

CONTENTS

Contributors

xi

Preface

xvii

Part I

Organismal Rhythms as Read-Outs of Clock Function

1. Studying Circadian Rhythm and Sleep Using Genetic Screens in *Drosophila*

3

Sofia Axelrod, Lino Saez, and Michael W. Young

1. Introduction: Studying Circadian Behavior in the Fruit Fly, *Drosophila melanogaster*

4

2. Screening for Circadian Rhythm and Sleep Mutants

5

3. Screening Techniques

10

Acknowledgments

21

References

22

2. Dissecting the Mechanisms of the Clock in *Neurospora*

29

Jennifer Hurley, Jennifer J. Loros, and Jay C. Dunlap

1. Introduction

29

2. Molecular Mechanism of the *Neurospora* Circadian Oscillator

34

3. Core Clock Components

37

4. Conclusion

46

References

47

3. High-Throughput and Quantitative Approaches for Measuring Circadian Rhythms in Cyanobacteria Using Bioluminescence

53

Ryan K. Shultzaberger, Mark L. Paddock, Takeo Katsuki, Ralph J. Greenspan, and Susan S. Golden

1. Theory

54

2. Build a Computer-Controlled Turntable

55

3. Use a Computer-Controlled Turntable

60

4. Analyzing Data from Turntable

63

5. Steps to Extract Reliable Quantitative Information from Bioluminescence Levels

64

Acknowledgments

71

References

71

4. Using Circadian Entrainment to Find Cryptic Clocks 73

Zheng Eelderink-Chen, Maria Olmedo, Jasper Bosman, and Martha Merrow

1. Introduction	74
2. Methods	78
3. Discussion	87
Acknowledgments	90
References	90

5. Wavelet-Based Analysis of Circadian Behavioral Rhythms 95

Tanya L. Leise

1. Introduction	96
2. Fourier and Wavelet Methods for Time Series Analysis	98
3. Computations	116
4. Concluding Remarks	116
References	117

6. Genetic Analysis of *Drosophila* Circadian Behavior in Seminatural Conditions 121

Edward W. Green, Emma K. O'Callaghan, Mirko Pegoraro,

J. Douglas Armstrong, Rodolfo Costa, and Charalambos P. Kyriacou

1. Introduction	122
2. Considerations for Studies Outside	126
3. Simulating Natural Conditions in the Laboratory	128
Acknowledgments	132
References	132

Part II

Characterization of Molecular Clock Components

7. Methods to Study Molecular Mechanisms of the *Neurospora* Circadian Clock 137

Joonseok Cha, Mian Zhou, and Yi Liu

1. Introduction	137
2. Description of Methods	140
3. Concluding Remarks	148
Acknowledgment	148
References	148

8. Detecting KaiC Phosphorylation Rhythms of the Cyanobacterial Circadian Oscillator *In Vitro* and *In Vivo* 153

Yong-Ick Kim, Joseph S. Boyd, Javier Espinosa, and Susan S. Golden

1. Theory	155
2. Equipment	155
3. Materials	157
4. Protocol	161
5. Step 1: Expression of KaiA or KaiB in <i>E. coli</i>	161
6. Step 2: Expression of KaiC in <i>E. coli</i>	162
7. Step 3: Purification of KaiA or KaiB	162
8. Step 4: Purification of KaiC	164
9. Step 5: <i>In vitro</i> Oscillation Reaction	165
10. Step 6: SDS-PAGE	166
11. Step 7: Densitometry	167
12. Detection of Protein Phosphorylation Forms from <i>In Vivo</i> Cell Extracts	168
13. Equipment	168
14. Materials	169
15. Protocol	170
16. Step 1: Preparation	170
17. Step 2: Electrophoresis and Blotting	172
Acknowledgments	172
References	173

9. The Role of Casein Kinase I in the *Drosophila* Circadian Clock 175

Jeffrey L. Price, Jin-Yuan Fan, Andrew Keightley, and John C. Means

1. Introduction	176
2. Expression of Mutant Forms of DBT with the GAL4/UAS Binary Expression Method	178
3. Expression of DBT in <i>Drosophila</i> S2 Cells for Analysis of DBT Kinase Activity	180
4. Proteomic Approaches	181
References	192

10. Purification and Analysis of PERIOD Protein Complexes of the Mammalian Circadian Clock 197

Jin Young Kim, Pieter Bas Kwak, Michael Gebert, Hao A. Duong, and Charles J. Weitz

1. General Strategy	198
2. Extraction and Characterization of PER Complexes from Mouse Tissues	201
References	210

11. Best Practices for Fluorescence Microscopy of the Cyanobacterial Circadian Clock	211
Susan E. Cohen, Marcella L. Erb, Joe Pogliano, and Susan S. Golden	
1. Introduction	211
2. Materials	212
3. Methods	213
Acknowledgments	220
References	220
12. Structural and Biophysical Methods to Analyze Clock Function and Mechanism	223
Martin Egli	
1. Introduction	224
2. Kai Protein Overexpression, Purification, Complex Formation, and Analysis by Denatured and Native Polyacrylamide Gel Electrophoresis	228
3. Analytical Ultracentrifugation	231
4. Dynamic Light Scattering	232
5. Thin Layer Chromatography	233
6. Mass Spectrometry	234
7. Site-Directed Mutagenesis	235
8. Fluorescence Techniques (Labeled Proteins, Anisotropy, and Fluorescence Resonance Energy Transfer)	236
9. Electron Microscopy	238
10. X-ray Crystallography	241
11. Small-Angle X-ray and Neutron Scattering	245
12. Nuclear Magnetic Resonance	249
13. Hydrogen-Deuterium Exchange	251
14. MD Simulations	253
15. Modeling the <i>In Vitro</i> Oscillator	255
16. Summary and Outlook	256
Acknowledgments	259
References	259
13. Identification of Small-Molecule Modulators of the Circadian Clock	267
Tsuyoshi Hirota and Steve A. Kay	
1. Introduction	268
2. Cell-Based Circadian Assay	268
3. High-Throughput Screening System	270

4. Circadian Screening	274
5. Conclusion	278
References	280

Part III

Circadian Regulation of Gene and Protein Expression

14. ChIP-seq and RNA-seq Methods to Study Circadian Control of Transcription in Mammals 285

Joseph S. Takahashi, Vivek Kumar, Prachi Nakashe, Nobuya Koike,
Hung-Chung Huang, Carla B. Green, and Tae-Kyung Kim

1. Critical Factors	288
2. ChIP-seq Method for Mouse Liver	290
3. RNA-Seq Method for Mouse Liver	303
References	319

15. ChIPping Away at the *Drosophila* Clock 323

Jian Zhou, Wangjie Yu, and Paul E. Hardin

1. Introduction	324
2. Equipment	326
3. Solutions	328
4. Protocol	333
5. Discussion	343
References	345

16. Considerations for RNA-seq Analysis of Circadian Rhythms 349

Jiajia Li, Gregory R. Grant, John B. Hogenesch, and Michael E. Hughes

1. Introduction	350
2. Results	353
3. Conclusions	361
4. Methods	362
Acknowledgments	363
References	363

17. RNA-seq Profiling of Small Numbers of *Drosophila* Neurons 369

Katharine Abruzzi, Xiao Chen, Emi Nagoshi, Abby Zadina,
and Michael Rosbash

1. Introduction	370
2. Results/Methods	371

3. Discussion	382
References	385
18. Analysis of Circadian Regulation of Poly(A)-Tail Length	387
Shihoko Kojima and Carla B. Green	
1. Introduction	388
2. Measurement of Poly(A)-Tail Length at a Genomewide Level	389
3. Measurement of Poly(A)-Tail Length at a Single-Gene Level	395
4. Materials	401
5. Concluding Remarks	401
Acknowledgments	402
References	402
19. Sample Preparation for Phosphoproteomic Analysis of Circadian Time Series in <i>Arabidopsis thaliana</i>	405
Johanna Krahmer, Matthew M. Hindle, Sarah F. Martin, Thierry Le Bihan, and Andrew J. Millar	
1. Introduction	406
2. Materials and Methods	408
3. Results	415
4. Discussion	423
5. Conclusions	428
Acknowledgments	429
References	429
Author Index	433
Subject Index	457