MOLECULAR BIOLOGY OF THE CELL

Bruce Alberts • Dennis Bray
Julian Lewis • Martin Raff • Keith Roberts
James D. Watson

Garland Publishing, Inc.
New York & London

List of Topics

PART I

Introduction to the Cell

CHAPTER 1

The Evolution of the Cell

From Molecules to the First Cell 4

Simple Biological Molecules Can Form Under Prebiotic Conditions 4

Polynucleotides Are Capable of Directing Their Own Synthesis 4

Self-replicating Molecules Undergo Natural Selection 6

Information Flows from Polynucleotides to Polypeptides 7

Membranes Defined the First Cell 8

Mycoplasmas Are the Simplest Living Cells 10

Summary 11

From Procaryotes to Eucaryotes 11

Procaryotic Cells Are Structurally Simple But Biochemically Diverse 11

Metabolic Reactions Evolve 12

Cyanobacteria Can Fix CO_2 and N_2 14

Bacteria Can Carry Out the Aerobic Oxidation of Food Molecules 15

Eucaryotic Cells Contain Several Distinctive Organelles 15

Eucaryotic Cells Depend on Mitochondria for Their Oxidative Metabolism 19

Chloroplasts May Be Descendants of Procaryotic Algae 19

Eucaryotic Cells Contain a Rich Array of Internal Membranes 21

Eucaryotic Cells Have a Cytoskeleton 21

Protozoa Include the Most Complex Cells Known 23

Genes Can Be Switched On and Off 23

Eucaryotic Cells Have Vastly More DNA Than They Need for the Specification of Proteins 26

In Eucaryotic Cells the Genetic Material Is Packaged in Complex Ways 26

Summary 27

From Single Cells to Multicellular Organisms 28

Single Cells Can Associate to Form Colonies 28

The Cells of a Higher Organism Become Specialized and Cooperate 29

Multicellular Organization Depends on Cohesion Between Cells 29

Epithelial Sheets of Cells Enclose a Sheltered Internal Environment 30

Cell-Cell Communication Controls the Spatial Pattern of Multicellular Organisms 31

Cell Memory Permits the Development of Complex Patterns 31

Basic Developmental Programs Tend to Be Conserved in Evolution 32

Eucaryotic Organisms Possess a Complex Machinery for Reproduction 32

The Cells of the Vertebrate Body Exhibit More Than 200 Different Modes of Specialization 33

Cells of the Immune System Are Specialized for the Task of Chemical Recognition 34

Nerve Cells Allow a Rapid Adaptation to a Changing World 35

Developing Nerve Cells Must Assemble to Form a Nervous System 36

Nerve Cell Connections Determine Patterns of Behavior 37

Summary 39

References 41

CHAPTER 2

Small Molecules, Energy, and Biosynthesis

The Chemical Components of a Cell 44

Cell Chemistry Is Based on Carbon Compounds 44

Cells Use Four Basic Types of Small Molecules 45

Sugars Are Food Molecules of the Cell 50
Fatty Acids Are Components of Cell Membranes 51
Amino Acids Are the Subunits of Proteins 56
Nucleotides Are the Subunits of DNA and RNA 56
Summary 62

Biological Order and Energy 62
Biological Order Is Made Possible by the Release of Heat Energy from Cells 62
Photosynthetic Organisms Use Sunlight to Synthesize Organic Compounds 63
Chemical Energy Passes from Plants to Animals 64
Cells Obtain Energy by the Oxidation of Biological Molecules 64
The Breakdown of Organic Molecules Takes Place in Sequences of Enzyme-catalyzed Reactions 65
Part of the Energy Released in Oxidation Reactions Is Coupled to the Formation of ATP 66
The Hydrolysis of ATP Generates Order in Cells 67
Summary 67

Food and the Derivation of Cellular Energy 67
Food Molecules Are Broken Down in Three Stages to Give ATP 67
Glycolysis Can Produce ATP Even in the Absence of Oxygen 69
Oxidative Catabolism Yields a Much Greater Amount of Usable Energy 71
Metabolism Is Dominated by the Citric Acid Cycle 72
The Transfer of Electrons to Oxygen Drives ATP Formation 73
Amino Acids and Nucleotides Are Part of the Nitrogen Cycle 74
Summary 75

Biosynthesis and the Creation of Order 75
The Energy for Biosynthesis Comes from the Hydrolysis of ATP 75
Biosynthetic Reactions Are Often Directly Coupled to ATP Hydrolysis 77
Coenzymes Are Involved in the Transfer of Specific Chemical Groups 79
Biosynthesis Requires Reducing Power 80
Biological Polymers Are Synthesized by Repetition of Elementary Dehydration Reactions 81
Summary 82

The Coordination of Catabolism and Biosynthesis 82
Metabolism Is Organized and Regulated 82
Metabolic Pathways Are Regulated by Changes in Enzyme Activity 84
Catabolic Reactions Can Be Reversed by an Input of Energy 84
Enzymes Can Be Switched On and Off by Covalent Modification 87
Reactions Are Compartmentalized Both Within Cells and Within Organisms 87
Summary 89

References 89

CHAPTER 3

Macromolecules: Structure, Shape, and Information

Molecular Recognition Processes 91
The Information Carried by a Macromolecule Is Expressed by Means of Weak Noncovalent Bonds 92
Diffusion Is the First Step to Molecular Recognition 93
Thermal Motions Not Only Bring Molecules Together But Also Pull Them Apart 96
Molecular Recognition Processes Can Never Be Perfect 97
Summary 98

Nucleic Acids 98
Genes Are Made of DNA 98
DNA Molecules Consist of Two Long Complementary Chains Held Together by Base Pairs 99
The Structure of DNA Provides an Explanation for Heredity 103
Errors in DNA Replication Cause Mutations 103
The Nucleotide Sequence of a Gene Determines the Amino Acid Sequence of a Protein 106
Portions of the DNA Sequence Are Copied into RNA 107
Sequences of Nucleotides in mRNA Are "Read" in Sets of Three 107
tRNA Molecules Match Amino Acids to Groups of Nucleotides 108
The RNA Message Is Read from One End to the Other by a Ribosome 109
Summary 111

Protein Structure 111
The Shape of a Protein Molecule Is Determined by Its Amino Acid Sequence 111
Common Folding Patterns Recur in Different Protein Chains 113
Proteins Are Enormously Versatile in Structure 115
Proteins Show Different Levels of Structural Organization 118
Relatively Few of the Many Possible Polypeptide Chains Would Be Useful 118
New Proteins Often Evolve by Minor Alterations of Old Ones 118
New Proteins Often Evolve Through the Combination of Different Polypeptide Domains 120
Protein Subunits Can Self-assemble into Large Structures in the Cell 121
A Single Type of Protein Subunit Can Interact with Itself to Form Geometrically Regular Aggregates 121
Self-assembling Aggregates Can Include Different Protein Subunits and Nucleic Acids 124

here Are Limits to Self-assembly 126
ummary 127

rotein Function 127

Protein's Conformation Determines Its Chemistry 128
ubstrate Binding Is the First Step in Enzyme atalysis 129
nzymes Accelerate Reaction Rates Without Shifting quilibria 131
Many Enzymes Make Reactions Proceed Preferentially One Direction by Coupling Them to ATP ydrolysis 132
ultienzyme Complexes Help to Increase the Rate of ell Metabolism 132
tracellular Membranes Increase the Rates of iffusion-limited Reactions 133
otein Molecules Can Reversibly Change Their Shape 134
llosteric Proteins Are Involved in Metabolic egulation 134
llosteric Proteins Are Vital for Cell Signaling 135
oteins Can Be Pushed or Pulled into Different hapes 135
nergy-driven Changes in Protein Conformations Can o Useful Work 137
TP-driven Membrane-bound Allosteric Proteins Can ct as Pumps 137
otein Molecules Can Harness Ion Gradients to Do seful Work 139
ummary 139

eferences 139

CHAPTER 4

low Cells Are Studied

licroscopy 143

ıe Light Microscope Can Resolve Details 0.2 μm ıart 144
fferent Components of the Cell Can Be Selectively ained 145
ssues Are Usually Fixed and Stained for Microscopy 145
ving Cells Can Be Seen in a Phase-Contrast or fferential-Interference-Contrast Microscope 146
ıe Electron Microscope Resolves the Fine Structure the Cell 148
ological Specimens Require Special Preparation for ı Electron Microscope 149
ree-dimensional Images Can Be Obtained by ctron Microscopy 151
eeze-Fracture and Freeze-Etch Electron Microscopy ovide Novel Views of the Cell 152
ividual Macromolecules Can Be Resolved in the ctron Microscope 154
e Detailed Structure of Molecules in a Crystalline ay Can Be Calculated from the Diffraction Patterns ey Create 154
Image Reconstruction Techniques Based on Diffraction Can Be Used to Extract Additional Information from Electron Micrographs 157
X-ray Diffraction Reveals the Three-dimensional Arrangement of the Atoms in a Molecule 158
Summary 159

Cell Culture 160

Cells Can Be Grown in a Culture Dish 161
Chemically Defined Media Permit Identification of Specific Growth Factors 162
Eucaryotic Cell Lines Are a Convenient Source of Homogeneous Cells 162
Cells Can Be Fused Together to Form Hybrid Cells 163
Summary 165

The Fractionation of Cells and Their Contents 165

Cells Can Be Isolated from a Tissue and Separated into Different Types 166
Organelles and Macromolecules Can Be Separated by Ultracentrifugation 166
The Molecular Details of Complex Cellular Processes Can Be Determined Only by Studies of Fractionated Cell Components 169
Proteins Can Be Separated by Chromatography 170
The Size and Subunit Composition of a Protein Can Be Determined by SDS Polyacrylamide-Gel Electrophoresis 173
More Than 1000 Proteins Can Be Resolved on a Single Gel by Two-dimensional Polyacrylamide-Gel Electrophoresis 175
Selective Cleavage of a Protein Generates a Distinctive Set of Peptide Fragments 177
Short Amino Acid Sequences Can Be Analyzed by Automated Machines 177
Summary 178

Tracing Cellular Molecules with Radioactive Isotopes and Antibodies 179

Radioactive Atoms Can Be Detected with Great Sensitivity 179
Radioisotopes Are Used to Trace Molecules in Cells and Organisms 179
Antibodies Can Be Used to Detect and Isolate Specific Molecules 181
Hybridoma Cell Lines Provide a Permanent Source of Monoclonal Antibodies 182
Antibodies and Other Macromolecules Can Be Injected into Living Cells 184
Summary 184

Recombinant DNA Technology 185

Restriction Nucleases Hydrolyze DNA Molecules at Specific Nucleotide Sequences 185
Selected DNA Sequences Are Produced in Large Amounts by Cloning 187
Copies of Specific mRNA Molecules Can Be Cloned 188

Cloned DNA Fragments Can Be Rapidly Sequenced 189
Nucleic Acid Hybridization Reactions Provide a Sensitive Way of Detecting Specific Nucleotide Sequences 189
In Situ Hybridization Techniques Are Used to Localize Specific Nucleic Acid Sequences in Chromosomes and Cells 191
Recombinant DNA Techniques Allow Even the Minor Proteins of a Cell to Be Studied 191
Mutant Genes Can Now Be Made to Order 192
Summary 194

References **194**

PART III

The Molecular Organization of Cells

CHAPTER 5

Basic Genetic Mechanisms

Protein Synthesis **199**
The Decoding of DNA into Protein 199
RNA Polymerase Copies DNA into RNA 200
Protein Synthesis Is Inherently Very Complex 202
Specific Enzymes Couple Each Amino Acid to Its Appropriate tRNA Molecule 205
Amino Acids Are Added to the Carboxyl-Terminal End of a Growing Polypeptide Chain 206
Each Amino Acid Added Is Selected by a Complementary Base-pairing Interaction Between Its Linked tRNA Molecule and an mRNA Chain 207
The Events in Protein Synthesis Are Catalyzed on the Ribosome 208
A Ribosome Moves Stepwise Along the mRNA Chain 210
A Protein Chain Is Released from the Ribosome Whenever One of Three Different Termination Codons Is Reached 211
The Initiation Process Sets the Reading Frame for Protein Synthesis 211
The Overall Rate of Protein Synthesis in Eucaryotes Is Controlled by Initiation Factors 212
Many Inhibitors of Procaryotic Protein Synthesis Are Useful as Antibiotics 212
Summary 213

DNA Repair Mechanisms **214**
DNA Sequences Are Maintained with a Very High Fidelity 214
Directly Detected Spontaneous-Mutation Rates Are Consistent with Evolutionary Estimates 214
Most Mutations in Proteins Are Deleterious and Are Eliminated by Natural Selection 215
Low Mutation Rates Mean That Related Organisms Must Be Made from Essentially the Same Proteins 216
If Left Uncorrected, Spontaneous DNA Damage Would Change DNA Sequences Rapidly 216
The Stability of Genes Is Due to DNA Repair 217
The Structure and Chemistry of the DNA Double Helix Make It Easy to Repair 220
Summary 221

DNA Replication Mechanisms **221**
Base-pairing Underlies DNA Replication as well as DNA Repair 221
The DNA Replication Fork Is Asymmetrical 223
The High Fidelity of DNA Replication Requires a "Proofreading" Mechanism 224
DNA Replication in the 5′-to-3′ Direction Is Intrinsically More Accurate 226
A Special Nucleotide Polymerizing Enzyme Is Needed to Synthesize Short Primer Molecules on the Lagging Strand 226
Special Proteins Help Open Up the DNA Double Helix in Front of the Replication Fork 227
Other Special Proteins Prevent DNA Tangling 228
The Replication of DNA in Eucaryotes and Procaryotes Is Basically Similar 230
Replication Forks Are Created at Replication Origins 231
Summary 232

Viruses **232**
Viruses Are Mobile Genes 232
The Genetic Component of a Virus Is Either DNA or RNA 233
The Outer Coat of a Virus May Be a Protein Capsid or a Membrane Envelope 233
Viral Chromosomes Usually Code for One or More Enzymes Involved in the Replication of the Viral Nucleic Acid 235
Viral Genomes Come in a Variety of Forms 235

Viral Chromosomes Can Integrate Themselves into Host Chromosomes 236
RNA Viruses Also Replicate Through the Formation of Complementary Chains 236
Viral Genetic Elements Can Make Cells Cancerous 238
How Did Viruses Evolve? 239
Summary 240

Genetic Recombination Mechanisms 240

General Recombination Processes Are Guided by Base-pairing Interactions Between Complementary Strands of Homologous DNA Helices 241
General Recombination Is Initiated at a Nick in One Strand of a DNA Double Helix 242
Special Proteins Enable DNA Single Strands to Pair with a Homologous Region of DNA Double Helix 243
Genetic Recombination Usually Involves a Cross-Strand Exchange 244
General Recombination Aids DNA Repair Processes 247
Site-specific Recombination Enzymes Move Special Sequences of DNA in and out of Genomes 247
Summary 250

References 250

CHAPTER 6

The Plasma Membrane

The Lipid Bilayer 256

Membrane Lipids Are Amphipathic Molecules That Spontaneously Form Bilayers 256
The Lipid Bilayer Is a Two-dimensional Fluid 258
The Fluidity of a Lipid Bilayer Depends on Its Composition 259
The Lipid Bilayer Serves as a Solvent for Membrane Proteins 260
The Lipid Bilayer of the Plasma Membrane Is Asymmetrical 261
Glycolipids Are Found on the Surface of All Plasma Membranes, But Their Function Is Unknown 262
Summary 263

Membrane Proteins 264

Many Membrane Proteins Are Held in the Bilayer by Hydrophobic Interactions with Lipid Molecules 264
The Use of SDS Polyacrylamide-Gel Electrophoresis Has Revolutionized the Study of Membrane Proteins 266
The Cytoplasmic Side of Membrane Proteins Can Be Studied in Red Blood Cell Ghosts 266
Spectrin Is Loosely Associated with the Cytoplasmic Side of the Red Blood Cell Membrane 268
Glycophorin Extends Through the Red Cell Lipid Bilayer as a Single α-Helix 268
Band III of the Human Red Blood Cell Membrane Is a Transport Protein 269
Bacteriorhodopsin Is a Proton Pump That Traverses the Bilayer as Seven α-Helices 271
Membrane Transport Proteins Can Be Visualized by Freeze-Fracture Electron Microscopy 272
Vectorial Labeling Reagents Can Be Used to Study Some Plasma Membrane Proteins of Nucleated Cells 274
When Two Cells Are Fused Together, Their Plasma Membrane Proteins Rapidly Mix 275
Membrane Proteins Cluster into Patches When They Are Cross-linked by Antibodies 276
Cross-linked Membrane Proteins Are Actively Swept to One Pole of the Cell in the Process of "Capping" 278
Conflicting Views on How Cells Cap: Flow Versus Pull Hypotheses 279
Antibody-induced Redistribution Can Be Used to Determine Whether Two Polypeptides Are Associated with Each Other in the Plasma Membrane 280
Lateral Diffusion Rates of Membrane Proteins Can Be Quantified 281
Cells Have Ways of Restricting the Lateral Mobility of Certain of Their Membrane Proteins 282
Summary 283

Membrane Carbohydrate 283

The Carbohydrate in Biological Membranes Is Confined to the Noncytoplasmic Surface 284
Cell-Surface Carbohydrate Is Suspected to Be Important in Cell-Cell Interactions, But This Has Been Difficult to Demonstrate 285
Summary 286

Membrane Transport of Small Molecules 286

Protein-free Lipid Bilayers Are Impermeable to Ions But Freely Permeable to Water 287
Membrane-bound Transport Proteins Transfer Specific Small Molecules Across Cell Membranes 287
Transport Proteins Form a Continuous Protein Pathway Across the Lipid Bilayer 289
Carrier Proteins Behave Like Membrane-bound Enzymes 289
The Membrane Potential That Exists Across the Plasma Membrane Is Maintained by a Na^+-K^+ Pump 290
The Ubiquitous Plasma Membrane Na^+-K^+ Pump Is an ATPase 291
The Na^+-K^+ ATPase Helps Control Cell Volume by Controlling the Solute Concentration Inside Cells 294
Some Ca^{2+} Pumps Are Also Membrane-bound ATPases 295
Membrane-bound Enzymes That Synthesize ATP Are Transport ATPases Working in Reverse 295
Active Transport Can Be Driven by Ion Gradients 295
Active Transport in Bacteria Can Occur by "Group Translocation" and Can Involve Water-soluble Binding Proteins 297
Some Transmembrane Protein Channels Are "Gated" and Open Only Transiently 298
Asymmetrically Distributed Ion Channels Can Generate Ion Currents That Polarize Cells 300
Ionophores Increase the Ion Permeability of Synthetic and Biological Membranes 301
Summary 301

Membrane Transport of Macromolecules and Particles: Exocytosis and Endocytosis **302**

Exocytosis Occurs by the Fusion of Intracellular Vesicles with the Plasma Membrane 303

Triggered Exocytosis Is a Localized Response of the Plasma Membrane and Its Underlying Cytoplasm 304

Membrane Fusion Involves Bilayer Adherence Followed by Bilayer Joining 305

Endocytosis Occurs Continually in Most Cells 306

Most Endocytotic Vesicles Ultimately Fuse with Lysosomes 307

Many Endocytotic Vesicles Are Coated 307

Coated Pits and Vesicles Provide a Specialized Pathway for Receptor-mediated Endocytosis of Specific Macromolecules 309

Many Cell-Surface Receptors Associate with Coated Pits Only After Ligand Binding 311

Some Macromolecules Can Penetrate Cell Membranes Directly 311

Specialized Phagocytic Cells Ingest Particles That Bind to Specific Receptors on Their Surface 311

Phagocytosis Is a Localized Response That Proceeds by a "Membrane-zippering" Mechanism 312

Membrane Vesicular Traffic: How Is It Powered, Guided, and Regulated? 313

Summary 314

References **314**

CHAPTER 7

Internal Membranes and the Synthesis of Macromolecules

The Compartmentalization of Higher Cells **320**

Large Eucaryotic Cells Need Internal Membranes 320

Internal Membranes Divide the Cell into Specialized Compartments 320

Even Complex Eucaryotic Cells Have Only a Few Major Intracellular Compartments 320

Intracellular Compartments Permit the Cell to Carry Out Many Incompatible Chemical Reactions Simultaneously 323

Viruses Reveal the Existence of Highly Organized Pathways Between Host Cell Compartments 323

Different Viruses Follow Different Pathways Through the Cell 325

Summary 326

The Cytosol **326**

Most Intermediary Metabolism Takes Place in the Cytosol 326

Many Proteins Are Synthesized by Ribosomes in the Cytosol 328

The Binding of Many Ribosomes to an Individual Messenger RNA Molecule Generates Polysomes 328

Protein Synthesis Is Blocked by Specific Inhibitors 330

Some Proteins Regulate the Rate of Their Own Synthesis by Binding to the Messenger RNA Molecules on Which They Are Made 330

In Eucaryotes Only One Species of Polypeptide Chain Can Be Synthesized on Each Messenger RNA Molecule 331

Polyproteins Are Often Made in Eucaryotes 332

Many Proteins Undergo Covalent Modification After Their Synthesis 333

Some Proteins Are Degraded Soon After They Have Been Synthesized 334

Not All Proteins Synthesized in the Cytosol Remain There 335

Summary 335

The Endoplasmic Reticulum **335**

Attached Ribosomes Define "Rough" Regions of ER 336

Smooth ER Is Abundant in Certain Specialized Cells 338

Rough and Smooth Regions of ER Can Be Physically Separated 339

Rough Regions of ER Contain Specific Proteins Responsible for the Binding of Ribosomes 340

Membrane-bound Ribosomes Synthesize Proteins That Pass Through the Membrane During Their Translation 341

Direct Evidence Favoring Vectorial Discharge Has Come from Experiments in Bacteria 342

Membrane-bound Ribosomes Are Derived from Free Ribosomes That Are Directed to the ER Membrane by Special Signal Sequences 343

There Is Genetic Evidence for the Signal Hypothesis 344

Some Proteins Cross Membranes by a Posttranslational Import Mechanism Rather Than by Vectorial Discharge 344

Most Proteins Synthesized in the Rough ER Are Glycosylated 345

The Oligosaccharide Is Added to the Growing Polypeptide Chain on the Luminal Side of the ER 347

The Oligosaccharide Is Donated to the Polypeptide by an Activated Lipid and Then Almost Immediately Modified 347

Lipids Are Synthesized in the ER Membrane 350

Lipid Biosynthesis Is Asymmetric 351

Special Proteins Transfer Phospholipids from the ER to Mitochondria 351

The Luminal Side of an Internal Organelle Is Topologically Equivalent to the Outside of the Cell 352

Membrane Growth by Continuous Expansion of the ER Ensures the Propagation of Transmembrane Asymmetry 353

Summary 354

The Golgi Apparatus **355**

The Golgi Apparatus Consists of Stacks of Disc-shaped Cisternae with Associated Small Vesicles 355

The Golgi Apparatus Is Structurally and Biochemically Polarized 356
The Golgi Apparatus Is Not Yet Understood in Biochemical Terms 356
Carbohydrate Structures Are Modified in the Golgi Apparatus 357
Oligosaccharide-trimming Pathways Are Elaborate and Precisely Programmed 359
The Correct Program of Trimming Is Set by an Irreversible "Switch" Thrown Early in Oligosaccharide Processing 359
Carbohydrate Modification in the Golgi Apparatus Can Be Detected by Autoradiography 359
Proteins Destined for Secretion Are Packaged into Golgi-associated Secretory Vesicles That Then Fuse with the Plasma Membrane 361
Membrane Components Are Recycled 362
Membrane Fusion and Recycling Can Be Demonstrated by Electron Microscopy 363
Coated Vesicles Are Thought to Play a Major Part in the Intracellular Sorting of Proteins 365
Why Have a Golgi Apparatus? 365
Summary 366

Lysosomes and Peroxisomes 367

Lysosomes Are the Principle Sites of Intracellular Digestion 367
Histochemical Staining Demonstrates That Lysosomes Are Heterogeneous Organelles 367
Primary Lysosomes Are Formed by Budding from the Golgi Apparatus 370
Lysosomal Function in Cultured Cells from Patients Having a Lysosomal Enzyme Deficiency Can Be Corrected by Adding the Missing Enzyme to the Culture Medium 370
Only Lysosomal Hydrolases with a Mannose-Phosphate-containing Oligosaccharide Are Internalized 371
Intracellular Hydrolases Are Probably Directed to Primary Lysosomes by the Mannose-Phosphate Marker 372
The Membrane of the Peroxisome Is Formed by Budding from the Smooth ER 373
Peroxisomes Use Oxygen to Carry Out Catabolic Reactions 373
The Enzyme Content of Peroxisomes Varies with Cell Type 374
A Sorting Mechanism Similar to That Used in the Lysosomal Pathway May Operate for Other Intracellular Compartments 374
Summary 376

Organelles with Double Membranes: The Nucleus, Mitochondria, and Chloroplasts 376

Bacteria with Inner and Outer Cell Membranes May Be Viewed as Models of the Double-Membrane Organelles 377
Membrane Proteins May Move Between the Inner and Outer Bilayers at Adhesion Sites 378
Adhesion Sites May Also Play a Part in Transporting Water-soluble Molecules into the Lumen of Double-Membrane Organelles 379
Summary 380

References 380

CHAPTER 8

The Cell Nucleus

The Organization of DNA into Chromosomes 386

Histones Are Among the Most Highly Conserved Proteins Known 386
The Association of Histones with DNA Leads to the Formation of Nucleosomes, the Unit Particles of Chromatin 388
Nucleosomes Are Packed Together to Form Regular Higher-Order Structures 388
Histone H1 Proteins Help Pack Nucleosomes Together 389
Not All Nucleosomes Are Packed in Precisely the Same Way 390
The DNA Helix Is Punctuated by Proteins That Bind to Specific DNA Sequences 392
Proteins Can Recognize Specific DNA Sequences by Hydrogen Bonding to Base Pairs and by Sensing Helix Geometry 394
Histones Restrict the Accessibility of DNA to Other DNA-binding Proteins and Can Thereby Affect Gene Regulation 395
Nucleosome Beads Can Be Nonrandomly Positioned in Chromatin 395
Each Chromosome Probably Contains One Very Long DNA Molecule Organized in a Series of Looped Domains 397
Bands on Mitotic Chromosomes Reveal an Even Higher Level of Organization 397
Interphase Genes Can Be Seen in Polytene Chromosomes 401
Each Looped Domain of Chromatin May Correspond to a Separate Unit of Function 402
Different Domains of Chromatin Appear to Contain Differently Organized Nucleosomes 404
Most Chromosomal DNA Does Not Code for Essential Proteins 405
Summary 406

RNA Synthesis and RNA Processing 406

Three Different RNA Polymerases Make RNA in Eucaryotes 407
RNA Polymerase II Transcribes Some DNA Sequences Much More Often Than Others 408
Transcription Occurs on DNA Bound Up in Nucleosomes 411
New RNA Is Packaged in Ribonucleoprotein Particles 411
The Precursors of Messenger RNA Are Covalently Modified at Both Ends 412

RNA Processing Removes Long Nucleotide Sequences from the Middle of RNA Molecules 414
Small Nuclear Ribonucleoprotein Particles May Help Guide RNA Processing 415
Multiple Intron Sequences Can Be Removed from a Single RNA Transcript 417
The Same RNA Transcript Can Be Processed in Different Ways to Produce mRNAs Coding for Several Proteins 418
Different Proteins May Be Made from a Single DNA Coding Sequence at Different Stages of Cell Development 419
What Is a Gene? 421
The Export of RNA from the Nucleus Requires Molecular Signals 422
Ribosomal RNAs and Transfer RNAs Are Made on Tandemly Arranged Sets of Identical Genes 422
The Nucleolus Is a Ribosome-producing Machine 424
The Nucleolus Has a Highly Organized Structure 426
The Nucleolus Is Reassembled on Specific Chromosomes After Each Mitosis 427
Summary 427

The Nuclear Envelope 429

The Nucleus Is Enclosed by a Double Membrane 429
The Nuclear Lamina Helps to Determine Nuclear Shape 430
Nuclear Transport Occurs Through Nuclear Pores 432
Nuclear Pores Probably Transport Large Particles Selectively 433
The Inner Surface of the Nuclear Lamina Helps to Organize the Chromosomes 434
Summary 435

Basic Elements in the Control of Gene Expression 435

The Cells of a Multicellular Organism All Contain the Same DNA 436
Different Cell Types Synthesize Different Sets of Proteins 436
Different Cell Types Transcribe Different Sets of Genes 437
Repressor Proteins Inhibit the Transcription of Specific Genes in Bacteria 438
Gene Activator Proteins Probably Predominate in Higher Eucaryotic Cells 440
Two Interacting Gene Regulatory Proteins That Repress Each Other's Synthesis Produce a Stable "Memory" in a Bacterial Cell 442
Gene Regulatory Proteins That Act on Many Genes Simultaneously Are Probably Used in Combinations to Generate Different Tissues in Eucaryotes 443
In Principle, Many Different Cell Types Can Be Efficiently Specified by Combinations of a Few Gene Regulatory Proteins 444
Input from Several Different Gene Regulatory Proteins May Be Needed to Turn On a Single Gene 445
In Bacteria, DNA Supercoiling Facilitates the Transcription of Genes 446
The Regulation of Gene Expression in Eucaryotic Cells Includes Types of Controls Not Found in Bacteria 447
Eucaryotic Gene Activation Mechanisms Appear to Loosen Chromatin Structure All Along a Gene 448
Eucaryotic Gene Activation Probably Occurs in Two Stages 449
Looped Chromatin Domains Unfold as They Are Transcribed in Polytene Chromosomes 450
Lampbrush Chromosomes Display Their Transcription Units in Extended Looped Domains 452
Altering the Ends of a Primary RNA Transcript Can Change the Protein Made 454
Are Important Controls Also Exerted by Alterations in the Specificity of RNA Processing and Export? 455
Summary 455

Gene Regulatory Mechanisms and Cell "Memory" 456

A Highly Condensed Fraction of Interphase Chromatin Contains Specially Inactivated Genes 456
Two Subclasses of Heterochromatin Can Be Distinguished 457
An Inactive X Chromosome Is Inherited 458
Eucaryotic Genes Can Be Turned Off by a Novel Heritable Mechanism 459
Cooperatively Bound Clusters of Gene Regulatory Proteins Can, in Principle, Be Directly Inherited 460
Directly Inherited Patterns of DNA Methylation Help to Control Some Mammalian Genes 460
In Special Cases, Local DNA Sequences Are Reversibly Rearranged to Turn Genes On and Off 462
Selected Portions of Chromosomes Can Be Either Deleted or Amplified in Somatic Cells 464
Major Rearrangements of Chromosomes Are Usually Deleterious 465
Summary 467

The Organization and Evolution of DNA Sequences 467

Genetic Recombination Drives Evolutionary Changes 468
A Large Fraction of the DNA of Most Eucaryotes Consists of Repeated Nucleotide Sequences 468
Frequent Genetic-Recombination Events Expand and Contract Serially Repeated Satellite DNA Sequences 468
The Evolution of Globins Shows How Random DNA Duplications Contribute to the Evolution of Organisms 470
Recombination Events Also Create New Types of Protein Chains by Joining Coding Domains 471
Short, Interspersed Repeated Sequences Are Common in Eucaryotic Genomes 472
The Concept of "Selfish" DNA 472
Cataclysmic Changes in Genomes Can Increase Biological Diversity 473
Summary 475

References 476

In Mammals, Mitochondrial Genes Are Maternally Inherited 533
Petite Mutants in Yeast Demonstrate the Overwhelming Importance of the Cell Nucleus in Mitochondrial Biogenesis 534
A Large Fraction of the DNA in Yeast Mitochondria Is Noncoding 535
The Complete Nucleotide Sequence of the Human Mitochondrial Genome Is Known, and It Has Some Surprising Features 536
Chloroplasts Have a More Complex Genome Than Yeast and Animal Mitochondria 537
The RNA Transcripts Made on Mitochondrial DNA *Are Extensively Processed After Their Synthesis* 537
Proteins Are Imported into Mitochondria and Chloroplasts by an Energy-requiring Process 538
Chloroplasts Make Most of Their Own Lipids, While Mitochondria Rely Mainly on Import 540
The Biosynthesis of Mitochondria and Chloroplasts Is Largely Controlled by the Nucleus 540
How Can Drugs That Inhibit Mitochondrial Protein Synthesis Be Used as Antibiotics Without Harming the Patient? 540
Mitochondria and Chloroplasts Have Probably Evolved from Endosymbiotic Bacteria 541
Why Do Mitochondria and Chloroplasts Have Their Own Genetic Systems? 542
Summary 543

References **544**

CHAPTER 10

The Cytoskeleton

Muscle Contraction **550**
A Myofibril Is the Contractile Element of a Skeletal Muscle Cell 550
Myofibrils Are Composed of Repeating Assemblies of Thick and Thin Filaments 550
Contraction Occurs as Filaments Slide Past Each Other 551
Thin Filaments Consist Principally of Actin 553
Thick Filaments Are Composed Principally of Myosin 553
ATP Hydrolysis Drives Muscle Contraction 554
Myosin is an Actin-activated ATPase 555
Myosin Heads Bind to Actin Filaments 555
Myosin Heads "Walk" Along Actin Filaments 556
Actin and Myosin Are Held in Position in the Myofibril by Other Proteins 558
Muscle Contraction Is Initiated by a Rise in Intracellular Ca^{2+} 558
Troponin and Tropomyosin Mediate the Ca^{2+} Regulation of Muscle Contraction 559
Smooth Muscle Myosin Is Activated by Ca^{2+}-dependent Phosphorylation 560
Summary 561

Ciliary Movement **561**
Cilia and Flagella Propagate Bending Movements 561
The Core of a Cilium Contains a Bundle of Parallel Microtubules in a "9 + 2" Arrangement 562
Microtubules Are Hollow Tubes Formed from Tubulin 563
The Ciliary Axoneme Contains Links, Spokes, and Sidearms Made of Protein 563
The Axoneme Moves by a Sliding Microtubule Mechanism 566
Dynein Is Responsible for the Sliding 567
Cilia Can Be Dissected Genetically 567
The Conversion of Microtubule Sliding to Ciliary Bending Depends on the Inner Sheath 569
Summary 569

General Features of Microtubules and Actin Filaments as Dynamic Assemblies **569**
Microtubules Are Highly Labile Structures That Are Sensitive to Specific Antimitotic Drugs 570
Actin Filaments Are Continually Formed and Broken Down in Cells 571
Specific Drugs Change the State of Actin Polymerization and Thereby Affect Cell Behavior 572
The Polymerization of Actin and Tubulin Can Be Studied *in Vitro* 573
Actin Filaments and Microtubules Are Polar Structures That Grow at Different Rates from Their Two Ends 574
Actin and Tubulin Polymers Can Undergo a "Treadmilling" of Subunits 576
Summary 577

Microtubule Organizing Centers and Microtubule-associated Proteins **578**
Cytoplasmic Microtubules Are Associated with Other Proteins 578
Microtubules Grow from Discrete Organizing Centers 578
Centrioles and Basal Bodies Are Structurally Identical and Functionally Interconvertible 579
Centrioles Usually Arise by Duplication 580
What Is the Function of Centrioles? 581
Summary 581

Actin Filaments and Actin-binding Proteins in Nonmuscle Cells **582**
Microvilli Contain Bundles of Actin Filaments 582
Rigid Arrays of Cross-linked Actin Filaments Play a Part in the Detection of Sound 583
Dynamic Actin-Filament Structures Occur on the Surface of Many Cells 584
Nonmuscle Cells Contain Small "Musclelike" Assemblies 586
The Assembly of Myosin in Nonmuscle Cells Is Ca^{2+}-dependent 587
Actin Filaments Are Often Anchored in Cell Membranes 588
Amoeboid and Fibroblast Locomotion Depend on Actin Filaments 588
Amoeboid Movement Is Accompanied by Gel-to-Sol Transitions in the Cytoplasm 589

Cross-linking Proteins Can Produce an Actin Gel 590
Fragmenting Proteins Can Produce a Ca^{2+}-dependent Liquefaction of Actin Gels 590
The Contraction of Cytoplasmic Gels Is Mediated by Myosin 591
Summary 591

Intermediate Filaments 593
Intermediate Filaments Consist of Fibrous Polypeptides That Vary Greatly in Size 593
Disassembly of Intermediate Filaments May Require Filament Destruction 594
Different Cell Types Contain Intermediate Filaments of Distinct Composition 594
Keratin Filaments Strengthen Epithelial Cell Sheets 595
Do Intermediate Filaments Have Nonstructural Functions? 596
Summary 596

Organization of the Cytoskeleton 597
The Cytoskeleton Can Be Seen in Three Dimensions in the Electron Microscope 597
Organelles and Soluble Proteins Can Be Associated with the Cytoskeleton 599
Extensive Changes in the Cytoskeleton Are Produced by a Single Gene of a Transforming Virus 600
Microtubules May Be the Overall Organizers of the Cytoskeleton 600
The Cytoskeleton Enables Cells to Respond to the Physical Nature of a Solid Surface 602
Cytoskeleton Organization Can Be Passed from a Parent Cell to Its Daughters 602
Cytoskeletal Organization Can Be Transmitted Across Cell Membranes 603
How Do Cells Move? 604
Summary 605

References 605

CHAPTER 11

Cell Growth and Division

The Control of Cell Division 611
The Cells in a Multicellular Organism Divide at Very Different Rates 612
Differences in Cell-Cycle Times Are Due Mainly to Variations in the Length of G_1 613
Determination of Cell-Cycle Times 613
A Special "Trigger Protein" May Control Cell Division 617
Cell Division Is Regulated by a Variety of "Feedback Control" Mechanisms 618
Normal Mammalian Cells Grown in Culture Will Stop Dividing When They Run Out of Space 618
Positional Signals Also Control Cell Division 619
Cancer Cells Have Lost Their Normal Growth Control 620
Summary 621

Tumor Viruses as Tools for Studying the Control of the Cell Cycle 621
Oncogenes Are Identified Through the Methods of Molecular Genetics 623
The Oncogenes of RNA Tumor Viruses Are the Best Understood 625
Oncogenes Frequently Code for Protein Kinases 626
Increased Levels of a Normal Cellular Protein Can Destroy Normal Cell Growth Regulation 627
Active Oncogenes Have Been Isolated Directly from Human Tumors 628
Summary 628

Events in the S Phase 629
During the S Phase, Clusters of Replication Forks Become Simultaneously Active on Each Chromosome 630
New Histones Are Assembled into Chromatin as DNA Replicates 632
The Orientation of Replication Origins Relative to Genes May Have Important Biological Consequences 635
Different Genetic Regions on the Same Chromosome Replicate at Distinct Times During the S Phase 635
Replication Times During the S Phase Are Correlated with Interphase Chromatin Structure 637
How Is the Timing of DNA Replication Controlled? 638
Chromatin-bound Factors Ensure That Each Region of the DNA Is Replicated Only Once During Each S Phase 638
Summary 639

The Logic of the Cycle 640
Most Proteins Are Synthesized Continuously Throughout Interphase 640
The Cell Cycle Proceeds by an Obligatory Series of Sequential Reactions 641
Controls Operating Early in the Cycle Largely Determine the Cell Mass at Mitosis 642
The G_2 Phase Serves to Prepare Cells for Mitosis 644
Cell-Cycle Times Can Be Drastically Shortened When Not Limited by the Rates of Biosynthesis 644
Summary 645

Cell Division 646
Cell Division Is Traditionally Divided into Six Stages 646
Some Events in Mitosis Depend on the Mitotic Spindle While Others Do Not 647
The Spindle Contains Organized Arrays of Microtubules in Dynamic Equilibrium Between Assembly and Disassembly 647
Spindle Formation at Prophase Is Largely Controlled by the Mitotic Centers That Define the Poles 652
Each Mitotic Chromosome Consists of Two Chromatids and a Pair of Kinetochores That Bind to the Ends of Microtubules 652
The Chromosome Alignment at Metaphase Is Generated by Interactions Between the Kinetochore and Polar Spindle Fibers 653
Metaphase Chromosomes Are Held in a Deceptively Static State by Balanced Bipolar Forces 654

The Sister Chromatids Separate Suddenly at Anaphase 655
At Anaphase the Chromosomes Are Pulled Toward the Poles and the Two Poles Are Pushed Apart 655
A Dyneinlike ATPase Is Thought to Generate the Force That Pushes the Poles Apart in Anaphase, But a Different Mechanism Seems to Move the Chromosomes to the Poles 658
At Telophase the Nuclear Envelope Initially Re-forms Around Individual Chromosomes 659
The Mitotic Spindle Determines the Site of Cytoplasmic Cleavage During Cytokinesis 660
Actin and Myosin Generate the Forces for Cleavage 662
Cytokinesis Occurs by a Completely Different Mechanism in Plant Cells with Cell Walls 663
Cytokinesis Must Allow the Cytoplasmic Organelles to Be Faithfully Inherited 666
The Elaborate Mitotic Process of Higher Organisms Evolved Gradually from the Procaryotic Fission Mechanisms 666
Summary 668

References **668**

CHAPTER 12

Cell-Cell Adhesion and the Extracellular Matrix

Intercellular Recognition and Cell Adhesion **674**
Myxobacteria Exploit the Advantages of Social Behavior 674
The Assembly of Slime Mold Amoebae into a Multicellular Slug Involves Chemotaxis and Specific Cell Adhesion 676
Species-specific Cell Aggregation in Sponges Is Mediated by a Large Extracellular Aggregation Factor 679
Dissociated Embryonic Vertebrate Cells Preferentially Associate with Cells of the Same Tissue 680
Cells from Different Tissues Display a Hierarchy of Adhesiveness 681
Summary 682

Cell Junctions **682**
Desmosomes Anchor Cells Together 683
Tight Junctions Form a Permeability Barrier Across Cell Sheets 684
Gap Junctions Allow Small Molecules to Pass Directly from Cell to Cell 686
Why Are So Many Cells Electrically and Metabolically Coupled via Gap Junctions? 688
Most Cells in Early Embryos Are Ionically Coupled 689
Cells May Control the Permeability of Their Gap Junctions 689
Gap Junctions Are Composed of Channels That Directly Connect the Interiors of the Interacting Cells 690
Summary 692

The Extracellular Matrix **692**
The Extracellular Matrix Consists Primarily of Fibrous Proteins Embedded in a Hydrated Polysaccharide Gel 692
Collagen Is the Major Protein of the Extracellular Matrix 693
Collagen Chains Have an Unusual Amino Acid Composition 696
During Secretion, Procollagen Molecules Are Cleaved to Form Collagen Molecules, Which Self-assemble into Fibrils 697
Once Formed, Collagen Fibrils Are Greatly Strengthened by Covalent Cross-linking 699
Elastin Is a Cross-linked, Random-Coil Protein That Gives Tissues Their Elasticity 701
Proteoglycans and Hyaluronic Acid Are Major Constituents of the Extracellular Matrix 702
Glycosaminoglycan Chains Occupy Vast Amounts of Space and Form Hydrated Gels 705
Glycosaminoglycan Chains May Be Highly Organized in the Extracellular Matrix 705
Fibronectin Is an Extracellular-Matrix Glycoprotein That Promotes Cell Adhesion 707
The Basal Lamina Is a Specialized Extracellular Matrix That Contains a Unique Type of Collagen 709
Basal Laminae Perform Diverse and Complex Functions 711
Intracellular Actin Filament Bundles Direct the Organization of Matrix Macromolecules on Cell Surfaces and Thereby Organize the Extracellular Matrix 711
In Reciprocal Fashion, an Ordered Extracellular Matrix Influences the Organization and Behavior of the Cells It Contains 712
Summary 713

References **713**

CHAPTER 13

Chemical Signaling Between Cells

Three Different Strategies of Chemical Signaling: Local Chemical Mediators, Hormones, and Neurotransmitters **718**
The Hypothalamus Is the Main Regulator of the Endocrine System 719
Different Cells Respond in Different Ways to the Same Chemical Signal 719
Some Cellular Responses to Chemical Signals Are Rapid and Transient, While Others Are Slow and Long-lasting 723
Signaling Molecules Can Be Either Water-soluble or Lipid-soluble 724
Local Chemical Mediators Are Rapidly Destroyed After They Are Secreted 724
Some Signaling Molecules Released by Nerve Terminals Probably Act as Local Chemical Mediators Rather Than as Neurotransmitters 726
Some Hormones and Local Chemical Mediators Act as Specific Growth Factors 727
Summary 729

Signaling Mediated by Intracellular Receptors: Mechanisms of Steroid Hormone Action 729
Steroid-Hormone-Receptor Complexes Bind to Chromatin and Regulate the Transcription of Specific Genes 730
Steroid Hormones Often Induce Both Primary and Secondary Responses 731
Steroid Hormones Regulate Different Genes in Different Target Cells 731
Summary 733

Signaling Mediated by Cell-Surface Receptors: Cyclic AMP and Calcium Ions as Second Messengers 733
The Use of Labeled Ligands Revolutionized the Study of Cell-Surface Receptors 733
Protein Hormones and Growth Factors Are Ingested by Receptor-mediated Endocytosis 734
Cell-Surface Receptors Act as Transducers by Regulating Enzymes or Ion Channels in the Plasma Membrane 735
Cyclic AMP Is a Ubiquitous Intracellular Mediator 736
Receptor and Adenylate Cyclase Molecules Are Separate Proteins that Functionally Interact in the Plasma Membrane 737
Receptors Activate Adenylate Cyclase Molecules Indirectly Through a GTP-binding Protein 738
Ca^{2+} Also Functions as a Ubiquitous Intracellular Mediator 739
The Activation of Some Cell-Surface Receptors Opens Membrane-bound Ca^{2+} Channels 742
Summary 742

The Mode of Action of Cyclic AMP and Calcium Ions as Second Messengers 743
Cyclic AMP Activates Intracellular Protein Kinases 743
Cyclic AMP Also Regulates the Dephosphorylation of Cellular Proteins 745
Phosphorylase Kinase Illustrates How an Enzyme Can Be Activated Only Transiently by an Extracellular Chemical Signal 746
The Reversible Covalent Modification of Proteins Regulates a Large Number of Cellular Processes 747
Ca^{2+} Alters the Conformation of Intracellular Ca^{2+}-binding Proteins 748
Does Cyclic GMP Act as an Intracellular Second Messenger? 749
Extracellular Signals Are Amplified Enormously by the Use of Second Messengers and Enzymatic Cascades 749
The Concentration of a Molecule Can Change Quickly If It Is Continuously Degraded or Removed at a Rapid Rate 750
Cells Can Respond Gradually or Suddenly to Signals 751
Summary 753

Target Cell Adaptation 754
Some Cells Become Desensitized by Endocytosing Their Surface Receptors 754
Some Cell-Surface Receptors Are Reversibly Inactivated by Prolonged Ligand Binding 754
Morphine-induced Target Cell Desensitization Is Not the Result of Receptor Inactivation 755
Chemical Signaling in Unicellular Organisms Provides Useful Models for Study 756
Bacterial Chemotaxis Is a Simple Kind of Intelligent Behavior 757
Chemotaxis-deficient Mutants Have Revealed Four Classes of Proteins Involved in Bacterial Chemotaxis 759
Protein Methylation Is Responsible for Adaptation 760
A Model for Bacterial Chemotaxis 761
Summary 763

References 763

PART III

From Cells to Multicellular Organisms

CHAPTER 14

Germ Cells and Fertilization

The Benefits of Sex 769
In Multicellular Animals the Diploid Phase Is Complex and Long, the Haploid Simple and Fleeting 770
Sexual Reproduction Gives a Competitive Advantage to Organisms in an Unpredictably Variable Environment 771
Sexual Reproduction Helps Establish Favorable Alleles in a Large Population 771
New Genes Evolve by Duplication and Divergence 773
Sexual Reproduction Keeps a Diploid Species Diploid 773
A Diploid Species Has a Spare Copy of Each Gene Free to Mutate to Serve a New Function 773

A Diploid Species Can Rapidly Enrich Its Genome by the Addition of New Genes 775
Summary 776

Meiosis 776
Meiosis Involves Two Nuclear Divisions Rather Than One 778
Genetic Reassortment Is Enhanced by Crossing Over Between Homologous Nonsister Chromatids 778
A Synaptonemal Complex Mediates Chromosome Pairing 779
Recombination Nodules Are Thought to Mediate the Chromatid Exchanges 786
Chiasmata Play an Important Part in Chromosome Segregation in Meiosis 786
Pairing of the Sex Chromosomes Ensures That They Also Segregate 787
Meiotic Division II Resembles a Normal Mitosis 787
Summary 788

Gametes 788
An Egg Is the Only Cell in a Higher Animal Able to Develop into a New Individual 788
Eggs are Highly Specialized Cells with Unique Features 789
Eggs Develop in Stages 790
Many Eggs Grow to Their Large Size Through Special Mechanisms 791
Hormones Induce Egg Maturation and Ovulation 793
Oogenesis Is Wasteful 795
Sperm Are Highly Adapted for Delivering Their DNA to an Egg 796
Sperm Are Produced Continuously in Many Mammals 797
Sperm Nuclei Are Haploid, But Sperm Cell *Differentiation Is Directed by the Diploid Genome* 797
Summary 800

Fertilization 801
A Sperm Must Be Activated Before It Can Fertilize an Egg 801
Sperm-Egg Adhesion Is Mediated by Species-specific Proteins 803
Egg Activation Is Mediated by Changes in Intracellular Ion Concentrations 804
The Rapid Depolarization of the Egg Membrane Prevents Further Sperm-Egg Fusions, Thereby Mediating the Fast Block to Polyspermy 805
The Cortical Reaction Is Responsible for the Late Block to Polyspermy 806
An Increase in Intracellular Free Ca^{2+} Initiates Egg Development 807
A Rise in the Intracellular pH in Some Organisms Induces the Late Synthetic Events of Egg Activation 808
Mammalian Eggs Can Be Fertilized in Vitro 808
Summary 809

References 811

CHAPTER 15

Cellular Mechanisms of Development

Cleavage and Blastula Formation 814
Cleavage Produces Many Cells from One 814
The Polarity of the Embryo Depends on the Polarity of the Egg 815
The Blastula Consists of an Epithelium Surrounding a Cavity 815
Summary 816

Gastrulation, Neurulation, and Somite Formation 816
Gastrulation Transforms a Hollow Ball of Cells into a Three-layered Structure 816
The Ability of Cells to Extend, Adhere, and Contract Is the Universal Basis of Morphogenetic Movement 818
Gastrulation in Amphibians 819
Movements Are Organized About the Blastopore 820
The Endoderm Will Form the Gut and Associated Organs Such as the Lungs and Liver 821
The Mesoderm Will Form Connective Tissues, Muscles, and the Vascular and Urogenital Systems 821
The Ectoderm Will Form the Epidermis and the Nervous System 822
The Neural Tube Is Formed Through Coordinated Changes in Cell Shape 822
Blocks of Mesoderm Cells Uncouple to Form Somites on Either Side of the Body Axis 823
The Vertebrate Body Plan Is First Formed in Miniature and Then Maintained as the Embryo Grows 824
Summary 824

Early Steps in Pattern Formation: The Mouse 825
Mammalian Development Involves an Added Complication 826
The Steps Before Gastrulation 826
Organogenesis and Growth Before Birth 828
Studies of Chimeras Show That All of the Cells of the Very Early Mammalian Embryo Are Functionally Equivalent 828
Position in the Morula Determines the Fate of a Cell 830
A Group of Founder Cells, Rather Than a Single Founder Cell, Gives Rise to a Particular Tissue or Organ 830
Teratomas Can Arise From Embryos That Develop in the Wrong Environment 831
Cells from Teratocarcinomas Can Cooperate with Normal Cells in a Chimera to Make a Normal Mouse 832
Summary 832

Determination and Differentiation 832
In Higher Eucaryotes, the Behavior of a Cell Depends on Its History as well as on Its Environment and Its Genome 834

Cells Often Become Determined for a Future Specialized Role Long Before They Differentiate Overtly 834
The Time of Cell Determination Can Be Discovered by Transplantation Experiments 835
The Genetic Control of Development Is Best Studied in *Drosophila* 836
The State of Determination of Imaginal Disc Cells Is Heritable 838
Groups of Cells Occasionally Transdetermine 838
Homoeotic Mutants Reveal Genes Whose Activities Control Cell Determination 840
The Bithorax Complex Controls Differences Between Thoracic and Abdominal Segments 841
The Larval Body Is Constructed by Modulation of a Fundamental Pattern of Repeating Segments 842
Mitotic Recombination Can Be Exploited to Produce Marked Mutant Clones of Cells 842
Sharp Demarcation Lines Separate Polyclonal Compartments 844
Different Sets of Genes Are Active in the Cells of Different Compartments 846
The State of Determination Is Built Up Combinatorially 846
The Extent of Cell Proliferation in *Drosophila* Is Not Determined by Counting Cell Divisions: Fast-growing Clones May Nearly Fill Their Compartment But Do Not Make It Big 847
Cell Determination in Vertebrates Resembles Cell Determination in *Drosophila* 848
Summary 848

Patterns in Space **849**

Cells Are Assigned Different Characters According to Their Positions 849
Localized Determinants Are Sometimes Identifiable in the Cytoplasm of an Egg 850
The Determinant of Germ-Cell Character Is Localized at One End of the *Drosophila* Egg 850
Cell Character Is Controlled by Spatial Cues 851
Sharp Differences of Character Emerge Gradually in an Initially Uniform Population 852
Positional Information Is Refined by Installments 852
Nonequivalence: Cells That Ultimately Differentiate in the Same Way Can Have Different Positional Information 853
Cells in Separate Fields May Be Supplied with Positional Information in the Same Way But Interpret It Differently 854
Since Embryonic Fields Are Small, Gross Features of the Adult Must Be Determined Early 855
Summary 855

Positional Information in Limb Development **856**

The Developing Chick Limb Can Be Analyzed in Terms of a Three-dimensional System of Coordinates 856
The Cells of the Polarizing Region Control the Antero-Posterior Patterning of Adjacent Tissue 858
Positional Information Along the Antero-Posterior Axis May Be Supplied by Gradations in the Magnitude of a Signal from the Polarizing-Region Cells 858
The Polarizing Region of a Mammal or a Reptile Is Effective in the Chick Also 860
The Parts of the Limb Are Laid Down in Succession Along the Proximo-Distal Axis 860
The Apical Ectodermal Ridge Delimits the Special Region of Mesenchyme from Which Successive Distal Parts Develop, But It Does Not Instruct the Mesenchyme as to Which Parts It Should Form 860
Positional Specification Along the Proximo-Distal Axis Depends on the Amount of Time Spent in the Progress Zone 861
Neighboring Mesenchyme Cells in Early Chick Limb Buds May Interact so as to Smooth Out Discontinuities in the Pattern of Positional Values 863
Some Limbs Can Regenerate 864
Cockroach Legs Undergo Intercalary Regeneration 864
The Same Pattern of Positional Values Is Repeated in Successive Segments of the Cockroach Leg 866
Circumferential Intercalation Obeys the Same Rule as Proximo-Distal Intercalation 866
Intercalation in the Epidermis Is a Two-dimensional Problem 867
Regeneration of a Two-dimensional Patch Obeys the Rule of Intercalation 868
The Rule of Intercalation May Apply to Many Different Systems 869
Summary 869

Inductive Interactions in the Development of Epithelia **870**

Mesoderm Induces Ectoderm to Form the Different Parts of the Neural Tube 870
The Dermis Controls the Nature and Pattern of the Structures That Form from the Epidermis 870
Epithelium Invades Mesenchyme to Form the Tubules of a Gland 872
Summary 872

Multicellular Development Studied Cell by Cell: The Nematode Worm **873**

Caenorhabditis elegans Is Anatomically and Genetically Simple 873
Nematode Development Is Essentially Invariant 874
Cytokinesis Is Not Required for Cell Differentiation 875
The Influence of Local Cell Interactions on the Behavior of Cells During Development Can Be Studied by Laser Microsurgery 876
An "Anchor Cell" Controls Vulva Development 876
A "Distal Tip Cell" Causes Continued Proliferation of Nearby Germ Cells 877
Cell Fate Can Be Controlled by Inhibitory Interactions Among Cells in an Equivalence Group 878
Summary 879

Migratory Cells **880**

Cells Wander: Selective Cohesion May Stop Them from Straying from Their Proper Place 880

Germ Cells Leave the Yolk Sac and Settle in the Genital Ridges 880
Muscle Cells in the Chick Limb Originate by Migration from the Somites 881
Cells Disperse from the Neural Crest and Form Many Different Tissues 881
The Pathways of Migration Are Defined by the Host Connective Tissue 882
The Differentiation of Neural Crest Cells Is Decided by the Local Environment 884
The Development of the Nervous System Poses Special Problems 884
Summary 885

References 885

CHAPTER 16

Differentiated Cells and the Maintenance of Tissues

Maintenance of the Differentiated State 891
Differentiated Cells Commonly Remember Their Character Even in Isolation: Pigment Epithelium of the Retina 892
The Extracellular Matrix That a Cell Secretes Helps Maintain the Cell's Differentiated State 893
Cell-Cell Interactions Can Modulate the Differentiated State 894
Some Structures Are Maintained by a Continuing Interaction Between Their Parts: Taste Buds and Their Nerve Supply 895
An Agent That Causes Changes in DNA Methylation Can Cause Radical Alterations of Differentiated Character 896
Summary 897

Tissues With Permanent Cells 897
The Cells at the Center of an Adult Lens Are Remnants of the Embryo 897
Most Permanent Cells Renew Their Parts: Photoreceptor Cells of the Retina 899
Summary 902

Renewal by Simple Duplication 902
The Liver Is an Interface Between the Digestive Tract and the Blood 903
Liver Cell Loss Stimulates Liver Cell Proliferation 905
Regeneration May Be Hindered by Uncoordinated Growth of the Components of a Mixed Tissue 906
Endothelial Cells Constitute the Fundamental Component of All Blood Vessels 906
New Endothelial Cells Are Generated by Simple Duplication of Existing Endothelial Cells 908
New Capillaries Form by Sprouting 908
Growth of the Capillary Network Is Controlled by Factors Released by the Surrounding Tissues 909
Summary 910

Renewal by Stem Cells: Epidermis 910
Stem Cells Have the Ability to Divide Without Limit and to Give Rise to Differentiated Progeny 911
The Epidermis Is Organized into Proliferative Units 912
Differentiating Epidermal Cells Synthesize a Sequence of Different Keratins as They Mature 914
For Each Proliferative Unit, There Is an "Immortal" Stem Cell 915
Stem Cell Potential May Be Maintained by Contact with the Basal Lamina 915
Basal Cell Proliferation Is Regulated According to the Thickness of the Epidermis 916
Secretory Cells of the Skin Are Secluded in Glands and Have Different Population Kinetics 916
Summary 918

Renewal by Pluripotent Stem Cells: Blood Cell Formation 918
New Blood Cells Are Generated in the Bone Marrow 921
Bone Marrow Contains Pluripotent Stem Cells That Can Establish Hematopoietic Colonies 923
The Number of Specialized Blood Cells Is Amplified by Divisions That Follow Commitment 924
Production of Erythrocytes Is Controlled Through Hormonal Regulation of the Cell Divisions That Follow Commitment 925
Specific Glycoprotein Hormones Control the Survival and Fate of the Different Classes of Committed Hematopoietic Precursor Cells 927
Summary 927

Quiescent Stem Cells: Skeletal Muscle 928
Skeletal Muscle Cells Do Not Divide 929
New Skeletal Muscle Cells Form by Fusion of Myoblasts 929
Muscle Differentiation Requires Coordinated Changes in the Expression of Many Different Genes 929
Some Myoblasts Persist as Satellite Cells in the Adult 931
The State of Differentiation of Skeletal Muscle Fibers Can Be Modulated by Electrical Stimulation 931
Summary 933

Soft Cells and Tough Matrix: Growth, Turnover, and Repair in Skeletal Connective Tissue 933
Cartilage Grows by Swelling 933
Osteoblasts Secrete Bone Matrix While Osteoclasts Erode It 934
Cartilage Is Eroded by Osteoclasts to Make Way for Bone 937
Summary 938

Territorial Stability in the Adult Body 938
Epithelial Organization Helps to Keep Cells in Their Proper Territories 939
Normal Somatic Cells Are Destined to Die for the Sake of the Survival of the Germ Cell 939
Cancer Cells Break the Rules of Altruistic Social Behavior 940
Summary 941

Appendix 941

References 947

CHAPTER 17

The Immune System

The Cellular Basis of Immunity 952

The Immune System Is Composed of Billions of Lymphocytes 952

B Lymphocytes Make Humoral Antibody Responses; T Lymphocytes Make Cell-mediated Immune Responses 952

All Lymphocytes Develop from Pluripotent Hemopoietic Stem Cells 954

Cell-Surface Markers Make It Possible to Distinguish and Separate T and B Cells 955

Most Lymphocytes Continuously Recirculate Between the Blood and Lymph 956

The Immune System Works by Clonal Selection 956

Most Antigens Stimulate Many Different Lymphocyte Clones 958

Immunological Memory Is Due to Clonal Expansion and Lymphocyte Differentiation 959

The Failure to Respond to Self Antigens Is Due to Acquired Immunological Tolerance 960

Immunological Tolerance to Foreign Antigens Can Also Be Induced in Mature Animals 962

Summary 963

The Functional Properties of Antibodies 963

The Antigen-specific Receptors on B Cells Are Antibody Molecules 964

B Cells Can Be Stimulated to Make Antibodies in a Culture Dish 964

Antibodies Have Two Identical Antigen-binding Sites 964

An Antibody Molecule Is Composed of Four Polypeptide Chains—Two Identical Light Chains and Two Identical Heavy Chains 965

There Are Five Different Classes of H Chains, Each with Different Biological Properties 966

Antibodies Can Have Either κ or λ Light Chains But Never Both 969

The Strength of an Antibody-Antigen Interaction Depends on Both the Affinity and the Number of Binding Sites 969

Antibody-Antigen Interactions Can Be Measured in Many Ways 971

The Size of the Antigen-Antibody Complexes Formed Depends on the Valence of the Antigen and on the Relative Concentrations of the Antigen and Antibody 972

Antibodies Recruit Complement and Various Cells to Fight Infection 973

Summary 974

The Fine Structure of Antibodies 974

Myeloma Proteins Are Homogeneous Antibodies Made by Plasma-Cell Tumors 974

L and H Chains Consist of Constant and Variable Regions 975

The L and H Chains Each Contain Three Hypervariable Regions That Together Form the Antigen-binding Site 975

The L and H Chains Are Folded into Repeating Domains 976

X-ray Diffraction Studies Have Revealed the Structure of Immunoglobulin Domains and Antigen-binding Sites in Three Dimensions 977

Summary 979

The Generation of Antibody Diversity 979

More than One Gene Segment Codes for Each L and H Chain 980

Two Gene Segments Code for the V Region of Each L Chain 981

Three Gene Segments Code for the V Region of Each H Chain 983

Antibody Diversity Is Increased by Somatic Recombination, by the Combinatorial Joining of Light and Heavy Chains, and by Somatic Mutation 983

The Mechanisms of Antibody Gene Expression Ensure That B Cells Are Monospecific 984

The Switch from a Membrane-bound to a Secreted Form of the Same Antibody Occurs Through a Change in the H-Chain RNA Transcripts 985

B Cells Can Switch the Class of Antibody They Make 986

Idiotypes on Antibody Molecules Form the Basis of an Immunological network 986

Summary 988

The Complement System 988

Complement Activation Involves a Sequential Proteolytic Cascade 988

The Classical Pathway Is Activated by Antibody-Antigen Complexes 989

The Alternative Pathway Can Be Directly Activated by Microorganisms 990

The Assembly of the Late Complement Components Generates a Transmembrane Lytic Complex 991

The Complement Cascade Is Tightly Regulated and Designed to Attack a Nearby Membrane 992

Summary 993

T Lymphocytes and Cell-mediated Immunity 993

The T-Cell-Receptor Enigma 993

Different T-Cell Responses Are Mediated by Different T-Cell Subpopulations 994

Cytotoxic T Cells Kill Virus-infected Cells 995

Helper T Cells Are Required for Most B Cells and T Cells to Respond to Antigen 995

Helper T Cells Activate Macrophages by Secreting Lymphokines 996

Suppressor T Cells Inhibit the Responses of Other Lymphocytes 997

Helper and Suppressor T Cells Can Recognize Foreign Antigens on the Target Lymphocyte Surface 997

Regulatory T Cells May Communicate with Their Target Lymphocytes by Secreting Soluble Helper or Suppressor Factors 998

Transplantation Reactions Are T-Cell-mediated Immune Responses 999

T Cells Appear to be Obsessed with Foreign MHC Antigens 999
There Are Two Classes of MHC Molecules 1000
Class I MHC Glycoproteins Are Found on Virtually All Nucleated Cells and Are Extremely Polymorphic 1000
The Genes-Coding for Class II MHC Glycoproteins Were Originally Discovered as Immune Response (*Ir*) Genes 1001
T Cells Recognize Foreign Antigens in Association with Self MHC Molecules 1002
MHC Glycoproteins May Serve as Guides for Activating the Appropriate Subpopulations of T Cells 1003
Helper T Cells May Recognize Fragments of Foreign Antigens on the Surface of Antigen-presenting Cells 1004
Why Are MHC Glycoproteins So Polymorphic? 1005
The Immune System Is Ineffective Against Most Tumors 1006
The Immune System Has Had to Solve Three Major Problems in Antigen Recognition 1006
Summary 1007

References 1008

CHAPTER 18

The Nervous System

Cells of the Nervous System: A Preliminary Sketch 1013
Nerve Cells Carry Electrical Signals 1015
Nerve Cells Communicate Chemically at Synapses 1016
Neural Tissue Consists of Neurons and Glial Cells 1016
Summary 1017

Voltage-gated Channels and the Action Potential 1018
The Na^+-K^+ Pump Charges the Battery That Powers the Action Potential 1018
The Membrane Potential Depends on Selective Membrane Permeability 1019
Ion Channels Are Characterized by Their Selectivity, Their Gating, and Their Sensitivity to Specific Toxins 1022
Depolarization Causes Na^+ Channels First to Open and Then to Become Inactivated 1023
Fluctuations in the Transmembrane Current Suggest That Individual Channels Are Opening and Closing Randomly 1023
Gated Channels Open and Close in an All-or-None Fashion 1024
The Membrane Electric Field Controls the Energies of the Different Channel Conformations 1025
Voltage-gated Na^+ Channels Are Responsible for the Action Potential 1027
Action Potentials Are All-or-None 1028
Voltage Changes Can Spread Passively Within a Neuron 1028
Action Potentials Provide for Rapid Long-Distance Communication 1030
Myelination Speeds Conduction 1032
Summary 1032

Synaptic Transmission 1035
The Neuromuscular Junction Is the Best Understood Synapse 1036
Voltage-gated Ca^{2+} Channels Couple Action Potentials to Exocytosis 1036
Neurotransmitter Release Is Quantal and Random 1038
Ligand-gated Channels Convert the Chemical Signal Back into Electrical Form 1040
The Acetylcholine Receptor Is a Ligand-gated Cation Channel 1040
Acetylcholine Is Removed from the Synaptic Cleft by Diffusion and by Hydrolysis 1041
Some Synapses Are Excitatory, Others Inhibitory 1042
Neurotransmitters at Some Synapses Act Through Intracellular Second Messengers Rather Than by Directly Gated Ion Flows 1044
Many Synaptic Inputs Combine to Drive a Single Neuron 1045
The Membrane Potential in the Cell Body Represents a Spatial Summation of Postsynaptic Potentials 1046
Temporal Summation Translates the Frequency of Presynaptic Signals into the Size of a PSP 1046
The Grand PSP Is Translated into Nerve Impulse Frequency for Long-Distance Transmission 1047
Encoding Requires a Combination of Different Voltage-sensitive Channels 1048
Early K^+ Channels Help to Make the Firing Rate Proportional to the Stimulus 1049
Adaptation Lessens the Response to an Unchanging Stimulus 1049
Not All Signals Are Delivered via the Axon 1050
Summary 1052

Channel Regulation and Memory 1052
The Distribution of Ion Channels in a Muscle Cell Changes in Response to Denervation 1053
The Receptivity of a Muscle Cell Can Be Controlled by Electrical Stimulation 1054
The Site of a Synaptic Contact Is Marked by a Persistent Specialization of the Basal Lamina 1054
Synaptic Plasticity Provides a Mechanism for Memory 1055
A Short-Term Memory Is Registered by Modification of Channel Proteins 1056
Memory Remains Mysterious 1058
Summary 1058

Sensory Input 1058
Stimulus Magnitude Is Reflected in the Receptor Potential 1059
Sense Receptors Are Tuned to Detect Specific Stimuli 1061
Rod Cells Can Detect a Single Photon 1063
The Visual World Is Mapped onto a Sequential Hierarchy of Arrays of Neurons 1064

Vacuoles Can Function as Storage Organelles 1124
Plant Cells Exocytose But Generally Seem Not to Endocytose Macromolecules 1124
Golgi Vesicles Deliver Cell-Wall Material to Specific Regions of Plasma Membrane 1125
Cellulose Synthesis Occurs at the Surface of Plant Cells 1128
Cortical Microtubules Orient the Extracellular Deposition of Cellulose Microfibrils 1128
The Movement of Materials in Large Plant Cells is Driven by Cytoplasmic Streaming 1130
The Interaction of Actin and Myosin Drives Cytoplasmic Streaming in Giant Algal Cells 1131
Regions of the Plant Cell Cytoskeleton Can Be Reorganized in Response to Local Stimuli 1132
Summary 1133

Cell Growth and Division 1133
Most New Plant Cells Arise in Special Areas Called Meristems 1134
The Shape of a Growing Plant Cell Is Determined by the Organization of Cellulose Microfibrils 1135
A Preprophase Band of Microtubules Marks the Future Plane of Cell Division 1136
Hormones Help Control the Growth and Shape of Plants 1139
Tissue Culture Facilitates Studies of Mechanisms of Cell Determination in Plants 1139
Plant Cells Without Their walls Can Be Manipulated Much Like Animal Cells 1142
Summary 1143

References 1144