

# METHODS IN MOLECULAR BIOLOGY™

*Series Editor*  
**John M. Walker**  
School of Life Sciences  
University of Hertfordshire  
Hatfield, Hertfordshire, AL10 9AB, UK

For further volumes:  
<http://www.springer.com/series/7651>



# Planar Cell Polarity

## Methods and Protocols

Edited by

**Kursad Turksen**

*Regenerative Medicine Program, Sprott Centre for Stem Cell Research,  
Ottawa Hospital Research Institute, Ottawa, ON, Canada*



---

## **Preface**

With the ever-increasing awareness of the importance of *Planar Cell Polarity* in not only normal development but also disease states, I felt that it was timely to gather a number of protocols related to this topic.

I believe that this collection of protocols will be valuable to both novices and experts in the field. I thank all of the contributors for their efforts in putting together the details of their favorite protocols. This volume would not have materialized without their efforts. I would also like especially to acknowledge Dr. Peng Chen who was extremely supportive of me at the initiation of this project.

As always, I am grateful to Dr. John Walker for his continuous support and for his commitment to maintaining such a high-quality series. My thanks also to Patrick Marton for his encouragements and help. Finally, a special thank you goes to David Casey for his tremendous help in the production of the volume.

*Ottawa, ON, Canada*

*Kursad Turksen, Ph.D.*



---

# Contents

<i>Preface</i> . . . . .	<i>v</i>
<i>Contributors</i> . . . . .	<i>ix</i>
1 Live Imaging of <i>Drosophila</i> Embryos: Quantifying Protein Numbers and Dynamics at Subcellular Locations . . . . .	1
<i>Daryl J.V. David, Melanie A. McGill, R.F. Andrew McKinley, and Tony J.C. Harris</i>	
2 Analyzing Frizzled Signaling Using Fixed and Live Imaging of the Asymmetric Cell Division of the <i>Drosophila</i> Sensory Organ Precursor Cell . . . . .	19
<i>Bertrand Jauffred and Yohanns Bellaïche</i>	
3 Protein–Protein Interaction Techniques: Dissect PCP Signaling in <i>Xenopus</i> . . . . .	27
<i>Yingqun Wang</i>	
4 Cuticle Refraction Microscopy: A Rapid and Simple Method for Imaging <i>Drosophila</i> Wing Topography, an Alternative Readout of Wing Planar Cell Polarity . . . . .	43
<i>David Neff, Justin Hogan, and Simon Collier</i>	
5 Analysis of Cell Shape and Polarity During Zebrafish Gastrulation. . . . .	53
<i>Douglas C. Weiser and David Kimelman</i>	
6 Analyzing Planar Cell Polarity During Zebrafish Gastrulation . . . . .	69
<i>Jason R. Jessen</i>	
7 Wnt/Planar Cell Polarity Signaling in the Regulation of Convergent Extension Movements During <i>Xenopus</i> Gastrulation . . . . .	79
<i>Gun-Hwa Kim, Edmond Changkyun Park, and Jin-Kwan Han</i>	
8 Using 32-Cell Stage <i>Xenopus</i> Embryos to Probe PCP Signaling . . . . .	91
<i>Hyun-Shik Lee, Sergei Y. Sokol, Sally A. Moody, and Ira O. Daar</i>	
9 Gene Loss-of-Function and Live Imaging in Chick Embryos. . . . .	105
<i>Anne C. Rios, Christophe Marcelle, and Olivier Serralbo</i>	
10 Activation and Function of Small GTPases Rho, Rac, and Cdc42 During Gastrulation . . . . .	119
<i>Courtney Mezzacappa, Yuko Komiya, and Raymond Habas</i>	
11 Convergent Extension Analysis in Mouse Whole Embryo Culture. . . . .	133
<i>Sophie E. Pryor, Valentina Massa, Dawn Savery, Nicholas D.E. Greene, and Andrew J. Copp</i>	
12 Analysis of PCP Defects in Mammalian Eye Lens . . . . .	147
<i>Yuki Sugiyama and John W. McAvoy</i>	
13 Examining Planar Cell Polarity in the Mammalian Cochlea . . . . .	157
<i>Helen May-Simera and Matthew W. Kelley</i>	

14 Role of Prickle1 and Prickle2 in Neurite Outgrowth in Murine Neuroblastoma Cells. . . . . 173  
*Lisa Fujimura and Masahiko Hatano*

15 The Planar Cell Polarity Pathway and Parietal Endoderm Cell Migration . . . . . 187  
*Kristi LaMonica and Laura Grabel*

16 Analysis of Wnt/Planar Cell Polarity Pathway in Cultured Cells . . . . . 201  
*Mitsubaru Endo, Michiru Nishita, and Yasuhiro Minami*

17 Regulation of Focal Adhesion Dynamics by Wnt5a Signaling . . . . . 215  
*Shinji Matsumoto and Akira Kikuchi*

18 The Embryonic Mouse Gut Tube as a Model for Analysis of Epithelial Polarity . . . . . 229  
*Makoto Matsuyama and Akibiko Shimono*

19 Assessing PCP in the Cochlea of Mammalian Ciliopathy Models . . . . . 239  
*Daniel J. Jagger and Andrew Forge*

20 Morphometric Analysis of Centrosome Position in Tissues . . . . . 249  
*Hester Happé, Emile de Heer, and Dorien J.M. Peters*

*Index* . . . . . 257



---

## Contributors

- YOHANNIS BELLAICHE • *Polarity Division and Morphogenesis, Institut Curie, CNRS UMR 3215, INSERM U934, Paris, France*
- SIMON COLLIER • *Department of Biological Sciences, Marshall University, Huntington, WV, USA*
- ANDREW J. COPP • *Neural Development Unit, Institute of Child Health, University College London, London, UK*
- IRA O. DAAR • *Laboratory of Cell and Developmental Signaling, National Cancer Institute-Frederick, Frederick, MD, USA*
- DARYL J.V. DAVID • *Department of Cell & Systems Biology, University of Toronto, Toronto, ON, Canada*
- MITSUOHARU ENDO • *Department of Physiology and Cell Biology, Graduate School of Medicine, Kobe University, Kobe, Japan*
- ANDREW FORGE • *Centre for Auditory Research, UCL Ear Institute, University College London, London, UK*
- LISA FUJIMURA • *Department of Biomedical Science, Graduate School of Medicine, Chiba University, Chiba City, Chiba, Japan*
- LAURA GRABEL • *Department of Biology, Wesleyan University, Middletown, CT, USA*
- NICHOLAS D.E. GREENE • *Neural Development Unit, Institute of Child Health, University College London, London, UK*
- RAYMOND HABAS • *Department of Biochemistry, UMDNJ-Robert Wood Johnson Medical School, Piscataway, NJ, USA; Department of Biology, College of Science and Technology, Temple University, Philadelphia, PA, USA*
- JIN-KWAN HAN • *Division of Molecular and Life Sciences, Department of Life Science, Pohang University of Science and Technology, Hyoja Dong, Pohang, Kyungbuk, Republic of Korea*
- HESTER HAPPÉ • *Department of Human Genetics and Pathology, Leiden University Medical Center, Leiden, The Netherlands*
- TONY J.C. HARRIS • *Department of Cell & Systems Biology, University of Toronto, Toronto, ON, Canada*
- MASAHIKO HATANO • *Department of Biomedical Science, Graduate School of Medicine, Chiba University, Chiba City, Chiba, Japan*
- EMILE DE HEER • *Department of Pathology, Leiden University Medical Center, Leiden, The Netherlands*
- JUSTIN HOGAN • *Department of Biological Sciences, Marshall University, Huntington, WV, USA*
- DANIEL J. JAGGER • *Centre for Auditory Research, UCL Ear Institute, University College London, London, UK*
- BERTRAND JAUFFRED • *Polarity Division and Morphogenesis, Institut Curie, CNRS UMR 3215, INSERM U934, Paris, France*
- JASON R. JESSEN • *Division of Genetic Medicine, Department of Medicine, Vanderbilt University Medical Center, Nashville, TN, USA*

- MATTHEW W. KELLEY • *Laboratory of Cochlear Development, National Institute on Deafness and other Communication Disorders, National Institutes of Health, Bethesda, MD, USA*
- AKIRA KIKUCHI • *Department of Molecular Biology & Biochemistry, Graduate School of Medicine, Faculty of Medicine, Osaka University, Osaka, Japan*
- GUN-HWA KIM • *Division of Life Science, Korea Basic Science Institute, Daejeon, Republic of Korea*
- DAVID KIMELMAN • *Department of Biochemistry, University of Washington, Seattle, WA, USA*
- YUKO KOMIYA • *Department of Biology, College of Science and Technology, Temple University, Philadelphia, PA, USA*
- KRISTI LAMONICA • *Department of Craniofacial Biology, School of Dental Medicine, Anschutz Medical Campus, University of Colorado Denver, Aurora, CO, USA*
- HYUN-SHIK LEE • *School of Life Sciences, College of Natural Sciences, Kyungpook National University, Daegu, South Korea*
- CHRISTOPHE MARCELLE • *EMBL Australia, Australian Regenerative Medicine Institute (ARMI), Monash University, Clayton, VIC, Australia*
- VALENTINA MASSA • *Dulbecco Telethon Institute at M. Tettamanti Research Center, Pediatric Department, University of Milano-Bicocca, Monza, Italy*
- SHINJI MATSUMOTO • *Department of Molecular Biology & Biochemistry, Graduate School of Medicine, Faculty of Medicine, Osaka University, Osaka, Japan*
- MAKOTO MATSUYAMA • *Division of Biochemistry, Aichi Cancer Center Research Institute, Nagoya, Japan*
- HELEN MAY-SIMERA • *Laboratory of Cochlear Development, National Institute on Deafness and other Communication Disorders, National Institutes of Health, Bethesda, MD, USA*
- JOHN W. McAVOY • *Sydney Hospital and Eye Hospital, Sydney, NSW, Australia*
- MELANIE A. MCGILL • *Department of Cell & Systems Biology, University of Toronto, Toronto, ON, Canada*
- R.F. ANDREW MCKINLEY • *Department of Cell & Systems Biology, University of Toronto, Toronto, ON, Canada*
- COURTNEY MEZZACAPPA • *Department of Biochemistry, UMDNJ-Robert Wood Johnson Medical School, Piscataway, NJ, USA*
- YASUHIRO MINAMI • *Department of Physiology and Cell Biology, School of Medicine, Kobe University, Kobe, Japan*
- SALLY A. MOODY • *Department of Anatomy and Regenerative Biology, The George Washington University Medical Center, Washington, DC, USA*
- DAVID NEFF • *Department of Biological Sciences, Marshall University, Huntington, WV, USA*
- MICHIRU NISHITA • *Department of Physiology and Cell Biology, Graduate School of Medicine, Kobe University, Kobe, Japan*
- EDMOND CHANGKYUN PARK • *Division of Life Science, Korea Basic Science Institute, Daejeon, Republic of Korea*
- DORIEN J.M. PETERS • *Department of Human Genetics, Leiden University Medical Center, Leiden, The Netherlands*

- SOPHIE E. PRYOR • *Neural Development Unit, Institute of Child Health,  
University College London, London, UK*
- ANNE C. RIOS • *EMBL Australia, Australian Regenerative Medicine Institute  
(ARMI), Monash University, Clayton, VIC, Australia*
- DAWN SAVERY • *Neural Development Unit, Institute of Child Health,  
University College London, London, UK*
- OLIVIER SERRALBO • *EMBL Australia, Australian Regenerative Medicine  
Institute (ARMI), Monash University, Clayton, VIC, Australia*
- AKIHIKO SHIMONO • *Centre of Life Sciences, Cancer Science Institute of Singapore,  
National University of Singapore, Singapore*
- SERGEI Y. SOKOL • *Department of Developmental and Regenerative Biology,  
Mount Sinai School of Medicine, New York, NY, USA*
- YUKI SUGIYAMA • *Save Sight Institute, The University of Sydney,  
Sydney, NSW, Australia*
- YINGQUN WANG • *Department of Pathology, Anatomy, and Cell Biology,  
Thomas Jefferson University, Philadelphia, PA, USA*
- DOUGLAS C. WEISER • *Department of Biological Sciences, University of the Pacific,  
Stockton, CA, USA*

