Methods in ENZYMOLOGY

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Mitochondrial Function, Part A: Mitochondrial Electron Transport Complexes and Reactive Oxygen Species

Edited by

William S. Allison

Immo E. Scheffler

VOLUME FOUR HUNDRED AND FIFTY-SIX

METHODS IN ENZYMOLOGY

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EDITED BY

WILLIAM S. ALLISON
Department of Chemistry and Biochemistry
University of California
San Diego, La Jolla, CA, USA

IMMO E. SCHEFFLER
Division of Biological Sciences - Molecular Biology
University of California
San Diego, La Jolla, CA, USA

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METHODS IN ENZYMOLOGY

Editors-in-Chief

JOHN N. ABELSON AND MELVIN I. SIMON

Division of Biology
California Institute of Technology
Pasadena, California, USA

Founding Editors

SIDNEY P. COLOWICK AND NATHAN O. KAPLAN

Contributors

Boominathan Amutha
Department of Pharmacology and Physiology, UMDNJ, New Jersey Medical School, Newark, New Jersey, USA

Matthew C. Altman
The Medical Research Council Mitochondrial Biology Unit, Cambridge, United Kingdom

Nikolai P. Belevich
Institute of Biotechnology, University of Helsinki, Helsinki, Finland

Giancarlo A. Biagini
Liverpool School of Tropical Medicine, Pembroke Place, Liverpool, United Kingdom

Egbert J. Boekema
Department of Biophysical Chemistry, Groningen Biomolecular Sciences and Biotechnology Institute, University of Groningen, Groningen, The Netherlands

Ulrich Brandt
Molecular Bioenergetics Group, Cluster of Excellence Frankfurt "Macromolecular Complexes," Medical School, Johann Wolfgang Goethe-University, Frankfurt am Main, Germany

Hans-Peter Braun
Institute for Plant Genetics, Faculty of Natural Sciences, Universität Hannover, Hannover, Germany

Christine A. Butler
Department of Molecular Biology and Genetics, Biotechnology Building, Cornell University, Ithaca, New York, USA

Roosevelt V. Campbell
Department of Neuroscience and Cell Biology, University of Texas Medical Branch, Galveston, Texas, USA

Joe Carroll
The Medical Research Council Mitochondrial Biology Unit, Cambridge, United Kingdom

Xiaowei Cen
Department of Biochemistry and Molecular Biology, Oklahoma State University, Stillwater, Oklahoma, USA

Jing Chen
Department of Pathology, Immunology, and Laboratory Medicine, College of Medicine, The University of Florida, Gainesville, Florida, USA

Helena M. Cochemé
Institute of Healthy Ageing, University College London, London, United Kingdom, and The Medical Research Council Mitochondrial Biology Unit, Cambridge, United Kingdom

Andrew Dancis
Department of Medicine, Division of Hematology-Oncology, University of Pennsylvania, Philadelphia, Pennsylvania, USA

Grazyna Debska-Vielhaber
Department of Neurology, University Magdeburg, Magdeburg, Germany, and Division of Neurochemistry, Department of Epileptology and Life & Brain Center, University Bonn, Bonn, Germany

Cindy E. J. Dieteren
Department of Paediatrics, Nijmegen Centre for Mitochondrial Disorders, and Department of Biochemistry, Nijmegen Centre for Molecular Life Sciences, Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands

Martina G. Ding
Department of Biochemistry, Dartmouth Medical School, Hanover, New Hampshire, USA

Jack E. Dixon
Departments of Pharmacology, Cellular and Molecular Medicine, and Chemistry and Biochemistry, and The Howard Hughes Medical Institute, University of California, San Diego, La Jolla, California, USA

Derek A. Drechsel
Department of Pharmaceutical Sciences, University of Colorado, Denver, Aurora, Colorado, USA

Stefan Dröse
Molecular Bioenergetics Group, Cluster of Excellence Frankfurt "Macromolecular Complexes," Medical School, Johann Wolfgang Goethe-University, Frankfurt am Main, Germany

Bill Durham
Department of Chemistry and Biochemistry, University of Arkansas, Fayetteville, Arkansas, USA

Ian M. Fearnley
The Medical Research Council Mitochondrial Biology Unit, Cambridge, United Kingdom

Nicholas Fisher
Liverpool School of Tropical Medicine, Pembroke Place, Liverpool, United Kingdom

Thomas D. Fox
Department of Molecular Biology and Genetics, Biotechnology Building, Cornell University, Ithaca, New York, USA

Terrence G. Frey
Department of Biology, San Diego State University, San Diego, California, USA

Alexander Galkin
The Wolfson Institute for Biomedical Research, The Cruciform Building, University College London, London, United Kingdom

Lois Geren
Department of Chemistry and Biochemistry, University of Arkansas, Fayetteville, Arkansas, USA

François Godard
Institut de Biochimie et Génétique Cellulaires (CNRS), Université Victor Segalen, Bordeaux, France

Donna M. Gordon
Department of Biological Sciences, Mississippi State University, Mississippi State, Mississippi, USA

Aaron M. Gusdon
Department of Pathology, Immunology, and Laboratory Medicine, College of Medicine, The University of Florida, Gainesville, Florida, USA

Mark A. Hink
Max Planck Institute for Molecular Physiology, Dortmund, Germany

Gregory Holmes-Hampton
Department of Chemistry, Texas A&M University, College Station, Texas, USA

Thomas R. Hurd
The Medical Research Council Mitochondrial Biology Unit, Cambridge, United Kingdom

Andrew M. James
The Medical Research Council Mitochondrial Biology Unit, Cambridge, United Kingdom

Werner J. H. Koopman
Department of Biochemistry, and Microscopical Imaging Centre, Nijmegen Centre for Molecular Life Sciences, Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands

Roman Kouřil
Department of Biophysical Chemistry, Groningen Biomolecular Sciences and Biotechnology Institute, University of Groningen, Groningen, The Netherlands

Alexei P. Kudin
Division of Neurochemistry, Department of Epileptology and Life & Brain Center, University Bonn, Bonn, Germany

Wolfram S. Kunz
Division of Neurochemistry, Department of Epileptology and Life & Brain Center, University Bonn, Bonn, Germany

Michael Lazarou
Department of Biochemistry, La Trobe University, Melbourne, Australia

Yanchun Li
Department of Neurology, Baylor College of Medicine, Houston, Texas, USA

Roland Lill
Institut für Zytobiologie and Zytopathologie, Philipps-Universität, Marburg, Germany

Kathryn S. Lilley
Department of Biochemistry, Cambridge System Biology Centre, University of Cambridge, Cambridge, United Kingdom

Paul A. Lindahl
Department of Biochemistry and Biophysics, and Department of Chemistry, Texas A&M University, College Station, Texas, USA

Dominika Malinska
Laboratory of Intracellular Ion Channels, Nencki Institute of Experimental Biology, Warsaw, Poland, and Division of Neurochemistry, Department of Epileptology and Life & Brain Center, University Bonn, Bonn, Germany

Douglas Marshall
The Johnson Research Foundation and Department of Biochemistry and Biophysics, University of Pennsylvania, Philadelphia, Pennsylvania, USA

Clayton E. Mathews
Department of Pathology, Immunology, and Laboratory Medicine, College of Medicine, The University of Florida, Gainesville, Florida, USA

Matthew McKenzie
Department of Biochemistry, La Trobe University, Melbourne, Australia

Ren Miao
Department of Chemistry, Texas A&M University, College Station, Texas, USA

Francis Millett
Department of Chemistry and Biochemistry, University of Arkansas, Fayetteville, Arkansas, USA

Jessica Garber Morales
Department of Chemistry, Texas A&M University, College Station, Texas, USA

Michael P. Murphy
The Medical Research Council Mitochondrial Biology Unit, Cambridge, United Kingdom

Leo G. J. Nijtmans
Department of Paediatrics, Nijmegen Centre for Mitochondrial Disorders, Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands

Esther Nübel
Cluster of Excellence "Macromolecular Complexes," Zentrum der Biologischen Chemie, Molekulare Bioenergetik, Goethe-Universität Frankfurt, Frankfurt, Germany

Debkumar Pain
Department of Pharmacology and Physiology, UMDNJ, New Jersey Medical School, Newark, New Jersey, USA

Manisha Patel
Department of Pharmaceutical Sciences, University of Colorado Denver, Aurora, Colorado, USA

Guy A. Perkins
National Center for Microscopy and Imaging Research, Center for Research in Biological Systems, University of California, San Diego, La Jolla, California, USA

Aparna Rachamallu
Department of Neuroscience and Cell Biology, University of Texas Medical Branch, Galveston, Texas, USA

Michael Radermacher
University of Vermont, Department of Molecular Physiology and Biophysics, Burlington, Vermont, USA

Jean-Paul di Rago
Institut de Biochimie et Génétique Cellulaires (CNRS), Université Victor Segalen, Bordeaux, France

Matthew J. Rardin
Gibson Lab,The Buck Institute for Age Research, Novato, California, USA

Peter R. Rich
Glynn Laboratory of Bioenergetics, Institute of Structural and Molecular Biology, University College London, London, United Kingdom

Michael T. Ryan
Department of Biochemistry, La Trobe University, Melbourne, Australia

Scott A. Saracco
Department of Molecular Biology and Genetics, Biotechnology Building, Cornell University, Ithaca, New York, USA

Hermann Schägger
Cluster of Excellence "Macromolecular Complexes," Zentrum der Biologischen Chemie, Molekulare Bioenergetik, Goethe-Universität Frankfurt, Frankfurt, Germany

Alex D. Sheftel
Institut für Zytobiologie and Zytopathologie, Philipps-Universität, Marburg, Germany

Wim Sluiter
Department of Biochemistry, Mitochondrial Research Unit, Erasmus MC, Dr. Molewaterplein 50-60, Rotterdam, The Netherlands

Oliver Stehling
Institut für Zytobiologie and Zytopathologie, Philipps-Universität, Marburg, Germany

Rosemary A. Stuart
Department of Biological Sciences, Marquette University, Milwaukee, Wisconsin, USA

Mei G. Sun
Department of Biology, San Diego State University, San Diego, California, USA

Stephanie Sunderhaus
Institute for Plant Genetics, Faculty of Natural Sciences, Universität Hannover, Hannover, Germany

Herman G. Swarts
Department of Biochemistry, Nijmegen Centre for Molecular Life Sciences, Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands

Bernard L. Trumpower
Department of Biochemistry, Dartmouth Medical School, Hanover, New Hampshire, USA

Marina L. Verkhovskaya
Institute of Biotechnology, University of Helsinki, Helsinki, Finland

Michael I. Verkhovsky
Institute of Biotechnology, University of Helsinki, Helsinki, Finland

Stefan Vielhaber
Department of Neurology, University Magdeburg, Magdeburg, Germany

Tatyana V. Votyakova
Department of Pediatrics, School of Medicine, University of Pittsburgh, Pennsylvania, USA

John E. Walker
The Medical Research Council Mitochondrial Biology Unit, Cambridge, United Kingdom

Ting Wang
Department of Microbiology and Immunology, University of Texas Medical Branch, Galveston, Texas, USA

Stephen A. Ward
Liverpool School of Tropical Medicine, Pembroke Place, Liverpool, United Kingdom

Ashley J. Warman
Liverpool School of Tropical Medicine, Pembroke Place, Liverpool, United Kingdom

Stanley J. Watowich
Department of Biochemistry and Molecular Biology, University of Texas Medical Branch, Galveston, Texas, USA

Steven A. Weinman
Department of Internal Medicine, University of Kansas Medical Center, Kansas City, Kansas, USA

Sandra E. Wiley
Departments of Pharmacology, Cellular and Molecular Medicine, and Chemistry and Biochemistry, University of California, San Diego, La Jolla, California, USA

Peter H. G. M. Willems
Department of Biochemistry and Microscopical Imaging Centre, Nijmegen Centre for Molecular Life Sciences, Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands

L. Elly. A. de Wit
Department of Biochemistry, Mitochondrial Research Unit, Erasmus MC, Dr. Molewaterplein 50-60, Rotterdam, The Netherlands

Ilka Wittig
Cluster of Excellence "Macromolecular Complexes," Zentrum der Biologischen Chemie, Molekulare Bioenergetik, Goethe-Universität Frankfurt, Frankfurt, Germany

Zibiernisha Wumaier
Cluster of Excellence "Macromolecular Complexes," Zentrum der Biologischen Chemie, Molekulare Bioenergetik, Goethe-Universität Frankfurt, Frankfurt, Germany

Di Xia
Laboratory of Cell Biology, Center for Cancer Research, National Cancer Institute, National Institutes of Health, Bethesda, Maryland, USA

Yuanzheng Yang
Department of Neuroscience and Cell Biology, University of Texas Medical Branch, Galveston, Texas, USA

Shaoqing Yang
Department of Biochemistry and Molecular Biology, Oklahoma State University, Stillwater, Oklahoma, USA

Ying Yin
Department of Biochemistry and Molecular Biology, Oklahoma State University, Stillwater, Oklahoma, USA

Linda Yu
Department of Biochemistry and Molecular Biology, Oklahoma State University, Stillwater, Oklahoma, USA

Chang-An Yu
Department of Biochemistry and Molecular Biology, Oklahoma State University, Stillwater, Oklahoma, USA

Fei Zhou
Department of Biochemistry and Molecular Biology, Oklahoma State University, Stillwater, Oklahoma, USA

PREFACE

Two volumes of *Methods in Enzymology* devoted to methods in mitochondrial research were published in 1995 and 1996 under the editorship of the late Guiseppe Attardi and Anne Chomyn. The emphasis of the earlier volumes was on mitochondrial biogenesis. Nevertheless, they also contained several articles describing methods for examining the structure and function of inner membrane complexes that participate in electron transport and ATP synthesis. In the intervening years, high-resolution crystal structures have been obtained for complexes II, III, and IV derived from the mitochondrial inner membrane. However, a crystal structure has yet to be obtained for a eukaryotic complex I (NADH: quinone oxidoreductase). Thus, several chapters in this volume of *Methods in Enzymology* describe alternative methods to characterize the structure and function of complex I. Other chapters are focused on the location and function of mitochondrial iron-sulfur complexes and the characterization of reactive oxygen species that are formed during mitochondrial electron transport in mammalian and yeast mitochondria.

Because a crystal structure has not been obtained for complex I isolated from mammalian or yeast mitochondria, several chapters in this volume (Chapters 1, 2, 6 and 7) describe methods that have been developed to examine structural characteristics of complex I. Other articles describe methods that have been developed to examine electron transport through complex I (Chapters 3 and 4) and to assay complex I in human cells (Chapter 9). Methods to characterize type II NADH: quinone oxidoreductases isolated from the parasites *Plasmodium falciparum* and *Mycobacterium tuberculosis* are described in Chapter 17. Three chapters describe methods for the isolation and characterization of electron transport super complexes from yeast (Chapters 10 and 11) and mammalian mitochondria (Chapter 8). Another chapter describes methods to examine the assembly of subunits encoded by mitDNA and nuclear DNA in the mitochondrial inner membrane of mammalian cells (Chapter 18). The use of ruthenium ion photooxidation and photoreduction to examine electron transfer in mitochondrial complex II and complex IV, respectively, are described in Chapters 5 and 28, respectively. Chapter 16 describes methods for examining mitochondrial mobility and protein diffusion within the mitochondrial matrix.

Although they were discovered in the 1970s, the biosynthesis and assembly iron-sulfur proteins have been examined more recently. Three chapters (Chapters 12, 14 and 15) describe methods to examine the

location, function, and assembly of iron-sulfur complexes in the mitochondrial inner membrane, whereas Chapter 13 describes the isolation and characterization an iron-sulfur protein located in the mitochondrial outer membrane.

Although they probably play a beneficial role at low concentrations, reactive oxygen species (ROS), which include superoxide anion ($O_2^{\bullet -}$) and hydrogen peroxide (H_2O_2), excessive ROS formation in mitochondria is invariably associated with pathologic conditions. Chapters 19 to 27 describe methods that have developed to detect, induce, or control formation of ROS during electron transport in mammalian and yeast mitochondria.

The methods described in this volume should provide investigators with techniques that can be used or modified to examine mitochondrial electron transport complexes, the location and function of iron-sulfur proteins, and the detection and control of reactive oxygen species that are formed during electron transport in mitochondria.

WILLIAM S. ALLISON AND IMMO E. SCHEFFLER

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