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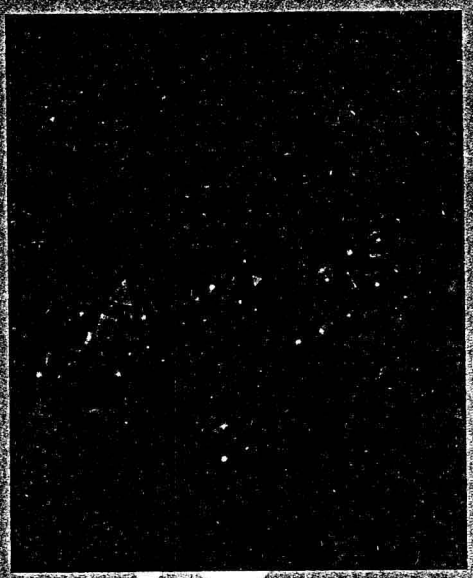
MOLECULAR CELL BIOLOGY



THIRD EDITION

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To our supportive families for their patience; to our teachers and students for their inspiration; and to the many scientists who generated the knowledge that made this book possible.

Preface

➤ *Our Traditional Strengths*

In the preface to the first edition of *Molecular Cell Biology* we asserted that the new techniques of molecular biology would soon unify all experimental biology. The next nine years confirmed the validity of our position. Although once separate, the fields of biochemistry, genetics, cell biology, physiology, developmental biology, and now even much of neurobiology have become wedded by fundamental experimental approaches. Their common quests are threefold:

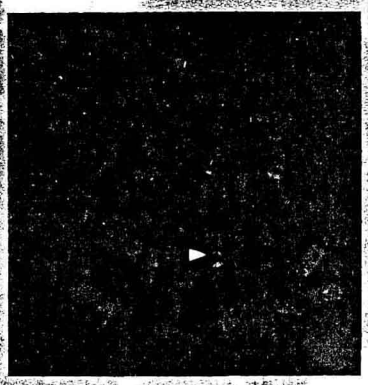


Figure 8-28a

• to understand how gene expression is controlled so that cells synthesize the right proteins at the right time in the right amounts

• to know the structure and function of proteins that carry out specific biologically important tasks: proteins that regulate ion flow, cause cell motility or contractility, catalyze a degradative or synthetic reaction, induce an embryonic structure, or send or receive a chemical signal

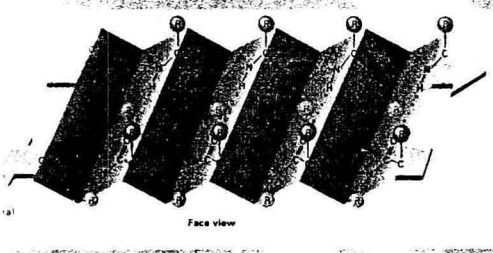


Figure 3-12a

• to understand how the properties of cells, tissues, and the other organisms relate to the properties of the proteins and other molecules that they contain.

Thus our focus continues to be the central dogma of the new integrated science of cell biology: the hierarchy of genes ↔ proteins ↔ organelles ↔ cells ↔ tissues ↔ organs ↔ organisms. Our emphasis on the fundamental experimental tools and tech-

> ➤ *"At the top of this hierarchy of biological organization lie the genes. They not only specify the structure of proteins but ultimately they define the organization of cells, direct cells to form tissues and organs, and maintain the integration of function that gives the organism its unity."*

niques that we regard as the necessary foundation of this new integrated science of *Molecular Cell Biology* has been maintained.

Part I introduces students to the basic concepts and experimental methodologies of molecular cell biology. Here we discuss the basic chemical and physical principles of cellular activity, essential features of DNA replication, transcription, and translation, the structure and function of proteins—the working molecules of cells—and the genetic and molecular technology needed to study cells at the molecular level.

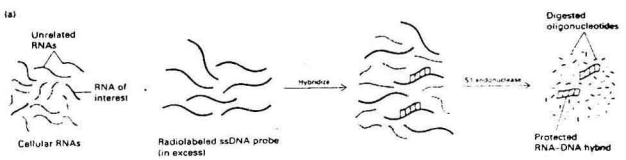
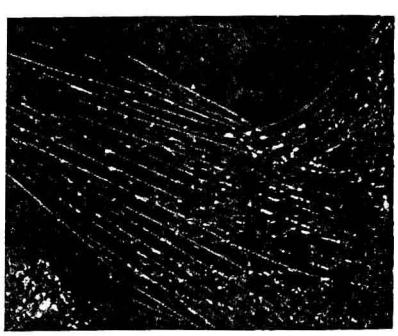


Figure 7-33a

In Part II the student is taught how genes work. Chapter 9 discusses the structure of genes and chromosomes and the genetic events that led to the evolution of present-day genomes. Chapter 10 explains the mechanisms of DNA replication, recombination, and repair. Chapter 11 focuses on the regulation of gene expression at the level of initiation of RNA synthesis, and Chapter 12 discusses post-transcriptional control of gene expression: the mechanism and control of RNA splicing, translation, and degradation. The regulation of gene expression during development of prokaryotes, eukaryotic microorganisms, and animals is the focus of Chapter 13.



Chapter 5 opener

In Part III we concentrate on the ways in which proteins work together to make a living cell. First, in Chapter 14, we describe the structure of cell membranes and of membrane proteins. Chapter 15 examines the role of membrane proteins in the transport of small molecules into and out of the cell. Chapters 16 and 19 describe the assembly of the membranes and organelles that make up a cell, focusing on the targeting of proteins to their correct destinations. Two chapters detail the generation of ATP in the cytosol and mitochondria (Chapter 17) and by photosynthesis (Chapter 18).

Part IV emphasizes the interactions of cells with each other, both in normal and abnormal situations. Cell surface receptors, intracellular "second messengers," and the complex network of cell-to-cell communication required to coordinate differentiation, growth, metabolism, and behavior are the focuses of Chapter 20. Chapter 21 focuses on signaling in the nervous system and on the generation and propagation of action potentials. Chapters 22 and 23 turn to the complex system of fibers—the cytoskeleton—that is responsible for the cell's shape and motility. Chapter 24 focuses on the many proteins, polysaccharides, proteoglycans, and other polymers that form the extracellular matrix surrounding animal cells and the plant cell wall and on how these molecules influence growth and differentiation of cells. Chapter 25, "Regulation of the Cell Cycle," emphasizes how cells integrate many internal and external signals in order to regulate their growth and division. In cancer these growth-regulatory circuits are impaired, and Chapter 26 explains how and why this happens. Chapter 27 integrates many of the themes of earlier chapters by showing how different cells function cooperatively to provide immunity from invading microorganisms.

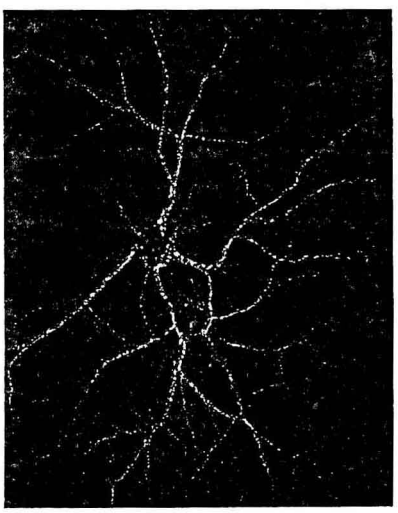


Figure 21-4

➤ *Much is New*

New Chapters: Chapters 1 through 8, which deal with molecules, cells, proteins, and experimental techniques, have been reorganized and expanded to help students master the essential concepts and experimental techniques in molecular and cellular biology:

• A new Chapter 1 introduces the cell, defines the hierarchy of biological organization: small molecules ↔ genes ↔ proteins ↔ organelles ↔ cells ↔ tissues ↔ organs ↔ organisms, and explains why the study of cells is at the very center of modern biological research.

• Chapter 3, "Protein Structure and Function," presents a cohesive view of the structure and function of proteins, the principal classes of proteins—including enzymes and antibodies—and the important (and some very new) ways to isolate and characterize proteins. Chapter 3 further under-scores the importance of proteins in all cellular activities.

> ➤ *"The facts in biology are only as good as the tools available for investigation."*

Two new chapters focus the book on the most powerful techniques used by modern cell biologists to research a wide range of topics:

• Chapter 7, "Recombinant DNA Technology," contains much new material on the polymerase chain reaction and on the techniques for isolating, mutating, and analyzing genes that are employed throughout modern biology.

• Chapter 8, "Genetic Analysis in Cell Biology," illustrates how an analysis of mutant cells or organisms can lead to a deep understanding of the roles of individual proteins. It shows how genetics is used to identify genes and proteins that are important in a developmental pathway, and to analyze the steps in a biosynthetic or signaling pathway. We learn how genes causing human genetic diseases are identified and cloned, and how to introduce mutant genes into living plants or animals and thus study the roles of the encoded proteins.

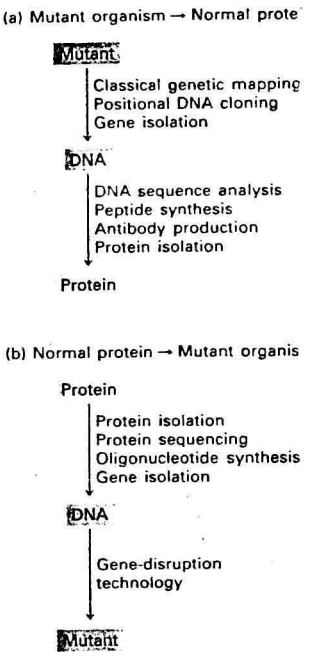


Figure 8-

• Chapter 11, "Regulation of Transcription Initiation," and Chapter 12, "Transcription Termination, RNA Processing and Posttranscriptional Control," allow greatly expanded coverage of these fast moving areas. Included is up-to-date coverage of the interactions of transcription initiation factors in regulating gene expression and newly uncovered mechanisms by which gene expression is con trolled at the levels of RNA chain elongation, RN/ splicing, and mRNA degradation. Examples range fron the control of sex determination in *Drosophila* to the control of expression of the HIV genome, the virus that causes AIDS.

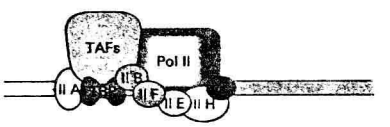


Figure 11-5

• Chapter 13, "Gene Control in Development," illustrate how differentiation and development in bacteria, yeasts and animals is controlle by selective gene expressior The unifying theme i switches in gene expression the decision to turn on o off a particular gene dur ing differentiation. Carefull chosen examples include the decision by bacteric phage λ to enter or not th lysogenic state, the contro

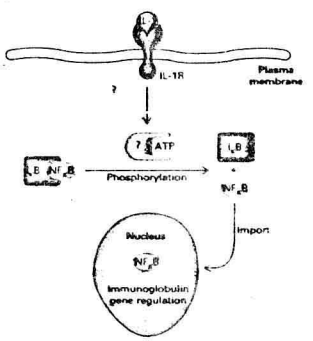


Figure 13-36

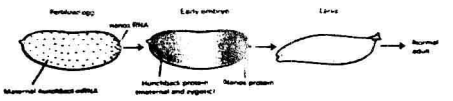


Figure 13-33

of gene expression during yeast mating, and the ways in which specific mRNAs are localized in the *Drosophila* egg and how this controls the patterning of the early embryo.

• Chapter 20, "Cell-to-Cell Signaling," contains new sections about the structure and function of cell receptors for growth factors, and how activation of a cell-surface receptor results in modification of specific transcription factors and induction of expression of specific genes.

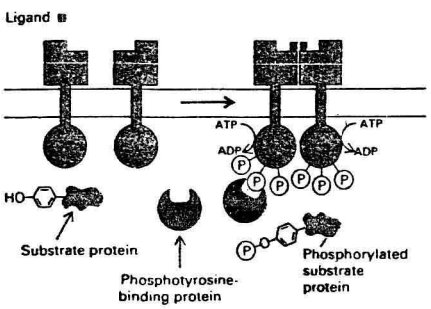


Figure 20-4g

• Chapter 24, "Multicellularity: Cell–Cell and Cell–Matrix Interactions," includes very recent developments that show how specific contacts between a cell and hormones bound to the extracellular matrix, or between proteins on the surface of two adjacent cells, trigger cell differentiation.

• The last several years have revealed much about how the cell cycle is regulated: how cells are triggered to begin DNA replication and how they regulate the start of mitosis. A totally new Chapter 25 details these discoveries, and also explains how cells integrate signals from many internal and external molecules.

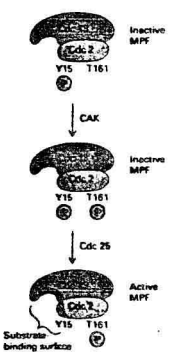


Figure 25-22

Complete Updating: All chapters that are not new to the text have been completely rewritten to incorporate the latest advances. Here are a few of the more prominent examples:

• Insights into the evolution of genes and genomes from the cloning and sequencing of many gene families (Chapter 9).

• New appreciation that some genetic diseases are due to alterations in mitochondrial DNA (Chapter 19).

• The proteins that cause intracellular vesicles to bud from and fuse with each other, and that sort and target proteins to their final destinations, and the importance of genetic analyses in identifying them (Chapters 16 and 19).

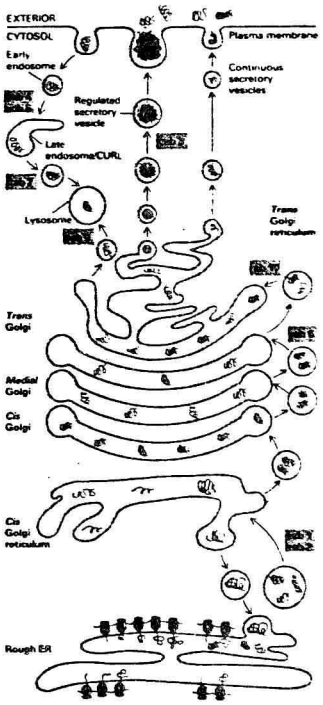


Figure 16-43b

• The folding of newly made proteins, and the roles of chaperones in assisting this process (Chapters 3, 16, and 19).

• Newly uncovered oncogenes and tumor suppressor genes: their roles in regulating the cell cycle and cell proliferation (Chapters 20 and 25) and the mutations in them that lead to malignancy (Chapter 26).

• New types of membrane transport proteins, such as water channels that control water flow and cell volume, and ATP-powered pumps (Chapter 15); and a molecular understanding of the function of voltage-gated ion channels in conducting nerve impulses (Chapter 21).

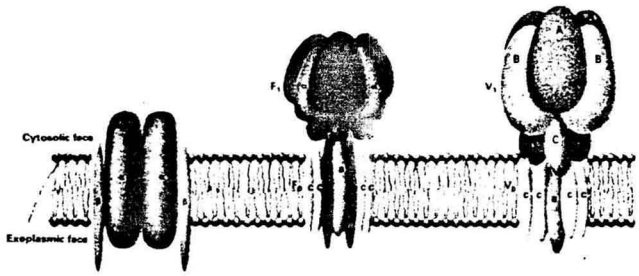


Figure 15-10

• The proteins that allow other proteins to cross membranes specifically and to insert in them (Chapters 16 and 19).

• New types of cell surface receptors, particularly in nerve cells, and their role in learning and memory (Chapter 21).

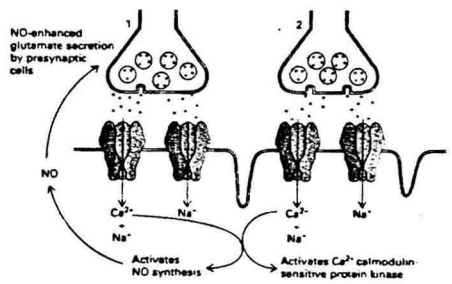


Figure 21-56

• The three-dimensional structure of actin and myosin, and the properties of newly identified proteins that regulate the structure and assembly of actin and other cytoskeletal proteins (Chapters 22 and 23)

Figure 22-21

New Authors: The exciting advances in every area of molecular cell biology during the four years since the second edition have driven these many changes in this third edition. The most obvious change, however, is the inclusion of three new authors, who are both outstanding teachers and experts in many of the fields that have been given increased coverage:

Arnold Berk has made major contributions to our understanding of proteins that control gene expression and also has developed some of the most widely used techniques for the study of messenger RNA.

Paul Matsudaira has employed a wide variety of experimental techniques to dissect the functions of cytoskeletal proteins and also has developed popular methods for sequencing proteins.

S. Lawrence Zipursky's studies on the fruit fly *Drosophila melanogaster* have led to new insights in how the developmental fate of cells is affected by their interactions with other cells.

All of us have worked together from the beginning to improve the overall structure of this edition and to better interrelate major concepts in each chapter. Reading and rewriting one another's chapters has been an education for all of us and, we hope, has given the book a greater cohesion and consistency.

➤ *New Study Tools*

In response to the advice of several dedicated teachers, Review Questions have been added to the third edition. We thank David Scicchitano of New York University, who so ably took on this task and created questions that review and reinforce the concepts and techniques to which students have been introduced. These end-of-chapter exercises will help students review and integrate the chapter's most important concepts and to integrate them with concepts learned in previous chapters. The text's cohesiveness is also improved by the new Chapter Introductions, which explain clearly how the material in that chapter relates to the text's overall hierarchy. For instance, the introduction to Chapter 8 explains *why* we need genetic tools to study cells. Similarly, short Summaries are now placed at critical points throughout each chapter, and Chapter-Ending Summaries have been rewritten to help students better focus on the chapter's major ideas and how they relate to the text's overall themes.

SUPPLEMENTS

We are pleased to announce that two outstanding supplements are available:

- The *Student Companion for Molecular Cell Biology*, first created to provide additional support for students using the second edition of *Molecular Cell Biology*, has been updated and revised in concert with the third edition. The revision, prepared by David Rintoul, Ruth Welti, Muriel Lederman, and Brian Storrie, once again is designed for use as an assigned text supplement or as a self-study guide for students taking molecular cell biology, reviewing and reinforcing the concepts, problems, and open questions of modern molecular cell biological research. Following a question and answer format (with all answers contained in an answer key), each chapter proceeds from simpler to more advanced problems that parallel a corresponding chapter in *Molecular Cell Biology*. Using this format, the *Companion* provides students of varying backgrounds a useful study resource.
- Full-color overhead transparencies of key figures from the text; available to qualified adopters.

For more information on the *Student Companion for Molecular Cell Biology* and the overhead transparencies, please contact Sales Support, W. H. Freeman and Company, 41 Madison Avenue, New York, NY 10010. Instructors in the Eastern United States and Canada may call 1-800-347-9411; those in the Western United States and Canada may call 1-800-347-9415.

ACKNOWLEDGMENTS

In updating, revising, and rewriting this book of well over a thousand pages, we were given invaluable help by many colleagues. We thank the following individuals who generously gave of their time and expertise by making major contributions to specific chapters in their areas of interest, or by reading and commenting on one or more chapters:

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Jeffrey H. Miller	*University of California at Los Angeles*
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Steven White	*San Jose State University*
Leslie Wilson	*University of California at Santa Barbara*
Alan T. Wortman	*University of Wisconsin at Lacrosse*
Ana-Belen Ybarronda	*The Scripps Research Institute*
Kenton Zavitz	*University of California at Los Angeles*

It is often hard for non-authors to realize that production of a book such as this requires the talents and devoted efforts of many people over several years. We have been fortunate to have many exceptional people at W. H. Freeman and Company work with us on this and the two previous editions. Our particular thanks go to Linda Chaput, former president, for nurturing this book from its inception 14 years ago, and for helping us to plan this revision. We thank Randi Rossignol and Janet Tannenbaum, our development editors, and Mary Shuford, director of development, for providing us with exceptional guidance and support on the art program and text—from our first to our last drafts; we appreciate their devoted and highly competent efforts throughout the revision process. Shana Ederer, also in the development group, deserves our special thanks for her continuing and unstinting support work. Travis Amos and Larry Marcus were extremely energetic and resourceful in locating and developing sources for photographs and molecular models.

The attention of the entire project editorial team—Penny Hull, Philip McCaffrey, Laura Spagnoli, and Kay Ueno—to editorial questions on both text and art has been indispensable. Kay Ueno, our project editor, directed the efforts of an expert team of copy editors. We thank them—Gloria Hamilton, Elmarie Hutchinson, Bill O'Neal, and especially Ruth Steyn, for their excellent contributions in clarifying and organizing our occasionally muddled text and figures. Walter Hadler, our proofreader, provided that necessary additional reading. We greatly appreciate the efforts of Ellen Cash, production manager, who expertly coordinated all aspects of production. The new "look" of the book, and the expert blending of text and illustrations on each page, are due to the efforts of Blake Logan, our designer, and our layout artists Stan Hatzakis and Natasha Sylvester. Chris McAuliffe, Bill Page, and Denise Wiles ably coordinated the exceptional work of our illustrators Tomo Narashima, Ian Worpole, and Network Graphics.

Our own office staff, including Lois Cousseau, Carol Eng, Katherine Kanamori, Maureen Murray, and Cynthia Peterson, provided invaluable support, ranging from locating specific information and assembling reprints to helping with corrections in the text. The help of Udayakumara Liyanage in locating references for many of the chapters is also appreciated.

All these people and many others made the enormous task of writing this new edition, if not actually pleasant, at least tolerable.

Again to our families we say simply, thank you.

We believe that a comprehension of modern biology is needed both by those who use biological concepts professionally and by the general public, who increasingly will be faced with decisions about integrating new biological understanding into the fabric of their lives. We hope that this edition of *Molecular Cell Biology* will help both groups to better comprehend the revolution in understanding of living systems that is being generated by research laboratories around the world.

Harvey Lodish
David Baltimore
Arnold Berk
S. Lawrence Zipursky
Paul Matsudaira
James Darnell

January 1995

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 Gareth White.]

Chapter 3 A two-dimensional crystalline array of 50S ribosomal subunits from *B. stearothermophilus*. Crystals were grown on phospholipid monolayers, rapidly frozen in vitreous ice, and imaged in a 100 kV cryoelectron microscope. In this 20 Å resolution projection map of the crystal, areas of high protein mass are displayed as bright colors. [Adapted from A. Avila-Sakar and W. Chiu, 1994, *J. Mol. Biol.* 239:689–697. Photograph courtesy of A. Avila-Sakar and W. Chiu.]

Chapter 4 This image shows *E. coli* RNA polymerase (the bright, round object) bound to a 580 base pair fragment of bacteriophage λ DNA containing a promoter sequence that specifies a transcriptional start site. In binding to the DNA, the polymerase bends the linear DNA at a 90° angle. The picture was obtained by a modification of electron microscopy called scanning force microscopy. [From W. A. Rees et al. 1993, *Science* 260:1646–1649; Photograph courtesy of R. W. Keller.]

Chapter 5 The actin filaments (red) and mitochondria (green) in a rat astrocyte. Astrocytes isolated from the cerebral cortex of a newborn rat were cultured for two weeks. They were fixed, permeabilized, and then stained with phalloidin, a protein that binds to actin filaments, that was tagged with rhodamine and that generates a red fluorescence. The cells were then stained with a fluorescein-tagged antibody (green fluorescence) specific to the mitochondrial enzyme cytochrome oxidase (antibody provided by Giampietro Schiavo and Cesare Montecucco). [Photomicrograph by Olaf Mundigl and Pietro DeCamilli.]

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 Two sibling mice at about 10 weeks of age. The larger mouse carries a chimeric gene, which includes the structural gene for rat growth hormone under the control of the mouse metallothionein promoter. This gene was introduced into the mouse germline using transgenic technology. Mice carrying the transgene grow 2–3 times as fast as their normal siblings and can reach nearly twice their size. Since mice carrying the transgene are fertile and the gene has been incorporated into chromosomes of the germline the transgene can be passed on to subsequent generations. The introduction of transgenes is an important technique used widely in the study of mouse development, physiology, and behavior. [From R. D. Palmiter et al., 1982, *Nature* 300:611–615; Photograph courtesy of R. L. Brinster.]

Chapter 9 Localization of the gastrin/cholecystokinin-B receptor gene to the tip of the short arm of chromosome 11 by fluorescence in situ hybridization. The gastrin/cholecystokinin-B receptor cDNA was labeled with biotinylated-dUTP, hybridized to propidium iodide stained human metaphase chromosomes, and detected with FITC-conjugated avidin and anti-avidin antibodies. [See D. B. Zimonjic et al., 1994. *Cytogenet. Cell Genet.* 65:184; Photograph courtesy of T. Matsui.]

Chapter 10 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 11 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 12 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 13 The expression of the myogenin gene in this mouse embryo was followed using a reporter transgene in which the promoter of the myogenin gene was fused to the structural gene encoding *E. coli* β-galactosidase. The blue color indicates the pattern of transgene expression. By joining different regions of the myogenin promoter to β-galactosidase investigators have discovered distinct elements that control expression in the limb buds and myotomes. [From T.-C Cheng, M. C. Wallace, J. P. Merlie, and E. N. Olson, 1993, *Science* 261:215–217; photograph courtesy of Eric N. Olson.]

Chapter 14 Model of a phospholipid bilayer derived from a molecular dynamics calculation. In cell membranes the fatty acid "tails" of the phospholipids are in a fluid-like state. To calculate a model for a bilayer in this state, the computer first generated energetically favored conformations of individual phospholipids, then allowed many to interact with each other in a model bilayer until a gel-like state of the hydrocarbon core was achieved. The carbon atoms in the fatty acids had the same ability to rotate about the C–C bonds as did carbon atoms in solution of pure hydrocarbon, such as $C_{16}H_{34}$. [Photograph courtesy of R. M. Venable and R. W. Pastor.]

Chapter 15 Immunofluorescence localization of the CHIP28/Aquaporin water channel protein (yellow-green fluorescence) in the outer medulla of rat kidney. The tissue section was stained with Evans Blue, which generates a red fluorescence. Aquaporin is localized to the apical (brush border) and basolateral plasma membranes of cells in the S3 segment of the proximal tubule, and is absent from other cells in this region of the kidney. [See I. Sabolic, et al., 1992, *Am. J. Physiol.* 263:C1225–C1233. Photograph courtesy of Dennis Brown.]

Chapter 16 Human skin fibroblasts were incubated at low temperature with a fluorescent (BODIPY) analog of the lipid sphingomyelin, labeling the plasma membrane of these cells. The cells were warmed to 37°C for 5 min to allow endocytosis to occur. The fluorescent lipid molecules remaining on the plasma membrane were then removed by incubating the cells with a protein solution, so that the hundreds of fluorescently labeled endosomal vesicles within the cell were readily visible. Each fluorescent dot is an endosome in the cytoplasm; the black hole in the center is the nucleus, which is unlabeled. Despite its appearance, this is not a photograph of a nebula from outer space. [Photomicrograph by Richard E. Pagano and Ona C. Martin, Carnegie Institution of Washington.]

Chapter 17 Two populations of mitochondria in a living mink fibroblast, treated with the lipophilic cation JC-1, fluoresce green and yellow. Formation of an aggregate of the dye within the mitochondria is caused by a high membrane potential and generates a red fluorescence; the red fluorescence combines with the green fluorescence from the unaggregated dye to generate a yellow fluorescence. Within single long mitochondria in this cell multiple regions with yellow fluorescence, separated by green fluorescence, are evident, indicating that within long continuous mitochondria heterogeneity in membrane potentials is possible. [See S. T. Smiley et al., 1991, *Proc. Nat'l. Acad. Sci. USA* 88:3671–3675; Photograph courtesy Lan Bo Chen.]

Chapter 18 Abundant chloroplasts, each about 10 μm in diameter, are seen in this unstained section of a leaf from the pond weed *Elodea*. [Photograph by Dwight R. Kuhn.]

Chapter 19 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 at 37°C, 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 fibroblast 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 20 PC12 cells overexpressing the epidermal growth factor (EGF) receptor respond to EGF by extending neurites. Activated forms of MAP kinase kinase injected into these cells lead to neurite extension also. In this experiment a dominant-interfering mutation was injected into a subpopulation of PC12 cells. Uninjected cells (red) extend neurites whereas injected cells (yellow/green) are inhibited. MAP kinase kinase is part of signal transduction cascade conserved from yeast to humans. [From S. Cowley and H. Paterson, P. Kemp and C. Marshall, 1994, *Cell* 77:841–852; photograph courtesy of H. Paterson, Chester Beatty Laboratories.]

Chapter 21 Long-term (18 day) culture of rat brain cells from the cortex. The field contains several mature neurons with long axons that extend beyond the end of the field; these axons contain a nerve-specific intermediate filament protein that is detected by a yellow/green fluorescing antibody. The field also contains many astrocytes, a type of glial cell. These cells contain the glial-cell-specific intermediate filament protein GFAP (Glial Fibrillary Acidic Protein) that is detected by the red fluorescing antibody. [Photograph courtesy of Nancy L. Kedersha.]

Chapter 22 Three-dimensional reconstructions of a human polymorphonuclear leukocyte as it moves across a glass slide. Taken 40 seconds apart, the time lapse sequence shows a leukocyte arching up and then descending to contact the surface. The bulk of the cytoplasm then flows to fill the pseudopodium, and the cell, now ellipsoidal in shape, repeats the sequence when it lifts the pseudopodium off the surface. The three-dimensional shape of the cell was calculated by merging into a continuous stack outlines of the cell taken at different levels of focus. The process is analogous to constructing a three-dimensional model from a two-dimensional contour map. [Adapted from J. Murray et al., 1992, *Cell Motil. Cytoskeleton* 22:211–223. This drawing is based upon a photograph by D. R. Soll.]

Chapter 23 An immunofluorescence micrograph showing the microtubule (light orange) and vimentin (purple) cytoskeletons in a human skin fibroblast. In many parts of the cell, the two systems of cytoskeletal fibers are co-linear (seen as red), indicating they may be directly connected; however, in other parts of the same cell, the fibers show different organizations. In Figure 23-53, the distribution of vimentin filaments, microtubules, and actin filaments in this same cell are shown in separate panels. [Photograph courtesy of V. Small.]

Chapter 24 Tight cell-cell contacts formed by cultured human squamous skin carcinoma cells. The permeabilized cells are stained with three fluorescent molecules. The green fluorescence results from a fluoresceine-coupled antibody that detects a monoclonal antibody specific for an as-yet uncharacterized cell-cell adhesion molecule; the staining is at the sites of cell-cell contacts. The red fluorescence results from a Texas-red coupled antibody that detects a monoclonal antibody specific for a cell protein mainly associated with the Golgi complex, and the Hoechst dye fluoresces blue when bound to DNA; here it stains the cell nuclei. [Photograph courtesy Nancy L. Kedersha.]

Chapter 25 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 26 Structure of the p53 protein bound to DNA. The structure was derived by X-ray crystallography. A short segment of DNA containing a p53-binding site (dark blue) was crystallized with a fragment of the p53 protein known to have the DNA binding site (light blue). Six p53 residues which are frequently mutated in human tumors are highlighted in yellow. They all turn out to be in the region of DNA contact of the protein. A red zinc atom is also indicated. [Photograph courtesy of Nikola P. Pavletich.]

Chapter 27 Peptides bound into the grooves on MHC class I molecules. Two peptides are shown, one from vesicular stomatitis virus (*left*, MHC shown in gold) and one from Sendai virus (*right*, MHC shown in light blue). The amino termini of the peptides are at the bottom. Peptide oxygens and nitrogens are shown in red and blue, respectively. Some structured water molecules are shown as larger blue spheres. The picture illustrates how deeply the peptide is buried in the MHC molecule. It shows that the antigens recognized by T cell receptors, which are peptides bound to MHC molecules, consist of just the upper surfaces of the bound peptides, actually representing no more than 25 percent of the total peptide surface. [From M. Matsumara et al., 1992, *Science* 257:927.]

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