

# Detailed Contents

## Chapter 1

### Introduction to Cell Signaling

#### WHAT IS CELL SIGNALING?

- All cells have the ability to respond to their environment 2
- Cells must perceive and respond to a wide range of signals 3
- Signaling systems need to solve a number of common problems 4

#### THE FUNDAMENTAL ROLE OF SIGNALING IN BIOLOGICAL PROCESSES

- Work in many different fields converged to reveal the underlying mechanisms of signaling 6
- Despite the diversity of signaling pathways and mechanisms, fundamental commonalities have emerged 7
- Signaling must operate at multiple scales in space and time 9

#### THE MOLECULAR CURRENCIES OF INFORMATION PROCESSING

- Information is transferred by changes in the state of proteins 11
- There is a limited number of ways in which the state of proteins can change 12
- Most changes in state involve simultaneous changes in several different currencies 14

#### LINKING SIGNALING NODES INTO PATHWAYS AND NETWORKS

- Information transfer involves linking different changes of state together 15
- Multiple state changes are linked together to generate pathways and networks 16
- Cellular information-processing systems have a hierarchical architecture 17
- Summary 18
- Questions 18
- References 19

## Chapter 2

### Principles and Mechanisms of Protein Interactions

#### PROPERTIES OF PROTEIN-PROTEIN INTERACTIONS

- Changes in protein binding have both direct and indirect functional consequences 22
- Protein binding can be mediated by broad interaction surfaces or by short, linear peptides 23

- The affinity and specificity of an interaction determine how likely it is to occur in the cell 24
- The strength of a binding interaction is defined by the dissociation constant ( $K_d$ ) 25
- The dissociation constant is related to the binding energy of the interaction 27
- The dissociation constant is also related to rates of binding and dissociation 28

#### PROTEIN INTERACTIONS IN THEIR CELLULAR AND MOLECULAR CONTEXT

- The apparent dissociation constant can be strongly affected by the local cellular environment and other binding partners 30
- Ideal affinity and specificity depends on biological function and ligand concentrations 31
- There are functional constraints on interaction affinities and specificities 32
- Interaction affinity and specificity can be independently modulated 34
- Cooperativity involves the coupled binding of multiple ligands 35
- Diverse molecular mechanisms underlie cooperativity 36
- Cooperative binding has a variety of functional consequences 36
- Protein assemblies differ in their stability and homogeneity 37
- Summary 38
- Questions 38
- References 40

## Chapter 3

### Signaling Enzymes and Their Allosteric Regulation

#### PRINCIPLES OF ENZYME CATALYSIS

- Enzymes have a number of properties that make them useful for transmitting signals in the cell 44
- Enzymes use a variety of mechanisms to enhance the rate of chemical reactions 45
- Enzymes can drive reactions in one direction by energetic coupling 46
- ALLOSTERIC CONFORMATIONAL CHANGES 47
- Conformational flexibility of proteins enables allosteric control 47

|   |    |   |           |
|---|----|---|-----------|
| Signaling proteins employ diverse classes of conformational rearrangements  | 48 | Questions   | 80        |
| PROTEIN PHOSPHORYLATION AS A REGULATORY MECHANISM   | 48 | References  | 82        |
| Phosphorylation can act as a regulatory mark  | 49 | <b>Chapter 4</b>  |           |
| Phosphorylation can either disrupt or induce protein structure  | 49 | <b>Role of Post-Translational Modifications in Signaling</b>  | <b>85</b> |
| PROTEIN KINASES   | 50 | THE LOGIC OF POST-TRANSLATIONAL REGULATION  | 85        |
| The structure and catalytic mechanism of protein kinases are conserved  | 52 | Proteins can be covalently modified by the addition of simple functional groups                         | 86        |
| The activation loop and C-helix are conserved molecular levers that conformationally control kinase activity                                  | 54 | Proteins can also be covalently modified by the addition of sugars, lipids, and even proteins           | 87        |
| Insulin receptor kinase activity is controlled via activation-loop phosphorylation  | 54 | Post-translational modifications can alter protein structure, localization, and stability               | 88        |
| Phosphorylation mediates long-range conformational regulation of Src family kinases   | 54 | Post-translational control machinery often works as part of "writer/eraser/reader" systems              | 90        |
| Multiple binding interactions regulate protein kinase substrate specificity   | 55 | Post-translational modifications allow very rapid signaling and transmission of spatial information     | 92        |
| Protein kinases can be divided into nine families   | 56 | INTERPLAY BETWEEN POST-TRANSLATIONAL MODIFICATIONS  | 92        |
| PROTEIN PHOSPHATASES  | 58 | A post-translational modification can promote or antagonize other modifications                         | 93        |
| Serine/threonine phosphatases are metalloenzymes  | 60 | p53 is tightly regulated by a wide variety of post-translational modifications                          | 95        |
| Most tyrosine phosphatases utilize a catalytic cysteine residue   | 60 | The level and activity of p53 are regulated by ubiquitylation and acetylation                           | 96        |
| Tyrosine phosphatases are regulated by modular domains while serine/threonine phosphatases often associate with regulatory accessory subunits | 62 | Additional modifications further fine-tune p53 activity   | 96        |
| G PROTEIN SIGNALING   | 64 | PROTEIN PHOSPHORYLATION   | 97        |
| G proteins are conformational switches controlled by two opposing enzymes   | 65 | Phosphorylation is often coupled with protein interactions  | 97        |
| The presence of the GTP $\gamma$ -phosphate determines the structure of G protein switch I and II regions                                     | 65 | Kinases and phosphatases vary in their substrate specificity  | 99        |
| There are two major classes of signaling G proteins   | 66 | Multiple phosphorylation of proteins can arise by different mechanisms                                  | 100       |
| Subfamilies of small G proteins regulate diverse biological functions   | 67 | Histidine and other amino acids can be phosphorylated, especially in prokaryotes                        | 101       |
| Many upstream receptors feed into a small set of common heterotrimeric G proteins   | 67 | Two-component systems and histidine phosphorylation are also present in eukaryotes                      | 103       |
| REGULATORY ENZYMES FOR G PROTEIN SIGNALING  | 68 | ADDITION OF UBIQUITIN AND RELATED PROTEINS  | 104       |
| G-protein-coupled receptors act as GEFs for heterotrimeric G proteins   | 70 | Specialized enzymes mediate the addition and removal of ubiquitin                                       | 104       |
| Distinct GEF and GAP domains regulate specific small G protein families   | 71 | E3 ubiquitin ligases determine which proteins will be ubiquitylated                                     | 105       |
| GEFs catalyze GDP/GTP exchange by deforming the nucleotide-binding pocket   | 71 | Ubiquitin-binding domains read ubiquitin-mediated signals in diverse cellular activities                | 106       |
| GAPs order the catalytic machinery for hydrolysis   | 73 | HISTONE ACETYLATION AND METHYLATION   | 107       |
| Regulators of G protein signaling (RGS) proteins act as GAPs for heterotrimeric G proteins  | 74 | Chromatin structure is regulated by post-translational modification of histones and associated proteins | 108       |
| Additional mechanisms are used to fine-tune the activity of G proteins  | 75 | Two writer/eraser/reader systems are based on protein methylation and acetylation                       | 109       |
| SIGNALING ENZYME CASCADES   | 75 | Chromatin modification in transcription is dynamic and leads to highly cooperative interactions         | 110       |
| The three-tiered MAP kinase cascade forms a signaling module in all eukaryotes  | 76 | Summary   | 112       |
| Scaffold proteins often organize MAPK cascades  | 77 | Questions   | 113       |
| G protein activity can also be regulated by signaling cascades  | 79 | References  | 114       |
| Summary   | 80 |   |           |



|  |            |  |
|--|------------|--|
| <b>Chapter 5</b>   |            |  |
| <b>Subcellular Localization of Signaling Molecules</b>   | <b>115</b> |  |
| LOCALIZATION AS A SIGNALING CURRENCY   | 115        |  |
| Changes in subcellular localization can transmit information   | 116        |  |
| Subcellular localization can be regulated by a variety of mechanisms                                     | 117        |  |
| CONTROL OF NUCLEAR LOCALIZATION  | 117        |  |
| Short, modular peptide motifs direct nuclear import and export   | 118        |  |
| Nuclear transport is controlled by shuttle proteins and the G protein Ran                                | 118        |  |
| Phosphorylation of transcription factor Pho4 regulates nuclear import and export                         | 119        |  |
| Nuclear import of STATs is regulated by phosphorylation and conformational change                        | 120        |  |
| Localization of MAP kinases is regulated by association with nuclear and cytosolic binding partners      | 121        |  |
| Notch nuclear localization is regulated by proteolytic cleavage  | 122        |  |
| CONTROL OF MEMBRANE LOCALIZATION   | 122        |  |
| Proteins can span the membrane or be associated with it peripherally                                     | 122        |  |
| Proteins can be covalently modified with lipids after translation  | 123        |  |
| Modular lipid-binding domains are important for regulated association of proteins with membranes         | 124        |  |
| Some lipid-modified proteins can reversibly associate with membranes                                     | 125        |  |
| Coupling effector protein activation to membrane recruitment is a common theme in signaling              | 126        |  |
| Akt kinase is regulated by membrane recruitment and phosphorylation                                      | 126        |  |
| MODULATION OF SIGNALING BY MEMBRANE TRAFFICKING  | 127        |  |
| Proteins can be internalized by a variety of mechanisms  | 127        |  |
| Internalization of receptors can modulate signal transduction  | 128        |  |
| TGF $\beta$ signaling output depends on the mechanism of receptor internalization                        | 129        |  |
| Retrograde signaling allows effects distant from the site of ligand binding                              | 130        |  |
| Ras isoforms in distinct subcellular locations have different signaling outputs                          | 130        |  |
| Summary  | 132        |  |
| Questions  | 132        |  |
| References   | 132        |  |
| <b>Chapter 6</b>   |            |  |
| <b>Second Messengers: Small Signaling Mediators</b>  | <b>135</b> |  |
| PROPERTIES OF SMALL SIGNALING MEDIATORS  | 135        |  |
| Small signaling mediators are controlled by an interplay of their production and elimination             | 136        |  |
| Small signaling mediators exert their effects by binding downstream effectors                            | 136        |  |
| Small signaling mediators can lead to fast, distant, and amplified signal transmission                   | 137        |  |
| Small signaling mediators can generate complex temporal and spatial patterns                             | 138        |  |
| CLASSES OF SMALL SIGNALING MEDIATORS   | 139        |  |
| Small signaling mediators have a wide range of physical properties                                       | 140        |  |
| The cyclic nucleotides cAMP and cGMP are produced by cyclase enzymes and destroyed by phosphodiesterases | 140        |  |
| Cyclic nucleotides regulate diverse cellular activities  | 141        |  |
| The regulatory (R) subunit of protein kinase A is a conformational sensor of cAMP binding                | 142        |  |
| Some small signaling mediators are derived from membrane lipids  | 143        |  |
| PLC generates two signaling mediators, IP $_3$ and DAG   | 144        |  |
| Activation of protein kinase C is regulated by IP $_3$ and DAG   | 144        |  |
| CALCIUM SIGNALING  | 145        |  |
| Activation of Ca $^{2+}$ channels is a common means of regulation  | 146        |  |
| Ca $^{2+}$ influx is rapid and local   | 147        |  |
| Calmodulin is a conformational sensor of intracellular calcium levels                                    | 147        |  |
| Signaling can lead to propagating Ca $^{2+}$ waves   | 148        |  |
| SPECIFICITY AND REGULATION   | 149        |  |
| Scaffold proteins can increase input and output specificity of small-molecule signaling                  | 150        |  |
| AKAP scaffold proteins can also regulate dynamics of cAMP signaling                                      | 150        |  |
| Summary  | 152        |  |
| Questions  | 152        |  |
| References   | 153        |  |
| <b>Chapter 7</b>   |            |  |
| <b>Membranes, Lipids, and Enzymes That Modify Them</b>   | <b>155</b> |  |
| BIOLOGICAL MEMBRANES AND THEIR PROPERTIES  | 155        |  |
| Biological membranes consist of a variety of polar lipids  | 156        |  |
| Structural properties of membrane lipids favor the formation of bilayers                                 | 157        |  |
| The composition of the membrane determines its physical properties                                       | 158        |  |
| There are fundamental differences between biochemistry in solution and on the membrane                   | 160        |  |
| LIPID-MODIFYING ENZYMES USED IN SIGNALING  | 161        |  |
| Cleavage of membrane lipids by phospholipases generates a variety of bioactive products                  | 161        |  |
| A variety of lipid kinases and phosphatases are involved in signaling                                    | 163        |  |

## EXAMPLES OF MAJOR LIPID SIGNALING PATHWAYS

|  |     |
|--|-----|
| Phosphoinositides can serve as membrane binding sites and as a source of signaling mediators       | 164 |
| Phosphoinositide species provide a set of membrane binding signals                                 | 166 |
| Phospholipase D generates the important signaling mediator, phosphatidic acid (PA)                 | 168 |
| Phospholipase D plays a role in mTOR signaling   | 169 |
| The metabolism of sphingomyelin generates a host of signaling mediators                            | 170 |
| Phospholipase A <sub>2</sub> generates the precursor for a family of potent inflammatory mediators | 172 |
| Summary  | 174 |
| Questions  | 174 |
| References   | 174 |

## Chapter 8

### Information Transfer Across the Membrane 177

#### PRINCIPLES OF TRANSMEMBRANE SIGNALING 177

|   |     |
|---|-----|
| The cell must process and respond to a diversity of environmental cues        | 178 |
| Three general strategies are used to transfer information across the membrane | 179 |
| Many drugs target receptors   | 180 |

#### TRANSDUCTION STRATEGIES USED BY TRANSMEMBRANE RECEPTORS 180

|   |     |
|---|-----|
| Receptors with multiple membrane-spanning segments undergo conformational changes upon ligand binding | 180 |
| Receptors with a single membrane-spanning segment form higher-order assemblies upon ligand binding    | 181 |
| Receptor clustering confers advantages for signal propagation   | 182 |

#### G-PROTEIN-COUPLED RECEPTORS 184

|   |     |
|---|-----|
| G-protein-coupled receptors have intrinsic enzymatic activity                 | 184 |
| Signaling by GPCRs can be very fast and lead to enormous signal amplification | 186 |

#### TRANSMEMBRANE RECEPTORS ASSOCIATED WITH ENZYMATIC ACTIVITY 186

|   |     |
|---|-----|
| Receptor tyrosine kinases control important cell fate decisions in multicellular eukaryotes                   | 186 |
| TGF $\beta$ receptors are serine/threonine kinases that activate transcription factors                        | 186 |
| Some receptors have intrinsic protein phosphatase or guanylyl cyclase activity                                | 187 |
| Noncovalent coupling of receptors to protein kinases is a common signaling strategy                           | 189 |
| Some receptors use complex activation pathways that involve both kinase activation and proteolytic processing | 193 |
| Wnt and Hedgehog are two important signaling pathways in development  | 194 |
| A variety of receptors couple to proteolytic activities   | 197 |
| <b>GATED CHANNELS</b>   | 199 |
| Gated channels share a similar overall structure  | 199 |

|  |     |
|--|-----|
| The voltage-gated potassium channel provides clues to mechanisms of gating and ion specificity | 200 |
| Ligand-gated ion channels play a central role in neurotransmission                             | 202 |

#### MEMBRANE-PERMEABLE SIGNALING 204

|  |     |
|--|-----|
| Nitric oxide mediates short-range signaling in the vascular system | 204 |
| O <sub>2</sub> binding regulates the response to hypoxia           | 205 |
| The receptors for steroid hormones are transcription factors       | 206 |

#### DOWN-REGULATION OF RECEPTOR SIGNALING 208

|  |     |
|--|-----|
| Ubiquitylation regulates the endocytosis, recycling, and degradation of cell-surface receptors | 209 |
| G protein coupled receptors are desensitized by phosphorylation and adaptor binding            | 211 |
| Summary  | 213 |
| Questions  | 213 |
| References   | 215 |

## Chapter 9

### Regulated Protein Degradation 217

#### GENERAL PROPERTIES AND EXAMPLES OF SIGNAL-REGULATED PROTEOLYSIS 217

|  |     |
|--|-----|
| Proteases are a diverse group of enzymes   | 218 |
| Blood coagulation is regulated by a cascade of proteases   | 219 |
| Regulated proteolysis by metalloproteases can generate signaling molecules and alter the extracellular environment | 220 |
| ADAMs regulate signaling pathways by cleaving membrane-associated proteins   | 221 |
| MMPs participate in remodeling the extracellular environment   | 222 |
| Proteolysis activates the thrombin receptor  | 223 |
| Regulated intramembrane proteolysis (RIP) is an essential step in signaling by some receptors                      | 224 |

#### UBIQUITIN AND THE PROTEASOME DEGRADATION PATHWAY 225

|  |     |
|--|-----|
| The proteasome is a specialized molecular machine that degrades intracellular proteins | 225 |
| The cell cycle is controlled by two large ubiquitin-conjugating complexes              | 226 |
| SCF recognizes specific phosphorylated proteins, targeting them for destruction        | 227 |
| Two APC species act at distinct points in the cell cycle                               | 228 |
| NF- $\kappa$ B is controlled by regulated degradation of its inhibitor                 | 230 |

#### CASPASE-MEDIATED CELL DEATH PATHWAYS 232

|  |     |
|--|-----|
| Apoptosis is an orderly and highly regulated form of cell death        | 232 |
| The activity of caspases is tightly regulated                          | 233 |
| The extrinsic pathway links cell death receptors to caspase activation | 235 |
| Mitochondria orchestrate the intrinsic cell death pathway              | 238 |
| Summary  | 241 |



|  |            |   |            |
|--|------------|---|------------|
| Questions  | 241        | Certain plant protein kinases are regulated by modular light-gated domains          | 267        |
| References   | 242        | Regulation of the neutrophil NADPH oxidase by modular interactions                  | 268        |
| <b>Chapter 10</b>  |            | <b>CREATING NEW FUNCTIONS THROUGH DOMAIN RECOMBINATION</b>                          | 269        |
| <b>The Modular Architecture and Evolution of Signaling Proteins</b>  | <b>243</b> | Some modular domain rearrangements can lead to cancer                               | 269        |
| <b>MODULAR PROTEIN DOMAINS</b>   | 244        | Modules can be recombined experimentally to engineer new signaling behaviors        | 270        |
| Protein domains usually have a globular structure  | 244        | Summary   | 272        |
| Bioinformatic approaches can identify protein domains  | 244        | Questions   | 272        |
| Domains can be composed of several smaller repeats   | 245        | References  | 273        |
| Protein domains often act as recognition modules   | 246        |   |            |
| <b>INTERACTION DOMAINS THAT RECOGNIZE POST-TRANSLATIONAL MODIFICATIONS</b>                                       | 249        | <b>Chapter 11</b>   |            |
| SH2 domains bind phosphotyrosine-containing sites  | 249        | <b>Information Processing by Signaling Devices and Networks</b>                     | <b>275</b> |
| Some SH2 domains are elements of larger binding structures   | 252        | <b>SIGNALING SYSTEMS AS INFORMATION-PROCESSING DEVICES</b>                          | 276        |
| Several different types of interaction domains recognize phosphotyrosine   | 252        | Signaling devices can be considered as state machines                               | 276        |
| Multiple domains recognize motifs phosphorylated on serine/threonine   | 254        | Signaling devices are organized in a hierarchical fashion                           | 277        |
| 14-3-3 proteins recognize specific phosphoserine/phosphothreonine motifs   | 254        | Signaling devices face a variety of challenges in input detection                   | 278        |
| Interaction domains recognize acetylated and methylated sites  | 255        | Proteins can function as simple signaling devices                                   | 279        |
| Ubiquitylation regulates protein-protein interactions  | 256        | <b>INTEGRATING MULTIPLE SIGNALING INPUTS</b>  | 281        |
| <b>INTERACTION DOMAINS THAT RECOGNIZE UNMODIFIED PEPTIDE MOTIFS OR PROTEINS</b>                                  | 257        | Logic gates process information from multiple inputs                                | 281        |
| Proline-rich sequences are favorable recognition motifs  | 257        | Simple peptide motifs can integrate multiple post-translational modification inputs | 282        |
| SH3 domains bind proline-rich motifs   | 258        | Cyclin-dependent kinase is an allosteric signal-integrating device                  | 283        |
| PDZ domains recognize C-terminal peptide motifs  | 258        | Modular signaling proteins can integrate multiple inputs                            | 284        |
| Protein interaction domains can form dimers or oligomers   | 259        | Transcriptional promoters can integrate input from multiple signaling pathways      | 285        |
| <b>INTERACTION DOMAINS THAT RECOGNIZE PHOSPHOLIPIDS</b>  | 260        | <b>RESPONDING TO THE STRENGTH OR DURATION OF AN INPUT</b>                           | 286        |
| PH domains form a major class of phosphoinositide-binding domains  | 260        | Signaling systems can respond to signal amplitude in a graded or a digital manner   | 288        |
| FYVE domains are phospholipid-binding domains found in endocytic proteins  | 261        | An enzyme can behave as a switch through cooperativity                              | 289        |
| BAR domains bind and stabilize curved membranes  | 262        | Networks can also yield switchlike activation                                       | 290        |
| <b>CREATING COMPLEX FUNCTIONS BY COMBINING INTERACTION DOMAINS</b>   | 262        | Signaling systems can distinguish between transient and sustained input             | 292        |
| Recombination of domains occurs through evolution  | 262        | <b>MODIFYING THE STRENGTH OR DURATION OF OUTPUT</b>                                 | 294        |
| Combinations of interaction domains or motifs can be used as a scaffold for the assembly of signaling complexes  | 263        | Signaling pathways often amplify signals as they are transmitted                    | 294        |
| Scaffold proteins containing PDZ domains organize cell-cell signaling complexes such as the postsynaptic density | 264        | Negative feedback allows fine-tuning of output                                      | 294        |
| Proteins with multiple phosphotyrosine motifs function as dynamically regulated scaffolds                        | 265        | Adaptation allows cells to control output duration                                  | 296        |
| <b>RECOMBINING INTERACTION AND CATALYTIC DOMAINS TO BUILD COMPLEX ALLOSTERIC SWITCH PROTEINS</b>                 | 266        | Feedback can cause output levels to oscillate between two stable states             | 299        |
| Many signaling enzymes are allosteric switches   | 266        | Bistable responses also underlie more permanent outputs                             | 301        |
| 14-3-3 Protein regulates the Raf kinase by coordinately binding two phosphorylation sites                        | 267        | Summary   | 303        |
|  |            | Questions   | 303        |
|  |            | References  | 303        |

## Chapter 12

### How Cells Make Decisions

#### VERTEBRATE VISION

- Organ: vertebrate eye
- Cell: photoreceptor cell
- Molecular Network: visual transduction cascade
- How does the photoreceptor cell detect light and convert it to a biochemical signal?
- How is the photoreceptor cell able to detect low light, even a single photon?
- How can the response be so rapid?
- How does the photoreceptor cell reset itself to enable detection of further changes in light?

#### Summary

#### References

#### PDGF SIGNALING

- Tissue: process of wound healing
- Cell: fibroblast response to wounding & platelet activation
- Molecular Network: control of fibroblast proliferation
- How do fibroblasts detect the local occurrence of a wound?
- How are PDGF signals propagated within the cell to generate outputs such as cell proliferation?
- How is misactivation of the proliferation response prevented?
- How is the proliferative response terminated?

#### Summary

#### References

#### THE CELL CYCLE

- Cell: distinct phases of the cell cycle
- Molecular Network: cyclin-dependent kinase (Cdk) is a central switch whose activity is modulated by the different cyclins
- How are sharp and committed transitions between cell cycle phases achieved?
- How does the cell cycle ensure that each transition proceeds only under appropriate conditions?

#### Summary

#### References

#### T CELL SIGNALING

- Organism: launching the adaptive immune response
- Cell: engagement of T cell and antigen presenting cell
- Molecular Network: T cell receptor (TCR) signaling network
- How does the T cell receptor transmit signals after peptide/MHC recognition?
- How does the T cell prevent misactivation?
- How does the T cell launch a robust response when stimulated by as few as ten antigenic peptide complexes?
- How might the T cell discriminate between antigenic and non-antigenic peptides?

#### Summary

#### References

## Chapter 13

### 305 Methods for Studying Signaling Proteins and Networks

345

#### 307 BIOCHEMICAL AND BIOPHYSICAL ANALYSIS OF PROTEINS

345

- 309 Analytical methods can determine quantitative binding parameters
- 310 Michaelis-Menten analysis provides a way to measure the catalytic power of enzymes
- 311 Methods to determine and analyze protein conformation are central to the study of signaling
- 312 X-ray crystallography provides high-resolution protein structures
- 314 Nuclear magnetic resonance (NMR) can reveal the dynamic structure of small proteins
- 315 Electron microscopy can map the shape of very large protein complexes
- 316 Specialized spectroscopic methods can be used to study protein dynamics

#### 317 MAPPING PROTEIN INTERACTIONS AND LOCALIZATION

355

- 318 Interacting proteins can be identified by isolating protein complexes from cell extracts
- 319 Binding partners can be identified by screening large libraries of genes
- 320 Direct protein-protein interactions can be detected by solid-phase screening
- 321 Fluorescent protein tags are used to locate and track proteins in living cells
- 322 Protein-protein interactions can be visualized directly in living cells

#### 323 METHODS TO PERTURB CELL SIGNALING NETWORKS AND MONITOR CELLULAR RESPONSES

360

- 325 Genetic and pharmacological methods can be used to perturb networks
- 326 Chemical dimerizers and optogenetic proteins provide a dynamic way to artificially activate pathways
- 329 cDNA microarrays and high-throughput sequencing are used to monitor the transcriptional state of a cell
- 331 Modification-specific antibodies provide a method to track post-translational changes
- 333 Mass spectrometry is the workhorse for identification of proteins and their modifications
- 336 Live-cell time-lapse microscopy provides a way to track the dynamics of single-cell responses
- 338 Biosensors allow signaling activity to be monitored in living cells
- 339 Flow cytometry provides a method to analyze rapidly single-cell responses

#### 340 Questions

#### 342 References

#### 344 Glossary

375

#### 344 Index

385