

VOLUME FOUR HUNDRED AND SEVENTY

# METHODS IN ENZYMOMOLOGY

Guide to Yeast Genetics:  
Functional Genomics, Proteomics,  
and Other Systems Analysis,  
2nd Edition

EDITED BY

JONATHAN WEISSMAN

*Howard Hughes Medical Institute  
Department of Cellular and Molecular Pharmacology  
University of California, San Francisco, California, USA*

CHRISTINE GUTHRIE

*Department of Biochemistry and Biophysics  
University of California, San Francisco, California, USA*

GERALD R. FINK

*Whitehead Institute for Biomedical Research/MIT  
Nine Cambridge Center, Cambridge, Massachusetts, USA*

Universitäts- und Landes-  
bibliothek Darmstadt  
Bibliothek Biologie

Inv.-Nr. 16764  
.....



ELSEVIER

AMSTERDAM • BOSTON • HEIDELBERG • LONDON  
NEW YORK • OXFORD • PARIS • SAN DIEGO  
SAN FRANCISCO • SINGAPORE • SYDNEY • TOKYO

Academic Press is an imprint of Elsevier



# CONTENTS

|                          |       |
|--------------------------|-------|
| <i>Contributors</i>      | xv    |
| <i>Preface</i>           | xxiii |
| <i>Volumes in Series</i> | xxv   |

## **Section I. Functional Genomics 1**

### **1. Analysis of Gene Function Using DNA Microarrays 3**

Andrew P. Capaldi

|                                         |    |
|-----------------------------------------|----|
| 1. Introduction and Experimental Design | 4  |
| 2. Methods                              | 8  |
| References                              | 16 |

### **2. An Introduction to Microarray Data Analysis and Visualization 19**

Gregg B. Whitworth

|                                                                        |    |
|------------------------------------------------------------------------|----|
| 1. Introduction                                                        | 20 |
| 2. Experimental Design: Single-Sample Versus Competitive Hybridization | 23 |
| 3. Image Analysis                                                      | 26 |
| 4. Preprocessing                                                       | 29 |
| 5. Visualizing Data Using Cluster Analysis                             | 38 |
| 6. Assessing the Statistical Evidence for Differential Expression      | 41 |
| 7. Exploring Gene Sets                                                 | 42 |
| 8. Managing Data                                                       | 44 |
| Acknowledgments                                                        | 49 |
| References                                                             | 49 |

### **3. Genome-Wide Approaches to Monitor Pre-mRNA Splicing 51**

Maki Inada and Jeffrey A. Pleiss

|                               |    |
|-------------------------------|----|
| 1. Introduction               | 52 |
| 2. Microarray Design          | 53 |
| 3. Sample Preparation         | 56 |
| 4. Microarray Data Collection | 68 |
| 5. Microarray Data Analysis   | 69 |
| 6. Future Methodologies       | 72 |
| Acknowledgments               | 73 |
| References                    | 74 |

|                                                                                                                                |            |
|--------------------------------------------------------------------------------------------------------------------------------|------------|
| <b>4. ChIP-Seq: Using High-Throughput DNA Sequencing for Genome-Wide Identification of Transcription Factor Binding Sites</b>  | <b>77</b>  |
| Philippe Lefrançois, Wei Zheng, and Michael Snyder                                                                             |            |
| 1. Introduction                                                                                                                | 78         |
| 2. Protocols                                                                                                                   | 81         |
| 3. Sequencing Data Management                                                                                                  | 93         |
| 4. Genome Analysis Pipeline                                                                                                    | 94         |
| 5. Examining Data Quality and Parsing Barcoded Data                                                                            | 95         |
| 6. Visualization in Genome Browser                                                                                             | 95         |
| 7. Conclusion and Future Directions                                                                                            | 102        |
| Acknowledgments                                                                                                                | 102        |
| References                                                                                                                     | 102        |
| <b>5. Genome-Wide Mapping of Nucleosomes in Yeast</b>                                                                          | <b>105</b> |
| Oliver J. Rando                                                                                                                |            |
| 1. Introduction                                                                                                                | 105        |
| 2. Isolation of Mononucleosomal DNA                                                                                            | 107        |
| 3. Variation in Titration Level Used for Nucleosome Purification                                                               | 112        |
| 4. Labeling of Mononucleosomal DNA for Tiling Microarray Analysis                                                              | 113        |
| 5. Generation of Nucleosomal DNA Libraries for Deep Sequencing                                                                 | 115        |
| References                                                                                                                     | 117        |
| <b>6. Genome-Wide Translational Profiling by Ribosome Footprinting</b>                                                         | <b>119</b> |
| Nicholas T. Ingolia                                                                                                            |            |
| 1. Introduction                                                                                                                | 120        |
| 2. Ribosome Footprint Generation and Purification                                                                              | 121        |
| 3. Sequencing Library Preparation                                                                                              | 128        |
| 4. Data Analysis                                                                                                               | 133        |
| 5. Solutions and Common Procedures                                                                                             | 138        |
| Acknowledgments                                                                                                                | 140        |
| References                                                                                                                     | 140        |
| <b>Section II. Systematic Genetic Analysis</b>                                                                                 | <b>143</b> |
| <b>7. Synthetic Genetic Array (SGA) Analysis in <i>Saccharomyces cerevisiae</i> and <i>Schizosaccharomyces pombe</i></b>       | <b>145</b> |
| Anastasia Baryshnikova, Michael Costanzo, Scott Dixon, Franco J. Vizeacoumar, Chad L. Myers, Brenda Andrews, and Charles Boone |            |
| 1. Introduction                                                                                                                | 146        |
| 2. Methodology                                                                                                                 | 148        |
| 3. Media and Stock Solutions                                                                                                   | 168        |
| 4. Applications of SGA Methodology                                                                                             | 171        |
| References                                                                                                                     | 176        |

|                                                                                     |            |
|-------------------------------------------------------------------------------------|------------|
| <b>8. Making Temperature-Sensitive Mutants</b>                                      | <b>181</b> |
| Shay Ben-Aroya, Xuewen Pan, Jef D. Boeke, and Philip Hieter                         |            |
| 1. Introduction                                                                     | 182        |
| 2. Diploid Shuffle—Plasmid Method                                                   | 183        |
| 3. Diploid Shuffle—Chromosome Method                                                | 194        |
| 4. Perspectives                                                                     | 202        |
| References                                                                          | 203        |
| <br>                                                                                |            |
| <b>9. Quantitative Genetic Interaction Mapping Using the E-MAP Approach</b>         | <b>205</b> |
| Sean R. Collins, Assen Roguev, and Nevan J. Krogan                                  |            |
| 1. Introduction                                                                     | 206        |
| 2. Selection of Mutations for Genetic Analysis                                      | 209        |
| 3. Generation and Measurement of Double Mutant Strains                              | 211        |
| 4. Data Processing and Computation of Scores                                        | 221        |
| 5. Extraction of Biological Hypotheses                                              | 224        |
| 6. Perspective                                                                      | 229        |
| References                                                                          | 229        |
| <br>                                                                                |            |
| <b>10. Exploring Gene Function and Drug Action Using Chemogenomic Dosage Assays</b> | <b>233</b> |
| Elke Ericson, Shawn Hoon, Robert P. St. Onge, Guri Giaever, and Corey Nislow        |            |
| 1. Introduction                                                                     | 234        |
| 2. Methodology                                                                      | 237        |
| 3. Experimental Considerations                                                      | 252        |
| 4. Perspectives                                                                     | 253        |
| Acknowledgments                                                                     | 254        |
| References                                                                          | 254        |
| <br>                                                                                |            |
| <b>Section III. Proteomics</b>                                                      | <b>257</b> |
| <br>                                                                                |            |
| <b>11. Yeast Expression Proteomics by High-Resolution Mass Spectrometry</b>         | <b>259</b> |
| Tobias C. Walther, Jesper V. Olsen, and Matthias Mann                               |            |
| 1. Introduction                                                                     | 260        |
| 2. Background, Methods, and Applications                                            | 261        |
| 3. Protocols                                                                        | 272        |
| Acknowledgments                                                                     | 278        |
| References                                                                          | 278        |

|                                                                                                                                                                          |            |
|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------|
| <b>12. High-Quality Binary Interactome Mapping</b>                                                                                                                       | <b>281</b> |
| Matija Dreze, Dario Monachello, Claire Lurin, Michael E. Cusick,<br>David E. Hill, Marc Vidal, and Pascal Braun                                                          |            |
| 1. Introduction                                                                                                                                                          | 282        |
| 2. High-Quality Binary Interactome Mapping                                                                                                                               | 283        |
| 3. High-Throughput Y2H Pipeline                                                                                                                                          | 292        |
| 4. Validation Using Orthogonal Binary Interaction Assays                                                                                                                 | 309        |
| 5. Conclusion                                                                                                                                                            | 312        |
| Acknowledgments                                                                                                                                                          | 312        |
| References                                                                                                                                                               | 313        |
| <b>13. Quantitative Analysis of Protein Phosphorylation on a<br/>System-Wide Scale by Mass Spectrometry-Based<br/>Proteomics</b>                                         | <b>317</b> |
| Bernd Bodenmiller and Ruedi Aebersold                                                                                                                                    |            |
| 1. Introduction                                                                                                                                                          | 318        |
| 2. Protocols                                                                                                                                                             | 319        |
| Acknowledgments                                                                                                                                                          | 331        |
| References                                                                                                                                                               | 332        |
| <b>14. A Toolkit of Protein-Fragment Complementation Assays<br/>for Studying and Dissecting Large-Scale and Dynamic<br/>Protein-Protein Interactions in Living Cells</b> | <b>335</b> |
| Stephen W. Michnick, Po Hien Ear, Christian Landry,<br>Mohan K. Malleshaiah, and Vincent Messier                                                                         |            |
| 1. Introduction                                                                                                                                                          | 336        |
| 2. General Considerations in Using PCA                                                                                                                                   | 337        |
| 3. DHFR PCA Survival-Selection for Large-Scale Analysis of PPIs                                                                                                          | 339        |
| 4. A Life and Death Selection PCA Based on the<br>Prodrug-Converting Cytosine Deaminase for Dissection of PPIs                                                           | 348        |
| 5. Visualizing the Localization of PPIs with GFP Family<br>Fluorescent Protein PCAs                                                                                      | 356        |
| 6. Studying Dynamics of PPIs with Luciferase Reporter PCAs                                                                                                               | 361        |
| References                                                                                                                                                               | 366        |
| <b>15. Yeast Lipid Analysis and Quantification by Mass Spectrometry</b>                                                                                                  | <b>369</b> |
| Xue Li Guan, Isabelle Riezman, Markus R. Wenk, and Howard Riezman                                                                                                        |            |
| 1. Introduction                                                                                                                                                          | 370        |
| 2. Methods                                                                                                                                                               | 373        |
| Acknowledgments                                                                                                                                                          | 389        |
| References                                                                                                                                                               | 389        |

|                                                                                    |                |
|------------------------------------------------------------------------------------|----------------|
| <b>16. Mass Spectrometry-Based Metabolomics of Yeast</b>                           | <b>393</b>     |
| Christopher A. Crutchfield, Wenyun Lu, Eugene Melamud,<br>and Joshua D. Rabinowitz |                |
| 1. Introduction                                                                    | 394            |
| 2. LC-MS Basics                                                                    | 395            |
| 3. Experimental Design                                                             | 395            |
| 4. Strains                                                                         | 398            |
| 5. Culture Conditions                                                              | 398            |
| 6. Metabolite Extraction                                                           | 402            |
| 7. Chemical Derivatization of Metabolites                                          | 405            |
| 8. LC-MS for Mixture Analysis                                                      | 406            |
| 9. Liquid Chromatography                                                           | 407            |
| 10. Electrospray Ionization                                                        | 410            |
| 11. Mass Spectrometry                                                              | 411            |
| 12. Targeted Data Analysis                                                         | 415            |
| 13. Untargeted Data Analysis                                                       | 418            |
| 14. Future Outlook                                                                 | 421            |
| References                                                                         | 422            |
| <br><b>Section IV. Systems Analysis</b>                                            | <br><b>427</b> |
| <b>17. Imaging Single mRNA Molecules in Yeast</b>                                  | <b>429</b>     |
| Hyun Youk, Arjun Raj, and Alexander van Oudenaarden                                |                |
| 1. Introduction                                                                    | 429            |
| 2. RNA FISH Protocol                                                               | 432            |
| 3. Example: <i>STL1</i> mRNA Detection in Response to NaCl Shock                   | 444            |
| 4. Conclusions                                                                     | 445            |
| Acknowledgments                                                                    | 445            |
| References                                                                         | 445            |
| <br><b>18. Reconstructing Gene Histories in <i>Ascomycota</i> Fungi</b>            | <br><b>447</b> |
| Ilan Wapinski and Aviv Regev                                                       |                |
| 1. Introduction                                                                    | 448            |
| 2. Synergy                                                                         | 451            |
| 3. Evaluating Orthogroup Quality                                                   | 459            |
| 4. Biological Analysis of Gene Histories                                           | 466            |
| 5. Analysis of Paralogous Genes                                                    | 478            |
| 6. Discussion and General Applicability                                            | 480            |
| References                                                                         | 482            |

|                                                                                                    |            |
|----------------------------------------------------------------------------------------------------|------------|
| <b>19. Experimental Evolution in Yeast: A Practical Guide</b>                                      | <b>487</b> |
| Maitreya J. Dunham                                                                                 |            |
| 1. Introduction                                                                                    | 488        |
| 2. Experiment Rationale                                                                            | 488        |
| 3. Experimental Evolution Approaches                                                               | 490        |
| 4. Experimental Design                                                                             | 493        |
| 5. Practical Considerations                                                                        | 496        |
| 6. Analysis Techniques                                                                             | 500        |
| 7. Example Protocol                                                                                | 502        |
| 8. Conclusions                                                                                     | 504        |
| Acknowledgments                                                                                    | 505        |
| References                                                                                         | 505        |
| <b>20. Enhancing Stress Resistance and Production Phenotypes Through Transcriptome Engineering</b> | <b>509</b> |
| Felix H. Lam, Franz S. Hartner, Gerald R. Fink, and Gregory Stephanopoulos                         |            |
| 1. Introduction                                                                                    | 510        |
| 2. Transcription Factor Selection                                                                  | 511        |
| 3. Plasmid Library Construction                                                                    | 512        |
| 4. Assessment of Phenotypic Diversity                                                              | 519        |
| 5. Selecting for Phenotypes of Interest                                                            | 524        |
| 6. Validation                                                                                      | 528        |
| 7. Concluding Remarks                                                                              | 529        |
| References                                                                                         | 530        |
| <b>Section V. Advances in Cytology/Biochemistry</b>                                                | <b>533</b> |
| <b>21. Visualizing Yeast Chromosomes and Nuclear Architecture</b>                                  | <b>535</b> |
| Peter Meister, Lutz R. Gehlen, Elisa Varela, Véronique Kalck, and Susan M. Gasser                  |            |
| 1. Introduction                                                                                    | 536        |
| 2. Strain Constructions and Image Acquisition for Nuclear Architecture Analysis in Living Cells    | 537        |
| 3. Data Analysis and Quantitative Measurements                                                     | 547        |
| 4. IF and FISH on Fixed Samples                                                                    | 557        |
| References                                                                                         | 566        |
| <b>22. Quantitative Localization of Chromosomal Loci by Immunofluorescence</b>                     | <b>569</b> |
| Donna Garvey Brickner, William Light, and Jason H. Brickner                                        |            |
| 1. Yeast Strain Construction                                                                       | 570        |
| 2. Immunofluorescence                                                                              | 572        |

|                                                                                            |            |
|--------------------------------------------------------------------------------------------|------------|
| 3. Fixing Cells                                                                            | 573        |
| 4. Spheroplasting                                                                          | 574        |
| 5. Preparing Slides                                                                        | 574        |
| 6. Antibody Incubations                                                                    | 575        |
| 7. Mounting and Storage of Slides                                                          | 575        |
| 8. Microscopy and Analysis                                                                 | 576        |
| Acknowledgments                                                                            | 578        |
| References                                                                                 | 579        |
| <b>23. Spinning-Disk Confocal Microscopy of Yeast</b>                                      | <b>581</b> |
| Kurt Thorn                                                                                 |            |
| 1. Introduction                                                                            | 582        |
| 2. Building a Spinning-Disk Confocal Microscope                                            | 583        |
| 3. Sample Preparation                                                                      | 594        |
| Acknowledgments                                                                            | 600        |
| References                                                                                 | 600        |
| <b>24. Correlative GFP-Immunoelectron Microscopy in Yeast</b>                              | <b>603</b> |
| Christopher Buser and Kent McDonald                                                        |            |
| 1. Introduction                                                                            | 604        |
| 2. Recent Advances in High-Pressure Freezing and Freeze-Substitution                       | 605        |
| 3. How to Prepare Yeast by HPF/FS                                                          | 606        |
| 4. Immunolabeling                                                                          | 612        |
| 5. Conclusions                                                                             | 613        |
| 6. Protocols                                                                               | 614        |
| Acknowledgments                                                                            | 617        |
| References                                                                                 | 617        |
| <b>25. Analyzing P-Bodies and Stress Granules in <i>Saccharomyces cerevisiae</i></b>       | <b>619</b> |
| J. Ross Buchan, Tracy Nissan, and Roy Parker                                               |            |
| 1. Introduction                                                                            | 620        |
| 2. Determining If a Specific Protein can Accumulate in P-Bodies or Stress Granules         | 622        |
| 3. Monitoring Messenger RNA in P-Bodies                                                    | 630        |
| 4. Determining If a Mutation/Perturbation Affects P-Body or Stress Granule Size and Number | 631        |
| 5. Quantification of P-Body Size and Number                                                | 635        |
| Acknowledgments                                                                            | 637        |
| References                                                                                 | 637        |

|                                                                                                            |            |
|------------------------------------------------------------------------------------------------------------|------------|
| <b>26. Analyzing mRNA Expression Using Single mRNA Resolution Fluorescent <i>In Situ</i> Hybridization</b> | <b>641</b> |
| Daniel Zenklusen and Robert H. Singer                                                                      |            |
| 1. Introduction                                                                                            | 642        |
| 2. Probe Design                                                                                            | 643        |
| 3. Probe Labeling                                                                                          | 645        |
| 4. Cell Fixation, Preparation, and Storage                                                                 | 647        |
| 5. Hybridization                                                                                           | 650        |
| 6. Image Acquisition                                                                                       | 653        |
| 7. Image Analysis                                                                                          | 654        |
| 8. Summary and Perspectives                                                                                | 657        |
| Acknowledgments                                                                                            | 658        |
| References                                                                                                 | 658        |
| <b>27. The Use of <i>In Vitro</i> Assays to Measure Endoplasmic Reticulum-Associated Degradation</b>       | <b>661</b> |
| Jeffrey L. Brodsky                                                                                         |            |
| 1. Introduction                                                                                            | 662        |
| 2. <i>In Vitro</i> ERAD Assays Using a Soluble Substrate, p $\alpha$ F                                     | 665        |
| 3. <i>In Vitro</i> Assays for Integral Membrane Proteins that are ERAD Substrates                          | 673        |
| References                                                                                                 | 677        |
| <b>28. A Protein Transformation Protocol for Introducing Yeast Prion Particles into Yeast</b>              | <b>681</b> |
| Motomasa Tanaka                                                                                            |            |
| 1. Introduction                                                                                            | 682        |
| 2. Purification of Bacterially Expressed Sup-NM                                                            | 683        |
| 3. Preparation of Different Conformations of <i>In Vitro</i> Sup-NM Amyloid                                | 684        |
| 4. Preparation of <i>In Vivo</i> Prions from Yeast                                                         | 685        |
| 5. Preparation of Lyticase                                                                                 | 686        |
| 6. Protein Transformation                                                                                  | 686        |
| 7. Determination of Prion Conversion Efficiency and Prion Strain Phenotypes                                | 688        |
| Acknowledgments                                                                                            | 692        |
| References                                                                                                 | 692        |
| <b>29. Overexpression and Purification of Integral Membrane Proteins in Yeast</b>                          | <b>695</b> |
| Franklin A. Hays, Zygy Roe-Zurz, and Robert M. Stroud                                                      |            |
| 1. Introduction                                                                                            | 696        |
| 2. General Considerations                                                                                  | 696        |

|                                                                                                               |            |
|---------------------------------------------------------------------------------------------------------------|------------|
| 3. Protocol—Molecular Biology                                                                                 | 697        |
| 4. Protocol—Cell Growth                                                                                       | 699        |
| 5. Protocol—Membrane Preparation and Solubilization                                                           | 699        |
| 6. Protocol—Protein Purification                                                                              | 701        |
| 7. Protocol—Protein Characterization                                                                          | 705        |
| 8. Conclusion                                                                                                 | 706        |
| Acknowledgments                                                                                               | 706        |
| References                                                                                                    | 706        |
| <br>                                                                                                          |            |
| <b>30. Biochemical, Cell Biological, and Genetic Assays to Analyze Amyloid and Prion Aggregation in Yeast</b> | <b>709</b> |
| Simon Alberti, Randal Halfmann, and Susan Lindquist                                                           |            |
| 1. Introduction                                                                                               | 710        |
| 2. Methods                                                                                                    | 712        |
| 3. Concluding Remarks                                                                                         | 731        |
| References                                                                                                    | 731        |
| <br>                                                                                                          |            |
| <b>Section VI. Other Fungi</b>                                                                                | <b>735</b> |
| <br>                                                                                                          |            |
| <b>31. Genetics and Molecular Biology in <i>Candida albicans</i></b>                                          | <b>737</b> |
| Aaron. D. Hernday, Suzanne. M. Noble, Quinn. M. Mitrovich, and Alexander. D. Johnson                          |            |
| 1. Homozygous Gene Disruption in <i>C. albicans</i>                                                           | 738        |
| 2. <i>C. albicans</i> DNA Transformation                                                                      | 743        |
| 3. <i>C. albicans</i> Total RNA Purification                                                                  | 745        |
| 4. C-Terminal Epitope Tagging in <i>C. albicans</i>                                                           | 746        |
| 5. <i>C. albicans</i> Chromatin Immunoprecipitation                                                           | 750        |
| References                                                                                                    | 758        |
| <br>                                                                                                          |            |
| <b>32. Molecular Genetics of <i>Schizosaccharomyces pombe</i></b>                                             | <b>759</b> |
| Sarah A. Sabatinos and Susan L. Forsburg                                                                      |            |
| 1. Introduction                                                                                               | 760        |
| 2. Biology, Growth, and Maintenance of Fission Yeast                                                          | 761        |
| 3. Genetics and Physiology                                                                                    | 767        |
| 4. Molecular Analysis                                                                                         | 776        |
| 5. Cell Biology                                                                                               | 785        |
| 6. Conclusion                                                                                                 | 791        |
| References                                                                                                    | 793        |

|                                                                                                                      |                |
|----------------------------------------------------------------------------------------------------------------------|----------------|
| <b>33. Applying Genetics and Molecular Biology to the Study of the Human Pathogen <i>Cryptococcus neoformans</i></b> | <b>797</b>     |
| Cheryl D. Chun and Hiten D. Madhani                                                                                  |                |
| 1. Introduction                                                                                                      | 798            |
| 2. Serotypes, Strains, and Sequences                                                                                 | 799            |
| 3. Life Cycle                                                                                                        | 800            |
| 4. Techniques for Basic Culture                                                                                      | 802            |
| 5. Basic Molecular Biology Techniques                                                                                | 804            |
| 6. Methods for Assaying Pathogenesis                                                                                 | 817            |
| 7. Concluding Remarks                                                                                                | 828            |
| Acknowledgments                                                                                                      | 829            |
| References                                                                                                           | 829            |
| <br><b>34. The Fungal Genome Initiative and Lessons Learned from Genome Sequencing</b>                               | <br><b>833</b> |
| Christina A. Cuomo and Bruce W. Birren                                                                               |                |
| 1. Introduction: Yeast Genomes and Beyond                                                                            | 834            |
| 2. Computational Prediction of Genes and Noncoding Elements                                                          | 841            |
| 3. Mechanisms of Genome Evolution                                                                                    | 843            |
| 4. Genomic Potential for Sex                                                                                         | 847            |
| 5. Gene Family Conservation and Evolution                                                                            | 848            |
| 6. Impact of Next-Generation Sequencing                                                                              | 849            |
| 7. Future Directions                                                                                                 | 851            |
| Acknowledgments                                                                                                      | 852            |
| References                                                                                                           | 853            |
| <br><b>35. Ultradian Metabolic Cycles in Yeast</b>                                                                   | <br><b>857</b> |
| Benjamin P. Tu                                                                                                       |                |
| 1. Introduction                                                                                                      | 857            |
| 2. Induction of Ultradian Cycles of Oxygen Consumption Using a Chemostat                                             | 858            |
| 3. Long-Period Cycles                                                                                                | 861            |
| 4. Short-Period Cycles                                                                                               | 862            |
| 5. Significance of Ultradian Cycles                                                                                  | 863            |
| Acknowledgments                                                                                                      | 865            |
| References                                                                                                           | 865            |
| <br><i>Author Index</i>                                                                                              | <br><i>867</i> |
| <i>Subject Index</i>                                                                                                 | <i>879</i>     |