

Contents

Preface VII

1	Basics of Cell Signaling	1
1.1	Cell Signaling: Why, When and Where?	1
1.2	Intercellular Signaling	3
1.2.1	Tools for Intercellular Signaling	3
1.2.2	Steps of Intercellular Signaling	5
1.2.3	Regulation of Intercellular Signaling	7
1.3	Hormones in Intercellular Signaling	8
1.3.1	Chemical Nature of Hormones	8
1.3.2	Hormone Analogs: Agonists and Antagonists	12
1.3.3	Endocrine, Paracrine and Autocrine Signaling	13
1.3.4	Direct Protein Modification by Signaling Molecules	15
1.4	Intracellular Signaling: Basics	15
1.4.1	Reception of External Signals	15
1.4.2	Activation and Deactivation of Signaling Proteins	16
1.4.3	Processing of Multiple Signals	17
1.4.4	Variability of Signaling Proteins	17
1.5	Molecular Tools for Intracellular Signaling	18
1.5.1	Receptors	18
1.5.2	Signaling Enzymes	21
1.5.3	Adaptors and Scaffolding Proteins	22
1.5.4	Diffusible Intracellular Messengers: Second Messengers	22
1.6	Basic Mechanisms of Intracellular Signaling	24
1.6.1	Regulatory Modifications	24
1.6.2	Recognition of Protein Modifications by Modification-specific Protein Modules	25
1.6.3	Multisite Protein Modification	26
1.6.4	Protein Interaction Domains	28
1.7	Modular Structure of Signaling Proteins and Signaling Complexes	32
1.7.1	Modules in Signaling Proteins	32
1.7.2	Modular Signaling Complexes	33

1.8	Organization of Signaling	34
1.8.1	Linear Signaling Pathways, Branching and Crosstalk	35
1.8.1.1	Linear Pathways	35
1.8.1.2	Branching and Crosstalk	37
1.8.1.3	Interactions between Signaling Paths	37
1.8.2	Signaling Networks	38
1.8.2.1	Complexity of Signaling Networks	39
1.8.2.2	Properties of Signaling Networks	39
1.8.2.3	Nodes and Junctions	41
1.8.2.4	Feedback Loops	42
1.8.3	Redundancy and Specificity of Signaling	46
1.8.4	Regulation of Signaling Pathways	47
1.8.5	Spatial Organization of Signaling Pathways	49
1.8.6	Compartmentalization and Transport	50
1.8.7	Evolution of Signaling Pathways	51
1.9	Variability and Cell-type Specificity of Signaling	51
1.10	References	53
2	Regulation of Enzyme Activity	55
2.1	Basis of Enzyme Catalysis	56
2.2	Basics of Allosteric Regulation	58
2.3	Regulation of Enzymes by Effector Molecules	60
2.4	Regulation of Enzyme Activity by Phosphorylation	62
2.5	"Ubiquitin (Ub)-Proteasome" Pathway	64
2.5.1	Components of the Ub System	65
2.5.2	Ub and Ub-like Proteins	66
2.5.3	Activation and Transacylation of Ub: E1 and E2 Enzymes	67
2.5.4	Transfer to the Target Protein with the Participation of E3	69
2.5.5	HECT Domain E3 Enzymes	71
2.5.6	RING Domain E3 Enzymes	72
2.5.6.1	N-end Rule Enzymes	73
2.5.6.2	Cbl Protein	73
2.5.6.3	Cullin-based E3 Enzymes, SCF and APC	74
2.5.6.4	Processing of Nuclear Factor (NF) κ B and Degradation of I κ B	76
2.5.7	Degradation in the Proteasome	78
2.5.8	Other Regulatory Functions of Ub Conjugation	80
2.5.9	Regulation of Proteins by Sumoylation	82
2.6	Lipidation of Signaling Proteins	83
2.6.1	Myristoylation	84
2.6.2	Palmitoylation	85
2.6.3	Farnesylation and Geranylation	86
2.6.4	Dual Lipidation	87

2.6.5	Cholesterol Membrane Anchor	88
2.6.6	Switch Function of Lipid Anchors	88
2.6.7	Glycosylphosphatidylinositol (GPI) Anchor	90
2.7	References	91
3	Regulation of Gene Expression	93
3.1	Basic Steps of Gene Expression	93
3.2	Components of the Eukaryotic Transcription Machinery	95
3.2.1	Basic Features of Eukaryotic Transcription	96
3.2.2	Elementary Steps of Eukaryotic Transcription	98
3.2.3	Eukaryotic RNA Polymerases	98
3.2.4	Structure of the Transcription Start Site and Regulatory Sequences	100
3.2.5	General Transcription Factors and the Core Transcription Apparatus	101
3.2.6	Holoenzyme Forms of RNA Pol II	103
3.2.7	Phosphorylation of RNA Pol II and the Onset of Transcription	104
3.2.8	TFIIH – A Pivotal Regulatory Protein Complex	106
3.2.9	Mediator Complex	107
3.3	Principles of Transcription Regulation	109
3.3.1	Elements of Transcription Regulation	109
3.3.2	Regulation of Eukaryotic Transcription by Sequence-specific DNA-binding Proteins	111
3.3.3	DNA Binding of Transcriptional Regulators	112
3.3.4	Structure of the Recognition Sequence and Quaternary Structure of DNA-binding Proteins	113
3.3.5	Communication with the Transcription Apparatus: Transactivation Domains	115
3.3.6	Families of Sequence-specific Transcription Factors and Homo- or Heterodimerization	116
3.4	Control of Transcription Factors	118
3.4.1	Mechanisms for the Control of Regulatory DNA-binding Proteins	118
3.4.2	Changes in the Concentration of Regulatory DNA-binding Proteins	119
3.4.3	Regulation by Binding of Effector Molecules	119
3.4.4	Posttranslational Modification of Transcription Regulators	120
3.4.5	Regulation by Phosphorylation	121
3.4.6	Regulation by Methylation, Acetylation and Redox Modification	125

3.4.7	Transcriptional Regulation in the Framework of Signal Transduction Networks	125
3.4.7.1	Constitutively Active Transcription Factors	126
3.4.7.2	Regulatory Transcription Factors	126
3.4.8	Repression of Transcription	128
3.4.9	Coregulators of Transcription	130
3.5	Chromatin Structure and Transcription Regulation	131
3.5.1	Nucleosome and Chromatin Structure	132
3.5.2	Histone Acetylation and Deacetylation	134
3.5.3	Histone Methylation	136
3.5.3.1	Histone Arginine Methylation	136
3.5.3.2	Arginine Demethylation/Citrullination	138
3.5.3.3	Histone Lysine Methylation	138
3.5.3.4	Histone Lysine Demethylation	139
3.5.4	Histone Phosphorylation	140
3.5.5	Histone Ubiquitination and Sumoylation	141
3.5.6	Histone Modification "Code"	141
3.5.7	Recognition of Histone Modifications by Protein Domains	143
3.5.8	DNA Methylation	144
3.5.9	Summary of the Regulatory Steps in Transcription	148
3.6	Posttranscriptional Regulation of Gene Expression	150
3.6.1	Control at the Level of Pre-mRNA Processing	151
3.6.2	Stability of mRNA	155
3.6.3	Regulation at the Level of Translation	157
3.6.3.1	General Mechanisms of Translational Control	157
3.6.3.2	mRNA-specific Regulation by 5' Sequences: Control of Ferritin mRNA Translation by Iron	159
3.6.3.3	mRNA-specific Translational Regulation by Protein Binding to 3'-Untranslated Regions	160
3.6.3.4	Global Translational Regulation of mRNAs by Targeting eIF-4E	162
3.6.3.5	Regulation of Translation via eIF-2	164
3.7	Regulation by RNA Interference (RNAi)	167
3.7.1	Small Regulatory RNAs	170
3.7.2	Incorporation of Small RNAs into RISC	172
3.7.3	Cleaving the Target RNA	173
3.7.4	Specificity and Target Selection in RNAi	175
3.7.5	Biological Functions of RNAi	176
3.7.5.1	Functions and Applications of siRNAs	176
3.7.5.2	Functions of miRNAs	176
3.7.5.3	RISCs and Chromatin Structure	177
3.8	References	179

4	Signaling by Nuclear Receptors	181
4.1	Ligands of Nuclear Receptors	181
4.2	Principles of Signaling by Nuclear Receptors	184
4.3	Classification and Structure of Nuclear Receptors	186
4.3.1	DNA-binding Elements of Nuclear Receptors: HREs	187
4.3.2	The DNA-binding Domain of Nuclear Receptors	189
4.3.3	HRE Recognition and Structure of the HRE-Receptor Complex	190
4.3.4	Ligand-binding Domain (LBD)	191
4.3.5	Transactivating Elements of the Nuclear Receptors	195
4.4	Mechanisms of Transcriptional Regulation by Nuclear Receptors	196
4.4.1	Steroid Receptor Coactivator (SRC)-1/p160 and Thyroid Hormone Receptor-activating Protein (TRAP) Coactivators	198
4.4.2	Corepressors of Nuclear Receptors	200
4.5	Regulation of Signaling by Nuclear Receptors	200
4.6	The Signaling Pathway of the Steroid Hormone Receptors	203
4.7	Signaling by Retinoids, Vitamin D ₃ and the T ₃ Hormone	205
4.8	Nongenomic Functions of Nuclear Receptors and their Ligands	207
4.9	References	211
5	G-protein-coupled Signal Transmission Pathways	213
5.1	Transmembrane (TM) Receptors: General Structure and Classification	213
5.2	Structural Principles of TM Receptors	215
5.2.1	The Extracellular Domain of TM Receptors	217
5.2.2	TM Domain	217
5.2.3	Intracellular Domain of Membrane Receptors	219
5.2.4	Regulation of Receptor Activity	221
5.3	GPCRs	222
5.3.1	Classification of GPCRs	223
5.3.2	Structure of GPCRs	225
5.3.3	Ligand Binding and Mechanism of Signal Transmission	227
5.3.4	Regulation of GPCRs	228
5.3.5	Switching Off and Desensitization of 7TM Receptors	229
5.3.6	Dimerization of GPCRs	233
5.4	Regulatory GTPases	233
5.4.1	The GTPase Superfamily: General Functions and Mechanism	234
5.4.2	Inhibition of GTPases by GTP Analogs	236

5.4.3	The G-domain as a Common Structural Element of the GTPases	237
5.4.4	GTPase Families	238
5.5	Heterotrimeric G-proteins	239
5.5.1	Classification of the Heterotrimeric G-proteins	240
5.5.2	Toxins as Tools in the Characterization of Heterotrimeric G-proteins	243
5.5.3	Functional Cycle of Heterotrimeric G-proteins	244
5.5.4	Structural and Mechanistic Aspects of the Switch Function of G-proteins	247
5.5.5	Structure and Function of the $\beta\gamma$ -Complex	253
5.5.6	Membrane Association of the G-proteins	255
5.5.7	Regulators of G-proteins: Phosducin and RGS Proteins	256
5.6	Receptor-independent Functions of Heterotrimeric G-proteins	259
5.7	Effector Molecules of G-proteins	259
5.7.1	Adenylyl Cyclase and cAMP as "Second Messenger"	260
5.7.2	PLC	264
5.8	GPCR Signaling via Arrestin	268
5.9	References	269
6	Intracellular Messenger Substances: "Second Messengers"	271
6.1	General Properties of Intracellular Messenger Substances	272
6.2	cAMP	273
6.3	cGMP and Guanylyl Cyclases	275
6.3.1	Guanylyl Cyclases	276
6.3.2	Targets of cGMP	277
6.4	Metabolism of Inositol Phospholipids and Inositol Phosphates	278
6.5	Storage and Release of Ca^{2+}	282
6.5.1	Release of Ca^{2+} from Ca^{2+} Storage	282
6.5.2	Influx of Ca^{2+} from the Extracellular Region	288
6.5.3	Removal and Storage of Ca^{2+}	288
6.5.4	Temporal and Spatial Changes in Ca^{2+} Concentration	289
6.6	Functions of Phosphoinositides	290
6.6.1	Messenger Function of $\text{PtdIns}(3,4,5)\text{P}_3$	291
6.6.2	Functions of $\text{PtdIns}(4,5)\text{P}_2$ and other Phosphoinositides	291
6.7	Ca^{2+} as a Signal Molecule	292
6.7.1	Calmodulin as a Ca^{2+} Sensor	295
6.7.2	Target Proteins of Ca^{2+} /Calmodulin	297
6.7.3	Other Ca^{2+} Sensors	298
6.8	DAG as a Signal Molecule	299
6.9	Other Lipid Messengers	300

6.10	NO Signaling Molecule	301
6.10.1	Reactivity of NO	302
6.10.2	Synthesis of NO	303
6.10.3	Physiological Functions of Nitrosylation	305
6.10.3.1	Nitrosylation of Metal Centers	305
6.10.3.2	Regulatory Functions of S-Nitrosylation	307
6.10.3.3	Toxic Action of NO and Nitrosative Stress	309
6.11	References	310
7	Ser/Thr-specific Protein Kinases and Protein Phosphatases	313
7.1	Classification, Structure and Characteristics of Protein Kinases	313
7.1.1	General Classification and Function of Protein Kinases	313
7.1.2	Classification of Ser/Thr-specific Protein Kinases	315
7.2	Structure and Regulation of Protein Kinases	316
7.2.1	Main Structural Elements of Protein Kinases	318
7.2.2	Substrate Binding and Recognition	321
7.2.3	Control of Protein Kinase Activity	322
7.2.4	Regulation of Protein Phosphorylation by Subcellular Localization and Specific Targeting Subunits	325
7.3	PKA	327
7.3.1	Structure and Substrate Specificity of PKA	327
7.3.2	Regulation of PKA	329
7.3.3	AKAPs	330
7.4	Phosphatidylinositol-3-kinase (PI3K)/Akt Pathway	332
7.4.1	PI3K	332
7.4.2	PKB/Akt Kinase	335
7.5	PKC	338
7.5.1	Classification and Structure	338
7.5.2	Activation of PKC by Cofactors	341
7.5.3	Regulation of PKC	341
7.5.4	Receptors for PKC and RACK (Receptors for Activated PKC) Proteins	344
7.5.5	Functions and Substrates of PKC	345
7.6	Ca ²⁺ /Calmodulin-dependent Protein Kinases (CaMKs)	346
7.6.1	Importance and General Function	346
7.6.2	CaMK II	347
7.7	Ser/Thr-specific Protein Phosphatases	351
7.7.1	Structure and Classification of Ser/Thr Protein Phosphatases	351
7.7.2	Regulation of Ser/Thr Protein Phosphatases	352
7.7.3	PP1	353
7.7.4	PP2A	354
7.7.5	PP2B (Calcineurin)	356
7.8	References	359

8	Signal Transmission via Transmembrane Receptors with Tyrosine-Specific Protein Kinase Activity	361
8.1	Structure and Function of RTKs	363
8.1.1	General Structure and Classification	363
8.1.2	Ligand Binding and Receptor Dimerization	365
8.1.3	Structure and Activation of the Tyrosine Kinase Domain	369
8.1.4	Effector Proteins of the RTKs	373
8.1.5	Attenuation and Termination of RTK Signaling	376
8.2	Protein Modules in Downstream Signaling of RTKs	379
8.2.1	Domains with Binding Specificity for Phosphotyrosine: SH2, PTB and C2 Domains	381
8.2.2	SH3 Domains	384
8.2.3	Membrane-targeting Domains: Pleckstrin Homology (PH) Domains and FYVE Domains	385
8.2.4	Phosphoserine/Threonine-binding Domains	386
8.2.5	PDZ Domains	387
8.3	Non-RTK-specific Protein Kinases	388
8.3.1	Structure and General Function of Non-RTKs	389
8.3.2	Src Tyrosine Kinase	390
8.3.3	Abl Tyrosine Kinase	392
8.4	PTPs	395
8.4.1	Structure and Classification of PTPs	396
8.4.2	Cooperation of PTPs and Protein Tyrosine Kinases	399
8.4.3	Regulation of PTPs	401
8.5	Adaptor Molecules of RTKs	404
8.6	References	408
9	Signal Transmission via Ras Proteins	411
9.1	Ras Superfamily of Monomeric GTPases	411
9.2	GAPs of the Monomeric GTPases	413
9.3	GEFs of the Monomeric GTPases	414
9.4	Inhibitors of G-nucleotide dissociation GDIs	416
9.5	Ras Family of Monomeric GTPases	417
9.5.1	General Properties of the Ras Protein	418
9.5.2	Structure of the GTP- and GDP-bound Forms of Ras Protein	419
9.5.3	GTP Hydrolysis Mechanism and Stimulation by GAP Proteins	420
9.5.4	Structure and Biochemical Properties of Transforming Mutants of Ras Protein	422
9.5.5	Membrane Localization of Ras Protein	423
9.5.6	GAPs in Ras Signal Transduction	424
9.5.7	GEFs in Ras Signal Transduction	424

- 9.6 Raf Kinase as an Effector of Signal Transduction by Ras Proteins 427
 - 9.6.1 Structure of Raf Kinase 427
 - 9.6.2 Mechanism of Activation and Regulation of Raf Kinase 428
 - 9.6.3 Oncogenic Activation of Raf 430
- 9.7 Further Ras Family Members: R-Ras, Ral and Rap 430
- 9.8 Reception and Transmission of Multiple Signals by Ras Protein 431
 - 9.8.1 Multiple Input Signals of Ras Protein 431
 - 9.8.2 Multiple Effector Molecules of Ras Proteins 432
- 9.9 Further Branches of the Ras Superfamily 434
 - 9.9.1 Rho/Rac Family 434
 - 9.9.2 Rab Family 435
 - 9.9.3 Ran Family 435
 - 9.9.4 Arf Family 436
- 9.10 Ras Protein Network and Crosstalk within the Ras Superfamily 436
- 9.11 References 439
- 10 Intracellular Signal Transduction: Protein Cascades of the Mitogen-activated Protein Kinase Pathways 441**
 - 10.1 Organization and Components of MAPK Pathways 443
 - 10.2 Regulation of MAPK Pathways by Protein Phosphatases and Inhibitory Proteins 446
 - 10.3 Specificity in MAPK Activation and Organization in Multiprotein Complexes 447
 - 10.4 Major MAPK Pathways of Mammals 450
 - 10.4.1 ERK Pathway 450
 - 10.4.2 JNK and p38 MAPK Pathways 451
 - 10.5 References 456
- 11 Membrane Receptors with Associated Tyrosine Kinase Activity 457**
 - 11.1 Cytokines and Cytokine Receptors 457
 - 11.2 Structure and Activation of Cytokine Receptors 458
 - 11.2.1 Activation of Cytoplasmic Tyrosine Kinases 465
 - 11.2.2 The JAK-STAT Pathway 468
 - 11.2.2.1 JAKs 468
 - 11.2.2.2 STAT Proteins 469
 - 11.2.3 Regulation of Cytokine Receptor Signaling 472
 - 11.3 T and B Cell Receptors (TCRs and BCRs) 474
 - 11.3.1 Receptor Structure 474
 - 11.3.2 Intracellular Signal Molecules of the TCRs and BCRs 476
 - 11.4 Signal Transduction via Integrins 478
 - 11.5 References 482

12	Other Transmembrane Receptor Classes	483
12.1	Receptors with Intrinsic Ser/Thr Kinase Activity: TGF- β Receptor and SMAD Protein Signaling	484
12.1.1	Family of TGF- β Cytokines	484
12.1.2	TGF- β Receptor	485
12.1.3	SMAD Proteins	486
12.2	Receptor Regulation by Intramembrane Proteolysis: Notch Receptor	491
12.3	References	494
13	Regulation of the Cell Cycle	495
13.1	Principles of Cell Cycle Control	496
13.2	Key Elements of the Cell Cycle Apparatus	499
13.2.1	CDKs	499
13.2.2	Cyclins	502
13.2.3	CKIs	505
13.3	CDK-Cyclin Complexes	507
13.3.1	Structure of CDKs and CDK-Cyclin complexes	508
13.3.2	Regulation of CDKs by Phosphorylation	509
13.3.3	Inhibition by CKIs	511
13.3.4	Substrates of CDKs	512
13.3.5	Multiple Regulation of CDKs	514
13.4	Regulation of the Cell Cycle by Proteolysis	515
13.4.1	Proteolysis mediated by the SCF Complex	516
13.4.2	Proteolysis mediated by the APC	517
13.5	G ₁ Progression and S-phase Entry	518
13.5.1	CDK4/6 and the D-type Cyclins	519
13.5.2	Central Function of CDK2-Cyclin E in S-phase Entry	521
13.5.3	Function of Rb in the Cell Cycle	522
13.5.4	E2F Transcription Factors and their Control by Rb	523
13.5.5	Negative Regulation of the G ₁ /S Transition	526
13.6	Cell Cycle Control of DNA Replication	527
13.7	The G ₂ /M Transition and CDC25C Phosphatase	529
13.8	Progression through the M phase: APC and the Metaphase-Anaphase Transition	531
13.9	Summary of Cell Cycle Progression	533
13.10	DNA Damage and DNA Replication Checkpoints	536
13.10.1	Components and Organization of DNA Damage Checkpoints	537
13.10.2	Mammalian G ₁ DNA Damage Checkpoint	539
13.10.3	S-phase Checkpoint Pathways	540
13.10.4	G ₂ /M Checkpoint	540
13.11	References	541

14	Malfunction of Signaling Pathways and Tumorigenesis: Oncogenes and Tumor Suppressor Genes	543
14.1	General Aspects of Tumor Formation	543
14.1.1	Characteristics of Tumor Cells	543
14.1.2	Genetic Changes in Tumor Cells	545
14.1.3	Epigenetic Changes in Tumor Cells	546
14.2	Signaling Proteins Mutated in Cancer: Oncogenes	548
14.2.1	Mechanisms of Oncogene Activation	549
14.2.2	Examples of the Functions of Oncogenes	552
14.3	Tumor Suppressor Genes: General Functions	558
14.4	Rb in Cancer	559
14.5	p16 ^{INK4a} Gene Locus and ARF	564
14.6	Tumor Suppressor Protein p53	565
14.6.1	Structure and Biochemical Properties of p53	565
14.6.2	Structure of p53 and its DNA Complex	567
14.6.3	Posttranslational Modification of p53	567
14.6.4	Genes Regulated by p53	570
14.6.5	Regulation of p53 by Ubiquitination and Proteasomal Degradation: The MDM2 Protein	573
14.6.6	Pathways Involved in Activation of p53	575
14.6.7	The MDM2-p53 Network and Cancer	578
14.7	Wnt/ β -Catenin Signaling and the Tumor Suppressor APC	578
14.8	Genomic instability and Tumor Formation: Roles of DNA Repair and DNA Damage Checkpoints	580
14.9	Common Physiologic Changes in Tumor Cells: Hallmarks of Cancer	582
14.9.1	Self-sufficiency in Growth Signals	583
14.9.2	Insensitivity to Antigrowth Signals	584
14.9.3	Evasion of Programmed Cell Death (Apoptosis)	585
14.9.4	Limitless Replicative Potential of Cancer Cells	586
14.10	References	587
15	Apoptosis	589
15.1	Basic Functions of Apoptosis	589
15.2	Overview of Apoptotic Pathways	591
15.3	Caspases: Death by Proteolysis	592
15.3.1	Initiator and Effector Caspases	593
15.3.2	Mechanism of Caspases	594
15.3.3	Caspase Activation and Regulation	596
15.4	Bcl-2 Proteins Family: Gatekeepers of Apoptosis	599
15.5	The Mitochondrial Pathway of Apoptosis	602

15.6	Death Receptor-triggered Apoptosis	606
15.6.1	Fas/CD95 Signaling Pathway	607
15.6.2	TNFR1 and Apoptosis	609
15.7	Links of Apoptosis to Cellular Signaling Pathways	610
15.7.1	Phosphatidylinositol-3-kinase (PI3K)/Akt Kinase and Apoptosis	611
15.7.2	p53 and Apoptosis	612
15.8	References	615

Subject Index	617
---------------	-----