

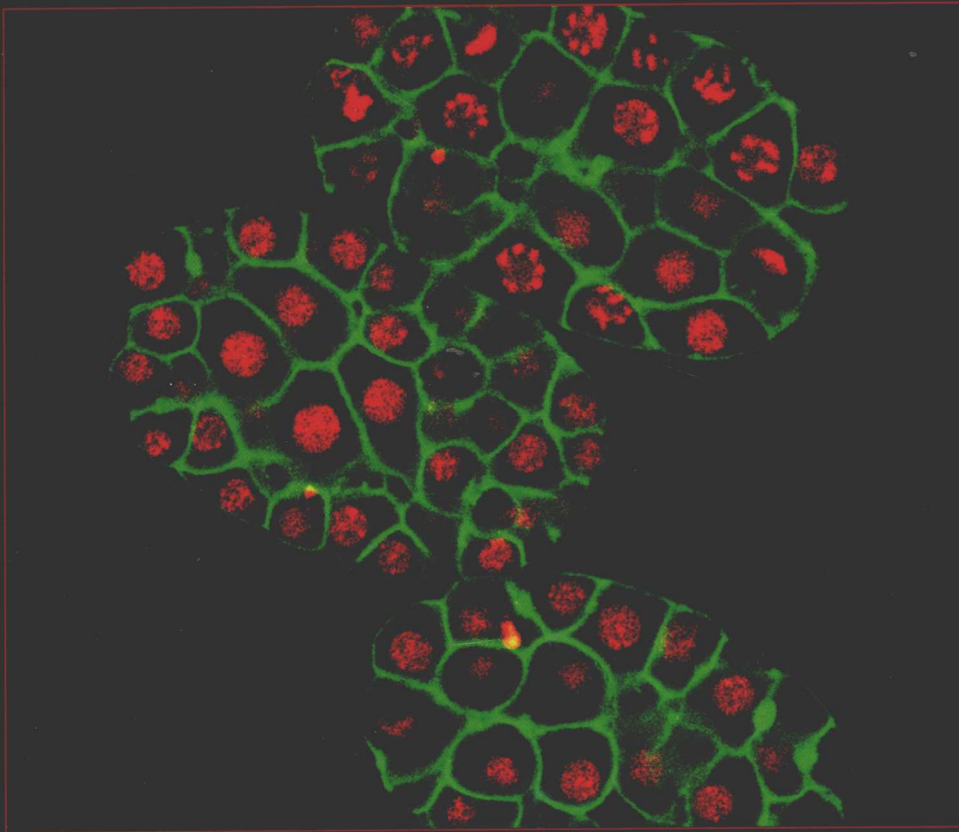
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Methods in Cell Biology • Volume 106

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# CAENORHABDITIS ELEGANS: MOLECULAR GENETICS AND DEVELOPMENT

SECOND EDITION



Edited by

Joel H. Rothman and Andrew Singson



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# Methods in Cell Biology

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## VOLUME 106

*Caenorhabditis elegans*: Molecular Genetics and Development  
2nd Edition

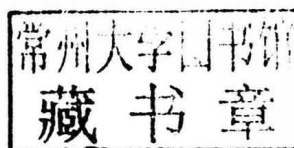
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# Methods in Cell Biology

**VOLUME 106**

*Caenorhabditis elegans*: Molecular Genetics and Development  
2nd Edition

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## 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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