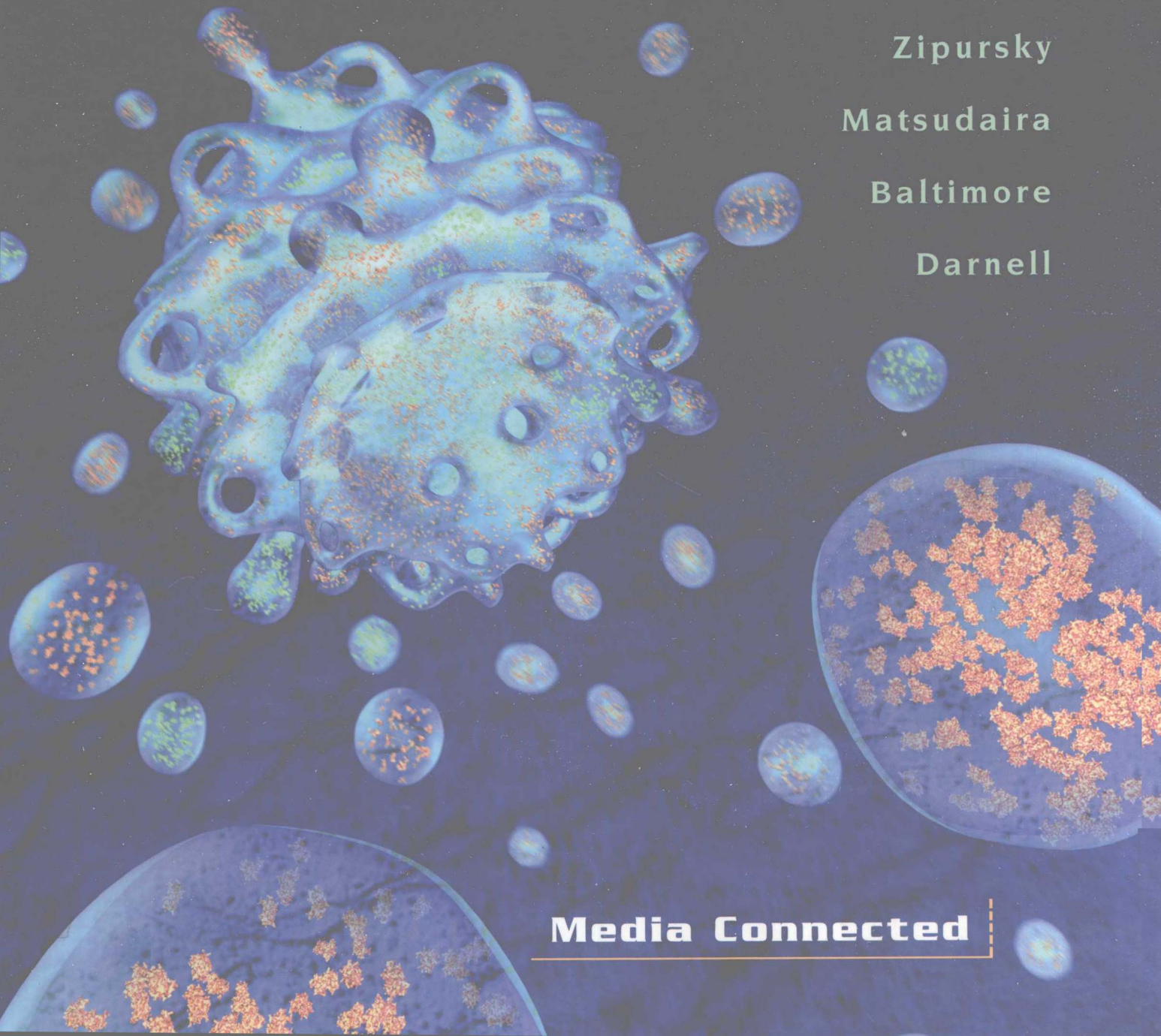
FOURTH EDITION

MOLECULAR CELL BIOLOGY

Lodish
Berk
Zipursky
Matsudaira
Baltimore
Darnell



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Harvey Lodish
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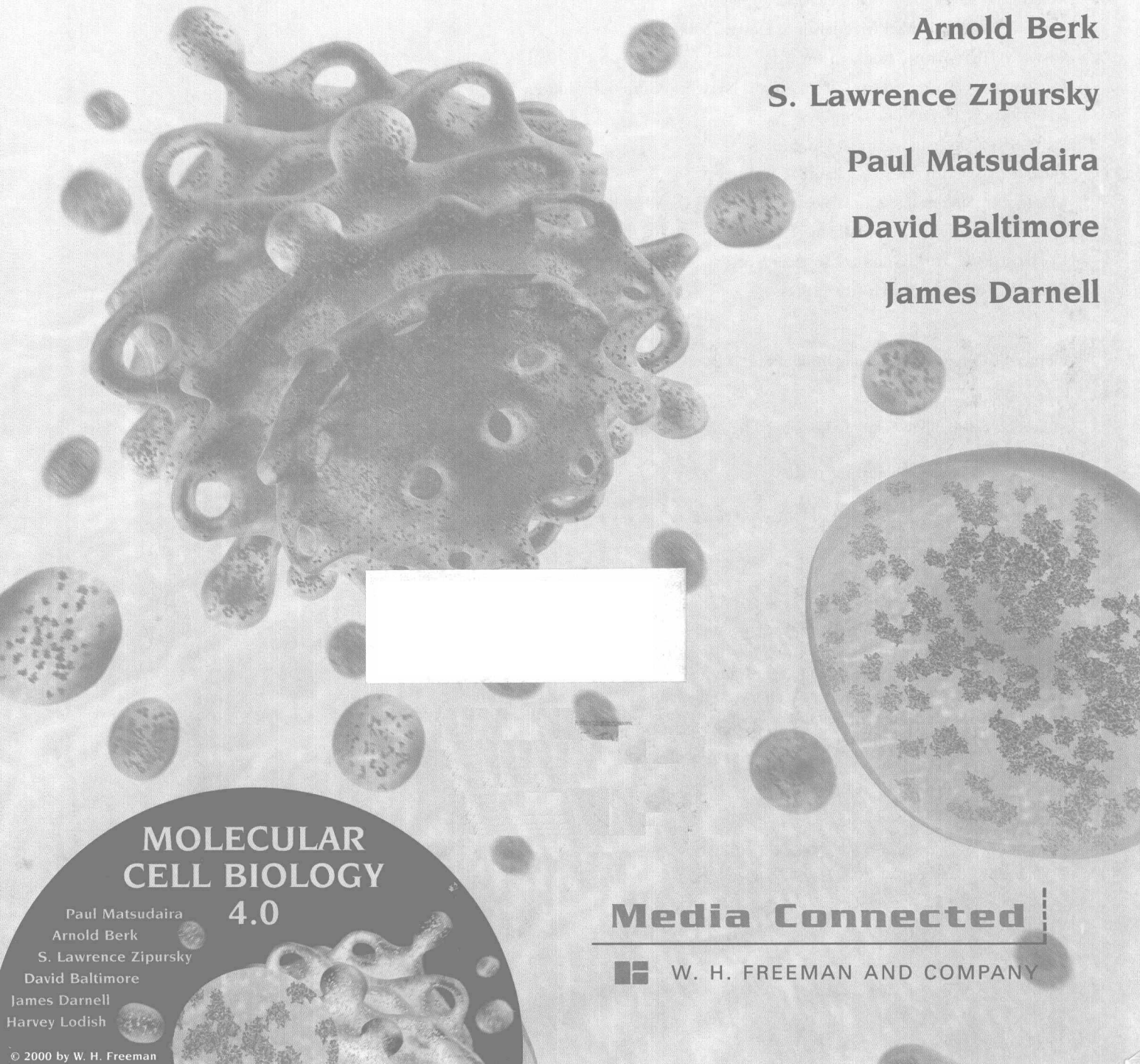

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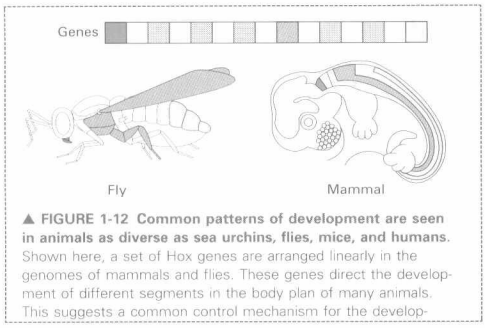
To our students and to our teachers,
from whom we continue to learn

Preface

Molecular Cell Biology for the 21st Century

Modern biology is rooted in an understanding of the molecules within cells and of the interactions between cells that allow construction of multicellular organisms. The more we learn about the structure, function, and development of different organisms, the more we recognize that **all life processes exhibit remarkable similarities.** *Molecular Cell Biology* concentrates on the macromolecules and reactions studied by biochemists, the processes described by cell biologists, and the gene control pathways identified by molecular biologists and geneticists. In this millennium, two gathering forces will reshape molecular cell biology: *genomics*, the complete DNA sequence of many organisms, and *proteomics*, a knowledge of all the possible shapes and functions that proteins employ.



▲ **FIGURE 1-12 Common patterns of development are seen in animals as diverse as sea urchins, flies, mice, and humans.** Shown here, a set of Hox genes are arranged linearly in the genomes of mammals and flies. These genes direct the development of different segments in the body plan of many animals. This suggests a common control mechanism for the develop-

All the concepts of molecular cell biology continue to be derived from experiments, and powerful experimental tools that allow the study of living cells and organisms at higher and higher levels of resolution are being developed constantly. In this fourth edition, we address the current state of molecular cell biology and look forward to what further exploration will uncover in the twenty-first century.

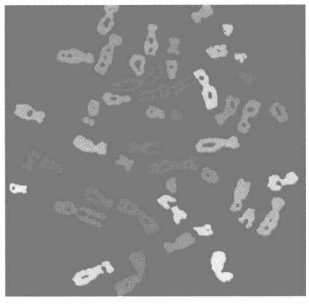
New Discoveries, New Methodologies

Since the publication of the third edition of this text in 1995, extraordinary developments have taken place:

- The **entire genomes** of yeast, a nematode, and many bacterial species have been sequenced (Chapter 7). Now researchers are racing, with corporate and government sponsorship, to sequence all 3 billion base pairs of the **human genome,** and thus identify the sequence of each of the ~70,000 proteins it encodes, by the year 2001. How do we store and use this new information? A rapidly developing area of computer science, **bioinformatics,** is devoted to collecting, organizing, and analyzing DNA and protein sequences (Chapter 7).

From Figure 7-39.

- The simultaneous expression of thousands of genes can now be analyzed using newly developed **DNA "chip" microarray technology,** enhancing our understanding of gene control during development and disease states (Chapter 7).
- **Macromolecular synthesis** is now understood to require large multiprotein machines. The detailed structure of the ribosome has advanced our knowledge of the steps in peptide bond formation (Chapter 4), and we now understand many of the proteins that participate in and regulate steps of DNA replication (Chapter 12) and messenger RNA synthesis (Chapter 10).



Chapter 9 opening photo; chromosome painting

- **Chromosome "painting,"** using fluorescent in situ hybridization, allows each human chromosome in a metaphase spread to be clearly distinguished on the basis of size and color (Chapter 9). This method greatly increases the sensitivity for detecting chromosomal translocations in cancer cells (Chapter 24).
- We now understand a great deal about how **chromatin structure** is modified by histone acetylation to **regulate gene expression,** and how the RNA polymerase II holoenzyme interacts with activators and coactivators to **regulate transcription** (Chapter 10).
- Significant advances have been made in understanding the processes that regulate **transport into and out of the nucleus** through nuclear pore complexes, and their interactions with importins, exportins, and the Ran-GTPase (Chapter 11).
- The function of many proteins essential for **vesicle budding and fusion,** and for **targeting proteins** to specific subcellular organelles is understood in detail, and our understanding of **vesicular transport through the Golgi** has undergone a major revision (Chapter 17).
- New findings about microfilaments and microtubule dynamics and the role of various motor proteins, including myosin V and kinesin-related proteins, give us a better understanding of **cell motility and mitosis** (Chapters 18 and 19).

• We now understand a great deal more about **apoptosis** (regulated cell death). New discoveries in the molecular mechanisms of cell death inform our understanding of the mechanisms of development; coverage of the pathways relevant to cell death sheds light on cancer and neurological diseases (Chapter 23).

• The essential features of intricate **cell signaling pathways** that start at cell surface receptors and control cell proliferation, growth, and motility have been identified. For example, the structures of G proteins and G-protein complexes have been determined through x-ray crystallography, and are providing important insights into the mechanisms by which G proteins regulate many aspects of cell function (Chapter 20).

Countless other new developments are incorporated in this completely rewritten, fully updated fourth edition.

The author team working together.

Streamlined Coverage

With each edition, and with all that is new, the question arises: **What should we cut out and what should we cover** to provide the most useful text possible to students and professors of this course? We have consulted with professors across the country and worked closely as an author team to streamline our coverage in each chapter and across the book. In streamlining coverage within a chapter we chose, where appropriate, to **illustrate key concepts with one key experiment rather than several.** In streamlining coverage across the book we have **reorganized and combined chapters** to reflect the connections we see between and among topics. And we have **added more pedagogical aids** to help the student succeed in this course.

The four-part structure of the previous edition continues to reflect our broader concept of the organization of this material. Changes in organization and coverage are noted below.

In Part I, "Laying the Groundwork," we present the scope of the book in an all-new Chapter 1. We lay the groundwork for understanding the experimental and conceptual basis of *Molecular Cell Biology* in Chapters 2–8. Coverage of membrane structure and cell organization is now presented early in the book (Chapter 5).

In Part II, "Nuclear Control of Cellular Activity," we teach students how genes work. We now discuss transcription and RNA processing (Chapters 10 and 11) before DNA replication, repair, and recombination (Chapter 12). We have moved the chapter on the eukaryotic cell cycle to this part because of its close relationship to those on DNA replication and control of gene expression. In our chapter on gene control in development (Chapter 14), we have added a full section on plant development in the model organism *Arabidopsis*.

In Part III, "Building and Fueling the Cell," we focus on the ways in which proteins work together to make a living cell. We have combined coverage of cellular energetics in plant and animal cells into a single chapter to focus attention on the commonalities in these processes: generation of a proton motive force and its utilization in ATP synthesis (Chapter 16). We have combined discussion of organelle biogenesis and protein secretion into a single chapter on protein sorting to emphasize the multiple mechanisms by which proteins are targeted to specific subcellular locations (Chapter 17).

In Part IV, "Cell Interactions," we emphasize how cells interact with each other, in normal and abnormal situations. We have consolidated the discussion of cell adhesion molecules and junctions (Chapter 22) and added a new chapter on cell interactions in development (Chapter 23) that includes expanded information on the role of the TGFβ pathway in determining the overall organization of early vertebrate embryos, and also coverage of regulated cell death. We conclude with a completely new chapter on cancer (Chapter 24), which integrates much material presented earlier in the book, on the cell cycle, cell-cell signaling, DNA repair, and interactions of cells with the extracellular matrix. For the first time, we cover immunology in a hot-linked chapter on our Web site to expose students to the original literature, and to gain the flexibility to update coverage of this fast-moving field.

Training the Scientists of Tomorrow

We have always believed that it is critical to present to students the **experimental basis of our understanding**—to show them *how* we know what we know. We hope that this will demonstrate the dynamic nature of science and prepare them not only to engage actively in scientific research and teaching but also to become educated members of a public that increasingly is asked to deal with complex issues such as environmental toxins, genetically modified foods, and human gene technology.

To further this end, we have added a new set of features to this edition: **Perspectives for the Future** and **Perspectives in the Literature.** Perspectives for the Future is a brief essay that gives us an opportunity to discuss potential future developments. Perspectives in the Literature is a related

PERSPECTIVES for the Future

During the past two decades remarkable progress has been made in understanding the mechanisms regulating gene transcription during development. Through a combination of genetic and biochemical studies, the DNA sequences regulating expression of many different genes in simple and higher eukaryotes and the transcription factors that bind to them have been identified. I
grams in various organisms
only on combinations of tr
specific cells but also on int
extracellular signals acting
through their direct interac
Continuation of such studie
into the way specific genes
and times in development

p. 573

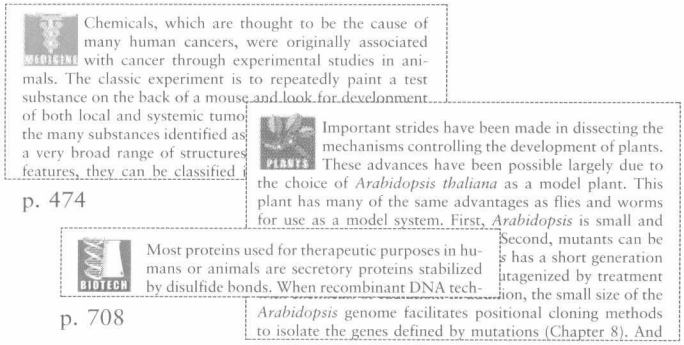
PERSPECTIVES in the Literature

You are working with a well-characterized in vitro system that allows you to induce myoblasts to differentiate into myotubes synchronously. Your long term goal is to describe the transcriptional regulatory networks that control muscle differentiation. An important step in your studies is to describe patterns of gene expression. You are interested in studying which genes are turned on and off and in what order during the transition from a myoblast to a differentiated myotube. Design a set of experiments that would allow you

p. 574

critical-thinking question that challenges students to solve a problem working with original research and review articles and resources available on the Web.

Of course, we want to show students not only how we know what we know but why we do what we do. As in previous editions, our coverage of **medical topics, biotechnology, and plant biology** is integrated throughout. We know that these topics may be of particular interest to your students, so we have highlighted them in context with clear icons.



Text, Figures, and Animations Developed Together

In this edition, we have worked simultaneously on the development of the text and of the animations available on our CD-ROM. Through careful planning and collaboration, we have developed a **CD that is an integral part of the text.** We have developed thirty-five new, aesthetically pleasing and pedagogically useful animations that are visually consistent with the figures in the book.

Acknowledgements

In updating, revising, and rewriting this book, we were given invaluable help by many colleagues. We thank the following people who generously gave of their time and expertise by making contributions to specific chapters in their areas of interest, providing us with detailed information about their courses, or by reading and commenting on one or more chapters:

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We thank Sara Tenney, our acquiring editor, for overseeing the seamless integration of our text and media package and for organizing our "group reads"—opportunities for us to work together on all of the chapters. Thanks to Kate Ahr and Ruth Steyn, our development editors, for their devoted efforts in streamlining the text and clarifying the figures. Thanks also to Kay Ueno for her extensive contributions to the initial planning of this edition. Jennifer MacMillan, our photo researcher, was particularly adroit at tracking down the photographs we needed.

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Special thanks to our families for inspiring us and for granting us the time it takes to work on such a book.

October 1999

Supplements

In preparing the supplements package for *Molecular Cell Biology*, we have drawn on our collective experience with the instruction of cellular and molecular biology at almost every level taught, both undergraduate and graduate, at Virginia Polytechnic Institute and State University.

The Virginia Tech supplements author team.

Together, we have written the end-of-chapter questions for the textbook, the self-test questions resident on the companion CD-ROM and Web site, the review questions and more challenging data analysis questions for the new study guide/problems book, *Working with Molecular Cell Biology: A Study Companion*, and the test bank questions that parallel the questions found in the study companion. We've worked to produce a package that is not only extremely useful to students and instructors but also closely integrated with the goals of text, and with each individual supplement.

Special thanks to our families for their support and encouragement. Muriel Lederman extends a special thanks to Jill Sible and Bruce Turner.

Brian Storrie
Muriel Lederman
Eric A. Wong
Richard Walker
Glenda Gillaspy

October 1999

For the Instructor

Print and Computer Test Banks *NEW*

Brian Storrie, Muriel Lederman, Eric A. Wong, Richard Walker, and Glenda Gillaspy, Virginia Polytechnic Institute and State University.

Print Test Bank: 0-7167-3601-2; Computer Test Bank CD-ROM (Windows/Macintosh hybrid) 0-7167-3603-9

Realizing that instructors would appreciate an occasional inspiration when writing tests, we've chosen to add a test bank to our instructor's materials. Questions parallel those posed in both the Study Companion and the end-of-chapter review—the same concept or principle is asked in different ways. Questions are also posed in a number of different formats: short-answer, essay, and multiple choice. The electronic version of the test bank allows instructors to edit and rearrange the questions, or add their own questions.

In addition, instructors can visit the password-protected **Online Instructor Test Bank** on the **Molecular Cell Biology Web Site Companion** to access favorite test questions submitted by their peers, or post their own questions.

Instructor's Resource CD-ROM *NEW*

 0-7167-3600-4

Contains all art from the text, plus all animations and videos, with a powerful and easy-to-use presentation manager application, Presentation Manager Pro. Source files for the resources are also provided for instructors using other presentation programs.

Overhead Transparency Set

0-7167-3605-5

275 full-color figures from the text, optimized for classroom projection, in one volume. Available free to qualified adopters.

Instructor's Solutions Manual *NEW*

Brian Storrie, Muriel Lederman, Eric A. Wong, Richard Walker, and Glenda Gillaspy, Virginia Polytechnic Institute and State University. 0-7176-3752-3

Contains answers for all end-of-chapter questions. Also includes a convenient print version of the User's Guide for the Instructor's Resource CD-ROM.

For the Student

Molecular Cell Biology 4.0 CD-ROM Companion *NEW*

(hybrid format for Windows and Macintosh)

Packaged with every copy of the textbook.

Animations authored by Paul Matsudaira, Arnold Berk, S. Lawrence Zipursky, James Darnell, and Harvey Lodish, with Tanya Awabdy.

With contributions from: David Marcey, California Lutheran University; Brian Storrie, Muriel Lederman, Eric A. Wong, Richard Walker, and Glenda Gillaspy, Virginia Polytechnic Institute and State University; Lisa Rezende, Harvard Medical School; Ruth Alscher, Virginia Polytechnic Institute and State University.

Visualization of cell and molecular processes helps students better understand the relationship between structure, function, and process. In addition to the **book animations,** the CD also features several valuable visual supplements:

- **Videos of cell processes** using cutting-edge techniques that vividly illustrate the dynamic nature of the cell;
- Interactive macromolecular biology **tutorials** by David Marcey, to help students better understand the relationship between structure and function in cell and molecular biology;
- Illustrated, printable **essays on classic experiments** in molecular and cell biology which treat the investigative side of classic groundbreaking experiments by exploring the process of asking questions and devising tests.

To help students put cell and molecular processes in context, the CD also features a visual **"Cell Navigation" interface.** Clicking on structures and organelles brings up animations, videos, and other resources pertaining to that cellular feature.

An interactive review tutorial also resides on the CD; additionally, for students intending graduate study, we provide a **timed MCAT/GRE prep exam** referenced to the text.

Molecular Cell Biology Web Site Companion: *NEW*

www.whfreeman.com/biology

Michael Klymkowsky, University of Colorado at Boulder

The companion Web site to the text provides students with a **bridge to the world of working cell and molecular biologists.** Updated links to Web sites referenced in the text, plus suggestions for how to explore topics in-depth using Internet resources, are just some of the features of the site.

We've also included valuable enrichment resources to bring students to the real world of molecular cell biology:

- ***Working with the Literature***—selected scientific papers on topics covered in the text, with questions based on the data and methods presented, to help students navigate though current scientific literature and better understand the experimental process;
- ***Classic Experiments*** **essays** which treat the investigative side of classic groundbreaking experiments by exploring the process of asking questions and devising tests;
- ***Analyzing Experiments*** **questions** test students' ability to apply concepts and understand data and experiments;
- ***Integrative Biology Topics*** presents printable and hypertext optional chapters on subjects related to molecular and cell biology, such as immunology, developmental biology, among others, for instructors who wish to cover these topics in their courses, and for students with an interest in the applications of cell and molecular biology.
- An **online self-test section** with text and study references, to reinforce key terms and concepts;
- **Links** to the Macromolecular Tutorial, plus a molecular viewer and modeler, to help students better understand the relationship between structure and function in Cell and Molecular Biology.

Working with Molecular Cell Biology: A Study Companion

Brian Storrie, Muriel Lederman, Eric A. Wong, Richard Walker, and Glenda Gillaspy, Virginia Polytechnic Institute and State University. 0-7167-3604-7

The study companion has been **reorganized to mirror the text in-chapter organization,** providing greater flexibility for instructors who prefer to teach topics in alternative sequences. Students can easily find the questions in the study companion which correspond to the material they're covering in class.

The study companion has also been **tailored to the needs of students at many levels.** The first part of each chapter, "Reviewing Concepts," serves as a study and review resource, posing questions on key principles and concepts. Students are encouraged to draw together the text, CD-ROM, and their own lecture notes to answer the questions. The second part of the chapter, "Analyzing Experiments," allows students to apply the knowledge they've gained to experimental situations, and to work with data sets. Since Molecular Cell Biology is used by students at many levels, we have coded each "Analyzing Experiments" question by a bullet system that denotes the level of difficulty: one bullet is appropriate for sophomore or junior level students, two bullets for junior or senior level, and three for advanced students. Worked-out answers for all questions are included, as well as answers for every other end-of-chapter question.

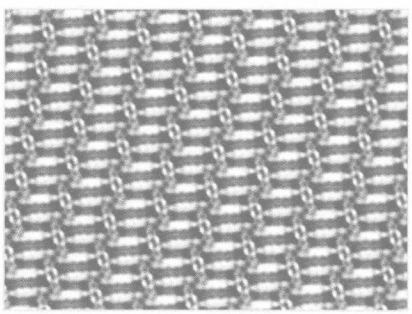
To make study more productive, we've also chosen to pose the sorts of questions students are most likely to encounter on tests—**questions in the study companion parallel those in the Test Bank** in terms of content, and the principles and concepts tested.

To the Student

Biology is a living science, in which changing knowledge continually generates fresh perspectives and fresh opportunities for productive impacts on our society. Our goal in this book is to provide you with the **experimental basis** of our current understanding, and to give you the tools to participate in the development of our future knowledge. We have provided 15 new text and visual aids to help you through each chapter.

3 Protein Structure and Function

Proteins, the working molecules of a cell, carry out the program of activities encoded by genes. This program requires the coordinated effort of many different types of proteins, which first evolved as rudimentary molecules that facilitated a limited number of chemical reactions. Gradually, many of these primitive proteins evolved into a wide array of enzymes capable of catalyzing an incredible range of intracellular and extracellular chemical reactions, with a speed and specificity that is nearly impossible to attain in a test tube. Other proteins acquired numerous structural, regulatory, and other functions. For a flavor of the various roles of proteins in today's organisms, we can look to the yeast *Saccharomyces cerevisiae*, a simple unicellular eukaryote. The yeast genome is predicted to encode about 6225 proteins (see Table 7-3). On the basis of their sequences, 17 percent are estimated to be involved in metabolism, the synthesis or degradation of cell building blocks; 30 percent, in cellular organization and biogenesis of cell organelles and membranes; and 10 percent, in transporting molecules across membranes.



A two-dimensional array of α-actinin molecules.

In this chapter, we will study how the structure of a protein gives rise to its function. The first section examines protein architecture: the structure and chemistry of amino acids, the linkage of amino acids to form a linear chain, and the forces that guide folding of the chain into higher orders of structure. In the next section, we learn about special proteins that aid in the folding of proteins, modifications that occur after the protein chain is synthesized, and mechanisms that degrade proteins. In the third section, we illustrate several key concepts in the functional design of proteins, using antibodies and enzymes as examples. A separate section is devoted to the general characteristics of membrane proteins, which reside in the lipid bilayer surrounding cells and organelles.

OUTLINE

MEDIA CONNECTIONS

Focus: Chaperone-Mediated Folding
Overview: Life Cycle of a Protein
Technique: SDS Gel Electrophoresis
Technique: Immunoblotting

p. 50

Chapter Outline

The chapter Outline lists the major section headings and the pages on which they can be found.

Numbered Headings

We know that your professor may only assign certain portions of a chapter. We have numbered the major section headings for easy reference.

Media Connections

We list the animations and Classic Experiments available on the CD-ROM that directly pertain to the chapter.

3.3 Functional Design of Proteins

A key concept in biology is that form and function are inseparable. This concept applies equally well to protein design as to other levels of biological organization (e.g., the morphology of cells and the organization of tissues). In fact, we can often guess how a protein works by looking at its structure. Perhaps the best way to illustrate this is by examining a few protein structures. For instance, a barrel-like nuclear pore, a complex of several proteins, sits in the nuclear membrane and acts as a channel through which molecules travel in or out of the nucleus (Figure 3-20a). In the cavity of a different barrel-like structure, the GroEL/ES chaperonin, protein folding takes place (Figure 3-20b). Some proteins have grooves in their surface, which are logical binding sites for a variety of molecules, especially rod-shaped or filamentous ones. An example is reverse transcriptase, which copies RNA into DNA; this enzyme has a groove on one side through which RNA slides along the surface of the protein (Figure 3-20c). Topoisomerase II, a DNA-binding enzyme, is an articulated enzyme that opens and closes at both ends like locks in a canal (Figure 3-20d). A delight in studying protein structure is uncovering the simple but ingenious ways that nature has built each protein to perform a particular function.

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Overview Paragraphs

Each major section begins with an overview that introduces the topic at hand.

SUMMARY Functional Design of Proteins

- The function of nearly all proteins depends on their ability to bind other molecules (ligands). Ligand-binding sites on proteins and the corresponding ligands are chemically and topologically complementary. The affinity of a protein for a particular ligand refers to the strength of binding; its specificity, to the restriction of binding to one or a few preferred ligands.
- Enzymes are catalytic proteins that accelerate the rate of cellular reactions by lowering the activation energy and stabilizing transition-state intermediates.
- Enzyme active sites comprise two functional parts: a substrate-binding region and a catalytic region. The amino acids composing the active site are not necessarily adjacent in the amino acid sequence, but are brought into proximity in the native conformation.
- The kinetics of many enzymes are described by the Michaelis-Menten equation. From plots of reaction rate versus substrate concentration, two characteristic parameters of an enzyme can be determined: the Michaelis constant K_m, a measure of the enzyme's affinity for substrate, and the maximal velocity V_{max} (see Figure 3-26).

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Summaries

Each major section concludes with a summary that reviews the important points covered in the section, both in the text and in the figures.

MEDICINE Another example of this approach involves the *BRCA-1* gene. Women who inherit a mutant form of this gene have a high probability of developing breast cancer before age 50. The *BRCA-1* gene was isolated by methods described in Chapter 8, and a cDNA of the *BRCA-1* mRNA was cloned and sequenced, revealing the amino acid sequence of the BRCA-1 protein. Sophisticated methods of sequence comparison revealed that [illegible] tein is distantly, but significantly related to th

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PLANTS Unlike animal cells, plant cells are surrounded by a cell wall and lack the extracellular matrix found in animal tissues. As a plant cell matures, new layers of wall are laid down just outside the plasma membrane, which is intimately involved in the assembly of cell walls (Figure 5-41). The walls are built prim

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BIOTECH This procedure is commonly used to purify the different types of white blood cells, each of which bears on its surface one or more distinctive proteins

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Applications in Context

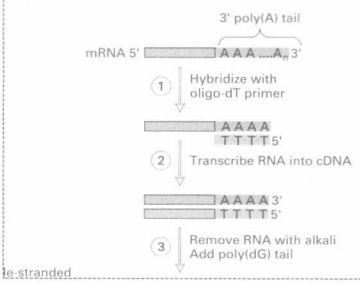
Medicine, biotechnology, and plants play increasingly important roles in our lives. We think it's important to discuss these issues in context, and we've highlighted them with clear icons for ready reference.

Figure Titles

We've started each legend with a clear description of the figure, in blue.

New, Clearer Art

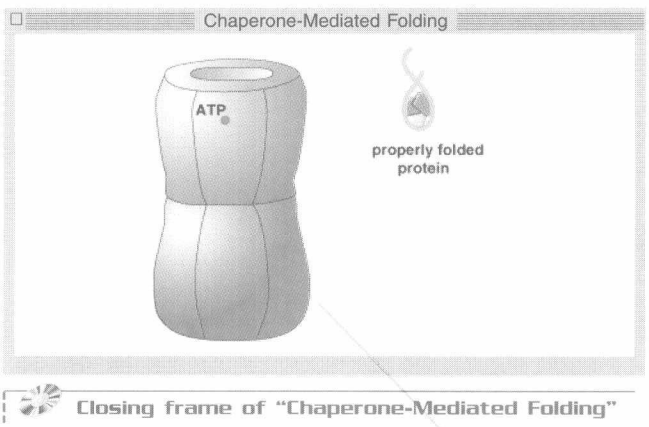
We have stepped out and numbered many experimental processes.



◀ FIGURE 7-15 Preparation of a bacteriophage λ cDNA library. A mixture of mRNAs, isolated as shown in Figure 7-14, is used to produce cDNAs corresponding to all the cellular mRNAs (steps ①–③). These single-stranded cDNAs (light green) are then converted into double-stranded cDNAs, which are treated with *Eco*RI methylase to prevent subsequent digestion by *Eco*RI (steps ④–⑥). The protected double-stranded cDNAs are ligated to a synthetic double-stranded *Eco*RI-site linker at both ends and then cleaved with the corresponding restriction enzyme, yielding cDNAs with sticky ends (red letters); these are incorporated into λ phage cloning vectors, and the resulting recombinant λ virions are plated on a lawn of *E. coli* cells (steps ⑦–⑨). See text for further discussion.

As scientists and educators, we know that some processes, life cycles, and techniques are easier to comprehend if you can see them in motion. We have chosen these subjects to animate.

It's easy to navigate your way from the book to the CD-ROM. In addition to the list of resources provided in the **Media Connections** section at the beginning of each chapter, we have tabbed those figures directly related to animations. We also list all the animations, videos, and macromolecular models on the endpapers of the book.

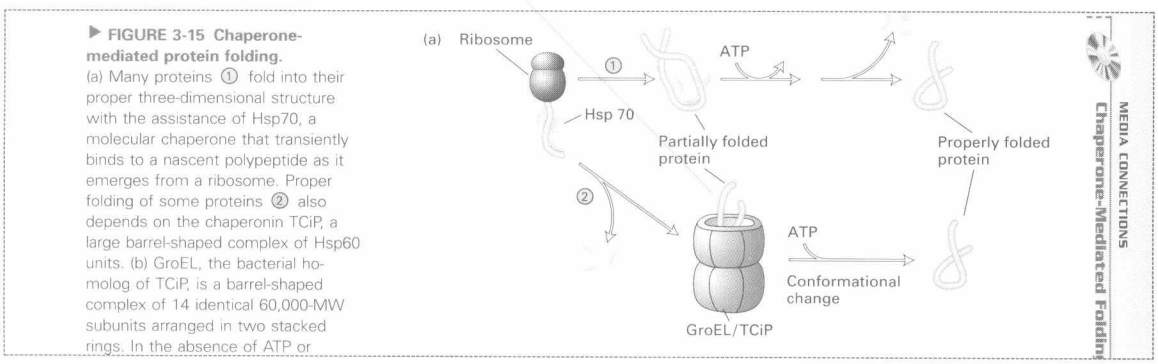


Figures and Animations Have the Same Look

The figures in the book and their related animations use a consistent color scheme and set of icons for representing like elements. This means you can use the CD and the book together without confusion.

Media Connections

Each figure that has a related animation is tabbed for ready reference. The title of the animation runs alongside the figure.



▶ **FIGURE 3-15 Chaperone-mediated protein folding.** (a) Many proteins ① fold into their proper three-dimensional structure with the assistance of Hsp70, a molecular chaperone that transiently binds to a nascent polypeptide as it emerges from a ribosome. Proper folding of some proteins ② also depends on the chaperonin TCiP, a large barrel-shaped complex of Hsp60 units. (b) GroEL, the bacterial homolog of TCiP, is a barrel-shaped complex of 14 identical 60,000-MW subunits arranged in two stacked rings. In the absence of ATP or

PERSPECTIVES for the Future

Researchers are seeking easier and more widely applicable tools and methods for characterizing the three-dimensional structures of proteins. As more and more sequence data are amassed in large data banks, the sequence and function of new proteins will increasingly be deduced from gene sequences and by comparison with known proteins. Development of an algorithm for predicting the three-dimensional structure of a protein from the sequence of its gene or mRNA would extend this approach even further. Such an algorithm seems attainable in view of our increasing knowledge about the rules that guide protein folding and the recurrent use of domains and motifs as modules of structure and function in different proteins. For novel proteins that exh mology to known proteins, improvements in x lography and NMR spectroscopy will permit an larger proteins. Advances in cryoelectron mic provide images of uncrystallizable proteins at comparable with that obtained with x-ray cry

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Future Possibilities

We have written two new sections at the end of each chapter: **Perspectives for the Future** and **Perspectives in the Literature.** These sections provide an opportunity for us to discuss what we think the future will hold, and give you a chance to explore original literature and Web resources in answering critical-thinking questions.

PERSPECTIVES in the Literature

Naturally occurring proteins are formed through an organization of modules, or domains, that dictates their structures and functions. We can create new proteins to have desired properties by choosing the combination of modules we use. Among the list of "parts" available to a protein designer are domains that perform structural, enzymatic, anchoring, and transport functions. Perhaps the best-studied type of domain is the multistranded coiled-coil. Using this domain, construct a protein that has a novel function. You might, for example, design the simplest protein that connects the nucleus to the cell membrane. The basic design would call for a long protein that is anchored at either end to the plasma and nuclear membranes. For a "parts" list of domains, including the coiled-coil, please see the following Web site on the struc-

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Testing Yourself on the Concepts

These questions help you review the key concepts covered in the chapter. Questions like this are also available in the Student Companion.

Testing Yourself on the Concepts

1. Describe the molecular features of the four hierarchical levels of structure that determine the shape or conformation of a protein.

2. Describe the structural and functional properties of a cellular enzyme.

3. Compare and contrast the properties of integral and peripheral membrane proteins.

4. Describe the methodologies for separating proteins based on their charge or mass.

MCAT/GRE-Style Questions

Key Concept Please read the sections titled "Enzymes Are Highly Efficient and Specific Catalysts" and "An Enzyme's Active Site Binds Substrates and Carries Out Catal-

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MCAT/GRE-Style Questions

These questions cover key concepts, applications, and experiments discussed in the chapter. Framed in the style of standardized tests, they are another useful method of review.

Key Terms

α helix *54*	motifs *58*
active site *71*	peptide bond *53*
amino acids *51*	polypeptides *54*
autoradiography *92*	primary structure *54*
β sheet *54*	protein *54*
chaperones *63*	quaternary structure *54*
conformation *51*	radioisotope *91*
disulfide bond *53*	secondary structure *54*
domains *60*	substrates *69*
electrophoresis *87*	tertiary structure *54*
enzymes *71*	V_{max} *73*
ligands *68*	Western blotting *90*

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Key Terms

We have provided a selective list of key terms covered in the chapter, along with the page on which they can be found.

References

General References

Stryer, L. 1995. *Biochemistry,* 4th ed. W. H. Freeman and Company, chaps. 1–4, 7–9, 11, 12, 14, 16, 31.

Web Sites

Entry site into the proteins, structures, genomes, and taxonomy
http://www.ncbi.nlm.nih.gov/Entrez/

The protein 3D structure database
http://www.rcsb.org/

Structural classifications of proteins
http://scop.mrc-lmb.cam.ac.uk/scop/

Sites containing general information about proteins
http://www.expasy.ch/
http://www.proweb.org/

Hierarchical Structure of Proteins

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References

References to the literature are organized by major section heading. We also list some pertinent Web sites you may be interested in.

Chapter Opening Illustrations

Chapter 1 An artist's rendition of the interior of a eukaryotic cell. Depicting a cell's interior is difficult because electron micrographs provide detailed pictures of only a thin slice of a cell, while the cell itself is a three-dimensional object with a very complex interior structure. Thus an artist can create a special sense of the cell's inner workings by using color and shading. Here the artist rendered the organelles inside the cell as he imagined them rather than as a faithful reconstruction from electron micrographs. The blue object is the cell's nucleus with the DNA visible inside as a coil. The red strands emerging from the nucleus are RNA molecules. In the rest of the cell is the cytoplasm, which contains many organelles like the red, kidney-shaped mitochondria and the sectioned orange vesicles. The green stack of flattened vesicles near the nucleus is the Golgi apparatus, and the other flat vesicles represent the cell's endoplasmic reticulum. All of these cellular elements are described in later chapters. [This picture, drawn by Tomo Narashima, originally appeared on the cover of the second edition of this book.]

Chapter 2 Three-dimensional model of an ATP molecule. The atoms are represented by spheres of the appropriate van der Waals radius; carbon atoms are gray, nitrogens are blue, phosphorus are yellow, and oxygen is red. The model was based on the three-dimensional coordinates of the atoms in several nucleotide protein complexes, derived from the crystalline structures of the molecules, in the computerized file of the Protein Data Bank. [Photograph courtesy of Sung Choe.]

Chapter 3 α-Actinin crosslinks actin filaments at the Z-line of muscle cells and at focal adhesions of non-muscle cells. To determine its structure, electron microscopists grow a two-dimensional crystal of the protein and solve its structure from images taken in a cryoelectron microscope. The micrograph shows the protein has the rough outline of a dumb-bell, an elongated molecule with globular domains at either end. [Micrograph courtesy of K. Taylor.]

Chapter 4 Ribosomes plus attached tRNA decode messenger RNA during translation, the process of assembling the amino acids in correct order to make a protein. This figure shows the two-lobed structure (small and large ribosomal subunits) of the *E. coli* ribosome deduced from various physical techniques. Highlighted here are the positions occupied by a tRNA with an attached amino acid (red) and the tRNA to which is attached the growing peptide chain (green). The messenger is not shown. [K. H. Nierhaus et al., 1998, *Proc. Nat'l Acad. Sci. USA* **95**:945–950; photograph courtesy of K. H. Nierhaus.]

Chapter 5 Human squamous carcinoma cells (SqCC/Y1) stained with fluorescent antibodies to the cytoskeletal proteins tubulin (green) and keratin-19 (red), and also with the dye DAPI that causes DNA to fluoresce blue. Note that the keratin fibers appear to concentrate around the nucleus, and terminate at adjacent sites of cell-cell contact. 125X magnification (to the slide). [Photograph courtesy of Nancy Kedersha.]

Chapter 6 Formation of syncytia in NIH 3T3 cells that express truncated Moloney murine leukemia virus envelope proteins. The nuclei are stained with Hoechst dye 33258 (blue) and the location of the envelope protein is indicated by the red rhodamine staining. Cultured NIH 3T3 cells were transfected by electroporation with DNA that encodes a truncated envelope protein that promotes cell-cell membrane fusion and formation of syncytia (multinucleated cells). The truncation is necessary for making the envelope protein competent for membrane fusion. After DNA transfection the cells were grown on glass cover slips and fixed 48 hours later with a 15-minute incubation with 4% paraformaldehyde in phosphate buffered saline. The cells were stained for 30 minutes with a rat monoclonal antibody, 83A25, directed against the gp70 envelope protein, and then with goat anti-rat immunoglobulin G antibodies coupled to rhodamine. The cells were then incubated for 2 minutes with Hoechst dye 33258 and the cover slips were mounted in Fluormount and viewed with a fluorescence microscope. [Photograph courtesy of David Sanders, Whitehead Institute for Biomedical Research.]

Chapter 7 Detection of HIV-1 nucleic acid in human lymphocytes by in situ PCR. Lymphocytes isolated from peripheral blood were fixed, permeabilized, and subjected to PCR with HIV-1 specific primers. Amplified DNA (green) was detected by hybridization to a complementary oligonucleotide probe conjugated with 5-carboxyfluorescein. Nuclei were counterstained (red) with propidium iodide. Green fluorescent cells were isolated with a fluorescence activated cell sorter and visualized by confocal microscopy. [Photograph courtesy of Bruce Patterson, M.D.]

Chapter 8 In mammals, only one X chromosome is active. A specific region of the X chromosome called Xic (X inactivation center) is required in cis for X inactivation. One gene within this region called Xist (in mouse) or XIST (in human) plays a crucial role in silencing expression of genes from the inactive X chromosome. The Xist gene does not encode a protein. Accumulation of Xist RNA is required for the spreading of X inactivation. In this figure the FISH technique was used to analyze the expression pattern of Xist RNA in female E7.0 mouse embryo nuclei. A probe to exonic sequence is shown in red and a probe for intronic sequence is shown in

green. The overlap of the two probes appears in yellow. These data demonstrate that at this stage in development Xist RNA is being synthesized from both the inactive and active X chromosomes, but only accumulates over the former. This results, in large part, through selective stabilization of Xist RNA on the inactive X chromosome. [From Panning et al., 1997, *Cell* **90**:907–916; photograph courtesy of Rudolf Jaenisch.]

Chapter 9 Male human chromosomes visualized by the method of chromosome "painting." Metaphase chromosomes were hybridized to multiple DNA probes specific for sequences along the length of each chromosome. A different combination of fluorochromes that fluoresce with different spectra was used to label the probes for each chromosome. Following hybridization, digital images of the fluorescently labeled chromosomes were collected using a charge-coupled device (CCD) camera and multiple exposures with separate optical filters specific for each of the fluorochromes. The images were then analyzed by computer and a composite image was generated in which each chromosome can be clearly distinguished from chromosomes of a similar size by a pseudocolor assigned on the basis of its fluorochrome composition. Note that there are two homologs of each chromosome. This same method can be used to recognize abnormal chromosomal translocations with great sensitivity (see Figure 9-38b). [See P. Lichter, 1997, *Trends Genet.* **13**:475–479; photograph courtesy of M. Speicher and D. C. Ward.]

Chapter 10 An active region of transcription producing a "puff" in a *Drosophila* polytene chromosome. Chromosomes were stained with fluorescently labeled antibodies against the heat shock transcription factor (red) and RNA polymerase II (green). Regions of overlap appear yellow. The transcription factor is concentrated near the 5′ end of the transcription unit comprising the puff and at additional positions along the polytene chromosomes. [Photograph courtesy of John R. Weeks and Arno L. Greenleaf.]

Chapter 11 The non-snRNP pre-mRNA splicing factor SC35 localizes in a speckled distribution in interphase nuclei (orange regions). HeLa cell SC35 was visualized by immunostaining with a fluorescently labeled antibody. An optical section of the immunostaining pattern is superimposed over a differential interference contrast image of the cells. [Photograph courtesy of David L. Spector, Cold Spring Harbor Laboratory.]

Chapter 12 During S phase of the cell cycle, DNA replication proteins assemble into large complexes or replication foci containing many replication forks (e.g., tens to hundreds) and thousands of replication proteins. During interphase these components are distributed uniformly throughout the nucleoplasm. In this figure antibodies to a DNA methylase were used to visualize replication foci in S-phase cells. Antibodies to other replication factors also show recruitment into these structures during S phase. [From H. Leohardt, A. Page, H.-U Weier, and T. H. Bestor, 1992, *Cell* **71**:865–873.]

Chapter 13 Metaphase in a cultured newt lung cell. Microtubules were visualized by indirect immunofluorescence. Chromosomes were stained with Hoechst 33342. [From J. C. Waters, R. W. Cole, and C. L. Reider, 1993, *J. Cell Biol.* **122**:361–372. Photograph courtesy of Conly L. Reider.]

Chapter 14 Different proteins are expressed in different cells in the developing spinal cord. The Hedgehog protein (yellow) is an extracellular signal specifically expressed in the ventral-most region of the spinal cord, called the floor plate (see Chapter 23). Hedgehog controls the identity of different neuronal precursor cells in the ventral spinal cord. The more dorsal population expresses the homeobox protein Pax-6 (green), while the more ventral population expresses the homeobox protein Nkx2.2 (red). Hedgehog represses Pax-6 and induces Nkx2.2 in the ventral-most progenitor cells. Motoneurons are derived from both populations of progenitor cells and express Isl1 (blue), another homeobox transcription factor. [See J. Ericson et al., 1997, *Cell* **90**: 169–180; photograph courtesy of T. M. Jessell.]

Chapter 15 Three-dimensional structure of a recombinant cardiac gap junction membrane channel determined by electron crystallography. These channels allow the direct exchange of ions and small molecules between adjacent cells. Each channel is formed by association of six connexin subunits, each of which contains four α helices, in one plasma membrane, with a similar structure in the plasma membrane of an adjacent cell. [From V. Unger et al., 1999, *Science* **283**:1176; courtesy of Mark Yeager.]

Chapter 16 Ubiquinone (orange) bound to the surface of the photosynthetic reaction center (white) from the bacterium *Rhodobacter spheroides*. Only one of the oxygen atoms in ubiquinone is visible (blue). [After C.-H Cheng et al., 1991, *Biochemistry* **30**:5352; courtesy of Dr. Lawren Wu.]

Chapter 17 Firefly luciferase, a peroxisomal matrix protein, is transported to peroxisomes of normal human fibroblasts, but remains cytoplasmic in cells from a Zellweger syndrome patient. The fibroblasts (on coverslips) were microinjected with mRNA encoding the luciferase. After overnight incubation in a humidified CO_2 incubator, the cells were fixed, permeabilized, and labeled with appropriate primary (rabbit anti-luciferase) and secondary (FITC anti-rabbit) antibodies. The punctate immunofluorescence observed in normal human HS68 cells *(left)* is indicative of peroxisomal luciferase. The fibrobrast cell line *(GM6231)* from the human patient *(right)* does not import luciferase into peroxisomes, but shows a cytoplasmic signal instead of the punctate signal. Magnification 165X. [See P. Walton et al., 1993, *Mol. Cell Biol.* **12**:531–541; photographs courtesy of Suresh Subramani.]

Chapter 18 A fish scale keratinocyte is one of the fastest moving cells. In this micrograph, the actin (blue) and myosin (red) molecules are labeled with specific fluorescent antibodies. [Courtesy of A. B. Verhovsky.]

Chapter 19 Macrophage cells stained for tubulin (green) and the K14 keratin subunit (red). Immunofluorescence micrograph shows the fibrillar distribution of microtubules and intermediate filaments in the same cell. Regions in which the two proteins are colocalized are colored yellow. [Photograph courtesy of Nancy Kedersha.]

Chapter 20 The compound eye of the fruit fly *Drosophila melanogaster* contains about 750 simple eyes, or ommatidia, each containing eight photoreceptor neurons. The eye develops from an epithelium called the eye imaginal disc. Cells in the disc assemble into clusters in a highly ordered fashion. A wave of morphogenesis sweeps across the disc from posterior (left) to anterior (right). At the leading edge of this wave, called the morphogenetic furrow, cells change shape and stain strongly with phalloidin, giving rise to a continuous band of staining (green). Within the furrow, cells form a reiterated pattern of clusters. This is correlated with activation of the Ras/MAP kinase pathway. Activated MAP kinase (red) was detected using an antibody that specifically recognizes the diphosphorylated (active) but not the unphosphorylated (inactive) form of MAP kinase. The overlap of the green (actin) and red (active MAP kinase) gives rise to the yellow clusters evenly spaced at the morphogenetic furrow. Each one of these clusters will give rise to an ommatidium. [From Kumar et al., 1998, *Development* **125**(**19**):3875–3885; courtesy of Kevin Moses.]

Chapter 21 Neonatal rat cortical brain cells, cultured for 25 days in vitro, stained with a fluorescent antibody to the cytoskeletal intermediate filament protein GFAP (Glial Fibrillary Acidic Protein, green) and with the dye DAPI that causes DNA to fluoresce blue. Two distinct types of astrocytes (green cells) are present in this culture, along with other types of cells (non-green) that appear as isolated blue nuclei. [Photograph courtesy of Nancy Kedersha.]

Chapter 22 A dense network of elastin and collagen fibers form the extracellular matrix of elastic cartilage. These fibers of the ECM are intimately connected to the plasma membrane of a chondrocyte. The membrane is supported by the network of filaments from the actin cytoskeleton. [Courtesy of R. Mecham and J. Heuser, Washington University School of Medicine.]

Chapter 23 As a single fertilized human egg divides, its progeny give rise to hundreds of different types of cells. During embryonic development, specific interactions between different types of cells, and between cells and different types of extracellular matrices, are essential for formation of each type of differentiated cell and its specific organization into tissues and organs. Shown is Emma Rachel Steinert soon after her birth, and her parents Heidi Lodish Steinert and Eric Steinert. [Photograph courtesy of Stephanie Lodish.]

Chapter 24 Human melanoma cells (cell line Hs695T) stained for a melanoma-specific cell surface glycoprotein in green and counterstained for myosin (antibody 5.15, red) and for DNA (Hoechst, blue). [Photograph courtesy of Nancy Kedersha.]