

METHODS IN MOLECULAR BIOLOGY

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

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

Lipid Signaling Protocols

Edited by

Mark G. Waugh

*University College London, School of Life and Medical Sciences,
London, United Kingdom*

 Humana Press

Editor

Mark G. Waugh
University College London
School of Life and Medical Sciences
London, United Kingdom

ISSN 1064-3745 ISSN 1940-6029 (electronic)
Methods in Molecular Biology
ISBN 978-1-4939-3169-9 ISBN 978-1-4939-3170-5 (eBook)
DOI 10.1007/978-1-4939-3170-5

Library of Congress Control Number: 2015947784

Springer New York Heidelberg Dordrecht London
© Springer Science+Business Media New York 2016

This work is subject to copyright. All rights are reserved by the Publisher, whether the whole or part of the material is concerned, specifically the rights of translation, reprinting, reuse of illustrations, recitation, broadcasting, reproduction on microfilms or in any other physical way, and transmission or information storage and retrieval, electronic adaptation, computer software, or by similar or dissimilar methodology now known or hereafter developed.

The use of general descriptive names, registered names, trademarks, service marks, etc. in this publication does not imply, even in the absence of a specific statement, that such names are exempt from the relevant protective laws and regulations and therefore free for general use.

The publisher, the authors and the editors are safe to assume that the advice and information in this book are believed to be true and accurate at the date of publication. Neither the publisher nor the authors or the editors give a warranty, express or implied, with respect to the material contained herein or for any errors or omissions that may have been made.

Printed on acid-free paper

Humana Press is a brand of Springer
Springer Science+Business Media LLC New York is part of Springer Science+Business Media (www.springer.com)

Preface

Lipids are of central importance in the regulation of many aspects of cell function including receptor signaling, vesicle trafficking, and motility. There are a very large number of therapeutically important G-protein-coupled receptors and receptor tyrosine kinases that activate lipid signaling pathways and a range of diseases including some cancers and neurodegenerative conditions that are associated with defective intracellular lipid metabolism. Furthermore, as our knowledge of the molecular basis of human genetic disease expands so too does the long list of rare but often devastating inherited diseases that arise from mutations in genes encoding enzymes involved in lipid signaling. Therefore, there is a requirement for sensitive, reliable, and quantitative laboratory methods to investigate this medically important area of biochemistry.

However, mainly because of their characteristic insolubility in aqueous buffers, lipids have collectively proven to be more difficult to study than other biomolecules such as proteins and nucleic acids. There are very few off-the-shelf commercially available kits to purify and quantify signaling lipids, such as for example the seven different phosphoinositide species, and compared to other bioscience disciplines there has then been less progress generally in the development of easy to use, inexpensive, and highly selective techniques to measure and detect these molecules in cultured cells. Nevertheless, despite these various hurdles, progress is being made in laboratories across the globe to develop robust protocols to study lipid signaling at the cellular level, and many of these cutting-edge techniques are described in detail in this volume.

In this second edition of *Lipid Signaling*, tried and tested methods are described to measure the synthesis of lipids such as the phosphoinositides, ceramides, and sphingomyelin, and to molecularly characterize the various kinases and phosphatases that regulate their levels in cells. As lipid signaling occurs within membranes and is known to be sensitive to alterations in membrane environment, there are also all several chapters detailing strategies to isolate, characterize and image receptor-initiated signaling cascades in detergent-resistant membrane domains and cholesterol-rich lipid rafts. These detailed experimental protocols are complemented by review chapters that highlight the technical considerations, challenges, and potential pitfalls associated with using these laboratory-based approaches.

As editor I have been struck by the enthusiasm of the lipid research community to contribute to this book. This completely rewritten second edition of *Lipid Signaling* has truly been an international project. The finished product represents the combined work of 47 authors from 4 continents and I am very grateful to all of them for their efforts. Finally I would like to thank Prof John Walker, *Methods in Molecular Biology* series editor at Springer, for his editorial guidance during the preparation of this book.

London, UK

Mark G. Waugh

Contents

<i>Preface</i>	<i>v</i>
<i>Contributors</i>	<i>ix</i>
1 Method for Assaying the Lipid Kinase Phosphatidylinositol-5-phosphate 4-kinase α in Quantitative High-Throughput Screening (qHTS) Bioluminescent Format	1
<i>Mindy I. Davis, Atsuo T. Sasaki, and Anton Simeonov</i>	
2 Assaying Ceramide Synthase Activity In Vitro and in Living Cells Using Liquid Chromatography-Mass Spectrometry.	11
<i>Xin Ying Lim, Russell Pickford, and Anthony S. Don</i>	
3 Fluorescent Assays for Ceramide Synthase Activity.	23
<i>Timothy A. Couttas and Anthony S. Don</i>	
4 Identification of the Interactome of a Palmitoylated Membrane Protein, Phosphatidylinositol 4-Kinase Type II Alpha	35
<i>Avanti Gokhale, Pearl V. Ryder, Stephanie A. Zlatic, and Victor Faundez</i>	
5 Measurement of Long-Chain Fatty Acyl-CoA Synthetase Activity	43
<i>Joachim Füllekrug and Margarete Poppelreuther</i>	
6 Qualitative and Quantitative In Vitro Analysis of Phosphatidylinositol Phosphatase Substrate Specificity.	55
<i>Laura Ren Huey Ip and Christina Anja Gewinner</i>	
7 Luciferase Reporter Assays to Assess Liver X Receptor Transcriptional Activity	77
<i>Matthew C. Gage, Benoit Pourcet, and Inés Pineda-Torra</i>	
8 Metabolically Biotinylated Reporters for Electron Microscopic Imaging of Cytoplasmic Membrane Microdomains	87
<i>Kimberly J. Krager and John G. Koland</i>	
9 Fluorescence Recovery After Photobleaching Analysis of the Diffusional Mobility of Plasma Membrane Proteins: HER3 Mobility in Breast Cancer Cell Membranes.	97
<i>Mitul Sarkar and John G. Koland</i>	
10 Isolation and Analysis of Detergent-Resistant Membrane Fractions	107
<i>Massimo Aureli, Sara Grassi, Sandro Sonnino, and Alessandro Prinetti</i>	
11 Detection of Isolated Mitochondria-Associated ER Membranes Using the Sigma-1 Receptor	133
<i>Abasha Lewis, Shang-Yi Tsai, and Tsung-Ping Su</i>	

12	Using Surface Plasmon Resonance to Quantitatively Assess Lipid–Protein Interactions	141
	<i>Kathryn Del Vecchio and Robert V. Stabelin</i>	
13	Analyzing Protein–Phosphoinositide Interactions with Liposome Flotation Assays.	155
	<i>Ricarda A. Busse, Andreea Scacioc, Amanda M. Schalk, Roswitha Krick, Michael Thumm, and Karin Kühnel</i>	
14	High-Throughput Fluorometric Assay for Membrane–Protein Interaction.	163
	<i>Wonhwa Cho, Hyunjin Kim, and Yusi Hu</i>	
15	Guidelines for the Use of Protein Domains in Acidic Phospholipid Imaging.	175
	<i>Matthieu Pierre Platre and Yvon Jaillais</i>	
16	Analysis of Sphingolipid Synthesis and Transport by Metabolic Labeling of Cultured Cells with [³ H]Serine.	195
	<i>Neale D. Ridgway</i>	
17	Determination and Characterization of Tetraspanin-Associated Phosphoinositide-4 Kinases in Primary and Neoplastic Liver Cells	203
	<i>Krista Rombouts and Vinicio Carloni</i>	
18	Analysis of the Phosphoinositide Composition of Subcellular Membrane Fractions	213
	<i>Deborah A. Sarkes and Lucia E. Rameh</i>	
19	Single-Molecule Imaging of Signal Transduction via GPI-Anchored Receptors.	229
	<i>Kenichi G.N. Suzuki</i>	
20	Measuring Phosphatidylinositol Generation on Biological Membranes.	239
	<i>Mark G. Waugh</i>	
21	Assay for CDP-Diacylglycerol Generation by CDS in Membrane Fractions	247
	<i>Mark G. Waugh</i>	
	<i>Index</i>	255

Contributors

- MASSIMO AURELI • *Department of Medical Biotechnology and Translational Medicine, University of Milan, Segrate, Milano, Italy*
- RICARDA A. BUSSE • *Department of Neurobiology, Max-Planck-Institute for Biophysical Chemistry, Göttingen, Germany*
- VINICIO CARLONI • *Department of Experimental and Clinical Medicine, Center for Research, Transfer and High Education, DENOthe, University of Florence, Florence, Italy*
- WONHWA CHO • *Department of Chemistry, University of Illinois at Chicago, Chicago, IL, USA*
- TIMOTHY A. COUTTAS • *Prince of Wales Clinical School, Faculty of Medicine, University of New South Wales, Sydney, NSW, Australia*
- MINDY I. DAVIS • *Division of Preclinical Innovation, National Center for Advancing Translational Sciences, National Institutes of Health, Rockville, MD, USA*
- ANTHONY S. DON • *Prince of Wales Clinical School, Faculty of Medicine, University of New South Wales, Sydney, NSW, Australia*
- VICTOR FAUNDEZ • *Department of Cell Biology, Emory University, Atlanta, GA, USA; Center for Social Translational Neuroscience, Emory University, Atlanta, GA, USA*
- JOACHIM FÜLLEKRUG • *Molecular Cell Biology Laboratory, Internal Medicine IV, University of Heidelberg, Heidelberg, Germany*
- MATTHEW C. GAGE • *Division of Medicine, Centre for Clinical Pharmacology, University College of London, London, UK*
- CHRISTINA ANJA GEWINNER • *Translational Innovation Group, UCL-Eisai Collaborative, University College London, London, UK*
- AVANTI GOKHALE • *Department of Cell Biology, Emory University, Atlanta, GA, USA*
- SARA GRASSI • *Department of Medical Biotechnology and Translational Medicine, University of Milan, Segrate, Milano, Italy*
- YUSI HU • *Department of Chemistry, University of Illinois at Chicago, Chicago, IL, USA*
- LAURA REN HUEY IP • *Research Department of Cancer Biology, UCL Cancer Institute, University College London, London, UK*
- YVON JAILLAIS • *Laboratoire de Reproduction et Développement des Plantes, CNRS, INRA, ENS Lyon, UCBL, Université de Lyon, Lyon Cedex, France*
- HYUNJIN KIM • *Department of Chemistry, University of Illinois at Chicago, Chicago, IL, USA*
- JOHN G. KOLAND • *Department of Pharmacology, Carver College of Medicine, The University of Iowa, Iowa City, IA, USA*
- KIMBERLY J. KRAGER • *Department of Pharmacology, Carver College of Medicine, The University of Iowa, Iowa City, IA, USA; Division of Radiation Health, College of Pharmacy, University of Arkansas for Medical Sciences, Little Rock, AR, USA*

- ROSWITHA KRICK • *Institute of Cellular Biochemistry, University Medicine, Georg-August University, Göttingen, Germany*
- KARIN KÜHNEL • *Department of Neurobiology, Max-Planck-Institute for Biophysical Chemistry, Göttingen, Germany*
- ABASHA LEWIS • *Cellular Pathobiology Section, IRP, DHHS, NIDA, NIH, Baltimore, MD, USA*
- XIN YING LIM • *Prince of Wales Clinical School, Faculty of Medicine, University of New South Wales, Sydney, NSW, Australia*
- RUSSELL PICKFORD • *Bioanalytical Mass Spectrometry Facility, University of New South Wales, Sydney, NSW, Australia*
- INÉS PINEDA-TORRA • *Division of Medicine, Centre for Clinical Pharmacology, University College of London, London, UK*
- MATTHIEU PIERRE PLATRE • *Laboratoire de Reproduction et Développement des Plantes, CNRS, INRA, ENS Lyon, UCBL, Université de Lyon, Lyon Cedex, France*
- MARGARETE POPPELREUTHER • *Molecular Cell Biology Laboratory Internal Medicine IV, University of Heidelberg, Heidelberg, Germany*
- BENOIT POURCET • *Division of Medicine, Centre for Clinical Pharmacology, University College of London, London, UK; UMR INSERM 1011, Institut Pasteur de Lille, Université Lille Nord de France, Université Lille 2, Lille Cedex, France*
- ALESSANDRO PRINETTI • *Department of Medical Biotechnology and Translational Medicine, University of Milan, Segrate, Milano, Italy*
- LUCIA E. RAMEH • *Department of Medicine, Boston University School of Medicine, Boston, MA, USA*
- NEALE D. RIDGWAY • *Department of Pediatrics, Dalhousie University, Halifax, NS, Canada; Department of Biochemistry and Molecular Biology, Dalhousie University, Halifax, NS, Canada*
- KRISTA ROMBOUITS • *Division of Medicine, Institute for Liver and Digestive Health, Royal Free Hospital, University College London (UCL), London, UK*
- PEARL V. RYDER • *Department of Cell Biology, Emory University, Atlanta, GA, USA*
- MITUL SARKAR • *Department of Pharmacology, Carver College of Medicine, The University of Iowa, Iowa City, IA, USA*
- DEBORAH A. SARKES • *U.S. Army Research Laboratory, Sensors and Electron Devices Directorate, Adelphi, MD, USA*
- ATSUO T. SASAKI • *Department of Internal Medicine, Division of Hematology Oncology UC Cancer Institute, University of Cincinnati, OH, USA; UC Neuroscience Institute, Department of Neurosurgery, Brain Tumor Center, UC Neuroscience Institute, College of Medicine, University of Cincinnati, Cincinnati, OH, USA*
- ANDREEA SCACIOC • *Department of Neurobiology, Max-Planck-Institute for Biophysical Chemistry, Göttingen, Germany*
- AMANDA M. SCHALK • *Department of Neurobiology, Max-Planck-Institute for Biophysical Chemistry, Göttingen, Germany; Department of Biochemistry and Molecular Genetics, University of Illinois at Chicago, Chicago, IL, USA*
- ANTON SIMEONOV • *Division of Preclinical Innovation, National Center for Advancing Translational Sciences, National Institutes of Health, Rockville, MD, USA*
- SANDRO SONNINO • *Department of Medical Biotechnology and Translational Medicine, University of Milan, Segrate, Milano, Italy*

ROBERT V. STAHELIN • *Department of Chemistry and Biochemistry, University of Notre Dame, Notre Dame, IN, USA; Department of Biochemistry and Molecular Biology, Indiana University School of Medicine-South Bend, South Bend, IN, USA*

TSUNG-PING SU • *Cellular Pathobiology Section, IRP, DHHS, NIDA, NIH, Baltimore, MD, USA*

KENICHI G.N. SUZUKI • *Institute for Integrated Cell-Material Sciences (WPI-iCeMS), Institute for Frontier Medical Sciences, Kyoto University, Kyoto, Japan; Institute for Stem Cell Biology and Regenerative Medicine (inStem), National Centre for Biological Sciences (NCBS), Bangalore, India*

MICHAEL THUMM • *Institute of Cellular Biochemistry, University Medicine, Georg-August University, Göttingen, Germany*

SHANG-YI TSAI • *Cellular Pathobiology Section, IRP, DHHS, NIDA, NIH, Baltimore, MD, USA*

KATHRYN DEL VECCHIO • *Department of Chemistry and Biochemistry, University of Notre Dame, Notre Dame, IN, USA*

MARK G. WAUGH • *University College London, School of Life and Medical Sciences, London, United Kingdom*

STEPHANIE A. ZLATIC • *Department of Cell Biology, Emory University, Atlanta, GA, USA*