
CONTENTS

PART 1 CELLS AND CELL GROWTH

1

Chapter 1 The Cell: An Introduction

3

MICROSCOPY

5

Fundamentals of Light Microscopy and Transmission

Electron Microscopy 5

Preparation of Materials for Microscopy 10

Specialized Applications of Transmission Electron
Microscopy 12

The Scanning Electron Microscope 20

Stereo Microscopy (Stereoscopy) 21

CELL STRUCTURE: A PREVIEW

27

Eukaryotic Cells: The Composite Animal Cell 27

The Endoplasmic Reticulum and Ribosomes 32

Eukaryotic Cells: The Composite Plant Cell 39

Prokaryotic Cells: Bacteria 42

Prokaryotic Cells: Cyanobacteria 45

Prokaryotic Cells: Mycoplasmas 45

VIRUSES

46

Structure of Viruses 46

Proliferative Cycle of a Virus 48

Classification of Viruses and the Nature of Viral Nucleic
Acids 51

VIROIDS

51

PRIONS 52

SUMMARY 52

REFERENCES AND SUGGESTED
READING 52

Chapter 2 Cell Growth and Proliferation

55

THE POPULATION GROWTH CURVE

56

Exponential Growth 56

Doubling Time 57

The Lag Phase of Growth 58

The Stationary Phase 58

The Death or Declining Phase 58

SENESCENCE 59

CONFLUENCE AND DENSITY-DEPENDENT
INHIBITION 59GROWTH CHARACTERISTICS OF
TRANSFORMED CELLS 59

THE QUANTITATION OF CELLS 60

Optical Enumeration of Cells 60

ELECTRONIC ENUMERATION OF
CELLS 60

Electronic Gating 60

Flow Cytometry 61

INTRACELLULAR PHOSPHATE TURNOVER	205
REDOX REACTIONS AND REDOX COUPLES	205
LIGHT AND THE TRANSDUCTION OF ENERGY	206
Vision	209
OTHER KINDS OF TRANSDUCTIONS SUMMARY	210
REFERENCES AND SUGGESTED READING	211
Chapter 10 Cell Metabolism	213
REVEALING THE INDIVIDUAL STEPS OF METABOLIC PATHWAYS	215
Marker and Tracer Techniques	215
Enzyme Techniques	216
Use of Enzyme Inhibitors and Enzyme-Deficient Cells	216
CARBOHYDRATE METABOLISM	216
GLYCOLYSIS	217
ANAEROBIC RESPIRATION AND FERMENTATION	217
OXIDATION OF PYRUVATE	221
OTHER PATHWAYS OF CARBOHYDRATE CATABOLISM	221
Pentose Phosphate Pathway	221
The Glyoxylate Pathway	224
Gluconeogenesis	224
Synthesis of Glycogen and Starch	227
LIPID METABOLISM	228
Triglycerides	228
NITROGEN METABOLISM	228
FUNCTIONS OF METABOLIC PATHWAYS	231
CALCULATIONS OF ENERGY CHANGE	232
SUMMARY	233
REFERENCES AND SUGGESTED READING	234

Chapter 11 Metabolic Regulation	235
MASS ACTION	235
REGULATION OF METABOLISM	237
GROUP I CONTROL MECHANISMS—IMMEDIATE REGULATION	237
Substrate Concentration Effects	237

Effects of Salts and pH	238
Covalent Bond Modification	238
Isozymes	239
GROUP II CONTROL MECHANISMS—ALLOSTERISM	240
METABOLIC PROCESSES CONTROLLED BY ALLOSTERIC ENZYMES	241
Alternate Pathways for Glycogen Synthesis and Degradation	241
The Regulation of Amino Acid Synthesis	242
GROUP III CONTROL MECHANISMS—REGULATION OF ENZYME SYNTHESIS IN PROKARYOTES	243
Constitutive and Inducible Enzymes	243
Repressible Enzymes	244
THE OPERON	245
The Lac Operon	245
Catabolic Repression	248
Translational Control	248
GROUP IV CONTROL MECHANISMS—REGULATION OF ENZYME PRODUCTION IN EUKARYOTIC CELLS	250
Z-DNA	250
Calcium and Calmodulin	250
Enzyme Induction by Hormones	251
Effects of Hormones on Gene Expression	254
Protein Phosphorylation and Metabolic Control	254
Amplification of Signals	254
Repression of the Genome in Eukaryotes	255
The Britten-Davidson Model of Gene Regulation in Eukaryotes	256
Compartmentalization	256
SUMMARY	257
REFERENCES AND SUGGESTED READING	258

PART 4 TOOLS AND METHODS OF CELL BIOLOGY 259

Chapter 12 Fractionation of Tissues and Cells	261
METHODS FOR DISRUPTING TISSUES AND CELLS	261
CENTRIFUGATION	262
Theory of Centrifugation	263
Sedimentation Rate and Sedimentation Coefficient	263

The Analytical Ultracentrifuge	264
Preparative Centrifugation	266
Differential Centrifugation	267
Density Gradient Centrifugation	269
ZONAL CENTRIFUGATION	272
Dynamically Unloaded Zonal Rotors	273
Reograd Zonal Rotors	275
EXAMINATION AND ANALYSIS OF SEPARATED CELL FRACTIONS	276
METHODS FOR SEPARATING WHOLE CELLS	276
Tissues Disaggregation	276
Adherence and Filtration	277
Conventional and Zonal Centrifugation	277
Centrifugal Elutriation (Counter-Streaming Centrifugation)	277
Unit Gravity Separation	277
Countercurrent Distribution	278
Electrophoresis	278
Fluorescence-Activated Cell Sorting	280
HARVESTING CELLS AND SUBCELLULAR COMPONENTS: CONTINUOUS-FLOW CENTRIFUGATION	281
SUMMARY	282
REFERENCES AND SUGGESTED READING	282

Chapter 13 The Isolation and Characterization of Cellular Macromolecules 283

SALTING IN AND SALTING OUT	284
ISOELECTRIC PRECIPITATION	285
DIALYSIS AND ULTRAFILTRATION	285
ULTRACENTRIFUGATION	286
ELECTROPHORESIS	286
Moving-Boundary Electrophoresis	288
Zone Electrophoresis	288
Immunoelectrophoresis	294
Isoelectric Focusing	294
COUNTERCURRENT DISTRIBUTION	296
PAPER CHROMATOGRAPHY	298
THIN-LAYER CHROMATOGRAPHY	299
ION-EXCHANGE CHROMATOGRAPHY	300
AFFINITY CHROMATOGRAPHY	303
GEL FILTRATION	303
HPLC	304
GAS CHROMATOGRAPHY	305
SUMMARY	306
REFERENCES AND SUGGESTED READING	306

Chapter 14 Radioactive Isotopes as Tracers in Cell Biology 309

ADVANTAGE OF THE RADIOISOTOPE TECHNIQUE 310

Determination of Molecular Fluxes under Conditions of Zero Net Exchange	310
Simplification of Chemical Analyses	310
"Isotope Dilution" Methods	311

PRECURSOR-PRODUCT RELATIONSHIPS 311

PROPERTIES OF RADIOACTIVE ISOTOPES 312

Types of Radiation Emitted by Radioisotopes	312
Energy of Radiation and Its Interaction with Matter	313
Half-Life	314

DETECTION AND MEASUREMENT OF RADIATION 315

Geiger-Muller Counters	315
Solid Scintillation Counters	317
Liquid Scintillation Counters	319

AUTORADIOGRAPHY 320

SUMMARY 322

REFERENCES AND SUGGESTED READING 323

PART 5 STRUCTURE AND FUNCTION OF THE MAJOR CELL ORGANELLES 325

Chapter 15 The Plasma Membrane and the Endoplasmic Reticulum 327

EARLY STUDIES ON THE CHEMICAL ORGANIZATION OF THE PLASMA MEMBRANE 328

Existence of Lipid in the Membrane	328
The Langmuir Trough	328
Gorter and Grendel's Bimolecular Lipid Leaflet Model	329

THE DANIELLI-DAVSON MEMBRANE MODEL 330

ROBERTSON'S UNIT MEMBRANE 331

FUNCTIONAL DOMAINS IN THE PLASMA MEMBRANE 332

THE FLUID-MOSAIC MODEL OF MEMBRANE STRUCTURE 333

FREEZE-FRACTURED MEMBRANES	334
MEMBRANE PROTEINS	336

Peripheral (Extrinsic) Proteins	336	Receptor-Mediated Endocytosis	370
Integral (Intrinsic) Proteins	336	Phagocytosis	373
Asymmetric Distribution of Membrane Proteins	337	EXOCYTOSIS	374
Mobility of Membrane Proteins	337	SUMMARY	374
Enzymatic Properties of Membrane Proteins	338	REFERENCES AND SUGGESTED	
Isolation and Characterization of Membrane Proteins	338	READING	375
MEMBRANE LIPIDS	339	Chapter 16 The Mitochondrion	377
Mobility of Membrane Lipids	339	DISCOVERY OF MITOCHONDRIA	377
Lipid Asymmetry	340	THE ORIGIN AND EVOLUTION OF	
MEMBRANE CARBOHYDRATE	340	MITOCHONDRIA	378
Possible Functions of Membrane Carbohydrate	341	STRUCTURE OF THE	
LECTINS, ANTIBODIES, ANTIGENS, AND THE		MITOCHONDRION	380
PLASMA MEMBRANE	342	The Mitochondrial Membranes	386
Lectins	342	Conformational States of Mitochondria	391
Antigens and Antibodies	343	TRICARBOXYLIC ACID (KREBS)	
ORIGIN OF THE PLASMA MEMBRANE AND ITS		CYCLE	393
PROTEIN AND LIPID ASYMMETRY	344	Oxidation of Pyruvate	397
Synthesis of Membrane Proteins and the "Signal Hypothesis"	344	The Krebs Cycle Reactions	397
Synthesis of Membrane Lipids	347	Summary of the TCA Cycle	399
CELL SURFACE PROPERTIES REVEALED BY		ELECTRON TRANSPORT SYSTEM	399
ERYTHROCYTES	348	OXIDATION-REDUCTION REACTIONS	400
CELL-CELL JUNCTIONS AND OTHER		CLASSES OF ELECTRON TRANSPORT SYSTEM	
SPECIALIZATIONS OF THE PLASMA		COMPOUNDS	401
MEMBRANE	350	Electron Transport Pathway	402
Tight Junctions	351	BALANCE OF ELECTRONS FROM GLYCOLYSIS	
Intermediate Junctions	351	AND TCA CYCLE METABOLISM	403
Spot Desmosomes	353	THE ENERGETICS OF ELECTRON	
Gap Junctions	353	TRANSPORT	403
Microvilli and Surface Ruffles	354	Transport of Protons	404
Plasmodesmata	356	Electron Transport Inhibitors	404
PASSIVE MOVEMENTS OF MATERIALS		NAD[P] ⁺ -transhydrogenases	405
THROUGH CELL MEMBRANES	358	OXIDATIVE PHOSPHORYLATION	405
Osmosis and Diffusion across Membranes	358	Molecular Events in Oxidative Phosphorylation	405
Osmosis and Diffusion across Cell Membranes	359	THE CHEMIOSMOTIC-COUPLING	
The Gibbs-Donnan Effect	363	HYPOTHESIS	407
FACILITATED (MEDIATED) DIFFUSION		OTHER FUNCTIONS OF	
THROUGH THE CELL MEMBRANE	364	MITOCHONDRIA	410
ACTIVE TRANSPORT	366	The Glyoxylate Cycle	410
The Na ⁺ /K ⁺ Exchange Pump	366	Fatty Acid Oxidation	410
Cotransport	367	Fatty Acid Chain Elongation	410
"Simple" Active Transport	368	Superoxide Dismutase and Catalase	410
BULK TRANSPORT INTO AND OUT OF		Amphibolic and Anaplerotic Reactions	410
CELLS	368	PERMEABILITY OF THE INNER	
ENDOCYTOSIS	369	MEMBRANE	411

CYTOSOL-MATRIX EXCHANGE OF NADH AND NADPH 412

- Glycerol Phosphate Shuttle 412
- Malate-Aspartate Shuttle 412
- Total ATP Production from the Catabolism of Glucose 412

SUMMARY 413
REFERENCES AND SUGGESTED
READING 415

Chapter 17 The Chloroplast 417

- Fine Structure of the Chloroplast 419
- Structure of the Thylakoid 423
- Stroma Structures 423

CHEMICAL COMPOSITION OF CHLOROPLASTS 424

- The Chlorophylls 425
- The Carotenoids 426
- Location and Arrangement of the Pigments 426

DEVELOPMENT OF CHLOROPLASTS 426

PHOTOSYNTHESIS—HISTORICAL

BACKGROUND 430

PHOTOSYNTHESIS—THE PHOTOCHEMICAL (LIGHT) REACTIONS 431

- The Absorption of Light by Chlorophyll 431
- Primary Photochemical Events in Photosynthesis 431
- Two Photosystems 432
- Sequence of Energy (Electron) Flow 432
- Redox Reactions 433
- Cyclic and Noncyclic Photophosphorylation 435
- Summary of the Light Reactions 435

PHOTOSYNTHESIS—SYNTHETIC (DARK) REACTIONS 436

- The Calvin Cycle 436
- C₄ Photosynthesis (Hatch-Slack Pathway) 440
- Crassulacean Acid metabolism 441

BACTERIAL PHOTOSYNTHESIS 441

OTHER PLASTIDS 442

SUMMARY 442
REFERENCES AND SUGGESTED
READING 444

Chapter 18 The Golgi Apparatus 445

STRUCTURE OF THE GOLGI

APPARATUS 446

ORIGIN AND DEVELOPMENT OF GOLGI

BODIES 449

FUNCTIONS OF THE GOLGI

APPARATUS 453

- Secretion 453
- Posttranslational Processing of Proteins 454
- Proliferation of Cellular Membranes 456
- Sorting Problems Faced by the Golgi Apparatus 458

RECYCLING OF PLASMA MEMBRANE

COMPONENTS 459

CELL-SPECIFIC FUNCTIONS OF THE GOLGI APPARATUS 459

- Formation of the Cell Plate and Cell Wall in Plant Tissues 459
- Acrosome Development in Sperm 461

SUMMARY 461

REFERENCES AND SUGGESTED
READING 461

Chapter 19 Lysosomes and Microbodies 463

LYSOSOMES 463

- Formation and Function of Lysosomes (the "Vacuolar System") 469
- Distribution of Lysosomes 471
- Plant Vacuoles 472
- Lysosome Precursors in Bacteria 472
- Regulation of Lysosome Production 472
- Disposition and Action of the Lysosomal Hydrolases 473

MICROBODIES 473

- Peroxisomes 473
- Glyoxysomes 475

SUMMARY 478

REFERENCES AND SUGGESTED
READING 478

Chapter 20 The Nucleus: Organization and Division 481

STRUCTURE OF THE NUCLEUS 481

CHROMOSOMES 483

- Fine Structure of Chromosomes 485

NUCLEOSOMES 485

- Bacterial and Viral Chromosomes 490
- Supercoiling 492
- Plasmids and Episomes 494
- Mitochondrial and Chloroplast DNA 494
- Promiscuous DNA 495

FORMS OF CHROMATIN	495	GENETIC ENGINEERING	534
Chromosome-Chromosome Associations	497	Preparing Donor DNA	535
Polytene Chromosomes	497	Preparing the Cloning Vehicle	535
THE NUCLEOLUS	497	Gene Cloning Methods Using Reverse Transcription	538
The Nuclear Envelope	498	Genetic Engineering of Mammalian Cells	540
MITOSIS	499	SUMMARY	541
MEIOSIS	504	REFERENCES AND SUGGESTED READING	541
MEIOTIC DIVISION I	504		
Prophase I	504	Chapter 22 Ribosomes and the Synthesis of Proteins	543
Metaphase I	509	PROTEIN TURNOVER IN CELLS	543
Anaphase I	509	A PRELIMINARY OVERVIEW OF PROTEIN BIOSYNTHESIS	544
Telophase I	509	STRUCTURE, COMPOSITION, AND ASSEMBLY OF RIBOSOMES	546
Interkinesis (or Interphase)	509	Prokaryotic Ribosomes	547
MEIOTIC DIVISION II	509	Assembly of Prokaryotic Ribosomes	549
Prophase II	509	Model of the Prokaryotic Ribosome	550
Metaphase II	509	Genes for Ribosomal RNA and Protein	551
Anaphase II	509	Eukaryotic Ribosomes	551
Telophase II	509	Model of the Eukaryotic Cytoplasmic Ribosome	556
CYTOKINESIS	511	Free and Attached Ribosomes	557
SUMMARY	512	VECTORIAL SYNTHESIS	559
REFERENCES AND SUGGESTED READING	512	RIBOSOMES OF ORGANELLES	563
		Chloroplast Ribosomes	563
Chapter 21 The Nucleus and Molecular Genetics	515	Mitochondrial Ribosomes	563
REPLICATION OF DNA	515	Protein Synthesis in Chloroplasts and Mitochondria	563
Replication as a "Semiconservative" Process	515	MECHANISM OF PROTEIN SYNTHESIS	564
Replication by Addition of Nucleotides in the 5'→3' Direction	517	Linearity and Direction of Polypeptide Chain Assembly—The Experiments of H. M. Dintzis	565
Unidirectional and Bidirectional Replication	517	Processing and Structure of the Transfer RNA	569
Visualization of Replication in <i>E. coli</i>	519	Activation of Amino Acids	573
The Replicon and the Replication Sequence	520	Processing and Structure of Messenger RNA	573
High Fidelity of Replication	523	Initiation of Polypeptide Chain Synthesis	577
CHROMOSOME REPLICATION IN EUKARYOTES	524	Chain Elongation	581
Semiconservative Replication in Eukaryotes	524	Chain Termination	582
Replicons of Eukaryotic Chromosomes	524	Polyribosomes (Polysomes)	584
Nucleosome Assembly and DNA Replication	524	Differences between Translation in Prokaryotic and Eukaryotic Cells	588
Repetitive DNA	524	Cotranslational and Postranslational Protein Modification	588
REPLICATION OF BACTERIOPHAGE DNA	527	Transfer RNA Specialization	591
Lambda (λ) Phage	527	INHIBITORS OF PROTEIN SYNTHESIS	591
φX174 Phage and Rolling Circle Replication	529	Inhibitors of Both Prokaryotic and Eukaryotic Protein Synthesis	591
DNA REPAIR	530	Inhibitors Specific for Prokaryotes	592
TRANSCRIPTION OF DNA	532		
BACTERIAL RECOMBINATION	534		

Inhibitors Specific for Eukaryotes	592
Inhibitors of Organellar Protein Synthesis	593
SUMMARY	593
REFERENCES AND SUGGESTED	
READING	594

Chapter 23 The Cytoplasmic Matrix 597

THE GROUND SUBSTANCE AND THE MICROTRABECULAR LATTICE 597

Structure of the Lattice	597
Chemistry of the Lattice	599
Functions of the Microtrabecular Lattice	600

CYTOPLASMIC FILAMENTS 600

Composition, Distribution, and Functions of Cytoplasmic Filaments	600
Cytokinesis	602
Plasma Membrane Movement	603
Amoeboid Movement	605

MICROTUBULES 607

Assembly and Functions of Microtubules	609
Centrioles	611
Cilia and Flagella	613
The Mitotic Spindle	618
Other Cell Movements	619

SUMMARY	619
REFERENCES AND SUGGESTED	
READING	619

PART 6 SPECIAL CELL FUNCTIONS 621

Chapter 24 Cellular Differentiation and Specialization 623

INTRINSIC (NUCLEOCYTOPLASMIC) INTERACTIONS 624

Transplantation of Cell Nuclei	624
Experiments with <i>Acetabularia</i>	625

EXTRINSIC (ENVIRONMENTAL) EFFECTS ON DIFFERENTIATION OF CELLS 627

RED BLOOD CELLS 628

Erythropoiesis	628
Genetic and Molecular Basis of Erythrocyte Differentiation	629
Morphological and Physiological Specialization of Red Blood Cells	630

DIFFERENTIATION OF B LYMPHOCYTES 631

MUSCLE CELLS 632

Muscle Contraction	635
--------------------	-----

NERVE CELLS (NEURONS) 638

Conduction of Nerve Impulses	639
Ion Gradients across the Membrane	640
Initiation of the Action Potential	641
Conduction of the Action Potential	641
Synaptic Transmission	642

SUMMARY	644
REFERENCES AND SUGGESTED	
READING	645

Chapter 25 Immunity and Cancer 647

NONADAPTIVE AND ADAPTIVE SYSTEMS OF DEFENSE AGAINST INFECTION 647

DUALITY OF THE IMMUNE SYSTEM 648

T Lymphocytes and B Lymphocytes	648
---------------------------------	-----

ANTIBODIES (IMMUNOGLOBULINS) AND THEIR DIVERSITY 649

Constant and Variable Domains of an Immunoglobulin	650
Antibody Diversity and the Genome	651

T-CELL RECEPTORS 653

CLONAL SELECTION 653

Antigenic Determinants and Haptens	654
------------------------------------	-----

MONOCLONAL ANTIBODIES AND HYBRIDOMAS 655

B LYMPHOCYTES AND THE IMMUNE RESPONSE 656

Precipitation of Soluble Antigens	657
Agglutination	658
Complement Fixation	659
Immunologic Memory	659
Autoimmune Diseases	660

MAJOR HISTOCOMPATIBILITY COMPLEXES 661

T LYMPHOCYTES AND THE IMMUNE RESPONSE 661

Cytotoxic T Cells	662
Helper T Cells	662
Acquired Immune Deficiency Syndrome (AIDS)	663

CANCER 663

Mutagenesis, Proto-oncogenes and Oncogenes	663
Cancer of the Immune System—Burkitt's	

Lymphoma	664	Tumor Detection and Tumor Immunotherapy	669
Cancer of the Immune System—T-Cell		SUMMARY	670
Leukemia	665	REFERENCES AND SUGGESTED	
Viruses and Cancer	665	READING	670
Oncogenes and Their Protein Products		GLOSSARY	673
Multiple Oncogene Activation and Cancer	668	INDEX	689
THE IMMUNE SYSTEM AND CANCER	669		
Natural Killer Cells	669		