

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>

Calpain

Methods and Protocols

Edited by

Jeannette S. Messer

*Department of Inflammation and Immunity, Lerner Research Institute, Cleveland Clinic,
Cleveland, OH, USA*

Editor

Jeannette S. Messer
Department of Inflammation and Immunity
Lerner Research Institute, Cleveland Clinic
Cleveland, OH, USA

ISSN 1064-3745 ISSN 1940-6029 (electronic)
Methods in Molecular Biology
ISBN 978-1-4939-8987-4 ISBN 978-1-4939-8988-1 (eBook)
<https://doi.org/10.1007/978-1-4939-8988-1>

Library of Congress Control Number: 2018965755

© Springer Science+Business Media, LLC, part of Springer Nature 2019

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. The publisher remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

This Springer imprint is published by the registered company Springer Science+Business Media, LLC, part of Springer Nature.

The registered company address is: 233 Spring Street, New York, NY 10013, U.S.A.

Dedication

This volume is dedicated to Dr. Hiro Sorimachi:

Dedication to Calpain research and determination to enjoy science have been messages that we have learned from Dr. Hiro Sorimachi. He has been a Calpain expert and, therefore, was a very tough as well as fair judge. Getting him convinced with our data always took some rounds of discussion, but when he cast off his doubt with a hearty laugh, all the hard work was enormously rewarded. It has been our privilege to have known him as our mentor. He will be dearly missed.

Yasuko Ono and Shoji Hata

Preface

The calpains have been something of an enigma in the world of proteases. They are a large family of cysteine proteases with limited proteolytic activity. This means that calpain-mediated cleavage events create protein fragments, often with novel functions, rather than degrade proteins. The two best-characterized calpains, calpain 1 and calpain 2, are ubiquitously expressed. However, there are now 15 characterized calpains, many of which are expressed exclusively or predominantly in specific tissue types. To add to the complexity of this system, calpains also have an endogenous inhibitor (calpastatin), and calpain cleavage events can be regulated by unrelated proteins. Understanding calpains and their complexity is crucial since calpains are involved in many fundamental developmental and homeostatic processes and have been implicated in a number of human diseases through disease-associated single nucleotide polymorphisms in calpains or calpain substrates.

The large number and wide reach of calpains has led to a strong need for many investigators to pursue calpain-related studies but also a high level of trepidation when approaching these studies. In this volume, we have attempted to provide a library of protocols useful both for scientists dedicated to calpain research and those whose work has led them to calpain in other ways. We have included experimental methods to study calpains, their inhibitors, and their substrates along with bioinformatic approaches to calpain substrate identification. We have also paid special attention to tricky or challenging aspects of these methods and included tips on how to overcome these issues in the Notes section of each protocol.

I would like to thank all of the authors who lent their experience and expertise to developing and writing these protocols. These types of endeavors are increasingly important as the complexity of techniques continues to increase while the space allotted to describe methods in journals has decreased. It is therefore my hope that these efforts will open up calpain investigations to new investigators and expand the experimental repertoire of seasoned calpain scientists. Perhaps most importantly, I hope that this volume will provide information and inspiration that leads to the new discoveries needed to diagnose and treat calpain-related diseases.

Cleveland, OH, USA

Jeannette S. Messer

Contents

<i>Dedication</i>	<i>v</i>
<i>Preface</i>	<i>vii</i>
<i>Contributors</i>	<i>xi</i>

PART I PRODUCTION AND PURIFICATION OF CALPAINS

1 Single-Step Purification of Calpain-1, Calpain-2, and Calpastatin Using Anion-Exchange Chromatography	3
<i>Ashim Kumar Biswas and Samarth Tandon</i>	
2 Bacterial Expression and Purification of Calpains	13
<i>Christian-Scott E. McCartney and Peter L. Davies</i>	

PART II DETERMINATION OF CALPAIN LOCALIZATION, EXPRESSION, AND ACTIVITY

3 Casein Zymography for Analysis of Calpain-1 and Calpain-2 Activity	31
<i>Ashim Kumar Biswas and Samarth Tandon</i>	
4 FRET-Based Assays to Determine Calpain Activity	39
<i>Christian-Scott E. McCartney and Peter L. Davies</i>	
5 In Vivo Monitoring of Calpain Activity by Forster Resonance Energy Transfer	57
<i>William Lostal, Daniel Stockholm, and Isabelle Richard</i>	
6 Determining Temporal and Spatial Expression of Calpains in Amphibians	67
<i>Anna Charalambous, Ioanna Antoniadis, Neophytos Christodoulou, Sara Zanardelli, and Paris A. Skourides</i>	
7 Immunohistochemical Localization of Calpains in the Amphibian <i>Xenopus laevis</i>	81
<i>Jean-Marie Exbrayat, Elara N. Moudilou, and Claire Brun</i>	
8 Expression and Activity of Calpain A in <i>Drosophila melanogaster</i>	93
<i>Maira Cardoso, Danielle Oliveira, and Helena Araujo</i>	
9 Use of the β -Glucuronidase (GUS) Reporter System to Localize Promoter Activities of the Endogenous Plant Calpain <i>DEFECTIVE KERNEL1 (DEK1)</i>	103
<i>Zhe Liang and Hilde-Gunn Opsahl-Sorteberg</i>	

PART III IDENTIFICATION OF CALPAIN SUBSTRATES AND CALPAIN-ACTIVATED PROTEIN FUNCTIONS

10 LabCaS for Ranking Potential Calpain Substrate Cleavage Sites from Amino Acid Sequence	111
<i>Yong-Xian Fan, Xiaoyong Pan, Yang Zhang, and Hong-Bin Shen</i>	

11 CalCleaveMKL: a Tool for Calpain Cleavage Prediction 121
David A. duVerle and Hiroshi Mamitsuka

12 Identification of Calpain-Activated Protein Functions 149
Maria del Carmen Lafita-Navarro and Maralice Conacci-Sorrell

PART IV INTERROGATION OF CALPASTATIN, THE ENDOGENOUS
CALPAIN INHIBITOR

13 Production and Purification of Recombinant Calpastatin 163
Roberta De Tullio and Monica Averna

14 Isolation of Endogenous Calpastatin 187
Roberta De Tullio and Monica Averna

15 Immunoblotting for Calpastatin Expression 195
Monica Averna and Roberta De Tullio

PART V MANIPULATION OF CALPAIN EXPRESSION

16 Experimental Manipulation of Calpain Activity In Vitro 209
Seiichi Kitagawa

17 In Vivo Calpain Knockdown Using Delivery of siRNA 219
Xiaoqian Liu and Guochang Hu

18 In Vivo Expression of Mutant Calpains in the Eye Using Lentivirus..... 233
Katherine J. Wert and Vinit B. Mahajan

19 Methods of Calpain Inhibition to Determine the Role of Calpains
in Embryo Development in Amphibians 249
*Ioanna Antoniadou, Anna Charalambous, Neophytos Christodoulou,
Sara Zanardelli, and Paris A. Skourides*

20 Genetic Models of Calpain Deficiency and Ectopic Expression 261
Yan Gao, Christine Hall, James MacLeod, and Peter A. Greer

Index 275

Contributors

- IOANNA ANTONIADES • *Department of Biological Sciences, University of Cyprus, Nicosia, Cyprus*
- HELENA ARAUJO • *Institute of Biomedical Sciences, Federal University of Rio de Janeiro, Rio de Janeiro, Brazil; Institute of Molecular Entomology, Rio de Janeiro, Brazil*
- MONICA AVERNA • *Department of Experimental Medicine (DIMES)—Biochemistry Section, University of Genova, Genova, Italy*
- ASHIM KUMAR BISWAS • *Division of Post-Harvest Technology, ICAR-Central Avian Research Institute, Bareilly, Uttar Pradesh, India*
- CLAIRE BRUN • *University of Lyon, UMRS 449: General Biology—Reproduction and Comparative Development, Lyon Catholic University (UCLy), Ecole Pratique des Hautes Etudes (EPHE, PSL), Lyon, France*
- MAIRA CARDOSO • *Institute of Biomedical Sciences, Federal University of Rio de Janeiro, Rio de Janeiro, Brazil*
- ANNA CHARALAMBOUS • *Department of Biological Sciences, University of Cyprus, Nicosia, Cyprus*
- NEOPHYTOS CHRISTODOULOU • *Department of Biological Sciences, University of Cyprus, Nicosia, Cyprus*
- MARALICE CONACCI-SORRELL • *Department of Cell Biology, University of Texas Southwestern Medical Center, Dallas, TX, USA; Harold C. Simmons Comprehensive Cancer Center, University of Texas Southwestern Medical Center, Dallas, TX, USA*
- PETER L. DAVIES • *Department of Biomedical and Molecular Sciences, Queen's University, Kingston, ON, Canada*
- ROBERTA DE TULLIO • *Department of Experimental Medicine (DIMES)—Biochemistry Section, University of Genova, Genova, Italy; Centre of Excellence for Biomedical Research (CEBR), University of Genova, Genova, Italy*
- MARIA DEL CARMEN LAFITA-NAVARRO • *Department of Cell Biology, University of Texas Southwestern Medical Center, Dallas, TX, USA; Harold C. Simmons Comprehensive Cancer Center, University of Texas Southwestern Medical Center, Dallas, TX, USA*
- DAVID A. DUVERLE • *Graduate School of Frontier Science, The University of Tokyo, Kashiwa, Chiba, Japan; Artificial Intelligence Research Center, AIST, Koto-ku, Tokyo, Japan*
- JEAN-MARIE EXBRAYAT • *University of Lyon, UMRS 449: General Biology—Reproduction and Comparative Development, Lyon Catholic University (UCLy), Ecole Pratique des Hautes Etudes (EPHE, PSL), Lyon, France*
- YONG-XIAN FAN • *Guangxi Key Laboratory of Trusted Software, Guangxi Colleges and Universities Key Laboratory of Intelligent Processing of Computer Images and Graphics, Guilin University of Electronic Technology, Guilin, China*
- YAN GAO • *Department of Pathology and Molecular Medicine, Queen's University, Kingston, ON, Canada; Division of Cancer Biology and Genetics, Cancer Research Institute, Kingston, ON, Canada*
- PETER A. GREER • *Department of Pathology and Molecular Medicine, Queen's University, Kingston, ON, Canada; Division of Cancer Biology and Genetics, Cancer Research Institute, Kingston, ON, Canada*

- CHRISTINE HALL • *Department of Pathology and Molecular Medicine, Queen's University, Kingston, ON, Canada; Division of Cancer Biology and Genetics, Cancer Research Institute, Kingston, ON, Canada*
- GUOCHANG HU • *Department of Anesthesiology, University of Illinois College of Medicine, Chicago, IL, USA; Department of Pharmacology, University of Illinois College of Medicine, Chicago, IL, USA*
- SEICHI KITAGAWA • *Department of Physiology, Graduate School of Medicine, Osaka City University, Osaka, Japan; Shitennoji Tamatsukurien Clinic, Osaka, Japan*
- ZHE LIANG • *Department of Biological Sciences, National University of Singapore, Singapore, Singapore*
- XIAOQIAN LIU • *Department of Anesthesiology, University of Illinois College of Medicine, Chicago, IL, USA*
- WILLIAM LOSTAL • *Généthon INSERM, U951, INTEGRARE Research Unit, University Paris–Saclay, Evry, France*
- JAMES MACLEOD • *Department of Pathology and Molecular Medicine, Queen's University, Kingston, ON, Canada; Division of Cancer Biology and Genetics, Cancer Research Institute, Kingston, ON, Canada*
- VINIT B. MAHAJAN • *Department of Ophthalmology, Byers Eye Institute, Stanford University School of Medicine, Palo Alto, CA, USA*
- HIROSHI MAMITSUKA • *Bioinformatics Center, Kyoto University, Uji, Kyoto, Japan; Department of Computer Science, Aalto University, Espoo, Finland*
- CHRISTIAN-SCOTT E. MCCARTNEY • *Department of Biomedical and Molecular Sciences, Queen's University, Kingston, ON, Canada*
- ELARA N. MOUDILOU • *University of Lyon, UMRS 449: General Biology—Reproduction and Comparative Development, Lyon Catholic University (UCLY), Ecole Pratique des Hautes Etudes (EPHE, PSL), Lyon, France*
- DANIELLE OLIVEIRA • *Department of Biochemistry, Institute of Chemistry, Federal University of Rio de Janeiro, Rio de Janeiro, Brazil*
- HILDE-GUNN OPSAHL-SORTEBERG • *Department of Plant Sciences, BIOVIT, Norwegian University of Life Sciences, Ås, Norway*
- XIAOYONG PAN • *Department of Medical Informatics, Erasmus MC, Rotterdam, The Netherlands*
- ISABELLE RICHARD • *Généthon INSERM, U951, INTEGRARE Research Unit, University Paris–Saclay, Evry, France*
- HONG-BIN SHEN • *Institute of Image Processing and Pattern Recognition, Shanghai Jiao Tong University, Shanghai, China; Key Laboratory of System Control and Information Processing, Ministry of Education of China, Shanghai, China*
- PARIS A. SKOURIDES • *Department of Biological Sciences, University of Cyprus, Nicosia, Cyprus*
- DANIEL STOCKHOLM • *Généthon INSERM, U951, INTEGRARE Research Unit, University Paris–Saclay, Evry, France*
- SAMARTH TANDON • *Division of Post-Harvest Technology, ICAR-Central Avian Research Institute, Bareilly, Uttar Pradesh, India*
- KATHERINE J. WERT • *Department of Ophthalmology, Byers Eye Institute, Stanford University School of Medicine, Palo Alto, CA, USA*
- SARA ZANARDELLI • *Department of Biological Sciences, University of Cyprus, Nicosia, Cyprus*
- YANG ZHANG • *Department of Computational Medicine and Bioinformatics, University of Michigan, Ann Arbor, MI, USA*