

Methods in Enzymology

Volume 325

*Regulators and Effectors of
Small GTPases*

*Part D
Rho Family*

EDITED BY

W. E. Balch

THE SCRIPPS RESEARCH INSTITUTE
LA JOLLA, CALIFORNIA

Channing J. Der

LINEBERGER COMPREHENSIVE CANCER CENTER
THE UNIVERSITY OF NORTH CAROLINA AT CHAPEL HILL
CHAPEL HILL, NORTH CAROLINA

Alan Hall

UNIVERSITY COLLEGE LONDON, LONDON, ENGLAND



ACADEMIC PRESS

San Diego London Boston New York Sydney Tokyo Toronto

Table of Contents

CONTRIBUTORS TO VOLUME 325	ix
PREFACE	xv
VOLUMES IN SERIES	xvii

Section I. Purification, Posttranslational Modification, and *in Vitro* Regulation

1. Purification and Biochemical Characterization of TC10	CHERYL L. NEUDAUER AND IAN G. MACARA	3
2. Expression and Purification of Rho/RhoGDI Complexes	PAUL W. READ AND ROBERT K. NAKAMOTO	15
3. Bacterial Expressed DH and DH/PH Domains	KENT L. ROSSMAN AND SHARON L. CAMPBELL	25
4. Biochemical Analysis of Regulation of Vav, a Guanine-Nucleotide Exchange Factor for Rho Family of GTPases	RAYMOND MOSTELLER, JAEWON HAN, BALAKA DAS, AND DANIEL BROEK	38
5. Activation of Rac1 by Human Tiam1	GIDEON BOLLAG, ANNE M. CROMPTON, DANIELLE PEVERLY-MITCHELL, GASTON G. M. HABETS, AND MARC SYMONS	51
6. Activation of Rho GEF Activity by G α_{13}	MATTHEW J. HART, WILLIAM ROSCOE, AND GIDEON BOLLAG	61
7. Purification and Characterization of Guanine Nucleotide Dissociation Stimulator Protein	JON P. HUTCHINSON, KATRIN RITTINGER, AND JOHN F. ECCLESTON	71
8. Purification and Biochemical Activity of <i>Salmonella</i> Exchange Factor SopE	ANDREA FRIEBEL AND WOLF-DIETRICH HARDT	82
9. Stimulation of Rho GDI Release by ERM Proteins	AKIKO MAMMOTO, KAZUO TAKAHASHI, TAKUYA SASAKI, AND YOSHIMI TAKAI	91

10. Expression and Activity of Human Prenylcysteine-Directed Carboxyl Methyltransferase	EDWIN CHOY AND MARK PHILIPS	101
11. Purification and Evaluation of Large Clostridial Cytotoxins That Inhibit Small GTPases of Rho and Ras Subfamilies	MICHAEL MOOS AND CHRISTOPH VON EICHEL-STREIBER	114
12. Rho GTPase-Activating Toxins: Cytotoxic Necrotizing Factors and Dermonecrotic Toxin	GUDULA SCHMIDT AND KLAUS AKTORIES	125

Section II. Purification and Activity of GTPase Targets

13. Measurement of GTPase Effector Affinities	DANNY MANOR	139
14. Purification and <i>in Vitro</i> Activity of Rho-Associated Kinase	MUTSUKI AMANO, YUKO FUKATA, HIROAKI SHIMOKAWA, AND KOZO KAIBUCHI	149
15. Purification and <i>in Vitro</i> Activities of p21-Activated Kinases	CHARLES C. KING, ABINA M. REILLY, AND ULLA G. KNAUS	155
16. Stimulation of Phospholipase C- β_2 by Rho GTPases	DARIA ILLENBERGER, ILONA STEPHAN, PETER GHERSCHIK, AND FRIEDER SCHWALD	167
17. Regulation of Phospholipase D1 Activity by Rho GTPases	MICHAEL A. FROHMAN, YASUNORI KANAHO, YUE ZHANG, AND ANDREW J. MORRIS	177
18. <i>In Vitro</i> Interaction of Phosphoinositide-4-phosphate 5-Kinases with Rac	KIMBERLEY TOLIAS AND CHRISTOPHER L. CARPENTER	190
19. Activity and Regulation of p35/Cdk5 Kinase Complex	MARGARETA NIKOLIC AND LI-HUEI TSAI	200
20. Actin Assembly Mediated by Arp2/3 Complex and WASP Family Proteins	R. DYCHE MULLINS AND LAURA M. MACHESKY	214
21. <i>In Vitro</i> Actin Polymerization Using Polymorphonuclear Leukocyte Extracts	SALLY H. ZIGMOND	237

Section III. Analysis of Rho GTPase Function

22. Determination of GTP Loading on Rac and Cdc42 in Platelets and Fibroblasts	ANSER C. AZIM, KURT L. BARKALOW, AND JOHN H. HARTWIG	257
23. Determination of GTP Loading on Rho	XIANG-DONG REN AND MARTIN ALEXANDER SCHWARTZ	264

24. Use and Properties of ROCK-Specific Inhibitor Y-27632	SHUH NARUMIYA, TOSHIMASA ISHIZAKI, AND MASAYOSHI UEHATA	273
25. Inducible Membrane Recruitment of Small GTP-Binding Proteins by Rapamycin-Based System in Living Cells	FLAVIA CASTELLANO AND PHILIPPE CHAVRIER	285
26. Expression of Rho GTPases Using Retroviral Vectors	FRITS MICHIELS, ROB A. VAN DER KAMMEN, LENNERT JANSSEN, GARRY NOLAN, AND JOHN G. COLLARD	295
27. Expression of Rho GTPases Using Adenovirus Vectors	DANIEL M. SULLIVAN AND TOREN FINKEL	303
28. <i>In Vivo</i> Activity of Wild-Type and Mutant PAKs	CHARLES C. KING, LURAYNNE C. SANDERS, AND GARY M. BOKOCH	315
29. Single Cell Assays for Rac Activity	LAURA J. TAYLOR, AMY B. WALSH, PATRICK HEARING, AND DAFNA BAR-SAGI	327
30. Effect of Rho GTPases on Na-H Exchanger in Mammalian Cells	SHERYL P. DENKER, WEIHONG YAN, AND DIANE L. BARBER	334
31. Actin Filament Assembly in Permeabilized Platelets	JOHN H. HARTWIG AND KURT L. BARKALOW	349
32. Rho GTPases: Secretion and Actin Dynamics in Permeabilized Mast Cells	MARK R. HOLT AND ANNA KOFFER	356
33. Isolation and <i>in Vitro</i> Contraction of Stress Fibers	KAZUO KATOH, YUMIKO KANO, AND KEIGI FUJIWARA	369
34. Inhibition of Rho GTPases Using Protein Geranylgeranyltransferase I Inhibitors	SAÏD M. SEBTI AND ANDREW D. HAMILTON	381
35. Imaging Spatiotemporal Dynamics of Rac Activation <i>in Vivo</i> with FLAIR	CHESTER E. CHAMBERLAIN, VADIM S. KRAYNOV, AND KLAUS M. HAHN	389

Section IV. Biological Assays of Rho GTPase Function

36. Rho-like GTPases in Tumor Cell Invasion	EVA E. EVERS, ROB A. VAN DER KAMMEN, JEAN P. TEN KLOOSTER, AND JOHN G. COLLARD	403
---	---	-----

37. Mouse Embryo Fibroblasts: A Genetic Model System for Studying Rho- and Ras-Dependent Cell Cycle Progression	GIOVANNA M. D'ABACO AND MICHAEL F. OLSON	415
38. Analyses of Transforming Activity of Rho Family Activators	PATRICIA A. SOLSKI, KARON ABE, AND CHANNING J. DER	425
39. Rho GTPases and Cell Migration—Fibroblast Wound Healing	CATHERINE D. NOBES	441
40. Rho GTPases and Cell Migration: Measurement of Macrophage Chemotaxis	GARETH E. JONES, ANNE J. RIDLEY, AND DANIEL ZICHA	449
41. Rho GTPases and Macrophage Phagocytosis	JAYESH C. PATEL, ALAN HALL, AND EMMANUELLE CARON	462
42. Rho GTPases and Axonal Growth Cone Collapse	ALYSON E. FOURNIER, ROBERT G. KALB, AND STEPHEN M. STRITTMATTER	473
43. Study of <i>in Situ</i> Function of Cytoskeletal Proteins in Lamellipodia and Filopodia Using Microscale Chromophore-Assisted Laser Inactivation	ERIC V. WONG AND DANIEL G. JAY	482
44. Modulation of Actin Cytoskeleton by <i>Salmonella</i> GTPase Activating Protein SptP	JORGE E. GALÁN AND YIXIN FU	496
AUTHOR INDEX		505
SUBJECT INDEX		531