

The **BIOMEDICAL ENGINEERING** Series

Series Editor Michael R. Neuman

Introduction to Molecular Biology, Genomics and Proteomics for Biomedical Engineers

Robert B. Northrop and Anne N. Connor



CRC Press
Taylor & Francis Group

Contents

Preface	xv
Authors.....	xix
Chapter 1 Introduction	1
1.1 Scope of the Text.....	1
1.2 Some Definitions	2
1.2.1 Molecular Biology	2
1.2.2 Genomics	2
1.2.3 Proteomics	3
1.2.4 Bioinformatics.....	3
1.2.5 Genetic Engineering and Biotechnology	4
1.2.6 Systems Biology.....	5
1.3 Complex Systems	5
1.3.1 Introduction.....	5
1.3.2 Properties of Nonlinear Systems.....	7
1.3.3 Parametric Control.....	8
1.3.4 Modeling Complex Systems	9
1.4 Optimum Use of Reference Resources	9
1.5 Summary	10
Chapter 1 Home Problems	10
Chapter 2 Life and Death.....	13
2.1 What Is Life?.....	13
2.1.1 Introduction.....	13
2.1.2 Properties of Life	14
2.2 The Domains and Kingdoms of Life: Their Origins	16
2.2.1 Introduction.....	16
2.2.2 The Domains of Life.....	19
2.3 Nonliving, Self-Replicating Genomic Machines	20
2.3.1 Introduction.....	20
2.3.2 Viruses	20
2.3.3 Viroids.....	23
2.3.4 Plasmids	24
2.3.5 Satellites and Virusoids	24
2.4 The Origins of Life	25
2.4.1 Introduction.....	25
2.4.1.1 Carbon Dating	26
2.4.2 The First Life	29
2.4.3 Discussion	34
2.5 Cell and Animal Death	34
2.5.1 Introduction.....	34
2.5.2 Cell Death by Apoptosis (Programmed Cell Death)	35
2.5.3 Cell Necroptosis.....	40
2.5.4 Metazoan Death: Failure of Critical Organ Systems.....	41

2.6	Summary.....	43
Chapter 2 Home Problems		44
Chapter 3	Review of Basic Cell Anatomy and Physiology	45
3.1	Introduction.....	45
3.2	The Cell Membrane	45
3.3	Inside the Cell Membrane	50
3.4	Prokaryotic Cells.....	50
3.4.1	Introduction.....	50
3.4.2	Archaea	51
3.4.3	Eubacteria	51
3.5	Eukaryotic Cells.....	53
3.5.1	Introduction.....	53
3.5.2	Single-Cell Eukaryotes (Protists)	54
3.5.3	Multicelled Eukaryotes	56
3.6	Anatomical Components of Eukaryotic Cells	56
3.6.1	The Cytosol.....	56
3.6.2	The Nucleus and Nucleolus.....	57
3.6.3	Ribosomes.....	59
3.6.4	Endoplasmic Reticulum.....	59
3.6.5	Golgi Apparatus.....	60
3.6.6	Lysosomes	61
3.6.7	Peroxisomes	61
3.6.8	Mitochondria.....	61
3.7	The Roles of Transmembrane Proteins.....	63
3.7.1	Cell Adhesion Molecules	63
3.7.2	Transport of Substances Across the Cell Membrane.....	65
3.7.2.1	Diffusion and Osmosis.....	65
3.7.2.2	Aquaporins	66
3.7.2.3	Ion and Molecular Pumps	68
3.7.2.4	Regulated Ion Channels in Membranes	69
3.7.2.5	The Basis for the DC Transmembrane Potential.....	73
3.7.2.6	Cell Signaling by Changes in Transmembrane Potential.....	76
3.7.3	Electrical Synapses: Gap Junctions	78
3.7.4	Chemical Synapses	80
3.7.4.1	Introduction	80
3.7.4.2	Neural Transmembrane Chemical Signal Receptors	83
3.7.4.3	G-Protein-Coupled Receptors	83
3.7.4.4	Summary	84
3.8	Review of Cell Reproduction	84
3.8.1	The Cell Cycle: Mitosis	84
3.8.2	Meiosis	86
3.9	Summary	90
Chapter 3 Home Problems		92
Chapter 4	Introduction to Physical Biochemistry and Biochemical Systems Modeling	95
4.1	Introduction to Chemical Reactions	95
4.1.1	Introduction.....	95
4.1.2	Chemical Bond Energy	95

4.1.3	Types of Chemical Bonds	97
4.1.4	Chemical Kinetics and Mass Action	98
4.2	Important Coupled Reactions	101
4.2.1	Introduction.....	101
4.2.2	Glycolysis.....	102
4.2.3	The Citric Acid Cycle	104
4.3	Other Metabolic Pathways	106
4.3.1	The Chemistry of Organic Redox Reactions	106
4.3.2	Review of Carbohydrate Metabolism	109
4.3.3	Review of Lipid Metabolism.....	112
4.3.4	Review of Lipid Catabolism	114
4.3.5	Review of Amino Acid Metabolism	115
4.3.6	Review of Amino Acid Catabolism.....	116
4.4	Photosynthesis.....	118
4.4.1	Introduction.....	118
4.4.2	The Pigments of Photosynthesis	118
4.4.3	Structure of the Chloroplast.....	121
4.4.4	Chemical Reactions and Energy in Photosynthesis.....	123
4.4.5	The Calvin Cycle	124
4.4.6	The Light Reactions.....	124
4.5	The Challenge of Modeling Complex Biochemical Systems	126
4.5.1	Introduction.....	126
4.5.2	Generalized Approaches to Modeling Biochemical Systems.....	127
4.5.3	Models for Biochemical Oscillators	128
4.5.4	The Hodgkin-Huxley Model (1952) for Action Potential Generation	136
4.6	Some Simulation Languages for Biochemical Systems.....	143
4.7	Summary.....	150
	Chapter 4 Home Problems	150
Chapter 5	The Basis of Genetic Inheritance.....	155
5.1	Introduction	155
5.2	Mendelian Inheritance	156
5.2.1	Gene Swapping during Reproduction and Genetic Variation.....	157
5.3	Epigenetic Inheritance	158
5.4	Genetic Imprinting	159
5.5	Nuclear and Mitochondrial DNA.....	159
5.6	Genes and the Genome: The Central Dogma	160
5.6.1	Introns and Exons	161
5.6.2	Protein Synthesis	164
5.6.2.1	Transcription	165
5.6.2.2	Translation.....	169
5.7	The Control of Development.....	170
5.8	Summary.....	172
	Chapter 5 Home Problems	173

Chapter 6	Nucleic Acids and Their Functions	177
6.1	Introduction.....	177
6.1.1	DNA: The Command and Control Nucleic Acid.....	177
6.1.2	RNA: The Executive Nucleic Acid	179

6.2	RNAs Coded by DNA.....	181
6.2.1	Introduction.....	181
6.2.2	Messenger RNAs	182
6.2.3	Transfer RNAs	182
6.2.4	Ribosomal RNAs	183
6.2.5	Small Nuclear RNAs	184
6.2.6	Small Nucleolar RNAs	185
6.2.7	Short Interfering RNAs (siRNAs)	185
6.2.8	Micro-RNAs (miRNAs).....	186
6.2.9	Ribozymes	187
6.2.10	XIST RNA	188
6.2.11	Discussion	188
6.3	DNA Repair	189
6.3.1	Introduction.....	189
6.3.2	DNA Repair Mechanisms.....	189
6.3.3	Discussion	191
6.4	Gene Regulation.....	191
6.4.1	Introduction.....	191
6.4.2	Gene Regulation in Prokaryotes	192
6.4.3	Some Molecular Factors in Gene Expression in Eukaryotes.....	193
6.4.4	Epigenetic Gene Regulation.....	194
6.5	Summary	195
	Chapter 6 Home Problems	195

Chapter 7	A Review of Proteins	197
7.1	Introduction.....	197
7.1.1	Amino Acids and the Peptide Linkage	197
7.1.2	Primary, Secondary, Tertiary, and Quaternary Structure of Proteins.....	199
7.2	Examples of Protein Functions	202
7.2.1	Introduction.....	202
7.2.2	Enzymes.....	203
7.2.2.1	The Physical Chemistry of Enzymes	203
7.2.2.2	The Regulation of Protein Enzymes	203
7.2.3	Protein Hormones and Intercellular Command, Communication, and Control (C ³).....	204
7.2.3.1	Introduction	204
7.2.3.2	Protein Hormones	204
7.2.4	Structural Proteins	206
7.2.4.1	Introduction	206
7.2.4.2	Microfilaments	206
7.2.4.3	Intermediate Filaments	206
7.2.4.4	Microtubules	206
7.2.4.5	Cilia and Flagella	206
7.2.4.6	Muscle Fibers	208
7.2.4.7	Histone Proteins	215
7.2.5	Cell Membrane Receptors and Ion Pumps	216
7.2.5.1	Second Messenger Systems.....	216
7.2.5.2	Ion Pumps.....	217
7.2.5.3	Photon-Driven Proton Pumps	218

7.2.6	Proteins in the Immune System.....	219
7.2.6.1	Introduction	219
7.2.6.2	A Summary of the Cells of the Immune System	219
7.2.6.3	Complement	228
7.2.6.4	Antigen Presentation	229
7.2.6.5	Autacoids: Immunocytokines, Proteins, and Glycoproteins Secreted by Immune System Cells.....	232
7.2.6.6	Discussion	240
7.2.7	Lectins	241
7.2.8	Glycoproteins	242
7.2.9	Blood Proteins.....	243
7.2.9.1	Introduction.....	243
7.2.9.2	Hemoglobin	243
7.2.9.3	Serum Proteins and Antibodies	245
7.2.9.4	Hemocyanins in Invertebrate Circulatory Systems.....	247
7.2.10	Myoglobin	248
7.3	Errors in Protein Structure.....	248
7.3.1	Introduction.....	248
7.3.2	Nongenetic Causes of Protein Errors.....	249
7.3.3	Heat Shock (Stress) Proteins	251
7.3.4	When Good Proteins Go Bad: Prions	252
7.4	Posttranscription Regulation of Gene Expression	257
7.4.1	Introduction.....	257
7.4.2	Inteins and Protein Engineering	257
7.5	Protein Destruction and AA Recycling	259
7.5.1	Proteolytic Enzymes	259
7.5.2	The Proteosome	259
7.5.3	Proteolysis in Apoptosis and Cell Necrosis	261
7.5.4	Autophagy	261
7.6	Summary	265
	Chapter 7 Home Problems	265

Chapter 8	The Genetic Basis for Certain Inheritable Diseases: Genomic Medicine	267
8.1	Introduction	267
8.2	Examples of Genetic Diseases	268
8.3	Genomic Medicine	270
8.3.1	Introduction	270
8.3.2	Gene Defects and Disease	273
8.3.3	Gene Therapy	274
8.3.4	GM Viruses Used to Fight Cancers	275
8.3.5	Recombinant Vaccines	276
8.4	Epigenetic Therapy for Cancer	278
8.4.1	Introduction	278
8.4.2	Epigenetics and Cancer	278
8.4.3	Epigenetic Therapies for Cancer	279
8.4.4	Some Other Diseases with Epigenetic Etiologies	279
8.5	Summary	281
	Chapter 8 Home Problems	281

Chapter 9	Some Instrumental Methods Used in Genomics, Proteomics, and Forensic Science.....	283
9.1	Introduction.....	283
9.2	Biotin, Avidin, and Streptavidin: Applications in Genomics and Proteomics	283
9.2.1	Introduction.....	283
9.2.2	Biotin.....	283
9.2.3	Avidin.....	283
9.2.4	Streptavidin.....	284
9.2.5	Immobilization in Microarrays: Applications of Avidin–Biotin Systems.....	284
9.2.5.1	Nonspecific Immobilization.....	285
9.2.5.2	Specific Immobilization	285
9.3	The Polymerase Chain Reaction (PCR) and Its Applications in Genomics and Forensic Science.....	286
9.3.1	Introduction.....	286
9.3.2	The PCR Process	286
9.3.3	Direct DNA Tests for Genetic Diseases.....	289
9.3.4	The “454” System	290
9.4	Fluorescent Molecular Tags	291
9.4.1	Introduction to Fluorescence	291
9.4.2	Fluorescent In Situ Hybridization (FISH)	293
9.4.3	Fluorescence Resonance Energy Transfer (FRET)	294
9.4.4	Phase Fluorometry and FLIM-FRET	295
9.5	Introduction to Forensic Applications of DNA Restriction Enzymes and STRs.....	298
9.5.1	Introduction.....	298
9.5.2	Restriction Fragment Length Polymorphism (RLFP) Analysis	298
9.5.3	Short Tandem Repeat Markers	299
9.5.4	Validity of STR DNA Evidence.....	301
9.6	Rolling-Circle Replication of ssDNA Circles	302
9.6.1	Introduction.....	302
9.6.2	RCA Protocols	302
9.6.3	The JGI RCA Protocol	303
9.6.4	Padlock Probes in RCA	304
9.7	Methods for the Analysis of Protein Structure	306
9.7.1	Introduction.....	306
9.7.2	Electrophoretic Separation of Proteins	306
9.7.3	Sequencing Protein AAs.....	307
9.7.4	ELISA Tests for Specific Proteins and Antibodies.....	307
9.7.5	Protein Microarrays	310
9.7.5.1	Introduction	310
9.7.5.2	The First Large-Scale Protein Microarrays	311
9.7.5.3	Antibody Microarrays	311
9.7.5.4	Improvements in Fluorescent Microarrays	312
9.7.6	The Immuno PCR Method	314
9.7.7	Bio-Bar Codes	316
9.7.8	Structural Analysis of Protein “Crystals” Using X-Ray Crystallography	317
9.7.8.1	Introduction	317
9.7.8.2	Protein Crystals	318
9.7.8.3	X-Ray Crystallography	320
9.7.9	Cryoelectron Microscopy	324

9.8	Summary.....	326
	Chapter 9 Home Problems.....	327
Chapter 10 Applications of Genomics		329
10.1	Introduction.....	329
10.2	Genetically Modified Organisms.....	329
10.2.1	Introduction.....	329
10.2.2	Examples of Transgenic Plants.....	330
10.2.2.1	The FlavrSavr™ Tomato.....	330
10.2.2.2	Golden Rice.....	332
10.2.2.3	Transgenic Maize	332
10.2.2.4	Genetically Modified Soybeans	333
10.2.2.5	Genetically Modified Cotton.....	333
10.2.2.6	Transgenic Arabidopsis for Detection of Explosives in the Soil.....	334
10.2.3	Transgenic Animals and Animal Cells.....	335
10.2.3.1	Introduction.....	335
10.2.3.2	Uses of TGAs and Cells	335
10.2.4	Recombinant DNA Technology.....	337
10.2.4.1	Introduction.....	337
10.2.4.2	rDNA Insertion Methods.....	337
10.2.4.3	Preparation of a Plasmid Vector.....	337
10.2.4.4	The Use of Phages as cDNA Vectors	338
10.2.4.5	Other Viral Vectors in Gene Therapy	339
10.2.4.6	The Gene Gun	339
10.2.4.7	Electroporation and the Effects of Nanosecond Pulsed Electric Fields on Cells	341
10.2.4.8	Ultrasonic Poration.....	342
10.2.5	Problems with GMOs	342
10.3	Animal Reproductive Cloning	345
10.3.1	Introduction.....	345
10.3.2	Altered Nuclear Transfer	345
10.3.3	The Roslin Technique	346
10.3.4	The Honolulu Technique.....	346
10.3.5	Problems in Cloning Mammals	347
10.3.6	Therapeutic Cloning	347
10.4	Stem Cells	347
10.4.1	Introduction.....	347
10.4.2	Types of Stem Cells	348
10.4.3	Sources of Stem Cells	349
10.4.4	Stem Cell Applications in Regenerative Medicine	353
10.4.5	Cancer Stem Cells.....	354
10.4.5.1	Introduction	354
10.4.5.2	New Pathways for Cancer Therapy	355
10.4.5.3	Summary	359
10.5	Parthenogenesis.....	359
10.5.1	Introduction.....	359
10.5.2	Artificial Parthenogenesis.....	359
10.5.3	Human Parthenotes?	360
10.6	Chimeras	362
10.6.1	Introduction.....	362

10.6.2	Cellular Chimeras	362
10.6.3	Somatic Chimeras	363
10.7	Summary	364
	Chapter 10 Home Problems	365
 Chapter 11 Ethical Issues in Genetic Engineering		 367
11.1	Introduction	367
11.2	Framework for Ethical Decision Making	367
11.2.1	Defining Ethics	367
11.2.2	Ethical Frameworks	368
11.2.3	Other Resources	369
11.3	Genetically Modified Food Crops	369
11.3.1	Rogue Herbicide Resistance	369
11.3.2	Antibiotic-Resistant Bacteria	370
11.4	Chimeras	370
11.4.1	Transgenic Organ Farming	370
11.4.2	How Human Is Too Human?	371
11.4.3	Religious Concerns	371
11.4.4	Retrovirus Transfer	371
11.5	Human Embryonic Stem Cells	372
11.5.1	Religious Concerns	372
11.5.1.1	Alternative Sources of Stem Cells	373
11.5.2	Embryonic versus Adult Stem Cells	373
11.6	Summary	374
	Chapter 11 Home Problems	375
 Appendix		 377
 Bibliography and References		 383
 Glossary		 407
 Index		 435