

# 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>



# **Rab GTPases**

## **Methods and Protocols**

Edited by

**Guangpu Li**

*Department of Biochemistry and Molecular Biology, University of Oklahoma Health Sciences Center,  
Oklahoma City, OK, USA; Peggy and Charles Stephenson Cancer Center, University of Oklahoma  
Health Sciences Center, Oklahoma City, OK, USA*

 **Humana Press**

*Editor*

Guangpu Li  
Department of Biochemistry and Molecular Biology  
University of Oklahoma Health Sciences Center  
Oklahoma City, OK, USA

Peggy and Charles Stephenson Cancer Center  
University of Oklahoma Health Sciences Center  
Oklahoma City, OK, USA

ISSN 1064-3745                      ISSN 1940-6029 (electronic)  
Methods in Molecular Biology  
ISBN 978-1-4939-2568-1              ISBN 978-1-4939-2569-8 (eBook)  
DOI 10.1007/978-1-4939-2569-8

Library of Congress Control Number: 2015934919

Springer New York Heidelberg Dordrecht London  
© Springer Science+Business Media New York 2015

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](http://www.springer.com))

---

## Preface

Rab GTPases are master regulators of intracellular membrane trafficking in all eukaryotes from the last eukaryotic common ancestor (LECA) to human. In the human genome, there are 66 Rab genes some of which are ubiquitously expressed in all tissues, while others are expressed only in specific tissues. Individual Rabs target to distinct organelles and vesicles to promote vesicular transport through the exocytic and endocytic pathways, which are fundamental to cell physiology and have great impact on other cellular processes, such as hormone secretion, signal transduction, cell migration, and cell growth/differentiation. Indeed, mutations and/or altered expression of Rabs has been implicated in various human diseases ranging from neurodegenerative diseases, diabetes to cancer.

This book covers the latest technological advances in the characterization of the biosynthesis and functions of Rab GTPases and their regulation by guanine nucleotide exchange factors (GEFs) and GTPase-activating proteins (GAPs). The methods are described in detail so that beginners and experts alike can explore the general biochemical principles of Rab GTPase cycle and membrane targeting in vesicular transport and the specific functions of individual Rabs in different cell types. As such, this book should provide a valuable resource for researchers and students interested in the field.

The book consists of 28 chapters, starting with an overview of the Rab GTPase family, which represents the largest branch of the Ras superfamily of small GTPases that are essentially everywhere in the cell and function as molecular switches in regulation of diverse cellular functions by alternating between active GTP-bound and inactive GDP-bound conformations. The next group of chapters describes systematic approaches to the identification and classification of Rabs and Rab GAPs as well as the detection of Rab isoprenylation and membrane distribution. The following chapters examine the biochemical and functional properties of individual Rabs in the order of exocytic, recycling, and endocytic Rabs. The techniques range from in vitro approaches using reconstituted systems to in vivo studies in yeast, *Drosophila*, and mammalian cells. These techniques may also be useful for the study of Rabs in other organisms, especially the large number of uncharacterized Rabs identified through genome sequencing projects.

*Oklahoma City, OK, USA*

*Guangpu Li*



---

# Contents

|  |           |
|--|-----------|
| <i>Preface</i> . . . . .   | <i>v</i>  |
| <i>Contributors</i> . . . . .  | <i>xi</i> |
| 1 Rab Family of GTPases . . . . .<br><i>Guangpu Li and M. Caleb Marlin</i>   | 1         |
| 2 Bioinformatic Approaches to Identifying and Classifying Rab Proteins . . . . .<br><i>Yoon Diekmann and José B. Pereira-Leal</i>  | 17        |
| 3 Rab-NANOPS: FRET Biosensors for Rab Membrane Nanoclustering<br>and Prenylation Detection in Mammalian Cells . . . . .<br><i>Arafath Kaja Najumudeen, Camilo Guzmán, Itziar M.D. Posada,<br/>and Daniel Abankwa</i> | 29        |
| 4 High-Throughput Assay for Profiling the Substrate Specificity<br>of Rab GTPase-Activating Proteins . . . . .<br><i>Ashwini K. Mishra and David G. Lambright</i>  | 47        |
| 5 Measuring Rab GTPase-Activating Protein (GAP) Activity in Live Cells<br>and Extracts . . . . .<br><i>Ryan M. Nottingham and Suzanne R. Pfeffer</i>   | 61        |
| 6 Analysis of the Interactions Between Rab GTPases and Class V Myosins . . . . .<br><i>Andrew J. Lindsay, Stéphanie Miserey-Lenkei, and Bruno Goud</i>   | 73        |
| 7 Assaying the Interaction of the Rab Guanine Nucleotide Exchange<br>Protein Sec2 with the Upstream Rab, a Downstream Effector,<br>and a Phosphoinositide . . . . .<br><i>Danièle Stalder and Peter J. Novick</i>    | 85        |
| 8 Kinetic Activation of Rab8 Guanine Nucleotide Exchange Factor Rabin8<br>by Rab11 . . . . .<br><i>Shanshan Feng, Bin Wu, Johan Peränen, and Wei Guo</i>   | 99        |
| 9 Ypt1 and TRAPP Interactions: Optimization of Multicolor<br>Bimolecular Fluorescence Complementation in Yeast. . . . .<br><i>Zhanna Lipatova, Jane J. Kim, and Nava Segev</i>                                       | 107       |
| 10 Identifying a Rab Effector on the Macroautophagy Pathway . . . . .<br><i>Juan Wang, Serena Cervantes, Saralin Davis, and Susan Ferro-Novick</i>   | 117       |
| 11 Functional Analysis of Rab27A and Its Effector Slp2-a<br>in Renal Epithelial Cells . . . . .<br><i>Takao Yasuda, Paulina S. Mrozowska, and Mitsunori Fukuda</i>   | 127       |
| 12 Small GTPases in Acrosomal Exocytosis . . . . .<br><i>Matias A. Bustos, Ornella Lucchesi, Maria C. Ruete, Luis S. Mayorga,<br/>and Claudia N. Tomes</i>   | 141       |
| 13 Rab Antibody Characterization: Comparison of Rab14 Antibodies . . . . .<br><i>Andrew J. Lindsay and Mary W. McCaffrey</i>   | 161       |

|    |  |     |
|----|--|-----|
| 14 | Selective Visualization of GLUT4 Storage Vesicles and Associated Rab Proteins Using IRAP-pHluorin . . . . .  | 173 |
|    | <i>Yu Chen and Jennifer Lippincott-Schwartz</i>  |     |
| 15 | 3D Time-Lapse Analysis of Rab11/FIP5 Complex: Spatiotemporal Dynamics During Apical Lumen Formation . . . . .  | 181 |
|    | <i>Anthony Mangan and Rytis Prekeris</i>   |     |
| 16 | In Vitro and In Vivo Characterization of the Rab11-GAP Activity of <i>Drosophila</i> Evi5 . . . . .  | 187 |
|    | <i>Carl Laflamme and Gregory Emery</i>   |     |
| 17 | Characterization of the Role Rab25 in Energy Metabolism and Cancer Using Extracellular Flux Analysis and Material Balance . . . . .                        | 195 |
|    | <i>Shreya Mitra, Jennifer Molina, Gordon B. Mills, and Jennifer B. Dennison</i>  |     |
| 18 | Measurement of Rab35 Activity with the GTP-Rab35 Trapper RBD35 . . . . .   | 207 |
|    | <i>Hotaka Kobayashi, Kan Etoh, Soujiro Marubashi, Norihiko Ohbayashi, and Mitsunori Fukuda</i>   |     |
| 19 | Analysis of Connecdenn 1–3 (DENN1A-C) GEF Activity for Rab35 . . . . .   | 217 |
|    | <i>Patrick D. Allaire, Peter S. McPherson, and Brigitte Ritter</i>   |     |
| 20 | Assay of Rab17 and Its Guanine Nucleotide Exchange Factor Rabex-5 in the Dendrites of Hippocampal Neurons . . . . .  | 233 |
|    | <i>Yasunori Mori and Mitsunori Fukuda</i>  |     |
| 21 | Methods for Analysis of AP-3/Rabin4' in Regulation of Lysosome Distribution . . . . .  | 245 |
|    | <i>Viorica Ivan and Peter van der Sluijs</i>   |     |
| 22 | Determination of Rab5 Activity in the Cell by Effector Pull-Down Assay. . . . .  | 259 |
|    | <i>Yaoyao Qi, Zhimin Liang, Zonghua Wang, Guodong Lu, and Guangpu Li</i>   |     |
| 23 | Identification of the Rab5 Binding Site in p110 $\beta$ : Assays for PI3K $\beta$ Binding to Rab5. . . . .   | 271 |
|    | <i>Rachel S. Salamon, Hashem A. Dbouk, Denise Collado, Jaclyn Lopiccolo, Anne R. Bresnick, and Jonathan M. Backer</i>                                      |     |
| 24 | Role of the Rab5 Guanine Nucleotide Exchange Factor, Rme-6, in the Regulation of Clathrin-Coated Vesicle Uncoating . . . . .                               | 283 |
|    | <i>Elizabeth Smythe</i>  |     |
| 25 | Differential Effects of Overexpression of Rab5 and Rab22 on Autophagy in PC12 Cells with or without NGF . . . . .  | 295 |
|    | <i>M. Caleb Marlin and Guangpu Li</i>  |     |
| 26 | Determining the Role of Rab7 in Constitutive and Ligand-Mediated Epidermal Growth Factor Receptor Endocytic Trafficking Using Single Cell Assays . . . . . | 305 |
|    | <i>Brian P. Ceresa</i>   |     |



27 Visualizing Directional Rab7 and TrkA Cotrafficking in Axons  
 by pTIRF Microscopy . . . . . 319  
*Kai Zhang, Praveen D. Chowdary, and Bianxiao Cui*

28 Quantitative Bead-Based Flow Cytometry for Assaying Rab7 GTPase  
 Interaction with the Rab-Interacting Lysosomal Protein (RILP)  
 Effector Protein . . . . . 331  
*Jacob O. Agola, Daniel Sivalingam, Daniel F. Cimino, Peter C. Simons,  
 Tione Buranda, Larry A. Sklar, and Angela Wandinger-Ness*

Erratum to. . . . . E1

*Index* . . . . . 355



---

## Contributors

- DANIEL ABANKWA • *Turku Centre for Biotechnology, Åbo Akademi University, Turku, Finland*
- JACOB O. AGOLA • *Department of Pathology, University of New Mexico HSC, Albuquerque, NM, USA; Cancer Center, University of New Mexico School of Medicine, Albuquerque, NM, USA; Department of Chemical and Biological Engineering, Center for Micro-Engineered Materials, School of Engineering, University of New Mexico, Albuquerque, NM, USA*
- PATRICK D. ALLAIRE • *Department of Biology, University of Utah, Salt Lake City, UT, USA*
- JONTHAN M. BACKER • *Department of Molecular Pharmacology, Albert Einstein College of Medicine, Bronx, NY, USA; Department of Biochemistry, Albert Einstein College of Medicine, Bronx, NY, USA*
- ANNE R. BRESNICK • *Department of Biochemistry, Albert Einstein College of Medicine, Bronx, NY, USA*
- TIONE BURANDA • *Department of Pathology, University of New Mexico HSC, Albuquerque, NM, USA; Cancer Center, University of New Mexico School of Medicine, Albuquerque, NM, USA*
- MATIAS A. BUSTOS • *Instituto de Histología y Embriología (IHEM, CONICET/UNCuyo), Facultad de Ciencias Médicas, CC56, Universidad Nacional de Cuyo, Mendoza, Argentina*
- BRIAN P. CERESA • *Department of Pharmacology and Toxicology, University of Louisville, Louisville, KY, USA*
- SERENA CERVANTES • *Department of Cellular and Molecular Medicine, Howard Hughes Medical Institute, University of California at San Diego, La Jolla, CA, USA*
- YU CHEN • *Cell Biology and Metabolism Program, NICHD, NIH, Bethesda, MD, USA*
- PRAVEEN D. CHOWDARY • *Department of Chemistry, Stanford University, Stanford, CA, USA*
- DANIEL F. CIMINO • *Department of Cell Biology and Physiology, University of New Mexico, Albuquerque, NM, USA; Cancer Center, University of New Mexico School of Medicine, Albuquerque, NM, USA*
- DENISE COLLADO • *Department of Molecular Pharmacology, Albert Einstein College of Medicine, Bronx, NY, USA*
- BIANXIAO CUI • *Department of Chemistry, Stanford University, Stanford, CA, USA*
- SARALIN DAVIS • *Department of Cellular and Molecular Medicine, Howard Hughes Medical Institute, University of California at San Diego, La Jolla, CA, USA*
- HASHEM A. DBOUK • *Department of Molecular Pharmacology, Albert Einstein College of Medicine, Bronx, NY, USA*
- JENNIFER B. DENNISON • *Department of Systems Biology, UT MD Anderson Cancer Center, Houston, TX, USA*
- YOAN DIEKMANN • *Research Department of Genetics, Evolution and Environment, University College London, London, UK*
- GREGORY EMERY • *Department of Pathology and Cell Biology, Faculty of Medicine, University of Montréal, Montréal, QC, Canada; Institute for Research in Immunology and Cancer, University of Montréal, Montréal, QC, Canada*

- KAN ETOH • *Laboratory of Membrane Trafficking Mechanisms, Department of Developmental Biology and Neurosciences, Graduate School of Life Sciences, Tohoku University, Sendai, Miyagi, Japan*
- SHANSHAN FENG • *Department of Biology, University of Pennsylvania, Philadelphia, PA, USA*
- SUSAN FERRO-NOVICK • *Department of Cellular and Molecular Medicine, Howard Hughes Medical Institute, University of California at San Diego, La Jolla, CA, USA*
- MITSUNORI FUKUDA • *Laboratory of Membrane Trafficking Mechanisms, Department of Developmental Biology and Neurosciences, Graduate School of Life Sciences, Tohoku University, Sendai, Miyagi, Japan*
- BRUNO GOUD • *Molecular Mechanisms of Intracellular Transport, Centre National de la Recherche Scientifique, Institut Curie, Paris, France*
- WEI GUO • *Department of Biology, University of Pennsylvania, Philadelphia, PA, USA*
- CAMILO GUZMÁN • *Turku Centre for Biotechnology, Åbo Akademi University, Turku, Finland*
- VIORICA IVAN • *Department of Molecular Cell Biology, Institute of Biochemistry of the Romanian Academy, Bucharest, Romania*
- JANE J. KIM • *Department of Biochemistry and Molecular Genetics, University of Illinois at Chicago, Chicago, IL, USA; Department of Biological Sciences, University of Illinois at Chicago, Chicago, IL, USA*
- HOTAKA KOBAYASHI • *Laboratory of Membrane Trafficking Mechanisms, Department of Developmental Biology and Neurosciences, Graduate School of Life Sciences, Tohoku University, Sendai, Miyagi, Japan*
- CARL LAFLAMME • *Department of Pathology and Cell Biology, Faculty of Medicine, University of Montréal, Montréal, QC, Canada; Institute for Research in Immunology and Cancer, University of Montréal, Montréal, QC, Canada*
- DAVID G. LAMBRIGHT • *Program in Molecular Medicine, University of Massachusetts Medical School, Worcester, MA, USA*
- GUANGPU LI • *Department of Biochemistry and Molecular Biology, University of Oklahoma Health Sciences Center, Oklahoma City, OK, USA; Peggy and Charles Stephenson Cancer Center, University of Oklahoma Health Sciences Center, Oklahoma City, OK, USA*
- ZHIMIN LIANG • *Department of Biochemistry and Molecular Biology, University of Oklahoma Health Sciences Center, Oklahoma City, OK, USA*
- ANDREW J. LINDSAY • *Molecular Cell Biology Laboratory, School of Biochemistry and Cell Biology, Biosciences Institute, University College Cork, Cork, Ireland*
- ZHANNA LIPATOVA • *Department of Biochemistry and Molecular Genetics, College of Medicine, University of Illinois at Chicago, Chicago, IL, USA*
- JENNIFER LIPPINCOTT-SCHWARTZ • *Cell Biology and Metabolism Program, NICHD, NIH, Bethesda, MD, USA*
- JACLYN LOPICCOLO • *Department of Molecular Pharmacology, Albert Einstein College of Medicine, Bronx, NY, USA*
- GUODONG LU • *Key Laboratory of Biopesticide and Chemical Biology, Ministry of Education, Fujian Agriculture and Forestry University, Fuzhou, China*
- ORNELLA LUCCHESI • *Instituto de Histología y Embriología (IHEM, CONICET/UNCuyo), Facultad de Ciencias Médicas, CC56, Universidad Nacional de Cuyo, Mendoza, Argentina*
- ANTHONY MANGAN • *Department of Cell and Developmental Biology, School of Medicine, University of Colorado Denver, Aurora, CO, USA*

- M. CALEB MARLIN • *Department of Biochemistry and Molecular Biology, University of Oklahoma Health Sciences Center, Oklahoma City, OK, USA*
- SOUJIRO MARUBASHI • *Laboratory of Membrane Trafficking Mechanisms, Department of Developmental Biology and Neurosciences, Graduate School of Life Sciences, Tohoku University, Sendai, Miyagi, Japan*
- LUIS S. MAYORGA • *Instituto de Histología y Embriología (IHEM, CONICET/UNCuyo), Facultad de Ciencias Médicas, CC56, Universidad Nacional de Cuyo, Mendoza, Argentina*
- MARY W. MCCAFFREY • *Molecular Cell Biology Laboratory, School of Biochemistry and Cell Biology, Biosciences Institute, University College Cork, Cork, Ireland*
- PETER S. MCPHERSON • *Department of Neurology and Neurosurgery, Montréal Neurological Institute, McGill University, Montréal, QC, Canada*
- GORDON B. MILLS • *Department of Systems Biology, UT MD Anderson Cancer Center, Houston, TX, USA*
- STÉPHANIE MISEREY-LENKEI • *Molecular Mechanisms of Intracellular Transport, Centre National de la Recherche Scientifique, Institut Curie, Paris, France*
- ASHWINI K. MISHRA • *Program in Molecular Medicine, University of Massachusetts Medical School, Worcester, MA, USA*
- SHREYA MITRA • *Department of Systems Biology, UT MD Anderson Cancer Center, Houston, TX, USA*
- JENNIFER MOLINA • *Department of Systems Biology, UT MD Anderson Cancer Center, Houston, TX, USA*
- YASUNORI MORI • *Laboratory of Membrane Trafficking Mechanisms, Department of Developmental Biology and Neurosciences, Graduate School of Life Sciences, Tohoku University, Sendai, Miyagi, Japan*
- PAULINA S. MROZOWSKA • *Laboratory of Membrane Trafficking Mechanisms, Department of Developmental Biology and Neurosciences, Graduate School of Life Sciences, Tohoku University, Sendai, Miyagi, Japan*
- ARAFATH KAJA NAJUMUDEEN • *Turku Centre for Biotechnology, Åbo Akademi University, Turku, Finland*
- RYAN M. NOTTINGHAM • *Department of Biochemistry, Stanford University School of Medicine, Stanford, CA, USA*
- PETER J. NOVICK • *Department of Cellular and Molecular Medicine, University of California San Diego, La Jolla, CA, USA*
- NORIHICO OHBAYASHI • *Laboratory of Membrane Trafficking Mechanisms, Department of Developmental Biology and Neurosciences, Graduate School of Life Sciences, Tohoku University, Sendai, Miyagi, Japan*
- JOHAN PERÄNEN • *Institute of Biotechnology, University of Helsinki, Viikinkaari, Finland*
- JOSÉ B. PEREIRA-LEAL • *Instituto Gulbenkian de Ciência, Oeiras, Portugal*
- SUZANNE R. PFEFFER • *Department of Biochemistry, Stanford University School of Medicine, Stanford, CA, USA*
- ITZIAR M.D. POSADA • *Turku Centre for Biotechnology, Åbo Akademi University, Turku, Finland*
- RYTIS PREKERIS • *Department of Cell and Developmental Biology, School of Medicine, University of Colorado Denver, Aurora, CO, USA*
- YAoyao QI • *Department of Biochemistry and Molecular Biology, University of Oklahoma Health Sciences Center, Oklahoma City, OK, USA; Key Laboratory of Biopesticide and Chemical Biology, Ministry of Education, Fujian Agriculture and Forestry University, Fuzhou, China*

- BRIGITTE RITTER • *Department of Biochemistry, Boston University School of Medicine, Boston, MA, USA*
- MARIA C. RUETE • *Instituto de Histología y Embriología (IHEM, CONICET/UNCuyo), Facultad de Ciencias Médicas, CC56, Universidad Nacional de Cuyo, Mendoza, Argentina*
- RACHEL S. SALAMON • *Department of Molecular Pharmacology, Albert Einstein College of Medicine, Bronx, NY, USA*
- NAVA SEGEV • *Department of Biochemistry and Molecular Genetics, College of Medicine, University of Illinois at Chicago, Chicago, IL, USA*
- PETER C. SIMONS • *Department of Pathology, Center for Molecular Discovery, University of New Mexico, Albuquerque, NM, USA; Cancer Center, University of New Mexico School of Medicine, Albuquerque, NM, USA*
- DANIEL SIVALINGAM • *Department of Biology, California State University, Northridge, CA, USA; Department of Neurobiology, David Geffen School of Medicine, University of California, Los Angeles, CA, USA*
- LARRY A. SKLAR • *Department of Pathology, Center for Molecular Discovery, University of New Mexico, Albuquerque, NM, USA; Cancer Center, University of New Mexico School of Medicine, Albuquerque, NM, USA*
- PETER VAN DER SLUIJS • *Department of Cell Biology, University Medical Center Utrecht, Utrecht, The Netherlands*
- ELIZABETH SMYTHE • *Department of Biomedical Science, Centre for Membrane Interactions and Dynamics, University of Sheffield, Sheffield, UK*
- DANIÈLE STALDER • *Department of Cellular and Molecular Medicine, University of California San Diego, La Jolla, CA, USA*
- CLAUDIA N. TOMES • *Instituto de Histología y Embriología (IHEM, CONICET/UNCuyo), Facultad de Ciencias Médicas, CC56, Universidad Nacional de Cuyo, Mendoza, Argentina*
- ANGELA WANDINGER-NESS • *Department of Pathology, University of New Mexico, Albuquerque, NM, USA; Cancer Center, University of New Mexico School of Medicine, Albuquerque, NM, USA*
- JUAN WANG • *Department of Cellular and Molecular Medicine, Howard Hughes Medical Institute, University of California at San Diego, La Jolla, CA, USA*
- ZONGHUA WANG • *Key Laboratory of Biopesticide and Chemical Biology, Ministry of Education, Fujian Agriculture and Forestry University, Fuzhou, China*
- BIN WU • *Department of Biology, University of Pennsylvania, Philadelphia, PA, USA*
- TAKAO YASUDA • *Laboratory of Membrane Trafficking Mechanisms, Department of Developmental Biology and Neurosciences, Graduate School of Life Sciences, Tohoku University, Sendai, Miyagi, Japan*
- KAI ZHANG • *Department of Biochemistry, University of Illinois at Urbana-Champaign, Urbana, IL, USA*