

Wnt Signaling

METHODS IN MOLECULAR BIOLOGY™

John M. Walker, SERIES EDITOR

475. Cell Fusion: *Overviews and Methods*, edited by *Elizabeth H. Chen*, 2008
474. Nanostructure Design: *Methods and Protocols*, edited by *Ehud Gazit and Ruth Nussinov*, 2008
473. Clinical Epidemiology: *Practice and Methods*, edited by *Patrick Parfrey and Brendon Barrett*, 2008
472. Cancer Epidemiology, Volume 2: *Modifiable Factors*, edited by *Mukesh Verma*, 2008
471. Cancer Epidemiology, Volume 1: *Host Susceptibility Factors*, edited by *Mukesh Verma*, 2008
470. Host-Pathogen Interactions: *Methods and Protocols*, edited by *Steffen Rupp and Kai Sohn*, 2008
469. Wnt Signaling, Volume 2: *Pathway Models*, edited by *Elizabeth Vincan*, 2008
468. Wnt Signaling, Volume 1: *Pathway Methods and Mammalian Models*, edited by *Elizabeth Vincan*, 2008
467. Angiogenesis Protocols: *Second Edition*, edited by *Stewart Martin and Cliff Murray*, 2008
466. Kidney Research: *Experimental Protocols*, edited by *Tim D. Hewison and Gavin J. Becker*, 2008
465. Mycobacteria, Second Edition, edited by *Tanya Parish and Amanda Claire Brown*, 2008
464. The Nucleus, Volume 2: *Physical Properties and Imaging Methods*, edited by *Ronald Hancock*, 2008
463. The Nucleus, Volume 1: *Nuclei and Subnuclear Components*, edited by *Ronald Hancock*, 2008
462. Lipid Signaling Protocols, edited by *Banafshe Larijani, Rudiger Woscholski, and Colin A. Rosser*, 2008
461. Molecular Embryology: *Methods and Protocols, Second Edition*, edited by *Paul Sharpe and Ivor Mason*, 2008
460. Essential Concepts in Toxicogenomics, edited by *Donna L. Mendrick and William B. Mattes*, 2008
459. Prion Protein Protocols, edited by *Andrew F. Hill*, 2008
458. Artificial Neural Networks: *Methods and Applications*, edited by *David S. Livingstone*, 2008
457. Membrane Trafficking, edited by *Ales Vancura*, 2008
456. Adipose Tissue Protocols, Second Edition, edited by *Kaiping Yang*, 2008
455. Osteoporosis, edited by *Jennifer J. Westendorf*, 2008
454. SARS- and Other Coronaviruses: *Laboratory Protocols*, edited by *Dave Cavanagh*, 2008
453. Bioinformatics, Volume II: *Structure, Function and Applications*, edited by *Jonathan M. Keith*, 2008
452. Bioinformatics, Volume I: *Data, Sequence Analysis and Evolution*, edited by *Jonathan M. Keith*, 2008
451. Plant Virology Protocols: *From Viral Sequence to Protein Function*, edited by *Gary Foster, Elisabeth Jobansen, Yiguo Hong, and Peter Nagy*, 2008
450. Germline Stem Cells, edited by *Steven X. Hou and Shree Ram Singh*, 2008
449. Mesenchymal Stem Cells: *Methods and Protocols*, edited by *Darwin J. Prockop, Douglas G. Phinney, and Bruce A. Brunnell*, 2008
448. Pharmacogenomics in Drug Discovery and Development, edited by *Qing Tan*, 2008
447. Alcohol: *Methods and Protocols*, edited by *Laura E. Nagy*, 2008
446. Post-translational Modification of Proteins: *Tools for Functional Proteomics*, Second Edition, edited by *Christoph Kannicht*, 2008
445. Autophagosome and Phagosome, edited by *Vojo Deretic*, 2008
444. Prenatal Diagnosis, edited by *Sinhue Hahn and Laird G. Jackson*, 2008
443. Molecular Modeling of Proteins, edited by *Andreas Kukol*, 2008
442. RNAi: Design and Application, edited by *Sailen Barik*, 2008
441. Tissue Proteomics: *Pathways, Biomarkers, and Drug Discovery*, edited by *Brian Liu*, 2008
440. Exocytosis and Endocytosis, edited by *Andrei I. Ivanov*, 2008
439. Genomics Protocols, Second Edition, edited by *Mike Starkey and Ramnanth Elavarapu*, 2008
438. Neural Stem Cells: *Methods and Protocols*, Second Edition, edited by *Leslie P. Weiner*, 2008
437. Drug Delivery Systems, edited by *Kewal K. Jain*, 2008
436. Avian Influenza Virus, edited by *Erica Spackman*, 2008
435. Chromosomal Mutagenesis, edited by *Greg Davis and Kevin J. Kayser*, 2008
434. Gene Therapy Protocols: Volume II: *Design and Characterization of Gene Transfer Vectors*, edited by *Joseph M. LeDoux*, 2008
433. Gene Therapy Protocols: Volume I: *Production and In Vivo Applications of Gene Transfer Vectors*, edited by *Joseph M. LeDoux*, 2008
432. Organelle Proteomics, edited by *Delphine Pflieger and Jean Rossier*, 2008
431. Bacterial Pathogenesis: *Methods and Protocols*, edited by *Frank DeLeo and Michael Otto*, 2008
430. Hematopoietic Stem Cell Protocols, edited by *Kevin D. Bunting*, 2008
429. Molecular Beacons: *Signalling Nucleic Acid Probes, Methods and Protocols*, edited by *Andreas Marx and Oliver Seitz*, 2008
428. Clinical Proteomics: *Methods and Protocols*, edited by *Antonia Vlabou*, 2008
427. Plant Embryogenesis, edited by *Maria Fernanda Suarez and Peter Bozhkov*, 2008
426. Structural Proteomics: *High-Throughput Methods*, edited by *Bostjan Kobe, Mitchell Guss, and Huber Thomas*, 2008
425. 2D PAGE: *Sample Preparation and Fractionation*, Volume II, edited by *Anton Posch*, 2008
424. 2D PAGE: *Sample Preparation and Fractionation*, Volume I, edited by *Anton Posch*, 2008
423. Electroporation Protocols: *Preclinical and Clinical Gene Medicine*, edited by *Shulin Li*, 2008
422. Phylogenomics, edited by *William J. Murphy*, 2008
421. Affinity Chromatography: *Methods and Protocols*, Second Edition, edited by *Michael Zachariou*, 2008

METHODS IN MOLECULAR BIOLOGY™

Wnt Signaling

Volume 1
Pathway Methods and Mammalian Models

Edited by

Elizabeth Vincan, PhD

University of Melbourne, Parkville, Victoria, Australia

 **Humana Press**

Editor

Elizabeth Vincan
University of Melbourne
Parkville, Victoria VIC 3010
Australia

Series Editor

John M. Walker
School of life Sciences
University of Hertfordshire
Hatfield, Hertfordshire
AL10 9AB, UK

ISBN: 978-1-58829-912-3 e-ISBN: 978-1-59745-249-6
ISSN: 1064-3745 e-ISSN: 1940-6029
DOI: 10.1007/978-1-59745-249-6

Library of Congress Control Number: 2008936263

© 2008 Humana Press, a part of Springer Science+Business Media, LLC

All rights reserved. This work may not be translated or copied in whole or in part without the written permission of the publisher (233 Spring Street, New York, NY 10013, USA), except for brief excerpts in connection with reviews or scholarly analysis. Use in connection with any form of information storage and retrieval, electronic adaptation, computer software, or by similar or dissimilar methodology now known or hereafter developed is forbidden.

The use in this publication of trade names, trademarks, service marks, and similar terms, even if they are not identified as such, is not to be taken as an expression of opinion as to whether or not they are subject to proprietary rights.

While the advice and information in this book are believed to be true and accurate at the date of going to press, neither the authors nor the editors nor the publisher can accept any legal responsibility for any errors or omissions that may be made. The publisher makes no warranty, express or implied, with respect to the material contained herein.

Cover illustration:

Printed on acid-free paper

9 8 7 6 5 4 3 2 1

springer.com

Preface

Since their discovery some 20 years ago, Wnt signaling molecules have been shown to control key events in embryogenesis, maintain tissue homeostasis in the adult and, when aberrantly activated, promote human degenerative diseases and cancer. Elucidation of Wnt signaling mechanisms has relied on both biochemical methodologies and vertebrate and invertebrate model systems. Therefore, I felt that an issue dedicated to Wnt signaling had to include both assays (biochemical readout) and model systems (functional readout) of Wnt signaling. It is not an exhaustive catalog, but rather a point of reference to current molecular protocols and the diverse model systems employed to study this signaling pathway. The issue is divided into two volumes. The first volume includes assays to measure activation of the diverse Wnt signaling pathways as well as models and strategies used to study mammalian Wnt/FZD function (from protein–protein interaction and simple cell line models to organoid cultures and mouse models). The second volume is dedicated to the diverse vertebrate and invertebrate models that have shaped the Wnt signaling field. It provides an entry point for the novice and an overview of the unique properties of each organism with respect to studying Wnt/FZD function (for example asymmetric cell division in *Caenorhabditis elegans*, epithelial morphogenesis in *Dictyostelium* and so on). Given the collective expertise and knowledge of the contributors, I anticipate that this two-volume issue will be an invaluable resource.

The Wnt field advances at an exceptionally rapid rate for several reasons. First, diverse fields of research converge on this pathway. Second, the Wnt community is very generous: reagents, knowledge, and ideas are shared freely. This is facilitated by informative web sites and regular Wnt meetings that are packed back-to-back with cutting-edge research. The “no-frills” approach to these meetings means that the whole community, including students, can participate. Equally important is the elusive nature of the Wnt pathway itself, which continues to intrigue and fascinate both novice and veteran researchers alike. This book is a testament to all these. It was steered by the generosity and enthusiasm of contributors from diverse fields. I thank them all. Special thanks to Randall Moon and Stefan Hoppler; their suggestions for authors and chapters helped shape this issue.

On a personal note, I would also like to take this opportunity to acknowledge Bill Boyle for being an inspirational mentor during my formative years; his infectious enthusiasm for research set me on this exciting and rewarding career path. I am indebted to Bob Thomas and Rob Ramsay for generously supporting my research into FZD7 function in colon cancer when funding in Australia for the Wnt field was scarce in the early years. Most importantly, I thank my very patient and accommodating children for allowing me to indulge myself!

I thank Tony Goodwin, Scott Bowden, and the University of Melbourne—without their assistance this book would not have been possible. John Walker and all at Humana Press, especially David Casey and Amina Ravi, for their generosity and for the opportunity to edit this issue—a truly rewarding experience.

Contents

<i>Preface</i>	v
<i>Contents</i>	vii
<i>Contributors</i>	ix

PART I. WNT SIGNALING PATHWAY METHODS

SECTION A. CANONICAL WNT/FZD SIGNALING

1. The Canonical Wnt/ β -Catenin Signalling Pathway	3
<i>Nick Barker</i>	
2. Isolation and Application of Bioactive Wnt Proteins	17
<i>Karl H. Willert</i>	
3. Purification and Wnt-Inhibitory Activities of Secreted Frizzled-Related Proteins	31
<i>Vladimir Wolf, Yoshimi Endo, and Jeffrey S. Rubin</i>	
4. Measuring GSK3 Expression and Activity in Cells	45
<i>Adam R. Cole and Calum Sutherland</i>	
5. Inhibition of Glycogen Synthase Kinase-3	67
<i>Andrei V. Ougolkov and Daniel D. Billadeau</i>	
6. Detection of Cytoplasmic and Nuclear Localization of Adenomatous Polyposis Coli (APC) Protein in Cells	77
<i>Mariana Brocardo and Beric R. Henderson</i>	
7. Detection of β -Catenin Localization by Immunohistochemistry	91
<i>Nick Barker and Maaïke van den Born</i>	
8: Assaying β -Catenin/TCF Transcription with β -Catenin/TCF Transcription-Based Reporter Constructs	99
<i>Travis L. Biechele and Randall T. Moon</i>	
9. Native Promoter Reporters Validate Transcriptional Targets	111
<i>Otto Schmalhofer, Simone Spaderna, and Thomas Brabletz</i>	

SECTION B. NON-CANONICAL SIGNALING

10. β -Catenin-Independent Wnt Pathways: Signals, Core Proteins, and Effectors	131
<i>Richard G. James, William H. Conrad, and Randall T. Moon</i>	
11. Image Analysis of Calcium Release Dynamics	145
<i>Christina M. Freisinger, Douglas W. Houston, and Diane C. Slusarski</i>	
12. Detecting PKC Phosphorylation as Part of the Wnt/Calcium Pathway in Cutaneous Melanoma	157
<i>Samudra K. Dissanayake and Ashani T. Weeraratna</i>	
13. Measuring CamKII Activity in <i>Xenopus</i> Embryos as a Read-out for Non-canonical Wnt Signaling	173
<i>Michael Kühl and Petra Pandur</i>	

14.	Analysis of Wnt7a-Stimulated JNK Activity and cJun Phosphorylation in Non-Small Cell Lung Cancer Cells	187
	<i>Lynn E. Heasley and Robert A. Winn</i>	
15.	ROCK Enzymatic Assay.	197
	<i>John D. Doran and Marc D. Jacobs</i>	
16.	Detection of Planar Polarity Proteins in Mammalian Cochlea	207
	<i>Mireille Montcouquiol, Jennifer M. Jones, and Nathalie Sans</i>	
PART II. MAMMALIAN MODEL SYSTEMS FOR WNT/FZD FUNCTION		
17.	Proteomic Analyses of Protein Complexes in the Wnt Pathway	223
	<i>Stephane Angers</i>	
18.	In Situ Hybridization to Evaluate the Expression of Wnt and Frizzled Genes in Mammalian Tissues	231
	<i>Kestutis Planutis, Marina Planutiene, and Randall F. Holcombe</i>	
19.	Assaying Wnt5A-Mediated Invasion in Melanoma Cells	243
	<i>Michael P. O'Connell, Amanda D. French, Poloko D. Leotlela, and Ashani T. Weeraratna</i>	
20.	Coculture Methodologies for the Study of Wnt Signals	255
	<i>Kestutis Planutis, Marina Planutiene, and Randall F. Holcombe</i>	
21.	Analysis of Wnt/FZD-Mediated Signalling in a Cell Line Model of Colorectal Cancer Morphogenesis	263
	<i>Elizabeth Vincan, Robert H. Whitehead, and Maree C. Faux</i>	
22.	Analysing Tissue and Gene Function in Intestinal Organ Culture	275
	<i>Helen E. Abud, Heather M. Young, and Donald F. Newgreen</i>	
23.	Genetics of Wnt Signaling During Early Mammalian Development	287
	<i>Terry P. Yamaguchi</i>	
24.	Tissue-Specific Transgenic, Conditional Knockout and Knock-In Mice of Genes in the Canonical Wnt Signaling Pathway	307
	<i>Koji Aoki and Makoto M. Taketo</i>	
	<i>Index</i>	333

Contributors

- HELEN E. ABUD, Ph.D. • *Department of Anatomy and Developmental Biology, Monash University, Clayton, Victoria, Australia*
- STEPHANE ANGERS, Ph.D. • *Leslie Dan Faculty of Pharmacy, University of Toronto, Toronto, Ontario, Canada*
- KOJI AOKI, Ph.D. • *Department of Pharmacology, Graduate School of Medicine, Kyoto University, Yoshida-Konoé-cho, Sakyo, Kyoto, Japan*
- NICK BARKER, Ph.D. • *Hubrecht Institute for Developmental Biology and Stem Cell Research, Utrecht, The Netherlands*
- TRAVIS L. BIECHELE, B.Sc. • *Howard Hughes Medical Institute and Department of Pharmacology and Institute for Stem Cell and Regenerative Medicine, University of Washington School of Medicine, Seattle, WA, USA*
- DANIEL D. BILLADEAU, Ph.D. • *Division of Oncology Research, Mayo Clinic College of Medicine, Rochester MN, USA*
- MAAIKE VAN DEN BORN. • *Hubrecht Institute for Developmental Biology and Stem Cell Research, Utrecht, The Netherlands*
- THOMAS BRABLETZ, M.D., Ph.D. • *Department of Surgery, University of Freiburg, Freiburg, Germany*
- MARIANA BROCARDO, Ph.D. • *Westmead Millennium Institute, The University of Sydney, Westmead, NSW, Australia*
- ADAM R. COLE, Ph.D. • *Pathology and Neurosciences, University of Dundee, Ninewells Hospital, Dundee, Scotland*
- WILLIAM H. CONRAD, Ph.D. • *Howard Hughes Medical Institute, Department of Pharmacology, and Institute for Stem Cell and Regenerative Medicine, University of Washington School of Medicine, Seattle, WA, USA*
- SAMUDRA K. DISSANAYAKE, Ph.D. • *Laboratory of Immunology, National Institutes of Health, National Institute on Aging, Gerontology Research Center, Baltimore, MD, USA*
- JOHN D. DORAN, Ph.D. • *Protein Biochemistry, Vertex Pharmaceuticals, Cambridge, MA, USA*
- YOSHIMI ENDO, M.D., Ph.D. • *National Cancer Institute, Bethesda, MD, USA*
- MAREE C. FAUX, Ph.D. • *Ludwig Institute for Cancer Research, Parkville, Victoria, Australia*
- CHRISTINA M. FREISINGER, Ph.D. • *Department of Biology, University of Iowa, Iowa City, IA, USA*
- AMANDA D. FRENCH, B.Sc. • *Laboratory of Immunology, National Institutes of Health, National Institute on Aging, Gerontology Research Center, Baltimore, MD, USA*
- LYNN E. HEASLEY, Ph.D. • *Department of Medicine, University of Colorado Health Sciences Centre, Denver, Colorado, USA*

- BERIC R. HENDERSON, Ph.D. • *Westmead Millennium Institute, The University of Sydney, Westmead, NSW, Australia*
- RANDALL F. HOLCOMBE, M.D. • *Division of Hematology/Oncology, University of California–Irvine, Orange, CA, USA*
- DOUGLAS W. HOUSTON, Ph.D. • *Department of Biology, University of Iowa, Iowa City, IA, USA*
- MARC D. JACOBS, Ph.D. • *Structural Biology, Vertex Pharmaceuticals, Cambridge, MA, USA*
- RICHARD G. JAMES, Ph.D. • *Howard Hughes Medical Institute, Department of Pharmacology, and Institute for Stem Cell and Regenerative Medicine, University of Washington School of Medicine, Seattle, WA, USA*
- JENNIFER M. JONES, Ph.D. • *Department of Otolaryngology, Washington University School of Medicine, St. Louis, MO, USA*
- MICHAEL KÜHL, Ph.D. • *University of Ulm, Institute for Biochemistry and Molecular Biology, Ulm, Germany*
- POLOKO D. LEOTLELA, Ph.D. • *Laboratory of Immunology, National Institutes of Health, National Institute on Aging, Gerontology Research Center, Baltimore, MD, USA*
- MIREILLE MONTCOUQUIOL, Ph.D. • *Equipe Avenir, Development Neurosciences, INSERM U862, Institut Francois Magendie, Université Bordeaux II, Bordeaux Cédex, France*
- RANDALL T. MOON, Ph.D. • *Howard Hughes Medical Institute, Department of Pharmacology, and Institute for Stem Cell and Regenerative Medicine, University of Washington School of Medicine, Seattle, WA, USA*
- DONALD F. NEWGREEN, Ph.D. • *Murdoch Children’s Research Institute Royal Children’s Hospital, Parkville, Victoria, Australia*
- MICHAEL P. O’CONNELL, Ph.D. • *Laboratory of Immunology, National Institutes of Health, National Institute on Aging, Gerontology Research Center, Baltimore, MD, USA*
- ANDREI V. OUGOLKOV, M.D., Ph.D. • *Division of Oncology Research, Mayo Clinic College of Medicine, Rochester, MN, USA*
- PETRA PANDUR, Ph.D. • *University of Ulm, Institute for Biochemistry and Molecular Biology, Ulm, Germany*
- MARINA PLANUTIENE, Ph.D. • *Division of Hematology/Oncology, University of California–Irvine, Orange, CA, USA*
- KESTUTIS PLANUTIS, Ph.D. • *Division of Hematology/Oncology, University of California–Irvine, Orange, CA, USA*
- JEFFERY S. RUBIN, M.D., Ph.D. • *National Cancer Institute, Bethesda, MD, USA*
- NATHALIE SANS, Ph.D. • *Equipe Avenir, Molecular Neurobiology, INSERM U862, Université Bordeaux II, Bordeaux Cedex, France*
- OTTO SCHMALHOFER, M.Sc. • *Department of Surgery, University of Freiburg, Freiburg, Germany*
- DIANE C. SLUSARSKI, Ph.D. • *Department of Biology, University of Iowa, Iowa City, IA, USA*

- SIMONE SPADERNA, Ph.D. • *Department of Surgery, University of Freiburg, Freiburg, Germany*
- CALUM SUTHERLAND, Ph.D. • *Pathology and Neurosciences, University of Dundee, Ninewells Hospital, Dundee, Scotland*
- MAKOTO M. TAKETO, M.D., Ph.D. • *Department of Pharmacology, Graduate School of Medicine, Kyoto University, Yoshida-Konoé-cho, Sakyo, Kyoto, Japan*
- ELIZABETH VINCAN, Ph.D. • *Cancer Biology Laboratory, Department of Anatomy and Cell Biology, University of Melbourne, Victoria, Australia*
- ASHANI T. WEERARATNA, Ph.D. • *Laboratory of Immunology, National Institutes of Health, National Institute on Aging, Gerontology Research Center, Baltimore, MD, USA*
- ROBERT H. WHITEHEAD, M.Sc., Ph.D. • *Departments of Medicine, Cancer Biology and Cell and Developmental Biology, Vanderbilt University, Nashville, TN, USA*
- KARL H. WILLERT, Ph.D. • *Cellular and Molecular Medicine, University of California–San Diego, La Jolla, CA, USA*
- ROBERT A. WINN, M.D. • *Veterans Affairs Medical Center, Denver, Colorado, USA*
- VLADIMIR WOLF, M.D. • *National Cancer Institute, Bethesda, MD, USA*
- TERRY P. YAMAGUCHI, Ph.D. • *Cancer and Developmental Biology Laboratory, Center for Cancer Research, National Cancer Institute–Frederick, the National Institutes of Health, Frederick, MD, USA*
- HEATHER M. YOUNG, Ph.D. • *Department of Anatomy and Cell Biology, University of Melbourne, Parkville, Victoria, Australia*

Contents of Volume 2

Preface
Contents
Contributors

PART I. INTRODUCTION

1. Evolution of the Wnt pathway
Jenifer C Croce and David R McClay

PART II. DICTYOSTELIUM

2. *Dictyostelium* Development: a Prototypic Wnt Pathway?
Adrian J Harwood
3. Monitoring Patterns of Gene Expression in *Dictyostelium*
by β -galactosidase Staining
Adrian J Harwood
4. Use of the *Dictyostelium* Stalk Cell Assay to Monitor GSK-3 Regulation
Adrian J Harwood

PART III. CNIDARIANS

5. Wnt Signaling in Cnidarians
Thomas W. Holstein
6. Detecting Expression Patterns of Wnt Pathway Components
in *Nematostella vectensis* Embryos
Shalika Kumburegama, Naveen Wijesena, and Athula H. Wikramanayake
7. Detection of Expression Patterns in *Hydra* Pattern Formation
Hans Bode, Tobias Lengfeld, Bert Hobmayer, and Thomas Holstein

PART IV. C. ELEGANS

- 8: Analysis of Wnt Signaling Pathways during *Caenorhabditis elegans*
Postembryonic Development
Samantha Van Hoffelen and Michael A. Herman
9. Wnt Signaling During *Caenorhabditis elegans* Embryonic Development
Daniel J Marston, Minna Roh, Amanda J Mikels, Roel Nusse, and Bob Goldstein

PART V. DROSOPHILA

10. Function of the Wingless Signaling Pathway in *Drosophila*
Foster C Gonsalves and Ramanuj DasGupta
11. Visualisation of PCP Defects in the Eye and Wing of *Drosophila Melanogaster*
Natalia Arbouzova and Helen McNeill
12. Wingless Signaling in *Drosophila* Eye Development
Kevin Legent and Jessica Treisman
13. High-Throughput RNAi Screen in *Drosophila*
Ramanuj DasGupta and Foster C Gonsalves

PART VI. SEA URCHIN

14. Wnt Signaling in Early Sea Urchin Development
Shalika Kumburegama and Athula H. Wikramanayake
15. Detecting Expression Patterns of Wnt Pathway Components
in Sea Urchin Embryos
Joanna M. Bince, Chieh-fu Peng, and Athula H. Wikramanayake
16. Functional Analysis of Wnt Signaling in the Early Sea Urchin Embryo
Using mRNA Microinjection
Joanna M Bince and Athula H. Wikramanayake

PART VII. ZEBRAFISH

17. Wnt Signaling Mediates Diverse Developmental Processes in Zebrafish
Heather Verkade and Joan K Heath
18. Determination of mRNA and Protein Expression Patterns in Zebrafish
Elizabeth L Christie, Adam C Parslow, and Joan K Heath
19. Manipulation of Gene Expression During Zebrafish Embryonic
Development Using Transient Approaches
Benjamin M. Hogan, Heather Verkade, Graham J. Lieschke, and Joan K. Heath
20. Neural Patterning and CNS Functions of Wnt in Zebrafish
Richard I Dorsky

PART VIII. XENOPUS

21. Studying Wnt Signaling in *Xenopus*
Stefan Hoppler

SECTION A: METHODS FOR STUDYING WNT SIGNALING IN XENOPUS EMBRYOS

22. Analysis of Gene Expression in *Xenopus* Embryos
Danielle L Lavery and Stefan Hoppler
23. Detection of Nuclear β -catenin in *Xenopus* Embryos
Francois Fagotto and Carolyn M Brown
24. Transgenic Reporter Tools Tracing Endogenous Canonical
Wnt Signaling in *Xenopus*
Tinneke Denayer, Hong Thi Tran, and Kris Vleminckx
25. Gain-of-Function and Loss-of-Function strategies in *Xenopus*
Danielle L Lavery and Stefan Hoppler
26. How the Mother Can Help: Studying Maternal Wnt Signaling
by Anti-sense-mediated Depletion of Maternal mRNAs
and the Host Transfer Technique
Adnan Mir and Janet Heasman
27. Inducible Gene Expression in Transient Transgenic *Xenopus* Embryos
Grant N Wheeler, Danielle L Lavery, and Stefan Hoppler
28. Wnt-Frizzled Interactions in *Xenopus*
Herbert Steinbeisser and Rajeeb K Swain

SECTION B: WNT SIGNALING FUNCTION IN *XENOPUS* DEVELOPMENT

29. Dorsal Axis Duplication as a Functional Readout for Wnt Activity
Michael Kühl and Petra Pandur
30. Regulation of Convergent Extension by Non-canonical Wnt Signaling
in the *Xenopus* Embryo
Lars F Petersen, Hiromasa Ninomiya, and Rudolf Winklbauer
31. Frizzled-7-Dependent Tissue Separation in the *Xenopus* Gastrula
Rudolf Winklbauer and Olivia Luu

Index