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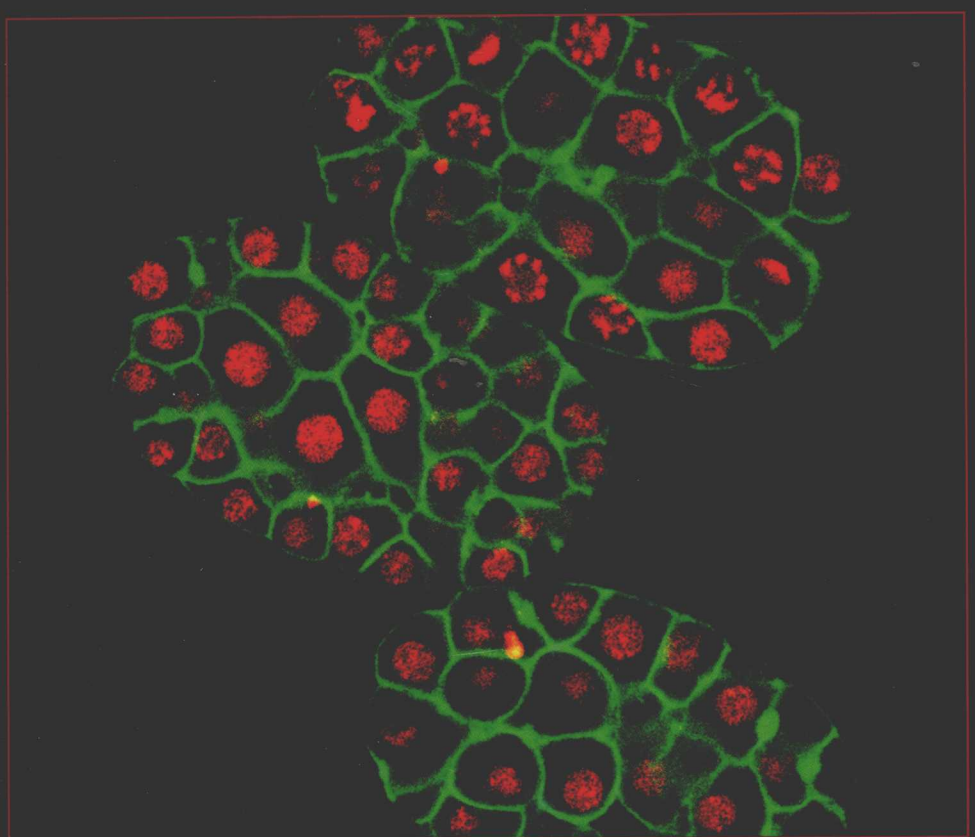
Caenorhabditis elegans: Molecular Genetics and Development

SECOND EDITION



Edited by

Joel H. Rothman and Andrew Singson

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Caenorhabditis elegans: Molecular Genetics and Development

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Edited by

Joel H. Rothman

Professor and Chair, Department of MCD Biology,
University of California, Santa Barbara, CA, USA

Andrew Singson

Associate Professor, Rutgers University,
Waksman Institute, Piscataway, NJ, USA

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Methods in Cell Biology

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Caenorhabditis elegans: Molecular Genetics and Development
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Series Editors

Leslie Wilson
Department of Molecular, Cellular and Developmental Biology
University of California
Santa Barbara, California

Paul Matsudaira
Department of Biological Sciences
National University of Singapore
Singapore

CONTRIBUTORS

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

Shirin Bahmanyar (289), Ludwig Institute for Cancer Research and Department of Cellular & Molecular Medicine, University of California San Diego, La Jolla, California, USA

Jean-Louis Bessereau (65), Ecole Normale Supérieure, Institut de Biologie de l'ENS, IBENS, Paris, France; INSERM U1024, Paris, France; CNRS, UMR 8197, Paris, France

Thomas Blumenthal (187), Department of Molecular, Cellular, and Developmental Biology, University of Colorado, Boulder, Colorado, USA

Gina Broitman-Maduro (253), Department of Biology, University of California at Riverside, Riverside, California, USA

Ana Carvalho (289), Ludwig Institute for Cancer Research and Department of Cellular & Molecular Medicine, University of California San Diego, La Jolla, California, USA

Shih-Peng Chan (219), Department of Molecular, Cellular and Developmental Biology, Yale University, New Haven, Connecticut, USA; Current address: Department of Microbiology, College of Medicine, National Taiwan University, Taipei, Taiwan

Iain Cheeseman (289), Whitehead Institute for Biomedical Research, MIT, Cambridge, MA, USA

Andrew D. Chisholm (325), Section of Cell and Developmental Biology, Division of Biological Sciences, University of California, San Diego, La Jolla, California, USA

Patricia G. Cipriani (89), Center for Genomics and Systems Biology; Department of Biology, New York University, New York, NY 10003

Arshad Desai (289), Ludwig Institute for Cancer Research and Department of Cellular & Molecular Medicine, University of California San Diego, La Jolla, California, USA

Julien Dumont (289), Institut Curie-UMR144, Paris, France

Brian D. Geldziler (343), Waksman Institute, Rutgers University, Department of Microbiology and Molecular Genetics, Piscataway, New Jersey, USA

Claudiu A. Giurumescu (325), Section of Cell and Developmental Biology, Division of Biological Sciences, University of California, San Diego, La Jolla, California, USA

Reto Gassmann (289), Ludwig Institute for Cancer Research and Department of Cellular & Molecular Medicine, University of California San Diego, La Jolla, California, USA

Jeff Hardin (377), Department of Zoology, University of Wisconsin-Madison, Madison, Wisconsin, USA

Pan-Young Jeong (445), Present address: Department of Molecular, Cellular and Developmental Biology, University of California Santa Barbara, Santa Barbara, California, USA

Yishi Jin (413), Howard Hughes Medical Institute, Neurobiology Section, Division of Biological Sciences, University of California, San Diego, La Jolla, California, USA

Martin R. Jones (23), Department of Medical Genetics, University of British Columbia, Vancouver, British Columbia, Canada

Hyoe-Jin Joo (445), Yonsei Proteome Research Center, Department of Biochemistry and Biomedical Science, College of Life Science and Biotechnology, World Class University Program, Graduate School, Yonsei University, Seoul, Korea

Heekyeong Kim (445), Yonsei Proteome Research Center, Department of Biochemistry and Biomedical Science, College of Life Science and Biotechnology, World Class University Program, Graduate School, Yonsei University, Seoul, Korea

Kwang-Youl Kim (445), Yonsei Proteome Research Center, Department of Biochemistry and Biomedical Science, College of Life Science and Biotechnology, World Class University Program, Graduate School, Yonsei University, Seoul, Korea

Eric J. Lambie (3), Biology Department II, Ludwig-Maximilians-University, Munich, Germany

Jeeyong Lee (445), Yonsei Proteome Research Center, Department of Biochemistry and Biomedical Science, College of Life Science and Biotechnology, World Class University Program, Graduate School, Yonsei University, Seoul, Korea

Zoe Lohn (23), Department of Medical Genetics, University of British Columbia, Vancouver, British Columbia, Canada

Paul Maddox (289), Institute for Research in Immunology and Cancer, Department of Pathology and Cell Biology, University of Montreal, Montreal, Quebec, Canada

Morris F. Maduro (161, 253), Department of Biology, University of California Riverside, Riverside, California, USA

Matthew R. Marcello (343), Waksman Institute, Rutgers University, Department of Microbiology and Molecular Genetics, Piscataway, New Jersey, USA; UMDNJ-Robert Wood Johnson Medical School, Piscataway, New Jersey, USA

J. Jason Morton (187), Department of Molecular, Cellular, and Developmental Biology, University of Colorado, Boulder, Colorado, USA

Sherry Niessen (289), The Skaggs Institute for Chemical Biology and Department of Chemical Physiology, The Center for Physiological Proteomics, The Scripps Research Institute, La Jolla, California, USA

Karen Oegema (289), Ludwig Institute for Cancer Research and Department of Cellular & Molecular Medicine, University of California San Diego, La Jolla, California, USA

Young-Ki Paik (445), Yonsei Proteome Research Center, Department of Biochemistry and Biomedical Science, College of Life Science and Biotechnology, World Class University Program, Graduate School, Yonsei University, Seoul, Korea; Rm#423, Industry-University Research Bldg., Yonsei University, 134, Shinchon-dong, Sudaemoon-ku, Seoul, Korea

Amy E. Pasquinelli (219), Department of Biology, University of California San Diego, La Jolla, California, USA

Fabio Piano (89), Center for Genomics and Systems Biology; Department of Biology, New York University, New York, NY 10003; New York University Abu Dhabi, Abu Dhabi, United Arab Emirates

Vida Praitis (161), Biology Department, Grinnell College, Grinnell, Iowa, USA

Valérie J.P. Robert (65), Ecole Normale Supérieure, Institut de Biologie de l'ENS, IBENS, Paris, France; INSERM U1024, Paris, France; CNRS, UMR 8197, Paris, France; Present address: Laboratory of Molecular and Cellular Biology, UMR5239 CNRS, Ecole Normale Supérieure de Lyon, Lyon, France

Ann M. Rose (23), Department of Medical Genetics, University of British Columbia, Vancouver, British Columbia, Canada

Diane C. Shakes (343), College of William and Mary, Department of Biology, Williamsburg, Virginia, USA

Andrew Singson (343), Waksman Institute, Rutgers University, Department of Microbiology and Molecular Genetics, Piscataway, New Jersey, USA

David R. Sherwood (113), Department of Biology, Duke University, Durham, North Carolina, USA

Frank J. Slack (219), Department of Molecular, Cellular and Developmental Biology, Yale University, New Haven, Connecticut, USA

Albertha J.M. Walhout (271), Program in Gene Function and Expression and Program in Molecular Medicine, University of Massachusetts Medical School, USA

Zheng Wang (113), Department of Biology, Duke University, Durham, North Carolina, USA

Priscilla M. Van Wynsberghe (219), Department of Biology, University of California San Diego, La Jolla, California, USA; Current address: Department of Biology, Colgate University, Hamilton, NY, USA

Dong Yan (413), Howard Hughes Medical Institute, Neurobiology Section, Division of Biological Sciences, University of California, San Diego, La Jolla, California, USA

John R. Yates III (289), The Skaggs Institute for Chemical Biology and Department of Chemical Physiology, The Center for Physiological Proteomics, The Scripps Research Institute, La Jolla, California, USA

Esther Zanin (289), Ludwig Institute for Cancer Research and Department of Cellular & Molecular Medicine, University of California San Diego, La Jolla, California, USA

PREFACE

Caenorhabditis elegans: Molecular Genetics and Development

The allure of a model organism comes not from any special fascination for the creature itself; it is doubtful that most researchers studying a simple and tiny animal, the nematode *Caenorhabditis elegans*, are particularly attracted to these modest creatures *per se*. Rather than any fondness for the animal, it is the exceptional experimental methodology available with these high-performance vehicles of biological discovery that entice those driven by intellectual curiosity about the living world to investigate their inner workings. As this millimeter-long creature has amply proven itself to be of enduring utility for biological discovery, it is of value to continue to assemble and update experimental methodology on its use.

A century, in fact a millennium, has turned since the last *C. elegans* volume was published in the Methods in Cell Biology series (as volume 48). That volume, "*Caenorhabditis elegans*: Modern Biological Analysis of an Organism," was the first major compendium of *C. elegans* methods and only the second complete published volume on this creature. Since that volume appeared, several other collections of methods have been published, notably a brief practical volume edited by I. Hope, a methods section edited by V. Ambros as a component of the online resource, Wormbook, and a published volume edited by K. Strange. Nonetheless, for over a decade and half the original *C. elegans* methods volume has served as an invaluable resource to both seasoned and new researchers who focus their scientific curiosity on *C. elegans*.

One might reasonably ask, in an era in which printed material is rapidly dissipating into cyberspace and vast information resources are available online, why a printed volume is of value. In our view, the accessibility of a printed form is still well-suited to the laboratory environment. Several copies of the 1995 *C. elegans* methods volume are stationed at ready access on our laboratory shelves. Members of our laboratories continually reach for the book, even many years after its publication. Just as laboratory notebooks have yet to be satisfactorily replaced by a digital medium, the ability to flip through the pages of a methods volume is not yet an anachronism in the setting of an experimental laboratory.

We are closing in on the 50th anniversary of Sydney Brenner's 1963 letter to the director of the Medical Research Council Laboratory of Molecular Biology, immortalized in the first Cold Spring Harbor Press *C. elegans* monograph, in which he proposed to adopt a *Caenorhabditis* worm as a model organism. The period since the predecessor of this volume was published in 1995 is a fraction of that interval and yet has seen the majority of the prominent discoveries made with the animal. Six Nobel laureates in the *C. elegans* community have been celebrated since the last volume in

this series was published and key discoveries that led to some of those prizes were made during this period. This era also saw the first complete animal genome sequence, the discovery of RNAi, the generality of miRNA-mediated control across biology, and many other fundamental advances that have emerged from the laboratories of *C. elegans* investigators and have proven to be broadly transformative to biology.While advances made with the worm are quantifiable, their full impact on science is inestimable.

The original volume made an effort to be fully comprehensive. In earlier days, it was possible to craft a single volume that fully addressed the state of the art. But the precipitous growth in the field would make such an undertaking unwieldy, if not impractical. 85% of all publications in the Pubmed database containing the text word "elegans" (currently approximately 20,000) were published since the last volume appeared, many by labs that have not traditionally focused on the animal. The number of *C. elegans* laboratories with strain designations (now over 850) is more than five times that in 1995. A compendium of methods that scaled similarly might contain over 170 chapters spread over as many as 10 volumes. Thus, the two volumes assembled here no longer attempt to serve as a single source book for *C. elegans* methodology. Rather, we have chosen to include chapters on many of the methods that have evolved or dramatically altered since the 1995 volume, with the recognition that more has been left out than included. It is inevitable that additional volumes will come in the future that fill and update voids left by the current collection.

Owing to the length of this updated collection, it is now distributed over two volumes. This volume (vol. 106 in the series) comprises genetics, molecular biology, and development, while the subsequent volume (vol. 107) will focus on imaging, cell biology, and physiology. Many methods from the original 1995 volume (e.g., basic culturing, mutagenesis, mosaic analysis, and so on) are still relevant and useful and the experimentalist is encouraged to consult that volume for such methods. It is inevitable that some of the methods in the earlier volume (e.g., the physical map, genomic and cDNA sequencing, and use of the extinct database structures that preceded Wormbase) have become obsolete. On the other hand, many methods have been improved or refined for specific applications, for example, genetic mapping techniques (Chapter 1), reverse genetic approaches (Chapters 3 and 4), transgenesis (Chapter 6), and *in situ* hybridization using RNA probes (Chapter 9), all of which are covered in this volume as revised or entirely new chapters. We note that, unlike the previous edition, we have not included comprehensive appendices, as this information is now readily available in a continually updated manner online through the internet resources listed in the single Appendix of this volume.

Mastery of the varied tools of *C. elegans* biology is enhanced by the experience gained in a lab connected to those that grew up during formative stages of the field. The lore, philosophy, and strategies one uses to dissect biological processes are not coherently incorporated in the literature, but can be effectively transmitted through a sort of apprenticeship in such labs.The worm field is famed for the large fraction of practitioners who trace lineal roots to the early pioneers in the field. However, over recent years, the prominence of the worm system has lured many researchers not

formally linked to the "worm pedigree" to adopt the animal as a useful tool for their favorite subjects of inquiry. Thus, rather than covering discrete methods *per se*, some of the chapters are designed to transmit strategies that are not easily gleaned from the literature (most prominently featured in Chapter 5, which describes genetic strategies used to deconstruct the pathways that drive cellular and developmental processes, and also in the chapters on mapping and on specialized chromosomes in Chapters 1 and 2.) We believe that these strategies will be of particular value to newcomers who learn worm biology without the benefits of apprenticeship in a seasoned worm lab.

Among the most notable of the advances in *C. elegans* technology since the first volume was published was the discovery of RNAi and subsequent methods for adapting RNAi to broad functional genomics screens, which have revolutionized discovery of gene function. Such approaches, and the integration of the "phenome" with informatics studies of functional relationships between gene activities, are covered in chapter 4, of value to aficionados and newcomers alike. Similarly, the recognition that miRNAs function at many levels across animal biology make chapter 8, on analysis of miRNAs, an essential component of this volume. In addition, since publication of the earlier volume, it has become clear that a large fraction of worms genes are organized in operons and are trans-spliced. Any worm molecular geneticist must be mindful of this complexity of gene organization in the animal and the methods for analyzing RNA processing (Chapter 7) are therefore important to any researcher considering the structure of genes and effects of mutations and RNAi on gene expression.

An overarching goal articulated by Sydney Brenner when he inaugurated *C. elegans* research was to obtain a complete description of the animal: this began with the comprehensive analysis of the cell lineage and anatomy and later the whole genome. More recently, this goal has been extended to the level of gene function and interaction by techniques covered in chapters that describe functional and transcriptional network analysis (Chapters 4 and 10). Genetic approaches have dominated *C. elegans* research; however, biochemical methods have become increasingly more significant, particularly as the pathway from *in vitro* discovery to *in vivo* validation has shortened, and methods for analyzing protein complexes and other proteomics approaches are covered in Chapters 11 and 16. The pre-eminent focus of Brenner's original vision to exploit *C. elegans* as a new model system was directed at unveiling the processes that drive development, the biological challenge that drew many researchers to the worm. In keeping with the predominance of this discipline, a major subdivision of this volume comprises five chapters (Chapters 12–16) that address varied approaches to problems in developmental biology, ranging from cell lineage analysis (including new advances in automated lineage analysis), fertilization, morphogenesis, nervous system development and regulation of the alternative developmental stage, the dauer larva.

Just as *C. elegans* develops rapidly, so do technological approaches to analyzing its biology. It is clear that we are able here to capture only an instant in this rapidly moving field, and methods have advanced even during the period in which these

chapters were being assembled and edited. For example, the tremendous advances in DNA sequencing technology is making whole-genome sequence identification of mutations inexpensive and routine, thereby superseding much of the traditional genetic mapping approaches. Moreover, effective new methods for generating genomic modifications based on synthetic nucleases have recently appeared, but came too late to include in the initial release of this volume.These and untold other technologies will no doubt occupy the pages of future editions in this series, devoted to this magnificent living tool for biological discovery.

Appreciation for the richness of technology available to *C. elegans* researchers, only partially captured in the current volumes, has been expressed in many ways, even beyond scientific activity. Two of the traditions at the biennial International *C. elegans* meeting are the Worm Show, an evening comedy variety show, and the Worm Art show, in which artistic members of the worm community pay homage to the animal through visual arts, films, and crafts, including clothing and even cuddly stuffed toys. Upon further reflection, perhaps those of us who have dedicated so many years to pursuing the wonderful mysteries of *C. elegans*, and appreciative of the many gifts that it has generously yielded, have indeed developed a deep and abiding fondness for the modest little creature after all.

Joel H. Rothman and Andrew Singson
August, 2011

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