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Cell Biology

A Laboratory Handbook

细胞生物学实验手册

4

大分子的转移、表达系统、基 因表达模型、蛋白质

Julio E. Celis



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Cell Biology

A Laboratory Handbook

Third Edition

Volume 4

细胞生物学实验手册

第三版

第4卷

Edited by

Julio E. Celis

Institute of Cancer Biology, Danish Cancer Society, Copenhagen, Denmark

Associate Editors

Nigel P. Carter

The Sanger Center, Wellcome Trust, Cambridge, UK

Kai Simons

Max-Planck Institute of Molecular Cell Biology and Genetics, Dresden, Germany

J. Victor Small

Austrian Academy of Sciences, Salzburg, Austria

Tony Hunter

The Salk Institute, La Jolla, California, USA

David M. Shotton

University of Oxford, UK



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细胞生物学技术方法领域中的“4 颗闪亮之星”

2002 年第 420 卷第 6916 期的 *Nature* 扉页写着“细胞生物学是一门大学科”(Cell Biology is a Big Science)。

或许我们可以从下述两段权威性讲话中,感受细胞生物学学科之“大”,及其在生命科学中的重要地位。

“地球上所有的生物都起源于 30 亿年前的一个原始细胞,从那时起细胞分裂从未间断。每一个人的生命都开始于单个细胞——受精卵,经不断分裂最终产生了我们整个身体 100 万亿(10^{14}) 个细胞。在我们身体内每秒钟都有成百上千个细胞在分裂。”这是本世纪第一个年头,即 2001 年生理学/医学诺贝尔奖颁奖仪式上,主持人安德斯·塞特博格的演讲。

“每一个生物学问题的关键最终必将从细胞中寻求(The key to every biological problem must finally be sought in the cell)”。这是上个世纪 20 年代细胞生物学和细胞遗传学一代宗师 Wilson E. B. 在他的不朽著作《细胞的发育与遗传》中的格言。

其实,除了相距近一个世纪的两位大师的格言之外,我们还可从细胞生物学研究领域之广阔,领略它在生命科学与医学中的重要地位。细胞生物学研究内容几乎涉及机体中的所有重要且基本的生命现象,其中包括细胞的生长、增殖、运动、发育、遗传、突变(尤其是癌变)、衰老和死亡(尤其是凋亡)等。由于细胞的生命现象是整个机体生命现象的基础与本质所在,其重要性是不言而喻的。因此,也毫不奇怪近些年来不少诺贝尔奖的工作大都属于细胞生物学范畴,或是与之密切相关。譬如 1999 年生理学/医学奖授予了细胞内信号系统的研究,2000 年授予神经细胞传导的研究,2001 年授予细胞周期的研究,2002 年授予细胞凋亡的研究,2003 年的化学奖授予细胞膜通道的研究,2006 年授予 RNA 干扰的研究,2007 年授予基因打靶的研究。

正是由于细胞生物学的重要地位,它的发展也极其迅猛,如今,细胞生物学的研究内容已突破了细胞本身的局限性,扩展到对细胞、亚细胞、分子等多个层次进行生命现象的探索。今天的细胞生物学家也不再是“纯粹”的细胞学家,他们集细胞生物学家、分子生物学家、免疫学家等于一身。同样,分子生物学家、免疫学家,甚至临床学家也努力地向细胞生物学靠拢,他们热切地希望了解细胞生物学知识,并期盼将细胞生物学技术引入他们的学科。在这种态势下,细胞生物学理论及技术方法倍受相关学科的青睐,于是有关细胞生物学研究方法的专著也应运而生。近年来最受人瞩目的专著或许要数下列几种:《精编细胞生物学实验指南》*Short Protocols in Cell Biology*、《当代细胞生物学实验方法全编》*Current Protocols in Cell Biology*。国内也有数种出版物,诚然,各种版本各具特色。

就实验手册的本质而言,无疑要求它们有如下几个根本的特征:

其一,内容要新,即所介绍的方法具有时代性、前沿性。我们认为,只有能反映与推动细胞生物学及相关学科继续发展的那些新技术才称得上具有时代性与前沿性。本书中有关干细胞、显像技术、大分子转移的内容都不乏新颖性与前沿性。

其二,可操作性。无疑,实验手册是授以操作技能的,因此方案的制定和步骤的描述都必须条理清晰,而理论基础则需言简意赅。本书有关操作步骤的描述该详则详,不尚空谈,相信我国多数读者,遵循其步骤“按图索骥”便会取得明显的功效,因此可适用于各个层次的实验人员。

其三,系统性。虽然实验手册不一定要如教科书那样有十分严密的顺序连贯性,但作为一个学科(如细胞生物学)的技术介绍仍有它的脉络联系,它必然逐次深入,或称作由表及里、由此及彼。本手册共四卷,就方法学讲也是有机联系的,即从细胞培养,结构分析,形态研究到最后的大分子研究。这种布局从形态到机能,从细胞到分子也反映出全书的逻辑性联系。

其四,人性化。这主要表现在各种方法步骤的描述皆以读者为本,处处考虑到能让读者理解与操作无

误，因此语言简洁、并配有必要的示意图。此外还特别指出可能会出现差错与“不尽人意”之处，并因此提出对策。读者无疑会在本手册中体会出这些特性，并感到特别实用甚至非常亲切。

本书分四卷，第一卷主要介绍细胞培养及相关的实验方法。其中有关线虫和其他非共生线虫的实验室培养是不可多得的资料（国内尚未发现相关信息）。第二卷主要介绍细胞器的分类与检测，方法之多、之新，是类似的其他手册不可企及的。第三卷介绍各种成像技术。除了常规的光学和电镜技术，还深入至组织矩阵、基因组学的研究等，这些内容无疑反映了“与时俱进”的特色。第四卷主要介绍大分子转移及表达系统的研究方法，其中包括体细胞核转移技术。可以认为本卷是分子生物学与细胞生物学理论与技术相结合的产物，或许可以认为是最具时代性的篇章。

由上也就不难看出本手册不仅适用于细胞生物学研究，对于从事分子生物学、生物化学、生物工程、发育生物学、病毒学、遗传学，甚至临床医学的研究者也是必不可缺的工具。

本书由 Elsevier Academic Press 出版。主编 Julio E. Celis 为世界著名肿瘤生物学家。副主编等人皆在各自领域中有所建树，并富有实验室经验。正如美国 Thomas Jefferson 大学的 Gregory J. B. 教授所评述的那样“4 颗闪亮之星——此 4 卷总结了几乎全部的现代细胞生物学方案”，我国学者清华大学吴畏教授、陈晔光教授对此书都有很高的评价，认为“非常细致、实用”、“是一套好书，其中的一些编著者也很有名，如 Tony Hunter 和 K. M. Simons 等。”

“他山之石，可以攻玉”，相信本手册为推动我国细胞生物学及相关学科的发展将起到一定的作用。

章静波

中国医学科学院中国协和医科大学基础医学研究所
二零零七年八月

前 言

当有新技术的创建，或是旧程序的改良时，常常会促成科学的进步。时至今日，随着从对单个分子的分析迅速地转向对复杂生物学问题的研究时，人们比以往更加需要有互补性的多种技术平台，以解决复杂的生物学问题。《细胞生物学实验手册》第3版萃集了236篇文章，涵盖了细胞学、分子生物学、蛋白质组学、基因组学和功能基因组学中的各种新技术和程序。其中165篇为新征集的文章，多数是应科学界的反馈意见而撰写的。

一如本书第二版那样，本版也分四卷。第一卷包括组织培养及其相关技术、病毒、抗体和免疫组织化学。第二卷包括细胞器和细胞结构，以及细胞生物学检测技术。第三卷涉及成像技术、电子显微镜、扫描探针和扫描电子显微镜、显微解剖、组织矩阵、细胞遗传学和原位杂交、基因组学、转基因、基因敲除和基因削减方法等。最后一卷包括大分子转移、表达系统、除各种蛋白质组学技术之外的基因表达模型。附录收集具代表性的培养细胞系及它们的特征、细胞生物学互联网资源、计算机模拟的蛋白质组分析系统中的生物信息资源。本手册能独到地提供从事生命科学研究所不可缺少的经典及最新的技术。若你身边缺乏专家，则本手册在你科研生涯的任何阶段，均能帮助你利用各种技术和模型系统进行生物学问题的研究。本书所介绍的技术都以一种人性化的、循序渐进的方式娓娓道来，并且还教你某些有用的小窍门以避免实验操作中可能遭遇到的小麻烦。

在此，我向那些辛勤工作、竭力支持、在遴选新技术方面具有远见卓识的副主编们表示由衷的感谢。我同样感谢 Elsevier 出版社的工作人员对出版本书的不懈支持和敬业精神。许多朋友也参与了本手册的出版，我特别要感谢的是 Lisa Tickner, Karen Dempsey, Angela Dooley, Carl Soares 以及 Tari Paschall，是他们通力协作并组织各卷的撰写。我还要表达对所有作者的谢意，乃因他们不惜时间与精力献身于本书的出版工程。

主编：Julio E. Celis

（章静波 译）

Contributors

Numbers in parenthesis indicate the volume (bold face) and page on which the authors' contribution begins.

Mads Aaboe (4: 83) Clinical Biochemical Department, Molecular Diagnostic Laboratory, Aarhus University Hospital, Skejby, Brendstrupgaardvej, Aarhus N, DK-8200, DENMARK

Tanja Aarvak (1: 239) Dynal Biotech ASA, PO Box 114, Smestad, N-0309, NORWAY

Harindra R. Abeyasinghe (3: 345) Department of Pathology and Laboratory Medicine, University of Rochester School of Medicine, 601 Elmwood Ave., Rm 1-6337, Rochester, NY 14642

Ruedi Aebersold (4: 437) The Institute for Systems Biology, 1441 North 34th Street, Seattle, WA 98103-8904

Ueli Aebi (3: 233, 241) ME Muller Institute for Microscopy, Biozentrum, University of Basel, Klingelbergstr. 50/70, Basel, CH-4056, SWITZERLAND

Cheol-Hee Ahn (4: 29) School of Materials Science and Engineering, Seoul National University, Seoul, 151-744, SOUTH KOREA

Natalie G. Ahn (4: 443) Department of Chemistry & Biochemistry, University of Colorado, 215 UCB, Boulder, CO 80309

Ramiro Alberio (4: 45) School of Biosciences, University of Nottingham, Sutton Bonington, Loughborough, Leics, LE12 5RD, UNITED KINGDOM

Donna G. Albertson (3: 445) Cancer Research Institute, Department of Laboratory Medicine, The University of California, San Francisco, Box 0808, San Francisco, CA 94143-0808

Heiner Albiez (1: 291) Department of Biology II, Ludwig-Maximilians University of Munich, Munich, GERMANY

Terence Allen (3: 325) CRC Structural Cell Biology Group, Paterson Institute for Cancer Research, Christie Hospital NHS Trust, Wilmslow Road, Withington, Manchester, M20 4BX, UNITED KINGDOM

Noona Ambartsumian (1: 363) Department of Molecular Cancer Biology, Danish Cancer Society, Institute of Cancer Biology, Strandboulevarden 49, Copenhagen, DK-2100, DENMARK

Øystein Åmellem (1: 239) Immunosystems, Dynal Biotech ASA, PO Box 114, Smestad, N-0309, NORWAY

Patrick Amstutz (1: 497) Department of Biochemistry, University of Zürich, Winterthurerstr. 190, Zurich, CH-8057, SWITZERLAND

Jens S. Andersen (4: 427) Protein Interaction Laboratory, University of Southern Denmark—Odense, Campusvej 55, Odense M, DK-5230, DENMARK

Mads Hald Andersen (1: 97) Tumor Immunology Group, Institute of Cancer Biology, Danish Cancer Society, Strandboulevarden 49, Copenhagen, DK-2100, DENMARK

Helena Andersson (4: 63) Bioscience at Novum, Karolinska Institutet, Hälsovägen 7-9, Huddinge, SE-141 57, SWEDEN

Peter W. Andrews (1: 183) Department of Biomedical Science, The University of Sheffield, Rm B2 238, Sheffield, S10 2TN, UNITED KINGDOM

Elsa Anes (2: 57) Faculdade de Farmacia, Universidade de Lisboa, Av. Forças Armadas, Lisboa, 1649-019, PORTUGAL

James M. Angelastro (1: 171) Department of Pathology and Center for Neurobiology and Behavior, Columbia University College of Physicians

and Surgeons, 630 West 168th Street, New York, NY 10032

Sergey V. Anisimov (4: 103) Molecular Cardiology Unit, National Institute on Aging, NIH, 5600 Nathan Shock Drive, Baltimore, MD 21224

Celia Antonio (2: 379) Department of Biochemistry & Molecular Biophysics, College of Physicians & Surgeons, Columbia University, 701 W 168ST HHSC 724, New York, NY 69117

Shigehisa Aoki (1: 411) Department of Pathology & Biodefence, Faculty of Medicine, Saga University, Nebeshima 5-1-1, Saga, 849-8501, JAPAN

Ron D. Appel (4: 207) Swiss Institute of Bioinformatics, CMU, Rue Michel Servet 1, Geneva 4, CH-1211, SWITZERLAND

Rolf Apweiler (4: 469) EMBL Outstation, European Bioinformatics Institute, Wellcome Trust Genome Campus, Hinxton, Cambridge, CB10 1SD, UNITED KINGDOM

Nobukazu Araki (2: 147) Department of Histology and Cell Biology, School of Medicine, Kagawa University, Mki, Kagawa, 761-0793, JAPAN

Christopher M. Armstrong (4: 295) Dana Faber Cancer Institute, Harvard University, 44 Binney Street, Boston, MA 02115

Anthony J. Ashford (2: 155) Antibody Facility, Max Planck Institute of Molecular Cell Biology and Genetics, Pfotenhauerstrasse 108, Dresden, D-01307, GERMANY

Daniel Axelrod (3: 19) Dept of Physics & Biophysics Research Division, University of Michigan, Ann Arbor, MI 48109-1055

Sheree Bailey (1: 475) Dept of Immunology, Allergy and Arthritis, Flinders Medical Centre and Flinders University, Bedford Park, Adelaide, SA, 5051, SOUTH AUSTRALIA

Nathalie Q. Balaban (2: 419) Department of Physics, The Hebrew University-Givat Ram, Racah Institute, Jerusalem, 91904, ISRAEL

William E. Balch (2: 209) Department of Cell and Molecular Biology, The Scripps Research Institute, 10550 North Torrey Pines Road, La Jolla, CA 92037

Debabrata Banerjee (1: 315) Department of Medicine, Cancer Institute of New Jersey, 195 Little Albany Street, New Brunswick, NJ 08903

Jiri Bartek (4: 253) Department of Cell Cycle and Cancer, Danish Cancer Society, Strandboulevarden 49, Copenhagen, DK-2100, DENMARK

Werner Baschong (3: 5) ME Muller Institute for Microscopy, Biozentrum, University of Basel, Klingelbergstrasse 50/70, Basel, CH-4056, SWITZERLAND

Philippe I. H. Bastiaens (3: 153) Cell Biology and Cell Biophysics Program, European Molecular Biology Laboratory, Meyerhofstrasse 1, Heidelberg, 69117, GERMANY

Jürgen C. Becker (1: 103) Department of Dermatology, University of Würzburg, Sanderring 2, Würzburg, 97070, GERMANY

Martin Béhé (4: 149) Department of Nuclear Medicine, Philipp's-University of Marburg, Baldingerstraße, Marburg/Lahn, D-35043, GERMANY

Thomas M. Behr (4: 149) Department of Nuclear Medicine, Philipp's-University of Marburg, Baldingerstraße, Marburg, D-35043, GERMANY

Stefanie Benesch (2: 399) Department of Cell Biology, Gesellschaft für Biotechnologische Forschung, Mascheroder Weg 1, Braunschweig, D-38124, GERMANY

Aaron Bensimon (3: 429) Laboratoire de Biophysique de l'ADN, Département des Biotechnologies, Institut Pasteur, 25 rue du Dr. Roux, Paris Cedex 15, F-75724, FRANCE

John J. M. Bergeron (2: 41) Department of Anatomy and Cell Biology, Faculty of Medicine, McGill University, STRATHCONA Anatomy & Dentistry Building, Montreal, QC, H3A 2B2, CANADA

Michael W. Berns (3: 351) Beckman Laser Institute, University of California, Irvine, 1002 Health Sciences Road E, Irvine, CA 92697-1475

Joseph R. Bertino (1: 315) The Cancer Institute of New Jersey, 195 Little Albany Street, New Brunswick, NJ 08901

Paulo Bianco (1: 79) Dipartimento di Medicina Sperimentale e Patologia, Università 'La Sapienza', Viale Regina Elena 324, Roma, I-00161, ITALY

Hans Kaspar Binz (1: 497) Department of Biochemistry, University of Zürich, Winterthurerstr. 190, Zürich, CH-8057, SWITZERLAND

R. Curtis Bird (1: 247) Department of Pathobiology, Auburn University, Auburn, Alabama 36849

Mina J. Bissell (1: 139) Life Sciences Division, Lawrence Berkeley National Laboratory, 1 Cyclotron Road, Bldg 83-101, Berkeley, CA 94720

Stephanie Blackwood (3: 445) Cancer Research Institute, University of California San Francisco, PO Box 0808, San Francisco, CA 94143-0808

Blagoy Blagoev (4: 427) Protein Interaction Laboratory, University of Southern Denmark—Odense, Campusvej 55, Odense M, DK-5230, DENMARK

Kenneth R. Boheler (4: 103) Laboratory of Cardiovascular Science, National Institute on Aging, NIH, 5600 Nathan Shock Drive, Baltimore, MD 21224-6825

Michelle A. Booden (1: 345) Lineberger Comprehensive Cancer Center, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599-7295

Gary G. Borisy (3: 277) Department of Cell and Molecular Biology, Northwestern University Medical School, Chicago, IL 6011-3072

Elliot Botvinick (3: 351) Beckman Laser Institute, University of California, Irvine, 1002 Health Sciences Road, East, Irvine, CA 92697-1475

Gérard Bouchet (4: 207) Swiss Institute of Bioinformatics (SIB), CMU, rue Michel-Servet 1, Genève 4, CH-1211, SWITZERLAND

Rosemary Boyle (4: 437) The Institute for Systems Biology, 1441 North 34th St., Seattle, WA 98109

Susanne Brandfass (1: 563) Department of Biochemistry and Cell Biology, Max Planck Institute of Biophysical Chemistry, Am Faßberg 11, Gottingen, D-37077, GERMANY

Pascal Braun (4: 73) Department of Chemistry and Chemical Biology, Harvard University, 12 Oxford Street, Cambridge, MA 02138

Steven A. Braut (4: 121) Department of Anatomy and Structural Biology, Golding # 601, Albert Einstein College of Medicine of Yeshiva University, 1300 Morris Park Avenue, Bronx, NY 10461

Alvis Brazma (4: 95) EMBL Outstation—Hinxton, European Bioinformatics Institute, Wellcome Trust Genome Campus, Hinxton, Cambridge, CB10 1SD, UNITED KINGDOM

J. David Briley (3: 471) Department of Genomic Sciences, Glaxo Wellcome Research and Development, 5 Moore Drive, Research Triangle Park, NC 27709-3398

Simon Broad (1: 133) Keratinocyte Laboratory, London Research Institute, 44 Lincoln's Inn Fields, London, WC2A 3PX, UNITED KINGDOM

Nicholas H. Brown (3: 77) Wellcome Trust/Cancer Research UK Institute and Department of Anatomy, University of Cambridge, Tennis Court Road, Cambridge, CB2 1QR, UNITED KINGDOM

Heather L. Brownell (2: 329, 341) Office of Technology Licensing and Industry Sponsored Research, Harvard Medical School, 25 Shattuck Street, Gordon Hall of Medicine, Room 414, Boston, MA 02115

Damien Brunner (3: 69) Cell Biology and Cell Biophysics Programme, European Molecular Biology Laboratory, Meyerhofstrasse 1, Heidelberg, D-69117, GERMANY

Suzannah Bumpstead (3: 463) Genotyping / Chr 20, The Wellcome Trust Sanger Institute, The Wellcome Trust Genome Campus, Hinxton, Cambridge, CB10 1SA, UNITED KINGDOM

Deborah C. Burford (3: 403) Wellcome Trust, Sanger Institute, The Wellcome Trust Genome Campus, Hinxton, Cambridge, CB10 1SA, UNITED KINGDOM

Gerald Burgstaller (2: 161) Department of Cell Biology, Institute of Molecular Biology, Austrian Academy of Sciences, Billrothstrasse 11, Salzburg, A-5020, AUSTRIA

Ian M. Caldicott (1: 157)

Angelique S. Camp (1: 457) Gene Therapy Centre, University of North Carolina at Chapel Hill, 7119 Thurston-Bowles (G44 Wilson Hall), Chapel Hill, NC 27599-7352

Keith H. S. Campbell (4: 45) School of Biosciences, Sutton Bonington, Loughborough, Leics, LE12 5RD, UNITED KINGDOM

Yihai Cao (1: 373) Microbiology & Tumor Biology Center, Karolinska Institute, Room: Skrivrum (G415), Box 280, Stockholm, SE-171 77, SWEDEN

Maria Carmo-Fonseca (2: 277, 3: 419) Institute of Molecular Medicine, Faculty of Medicine, University of Lisbon, Av. Prof. Egas Moniz, Lisbon, 1649-028, PORTUGAL

T. Carneiro (3: 419) Faculty of Medicine, Institute of Molecular Medicine, University of Lisbon, Av. Prof. Egas Moniz, Lisboa, 1649-028, PORTUGAL

Nigel P. Carter (2: 133) The Wellcome Trust, Sanger Institute, The Wellcome Trust, Genome Campus, Hinxton, Cambridge, CB10 1SA, UNITED KINGDOM

Célia Carvalho (3: 419) Faculty of Medicine, Institute of Molecular Medicine, University of Lisbon, Av. Prof. Egas Moniz, Lisboa, 1649-028, PORTUGAL

Lucy A. Carver (2: 11) Cellular and Molecular Biology Program, Sidney Kimmel Cancer Center, 10835 Altman Row, San Diego, CA 92121

Doris Cassio (1: 231, 3: 387) INSERM U-442: Signalisation cellulaire et calcium, Bat 443, Université Paris-Sud, Street George Clemenceau Pack, 444, Orsay, Cedex, F-91405, FRANCE

Chris Catton (3: 207) Department of Zoology, University of Oxford, South Parks Road, Oxford, OX1 3PS, UNITED KINGDOM

Julio E. Celis (1: 527, 4: 69, 165, 219, 243, 289) Danish Cancer Society, Institute of Cancer Biology and Danish Centre for Translational Breast Cancer Research, Strandboulevarden 49, Copenhagen O, DK-2100, DENMARK

Pierre Chambon (3: 501) Institut de Génétique et de Biologie Moléculaire et Cellulaire, 1 rue Laurent Fries, B.P.10142, Illkirch CEDEX, F-67404, FRANCE

Francis Ka-Ming Chan (2: 355) Department of Pathology, University of Massachusetts Medical School, Room S2-125, 55 Lake Avenue North, Worcester, MA 01655

Ming-Shien Chang (3: 87) Department of Physics, Duke University, 107 Physics Bldg, Durham, NC 27708-1000

Samit Chatterjee (2: 241) Margaret M. Dyson Vision Research Institute, Department of Ophthalmology, Weill Medical College of Cornell University, 1300 York Avenue, New York, NY 10021

Sandeep Chaudhary (1: 121) Veterans Affairs Medical Center, San Diego (V111G), 3350 La Jolla Village Drive, San Diego, CA 92161

Jingwen Chen (3: 471) Department of Genomic Sciences, Glaxo Wellcome Research and Development, 5 Moore Drive, Research Triangle Park, NC 27709

Yonglong Chen (1: 191) Institute for Biochemistry and Molecular Cell Biology, University of Goettingen, Justus-von-Liebig-Weg 11, Göttingen, D-37077, GERMANY

Yong Woo Cho (4: 29) Akina, Inc., Business & Technology Center, 1291 Cumberland Ave., #E130, West Lafayette, IN 47906

Juno Choe (1: 269) Institute for Systems Biology, 1441 N. 34th St, Seattle, WA 98103

Claus R. L. Christensen (1: 363) Department of Molecular Cancer Biology, Danish Cancer Society, Institute of Cancer Biology, Strandboulevarden 49, Copenhagen, DK-2100, DENMARK

Theodore Ciaraldi (1: 121) Veterans Affairs Medical Center, University of California, San Diego, 9500 Gilman Drive, La Jolla, CA 92093-9111

Aaron Ciechanover (4: 351) Center for Tumor and Vascular Biology, The Rappaport Faculty of Medicine and Research Institute, Technion-Israel Institute of Technology, POB 9649, Efron Street, Bat Galim, Haifa, 31096, ISRAEL

Mark S. F. Clarke (2: 233, 4: 5) Department of Health and Human Performance, University of Houston, 3855 Holman Street, Garrison—Rm 104D, Houston, TX 77204-6015

Martin Clynes (1: 335) National Institute for Cellular Biotechnology, Dublin City University, Glasnevin, Dublin, 9, IRELAND

Philippe Collas (1: 207) Institute of Medical Biochemistry, University of Oslo, PO Box 1112 Blindern, Oslo, 0317, NORWAY

Kristen Correia (4: 35) Krumlauf Lab, Stowers Institute for Medical Research, 1000 East 50th Street, Kansas City, MO 64110

Pascale Cossart (2: 407) Unite des Interactions Bacteries-Cellules/Unité INSERM 604, Institut Pasteur, 28, rue du Docteur Roux, Paris Cedex 15, F-75724, FRANCE

Thomas Cremer (1: 291) Department of Biology II, Ludwig-Maximilians University of Munich, Munich, 80333, GERMANY

Robert A. Cross (2: 371) Molecular Motors Group, Marie Curie Research Institute, The Chart, Oxted, Surrey, RH8 0TE, UNITED KINGDOM

Matthew E. Cunningham (1: 171) Hospital for Special Surgery, New York Hospital, 520 E. 70th Street, New York, NY 10021

Noélia Custódio (3: 419) Faculty of Medicine, Institute of Molecular Medicine, University of Lisbon, Av. Prof. Egas Moniz, Lisboa, 1649-028, PORTUGAL

Zbigniew Darzynkiewicz (1: 279) The Cancer Research Institute, New York Medical College, 19 Bradhurst Avenue, Hawthorne, NY 10532

Ilan Davis (3: 187) Wellcome Trust Centre for Cell Biology, Institute of Cell and Molecular Biology, The University of Edinburgh, Michael Swann Building, The King's Buildings, Mayfield Road, Edinburgh, EH9 3JR, SCOTLAND

Stephen C. De Rosa (1: 257) Vaccine Research Center, National Institutes of Health, 40 Convent Dr., Room 5610, Bethesda, MD 20892-3015

Nicholas M. Dean (3: 523) Functional Genomics, GeneTrove, GeneTrove (a division of Isis Isis Pharmaceuticals, Inc.), 2292 Faraday Avenue, Carlsbad, CA 92008

Anne Dell (4: 415) Department of Biological Sciences, Biochemistry Building, Imperial College of Science, Technology & Medicine, Biochemistry Building, London, SW7 2AY, UNITED KINGDOM

Panos Deloukas (3: 463) The Wellcome Trust, Sanger Institute, Hinxton, Cambridge, CB10 1SA, UNITED KINGDOM

Nicolas Demaurex (3: 163) Department of Cell Physiology and Metabolism, University of Geneva Medical Center, 1 Michel-Servet, Geneva, CH-1211, SWITZERLAND

Chris Denning (4: 45) Division of Animal Physiology, School of Biosciences, Institute of Genetics Room C15, University of Nottingham, Queens Medical Centre, Nottingham, NG7 2UH, UNITED KINGDOM

Ami Deora (2: 241) Margaret M. Dyson Vision Research Institute, Department of Ophthalmology, Weill Medical College of Cornell University, 1300 York Avenue, New York, NY 10021

Julien Depollier (4: 13) Centre de Recherche en Biochimie Macromoléculaire (UPR 1086), Centre National de la Recherche Scientifique (CNRS), 1919 Route de Mende, Montpellier Cedex 5, F-34293, FRANCE

Channing J. Der (1: 345) Department of Pharmacology, University of North Carolina at Chapel Hill, Lineberger Comprehensive Cancer Center, Chapel Hill, NC 27599

Bart Devreese (4: 259) Department of Biochemistry, Physiology and Microbiology, University of Ghent, K.L. Ledeganckstraat 35, Ghent, B-9000, BELGIUM

Alberto Diaspro (3: 201) Department of Physics, University of Genoa, Via Dodecaneso 33, Genoa, I-16146, ITALY

James Fred Dice (4: 345) Department Physiology, Tufts University School of Medicine, 136 Harrison Ave, Boston, MA 02111

Thomas J. Diefenbach (4: 307) Department of Physiology, Tufts University School of Medicine, 136 Harrison Avenue, Boston, MA 02111

Chris Dinant (2: 121) Biomolecular Sciences, UMIST, PO Box 88, Manchester, M60 1QD, UNITED KINGDOM

Da-Qiao Ding (3: 171) Structural Biology Section and CREST Research Project, Kansai Advanced Research Center, Communications Research Laboratory, 588-2 Iwaoka, Iwaoka-cho, Nishi-ku, Kobe, 651-2492, JAPAN

Gilles Divita (4: 13) Centre de Recherche en Biochimie Macromoléculaire (UPR 1086), Centre National de la Recherche Scientifique (CNRS), 1919 Route de Mende, Montpellier Cedex 5, F-34293, FRANCE

Eric P. Dixon (1: 483) TriPath Oncology, 4025 Stirrup Creek Drive, Suite 400, Durham, NC 27703

Bernhard Dobberstein (2: 215) Zentrum für Molekulare Biologie, Universität Heidelberg, Im Neuenheimer Feld 282, Heidelberg, D-69120, GERMANY

Lynda J. Donald (4: 457) Department of Chemistry, University of Manitoba, Room 531 Parker Building, Winnipeg, MB, R3T 2N2, CANADA

Wolfgang R. G. Dostmann (2: 299) Department of Pharmacology, University of Vermont, Health Science Research Facility 330, Burlington, VT 05405-0068

Adam Douglass (3: 129) Department of Cellular and Molecular Pharmacology, The University of California, San Francisco, School of Medicine, Medical Sciences Building, Room S1210, 513 Parnassus Avenue, San Francisco, CA 94143-0450

Kate Downes (3: 463) Genotyping / Chr 20, The Wellcome Trust, Sanger Institute, The Wellcome Trust Genome Campus, Hinxton, Cambridge, CB10 1SA, UNITED KINGDOM

Harry W. Duckworth (4: 457) Department of Chemistry, University of Manitoba, Room 531 Parker Building, Winnipeg, MB, R3T 2N2, CANADA

Derek M. Dykxhoorn (3: 511) CBR Institute for Biomedical Research, Harvard Medical School, 200 Longwood Ave, Boston, MA 02115

Lars Dyrskjøt (4: 83) Clinical Biochemical Department, Molecular Diagnostic Laboratory, Aarhus University Hospital, Skejby, Brendstrupgaardvej, Aarhus N, DK-8200, DENMARK

Christoph Eckerskorn (4: 157) Protein Analytics, Max Planck Institute for Biochemistry, Klopferspitz 18, Martinsried, D-82152, GERMANY

Glenn S. Edwards (3: 87) Department of Physics, Duke University, 221 FEL Bldg, Box 90305, Durham, NC 27708-0305

Andreas A. O. Eggert (1: 103) Department of Dermatology, Julius-Maximilians University, Josef-Schneider-Str. 2, Würzburg, 97080, GERMANY

Maria Ekström (4: 63) Bioscience at Novum, Karolinska Institutet, Huddinge, SE-141 57, SWEDEN

Andreas Engel (3: 317) Maurice E. Müller Institute for Microscopy at the Biozentrum, University of Basel, Klingelbergstrasse 70, Basel, CH-4056, SWITZERLAND

Anne-Marie Engel (1: 353) Bartholin Institute, Bartholinsgade 2, Copenhagen K, DK-1356, DENMARK

José A. Enríquez (2: 69) Department of Biochemistry and Molecular and Cellular Biology, Universidad de Zaragoza, Miguel Servet, 177, Zaragoza, E-50013, SPAIN

Rachel Errington (1: 305) Department of Medical Biochemistry and Immunology, University of Wales College of Medicine, Heath Park, Cardiff, CF14 4XN, UNITED KINGDOM

Virginia Espina (3: 339) Microdissection Core Facility, Laboratory of Pathology, National Cancer Institute, 9000 Rockville Pike, Building 10, Room B1B53, Bethesda, MD 20892

H. Dariush Fahimi (2: 63) Department of Anatomy and Cell Biology II, University of Heidelberg, Im Neuenheimer Feld 307, Heidelberg, D-69120, GERMANY

Federico Federici (3: 201) Department of Physics, University of Genoa, Via Dodecaneso 33, Genoa, I-16146, ITALY

Daniel L. Feeback (2: 233, 4: 5) Space and Life Sciences Directorate, NASA-Johnson Space Center, 3600 Bay Area Blvd, Houston, TX 77058

Patricio Fernández-Silva (2: 69) Dept of Biochemistry and Molecular and Cellular Biology, Universidad de Zaragoza, Miguel Servet 177, Zaragoza, E-50013, SPAIN

Erika Fernández-Vizarra (2: 69) Dept of Biochemistry and Molecular and Cellular Biology, Universidad de Zaragoza, Miguel Servet, 177, Zaragoza, E-50013, SPAIN

Patrick F. Finn (4: 345) Department of Physiology, Tufts University School of Medicine, 136 Harrison Ave, Boston, MA 02111

Kevin L. Firth (2: 329, 2: 341) ASK Science Products Inc., 487 Victoria St, Kingston, Ontario, K7L 3Z8, CANADA

Raluca Flükiger-Gagescu (2: 27) Unitec—Office of Technology Transfer, University of Geneva and University of Geneva Hospitals, 24, Rue Général-Dufour, Geneva 4, CH-1211, SWITZERLAND

Leonard J. Foster (4: 363, 427) Protein Interaction Laboratory, University of Southern Denmark, Odense, Campusvej 55, Odense M, DK-5230, DENMARK

Dimitrios Fotiadis (3: 317) M. E. Müller Institute for Microscopy at the Biozentrum, University of Basel, Klingelbergstrasse 70, Basel, CH-4056, SWITZERLAND

Patrick L. T. M. Frederix (3: 317) M. E. Müller Institute for Microscopy at the Biozentrum, University of Basel, Klingelbergstrasse 70, Basel, CH-4056, SWITZERLAND

Marcus Frohme (4: 113) Functional Genome Analysis, German Cancer Research Center, Deutsches Krebsforschungszentrum, Im Neuenheimer Feld 580, Heidelberg, D-69120, GERMANY

Masanori Fujimoto (4: 197) Department of Biochemistry and Biomolecular Recognition, Yamaguchi University School of Medicine, 1-1-1, Minami-kogushi, Ube, Yamaguchi, 755-8505, JAPAN

Margarida Gama-Carvalho (2: 277) Faculty of Medicine, Institute of Molecular Medicine, University of Lisbon, AV. Prof. Egas Moniz, Lisbon, 1649-028, PORTUGAL

Henrik Garoff (1: 419, 4: 63) Unit for Cell Biology, Center for Biotechnology, Karolinska Institute, Huddinge, SE-141 57, SWEDEN

Susan M. Gasser (2: 359) Friedrich Miescher Institute for Biomedical Research, Maulbeerstrasse 66, Basel, CH-1211, SWITZERLAND

Kristine G. Gaustad (1: 207) Institute of Medical Biochemistry, University of Oslo, PO Box 1112 Blindern, Oslo, 0317, NORWAY

Benjamin Geiger (2: 419) Dept. of Molecular Cell Biology, Weizman Institute of Science, Wolfson Building, Rm 617, Rehovot, 76100, ISRAEL

Kris Gevaert (4: 379, 4: 457) Dept. Medical Protein Research, Flanders Interuniversity Institute for Biotechnology, Faculty of Medicine and Health Sciences, Ghent University, Instituut Rommelaere—Blok D, Albert Baertsoenkaai 3, Gent, B-9000, BELGIUM

Jilur Ghorri (3: 463) Genotyping / Chr 20, The Wellcome Trust, Sanger Institute, The Wellcome Trust, Genome Campus, Hinxton, Cambridge, CB10 1SA, UNITED KINGDOM

Alasdair J. Gibb (1: 395) Department of Pharmacology, University College London, Gower Street, London, WC1E 6BT, UNITED KINGDOM

Mario Gimona (1: 557, 2: 161, 4: 145) Department of Cell Biology, Institute of Molecular Biology, Austrian Academy of Sciences, Billrothstrasse 11, Salzburg, A-5020, AUSTRIA

David A. Glesne (1: 165) Biosciences Division, Argonne National Laboratory, 9700 South Cass Avenue, Argonne, IL 60439-4844

Martin Goldberg (3: 325) Science Laboratories, University of Durham, South Road, Durham, DH1 3LE, UNITED KINGDOM

Kenneth N. Goldie (3: 267) Structural and Computational Biology Programme, EMBL, Meyerhofstrasse 1, Heidelberg, D-69117, GERMANY

Jon W. Gordon (3: 487) Geriatrics and Adult Development, Mount Sinai School of Medicine, One Gustave L. Levy Place, New York, NY 10029

Angelika Görg (4: 175) Fachgebiet Proteomik, Technische Universität München, Am Forum 2, Freising Weihenstephan, D-85350, GERMANY

Martin Gotthardt (4: 149) Department of Nuclear Medicine, Philipp's-University of Marburg, Baldingerstraße, Marburg/Lahn, D-35043, GERMANY

Frank L. Graham (1: 435) Department of Biology, McMaster University, Life Sciences Building, Room 430, Hamilton, Ontario, L8S 4K1, CANADA

Claude Granier (1: 519) UMR 5160, Faculté de Pharmacie, 15 Av. Charles Flahault, Montpellier Cedex 5, BP 14491, 34093, FRANCE

Lloyd A. Greene (1: 171) Department of Pathology and Center for Neurobiology and Behavior, Columbia University, College of Physicians and Surgeons, 630 W. 168th Street, New York, NY 10032

Susan M. Gribble (3: 403) Sanger Institute, The Wellcome Trust, The Wellcome Trust Genome Campus, Hinxton, Cambridge, CB10 1SA, UNITED KINGDOM

Gareth Griffiths (2: 57, 3: 299) Department of Cell Biology, EMBL, Postfach 102209, Heidelberg, D-69117, GERMANY

Sergio Grinstein (3: 163) Cell Biology Program, Hospital for Sick Children, 555 University Avenue, Toronto, Ontario, M5G 1X8, CANADA

Pavel Gromov (1: 527, 4: 69, 165, 243, 289) Institute of Cancer Biology and Danish Centre for Translational Breast Cancer Research, Danish Cancer Society, Strandboulevarden 49, Copenhagen, DK-2100, DENMARK

Irina Gromova (4: 219) Department of Medical Biochemistry and Danish Centre for Translational Breast Cancer Research, Danish Cancer Society, Strandboulevarden 49, Copenhagen, DK-2100, DENMARK

Dale F. Gruber (1: 33) Cell Culture Research and Development, GIBCO/Invitrogen Corporation, 3175 Staley Road, Grand Island, NY 14072

Markus Grubinger (4: 145) Institute of Physics and Biophysics, University of Salzburg, Hellbrunnerstr. 34, Salzburg, A-5020, AUSTRIA

Jean Gruenberg (2: 27, 201) Department of Biochemistry, University of Geneva, 30, quai Ernest Ansermet, Geneva 4, CH-1211, SWITZERLAND

Stephanie L. Gupton (3: 137) 10550 North Torrey Pines Road, CB 163, La Jolla, CA 92037

Cemal Gurkan (2: 209) Department of Cell and Molecular Biology, The Scripps Research Institute, 10550 North Torrey Pines Road, La Jolla, CA 92037

Martin Guttenberger (4: 131) Zentrum für Molekulariologie der Pflanzen, Universität Tübingen, Entwicklungs-genetik, Auf der Morgenstelle 3, Tübingen, D-72076, GERMANY

Thomas Haaf (3: 409) Institute for Human Genetics, Johannes Gutenberg-Universität Mainz, 55101, Mainz, D-55131, GERMANY

Christine M. Hager-Braun (1: 511) Health and Human Services, NIH National Institute of Environmental Health Sciences, MD F0-04, PO Box 12233, Research Triangle Park, NC 27709

Anne-Mari Håkelién (1: 207) Institute of Medical Biochemistry, Institute of Medical Biochemistry, University of Oslo, PO Box 1112 Blindern, Oslo, 0317, NORWAY

Fiona C. Halliday (1: 395) GlaxoSmithKline, Greenford, Middlesex, UB6 OHE, UNITED KINGDOM

Gerald Hammond (2: 223) Molecular Neuropathobiology Laboratory, Cancer Research UK London Research Institute, 44 Lincoln's Inn Fields, London, WC2A 3PX, UNITED KINGDOM

Klaus Hansen (4: 253)

Hironobu Harada (1: 367) Department of Neurosurgery, Ehime University School of Medicine, Shitsukawa, Toon-shi, Ehime, 791-0295, JAPAN

Robert J. Hay (1: 43, 49, 573) Viitro Enterprises Incorporated, 1113 Marsh Road, PO Box 328, Bealeton, VA 22712

Izumi Hayashi (1: 151) National Medical Center and Beckman Research Institute, Division of Neurosciences, City of Hope, 1500 E. Duarte Rd, Duarte, CA 91010-3000

Timothy A. Haystead (4: 265) Department of Pharmacology and Cancer Biology, Duke University Medical Center, Box 3813 Med Ctr, Durham, NC 27710

Rebecca Heald (2: 379) Molecular and Cell Biology Department, University of California, Berkeley, Berkeley, CA 94720-3200

Florence Hediger (2: 359) Department of Molecular Biology, University of Geneva, 30, Quai Ernest Ansermet, Geneva, CH-1211, SWITZERLAND

Rainer Heintzmann (3: 29) Randall Division of Cell and Molecular Biophysics, King's College London, Guy's Campus, London, SE1 1UL, UNITED KINGDOM

Frederic Heitz (4: 13) Centre de Recherche en Biochimie Macromoléculaire (UPR 1086), Centre National de la Recherche Scientifique (CNRS), 1919 Route de Mende, Montpellier Cedex 5, F-34293, FRANCE

Johannes W. Hell (2: 85) Department of Pharmacology, University of Iowa, 2152 Bowen Science Building, Iowa City, IA 52242

Kai Hell (4: 269) Adolf-Butenandt-Institut für Physiologische Chemie, Lehrstuhl: Physiologische Chemie, Universität München, Butenandtstr. 5, Gebäude B, München, D-81377, GERMANY

Robert R. Henry (1: 121) Veterans Affairs Medical Center, San Diego (V111G), 3350 La Jolla Village Drive, San Diego, CA 92161

Johan Hiding (2: 45) Göteborg University, Institute of Medical Biochemistry, PO Box 440, Göteborg, SE-403-50, SWEDEN

Yasushi Hiraoka (3: 171) Structural Biology Section and CREST Research Project, Kansai Advanced Research Center, Communications Research Laboratory, 588-2 Iwaoka, Iwaoka-cho, Nishi-ku, Kobe, 651-2492, JAPAN

Mary M. Hitt (1: 435) Department of Pathology & Molecular Medicine, McMaster University, 1200 Main Street West, Hamilton, Ontario, L8N 3Z5, CANADA

Julie Hodgkinson (3: 307) School of Crystallography, Birkbeck College, University of London, Malet Street, London, WC1E 7HX, UNITED KINGDOM

Klaus P. Hoeflich (2: 307) Division of Molecular and Structural Biology, Ontario Cancer Institute, Department of Medical Biophysics, University of Toronto, 610 University Avenue, 7-707A, Toronto, Ontario, M5G 2M9, CANADA

Tracy L. Hoffman (1: 21) ATCC, P.O. Box 1549, Manassas, VA 20108

Jörg D. Hoheisel (4: 113) Functional Genome Analysis, German Cancer Research Center, Deutsches Krebsforschungszentrum, Im Neuenheimer Feld 580, Heidelberg, D-69120, GERMANY

Thomas Hollemann (1: 191) Institute for Biochemistry and Molecular Cell Biology, University of Göttingen, Justus-von-Liebig-Weg 11, Göttingen, D-37077, GERMANY

Caterina Holz (4: 57) PSF biotech AG, Huebnerweg 6, Berlin, D-14059, GERMANY

Akira Honda (2: 299) Department of Pharmacology, University of Vermont, Health Science Research Facility 330, Burlington, VT 05405-0068

Masanori Honsho (2: 5) Max Planck Institute of Molecular Cell Biology and Genetics, Pfotenhauerstrasse 108, Dresden, D-01307, GERMANY

Andrew N. Hoofnagle (4: 443) School of Medicine, University of Colorado Health Sciences Center, Denver, CO 80262

Eliezer Huberman (1: 165) Gene Expression and Function Group, Argonne National Laboratory, 9700 South Cass Avenue, Argonne, IL 60439-4844

M. Shane Hutson (3: 87) Department of Physics, Duke University, 107 Physics Bldg, Durham, NC 27708-1000

Andreas Hüttmann (1: 115) Abteilung für Hämatologie, Universitätskrankenhaus Essen, Hufelandstr. 55, Essen, 45122, GERMANY

Anthony A. Hyman (2: 155) Max Planck Institute of Molecular Cell Biology and Gene Technology, Pfotenhauerstrasse 108, Dresden, D-01307, GERMANY

Sherrif F. Ibrahim (1: 269) Institute for Systems Biology, 1441 N. 34th St, Seattle, WA 98103

Kazuo Ikeda (1: 151) National Medical Center and Beckman Research Institute, Division of Neurosciences, City of Hope, 1500 East Duarte Road, Duarte, CA 91010-3000

Elina Ikonen (2: 181) The LIPID Cell Biology Group, Department of Biochemistry, The Finnish National Public Health Institute, Mannerheimintie 166, Helsinki, FIN-00300, FINLAND

Pranvera Ikononi (1: 49) Director, Cell Biology, American Type Culture Collection (ATCC), 10801 University Blvd., Manassas, VA 20110-2209

Mitsuhiko Ikura (2: 307) Division of Molecular and Structural Biology, Ontario Cancer Institute, Department of Medical Biophysics, University of Toronto, 610 University Avenue 7-707A, Toronto, Ontario, M5G 2M9, CANADA

Arup Kumar Indra (3: 501) Institut de Génétique et de Biologie Moléculaire et Cellulaire (IGBMC), 1 rue Laurent Fries, B.P.10142, Illkirch CEDEX, F-67404, FRANCE

Takayoshi Inoue (4: 35) National Institute for Neuroscience, 4-1-1 Ogawahigashi, Kodaira, Tokyo, 187-8502, JAPAN

Kumiko Ishii (2: 139) Supra-Biomolecular System Research Group, RIKEN (Institute of Physical and Chemical Research), 2-1, Hirosawa, Wako-shi, Saitama, 351-0198, JAPAN

Dean A. Jackson (2: 121) Department of Biomolecular Sciences, UMIST, PO Box 88, Manchester, M60 1QD, UNITED KINGDOM

Reinhard Jahn (2: 85) Department of Neurobiology, Max-Planck-Institut für Biophysikalische Chemie, Am Faßberg 11, Gottingen, D-37077, GERMANY

Kim D. Janda (1: 491) Department of Chemistry, BCC-582, The Scripps Research Institute, 10550 N. Torrey Pines Road, La Jolla, CA 92037

Harry W. Jarrett (4: 335) Department of Biochemistry, University of Tennessee Health Sciences Center, Memphis, TN 38163

Daniel G. Jay (4: 307) Dept. Physiology, Tufts University School of Medicine, 136 Harrison Avenue, Boston, MA 02111

David W. Jayme (1: 33) Cell Culture Research and Development, GIBCO/Invitrogen Corporation, 3175 Staley Road, Grand Island, NY 14072

Ole Nørregaard Jensen (4: 409) Protein Research Group, Department of Biochemistry and Molecular Biology, University of Southern Denmark, Campusvej 55, Odense M, DK-5230, DENMARK

Jae Hyun Jeong (4: 29) Department of Chemical & Biomolecular Engineering, Center for Ultramicrochemical Process Systems, Korea Advanced Institute of Science and Technology, Daejeon, 305-701, SOUTH KOREA

Jeff A. Jones (2: 233) Space and Life Sciences Directorate, NASA-Johnson Space Center, TX 77058

Gloria Juan (1: 279) Research Pathology Division, Room S-830, Memorial Sloan-Kettering Cancer Center, 1275 York Avenue, New York, NY 10021

Melissa S. Jurica (2: 109) Molecular, Cell & Developmental Biology, Center for Molecular Biology of RNA, UC Santa Cruz, 1156 High Street, Santa Cruz, CA 95064

Eckhart Kämpgen (1: 103) Department of Dermatology, Friedrich Alexander University, Hartmannstr. 14, Erlangen, D-91052, GERMANY

Roger Karlsson (2: 165) Department of Cell Biology, The Wenner-Gren Institute, Stockholm University, Stockholm, S-10691, SWEDEN

Fredrik Kartberg (2: 45) Göteborg University, Institute of Medical Biochemistry, PO Box 440, Gothenburg, SE, 403-50, SWEDEN

Irina N. Kaverina (3: 111) Institute of Molecular Biotechnology, Austrian Academy of Sciences, Dr. Bohrgasse 3-5, Vienna, A-1030, AUSTRIA

Ralph H. Kehlenbach (2: 267) Hygiene-Institut-Abteilung Virologie, Universität Heidelberg, Im Neuenheimer Feld 324, Heidelberg, D-69120, GERMANY

Daniel P. Kiehart (3: 87) Department of Biology, Duke University, B330g Levine Sci Bldg, Box 91000, Durham, NC 27708-1000

Katherine E. Kilpatrick (1: 483) Senior Research Investigator, TriPath Oncology, 4025 Stirrup Creek Drive, Suite 400, Durham, NC 27703

Jong-Duk Kim (4: 29) Department of Chemical & Biomolecular Engineering, Center for Ultramicrochemical Process Systems, Korea Advanced Institute of Science and Technology, Daejeon, 305-701, SOUTH KOREA

Maurice Kléber (1: 69) Institute of Cell Biology, Department of Biology, Swiss Federal Institute of Technology, ETH—Hönggerberg, Zurich, CH-8093, SWITZERLAND

Toshihide Kobayashi (2: 139) Supra-Biomolecular System Research Group, RIKEN (Institute of Physical and Chemical Research) Frontier Research System, 2-1, Hirosawa, Wako-shi, Saitama, 351-0198, JAPAN

Stefan Kochanek (1: 445) Division of Gene Therapy, University of Ulm, Helmholtz Str. 8/I, Ulm, D-89081, GERMANY

Anna Koffer (2: 223) Physiology Department, University College London, 21 University Street, London, WC1E 6JJ, UNITED KINGDOM

Antonius Koller (4: 383) Department of Cell Biology, Torrey Mesa Research Institute, 3115 Merryfield Row, San Diego, CA 92121

Erich Koller (3: 523) Functional Genomics, GeneTrove, Isis Pharmaceuticals, Inc., 2292 Faraday Ave., Carlsbad, CA 92008

Robert L. Kortum (1: 215) The Eppley Institute for Research in Cancer, The University of Nebraska Medical Center, 986805 Nebraska Medical Center, Omaha, NE 68198-6805

Irina Kratchmarova (4: 427) Protein Interaction Laboratory, University of Southern Denmark—Odense, Campusvej 55, Odense M, DK-5230, DENMARK

Geri E. Kreitzer (2: 189) Cell and Developmental Biology, Weill Medical College of Cornell University, LC-300, New York, NY 10021

Florian Kreppel (1: 445) Division of Gene Therapy, University of Ulm, Helmholtz Str. 8/I, Ulm, D-89081, GERMANY

Mogens Kruhøffer (4: 83) Molecular Diagnostic Laboratory, Clinical Biochemical Department, Aarhus University Hospital, Skejby, Brendstrupgaardvej, Aarhus N, DK-8200, DENMARK

Robb Krumlauf (4: 35) Stowers Institute for Medical Research, 1000 East 50th Street, Kansas City, MO 64110

Michael Kühl (1: 191) Development Biochemistry, University of Ulm, Albert-Einstein-Allee 11, Ulm, D-89081, GERMANY

Mark Kühnel (2: 57) Department of Cell Biology, EMBL, Postfach 102209, Heidelberg, D-69117, GERMANY

Anuj Kumar (3: 179) Dept. of Molecular, Cellular, and Developmental Biology and Life Sciences Institute, University of Michigan, 210 Washtenaw Avenue, Ann Arbor, MI 48109-2216

Thomas Küntziger (1: 207) Institute of Medical Biochemistry, Institute of Medical Biochemistry, University of Oslo, PO Box 1112 Blindern, Oslo, 0317, NORWAY

Yasuhiro Kuramitsu (4: 197) Department of Biochemistry and Biomolecular Recognition, Yamaguchi University School of Medicine, 1-1-1 Minami-kogushi, Ube, Yamaguchi, 755-8505, JAPAN

Sergei A. Kuznetsov (1: 79) Craniofacial and Skeletal Disease Branch, NIDCR, NIH, Department of Health and Human Services, 30 Convent Drive MSC 4320, Bethesda, MD 20892

Joshua Labaer (4: 73) Harvard Institute of Proteomics, 320 Charles Street, Boston, MA 02141-2023

Frank Lafont (2: 181) Department of Biochemistry, University of Geneva, 30, quai Ernest-Ansermet 1211, Geneva 4, CH-1211, SWITZERLAND

Yun Wah Lam (2: 103, 115) Wellcome Trust Biocentre, MSI/WTB Complex, University of Dundee, Dow Street, Dundee, DD1 5EH, UNITED KINGDOM

Angus I. Lamond (2: 103, 115) Wellcome Trust Biocentre, MSI/WTB Complex, University of Dundee, Dow Street, Dundee, DD1 5EH, UNITED KINGDOM

Lukas Landmann (3: 5) Institute for Anatomy (LL), Anatomisches Institut, University of Basel, Pestalozzistrasse 20, Basel, CH-4056, SWITZERLAND

Helga B. Landsverk (1: 207) Institute of Medical Biochemistry, Institute of Medical Biochemistry, University of Oslo, PO Box 1112 Blindern, Oslo, 0317, NORWAY

Christine Lang (4: 57) Department of Microbiology and Genetics, Berlin University of Technology, Gustav-Meyer-Allee 25, Berlin, D-13355, GERMANY

Paul LaPointe (2: 209) Department of Cell and Molecular Biology, The Scripps Research Institute, 10550 North Torrey Pines Road, La Jolla, CA 92037

Martin R. Larsen (4: 371) Department of Biochemistry and Molecular Biology, University of Southern Denmark, Campusvej 55, Odense M, DK-5230, DENMARK

Pamela L. Larsen (1: 157) Department of Cellular and Structural Biology, University of Texas Health Science Center at San Antonio, San Antonio, TX 78229-3900

Eugene Ngo-Lung Lau (1: 115) Leukaemia Foundation of Queensland Leukaemia Research Laboratories, Queensland Institute of Medical Research, Royal Brisbane Hospital Post Office, Brisbane, Queensland, Q4029, AUSTRALIA

Sabrina Laugesen (4: 371) Department of Biochemistry and Molecular Biology, University of Southern Denmark, Campusvej 55, Odense M, DK-5230, DENMARK

Daniel Laune (1: 519) Centre de Pharmacologie et Biotechnologie pour la Santé, CNRS UMR 5160, Faculté de Pharmacie, Avenue Charles Flahault, Montpellier Cedex 5, F-34093, FRANCE

Andre Le Bivic (2: 241) Groupe Morphogenese et Compartimentation Membranaire, UMR 6156, IBDM, Faculte des Sciences de Luminy, case 907, Marseille cedex 09, F-13288, FRANCE

Ronald Lebofsky (3: 429) Laboratoire de Biophysique de l'ADN, Departement des Biotechnologies, Institut Pasteur, 25 rue du Dr. Roux, Paris Cedex 15, F-75724, FRANCE

Chuan-PU Lee (2: 259) The Department of Biochemistry and Molecular Biology, Wayne State University School of Medicine, 4374 Scott Hall, 540 E. Canfield, Detroit, MI 48201

Eva Lee (1: 139) Life Sciences Division, Lawrence Berkeley National Laboratory, 1 Cyclotron Road, Bldg 83-101, Berkeley, CA 94720

Joon-Hee Lee (4: 45) School of Biosciences, University of Nottingham, Sutton Bonington, Loughborough, Leics, LE12 5RD, UNITED KINGDOM

Kwangmoon Lee (1: 215) The Eppley Institute for Research in Cancer, The University of Nebraska Medical Center, 986805 Nebraska Medical Center, Omaha, NE 68198-6805

Thomas Lee (4: 443) Dept of Chemistry and Biochemistry, Univ of Colorado, 215 UCB, Boulder, CO 80309-0215

Margaret Leversha (3: 395) Memorial Sloan Kettering Cancer Center, 1275 York Avenue, New York, NY 10021

Jeffrey M. Levsky (4: 121) Department of Anatomy and Structural Biology, Golding # 601, Albert Einstein College of Medicine of Yeshiva University, 1300 Morris Park Avenue, Bronx, NY 10461

Alexandre Lewalle (3: 37) Randall Centre, New Hunt's House, Guy's Campus, London, SE1 1UL, UNITED KINGDOM

Chung Leung Li (1: 115) Experimental Haematology Laboratory, Stem Cell Program, Institute of Zoology/Genomics Research Center, Academia Sinica, Nankang 115, Nankang, Taipei, 11529, R.O.C.