

Methods in
ENZYMOLOGY

Volume 535

**Endosome Signaling
Part B**

Edited by

P. Michael Conn



VOLUME FIVE HUNDRED AND THIRTY FIVE

METHODS IN ENZYMOLGY

Endosome Signaling Part B

Edited by

P. MICHAEL CONN

Senior Vice President for Research

Associate Professor

Professor of Internal Medicine and Cell Biology

Texas Tech University Health Sciences Center

Lubbock, TX 79430, USA



ELSEVIER

AMSTERDAM • BOSTON • HEIDELBERG • LONDON
NEW YORK • OXFORD • PARIS • SAN DIEGO
SAN FRANCISCO • SINGAPORE • SYDNEY • TOKYO

Academic Press is an imprint of Elsevier



Academic Press is an imprint of Elsevier
525 B Street, Suite 1800, San Diego, CA 92101-4495, USA
225 Wyman Street, Waltham, MA 02451, USA
Radarweg 29, PO Box 211, 1000 AE Amsterdam, The Netherlands
The Boulevard, Langford Lane, Kidlington, Oxford, OX5 1GB, UK
32 Jamestown Road, London NW1 7BY, UK

First edition 2014

Copyright © 2014 Elsevier Inc. All Rights Reserved.

No part of this publication may be reproduced, stored in a retrieval system or transmitted in any form or by any means electronic, mechanical, photocopying, recording or otherwise without the prior written permission of the publisher

Permissions may be sought directly from Elsevier's Science & Technology Rights Department in Oxford, UK: phone (+44) (0) 1865 843830; fax (+44) (0) 1865 853333; email: permissions@elsevier.com. Alternatively you can submit your request online by visiting the Elsevier web site at <http://elsevier.com/locate/permissions>, and selecting *Obtaining permission to use Elsevier material*

Notice

No responsibility is assumed by the publisher for any injury and/or damage to persons or property as a matter of products liability, negligence or otherwise, or from any use or operation of any methods, products, instructions or ideas contained in the material herein. Because of rapid advances in the medical sciences, in particular, independent verification of diagnoses and drug dosages should be made

For information on all Academic Press publications
visit our website at store.elsevier.com

ISBN: 978-0-12-397925-4

ISSN: 0076-6879

Printed and bound in United States of America

14 15 16 17 11 10 9 8 7 6 5 4 3 2 1



Working together
to grow libraries in
developing countries

www.elsevier.com • www.bookaid.org



VOLUME FIVE HUNDRED AND THIRTY FIVE

**METHODS IN
ENZYMOMOLOGY**

Endosome Signaling Part B

METHODS IN ENZYMOLOGY

Editors-in-Chief

JOHN N. ABELSON and MELVIN I. SIMON

*Division of Biology
California Institute of Technology
Pasadena, California*

ANNA MARIE PYLE

*Departments of Molecular, Cellular and Developmental
Biology and Department of Chemistry
Investigator, Howard Hughes Medical Institute
Yale University*

GREGORY L. VERDINE

*Department of Chemistry and Chemical Biology
Harvard University*

Founding Editors

SIDNEY P. COLOWICK and NATHAN O. KAPLAN

CONTRIBUTORS

Veronica Aran

Division of Cellular and Molecular Physiology, Institute of Translational Medicine, University of Liverpool, Liverpool, United Kingdom

François Authier

Service information scientifique et technique (IST) de l'Inserm, Délégation régionale Inserm Paris V, Paris, France

Masahiro Azuma

Department of Microbiology and Immunology, Hokkaido University Graduate School of Medicine, Kita-ku, Sapporo, Japan

Tadashi Baba

Department of Microbiology and Infection Control Science, Juntendo University Graduate School of Medicine, Tokyo, Japan

Rachel Barrow

Centre for Tumour Biology, Barts Cancer Institute – A Cancer Research UK Centre of Excellence, Queen Mary University of London, John Vane Science Centre, London, United Kingdom

John J.M. Bergeron

Department of Medicine, and Department of Cell Biology, McGill University, Montreal, Quebec, Canada

Rose Cairns

Faculty of Pharmacy, University of Sydney, Sydney, New South Wales, Australia

Eric C. Chang

Lester and Sue Smith Breast Center, Department of Molecular and Cellular Biology, Baylor College of Medicine, Houston, Texas, USA

Xue Chen

Atopy (Allergy) Research Center, Juntendo University Graduate School of Medicine, Tokyo, Japan, and Department of Dermatology, Peking University People's Hospital, Beijing, China

Helen R. Clark

Virginia Bioinformatics Institute, and Department of Biochemistry, Virginia Tech, Blacksburg, Virginia, USA

Bernard Desbuquois

Inserm U567, Institut Cochin, CNRS UMR 8104, Université Paris-Descartes, Paris, France

Gianni M. Di Guglielmo

Department of Physiology and Pharmacology, Western University, London, Ontario, Canada

Nikolai Engedal

Prostate Cancer Research Group, Centre for Molecular Medicine Norway, Nordic EMBL Partnership, University of Oslo and Oslo University Hospital, Oslo, Norway

Carlos Enrich

Departament de Biologia Cellular, Immunologia i Neurociències, IDIBAPS, Facultat de Medicina, Universitat de Barcelona, Barcelona, Spain

Marco Falasca

Inositide Signalling Group, Centre for Diabetes, Blizard Institute, Barts and The London School of Medicine and Dentistry, Queen Mary University of London, London, United Kingdom

Gareth W. Fearnley

Endothelial Cell Biology Unit, School of Molecular & Cellular Biology, University of Leeds, Leeds, United Kingdom

Theodore Fotsis

Laboratory of Biological Chemistry, Medical School, University of Ioannina, and Department of Biomedical Research, Foundation for Research & Technology – Hellas, Institute of Molecular Biology & Biotechnology, University Campus of Ioannina, Ioannina, Greece

Jürgen Fritsch

Institute of Immunology, Christian-Albrechts-University of Kiel, Kiel, Germany

Kenji Funami

Department of Microbiology and Immunology, Hokkaido University Graduate School of Medicine, Kita-ku, Sapporo, Japan

Mariona Gelibert-Baldrich

Departament de Biologia Cellular, Immunologia i Neurociències, IDIBAPS, Facultat de Medicina, Universitat de Barcelona, Barcelona, Spain

Thomas Grewal

Faculty of Pharmacy, University of Sydney, Sydney, New South Wales, Australia

Neil Grimsey

Department of Pharmacology, School of Medicine, University of California, La Jolla, California, USA

Mutsuko Hara

Atopy (Allergy) Research Center, Juntendo University Graduate School of Medicine, Tokyo, Japan

Michael A. Harrison

School of Biomedical Sciences, University of Leeds, Leeds, United Kingdom

Tristan A. Hayes

Virginia Bioinformatics Institute, and Department of Biological Sciences, Virginia Tech, Blacksburg, Virginia, USA

Carina Hellberg

University of Birmingham, School of Biosciences, Birmingham, United Kingdom

Maria Hernandez-Valladares

Division of Cellular and Molecular Physiology, Institute of Translational Medicine, University of Liverpool, Liverpool, United Kingdom

Keiichi Hiramatsu

Department of Microbiology and Infection Control Science, Juntendo University Graduate School of Medicine, Tokyo, Japan

Monira Hoque

Faculty of Pharmacy, University of Sydney, Sydney, New South Wales, Australia

Lukas A. Huber

Biocenter, Division of Cell Biology, Innsbruck Medical University, Innsbruck, Austria

Shigaku Ikeda

Atopy (Allergy) Research Center, Juntendo University Graduate School of Medicine, Tokyo, Japan, and Department of Dermatology and Allergology, Juntendo University Graduate School of Medicine, Tokyo, Japan

Roshanak Irannejad

Department of Psychiatry and, Department of Cellular & Molecular Pharmacology, University of California School of Medicine, San Francisco, California, USA

Kamil Jastrzębski

Laboratory of Cell Biology, International Institute of Molecular and Cell Biology, Warsaw, Poland

Carine Joffre

Centre for Tumour Biology, Barts Cancer Institute – A Cancer Research UK Centre of Excellence, Queen Mary University of London, John Vane Science Centre, London, United Kingdom, and UNITE 830 INSERM, Institut Curie Centre de Recherche, Paris Cedex 05, France

Shiv D. Kale

Virginia Bioinformatics Institute, Virginia Tech, Blacksburg, Virginia, USA

Seiji Kamijo

Atopy (Allergy) Research Center, Juntendo University Graduate School of Medicine, Tokyo, Japan

Junko Kawasaki

Atopy (Allergy) Research Center, and Department of Dermatology and Allergology, Juntendo University Graduate School of Medicine, Tokyo, Japan

Stéphanie Kermorgant

Centre for Tumour Biology, Barts Cancer Institute – A Cancer Research UK Centre of Excellence, Queen Mary University of London, John Vane Science Centre, London, United Kingdom

Hirokazu Kinoshita

Atopy (Allergy) Research Center, and Department of Dermatology and Allergology, Juntendo University Graduate School of Medicine, Tokyo, Japan

Eleftherios Kostaras

Laboratory of Biological Chemistry, Medical School, University of Ioannina, and Department of Biomedical Research, Foundation for Research & Technology – Hellas, Institute of Molecular Biology & Biotechnology, University Campus of Ioannina, Ioannina, Greece

Sarah J. Kotowski

Department of Psychiatry and, Department of Cellular & Molecular Pharmacology, University of California School of Medicine, San Francisco, California, USA

Antony M. Latham

Endothelial Cell Biology Unit, School of Molecular & Cellular Biology, University of Leeds, Leeds, United Kingdom

Tuan Anh Le

Atopy (Allergy) Research Center; Department of Dermatology and Allergology, Juntendo University Graduate School of Medicine, Tokyo, Japan, and Department of Dermatology and Allergology, Institute of Clinical Medical and Pharmaceutical Sciences 108, Hanoi, Vietnam

Huilan Lin

Department of Pharmacology, School of Medicine, University of California, La Jolla, California, USA

Tania Maffucci

Inositide Signalling Group, Centre for Diabetes, Blizard Institute, Barts and The London School of Medicine and Dentistry, Queen Mary University of London, London, United Kingdom

Bénédicte Manoury

INSERM, Unité 1013, and Université Paris Descartes, Sorbonne Paris Cité, Faculté de médecine, Paris, France

Misako Matsumoto

Department of Microbiology and Immunology, Hokkaido University Graduate School of Medicine, Kita-ku, Sapporo, Japan

Sarah McLean

Department of Anatomy and Cell Biology, Department of Physiology and Pharmacology, Western University, London, Ontario, Canada

Marta Miaczynska

Laboratory of Cell Biology, International Institute of Molecular and Cell Biology, Warsaw, Poland

Ian G. Mills

Prostate Cancer Research Group, Centre for Molecular Medicine Norway, Nordic EMBL Partnership, University of Oslo; Department of Cancer Prevention, Institute of Cancer Research; Department of Urology, Oslo University Hospital, Oslo, Norway, and Uro-Oncology Research Group, Cambridge Research Institute, University of Cambridge, Cambridge, United Kingdom

Ludovic Ménard

Centre for Tumour Biology, Barts Cancer Institute – A Cancer Research UK Centre of Excellence, Queen Mary University of London, John Vane Science Centre, London, United Kingdom

Jessica G. Moreland

Division of Critical Care, Department of Pediatrics and the Inflammation Program, The University of Iowa, Iowa City, Iowa, USA

Shunsuke Mori

Department of Oncogene Research, Research Institute for Microbial Diseases, Osaka University, Suita, Osaka, Japan

Carol Murphy

Department of Biomedical Research, Foundation for Research & Technology – Hellas, Institute of Molecular Biology & Biotechnology, University Campus of Ioannina, Ioannina, Greece

Shigeyuki Nada

Department of Oncogene Research, Research Institute for Microbial Diseases, Osaka University, Suita, Osaka, Japan

Adam F. Odell

Endothelial Cell Biology Unit, School of Molecular & Cellular Biology, University of Leeds, Leeds, United Kingdom

Hideoki Ogawa

Atopy (Allergy) Research Center, Juntendo University Graduate School of Medicine, Tokyo, Japan, and Department of Dermatology and Allergology, Juntendo University Graduate School of Medicine, Tokyo, Japan

Masato Okada

Department of Oncogene Research, Research Institute for Microbial Diseases, Osaka University, Suita, Osaka, Japan

Ko Okumura

Atopy (Allergy) Research Center, Juntendo University Graduate School of Medicine, Tokyo, Japan

A. Paige Davis Volk

Division of Critical Care, Department of Pediatrics and the Inflammation Program, The University of Iowa, Iowa City, Iowa, USA

Nina Marie Pedersen

Centre for Cancer Biomedicine, Faculty of Medicine, University of Oslo, Oslo, Norway

Albert Pol

Departament de Biologia Cel·lular, Immunologia i Neurociències, IDIBAPS, Facultat de Medicina, Universitat de Barcelona, and ICREA, Institució Catalana de Recerca Avançada, Barcelona, Spain

Sreenivasan Ponnambalam

Endothelial Cell Biology Unit, School of Molecular & Cellular Biology, University of Leeds, Leeds, United Kingdom

Barry I. Posner

Department of Medicine, and Department of Cell Biology, McGill University, Montreal, Quebec, Canada

Ian A. Prior

Division of Cellular and Molecular Physiology, Institute of Translational Medicine, University of Liverpool, Liverpool, United Kingdom

Elżbieta Purta

Laboratory of Bioinformatics and Protein Engineering, International Institute of Molecular and Cell Biology, Warsaw, Poland

Carles Rentero

Departament de Biologia Celular, Immunologia i Neurociències, IDIBAPS, Facultat de Medicina, Universitat de Barcelona, Barcelona, Spain

Łukasz Sadowski

Laboratory of Cell Biology, International Institute of Molecular and Cell Biology, Warsaw, Poland

Julia M. Scheffler

Biocenter, Division of Cell Biology, Innsbruck Medical University, Innsbruck, Austria

Natalia Schiefermeier

Biocenter, Division of Cell Biology, Innsbruck Medical University, Innsbruck, Austria

Stefan Schütze

Institute of Immunology, Christian-Albrechts-University of Kiel, Kiel, Germany

Tsukasa Seya

Department of Microbiology and Immunology, Hokkaido University Graduate School of Medicine, Kita-ku, Sapporo, Japan

Sudha K. Shenoy

Department of Medicine, Duke University Medical Center, Durham, North Carolina, USA

Gina A. Smith

Endothelial Cell Biology Unit, School of Molecular & Cellular Biology, University of Leeds, Leeds, United Kingdom

Harald Stenmark

Centre for Cancer Biomedicine, Faculty of Medicine, University of Oslo, Oslo, Norway

Yusuke Takahashi

Department of Oncogene Research, Research Institute for Microbial Diseases, Osaka University, Suita, Osaka, Japan

Toshiro Takai

Atopy (Allergy) Research Center, Juntendo University Graduate School of Medicine, Tokyo, Japan

Megumi Tatematsu

Department of Microbiology and Immunology, Hokkaido University Graduate School of Medicine, Kita-ku, Sapporo, Japan

Vladimir Tchikov

Institute of Immunology, Christian-Albrechts-University of Kiel, Kiel, Germany

Francesc Tebar

Departament de Biologia Cel·lular, Immunologia i Neurociències, IDIBAPS, Facultat de Medicina, Universitat de Barcelona, Barcelona, Spain

Mira Tohmé

INSERM, Unité 1013; Université Paris Descartes, Sorbonne Paris Cité, Faculté de médecine, and INSERM, Unité 932, Institut Curie, Paris, France

Darren C. Tomlinson

Biomedical Health Research Centre & Astbury Centre for Structural Molecular Biology, University of Leeds, Leeds, United Kingdom

JoAnn Trejo

Department of Pharmacology, School of Medicine, University of California, La Jolla, California, USA

Hiroko Ushio

Atopy (Allergy) Research Center, Juntendo University Graduate School of Medicine, Tokyo, Japan

Mark von Zastrow

Department of Psychiatry and, Department of Cellular & Molecular Pharmacology, University of California School of Medicine, San Francisco, California, USA

Anh Tuan Vu

Atopy (Allergy) Research Center; Department of Dermatology and Allergology, Juntendo University Graduate School of Medicine, Tokyo, Japan, and Quynhoa National Leprosy-Dermatology Hospital, Quynhon, Vietnam

Stephen B. Wheatcroft

Division of Diabetes and Cardiovascular Research, Faculty of Medicine & Health, University of Leeds, Leeds, United Kingdom

Yang Xie

Atopy (Allergy) Research Center, Juntendo University Graduate School of Medicine, Tokyo, Japan, and Department of Dermatology, The 3rd Affiliated Hospital of Sun Yat-sen University, Guangzhou, China

Ahmed Zahraoui

Phagocytosis and Bacterial Invasion Laboratory, INSERM U.1016-CNRS UMR8104, Institut Cochin, Université Paris Descartes, Paris, France

Ze-Yi Zheng

Lester and Sue Smith Breast Center, Department of Molecular and Cellular Biology, Baylor College of Medicine, Houston, Texas, USA

PREFACE

Endosomes are membrane-bound compartments that transport internalized material from the plasma membrane to the lysosome and elsewhere. These compartments, often about 500 nm, but ranging in size, have the capability to sort molecules, routing some contents to the lysosomes for degradation, and recycling other materials back to the plasma membrane. The Golgi apparatus also provides molecules to the endosome, some of which are delivered to lysosomes and others are recycled back to the Golgi. Because of this ability to differentially deliver molecules, the endosome is viewed as a pre-sorting structure.

Endosomes are categorized by size, enzymatic content, morphology, and by other criteria such as the length of time it takes internalized material to reach them. Endosomes may provide platforms for cross talk between signaling systems, and this consideration has provided them elite status among cellular components that contribute to signaling.

This volume provides descriptions of the range of methods used to analyze and evaluate these important compartments. The authors explain how these methods are able to provide important biological insights in the context of particular models.

Authors were selected based on both their research contributions and on their ability to describe their methodological contributions in a clear and reproducible way. They have been encouraged to make use of graphics, comparisons to other methods, and to provide tricks and approaches not revealed in prior publications that make it possible to adapt their methods to other systems.

The editor wants to express appreciation to the contributors for providing their contributions in a timely fashion, to the senior editors for guidance, and to the staff at Academic Press for helpful input.

P. MICHAEL CONN
Lubbock, TX, USA

CONTENTS

Contributors

xiii

Preface

xxi

Section I

SIGNALING

1. Assessment of Insulin Proteolysis in Rat Liver Endosomes: Its Relationship to Intracellular Insulin Signaling	3
François Authier and Bernard Desbuquois	
1. Introduction	4
2. Assays for Insulin Degradation	5
3. Assay for Endosomal Proteolysis of Insulin	7
4. Sites of Cleavage and Degradation Products of Insulin within Endosomes	12
5. Endosomal Insulinases	14
6. Effects of Insulin and Slowly Processed Insulin Analogs on Insulin Signaling in Liver Endosomes	15
7. Involvement of IR Endocytosis in Positive Regulation of Insulin Signaling	20
References	21
2. A Bimolecular Fluorescent Complementation Screen Reveals Complex Roles of Endosomes in Ras-Mediated Signaling	25
Ze-Yi Zheng and Eric C. Chang	
1. Introduction	26
2. Detect Ras–effector Interactions by BiFC	30
3. Screen for Ras-binding Proteins by BiFC	33
4. Concluding Remarks	36
Acknowledgments	37
References	37
3. TGFβ in Endosomal Signaling	39
Sarah McLean and Gianni M. Di Guglielmo	
1. Introduction	40
2. Endocytosis	40
3. The Role of the Early Endosome in TGF β Signal Transduction	45

4. Readouts of TGF β Signal Transduction	50
Acknowledgments	52
References	52
4. Annexins and Endosomal Signaling	55
Francesc Tebar, Mariona Gelabert-Baldrich, Monira Hoque, Rose Cairns, Carles Rentero, Albert Pol, Thomas Grewal, and Carlos Enrich	
1. Introduction	56
2. Isolation of Endocytic Compartments	59
3. Targeting Raf-1 Signaling to Early Endosomes	63
4. Monitoring Endosomal Signaling by Fluorescence Resonance Energy Transfer Microscopy	68
5. Targeting Annexins to Endosomes and Other Cellular Compartments	69
6. Summary	70
Acknowledgments	70
References	71
5. Analysis, Regulation, and Roles of Endosomal Phosphoinositides	75
Tania Maffucci and Marco Falasca	
1. Introduction	76
2. Endosomal PIs	76
3. Analysis of PtdIns3P Levels	78
4. Monitoring PtdIns3P Intracellular Localization	82
5. Endosomal PtdIns3P	85
6. Phosphatidylinositol 3,5-Bisphosphate	87
Acknowledgments	88
References	88
6. Mild Fixation and Permeabilization Protocol for Preserving Structures of Endosomes, Focal Adhesions, and Actin Filaments During Immunofluorescence Analysis	93
—Julia M. Scheffler, Natalia Schiefermeier, and Lukas A. Huber	
1. Introduction	94
2. Molecular Tools	95
3. Saponin Treatment Enhances the Preservation of Peripheral Endosomes After Fixation	96
4. Mild Fixation Allows Preserving the mCherry and GFP Localized to Focal Adhesions and Late Endosomes	98
5. Summary	101

Acknowledgment	101
References	101
7. Characterizing and Measuring Endocytosis of Lipid-Binding Effectors in Mammalian Cells	103
Helen R. Clark, Tristan A. Hayes, and Shiv D. Kale	
1. Introduction	104
2. Culturing and Maintenance of Mammalian Cell Lines	105
3. Transfection of Mammalian Cells	106
4. Protein Purification and Preparation	108
5. Treatment of Cells	110
6. Tracking Endocytosis by Confocal Microscopy	110
7. Quantification of Cell Entry by Fluorescent Microtiter Plate Reader	113
8. Quantification of Cell Entry by Flow Cytometry	115
9. Summary	117
Acknowledgments	118
References	118
8. Measuring the Role for Met Endosomal Signaling in Tumorigenesis	121
Rachel Barrow, Carine Joffre, Ludovic Ménard, and Stéphanie Kermorgant	
1. Introduction	122
2. Analyzing Met Trafficking with Fluorescence and Confocal Microscopy	123
3. Analyzing Met Internalization Using Flow Cytometry	127
4. Analyzing Met Internalization, Recycling, and Degradation with Surface Met Biotinylation	130
5. Analyzing Met Endosomal Signaling <i>In Vitro</i> and <i>In Vivo</i>	134
6. Summary	138
References	139
9. Intracellular Toll-Like Receptor Recruitment and Cleavage in Endosomal/Lysosomal Organelles	141
Mira Tohmé and Bénédicte Manoury	
1. Introduction	142
2. Purification of Endosomes and Lysosomes	142
3. Proteases Assays	145
4. Intracellular TLR Processing	145
References	146

10. Assessment of the Toll-Like Receptor 3 Pathway in Endosomal Signaling	149
Misako Matsumoto, Kenji Funami, Megumi Tatematsu, Masahiro Azuma, and Tsukasa Seya	
1. Introduction	150
2. Analyses of TLR3 Expression and Localization	151
3. Assay for TLR3-Mediated Signaling	154
4. Assay for DC-Mediated NK Activation	158
5. Assay for DC-Mediated CTL Activation	160
6. Summary	162
Acknowledgments	162
References	162
11. Labeling of Platelet-Derived Growth Factor by Reversible Biotinylation to Visualize Its Endocytosis by Microscopy	167
Łukasz Sadowski, Kamil Jastrzębski, Elżbieta Purta, Carina Hellberg, and Marta Miaczynska	
1. Introduction	168
2. PDGF Labeling with Sulfo- <i>N</i> -Hydroxysuccinimide-SS-Biotin	169
3. Determination of the Extent of PDGF Biotinylation with Mass Spectrometry	171
4. Determination of a Biological Activity of bt-PDGF	172
5. Stimulation of Cells with bt-PDGF and Removal of Extracellular Biotins	173
6. Validation and Detection of bt-PDGF in Microscopical Assays	174
7. Conclusion	175
Acknowledgments	176
References	176
12. Endosomal Signaling and Oncogenesis	179
Nikolai Engedal and Ian G. Mills	
1. Introduction	180
2. Receptors	181
3. Adaptor Proteins and Oncogenesis	188
References	195
13. ROS-Containing Endosomal Compartments: Implications for Signaling	201
A. Paige Davis Volk and Jessica G. Moreland	
1. Introduction	202
2. Overview of Endocytosis	203