

## Contents

Preface XIX

List of Contributors XXI

Abbreviations XXV

### Part I Fundamentals of Cellular and Molecular Biology 1

#### 1 The Cell as the Basic Unit of Life 3

*M. Wink*

#### 2 Structure and Function of Cellular Macromolecules 7

*M. Wink*

2.1 Structure and Function of Sugars 8

2.2 Structure of Membrane Lipids 10

2.3 Structure and Function of Proteins 14

2.4 Structure of Nucleotides and Nucleic Acids (DNA and RNA) 21

2.5 References 27

#### 3 Structure and Functions of a Cell 29

*M. Wink*

3.1 Structure of a Eukaryotic Cell 29

3.1.1 Structure and Function of the Cytoplasmic Membrane 29

3.1.1.1 Membrane Permeability 30

3.1.1.2 Transport Processes across Biomembranes 31

3.1.1.3 Receptors and Signal Transduction at Biomembranes 33

3.1.2 Endomembrane System in a Eukaryotic Cell 38

3.1.3 Mitochondria and Chloroplasts 40

3.1.4 Cytoplasm 45

3.1.5 Cytoskeleton 47

3.1.6 Cell Walls 49

3.2 Structure of Bacteria 50

3.3 Structure of Viruses 51

3.4 Differentiation of Cells 52

#### 4 Biosynthesis and Function of Macromolecules (DNA, RNA, and Proteins) 57

*M. Wink*

4.1 Genomes, Chromosomes, and Replication 57

4.1.1 Genome Size 57

4.1.2 Composition and Function of Chromosomes 62

4.1.3 Mitosis and Meiosis 64

4.1.4 Replication 66

4.1.5 Mutations and Repair Mechanisms 66

4.2	Transcription: From Gene to Protein	71
4.3	Protein Biosynthesis (Translation)	76
<b>5</b>	<b>Distributing Proteins in the Cell (Protein Sorting)</b>	<b>81</b>
	<i>M. Wink</i>	
5.1	Import and Export of Proteins via the Nuclear Pore	82
5.2	Import of Proteins in Mitochondria and Chloroplasts	83
5.3	Protein Transport into the Endoplasmic Reticulum	85
5.4	Vesicle Transport from the ER via the Golgi Apparatus to the Cytoplasmic Membrane	86
<b>6</b>	<b>Evolution and Diversity of Organisms</b>	<b>91</b>
	<i>M. Wink</i>	
6.1	Prokaryotes	91
6.2	Eukaryotes	91
<b>Part II</b>	<b>Standard Methods in Molecular Biotechnology</b>	<b>99</b>
<b>7</b>	<b>Isolation and Purification of Proteins</b>	<b>101</b>
	<i>T. Wieland, M. Lutz</i>	
7.1	Introduction	101
7.2	Producing a Protein Extract	102
7.3	Gel Electrophoretic Separation Methods	103
7.3.1	Principles of Electrophoresis	103
7.3.2	Native Gel Electrophoresis	104
7.3.3	Discontinuous Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE)	104
7.3.4	Two-Dimensional (2D) Gel Electrophoresis, Isoelectric Focusing (IEF)	105
7.3.5	Detecting Proteins in Gels	105
7.4	Methods of Protein Precipitation	106
7.5	Column Chromatography Methods	107
7.5.1	General Principles of Separation	107
7.5.1.1	Size Exclusion Chromatography (Gel Filtration)	107
7.5.1.2	Hydrophobic Interaction Chromatography	108
7.5.1.3	Ion Exchange Chromatography	109
7.5.1.4	Hydroxyapatite Chromatography	110
7.5.2	Group-specific Separation Techniques	110
7.5.2.1	Chromatography on Protein A or Protein G	110
7.5.2.2	Chromatography on Cibacron Blue (Blue Gel)	111
7.5.2.3	Chromatography on Lectins	111
7.5.2.4	Chromatography on Heparin	111
7.5.3	Purification of Recombinant Fusion Proteins	112
7.5.3.1	Chromatography on Chelating Agents	112
7.5.3.2	Chromatography on Glutathione Matrices	112
7.6	Examples	113
7.6.1	Example 1: Purification of Nucleoside Diphosphate Kinase from the Cytosol of Bovine Retina Rod Cells	113
7.6.2	Example 2: Purification of Recombinant His <sub>6</sub> -RGS16 after Expression in <i>E. coli</i>	114
<b>8</b>	<b>Peptide and Protein Analysis with Electrospray Tandem Mass Spectrometry</b>	<b>115</b>
	<i>A. Schlosser, W.D. Lehmann</i>	
8.1	Introduction	115
8.2	Principles of Mass Spectrometry	115
8.3	Mass Precision, Resolution, and Isotope Distribution	116

8.4	Principles of ESI	116
8.5	Tandem Mass Spectrometers	117
8.5.1	Mass Analyzers	117
8.5.2	Triple Quadrupole	118
8.5.3	Linear Trap Quadrupole (LTQ) and LTQ Orbitrap	118
8.5.4	Q-TOF	119
8.5.5	Q-FT-ICR	119
8.6	Peptide Sequencing with MS/MS	119
8.7	Identifying Proteins with MS/MS Data and Protein Databases	120
8.7.1	Database Search with MS/MS Raw Data	120
8.8	Determining Protein Molecular Mass	121
8.9	Analysis of Covalent Protein Modification	122
8.10	Relative and Absolute Quantification	123
<b>9</b>	<b>Isolation of DNA and RNA</b>	<b>125</b>
	<i>H. Weiher, R. Zwacka, I. Herr</i>	
9.1	Introduction	125
9.2	DNA Isolation	125
9.3	RNA Isolation	127
9.3.1	Enrichment of mRNA	127
<b>10</b>	<b>Chromatography and Electrophoresis of Nucleic Acids</b>	<b>129</b>
	<i>H. Weiher, R. Zwacka, I. Herr</i>	
10.1	Introduction	129
10.2	Chromatographic Separation of Nucleic Acids	129
10.3	Electrophoresis	130
10.3.1	Agarose Gel Electrophoresis: Submarine Electrophoresis	130
10.3.2	Pulsed Field Agarose Gel Electrophoresis	131
10.3.3	Polyacrylamide Gel Electrophoresis (PAGE)	131
<b>11</b>	<b>Hybridization of Nucleic Acids</b>	<b>133</b>
	<i>H. Weiher, R. Zwacka, I. Herr</i>	
11.1	Significance of Base Pairing	133
11.2	Experimental Hybridization: Kinetic and Thermodynamic Control	133
11.3	Analytical Techniques	134
11.3.1	Clone Detection, Southern Blotting, Northern Blotting, and Gene Diagnosis	134
11.3.2	Systematic Gene Diagnosis and Expression Screening based on Gene Arrays	135
11.3.3	<i>In Situ</i> Hybridization	135
<b>12</b>	<b>Use of Enzymes in the Modification of Nucleic Acids</b>	<b>137</b>
	<i>A. Groth, R. Zwacka, H. Weiher, I. Herr</i>	
12.1	Restriction Enzymes (Restriction Endonucleases)	137
12.2	Ligases	139
12.3	Methyltransferases	139
12.4	DNA Polymerases	140
12.5	RNA Polymerases and Reverse Transcriptase	141
12.6	Nucleases	141
12.7	T4 Polynucleotide Kinase	141
12.8	Phosphatases	142
<b>13</b>	<b>Polymerase Chain Reaction</b>	<b>143</b>
	<i>A. Mohr, H. Weiher, I. Herr, R. Zwacka</i>	
13.1	Introduction	143
13.2	Techniques	143

13.2.1	Standard PCR	143
13.2.2	RT-PCR	144
13.2.3	Quantitative/Real-Time PCR	145
13.2.4	Rapid Amplification of cDNA Ends (RACE)	146
13.3	Areas of Application	146
13.3.1	Genome Analysis	146
13.3.2	Cloning Techniques	147
13.3.3	Expression Studies	147
<b>14</b>	<b>DNA Sequencing</b>	<b>149</b>
	<i>R. Zwacka, A. Mohr, I. Herr, H. Weiher</i>	
14.1	Introduction	149
14.2	DNA Sequencing Methods	149
14.2.1	Chemical Sequencing Method (MaxamGilbert Method)	150
14.2.2	Enzymatic Sequencing (SangerCoulson Method)	150
14.2.3	Pyrosequencing	151
14.3	Strategies for Sequencing the Human Genome	151
14.4	Practical Significance of DNA	152
<b>15</b>	<b>Cloning Procedures</b>	<b>153</b>
	<i>T. Wieland, S. Lutz</i>	
15.1	Introduction	153
15.2	Construction of Recombinant Vectors	153
15.2.1	Insert	154
15.2.2	Vector	156
15.2.3	Essential Components of Vectors	156
15.2.3.1	Bacterial Origin of Replication ( <i>ori</i> )	156
15.2.3.2	Antibiotic Resistance	156
15.2.3.3	Polylinkers	157
15.2.4	Cloning Using Recombination Systems	157
15.2.5	Further Components of Vectors for Prokaryotic Expression Systems	158
15.2.5.1	Promoter	158
15.2.5.2	Ribosome-Binding Site	159
15.2.5.3	Termination Sequence	159
15.2.5.4	Fusion Sequence	159
15.2.6	Further Components of Eukaryotic Expression Vectors	159
15.2.6.1	Eukaryotic Expression Vectors: Yeast	160
15.2.6.2	Eukaryotic Expression Vectors for Mammal Cells	161
15.2.6.3	Viral Expression Systems for Mammalian Cells	163
15.2.7	Nonviral Introduction of Heterologous DNA to Host Organisms (Transformation, Transfection)	165
15.2.7.1	Transformation of Prokaryotes	165
15.2.7.2	Transformation of Yeast Cells	166
15.2.7.3	Transfection of Mammal Cells	166
<b>16</b>	<b>Expression of Recombinant Proteins</b>	<b>169</b>
	<i>T. Wieland, S. Lutz</i>	
16.1	Introduction	169
16.2	Expression of Recombinant Proteins in Host Organisms	170
16.2.1	Expression in <i>E. coli</i>	173
16.2.2	Expression in Yeasts	174
16.2.3	Expression in Insect Cells	176
16.2.3.1	Expression Based on Recombinant Baculoviruses	176
16.2.3.2	Expression of Proteins in Stably Transfected Insect Cells	177
16.2.4	Expression of Proteins in Mammalian Cells	177
16.3	Expression in Cell-Free Systems	178

- 16.3.1 Expression of Proteins in Reticulocyte Lysates 179
- 16.3.2 Protein Expression Using *E. coli* Extracts 179
- 17 Patch Clamp Method 181**  
*R. Kraft*
- 17.1 Biological Membranes and Ion Channels 181
- 17.2 Physical Foundations of the Patch Clamp Method 182
- 17.3 Patch Clamp Configurations 182
- 17.4 Applications of the Patch Clamp Method 184
- 18 Cell Cycle Analysis 187**  
*S. Wöfl, A. Kitanovic*
- 18.1 Analyzing the Cell Cycle 187
- 18.2 Experimental Analysis of the Cell Cycle 189
  - 18.2.1 Preparing Synchronized Cell Cultures of *S. cerevisiae* 190
    - 18.2.1.1 Centrifugal Elutriation 190
    - 18.2.1.2 Cell Cycle Arrest Using  $\alpha$ -Factor 191
  - 18.2.2 Identification of Cell Cycle Stages 191
    - 18.2.2.1 Budding Index 192
    - 18.2.2.2 Fluorescent Staining of the Nucleus 192
- 19 Microscopic Techniques 197**  
*S. Diekmann*
- 19.1 Electron Microscopy 197
  - 19.1.1 Cryo-electron Microscopy 199
  - 19.1.2 Electron Tomography 200
- 19.2 Atomic or Scanning Force Microscopy 200
  - 19.2.1 Force Spectroscopy 201
  - 19.2.2 Advantages and Disadvantages 201
- 19.3 Light Microscopy 202
  - 19.3.1 Deconvolution 203
  - 19.3.2 Confocal Microscopy 203
  - 19.3.3 Why Fluorescence? 204
  - 19.3.4 Nanoscopy 204
- 19.4 Microscopy in the Living Cell 206
  - 19.4.1 Analysis of Fluorescently Labeled Proteins *In Vivo* 207
  - 19.4.2 Fluorescence Recovery after Photobleaching 208
  - 19.4.3 Fluorescence Correlation Spectroscopy 208
  - 19.4.4 Förster Resonance Energy Transfer and Fluorescence Lifetime Imaging Microscopy 209
  - 19.4.5 Single-Molecule Fluorescence 209
- 20 Laser Applications 211**  
*M. Vogel, R. Fink*
- 20.1 Principles of Laser Technology 211
- 20.2 Properties of Laser Radiation 213
- 20.3 Types of Lasers and Setups 213
- 20.4 Applications 214
  - 20.4.1 Laser Scanning Microscopy 214
  - 20.4.2 Optical Tweezers 215
  - 20.4.3 Laser Microdissection 215

**Part III Key Topics 217**

<b>21</b>	<b>Genomics and Functional Genomics 219</b>
	<i>S. Wiemann, M. Frohme</i>
21.1	Introduction 219
21.2	Technological Developments in DNA Sequencing 221
21.3	Genome Sequencing 222
21.3.1	Mapping 222
21.3.1.1	Restriction Mapping and Restriction Fingerprinting 224
21.3.1.2	BAC End Sequencing 224
21.3.1.3	Genetic Mapping 226
21.3.1.4	Radiation Hybrid Mapping 227
21.3.1.5	HAPPY Mapping 228
21.3.1.6	Mapping Through Hybridization 228
21.3.1.7	Sequence Tagged Sites, Expressed Sequence Tags, Single Nucleotide Polymorphisms, and Sequence Length Polymorphisms (Amplified Fragment Length Polymorphisms) 231
21.3.1.8	Fluorescence <i>In Situ</i> Hybridization, Fiber Fish, Optical Mapping, and Comparative Genome Hybridization 232
21.3.2	Timeline of Genome Sequencing 233
21.3.3	Genome Sequencing Strategies 234
21.3.3.1	Conventional Approach: Random Shotgun Strategy 234
21.3.3.2	Whole-Genome Shotgun Strategy 235
21.3.3.3	Sequencing of the Human Genome 237
21.3.4	Outlook for Genome Sequencing 238
21.4	cDNA Projects 238
21.4.1	cDNA Libraries Represent the Cell's mRNA 238
21.4.2	Production of cDNA Libraries 240
21.4.3	EST Projects for Gene Identification 243
21.4.3.1	What is an EST? 243
21.4.4	Full-length Projects for the Production of Resources for Functional Genomics 245
21.5	Functional Genomics 246
21.6	Identification and Analysis of Individual Genes 248
21.6.1	Positional Cloning 248
21.6.2	Gene Trap 251
21.6.3	DNA/RNA <i>In Situ</i> Hybridization 251
21.6.4	Tissue Arrays 252
21.7	Investigation of Transcriptional Activity 253
21.7.1	Serial Analysis of Gene Expression 253
21.7.2	Subtractive Hybridization 254
21.7.3	RNA Fingerprinting 257
21.7.4	Array-based Techniques 258
21.7.4.1	Macroarrays 261
21.7.4.2	Microarrays 262
21.7.4.3	Global and Specific Arrays 264
21.7.5	Specificity and Sensitivity 265
21.8	Cell-based Methods 266
21.8.1	Green Fluorescence Protein Techniques 266
21.8.2	Alternatives to Green Fluorescence Protein 267
21.8.3	Fluorescence Resonance Energy Transfer 268
21.8.4	Fluorescence Recovery After Photobleaching 269
21.8.5	Cell-based Assays 269
21.8.5.1	Assay Design 270
21.8.5.2	Pipetting Systems 270
21.8.5.3	Reading and Recording of Data 270
21.8.5.4	Data Analysis 271

21.9	Functional Analysis of Entire Genomes	272
21.9.1	Genotypic Screening in Yeast	272
21.9.2	Phenotypic Screening in the Mouse	273
<b>22</b>	<b>Bioinformatics</b>	275
	<i>B. Brors, K. Fellenberg</i>	
22.1	Introduction	275
22.2	Data Sources	276
22.2.1	Primary Databases: EMBL/GenBank/DDBJ, PIR, Swiss-Prot	276
22.2.2	Genome Databases: Ensembl, GoldenPath	276
22.2.3	Genome Databases: Ensembl, GoldenPath	277
22.2.4	Molecular Structure Databases: PDB, SCOP	277
22.2.5	Transcriptome Databases: SAGE, ArrayExpress, GEO	277
22.2.6	Reference Databases: PubMed, OMIM, GeneCards	278
22.2.7	Pathway Databases and Gene Ontology	278
22.3	Sequence Analysis	279
22.3.1	Kyte-Doolittle Plot, Helical Wheel Analysis, Signal Sequence Analysis	279
22.3.2	Pairwise Alignment	280
22.3.2.1	Local/Global	281
22.3.2.2	Optimal/Heuristic	281
22.3.3	Alignment Statistics	282
22.3.4	Multiple Alignment	282
22.4	Evolutionary Bioinformatics	283
22.4.1	Statistical Models of Evolution	284
22.4.2	Relation to Score Matrices	285
22.4.3	Phylogenetic Analysis	285
22.5	Gene Prediction	287
22.5.1	Neural Networks or HMMs Based on Hexanucleotide Compo- sition	287
22.5.2	Comparison with Expressed Sequence Tags or other Genomes ( <i>Fugu</i> , Mouse)	288
22.6	Bioinformatics in Transcriptome and Proteome Analysis	288
22.6.1	Preprocessing, Normalization	288
22.6.2	Feature Selection	290
22.6.3	Similarity Measures: Euclidean Distance, Correlation, Manhattan Distance, Mahalanobis Distance, Entropy Measures	290
22.6.4	Unsupervised Learning Procedures: Clustering, Principal Component Analysis, Multidimensional Scaling, Correspondence Analysis	291
22.6.5	Supervised Learning Procedures: Linear Discriminant Analysis, Decision Trees, Support Vector Machines, ANNs	291
22.6.6	Analysis of Over-Representation of Functional Categories	293
22.7	Bioinformatic Software	293
<b>23</b>	<b>Cellular Systems Biology</b>	295
	<i>H. Schmidt-Gienewinkel, S. Legewie, B. Brors, R. König</i>	
23.1	Introduction	295
23.2	Analysis of Cellular Networks by Top-Down Approaches	296
23.2.1	Motivation	296
23.2.2	Definitions and Reconstruction of the Networks	296
23.2.3	Gene Set Enrichment Tests	297
23.2.4	Network Descriptors	298
23.2.4.1	Scale-Free Networks	299
23.2.4.2	Triangle Motifs in Networks	299
23.2.4.3	Centrality and Further Topology Features	300

23.2.5	Detecting Essential Enzymes with a Machine Learning Approach	301
23.2.6	Elementary Flux Modes	301
23.2.7	Inference of Regulatory Networks: Boolean and Bayesian Networks	303
23.3	Overview of Bottom-Up Modeling of Biochemical Networks	304
23.3.1	Motivation	304
23.3.2	Choosing Model Complexity	305
23.3.3	Model Construction	305
23.3.4	Model Simulation	306
23.3.5	Model Calibration	307
23.3.6	Model Verification and Analysis	309
23.4	Biological Examples	309
<b>24</b>	<b>Protein–Protein and Protein–DNA Interaction</b>	<b>315</b>
	<i>P. Uetz, E. Pohl</i>	
24.1	Protein–Protein Interactions	315
24.1.1	Classification and Specificity: Protein Domains	316
24.1.2	Protein Networks and Complexes	316
24.1.3	Structural Properties of Interacting Proteins	318
24.1.4	Which Forces Mediate Protein–Protein Interactions?	319
24.1.4.1	Thermodynamics	319
24.1.4.2	Energetics	320
24.1.5	Methods to Examine Protein–Protein Interactions	320
24.1.6	Regulation of Protein–Protein Interactions	322
24.1.7	Theoretical Prediction of Protein–Protein Interactions	323
24.1.7.1	Predicting Interacting Proteins by their Genome Sequence	323
24.1.7.2	Phylogenetic Profiles	324
24.1.8	Biotechnological and Medical Applications of Protein–Protein Interactions	324
24.2	Protein–DNA Interactions	324
24.2.1	Sequence-Specific DNA Binding	324
24.2.2	Thermodynamic Considerations Regarding Protein–DNA Complexes	325
24.2.3	Methods to Study Protein–DNA Interactions	325
24.2.3.1	Structural Classification of Protein–DNA Complexes	326
24.2.4	Regulatory Networks and Systems Biology	327
24.2.4.1	Medical Relevance of Protein–DNA Interactions	328
24.2.5	Biotechnological Applications of Protein–DNA Interactions	328
24.2.5.1	Synthetic Biology	328
<b>25</b>	<b>Drug Research</b>	<b>331</b>
	<i>M. Koegl, R. Tolle, U. Deuschle, C. Kremoser</i>	
25.1	Introduction	331
25.2	Active Compounds and their Targets	331
25.2.1	Identification of Potential Targets in the Human Genome	332
25.2.2	Comparative Genome Analysis	334
25.2.3	Experimental Target Identification: <i>In Vitro</i> Methods	334
25.2.4	Experimental Identification of Targets: Model Organisms	335
25.2.5	Experimental Target Identification in Humans	336
25.2.6	Difference between Target Candidates and Genuine Targets	337
25.2.7	Biologicals	337
25.2.8	DNA and RNA in New Therapeutic Approaches	339
25.2.9	Patent Protection for Targets	339
25.2.10	Compound Libraries as a Source of Drug Discovery	340
25.2.11	High-Throughput Screening	341
25.2.12	High-Quality Paramounts in Screening Assays	343



25.2.13	Virtual Ligand Screening	343
25.2.14	Activity of Drugs Described in Terms of Efficacy and Potency	344
25.2.15	Chemical Optimization of Lead Structures	344
25.3	Preclinical Pharmacology and Toxicology	344
25.4	Clinical Development	346
25.5	Clinical Testing	346
<b>26</b>	<b>Drug Targeting and Prodrugs</b>	<b>349</b>
	<i>G. Fricker</i>	
26.1	Drug Targeting	349
26.1.1	Passive Targeting by Exploiting Special Physiological Properties of the Target Tissue	350
26.1.2	Physical Targeting	350
26.1.3	Active Targeting	351
26.1.4	Cellular Carrier Systems	354
26.2	Prodrugs	355
26.2.1	Prodrugs to Improve Drug Solubility	355
26.2.2	Prodrugs to Increase Stability	355
26.3	Penetration of Drugs through Biological Membranes	356
26.4	Prodrugs to Extend Duration of Effect	357
26.5	Prodrugs for the Targeted Release of a Drug	357
26.6	Prodrugs to Minimize Side Effects	358
<b>27</b>	<b>Molecular Diagnostics in Medicine</b>	<b>359</b>
	<i>S. Wölfel, R. Gessner</i>	
27.1	Uses of Molecular Diagnostics	359
27.1.1	Introduction	359
27.1.2	Monogenic and Polygenic Diseases	359
27.1.3	Individual Variability in the Genome: Forensics	362
27.1.4	Individual Variability in the Genome: HLA Typing	362
27.1.5	Individual Variability in the Genome: Pharmacogenomics	362
27.1.6	Individual Variability in the Genome: Susceptibility to Infectious Diseases	363
27.1.7	Viral Diagnosis	363
27.1.8	Microbial Diagnosis and Resistance Diagnosis	364
27.2	Which Molecular Variations Should be Detected	364
27.2.1	Point Mutations	365
27.2.2	Insertions and Deletions	366
27.2.3	Nucleotide Repeats	366
27.2.4	Deletion or Duplication of Genes	366
27.2.5	Recombination between Chromosomes	366
27.2.6	Heading3	367
27.3	Molecular Diagnostic Methods	367
27.3.1	DNA/RNA Purification	367
27.3.2	Determination of Known Sequence Variations	368
27.3.2.1	Length Polymorphism	368
27.3.2.2	Restriction Fragment Length Polymorphism (RFLP)	368
27.3.2.3	Amplification-Created Restriction Sites (ACRS)	369
27.3.2.4	Amplification Refractory Mutation System (ARMS)	369
27.3.2.5	Mutationally Separated (MS)-PCR	369
27.3.2.6	Allele-Specific Hybridization	369
27.3.2.7	Ligase Chain Reaction (LCR)	371
27.3.2.8	Minisequencing	371
27.3.2.9	Pyrosequencing	371
27.3.2.10	Quantitative PCR	371
27.3.2.11	Chip Technology	372

27.3.2.12	Production and Manufacture of Microarrays	373
27.3.2.13	Determination of Unknown Mutations	374
27.4	Outlook	375
<b>28</b>	<b>Recombinant Antibodies and Phage Display</b>	<b>377</b>
	<i>S. Dübel</i>	
28.1	Introduction	377
28.2	Why Recombinant Antibodies?	379
28.2.1	Recombinant Antibodies are Available <i>In Vitro</i> without Immunization	379
28.2.2	Antibodies with New Characteristics Can Be Created	379
28.3	Obtaining Specific Recombinant Antibodies	379
28.3.1	Preparation of the Variety of Antibody Genes	380
28.3.2	Selection Systems for Recombinant Antibodies	380
28.3.2.1	Transgenic Mice	380
28.3.2.2	<i>In Vitro</i> Selection Systems	382
28.4	Production of Recombinant Antibodies	384
28.4.1	Recombinant Production Systems	384
28.4.2	Purification of Recombinant Antibodies and their Fragments	385
28.5	Formats for Recombinant Antibodies	386
28.5.1	Monospecific Antibody Fragments	386
28.5.1.1	Fab Fragments	388
28.5.1.2	Fv Fragments	388
28.5.1.3	Single-Chain Antibody Fragments (scFv)	388
28.5.1.4	Single-Chain Fab Fragments (scFab)	389
28.5.1.5	Disulfide-Stabilized Fv Fragments (dsFv)	389
28.5.1.6	V <sub>H</sub> and Camel Antibodies	389
28.5.2	Multivalent Antibody Fragments	389
28.5.2.1	Bifunctional Antibodies	390
28.5.2.2	Bispecific Antibodies	390
28.6	Applications of Recombinant Antibodies	392
28.6.1	Clinical Applications	392
28.6.2	Applications in Research and <i>In Vitro</i> Diagnostics	392
28.6.2.1	Recombinant Antibodies Selected to Avoid Cross-Reactivity	393
28.6.2.2	Intracellular Antibodies	394
28.6.2.3	Recombinant Antibodies as Binding Molecules for Arrays	394
28.7	Outlook	394
<b>29</b>	<b>Transgenic and Gene-Targeted Mice and their Impact in Medical Research</b>	<b>395</b>
	<i>R. Sprengel</i>	
29.1	Overview	395
29.2	Transgenic Mice	395
29.2.1	Retroviral Infection	396
29.2.2	Pronuclear Injection	397
29.3	Homologous Recombination: knock-out (-in) mice	398
29.4	Conditionally Regulated Gene Expression	399
29.5	Impact of Genetically Modified Mice in Biomedicine	400
29.5.1	Alzheimer's Disease	401
29.5.2	Amyotrophic Lateral Sclerosis (ALS)	401
29.5.3	Psychological Disorders	402
29.6	Outlook	402
<b>30</b>	<b>Gene Therapy: Strategies and Vectors</b>	<b>403</b>
	<i>A. Groth, I. Herr</i>	
30.1	Introduction	403
30.2	Principles of Somatic Gene Therapy	404

30.3	Germ Line Therapy	405
30.4	Setbacks in Gene Therapy	406
30.5	Vectors for Gene Therapy	406
30.5.1	Retroviral Vectors	407
30.5.2	Adenoviral Vectors	410
30.5.3	Adeno-associated Virus (AAV)	411
30.5.4	Other Viral Vectors	413
30.6	Specific Expression	413
<b>31</b>	<b>RNA Interference, Modified DNA, Peptide Nucleic Acid, and Applications in Medicine and Biotechnology</b>	<b>415</b>
	<i>N. Metzler-Nolte, A. Sosniak</i>	
31.1	Introduction	415
31.2	Modified Nucleic Acids	416
31.2.1	Phosphorothioate	416
31.2.2	Methylphosphonate	417
31.2.3	Peptide Nucleic Acids (PNAs)	418
31.3	Interactions of DNA Analogs with Complementary DNA and RNA	419
31.3.1	Melting Temperature	419
31.3.2	Mismatch Sensitivity	421
31.4	RNAi	421
31.4.1	Biogenesis of Small RNAs	422
31.4.1.1	Biogenesis of siRNAs	422
31.4.1.2	Biogenesis of miRNAs	422
31.4.2	Incorporation into RISC	423
31.4.3	Posttranscriptional Repression by miRNA und siRNA	424
31.5	Applications	424
31.5.1	Antisense Technology with DNA Analogs	424
31.5.2	siRNA in Biotechnological Applications	426
31.5.2.1	Design of siRNAs	426
31.5.2.2	Nonvectorial Applications	427
31.5.2.3	Vectorial Applications	427
31.5.2.4	Other Applications for PNA	428
31.5.3	Comparison of RNAi with DNA Analogs for Antisense Applications	429
<b>32</b>	<b>Plant Biotechnology</b>	<b>431</b>
	<i>H. Hillebrand, R. Hell</i>	
32.1	Introduction	431
32.1.1	Green Genetic Engineering – A New Method Towards Traditional Goals	431
32.1.2	Challenges in Plant Biotechnology	432
32.2	Gene Expression Control	433
32.3	Production of Transgenic Plants	434
32.3.1	Transformation Systems	434
32.3.1.1	<i>Agrobacterium</i> as a Natural Transformation System	435
32.3.1.2	Biolistic Method: Gene Gun	436
32.3.1.3	Plastid Transformation	438
32.3.1.4	Viral Systems	439
32.4	Selection of Transformed Plant Cells	439
32.4.1	Requirements for an Optimal Selection Marker System	440
32.4.2	Negative Selection Marker Systems	441
32.4.3	Positive Selection Marker Systems	442
32.4.4	Counter-Selection using Bifunctional Marker Genes	443
32.4.5	Visual Markers	443

32.4.6	Selection Systems, Genetic Engineering Safety, and Marker-Free Plants	443
32.5	Regeneration of Transgenic Plants	445
32.5.1	Regeneration Procedures	445
32.5.2	Composition of Regeneration Media	446
32.6	Plant Analysis: Identification and Characterization of Genetically Engineered Plants	446
32.6.1	DNA and RNA Verification	446
32.6.2	Protein Analysis	448
32.6.3	Genetic and Molecular Maps	448
32.6.4	Stability of Transgenic Plants	449
<b>33</b>	<b>Biocatalysis in the Chemical Industry</b>	<b>451</b>
	<i>M. Breuer, B. Hauer</i>	
33.1	Introduction	451
33.2	Bioconversion/Enzymatic Procedures	454
33.3	Development of an Enzyme for Industrial Biocatalysis	456
33.3.1	Identification of Novel Biocatalysts	456
33.3.2	Improvement of Biocatalysts	458
33.3.3	Production of Biocatalysts	458
33.3.4	Outlook	459
33.3.5	Case Study 1: Screening for New Nitrilases	459
33.3.6	Case Study 2: Use of Known Enzymes for New Reactions: Lipases for the Production of Optically Active Amines and Alcohols	460
33.3.7	Case Study 3: Enzyme Optimization with Rational and Evolutionary Methods	461
33.4	Fermentative Procedures	462
33.4.1	Improvement of Fermentation Processes	462
33.4.2	Classical Strain Optimization	463
33.4.3	Metabolic Engineering	464
33.4.4	Case Study 4: Fermentative Production of <i>n</i> -Butanol	465
33.4.5	Case Study 5: Production of Glutamic Acid with <i>C. glutamicum</i>	466
33.4.5.1	Molecular Mechanism of Glutamate Overproduction	467
33.4.6	Case Study 6: Production of Lysine with <i>C. glutamicum</i>	468
33.4.6.1	Molecular Mechanism of Lysine Biosynthesis	468
33.4.6.2	Deregulation of the Key Enzyme Aspartate Kinase	469
33.4.7	Genomic Research and Functional Genomics	470
33.4.8	Case Study 7: Fermentative Penicillin Production	470
33.4.9	Case Study 8: Vitamin B <sub>2</sub> Production	471
33.4.9.1	Riboflavin Biosynthesis	471
33.4.9.2	Classical Strain Development	472
<b>Part IV</b>	<b>Biotechnology in Industry</b>	<b>473</b>
<b>34</b>	<b>Industrial Application: Biotech Industry, Markets, and Opportunities</b>	<b>475</b>
	<i>J. Schüller</i>	
34.1	Historical Overview and Definitions of Concepts	475
34.2	Areas of Industrial Application of Molecular Biotechnology	476
34.2.1	Red Biotechnology	477
34.2.1.1	Biopharmaceutical Drug Development	477
34.2.1.2	Drug Delivery	479
34.2.1.3	Cell and Gene Therapy	479
34.2.1.4	Tissue Engineering/Regenerative Medicine	481
34.2.1.5	Pharmacogenomics and Personalized Medicine	481
34.2.1.6	Molecular Diagnostic Agents	482

34.2.1.7	Systems Biology	483
34.2.2	Green Biotechnology	483
34.2.2.1	Transgenic Plants	483
34.2.2.2	Genomic Approaches in Green Biotechnology	484
34.2.2.3	Novel Food and Functional Food	484
34.2.2.4	Livestock Breeding	484
34.2.3	White and Gray Biotechnology	485
34.3	Status Quo of the Biotech Industry World-Wide	485
34.3.1	Global Overview	485
34.3.2	United States	486
34.3.3	Europe	486
<b>35</b>	<b>Patents in the Molecular Biotechnology Industry: Legal and Ethical Issues</b>	<b>487</b>
	<i>David B. Resnik</i>	
35.1	Patent Law	487
35.1.1	What is a Patent?	487
35.1.2	How Does One Obtain a Patent?	488
35.1.3	What is the Proper Subject Matter for a Patent?	489
35.1.4	Types of Patents in Pharmaceutical and Molecular Biotechnology	490
35.1.5	Patent Infringement	490
35.1.6	International Patent Law	491
35.2	Ethical and Policy Issues in Biotechnology Patents	492
35.2.1	No Patents on Nature	492
35.2.2	Threats to Human Dignity	493
35.2.3	Problems with Access to Technology	494
35.2.4	Benefit Sharing	497
35.3	Conclusions	498
<b>36</b>	<b>Drug Approval in the European Union and United States</b>	<b>499</b>
	<i>G. Walsh</i>	
36.1	Introduction	499
36.2	Regulation within the European Union	499
36.2.1	EU Regulatory Framework	499
36.2.2	EMA	500
36.2.3	New Drug Approval Routes	501
36.2.3.1	Centralized Procedure	502
36.2.3.2	Mutual Recognition	503
36.3	Regulation in the United States	503
36.3.1	CDER and CBER	504
36.3.2	Approval Procedure	504
36.4	Advent and Regulation of Biosimilars	506
36.5	International Regulatory Harmonization	506
<b>37</b>	<b>Emergence of a Biotechnology Industry</b>	<b>509</b>
	<i>C. Kremoser</i>	
<b>38</b>	<b>The 101 of Founding a Biotech Company</b>	<b>517</b>
	<i>C. Kremoser</i>	
38.1	First Steps Towards Your Own Company	517
38.2	Employees: Recruitment, Remuneration, Participation	522

**39 Marketing 527**

*C. Kremoser*

39.1 Introduction 527

39.2 What Types of Deals are Possible? 528

39.3 What Milestone or License Fees are Effectively Paid in a Biotech/  
Pharma Cooperation? 529

39.4 PR and IR in Biotech Companies 530

**Appendix 533**

**Further Reading 535**

**Glossary 551**

*M. Wink*

**Subject Index 587**