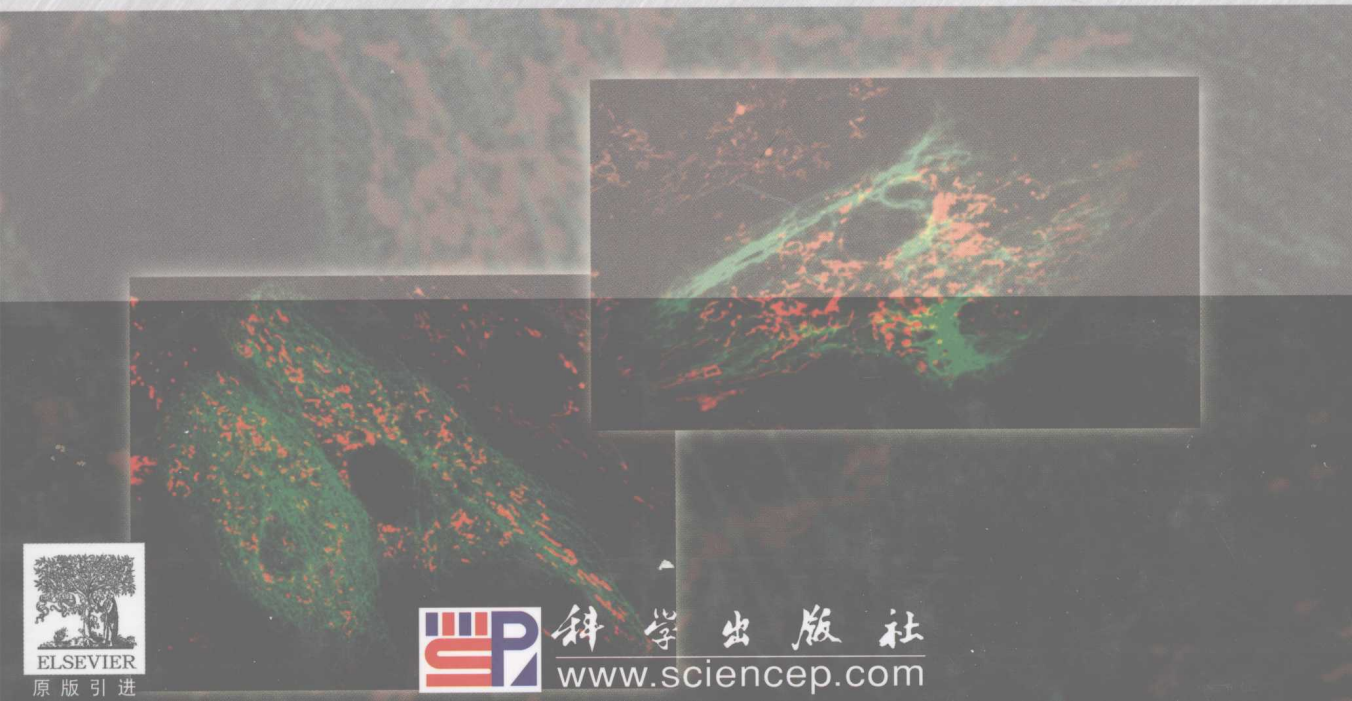
科爱传播
KE'AI COMMUNICATIONS
生命科学
导读版

Mitochondria
线粒体
（第二版）

Liza A. Pon, Eric A. Schon



ELSEVIER
原版引进
科学出版社
www.sciencep.com

Methods in Cell Biology Volume 80

细胞生物学方法　第80卷

Mitochondria

Second Edition

线　粒　体

第二版

Edited by

Liza A. Pon
Department of Anatomy and Cell Biology
College of Physicians and Surgeons
Columbia University
New York, New York

Eric A. Schon
Department of Neurology and Department of
Genetics and Development
Columbia University
New York, New York

科学出版社
北京

图字:01-2007-4862 号

This is an annotated version of
Methods in Cell Biology, Volume 80, Mitochondria, 2nd Edition by Liza A. Pon, Eric A. Schon.

ISBN-13: 978-0-12-544173-5
ISBN-10: 0-12-544173-8

图书在版编目(CIP)数据

线粒体:第 2 版 = Mitochondria, Second Edition:英文/(美)庞(Pon, L. A.),(美)舍恩(Schon, E. A.)编著. —北京:科学出版社,2007

(细胞生物学方法;80)

ISBN 978-7-03-020387-8

Ⅰ. 线… Ⅱ. ①庞…②舍… Ⅲ. 线粒体-英文 Ⅳ. Q24

中国版本图书馆 CIP 数据核字(2007)第 155611 号

责任编辑:孙红梅 李小汀
责任印制:钱玉芬/封面设计:耕者设计工作室

科学出版社 出版
北京东黄城根北街16号
邮政编码:100717
http://www.sciencep.com
中国科学院印刷厂印刷
科学出版社发行 各地新华书店经销
*
2008 年 1 月第 一 版 开本:787×1092 1/16
2008 年 1 月第一次印刷 印张:60 插页:4
印数:1—1 000 字数:1 423 000

定价:198.00 元

(如有印装质量问题,我社负责调换〈科印〉)

导　读

在生物学领域，实验技术对于学科发展的推动作用日益明显。有鉴于此，Elsevier 公司出版了《细胞生物学方法》系列丛书，分不同主题详细介绍了细胞生物学及生物化学、分子生物学等相关学科的常用实验技术。2007 年该丛书出版了第八十卷《线粒体》分册的第二版。该书汇编了研究线粒体的经典及现代方法，并着重介绍了近年来最新的技术进展和领域前沿，反映了当前线粒体研究中的新热点，有助于科技工作者从不同方面深入研究线粒体的结构与功能。

线粒体（mitochondria）是普遍存在于真核细胞中的细胞器，它通过氧化磷酸化进行能量转换，合成能源物质——三磷酸腺苷（ATP），为细胞的各项活动提供 90％以上的能量，是细胞能量代谢的中心，因此有“细胞动力工厂”之称。近年来发现，线粒体还具有其他重要的生物学功能，包括调控细胞凋亡、参与细胞信号转导、生成活性氧等。在细胞中，线粒体不断进行分裂（fission）、融合（fusion）、增殖和降解，它的生物发生（biogenesis）是在细胞核和线粒体两个遗传体系的共同控制下完成的；而线粒体功能低下（dysfunction）与机体的衰老以及肿瘤、糖尿病、心脑血管病等重大疾病的发生密切相关。

线粒体发现至今已超过百年，一直是最受关注的细胞器之一，近年来先后有 Paul D. Boyer，John E. Walker 等科学家因为在这一研究领域中的重大发现而获得诺贝尔奖。线粒体的研究大体可分为以下五个阶段：

1. 早期的细胞学研究。从 18 世纪末到 19 世纪初，主要通过光学显微镜观察真核细胞中的线粒体，确定线粒体是具有一定结构的细胞器。

2. 早期关于线粒体与细胞能量代谢的研究。在 20 世纪 40～50 年代，通过生物化学方法开展了线粒体脂肪酸氧化和三羧酸循环、电子传递链和氧化磷酸化方面的研究，确定了线粒体是细胞能量代谢的中心。

3. 线粒体超微结构与氧化磷酸化机理的研究。自 20 世纪 50 年代开始，电子显微镜技术的发展为探索线粒体的超微结构提供了有力武器。通过与生物化学、生物物理技术相结合，针对线粒体呼吸链氧化磷酸化基本结构和功能单元的研究不断取得进展。

4. 线粒体 DNA 与线粒体遗传学。自 1965 年首次从线粒体中鉴定出 DNA 以来，线粒体 DNA 与线粒体遗传学不断取得新的进展。人的线粒体 DNA（mitochondrial DNA；mtDNA）是一个长 16.6 kb 的环形双股分子，编码线粒体内膜氧化磷酸化酶系的 13 种多肽，两种 tRNA，以及两种 rRNA。mtDNA 的突变与多种线粒体病有密切关系，目前关于线粒体 DNA 与线粒体遗传学的研究正方兴未艾。

5. 线粒体调控细胞凋亡、参与细胞信号转导的相关研究。1998 年华裔科学家王晓东博士首次发现线粒体释放的细胞色素 c 是介导细胞凋亡的重要信号分子，这一重大发现表明线粒体不仅是细胞能量代谢中心，更是细胞信号转导的枢纽，参与包括细胞凋亡、机体衰老及氧化应激等重要生理和病理过程。由此引发了继线粒体氧化磷酸化、线粒体

DNA 及线粒体遗传学之后的新一轮线粒体研究热潮。

回顾以上线粒体研究的五个阶段，我们不难发现，重大理论创新都需要新实验技术作为基础，而理论创新又会大大促进实验技术的创新与发展。如光学显微技术是确定线粒体基本形态的基础，电子显微技术为观察线粒体的超微结构提供了可能，蛋白质工程技术、X 射线晶体学技术是研究线粒体重要功能蛋白的有力武器，而分子生物学技术则是研究线粒体基因组基因表达与调控的基本保障。

本世纪以来线粒体的研究进入了“全面复兴阶段”，*Science* 和 *Nature* 连续发表系列论文，充分表明线粒体的研究是当代生命科学和分子医学最活跃的新生长点和前沿之一。线粒体研究的外延不断扩大，内涵不断深入，多学科的交叉与融合日益明显，有越来越多的科技工作者从生物化学、细胞生物学、分子生物学、生物物理学、发育生物学等不同方面从事线粒体的相关研究，他们迫切需要一本系统、全面介绍线粒体研究的方法学工具书，使他们能够快速掌握最新及经典的实验技术。《线粒体》(第二版)集线粒体相关实验技术之大成，正是相关领域研究人员不可或缺的工具书。

本书的两位主编(Liza A. Pon 博士和 Eric A. Schon 博士)均来自美国哥伦比亚大学，具有丰富的线粒体研究经验。Pon 博士的主要研究方向集中在线粒体运动性方面，以出芽酵母为实验模型，系统研究了细胞分裂过程中线粒体的运动与分裂，发现细胞骨架、细胞周期相关信号通路均参与了线粒体的运动；Schon 博士的主要研究方向是线粒体遗传学与线粒体病，现正致力于用转线粒体小鼠(异源线粒体小鼠)发展神经退行性疾病模型。本书正文部分根据研究方向分为 7 个部分共 36 章，由多名来自线粒体研究与教学一线、具有扎实理论基础和丰富实践经验的名家(包括美国科学院院士、著名线粒体专家 Giuseppe Attardi 等)撰写，通过彼此独立又相互联系的 36 个科研课题，系统介绍了研究线粒体结构与功能的最新及经典实验技术。下面对本书的内容做一简介。

第一部分共 3 章，详述了从动物、植物和真菌组织中提取线粒体、纯化亚线粒体组分的方法。实验方法不仅涵盖了经典线粒体研究常用的哺乳动物、高等植物，还扩展到啤酒酵母、果蝇、秀丽线虫等模式生物。

第二部分共 10 章，汇编了研究线粒体生化性质的经典及现代实验方法，包括测试线粒体呼吸链酶的活性、分析线粒体中脂肪酸 β-氧化等经典生物化学实验技术；同时还详细介绍了在整体细胞水平观察线粒体的光学成像方法，包括对线粒体重要功能蛋白进行细胞内定位的组织化学方法和免疫组织化学方法、检测线粒体 DNA 的原位杂交技术等。此外，还介绍了线粒体电位依赖性阴离子通道(VDAC)通透性、线粒体铁代谢等相关新兴研究方向所需的实验技术。

第三部分共 3 章，详细介绍了用光学成像等新技术研究活细胞中的线粒体活性的现代实验方法和最新技术进展。其中，第 14 章除了介绍采用阳离子荧光探针定量测定线粒体跨膜电位的实验技术外，还介绍了应用荧光共振能量转移(FRET)技术检测去极化线粒体的方法，这一新型技术具有极高的灵敏度，可以检测出一个细胞内数百个线粒体中的单个去极化线粒体，因此在研究细胞凋亡的线粒体通路方面有望获得广泛应用；第 15 章介绍了应用融合蛋白、报告基因等生物传感器测定线粒体内钙离子和氢离子浓度(pH 值)的新技术；第 16 章则介绍了研究细胞凋亡中线粒体通透性改变的实验方法。

第四部分共4章，是第二版新增的内容，系统介绍了研究线粒体与氧化应激（oxidative stress）相互关系的实验方法，包括在细胞及线粒体中检测活性氧（reactive oxygen species）、测定线粒体抗氧化活性、研究一氧化氮调控线粒体呼吸等前沿领域。线粒体作为细胞通过氧化磷酸化进行能量转换的中心，也是最主要的产生活性氧的场所，由线粒体产生的活性氧如何影响线粒体的功能、对机体会造成何种影响一直是科学家关注的课题，也正成为线粒体研究领域内的新热点之一。在本书介绍的最新实验技术的指导下，有望在此方向获得更多具有创新性的成果。

第五部分共7章，系统、全面地介绍了研究线粒体DNA及线粒体遗传所需的实验技术，包括检测线粒体DNA突变、检测线粒体DNA复制及转录的基本实验技术，还着重介绍了转线粒体技术。线粒体是一种半自主的细胞器，其遗传特性有别于细胞核，依赖于母细胞，而缺失线粒体DNA的rho^0细胞则是研究线粒体DNA功能的有力武器。第25章详细介绍了产生哺乳动物rho^0细胞的实验技术，以及向rho^0细胞转染线粒体DNA、构建胞质杂合体（cybrid）的实验技术，使细胞生物学科研工作者能够顺利从事线粒体遗传学的研究。第26章以啤酒酵母和莱茵衣藻作为模式生物，介绍了转线粒体技术。第27章则详细介绍了转线粒体小鼠（异源线粒体小鼠）的相关实验技术，以及将转线粒体小鼠用于神经退行性疾病模型的探索。第28章主要介绍了在体（*in vivo*）及在线粒体水平（*in organello*）研究线粒体内蛋白质合成的实验方法。

第六部分共4章，综述了观测线粒体形态及运动性的实验技术，特别介绍了在活细胞中实时、动态观察线粒体形态变化、线粒体分裂与融合、线粒体运动的光学成像技术；还介绍了在无细胞体系中观察线粒体与细胞骨架相互作用的实验方法。

第七部分共4章，主要介绍了研究线粒体中蛋白输送与蛋白定位的实验技术，包括用非变性聚丙烯酰胺电泳和二维电泳分析分离蛋白质复合物、用免疫共沉淀等方法研究线粒体中蛋白质相互作用等新技术，还介绍了筛选、分析可能定位于线粒体的蛋白质的生物信息学工具。

与正文部分相得益彰的是本书的6篇附录，它们概述了线粒体的基本特性，列出了人类和重要模式生物的线粒体基因组图和线粒体遗传密码，汇编了已被确证或可能的线粒体蛋白质。应对于“组学”的蓬勃发展，附录还汇编了用微阵列技术（基因芯片）、蛋白质组学方法分析各种条件下线粒体功能变化的数据，充分反映了线粒体及相关领域的最新研究进展。

纵观全书，内容丰富全面，基本涵盖了线粒体研究的各个方面；系统性突出，根据不同研究方向介绍了研究线粒体所需的实验方法；重点明确、时效性强，第二版特别增补了近年来线粒体研究的最新进展（如线粒体与细胞凋亡、线粒体与氧化应激、线粒体与衰老等），同时也兼顾线粒体氧化磷酸化等经典研究内容。每一章都向读者详尽介绍了数种从事线粒体研究所需的经典和最新的实验技术，特别强调实验方法的适用性，并详述了特别需要注意的环节，实验方案具有很强的可操作性。每一章还深入浅出地介绍了研究背景和实验原理，让读者不仅能够掌握实验技术，更能领会实验原理。全书虽由众多科研人员共同编纂，但都力求保持脉络清晰、行文流畅、图表简明、图文并茂的统一风格；文字表达规范，易于理解。编者在纂写过程中注重理论和应用相结合，使本书不仅是一本指导性极强的实验指南，更是一本汇编了线粒体研究最新进展的优秀学术专著。我们相信，本书非

常适于在科研一线从事线粒体相关研究的科技工作者和研究生阅读、参考，并将对他们的科研工作提供巨大的帮助。

卫涛涛　杨福愉
中国科学院生物物理研究所
2007 年 8 月

前　言

在过去15年间，线粒体研究进入到一个全面复兴的阶段。与1985年相比，研究方向延伸到了探索线粒体功能与细胞凋亡、衰老，线粒体功能障碍与人类疾病关系等全新领域。此外，针对线粒体转运、运动、分裂、融合、遗传，以及线粒体与细胞核及其他细胞器的相互作用等基本现象的研究工作也取得了里程碑式的重大进展。

伴随着这些理论上的重大进展，一系列新的研究线粒体结构与功能的实验手段也发展起来。除了传统的分离纯化线粒体、分析其生化性质外，我们现在还掌握了新的有力工具，可以观察、检测和干预线粒体的功能；还能够分析这些干预措施对遗传造成的影响。

在2001年，我们编写了《细胞生物学方法》丛书中的《线粒体》分册，汇编了研究线粒体的许多经典和现代的实验方法，使得读者不论从生物化学、形态学还是遗传学等角度研究这一细胞器，都能够从此书中受益。此书的畅销激励我们编撰第二版，以便介绍近年来最新的技术进展和领域前沿。第二版的篇幅从25章扩展到36章，原有的内容也做了大幅度修改，删除了个别章节，补充了全新的14章，内容涵盖了分析乳酸盐、丙酮酸盐、β-氧化、三羧酸循环中间产物、心磷脂、铁硫中心和线粒体通透性的方法。我们还加入了一个新的部分共四章分析氧化应激（包括分析活性氧、自由基清除剂、一氧化氮及蛋白质巯基修饰）的内容，以及两章分析线粒体分裂、融合以及活细胞中线粒体运动的内容，这些都反映了当前线粒体研究中的新热点。

本书的附录与这些实验方法相得益彰，汇编了不同来源线粒体的基本数据。附录列出了所有已被确定的和可能的线粒体蛋白（与上一版相比有50%的蛋白是新列入的）、几个常见物种的线粒体基因组图，以及干预线粒体呼吸的试剂。应对于各种“组学”（“omics”）的蓬勃发展，新的附录还给出了用微阵列技术（基因芯片）、蛋白质组学方法分析各种条件下线粒体功能变化的数据。

我们谨将本书献给Giuseppe Attardi——线粒体研究、特别是哺乳动物线粒体遗传研究的先驱。他最大的学术贡献在于阐明了人类线粒体基因组的转录特性，发展了哺乳动物 ρ^0 细胞和胞质杂合体。Giuseppe是我们灵感的来源。

Liza A. Pon
Eric A. Schon

（卫涛涛　杨福愉 译）

CONTRIBUTORS

Numbers in parentheses indicate the pages on which the authors' contributions begin.

R. Acín-Pérez (571), Departamento de Bioquímica y Biología Molecular y Celular, Universidad de Zaragoza, Zaragoza 50013, Spain

J. S. Armstrong (355), Department of Biochemistry, Yong Loo Lin School of Medicine, National University of Singapore, Republic of Singapore 117597, Singapore

Giuseppe Attardi (121), Division of Biology, California Institute of Technology, Pasadena, California 91125

Sandra R. Bacman (503), Department of Neurology, University of Miami Miller School of Medicine, Miami, Florida 33136

Christopher J. Bell (341), Department of Biochemistry, Henry Wellcome Signalling Laboratories, School of Medical Sciences, University Walk, University of Bristol, Bristol BS8 1TD, United Kingdom

Michael J. Bennett (179), Department of Pathology and Laboratory Medicine, University of Pennsylvania, Metabolic Disease Laboratory, The Children's Hospital of Philadelphia, Philadelphia 19104

Istvan R. Boldogh (45, 683), Department of Anatomy and Cell Biology, College of Physicians and Surgeons, Columbia University, New York, New York 10032

Eduardo Bonilla (135), Department of Pathology and Department of Neurology, Columbia University, New York, New York 10032

Nathalie Bonnefoy (525), Centre de Génétique Moléculaire, CNRS UPR2167, Avenue de la Terrasse, 91198 Gif-sur-Yvette cedex, France

Paul S. Brookes (395), Department of Anesthesiology, University of Rochester Medical Center, Rochester, New York 14642

Marco Colombini (241), Department of Biology, University of Maryland, College Park, Maryland 20742

Victor M. Darley-Usmar (395), Cellular and Molecular Pathology, University of Alabama at Birmingham, Birmingham, Alabama 35294

Kurt J. De Vos (627, 683), Department of Neuroscience, MRC Centre for Neurodegeneration Research, The Institute of Psychiatry, King's College London, De Crespigny Park, Denmark Hill, London SE5 8AF, United Kingdom

Rachel DeVay (707), Department of Molecular and Cellular Biology, University of California Davis, Davis, California 95616

Orly Elpeleg (199), The Metabolic Disease Unit, Hadassah-Hebrew University Medical Centre, Jerusalem 91120, Israel

J. A. Enriquez (571), Departamento de Bioquímica y Biología Molecular y Celular, Universidad de Zaragoza, Zaragoza 50013, Spain

P. Fernández-Silva (571), Departamento de Bioquímica y Biología Molecular y Celular, Universidad de Zaragoza, Zaragoza 50013, Spain

E. Fernández-Vizarra (571), Departamento de Bioquímica y Biología Molecular y Celular, Universidad de Zaragoza, Zaragoza 50013, Spain

Thomas D. Fox (525), Department of Molecular Biology and Genetics, Cornell University, Ithaca, New York 14853

Elena Garcia-Arumi (379), Centre d'Investigacions en Bioquímica i Biología Molecular (CIBBIM), Institut de Recerca Hospital Universitari Vall d'Hebrón, Barcelona, Spain

Anna C. Gay (591), Department of Anatomy and Cell Biology, College of Physicians and Surgeons, Columbia University, New York, New York 10032

Elinor J. Griffiths (341), Department of Biochemistry, Henry Wellcome Signalling Laboratories, School of Medical Sciences, University Walk, University of Bristol, Bristol BS8 1TD, United Kingdom

Shukry J. Habib (761), Institut für Physiologische Chemie, Universität München, D-81377 Munich, Germany

Johannes M. Herrmann (743), Institut für Zellbiologie, Universität Kaiserslautern, 67663 Kaiserslautern, Germany

Elena Ingerman (707), Department of Molecular and Cellular Biology, University of California Davis, Davis, California 95616

Michelle S. Johnson (417), Department of Pathology, University of Alabama at Birmingham, Birmingham, Alabama 35294; Center for Free Radical Biology, University of Alabama at Birmingham, Birmingham, Alabama 35294

Denise M. Kirby (93), Mitochondrial Research Group, School of Neurology, Neurobiology and Psychiatry, The Medical School, Framlington Place, Newcastle University, Newcastle upon Tyne, NE2 4HH, United Kingdom; Mitochondrial Research Laboratory, Murdoch Childrens Research Institute, Royal Children's Hospital, Parkville, Victoria 3052, Australia

Guido Kroemer (327), INSERM U848, Université Paris Sud, Institut Gustave Roussy, F-94805 Villejuif, France

Aimee Landar (417), Department of Pathology, University of Alabama at Birmingham, Birmingham, Alabama 35294; Center for Free Radical Biology, University of Alabama at Birmingham, Birmingham, Alabama 35294

C. J. Leaver (65), Department of Plant Sciences, University of Oxford, Oxford OX1 3RB, United Kingdom

John J. Lemasters (283), Center for Cell Death, Injury and Regeneration, and Departments of Pharmaceutical Sciences and Biochemistry & Molecular Biology, Medical University of South Carolina, Charleston, South Carolina 29425

Giorgio Lenaz (3), Dipartimento di Biochimica "G. Moruzzi", Università degli Studi di Bologna, Bologna, Italy

A. Liddell (65), Department of Plant Sciences, University of Oxford, Oxford OX1 3RB, United Kingdom

Roland Lill (261), Institut für Zytobiologie und Zytopathologie, Philipps-Universität Marburg, 35033 Marburg, Germany

Nevi Mackay (173), Department of Pediatric Laboratory Medicine and the Research Institute, Hospital for Sick Children, University of Toronto, Toronto, Ontario, Canada; Department of Biochemistry, University of Toronto, Toronto, Ontario, Canada

Carine Maisse (327), INSERM U848, Université Paris Sud, Institut Gustave Roussy, F-94805 Villejuif, France

Giovanni Manfredi (155, 341), Department of Neurology and Neuroscience, Weill Medical College of Cornell University, New York, New York 10021

Shelly Meeusen (707), Department of Molecular and Cellular Biology, University of California Davis, Davis, California 95616

Didier Métivier (327), INSERM U848, Université Paris Sud, Institut Gustave Roussy, F-94805 Villejuif, France

A. H. Millar (65), ARC Centre of Excellence in Plant Energy Biology, The University of Western Australia, Crawley 6009, Western Australia, Australia

Sabine Molik (261), Institut für Zytobiologie und Zytopathologie, Philipps-Universität Marburg, 35033 Marburg, Germany

Carlos T. Moraes (481, 503), Department of Neurology, University of Miami Miller School of Medicine, Miami, Florida 33136

Ulrich Mühlenhoff (261), Institut für Zytobiologie und Zytopathologie, Philipps-Universität Marburg, 35033 Marburg, Germany

Ali Naini (437), H. Houston Merritt Clinical Research Center for Muscular Dystrophy and Related disorders, Department of Neurology, College of Physicians and Surgeons, Columbia University, New York, New York 10032

Walter Neupert (761), Institut für Physiologische Chemie, Universität München, D-81377 Munich, Germany

Jodi Nunnari (707), Department of Molecular and Cellular Biology, University of California Davis, Davis, California 95616

JooYeun Oh (417), Department of Pathology, University of Alabama at Birmingham, Birmingham, Alabama 35294; Center for Free Radical Biology, University of Alabama at Birmingham, Birmingham, Alabama 35294

A. Pérez-Martos (571), Departamento de Bioquímica y Biología Molecular y Celular, Universidad de Zaragoza, Zaragoza 50013, Spain

Francesco Pallotti (3), Dipartimento di Scienze Biomediche Sperimentali e Cliniche, Università degli Studi dell'Insubria, 21100 Varese, Italy

Nikolaus Pfanner (783), Institut für Biochemie und Molekularbiologie, Zentrum für Biochemie und Malekulare Zellforschung, Universität Freiburg, Hermann-Herder-Straße 7, D-79104 Freiburg, Germany

Carl A. Pinkert (549), Department of Pathobiology, College of Veterinary Medicine, Auburn University, Auburn, Alabama 36849

Paolo Pinton (297), Department of Experimental and Diagnostic Medicine, Section of General Pathology, Interdisciplinary Center for the Study of Inflammation (ICSI) and Emilia-Romagna Laboratory for Genomics and Biotechnology (ER-Gentech), University of Ferrara, I-44100 Ferrara, Italy

Liza A. Pon (45, 591, 683), Department of Anatomy and Cell Biology, College of Physicians and Surgeons, Columbia University, New York, New York 10032

Andrea Prandini (297), Department of Experimental and Diagnostic Medicine, Section of General Pathology, Interdisciplinary Center for the Study of Inflammation (ICSI) and Emilia Romagna Laboratory for Genomics and Biotechnology (ER-Gentech), University of Ferrara, I-44100 Ferrara, Italy

Venkat K. Ramshesh (283), Center for Cell Death, Injury and Regeneration, and Departments of Pharmaceutical Sciences and Biochemistry & Molecular Biology, Medical University of South Carolina, Charleston, South Carolina 29425

Doron Rapaport (761), Institut für Physiologische Chemie, Universität München, D-81377 Munich, Germany

Ann Saada Reisch (199), The Metabolic Disease Unit, Hadassah-Hebrew University Medical Centre, Jerusalem 91120, Israel; Ben Gurion University of the Negev, Beer Sheva 84105, Israel

Claire Remacle (525), Laboratoire de Génétique des Microorganismes, Département des Sciences de la vie, Université de Liège, B-4000 Liège, Belgium

Alessandro Rimessi (297), Department of Experimental and Diagnostic Medicine, Section of General Pathology, Interdisciplinary Center for the Study of Inflammation (ICSI) and Emilia Romagna Laboratory for Genomics and Biotechnology (ER-Gentech), University of Ferrara, I-44100 Ferrara, Italy

Rosario Rizzuto (297), Department of Experimental and Diagnostic Medicine, Section of General Pathology, Interdisciplinary Center for the Study of Inflammation (ICSI) and Emilia Romagna Laboratory for Genomics and Biotechnology (ER-Gentech), University of Ferrara, I-44100 Ferrara, Italy

Brian H. Robinson (173), Department of Pediatric Laboratory Medicine and the Research Institute, Hospital for Sick Children, University of Toronto, Toronto, Ontario, Canada; Department of Biochemistry, University of Toronto, Toronto, Ontario, Canada

Anna Romagnoli (297), Department of Experimental and Diagnostic Medicine, Section of General Pathology, Interdisciplinary Center for the Study of Inflammation (ICSI) and Emilia Romagna Laboratory for Genomics and Biotechnology (ER-Gentech), University of Ferrara, I-44100 Ferrara, Italy

Guy A. Rutter (341), Department of Biochemistry, Henry Wellcome Signalling Laboratories, School of Medical Sciences, University Walk, University of Bristol, Bristol BS8 1TD, United Kingdom; Department of Cell Biology, Faculty of Medicine, Imperial College London, London SW7 2AZ, United Kingdom

Hermann Schägger (723), Molekulare Bioenergetik, ZBC, Universitätsklinikum Frankfurt, 60590 Frankfurt am Main, Germany

Michael Schlame (223), Department of Anesthesiology and Department of Cell Biology, New York University School of Medicine, New York, New York 10016

Bonnie L. Seidel-Rogol (465), Department of Biochemistry, Emory University School of Medicine, Atlanta, Georgia 30322

Gerald S. Shadel (465), Department of Biochemistry, Emory University School of Medicine, Atlanta, Georgia 30322

Sara Shanske (437), H. Houston Merritt Clinical Research Center for Muscular Dystrophy and Related disorders, Department of Neurology, College of Physicians and Surgeons, Columbia University, New York, New York 10032

Michael P. Sheetz (627, 683), Department of Biological Sciences, Columbia University, New York, New York 10025

Sruti Shiva (395), Vascular Medicine Branch, NHLBI, NIH, Bethesda, Maryland 20892
Anatoly Starkov (379), Department of Neurology and Neuroscience, Weill Medical College, Cornell University, New York, New York 10021
Diana Stojanovski (783), Institut für Biochemie und Molekularbiologie, Zentrum für Biochemie und Molekulare Zellforschung, Universität Freiburg, Hermann-Herder-Straße 7, D-79104 Freiburg, Germany
Theresa C. Swayne (591), Department of Anatomy and Cell Biology, College of Physicians and Surgeons, Columbia University, New York, New York 10032
Kurenai Tanji (135), Department of Pathology, Columbia University, New York, New York 10032
Robert W. Taylor (93), Mitochondrial Research Group, School of Neurology, Neurobiology and Psychiatry, The Medical School, Framlington Place, Newcastle University, Newcastle upon Tyne, NE2 4HH, United Kingdom
David R. Thorburn (93), Mitochondrial Research Laboratory, Murdoch Childrens Research Institute, Royal Children's Hospital, Parkville, Victoria 3052, Australia
Ian A. Trounce (549), Centre for Neuroscience, University of Melbourne, Victoria, 3010 Australia
Douglass M. Turnbull (93), Mitochondrial Research Group, School of Neurology, Neurobiology and Psychiatry, The Medical School, Framlington Place, Newcastle University, Newcastle upon Tyne, NE2 4HH, United Kingdom
Gaetano Villani* (121), Division of Biology, California Institute of Technology, Pasadena, California 91125
Cristofol Vives-Bauza (155, 379), Department of Neurology and Neuroscience, Weill Medical College of Cornell University, New York, New York 10021; Centre d'Investigacions en Bioquímica i Biología Molecular (CIBBIM), Institut de Recerca Hospital Universitari Vall d'Hebrón, Barcelona, Spain
Benedikt Westermann (743), Institut für Zellbiologie, Universität Bayreuth, 95440 Bayreuth, Germany
M. Whiteman (355), Department of Biochemistry, Yong Loo Lin School of Medicine, National University of Singapore, Republic of Singapore 117597, Singapore
Nils Wiedemann (783), Institut für Biochemie und Molekularbiologie, Zentrum für Biochemie und Molekulare Zellforschung, Universität Freiburg, Hermann-Herder-Straße 7, D-79104 Freiburg, Germany
Sion L. Williams (481), Department of Neurology, University of Miami Miller School of Medicine, Miami, Florida 33136
Ilka Wittig (723), Molekulare Bioenergetik, ZBC, Universitätsklinikum Frankfurt, 60590 Frankfurt am Main, Germany
Lichuan Yang (155), Department of Neurology and Neuroscience, Weill Medical College of Cornell University, New York, New York 10021
Naoufal Zamzami (327), INSERM U848, Université Paris Sud, Institut Gustave Roussy, F-94805 Villejuif, France

* Current address: Department of Medical Biochemistry and Biology, University of Bari, Bari, Italy.

PREFACE

The field of "mitochondriology" has undergone a renaissance in the last 15 years. Compared to the state of the field as recently as 1985, entirely new areas of investigation have emerged, such as the role of mitochondrial function in apoptosis, aging, and mitochondrial dysfunction in human disease. Furthermore, there have been landmark discoveries and tremendous advances in our understanding of basic phenomena, including mitochondrial import, movement, fission, fusion, inheritance, and interactions with the nucleus and other organelles.

Concomitant with these conceptual advances has been the remarkable increase in the types of tools available to study mitochondrial structure and function. In addition to the traditional methods for isolating mitochondria and assaying their biochemical properties, we now have new and powerful ways to visualize, monitor, and perturb mitochondrial function and to assess the genetic consequences of those perturbations.

In 2001, we assembled the first volume in the Methods in Cell Biology series to bring together those methods—both "classic" and modern—that would enable anyone to study this organelle, be it from a biochemical, morphological, or genetic point of view. The popularity of that volume has inspired us to assemble a second edition that would reflect technical advances of the last few years, as well as new or emerging areas of investigation. Not only has the number of chapters grown from 25 to 36, but much of the material presented in the first volume has been revamped. A few chapters have been eliminated. Fourteen chapters are completely new and cover methods for assaying lactate, pyruvate, β-oxidation, TCA cycle intermediates, cardiolipin, iron–sulfur clusters, and the permeability transition. We have also added a new section on measurements of oxidative stress (assays of reactive oxygen species, free radical scavengers, nitric oxide, and protein thiol modification) and new chapters on examining mitochondrial fission/fusion and on measuring mitochondrial movement in living cells—two related emerging "hot" topics.

A noteworthy, and we believe useful, adjunct to the methods are the appendices, which bring together fundamental information regarding mitochondria that are not found in any single source. These include lists of every known or suspected mitochondrial protein (with approximately 50% more entries in this edition compared to the last), maps of mitochondrial genomes from several commonly-used organisms, and information on agents that perturb respiratory function. Reflecting the boom in "omics," a new appendix has been added describing microarray and proteomic analyses of alterations in mitochondrial function under various conditions.

We dedicate this volume to Giuseppe Attardi, a pioneer in the field of mitochondria in general and mammalian mitochondrial genetics in particular. Among his many notable achievements are the elucidation of the transcription of the human mitochondrial genome and the development of mammalian ρ^0 cells and cybrids. Giuseppe is an inspiration to us all.

Liza A. Pon
Eric A. Schon

目　录

第二部分 线粒体活性的生化分析