Cell Migration

METHODS IN MOLECULAR BIOLOGY™

John M. Walker, SERIES EDITOR

- 309 RNA Silencing: Methods and Protocols**, edited by Gordon Carmichael, 2005
- 308 Therapeutic Proteins: Methods and Protocols**, edited by C. Mark Smales and David C. James, 2005
- 307 Phosphodiesterase Methods and Protocols**, edited by Claire Lugnier, 2005
- 306 Receptor Binding Techniques: Second Edition**, edited by Anthony P. Davenport, 2005
- 305 Protein–Ligand Interactions: Methods and Protocols**, edited by G. Ulrich Nienhaus, 2005
- 304 Human Retrovirus Protocols: Virology and Molecular Biology**, edited by Tuofu Zhu, 2005
- 303 NanoBiotechnology Protocols**, edited by Sandra J. Rosenthal and David W. Wright, 2005
- 302 Handbook of ELISPOT: Methods and Protocols**, edited by Alexander E. Kalyuzhny, 2005
- 301 Ubiquitin–Proteasome Protocols**, edited by Cam Patterson and Douglas M. Cyr, 2005
- 300 Protein Nanotechnology: Protocols, Instrumentation, and Applications**, edited by Tuan Vo-Dinh, 2005
- 299 Amyloid Proteins: Methods and Protocols**, edited by Einar M. Sigurdsson, 2005
- 298 Peptide Synthesis and Application**, edited by John Howl, 2005
- 297 Forensic DNA Typing Protocols**, edited by Angel Carracedo, 2005
- 296 Cell Cycle Protocols**, edited by Tim Humphrey and Gavin Brooks, 2005
- 295 Immunochemical Protocols, Third Edition**, edited by Robert Burns, 2005
- 294 Cell Migration: Developmental Methods and Protocols**, edited by Jun-Lin Guan, 2005
- 293 Laser Capture Microdissection: Methods and Protocols**, edited by Graeme I. Murray and Stephanie Curran, 2005
- 292 DNA Viruses: Methods and Protocols**, edited by Paul M. Lieberman, 2005
- 291 Molecular Toxicology Protocols**, edited by Phouthone Keohavong and Stephen G. Grant, 2005
- 290 Basic Cell Culture, Third Edition**, edited by Cheryl D. Helgason and Cindy Miller, 2005
- 289 Epidermal Cells, Methods and Applications**, edited by Kursad Turksen, 2005
- 288 Oligonucleotide Synthesis, Methods and Applications**, edited by Piet Herdewijn, 2005
- 287 Epigenetics Protocols**, edited by Trygve O. Tollefsbol, 2004
- 286 Transgenic Plants: Methods and Protocols**, edited by Leandro Peña, 2005
- 285 Cell Cycle Control and Dysregulation Protocols: Cyclins, Cyclin-Dependent Kinases, and Other Factors**, edited by Antonio Giordano and Gaetano Romano, 2004
- 284 Signal Transduction Protocols, Second Edition**, edited by Robert C. Dickson and Michael D. Mendenhall, 2004
- 283 Bioconjugation Protocols**, edited by Christof M. Niemeyer, 2004
- 282 Apoptosis Methods and Protocols**, edited by Hugh J. M. Brady, 2004
- 281 Checkpoint Controls and Cancer, Volume 2: Activation and Regulation Protocols**, edited by Axel H. Schönthal, 2004
- 280 Checkpoint Controls and Cancer, Volume 1: Reviews and Model Systems**, edited by Axel H. Schönthal, 2004
- 279 Nitric Oxide Protocols, Second Edition**, edited by Aviv Hassid, 2004
- 278 Protein NMR Techniques, Second Edition**, edited by A. Kristina Downing, 2004
- 277 Trinucleotide Repeat Protocols**, edited by Yoshinori Kohwi, 2004
- 276 Capillary Electrophoresis of Proteins and Peptides**, edited by Mark A. Strege and Avinash L. Lagu, 2004
- 275 Chemoinformatics**, edited by Jürgen Bajorath, 2004
- 274 Photosynthesis Research Protocols**, edited by Robert Carpentier, 2004
- 273 Platelets and Megakaryocytes, Volume 2: Perspectives and Techniques**, edited by Jonathan M. Gibbins and Martyn P. Mahaut-Smith, 2004
- 272 Platelets and Megakaryocytes, Volume 1: Functional Assays**, edited by Jonathan M. Gibbins and Martyn P. Mahaut-Smith, 2004
- 271 B Cell Protocols**, edited by Hua Gu and Klaus Rajewsky, 2004
- 270 Parasite Genomics Protocols**, edited by Sara E. Melville, 2004
- 269 Vaccina Virus and Poxvirology: Methods and Protocols**, edited by Stuart N. Isaacs, 2004
- 268 Public Health Microbiology: Methods and Protocols**, edited by John F. T. Spencer and Alicia L. Ragout de Spencer, 2004
- 267 Recombinant Gene Expression: Reviews and Protocols, Second Edition**, edited by Paulina Balbas and Argelia Johnson, 2004
- 266 Genomics, Proteomics, and Clinical Bacteriology: Methods and Reviews**, edited by Neil Woodford and Alan Johnson, 2004
- 265 RNA Interference, Editing, and Modification: Methods and Protocols**, edited by Jonatha M. Gott, 2004
- 264 Protein Arrays: Methods and Protocols**, edited by Eric Fung, 2004
- 263 Flow Cytometry, Second Edition**, edited by Teresa S. Hawley and Robert G. Hawley, 2004
- 262 Genetic Recombination Protocols**, edited by Alan S. Waldman, 2004
- 261 Protein–Protein Interactions: Methods and Applications**, edited by Haian Fu, 2004

METHODS IN MOLECULAR BIOLOGY™

Cell Migration

Developmental Methods and Protocols

Edited by

Jun-Lin Guan

*The Department of Molecular Medicine,
College of Veterinary Medicine, Cornell University, Ithaca, NY*

HUMANA PRESS TOTOWA, NEW JERSEY

© 2005 Humana Press Inc.
999 Riverview Drive, Suite 208
Totowa, New Jersey 07512

www.humanapress.com

All rights reserved. No part of this book may be reproduced, stored in a retrieval system, or transmitted in any form or by any means, electronic, mechanical, photocopying, microfilming, recording, or otherwise without written permission from the Publisher. Methods in Molecular Biology™ is a trademark of The Humana Press Inc.

All papers, comments, opinions, conclusions, or recommendations are those of the author(s), and do not necessarily reflect the views of the publisher.

This publication is printed on acid-free paper.
ANSI Z39.48-1984 (American Standards Institute)

Permanence of Paper for Printed Library Materials.

Production Editor: C. Tirpak
Cover design by Patricia F. Cleary

Cover Illustration: From Fig. 1, Chapter 7, "Cell-Scatter Assay," by Hong-Chen Chen.

For additional copies, pricing for bulk purchases, and/or information about other Humana titles, contact Humana at the above address or at any of the following numbers: Tel: 973-256-1699; Fax: 973-256-8341; E-mail: humana@humanapr.com; or visit our Website: www.humanapress.com

Photocopy Authorization Policy:

Authorization to photocopy items for internal or personal use, or the internal or personal use of specific clients, is granted by Humana Press Inc., provided that the base fee of US \$25.00 per copy is paid directly to the Copyright Clearance Center at 222 Rosewood Drive, Danvers, MA 01923. For those organizations that have been granted a photocopy license from the CCC, a separate system of payment has been arranged and is acceptable to Humana Press Inc. The fee code for users of the Transactional Reporting Service is: [1-58829-382-3/05 \$25.00].

Printed in the United States of America. 10 9 8 7 6 5 4 3 2 1

ISSN 1064-3745

E-ISBN 1-59259-860-9

Cell migration : developmental methods and protocols / edited by Jun-Lin Guan.

p. ; cm. -- (Methods in molecular biology ; 294)

Includes bibliographical references and index.

ISBN 1-58829-382-3 (alk. paper)

1. Cell migration--Laboratory manuals. I. Guan, Jun-Lin. II. Series:

Methods in molecular biology (Clifton, N.J.) ; v. 294.

[DNLN: 1. Cell Movement--physiology--Laboratory Manuals. 2. Biological Assay--methods--Laboratory Manuals. 3. Cell Movement--genetics--Laboratory Manuals. W1 ME9616J v.294 2005 / QH 647 C3929 2005]

QH647.C4385 2005

571.6--dc22

2004016499

Preface

Cell migration plays an essential role in a variety of biological processes, including embryonic development, homeostasis, immune responses, wound healing, and cancer metastasis. Indeed, one of the defining features of life is the ability to move, which occurs at different levels from the macromolecular machines in a cell, to individual or populations of cells, and to the whole organism. The dramatic movements of layers of cells in gastrulation, a critical period of embryonic development of many animals, as well as the swimming of simple unicellular amoeba in search of nutrients, have fascinated biologists for years.

Cell migration is a complex process and has attracted the interest of researchers from diverse disciplines in biology. However, like many other areas of investigation, the recent exciting and explosive growth of cell migration studies are critically linked to the rapid advance of cell and molecular biology and its increasing influence on all the other fields of life sciences and biomedical research. Thanks to rapid progress at the cellular and molecular levels, unprecedented knowledge has been gathered regarding the components and interactions of the macromolecular machinery of cell movement, the signaling mechanisms and pathways involved in cellular responses to chemotactic and haptotactic stimulations, and the regulatory mechanisms that link the signaling pathways to the cell movement machinery.

A second major contributor to our modern understanding of, and research on, cell migration is the recent creation and success in using various model organisms in the study of cell migration. The power of genetics in these systems allowed for the discovery of novel molecules and signaling pathways that are important in the regulation of cell migration. The conservative nature of many of these molecules and pathways has also allowed researchers to move from one system to another when putting together the various puzzle pieces of the regulatory mechanisms, taking advantage of our knowledge in each of these different systems. Last but not least, the use of model organisms has also allowed scientists to study the intrinsically interesting biology of cell migration in the context of development and other biological processes *in vivo*.

A third emerging trend of modern cell migration research is the increasing use of more sophisticated imaging and other novel genetic approaches. However, it is important to note that many of the simple and classic cell migration assays developed in earlier times are still part of toolboxes of cell

migration researchers, and indeed they provide the basis from which the newer variations and novel approaches are developed. The combination of this long history with the recent expansion has ensured the development of a large number of methodologies and experimental protocols in the cell migration field that are derived from diverse biological disciplines, and sometimes as results of interdisciplinary efforts.

The aim of *Cell Migration: Developmental Methods and Protocols* is to bring together a wide range of novel and state-of-the-art methodologies as well as various classic methods in cell migration research in cultured cells, different model organisms, and as applied to specialized cells in normal development and in disease. Each chapter is presented in the user friendly format of the highly successful *Method in Molecular Biology* series by leading experts in their field who have contributed significantly to the development of the methods and/or applied the methods successfully in their research on various aspects of cell migration. This volume begins with an overview of the cell migration field that also discusses the methods presented. Part II (Chapters 2–8) describes various basic assays that apply to all cell migration studies in vitro. Part III (Chapters 9–12) includes cell migration assays for cancer cells, endothelial cells, and neurons both in vitro and in animal models. Part IV (Chapters 13–19) discusses cell migration assays in various model organisms. Part V (Chapters 20–24) combines chapters on biochemical methods to study the mechanisms of cell migration and several new approaches to the study of cell migration.

The overall goal of *Cell Migration: Developmental Methods and Protocols* is to provide comprehensive coverage on all relevant methods in one volume so that anyone who is interested in cell migration research can rely on it as a useful reference. It is intended for both beginning students and active researchers in the field. The primary target audience is cell and developmental biologists. However, it should also be very useful to geneticists, biochemists, and molecular biologists, as well as clinicians and others who are interested in looking at the mechanisms of cell migration or use of cell migration as biological assays in their studies of molecular mechanisms or disease processes.

The publication of *Cell Migration: Developmental Methods and Protocols* would not have been possible without outstanding contributions from all the authors. I am extremely grateful for their generosity in sharing with the readers their expertise and perspective on the various methods and protocols described. My heartfelt thanks also go to Cindy Westmiller, who has provided tremendous assistance in proofreading all the chapters and organizing the manuscripts. I would also like to acknowledge the series editor, Dr. John Walker, for his valuable input and guidance during the editing process.

We all share the hope that this book will find its place on the shelves on many laboratories and be used as a frequent reference by students and experienced researchers alike in the exciting field of cell migration studies.

Jun-Lin Guan

Contents

Preface	v
Contributors	xiii

PART I. INTRODUCTION

1 Cell Migration: <i>An Overview</i> <i>Donna J. Webb, Huaye Zhang, and Alan F. Horwitz</i>	3
--	---

PART II. BASIC CELL MIGRATION AND RELATED ASSAYS

2 Boyden Chamber Assay <i>Hong-Chen Chen</i>	15
3 Wound-Healing Assay <i>Luis G. Rodriguez, Xiaoyang Wu, and Jun-Lin Guan</i>	23
4 Analysis of Cell Migration Using the Dunn Chemotaxis Chamber and Time-Lapse Microscopy <i>Claire M. Wells and Anne J. Ridley</i>	31
5 Cell-Adhesion Assays <i>Dennis F. Kucik and Chuanyue Wu</i>	43
6 Cell-Spreading Assays <i>Allison L. Berrier and Susan E. LaFlamme</i>	55
7 Cell-Scatter Assay <i>Hong-Chen Chen</i>	69
8 Cell Migration Analyses Within Fibroblast-Derived 3-D Matrices <i>Edna Cukierman</i>	79

PART III. ANALYSIS OF CELL MIGRATION IN SPECIALIZED CELL SYSTEMS

9 Tumor Cell Invasion Assays <i>Leslie M. Shaw</i>	97
10 Analysis of Endothelial Cell Migration Under Flow <i>Song Li</i>	107
11 Angiogenesis Assays in the Chick CAM <i>Chris Storgard, David Mikolon, and Dwayne G. Stupack</i>	123

- 12 Investigations of Neuronal Migration in the Central Nervous System
Michael E. Ward and Yi Rao 137

PART IV. ANALYSIS OF CELL MIGRATION IN MODEL ORGANISMS

- 13 Analysis of Cell Migration in *Caenorhabditis elegans*
M. Afaq Shakir and Erik A. Lundquist 159
- 14 Analysis of Cell Migration Using *Drosophila* as a Model System
Jocelyn A. McDonald and Denise J. Montell 175
- 15 Imaging Cell Movement During Dorsal Closure in *Drosophila* Embryos
William Wood and Antonio Jacinto 203
- 16 Analysis of Cell Movements in Zebrafish Embryos: Morphometrics and Measuring Movement of Labeled Cell Populations In Vivo
Diane S. Sepich and Lilianna Solnica-Krezel 211
- 17 The *Xenopus* Embryo as a Model System for Studies of Cell Migration
Douglas W. DeSimone, Lance Davidson, Mungo Marsden, and Dominique Alfandari 235
- 18 Neural Crest Migration Methods in the Chicken Embryo
Maria Elena de Bellard and Marianne Bronner-Fraser 247
- 19 In Vivo and In Vitro Models of Mammalian Angiogenesis
Mien V. Hoang and Donald R. Senger 269

PART V. BIOCHEMICAL AND NOVEL APPROACHES IN CELL MIGRATION

- 20 Measurement of Protein Tyrosine Phosphorylation in Cell Adhesion
Vidhya V. Iyer, Christoph Ballestrem, Joachim Kirchner, Benjamin Geiger, and Michael D. Schaller 289
- 21 Cell-Adhesion Assays: Fabrication of an E-Cadherin Substratum and Isolation of Lateral and Basal Membrane Patches
Frauke Drees, Amy Reilein, and W. James Nelson 303
- 22 Application of Microscope-Based FRET to Study Molecular Interactions in Focal Adhesions of Live Cells
Christoph Ballestrem and Benjamin Geiger 321
- 23 Use of Multiphoton Imaging for Studying Cell Migration in the Mouse
Andrea Flesken-Nikitin, Rebecca M. Williams, Warren R. Zipfel, Watt W. Webb, and Alexander Yu. Nikitin 335

24 Using Microfluidic Channel Networks to Generate Gradients
for Studying Cell Migration
*Daniel S. Rhoads, Sharvari M. Nadkarni, Loling Song,
Camilla Voeltz, Eberhard Bodenschatz,
and Jun-Lin Guan* 347

Index 359

Contributors

DOMINIQUE ALFANDARI • *Department of Cell Biology, University of Virginia School of Medicine, Charlottesville, VA*

CHRISTOPH BALLESTREM • *Department of Molecular Cell Biology, The Weizmann Institute of Science, Rehovot, Israel*

ALLISON L. BERRIER • *LSU School of Dentistry, Department of Cell Biology and Anatomy, New Orleans, LA*

EBERHARD BODENSCHATZ • *Department of Physics, Cornell University, Ithaca, NY*

MARIANNE BRONNER-FRASER • *Division of Biology, California Institute of Technology, Pasadena, CA*

HONG-CHEN CHEN • *Department of Life Science, National Chung Hsing University, Taichung, Taiwan*

EDNA CUKIERMAN • *Tumor Cell Biology, Basic Science, Fox Chase Cancer Center, Philadelphia, PA*

LANCE DAVIDSON • *Department of Cell Biology, University of Virginia School of Medicine, Charlottesville, VA*

MARIA ELENA DE BELLARD • *Division of Biology, California Institute of Technology, Pasadena, CA*

DOUGLAS W. DESIMONE • *Department of Cell Biology, University of Virginia School of Medicine, Charlottesville, VA*

FRAUKE DREES • *Department of Molecular and Cellular Physiology, Beckman Center for Molecular and Genetic Medicine, Stanford University School of Medicine, Stanford, CA*

ANDREA FLESKEN-NIKITIN • *Department of Biomedical Sciences, Cornell University, Ithaca, NY*

BENJAMIN GEIGER • *Department of Molecular Cell Biology, The Weizmann Institute of Science, Rehovot, Israel*

JUN-LIN GUAN • *Department of Molecular Medicine, Cornell University College of Veterinary Medicine, Ithaca, NY*

MIEN V. HOANG • *Division of Cancer Biology and Angiogenesis, Department of Pathology, Beth Israel Deaconess Medical Center and Harvard Medical School, Boston, MA*

ALAN F. HORWITZ • *Department of Cell Biology, University of Virginia, Charlottesville, VA*

VIDHYA V. IYER • *Department of Cell and Developmental Biology, University of North Carolina-Chapel Hill, Chapel Hill, NC*

- ANTONIO JACINTO • *Instituto Gulbenkian de Ciência, Rua da Quinta Grande, Oeiras, Portugal*
- JOACHIM KIRCHNER • *Department of Molecular Cell Biology, The Weizmann Institute of Science, Rehovot, Israel*
- DENNIS F. KUCIK • *Research Service, Birmingham VA Medical Center, and Department of Pathology, University of Alabama at Birmingham, Center for Biophysical Sciences and Engineering, Pittsburgh, PA*
- SUSAN E. LAFLAMME • *Center for Cell Biology and Cancer Research, Albany Medical College, Albany, NY*
- SONG LI • *Department of Bioengineering and The Center for Tissue Engineering, University of California, Berkeley, CA*
- ERIK A. LUNDQUIST • *Department of Molecular Biosciences, University of Kansas, Lawrence, KS*
- MUNGO MARSDEN • *Department of Cell Biology, University of Virginia School of Medicine, Charlottesville, VA*
- JOCELYN A. McDONALD • *Department of Biological Chemistry, The Johns Hopkins University School of Medicine, Baltimore, MD*
- DAVID MIKOLON • *Department of Immunology, The Scripps Research Institute, La Jolla, CA*
- DENISE J. MONTELL • *Department of Biological Chemistry, The Johns Hopkins University School of Medicine, Baltimore, MD*
- SHARVARI M. NADKARNI • *Department of Physics, Cornell University, Ithaca, NY*
- W. JAMES NELSON • *Department of Molecular and Cellular Physiology, Beckman Center for Molecular and Genetic Medicine, Stanford University School of Medicine, Stanford, CA*
- ALEXANDER YU. NIKITIN • *Department of Biomedical Sciences, Cornell University, Ithaca, NY*
- YI RAO • *Department of Anatomy and Neurobiology, Washington University School of Medicine, St. Louis, MO*
- AMY REILEIN • *Department of Molecular and Cellular Physiology, Beckman Center for Molecular and Genetic Medicine, Stanford University School of Medicine, Stanford, CA*
- DANIEL S. RHOADS • *Department of Molecular Medicine, Cornell University, Ithaca, NY*
- ANNE J. RIDLEY • *Department of Biochemistry and Molecular Biology, University College London, London, UK and Ludwig Institute for Cancer Research, Royal Free and University College Medical School Branch, London, UK*
- LUIS G. RODRIGUEZ • *Department of Molecular Medicine, Cornell University College of Veterinary Medicine, Ithaca, NY*

- MICHAEL D. SCHALLER • *Department of Cell and Developmental Biology, University of North Carolina-Chapel Hill, Chapel Hill, NC*
- DONALD R. SENGER • *Division of Cancer Biology and Angiogenesis, Department of Pathology, Beth Israel Deaconess Medical Center and Harvard Medical School, Boston, MA*
- DIANE S. SEPICH • *Department of Biological Sciences, Vanderbilt University, Nashville, TN*
- M. AFAQ SHAKIR • *Department of Molecular Biosciences, University of Kansas, Lawrence, KS*
- LESLIE M. SHAW • *Division of Cancer Biology and Angiogenesis, Department of Pathology, Beth Israel Deaconess Medical Center and Harvard Medical School, Boston, MA*
- LILIANNA SOLNICA-KREZEL • *Department of Biological Sciences, Vanderbilt University, Nashville, TN*
- LOLING SONG • *Department of Physics, Cornell University, Ithaca, NY*
- CHRIS STORGARD • *Department of Immunology, The Scripps Research Institute, La Jolla, CA*
- DWAYNE G. STUPACK • *Department of Immunology, The Scripps Research Institute, La Jolla, CA*
- CAMILLA VOELTZ • *Department of Physics, Cornell University, Ithaca, NY*
- MICHAEL E. WARD • *Department of Anatomy and Neurobiology, Washington University School of Medicine, St. Louis, MO*
- DONNA J. WEBB • *Department of Cell Biology, University of Virginia, Charlottesville, VA*
- WATT W. WEBB • *Department of Applied and Engineering Physics, Cornell University, Ithaca, NY*
- CLAIRE M. WELLS • *Randall Centre, King's College London, London, UK*
Ludwig Institute for Cancer Research, Royal Free and University College Medical School Branch, London, UK
- REBECCA M. WILLIAMS • *Department of Applied and Engineering Physics, Cornell University, Ithaca, NY*
- WILLIAM WOOD • *Instituto Gulbenkian de Ciência, Rua da Quinta Grande, Oeiras, Portugal*
- CHUANYUE WU • *Department of Pathology, University of Pittsburgh, Pittsburgh, PA*
- XIAOYANG WU • *Department of Molecular Medicine, Cornell University College of Veterinary Medicine, Ithaca, NY*
- HUAYE ZHANG • *Department of Cell Biology, University of Virginia, Charlottesville, VA*
- WARREN R. ZIPFEL • *Department of Applied and Engineering Physics, Cornell University, Ithaca, NY*