

CONTENTS

Contributors	xi
Preface	xv

SECTION I Background

1. From Central to Rudimentary to Primary: The History of an Underappreciated Organelle Whose Time Has Come. The Primary Cilium <i>Robert A. Bloodgood</i>	
I. Introduction	4
II. Technical Issues in the Discovery and Study of Primary Cilia	6
III. Origins: 1844–1910	7
IV. The Henneگوی–Lenhossek Hypothesis and the Role of Primary Cilia	19
V. The Middle Period (1910–1935)	26
VI. Origin of Photoreceptor Connecting Cilia from Primary Cilia	28
VII. The Dark Ages of Primary Cilia (1935–1955)	30
VIII. The 1950s and 1960s: Transmission Electron Microscopy and the “Renaissance” of the Primary Cilium	31
IX. Primary Cilia and the Cell Cycle	34
X. The Golden Age of Primary Cilia (2000–Onward)	39
XI. Conclusions: The Morals of this Story	42
References	45
2. Origin of the Cilium: Novel Approaches to Examine a Centriolar Evolution Hypothesis <i>Mark C. Alliegro and Peter Satir</i>	
I. Introduction	54
II. Methods	58
III. Viral Relatives of cnRNAs and Other Centriolar and Centrosomal Components	60
IV. Conclusions	62
References	63

SECTION II Cell Biology/Biochemistry

3. Using quantitative PCR to Identify Kinesin-3 Genes that are Upregulated During Growth Arrest in Mouse NIH3T3 Cells
Rikke I. Thorsteinsson, Søren T. Christensen, and Lotte B. Pedersen
 - I. Introduction 68
 - II. Rationale 70
 - III. Materials 70
 - IV. Methods 72
 - V. Results and Discussion 80
 - VI. Summary 83
 - References 83

4. Methods for the Isolation of Sensory and Primary Cilia—An Overview
Kimberly A.P. Mitchell, Gabor Szabo, and Angela de S. Otero
 - I. Introduction 88
 - II. Isolation of Olfactory Cilia 88
 - III. Isolation of Rod Photoreceptor Cilia 91
 - IV. Isolation of Primary Cilia 92
 - References 98

5. Isolation of Primary Cilia for Morphological Analysis
Bing Huang, Tatyana Masyuk, and Nicholas LaRusso
 - I. Introduction 104
 - II. Materials and Instrumentations 105
 - III. Methods 106
 - IV. Results and Discussion 110
 - V. Summary 114
 - References 114

6. Analyzing Primary Cilia by Multiphoton Microscopy
Cornelia E. Farnum, Rebecca M. Williams, and Eve Donnelly
 - I. Introduction 118
 - II. Materials and Solutions 120
 - III. Methods 121
 - IV. Results and Discussion 128
 - References 133

7. Primary Cilia and the Cell Cycle	
<i>Olga V. Plotnikova, Elena N. Pugacheva, and Erica A. Golemis</i>	
I. Introduction	138
II. Signaling Systems Regulating Ciliary Protusion and Resorption	141
III. Methods	150
References	154

SECTION III Function

8. Utilization of Conditional Alleles to Study the Role of the Primary Cilium in Obesity	
<i>Robert A. Kesterson, Nicolas F. Berbari, Raymond C. Pasek, and Bradley K. Yoder</i>	
I. Introduction	164
II. CNS Mechanisms of Energy Balance	164
III. Human Ciliopathies and Obesity	165
IV. Cilia Mutant Mouse Models of Obesity	167
V. Neuronal Cilia	171
VI. Strategies and Methods of Analyzing Obesity in Cilia Mouse Models	172
References	176
9. Using Nucleofection of siRNA Constructs for Knockdown of Primary Cilia in P19.CL6 Cancer Stem Cell Differentiation into Cardiomyocytes	
<i>Christian A. Clement, Lars A. Larsen, and Søren T. Christensen</i>	
I. Introduction	182
II. Rationale	184
III. Materials	184
IV. Methods	186
V. Results and Discussion	190
VI. Summary	195
References	196
10. The Primary Cilium as a Hedgehog Signal Transduction Machine	
<i>Sarah C. Goetz, Polloneal J.R. Ocbina, and Kathryn V. Anderson</i>	
I. Introduction	200
II. Summary	215
References	216

11. Detecting the Surface Localization and Cytoplasmic Cleavage of Membrane-Bound Proteins	
<i>Hannah C. Chapin, Vanathy Rajendran, Anna Capasso, and Michael J. Caplan</i>	
I. Introduction	224
II. Assay Rationale and History	225
III. Materials	228
IV. Methods	230
V. Discussion	236
VI. Summary	238
References	238
12. Assay for <i>In Vitro</i> Budding of Ciliary-Targeted Rhodopsin Transport Carriers	
<i>Dusanka Deretic and Jana Mazelova</i>	
I. Introduction	242
II. Methods	243
III. Materials	249
IV. Results and Discussion	250
V. Summary	255
References	255
13. Immunoelectron Microscopy of Vesicle Transport to the Primary Cilium of Photoreceptor Cells	
<i>Tina Sedmak, Elisabeth Sehn, and Uwe Wolfrum</i>	
I. Introduction and Rationale	260
II. Materials	262
III. Methods and Procedures	264
IV. Results and Discussion	267
References	270
14. Polycystic Kidney Disease, Cilia, and Planar Polarity	
<i>Luis F. Menezes and Gregory G. Germino</i>	
I. Cystic Kidney Diseases	274
II. The Primary Cilium and Cystic Kidney Diseases	276
III. Explanatory Models for Cystic Kidney Diseases	279
IV. Final Remarks	292
References	292

15. Constructing and Deconstructing Roles for the Primary Cilium in Tissue Architecture and Cancer

E. Scott Seeley and Maxence V. Nachury

I. Introduction	300
II. Sensory Modalities	300
III. Flow and Mechanosensation	301
IV. Low-Abundance Ligands	301
V. Concentration Shifts	304
VI. Ciliary Receptors	304
VII. Growth Factor Receptors	304
VIII. Morphogens	305
IX. Hormone and G-Protein-Coupled Receptors	306
X. Tissue Homeostasis and Architecture	306
XI. Cancer	307
XII. Summary	310
References	311

SECTION IV Posttranslational Modifications

16. Polyglutamylation and the *fleer* Gene

Narendra H. Pathak and Iain A. Drummond

I. Introduction	317
II. Significance of Polyglutamylation	318
III. Biochemistry of Tubulin Glutamylation	319
IV. Functional Significance of Tubulin Glutamylase	319
V. The Zebrafish <i>fleer</i> Mutant as a Paradigm for Analysis of Cilia Tubulin Glutamylation	320
VI. Situs Inversus in <i>fleer</i> is Caused by Motility and Length Defects of Kupffer's Vesicle Cilia	322
VII. Pronephric Cysts in <i>fleer</i> Result from Defects of Cilia Motility and Length	322
VIII. Olfactory Placode Cilia	325
IX. Abnormal Otolith Numbers are Caused by Defects of Motile Otic Placode Cilia	325
X. Retinal Degeneration in <i>fleer</i> Results from Defects of the Connecting Cilium in Photoreceptors	326
XI. <i>fleer</i> -Like Phenotypes in Other Species	326
XII. Methods	327
References	330

17. Regulation of Cilia assembly, Disassembly, and Length by Protein Phosphorylation	
<i>Muqing Cao, Guihua Li, and Junmin Pan</i>	
I. Introduction	334
II. Protein Phosphorylation in Cilia Assembly	335
III. Protein Phosphorylation in Cilia Disassembly	338
IV. Protein Phosphorylation in Cilia Length Control	340
V. Conclusion	342
References	343
18. Posttranslational Protein Modifications in Cilia and Flagella	
<i>Roger D. Sloboda</i>	
I. Introduction	348
II. Posttranslational Protein Modification in Cilia and Flagella	350
III. Methods for the Detection of PTM in Cilia and Flagella using the Electron Microscope	354
References	360
Index	365
Volume in Series	379