

BT-C-753

2257-3276

*Methods in Enzymology*

*Volume 407*

*Regulators and Effectors of  
Small GTPases: Ras Family*

EDITED BY

*William E. Balch*

DEPARTMENT OF CELL BIOLOGY  
THE SCRIPPS RESEARCH INSTITUTE  
LA JOLLA, CALIFORNIA

*Channing J. Der*

DEPARTMENT OF PHARMACOLOGY  
THE UNIVERSITY OF NORTH CAROLINA  
CHAPEL HILL, NORTH CAROLINA

*Alan Hall*

CRC ONCOGENE AND SIGNAL TRANSDUCTION GROUP  
MRC LABORATORY FOR MOLECULAR CELL BIOLOGY  
UNIVERSITY COLLEGE LONDON  
LONDON, ENGLAND



ELSEVIER

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

Academic Press is an imprint of Elsevier



## Table of Contents

CONTRIBUTORS TO VOLUME 407 . . . . .		xi
PREFACE . . . . .		xix
VOLUMES IN SERIES . . . . .		xxi
1. Ras Superfamily and Interacting Proteins Database	ANDRE BERNARDS	1
2. Real-Time <i>In Vitro</i> Measurement of Intrinsic and Ras GAP-Mediated GTP Hydrolysis	ADAM SHUTES AND CHANNING J. DER	9
3. Schwann Cell Preparation from Single Mouse Embryos: Analyses of Neurofibromin Function in Schwann Cells	NANCY RATNER, JON P. WILLIAMS, JENNIFER J. KORDICH, AND HAESUN A. KIM	22
4. Regulation of the Nucleotide State of Oncogenic Ras Proteins by Nucleoside Diphosphate Kinase	MICHAEL A. FISCHBACH AND JEFFREY SETTLEMAN	33
5. Measurements of TSC2 GAP Activity Toward Rheb	YONG LI, KEN INOKI, HARIS VIKIS, AND KUN-LIANG GUAN	46
6. Characterization of AND-34 Function and Signaling	KYRIACOS FELEKKIS, LAWRENCE A. QUILLIAM, AND ADAM LERNER	55
7. Studying the Spatial and Temporal Regulation of Ras GTPase-Activating Proteins	SABINE KUPZIG, DALILA BOUYOUCEF, GYLES E. COZIER, AND PETER J. CULLEN	64
8. Activation of Ras Proteins by Ras Guanine Nucleotide Releasing Protein Family Members	QUE T. LAMBERT AND GARY W. REUTHER	82
9. Ras and Rap1 Activation of PLC $\epsilon$ Lipase Activity	HIRONORI EDAMATSU, TAKAYA SATOH, AND TOHRU KATAOKA	99
10. Specificity and Expression of RalGPS as RalGEFs	LAWRENCE A. QUILLIAM	108

11. Biochemical and Biological Analyses of Rgr RalGEF Oncogene	LAURA A. MARTELLO AND ANGEL PELLICER	115
12. Analysis of Ras Activation in Living Cells with GFP-RBD	TREVER G. BIVONA, STEVEN QUATELA, AND MARK R. PHILIPS	128
13. Genetic and Pharmacologic Analyses of the Role of <i>Icm1</i> in Ras Membrane Association and Function	ANNIKA W. SVENSSON, PATRICK J. CASEY, STEPHEN G. YOUNG, AND MARTIN O. BERGO	144
14. Characterization of the Activation of the Rap-Specific Exchange Factor Epac by Cyclic Nucleotides	HOLGER REHMANN	159
15. Biochemistry of the Rap-Specific Guanine Nucleotide Exchange Factors PDZ-GEF1 and -2	H. BEA KUIPERIJ, HOLGER REHMANN, AND FRIED J. T. ZWARTKRUIS	174
16. Characterization of Interactions Between Ras Family GTPases and Their Effectors	PABLO RODRIGUEZ-VICIANA AND FRANK MCCORMICK	187
17. Genetic and Pharmacologic Dissection of Ras Effector Utilization in Oncogenesis	PAUL M. CAMPBELL, ANURAG SINGH, FALINA J. WILLIAMS, KAREN FRANTZ, AYLIN S. ÜLKÜ, GRANT G. KELLEY, AND CHANNING J. DER	195
18. Sequencing Analysis of BRAF Mutations in Human Cancers	RICHARD WOOSTER, ANDREW P. FUTREAL, AND MICHAEL R. STRATTON	218
19. KSR Regulation of the Raf-MEK-ERK Cascade	DANIEL A. RITT, IRA O. DAAR, AND DEBORAH K. MORRISON	224
20. Ras-Sensitive IMP Modulation of the Raf/MEK/ERK Cascade Through KSR1	SHARON A. MATHENY AND MICHAEL A. WHITE	237
21. Raf Kinase Inhibitor Protein Regulation of Raf and MAPK Signaling	SUZANNE HAGAN, REY GARCIA, AMARDEEP DHILLON, AND WALTER KOLCH	248
22. Harnessing RNAi for Analyses of Ras Signaling and Transformation	ANGELIQUE W. WHITEHURST AND MICHAEL A. WHITE	259
23. The Rac Activator Tiam1 and Ras-Induced Oncogenesis	KRISTIN STRUMANE, TOMASZ P. RYGIEL, AND JOHN G. COLLARD	269

24. Phospholipase C $\alpha$ Guanine Nucleotide Exchange Factor Activity and Activation of Rap1	TAKAYA SATOH, HIRONORI EDAMATSU, AND TOHRU KATAOKA	281
25. Nore1 and RASSF1 Regulation of Cell Proliferation and of the MST1/2 Kinases	JOSEPH AVRUCH, MARIA PRASKOVA, SARA ORTIZ-VEGA, MATTHEW LIU, AND XIAN-FENG ZHANG	290
26. RASSF Family Proteins and Ras Transformation	MICHELE D. VOS AND GEOFFREY J. CLARK	311
27. Ras and the RAIN/RasIP1 Effector	NATALIA MITIN, STEPHEN F. KONIECZNY, AND ELIZABETH J. TAPAROWSKY	322
28. The RIN Family of Ras Effectors	JOANNE M. BLISS, BYRAPPA VENKATESH, AND JOHN COLICELLI	335
29. Rap1 Regulation of RIAM and Cell Adhesion	ESTHER LAFUENTE AND VASSILIKI A. BOUSSIOTIS	345
30. Regulation of Cell-Cell Adhesion by Rap1	YASUYUKI FUJITA, CATHERINE HOGAN, AND VANIA M. M. BRAGA	359
31. Effects of Ras Signaling on Gene Expression Analyzed by Customized Microarrays	OLEG I. TCHERNITSA, CHRISTINE SERS, ANITA GEFLITTER, AND REINHOLD SCHÄFER	373
32. Protein-Fragment Complementation Assays (PCA) in Small GTPase Research and Drug Discovery	JOHN K. WESTWICK AND STEPHEN W. MICHNICK	388
33. Ras Up-Regulation of Cyclooxygenase-2	MICHAEL G. BACKLUND, JASON R. MANN, DINGZHI WANG, AND RAYMOND N. DUBOIS	401
34. Regulation of the Expression of Tropomyosins and Actin Cytoskeleton by <i>ras</i> Transformation	G. L. PRASAD	410
35. Regulation of Par-4 by Oncogenic Ras	KRISHNA MURTHI VASUDEVAN, PADHMA RANGANATHAN, AND VIVEK M. RANGNEKAR	422

36. Using <i>Drosophila</i> and Yeast Genetics to Investigate a Role for the Rheb GTPase in Cell Growth	PARTHIVE H. PATEL AND FUYUHIKO TAMANOI	443
37. Biochemistry and Biology of ARHI (DIRAS3), an Imprinted Tumor Suppressor Gene Whose Expression Is Lost in Ovarian and Breast Cancers	YINHUA YU, ROBERT LUO, ZHEN LU, WEI WEI FENG, DONNA BADGWELL, JEAN-PIERRE ISSA, DANIEL G. ROSEN, JINSONG LIU, AND ROBERT C. BAST, JR.	455
38. Gem Protein Signaling and Regulation	YVONA WARD AND KATHLEEN KELLY	468
39. Analyses of Rem/RGK Signaling and Biological Activity	DOUGLAS A. ANDRES, SHAWN M. CRUMP, ROBERT N. CORRELL, JONATHAN SATIN, AND BRIAN S. FINLIN	484
40. Analysis of Rit Signaling and Biological Activity	DOUGLAS A. ANDRES, JENNIFER L. RUDOLPH, TOMOKO SENGOKU, AND GENG-XIAN SHI	499
41. Characterization of <i>RERG</i> : An Estrogen-Regulated Tumor Suppressor Gene	MEGAN D. KEY, DOUGLAS A. ANDRES, CHANNING J. DER, AND GRETCHEN A. REPASKY	513
42. Inhibition of Transcription Factor NF- $\kappa$ B Activation by $\kappa$ B-Ras	TOM HUXFORD AND GOURISANKAR GHOSH	527
43. Analysis of Rhes Activation State and Effector Function	JUAN BERNAL AND PIERO CRESPO	535
44. Rheb Activation of mTOR and S6K1 Signaling	JESSIE HANRAHAN AND JOHN BLENIS	542
45. Use of Retrovirus Expression of Interfering RNA to Determine the Contribution of Activated K-Ras and Ras Effector Expression to Human Tumor Cell Growth	ANTONIO T. BAINES, KIAN-HUAT LIM, JANIEL M. SHIELDS, JOHN M. LAMBERT, CHRISTOPHER M. COUNTER, CHANNING J. DER, AND ADRIENNE D. COX	556
46. Using Inhibitors of Prenylation to Block Localization and Transforming Activity	ANASTACIA C. BERZAT, DONITA C. BRADY, JAMES J. FIORDALISI, AND ADRIENNE D. COX	575

47. Sorafenib (BAY 43-9006, Nexavar <sup>®</sup> ), a Dual-Action Inhibitor That Targets RAF/MEK/ERK Pathway in Tumor Cells and Tyrosine Kinases VEGFR/PDGFR in Tumor Vasculature	LILA ADNANE, PAMELA A. TRAIL, IAN TAYLOR, AND SCOTT M. WILHELM	597
48. Yeast Screens for Inhibitors of Ras-Raf Interaction and Characterization of MCP Inhibitors of Ras-Raf Interaction	VLADIMIR KHAZAK, JURAN KATO-STANKIEWICZ, FUYU TAMANOI, AND ERICA A. GOLEMIS	612
49. A Tagging-via-Substrate Technology for Genome-Wide Detection and Identification of Farnesylated Proteins	SUNG CHAN KIM, YOONJUNG KHO, DEB BARMA, JOHN FALCK, AND YINGMING ZHAO	629
50. A Genetically Defined Normal Human Somatic Cell System to Study Ras Oncogenesis <i>In Vivo</i> and <i>In Vitro</i>	KEVIN M. O'HAYER AND CHRISTOPHER M. COUNTER	637
51. Analysis of Ras Transformation of Human Thyroid Epithelial Cells	ZARUHI POGHOSYAN AND DAVID WYNFORD-THOMAS	648
52. Use of Ras-Transformed Human Ovarian Surface Epithelial Cells as a Model for Studying Ovarian Cancer	DANIEL G. ROSEN, GONG YANG, ROBERT C. BAST, JR., AND JINSONG LIU	660
53. Physiological Analysis of Oncogenic K-Ras	PEDRO ANTONIO PÉREZ-MANCERA AND DAVID A. TUVESON	676
54. Use of Conditionally Active Ras Fusion Proteins to Study Epidermal Growth, Differentiation, and Neoplasia	JASON A. REUTER AND PAUL A. KHAVARI	691
55. Pancreatic Duct Epithelial Cell Isolation and Cultivation in Two-Dimensional and Three-Dimensional Culture Systems	CRISTINA AGBUNAG, KYOUNG EUN LEE, SERENA BUONTEMPO, AND DAFNA BAR-SAGI	703
56. Analyses of RAS Regulation of Eye Development in <i>Drosophila melanogaster</i>	LUCY C. FIRTH, WEI LI, HUI ZHANG, AND NICHOLAS E. BAKER	711
AUTHOR INDEX . . . . .		723
SUBJECT INDEX . . . . .		765