

METHODS IN MOLECULAR BIOLOGY™

Series Editor
John M. Walker
School of Life Sciences
University of Hertfordshire
Hatfield, Hertfordshire, AL10 9AB, UK

For other titles published in this series, go to
www.springer.com/series/7651

Cystic Fibrosis

Diagnosis and Protocols, Volume I: Approaches to Study and Correct CFTR Defects

Edited by

Margarida D. Amaral

*Centre for Biodiversity and Functional and Integrative Genomics,
Faculty of Sciences, University of Lisboa,
Lisboa, Portugal*

Karl Kunzelmann

*Department of Physiology, University of Regensburg,
Regensburg, Germany*

Editors

Margarida D. Amaral
Centre for Biodiversity & Functional
and Integrative Genomics
University of Lisboa
Lisboa 1749-016, Portugal
mdamaral@fc.ul.pt

Karl Kunzelmann
Department of Physiology
University of Regensburg
Regensburg 93053, Germany
karl.kunzelmann@vkl.uni-regensburg.de

ISSN 1064-3745

e-ISSN 1940-6029

ISBN 978-1-61779-116-1

e-ISBN 978-1-61779-117-8

DOI 10.1007/978-1-61779-117-8

Springer New York Dordrecht Heidelberg London

Library of Congress Control Number: 2011925926

© Springer Science+Business Media, LLC 2011

All rights reserved. This work may not be translated or copied in whole or in part without the written permission of the publisher (Humana Press, c/o Springer Science+Business Media, LLC, 233 Spring Street, New York, NY 10013, USA), except for brief excerpts in connection with reviews or scholarly analysis. Use in connection with any form of information storage and retrieval, electronic adaptation, computer software, or by similar or dissimilar methodology now known or hereafter developed is forbidden.

The use in this publication of trade names, trademarks, service marks, and similar terms, even if they are not identified as such, is not to be taken as an expression of opinion as to whether or not they are subject to proprietary rights.

While the advice and information in this book are believed to be true and accurate at the date of going to press, neither the authors nor the editors nor the publisher can accept any legal responsibility for any errors or omissions that may be made. The publisher makes no warranty, express or implied, with respect to the material contained herein.

Printed on acid-free paper

Humana Press is part of Springer Science+Business Media (www.springer.com)

Foreword

This book represents a milestone in the worldwide cystic fibrosis (CF) community's efforts to continue to pave the way toward the development of new and innovative therapies that address the basic defect in CF. But no book on this subject would be possible without the invaluable contributions of the many patients, families, and disease-related organizations that played a key role in creating the science outlined in these chapters. As an orphan disease, CF does not receive sufficient funding from traditional supporting agencies but depends instead on a vast network of people who selflessly give their time and energy to raise the dollars to support the research that will lead to new treatments and a cure. Much of the science described in the pages that follow is the result of funds raised by the CF community, as well as the willingness of patients to provide tissue specimens, share their data in patient registries, and participate in clinical studies. These contributions have been critical to the success that CF research is experiencing worldwide.

The global cystic fibrosis community is clearly unique and has often been described as a "culture of research." Shared among the many patient groups that represent about 70,000 people with CF worldwide is the belief that we will ultimately cure this disease through research. The constancy of this shared mission to cure CF by focusing on research is part of what sets the CF community apart from other rare diseases. The clear promise of small molecules and the excitement over gene therapy have kindled a sense of optimism that is critical to sustaining the momentum toward finding a cure.

In addition to the consistent focus on research, there are a number of other unique traits that the CF community around the world possesses that make it one of a kind. Some of the distinguishing qualities include the following:

- *Willingness to share:* Because of the insidious nature of CF, there is a rare sense of cooperative spirit among scientists, physicians, caregivers, patients, and families all over the world who are dedicated to a cure. CF research data know no borders, and waiting until data are published is not part of the CF research culture. The advancement of science is an open book, and the rapid exchange of new ideas and approaches is a mainstay of CF conferences and workshops in North America and overseas.
- *Willingness to take risks:* The search for the gene in the 1980s is an example of the risks and rewards of the pioneering work of the global CF populace. In the early 1980s, CF communities began to collect blood and tissue samples from families with multiply-affected individuals with CF. With newly evolved technologies (such as chromosome jumping), which could be quickly applied to these samples, and the rapid exchange of information, numerous efforts to find the gene were launched. The discovery of the CF gene in 1989 was the result of an intricate and highly successful international collaboration and is hailed today as one of the major milestones in modern medical research. Because of the involvement of CF families, patients, and the US Cystic Fibrosis Foundation, this groundbreaking discovery occurred over 14 years prior to the publication of the human genome. Importantly, it gave scientists an opportunity to explore the relationship of the genetic defect with the pathogenesis associated with CF. This discovery was a prelude to the effort to move from a knowledge acquisition

mode of research to the current CF research efforts, whereby scientists are using acquired knowledge about CF to develop new approaches to treat the disease.

- *Willingness to take responsibility for its own destiny:* Because there are only 70,000 CF patients throughout the world, there has been reluctance in the biopharmaceutical arena to enter the field of CF. Without their involvement, the ability to develop novel therapeutics is limited. In the late 1990s, frustrated by this fact, the US CF Foundation dramatically changed its business model and created a program to reduce risk of industry involvement in CF research by providing early funding and access to scientific and clinical expertise. This successful array of alliances with industry has led to an exciting clinical pipeline of products, including small molecules that are now being tested in centers worldwide, any of which could have a profound impact on individuals with CF.

Similarly, upon realizing industry's dwindling interest in gene therapy for CF, the British Trust launched a Gene Therapy Consortium that has painstakingly worked through some of the critical issues and problems associated with applying this pioneering mode of therapy for CF. As a result, and with a significant financial investment by the British Trust, the most comprehensive gene therapy clinical trial process is underway in the British Isles.

More recently, clinical trial networks have been established throughout North America and Europe to facilitate the evaluation of new clinical entities in order to hasten the regulatory process leading to drug approval. These clinical trial networks are linked through the sharing of data, expertise, and experience to contribute to the worldwide clinical trial efforts.

These are just a few examples of the willingness of the CF community to make strategic investments, some of which, in the case of other diseases, would be taken by industry or the government to bring us closer to accomplishing our mission.

- *Willingness to accept responsibility for the coordinating role in the areas of care, teaching, and research:* In many countries, scientists and clinicians look to the government for funding – agencies like the US National Institutes of Health and other equivalent organizations. However, not only is the science of CF frequently funded by CF organizations, but its direction is often defined by these entities as well. Similarly, the outstanding care that is provided all over the world is driven by the standards and guidelines set by these universal CF organizations. These guidelines are commonly established in international forums sponsored by CF groups. Once again, the community looks to CF organizations for leadership.

These volumes are the result of a distinct and worldwide undertaking. The environment, funding, and culture that have been put in place by patient organizations, coupled with the ability to bring the best minds to the field of CF, have made the science described in this book possible. This publication will be a useful tool as we continue to translate the knowledge acquired during decades of basic research to the development of new therapies that will modify and change the course of the disease in CF patients in the years ahead. *Cystic Fibrosis: Diagnosis and Protocols* is the fulfillment of decades of hard work by the volunteers and staff of the patient groups and organizations that have helped to pave the way toward our ultimate goal: a cure and control for cystic fibrosis.

President and Chief Executive Officer
Cystic Fibrosis Foundation

Robert J. Beall

Preface

More than 20 years have passed since the identification of the gene responsible for cystic fibrosis (CF) and undoubtedly many milestones have highlighted this area of research. But we have to admit it, progress towards finding a way of curing the disease has been slower than we initially expected and wished.

Apparently, this is not due to a lack of research efforts in the field, since in recent years the CF research community has been producing on average ~1,500 papers annually. So, probably we still need to dig deeper and with better tools to understand further the basic biological mechanisms underlying this complex disease. Nevertheless, it is increasingly difficult to grasp and use the already wide and still growing range of diverse methods currently employed to study CF so as to understand it in its multidisciplinary nature.

The aim of these *Cystic Fibrosis: Diagnosis and Protocols* volumes is thus to provide the CF research community (and that in related fields) with a very wide range of high-quality experimental tools, as an easy way to grasp and use classical and novel methods applied to CF. Hence, it is expected that it will contribute to accelerate the advancement of knowledge in this area. The purpose is thus to offer selected “good practice protocols” with a level of technical detail which is rarely published in peer-reviewed journals. Moreover, it is expected that this information will also enable researchers to identify subtle differences regarding techniques in their own laboratories, which often account for apparently “contradictory” data in the literature. Co-authorship from both sides of the Atlantic was particularly encouraged.

In the 2002 edition of this volume and in another previous comprehensive compilation of *Methods for Cystic Fibrosis and CFTR Research*¹, a large set of classical techniques used for CF research were already covered. So, here the focus is placed on innovative methodologies (some revolutionizing our way of doing science) by describing in detail how to perform and exploit these emergent techniques applied to CF. Moreover, a complete section has been devoted to available resources such as useful software and databases, as well as cell lines and animal models, reviewed for their usefulness towards multiple purposes. Notwithstanding, the more “classical” methods can also undergo improvements and thus their most up-to-date and revised versions are also recapped here by the leading experts. All book sections are introduced by an overview discussing the applicability and practicality of the protocols with examples.

It is hoped that the methods presented and revised here will provide users with optimal working tools to address their pressing questions in the best technical way while helping all of us, as a research and clinical community, to move faster hand-in-hand towards unravelling the secrets of this (and possibly other) challenging disorder(s) and cure it.

Finally, we wish to thank all authors for their enthusiasm in joining us in this project by contributing with their best protocols to this book and also for their patience with

¹ Journal of Cystic Fibrosis (2004), volume 3 (Supplement 2), a special issue focused on “Methods for Cystic Fibrosis and CFTR Research” and The online “Virtual Repository of the Cystic Fibrosis European Network” at <http://central.igc.gulbenkian.pt/cfr/vr/index.htm>

our multiple requests. Special thanks to Renata Vincent for her help in dealing with the manuscripts. Moreover, we would like to express our gratitude to the whole CF community in general, researchers, clinicians and all caregivers and other professionals, not forgetting CF patients and their families, for their continuous efforts towards finding a way out of this still devastating disease. We believe that we will be there soon and we hope this book somehow contributes to getting there sooner. Then, when our goals are met, all efforts will have been worthwhile, or as the Portuguese poet Fernando Pessoa has put it, “All is worthwhile if the soul is not small”.

Margarida D. Amaral
Karl Kunzelmann

Contents

<i>Foreword</i>	<i>v</i>
<i>Preface</i>	<i>vii</i>
<i>Contributors</i>	<i>xiii</i>

SECTION I: STRATEGIES TO CORRECT THE BASIC DEFECT IN CF AND ASSESS EFFICACY IN HUMAN CLINICAL TRIALS

1. Introduction to Section I: The Relevance of CF Diagnostic Tools for Measuring Restoration of CFTR Function After Therapeutic Interventions in Human Clinical Trials	3
<i>Kris De Boeck and Melissa Ashlock</i>	
2. High-Throughput Screening of Libraries of Compounds to Identify CFTR Modulators	13
<i>Nicoletta Pedemonte, Olga Zegarra-Moran, and Luis J.V. Galiotta</i>	
3. Repair of CFTR Folding Defects with Correctors that Function as Pharmacological Chaperones	23
<i>Tip W. Loo and David M. Clarke</i>	
4. Use of Primary Cultures of Human Bronchial Epithelial Cells Isolated from Cystic Fibrosis Patients for the Pre-clinical Testing of CFTR Modulators	39
<i>Timothy Neuberger, Bill Burton, Heather Clark, and Fredrick Van Goor</i>	
5. Design of Gene Therapy Trials in CF Patients	55
<i>Jane C. Davies and Eric W.F.W. Alton</i>	
6. Nasal Potential Difference Measurements to Assess CFTR Ion Channel Activity	69
<i>Steven M. Rowe, John Paul Clancy, and Michael Wilschanski</i>	
7. Measurement of Ion Transport Function in Rectal Biopsies	87
<i>Martin J. Hug, Nico Dericks, Inez Bronsveld, and Jean Paul Clancy</i>	

SECTION II: RNA METHODS TO APPROACH CFTR EXPRESSION

8. Introduction to Section II: RNA Methods to Approach <i>CFTR</i> Expression	111
<i>Ann Harris</i>	
9. Quantification of CFTR Transcripts	115
<i>Anabela S. Ramalho, Luka A. Clarke, and Margarida D. Amaral</i>	
10. Nonsense-Mediated mRNA Decay and Cystic Fibrosis	137
<i>Liat Linde and Batsheva Kerem</i>	

11. Approaches to Study CFTR Pre-mRNA Splicing Defects 155
Elisa Goïna, Eugenio Fernandez-Alanis, and Franco Pagani

12. Impact of MicroRNA in Normal and Pathological Respiratory Epithelia 171
Lisa Giovannini-Chami, Nathalie Grandvaux, Laure-Emmanuelle Zaragosi, Karine Robbe-Sermesant, Brice Marcet, Bruno Cardinaud, Christelle Coraux, Yves Berthiaume, Rainer Waldmann, Bernard Mari, and Pascal Barbry

13. Genomic Approaches to Studying CFTR Transcriptional Regulation 193
Christopher J. Ott and Ann Harris

SECTION III: CFTR PROTEIN BIOGENESIS, FOLDING, DEGRADATION, AND TRAFFIC

14. Introduction to Section III: Biochemical Methods to Study CFTR Protein 213
Margarida D. Amaral and Gergely L. Lukacs

15. Analysis of CFTR Folding and Degradation in Transiently Transfected Cells 219
Diane E. Grove, Meredith F.N. Rosser, Richard L. Watkins, and Douglas M. Cyr

16. In Vitro Methods for CFTR Biogenesis 233
Yoshihiro Matsumura, LeeAnn Rooney, and William R. Skach

17. Analysis of CFTR Interactome in the Macromolecular Complexes 255
Chunying Li and Anjaparavanda P. Naren

18. Methods to Monitor Cell Surface Expression and Endocytic Trafficking of CFTR in Polarized Epithelial Cells 271
Jennifer M. Bomberger, William B. Guggino, and Bruce A. Stanton

19. Segmental and Subcellular Distribution of CFTR in the Kidney 285
François Jouret, Pierre J. Courtoy, and Olivier Devuyst

20. Endocytic Sorting of CFTR Variants Monitored by Single-Cell Fluorescence Ratiometric Image Analysis (FRIA) in Living Cells 301
Herve Barrière, Pirjo Apaja, Tsukasa Okiyoneda, and Gergely L. Lukacs

SECTION IV: CFTR STRUCTURE

21. Introduction to Section IV: Biophysical Methods to Approach CFTR Structure 321
Juan L. Mendoza, André Schmidt, and Philip J. Thomas

22. CFTR Three-Dimensional Structure 329
Robert C. Ford, James Birtley, Mark F. Rosenberg, and Liang Zhang

23. Molecular Modeling Tools and Approaches for CFTR and Cystic Fibrosis 347
Adrian W.R. Serohijos, Patrick H. Thibodeau, and Nikolay V. Dokholyan

24. Biochemical and Biophysical Approaches to Probe CFTR Structure 365
André Schmidt, Juan L. Mendoza, and Philip J. Thomas

25. NMR Spectroscopy to Study the Dynamics and Interactions of CFTR 377
Voula Kanelis, P. Andrew Chong, and Julie D. Forman-Kay

SECTION V: CFTR FUNCTION

26. Introduction to Section V: Assessment of CFTR Function 407
Karl Kunzelmann

27. Application of High-Resolution Single-Channel Recording to Functional
 Studies of Cystic Fibrosis Mutants 419
*Zhiwei Cai, Yoshiro Sobma, Silvia G. Bompadre,
 David N. Sheppard, and Tzyh-Chang Hwang*

28. Electrophysiological, Biochemical, and Bioinformatic Methods for
 Studying CFTR Channel Gating and Its Regulation 443
László Csanády, Paola Vergani, Attila Gulyás-Kovács, and David C. Gadsby

29. CFTR Regulation by Phosphorylation 471
Rodrigo Alzamora, J Darwin King, and Kenneth R. Hallows

30. How to Measure CFTR-Dependent Bicarbonate Transport: From Single
 Channels to the Intact Epithelium 489
Martin J. Hug, Lane L. Clarke, and Michael A. Gray

Index 511

Contributors

- ERIC W.F.W. ALTON • *Department of Gene Therapy, Imperial College London, London, UK*
- RODRIGO ALZAMORA • *Renal-Electrolyte Division, School of Medicine, University of Pittsburgh, Pittsburgh, PA, USA*
- MARGARIDA D. AMARAL • *Faculty of Sciences, BioFiG-Centre for Biodiversity and Functional and Integrative Genomics, University of Lisboa, Lisbon, Portugal; Department of Genetics, National Institute of Health, Lisbon, Portugal; Centre of Human Genetics, National Institute of Health, Lisboa, Portugal; EMBL Heidelberg, Heidelberg, Germany*
- P. ANDREW CHONG • *Molecular Structure and Function, Hospital for Sick Children, Toronto, ON, Canada*
- PIRJO APAJA • *Department of Physiology, McGill University, Montréal, QC, Canada*
- MELISSA ASHLOCK • *Cystic Fibrosis Foundation Therapeutics, Bethesda, MD, USA*
- PASCAL BARBRY • *CNRS, Université de Nice Sophia Antipolis, IPMC, UMR6097, Sophia Antipolis, France*
- HERVE BARRIÈRE • *Department of Physiology, McGill University, Montréal, QC, Canada*
- YVES BERTHIAUME • *Centre de Recherche du Centre Hospitalier de l'Université de Montréal, Université de Montréal, Hôtel Dieu, Montréal, QC, Canada*
- JAMES BIRTLEY • *Faculty of Life Sciences, Manchester Interdisciplinary Biocentre, The University of Manchester, Manchester, UK*
- JENNIFER M. BOMBERGER • *Department of Microbiology and Immunology, Dartmouth Medical School, Hanover, NH, USA*
- SILVIA G. BOMPADRE • *Department of Medical Pharmacology and Physiology, Dalton Cardiovascular Research Center, University of Missouri-Columbia, Columbia, MO, USA*
- INEZ BRONSVELD • *Department of Pulmonology and Tuberculosis, University Medical Center Utrecht, Utrecht, The Netherlands*
- BILL BURTON • *Vertex Pharmaceuticals Incorporated, San Diego, CA, USA*
- ZHIWEI CAI • *Department of Physiology and Pharmacology, School of Medical Sciences, University of Bristol, Bristol, UK*
- BRUNO CARDINAUD • *CNRS, Université de Nice Sophia Antipolis, IPMC, UMR6097, Sophia Antipolis, France*
- JOHN PAUL CLANCY • *Departments of Medicine, Pediatrics, Physiology, Biophysics MCLM, University of Alabama, Birmingham, AL, USA*
- JEAN PAUL CLANCY • *Department of Pediatrics, University of Alabama, Birmingham, AL, USA*
- HEATHER CLARK • *Vertex Pharmaceuticals Incorporated, San Diego, CA, USA*
- LUKA A. CLARKE • *Faculty of Sciences, BioFiG-Centre for Biodiversity and Functional and Integrative Genomics, University of Lisboa, Lisbon, Portugal*
- LANE L. CLARKE • *Department of Biomedical Sciences, Dalton Cardiovascular Research Center, University of Missouri, Columbia, MO, USA*

- DAVID M. CLARKE • *Departments of Medicine and Biochemistry, University of Toronto, Toronto, ON, Canada*
- CHRISTELLE CORAUX • *INSERM UMRS 903, CHU Maison Blanche, Reims, France*
- PIERRE J. COURTOY • *CELL Unit, de Duve Institute, Université Catholique de Louvain Medical School, Brussels, Belgium*
- LÁSZLÓ CSANÁDY • *Department of Medical Biochemistry, Semmelweis University, Budapest, Hungary*
- DOUGLAS M. CYR • *Department of Cell and Developmental Biology, School of Medicine, The UNC-Cystic Fibrosis Center, University of North Carolina, Chapel Hill, NC, USA*
- JANE C. DAVIES • *Department of Gene Therapy, Imperial College London, London, UK*
- KRIS DE BOECK • *Department of Pediatrics, University of Leuven, Leuven, Belgium*
- NICO DERICHS • *Cystic Fibrosis Center, Pediatric Pulmonology and Neonatology, Medizinische Hochschule Hannover, Hannover, Germany, CFTR Biomarker Center, Christiane-Herzog-Zentrum für Mukoviszidose, Charité Universitätsmedizin Berlin, Berlin*
- OLIVIER DEVUYST • *Division of Nephrology, Université Catholique de Louvain Medical School, Brussels, Belgium*
- NIKOLAY V. DOKHOLYAN • *Department of Biochemistry and Biophysics, University of North Carolina, Chapel Hill, NC, USA*
- EUGENIO FERNANDEZ-ALANIS • *Human Molecular Genetics, International Centre for Genetic Engineering and Biotechnology, Trieste, Italy*
- ROBERT C. FORD • *Faculty of Life Sciences, Manchester Interdisciplinary Biocentre, The University of Manchester, Manchester, UK*
- JULIE D. FORMAN-KAY • *Molecular Structure and Function, Hospital for Sick Children, Toronto, ON, Canada; Department of Biochemistry, University of Toronto, Toronto, ON, Canada*
- DAVID C. GADSBY • *Laboratory of Cardiac/Membrane Physiology, The Rockefeller University, New York, NY, USA*
- LUIS J.V. GALIETTA • *Laboratorio di Genetica Molecolare, Istituto Giannina Gaslini, Genova, Italy*
- LISA GIOVANNINI-CHAMI • *CNRS, Université de Nice Sophia Antipolis, IPMC, UMR6097, Sophia Antipolis, France; Service de Pédiatrie, Unité de Pneumo-Allergologie, CHU de Nice, France*
- ELISA GOINA • *Human Molecular Genetics, International Centre for Genetic Engineering and Biotechnology, Trieste, Italy*
- NATHALIE GRANDVAUX • *Centre de Recherche du Centre Hospitalier de l'Université de Montréal, Université de Montréal, Hôpital Saint-Luc, PEA, Montréal, QC, Canada*
- MICHAEL A. GRAY • *Epithelial Research Group, Institute for Cell and Molecular Biosciences, Newcastle University, Newcastle Upon Tyne, UK*
- DIANE E. GROVE • *Department of Cell and Developmental Biology, School of Medicine, The UNC-Cystic Fibrosis Center, University of North Carolina, Chapel Hill, NC*
- WILLIAM B. GUGGINO • *Department of Physiology, Johns Hopkins University School of Medicine, Baltimore, MD, USA*
- ATTILA GULYÁS-KOVÁCS • *Laboratory of Cardiac/Membrane Physiology, The Rockefeller University, New York, NY, USA*
- KENNETH R. HALLOWS • *Renal-Electrolyte Division, School of Medicine, University of Pittsburgh, Pittsburgh, PA, USA*

- ANN HARRIS • *Human Molecular Genetics Program, Children's Memorial Research Center, Northwestern University Feinberg School of Medicine, Chicago, IL; Department of Pediatrics, Northwestern University Feinberg School of Medicine, Chicago, IL, USA*
- MARTIN J. HUG • *Pharmacy, University Medical Center Freiburg, Freiburg, Germany*
- TZYH-CHANG HWANG • *Department of Medical Pharmacology and Physiology, Dalton Cardiovascular Research Center, University of Missouri-Columbia, Columbia, MO, USA*
- FRANÇOIS JOURET • *Division of Nephrology, Université Catholique de Louvain Medical School, Brussels, Belgium*
- VOULA KANELIS • *Department of Chemical and Physical Sciences, University of Toronto Mississauga, Mississauga, ON, Canada*
- BATSHEVA KEREM • *Department of Genetics, The Life Sciences Institute, The Hebrew University, Jerusalem, Israel*
- J DARWIN KING • *Renal-Electrolyte Division, School of Medicine, University of Pittsburgh, Pittsburgh, PA, USA*
- KARL KUNZELMANN • *Department of Physiology, University of Regensburg, Regensburg, Germany*
- CHUNYING LI • *Department of Biochemistry and Molecular Biology, Wayne State University School of Medicine, Detroit, MI, USA*
- LIAT LINDE • *Department of Genetics, The Life Sciences Institute, The Hebrew University, Jerusalem, Israel*
- TIP W. LOO • *Departments of Medicine and Biochemistry, University of Toronto, Toronto, ON, Canada*
- GERGELY L. LUKACS • *Department of Physiology, McGill University, Montréal, QC, Canada*
- BRICE MARCET • *CNRS, Université de Nice Sophia Antipolis, IPMC, UMR6097, Sophia Antipolis, France*
- BERNARD MARI • *CNRS, Université de Nice Sophia Antipolis, IPMC, UMR6097, Sophia Antipolis, France*
- YOSHIHIRO MATSUMURA • *Department of Biochemistry and Molecular Biology, Oregon Health and Science University, Portland, OR, USA*
- JUAN L. MENDOZA • *Department of Physiology, University of Texas Southwestern Medical Center, Dallas, TX, USA*
- ANJAPARAVANDA P. NAREN • *Department of Physiology, University of Tennessee Health Science Center, Memphis, TN, USA*
- TIMOTHY NEUBERGER • *Vertex Pharmaceuticals Incorporated, San Diego, CA, USA*
- TSUKASA OKIYONEDA • *Department of Physiology, McGill University, Montréal, QC, Canada*
- CHRISTOPHER J. OTT • *Human Molecular Genetics Program, Children's Memorial Research Center, Northwestern University Feinberg School of Medicine, Chicago, IL, USA; Department of Pediatrics, Northwestern University Feinberg School of Medicine, Chicago, IL, USA*
- FRANCO PAGANI • *Human Molecular Genetics, International Centre for Genetic Engineering and Biotechnology, Trieste, Italy*
- NICOLETTA PEDEMONTE • *Laboratorio di Genetica Molecolare, Istituto Giannina Gaslini, Genova, Italy*

- ANABELA S. RAMALHO • *Faculty of Sciences, BioFiG-Centre for Biodiversity and Functional and Integrative Genomics, University of Lisboa, Lisbon, Portugal; Department of Genetics, National Institute of Health, Lisbon, Portugal*
- KARINE ROBBE-SERMESANT • *CNRS, Université de Nice Sophia Antipolis, IPMC, UMR6097, Sophia Antipolis, France*
- LEEANN ROONEY • *Department of Biochemistry and Molecular Biology, Oregon Health and Science University, Portland, OR, USA*
- MARK F. ROSENBERG • *Faculty of Life Sciences, Manchester Interdisciplinary Biocentre, The University of Manchester, Manchester, UK*
- MEREDITH F.N. ROSSER • *Department of Cell and Developmental Biology, School of Medicine, The UNC-Cystic Fibrosis Center, University of North Carolina, Chapel Hill, NC, USA*
- STEVEN M. ROWE • *Departments of Medicine, Pediatrics, and Physiology and Biophysics MCLM, University of Alabama, Birmingham, AL, USA*
- ANDRÉ SCHMIDT • *Department of Physiology, University of Texas Southwestern Medical Center, Dallas, TX, USA*
- ADRIAN W.R. SEROHIJOS • *Department of Physics and Astronomy, Program in Molecular and Cellular Biophysics, University of North Carolina, Chapel Hill, NC, USA; Department of Chemistry and Chemical Biology, Harvard University, Cambridge, MA, USA*
- DAVID N. SHEPPARD • *Department of Physiology and Pharmacology, School of Medical Sciences, University of Bristol, Bristol, UK*
- WILLIAM R. SKACH • *Department of Biochemistry and Molecular Biology, Oregon Health and Science University, Portland, OR, USA*
- YOSHIRO SOHMA • *Department of Pharmacology and Neuroscience, Keio University School of Medicine, Tokyo, Japan*
- BRUCE A. STANTON • *Department of Microbiology and Immunology, Dartmouth Medical School, Hanover, NH, USA*
- PATRICK H. THIBODEAU • *Department of Cell Biology and Physiology, University of Pittsburgh School of Medicine, Pittsburgh, PA, USA*
- PHILIP J. THOMAS • *Department of Physiology, University of Texas Southwestern Medical Center, Dallas, TX, USA*
- FREDRICK VAN GOOR • *Vertex Pharmaceuticals Incorporated, San Diego, CA, USA*
- PAOLA VERGANI • *Department of Neuroscience, Physiology and Pharmacology, University College London, London, UK*
- RAINER WALDMANN • *CNRS, Université de Nice Sophia Antipolis, IPMC, UMR6097, Sophia Antipolis, France*
- RICHARD L. WATKINS • *Department of Cell and Developmental Biology, School of Medicine, The UNC-Cystic Fibrosis Center, University of North Carolina, Chapel Hill, NC, USA*
- MICHAEL WILSCHANSKI • *Pediatric Gastroenterology, Hadassah University Hospitals, Jerusalem, Israel*
- LAURE-EMMANUELLE ZARAGOSI • *CNRS, Université de Nice Sophia Antipolis, IPMC, UMR6097, Sophia Antipolis, France*
- OLGA ZEGARRA-MORAN • *Laboratorio di Genetica Molecolare, Istituto Giannina Gaslini, Genova, Italy*
- LIANG ZHANG • *Department of Cell Biology and Physiology, University of Pittsburgh, Pittsburgh, PA, USA*