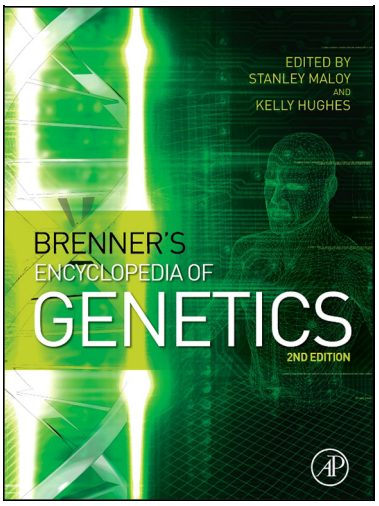

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Caenorhabditis elegans (Nematode)

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Glossary

Dauer An alternative larval form that *Caenorhabditis elegans* animals enter under conditions of environmental stress, nutrient deprivation, and/or overcrowding.
Diapause A stage of arrested development or quiescence.
Differential interference contrast (DIC) microscopy Also known as Nomarski microscopy; a phase-contrast approach taking advantage of different refractive indexes within the sample to visualize transparent structures.
Gonochorism Method of reproduction in which male and female germ cells are provided by two sexes and fertilized within the female; the opposite of hermaphroditism.
Inbreeding depression The reduced fitness of individual progeny within a population, resulting from breeding of genetically related parents.
Lethargus Animal behavioral state in which nematodes become generally inactive for a period of time, usually prior to shedding of the cuticle.
Molting The end of each larval stage of *C. elegans* is marked with a molt where new cuticle is synthesized and the old one is shed. Before molting, the animal enters a brief lethargus stage.
Nictation A dauer larva-specific behavioral pattern, where animals crawl up on the substrate and wave back and forth while standing on their tail (dispersal behavior).
Polycistronic mRNA Encodes several different proteins. These proteins are usually functionally related and are coregulated in an operon.
RNA interference (RNAi) A gene silencing phenomenon, whereby double-stranded RNA fragments elicit degradation of cognate mRNAs. RNAi provides an effective approach for gene knockdown and assessment of loss-of-function phenotypes.
Transgenesis A process of introducing exogenous genes (transgenes) into a living organism. Transgenes are typically transmitted to the offspring.

Introduction

Caenorhabditis elegans is a small, soil dwelling, and free-living (nonparasitic) nematode worm (phylum Nematoda, commonly known as roundworms). In the wild, *C. elegans* and other *Caenorhabditis* species are found on most continents and many isolated territories. Animals of the *Caenorhabditis* genus preferentially colonize various microbe-rich habitats, such as rotting plant material. More than 25 species of the *Caenorhabditis* genus have been characterized up to date. Although, *Caenorhabditis* species exhibit many morphological similarities, they are highly divergent at the genetic level. Genetic divergence reflects ecological specialization and territorial distribution of different species.

The *C. elegans* adult reaches a length of about 1 mm and a diameter of 80 µm. In the laboratory, animals can be grown in liquid medium or on agar plates seeded with *Escherichia coli* and can be easily cultivated in large numbers. *C. elegans* has five pairs of autosomes and one pair of sex chromosomes (XX). Most species of the phylum Nematoda are gonochoristic, with separate female and male sexes that reproduce through cross-fertilization. Hermaphroditic species also exist, which reproduce by self-fertilization. *C. elegans* is primarily a hermaphroditic species but males also exist (Figures 1 and 2, respectively). The ratio of sex chromosomes to autosomes determines sex. Hermaphrodites, the dominant sexual form, are diploid for the sex chromosome (XX), whereas males, which arise sporadically at a frequency of 0.1%, have only one X chromosome (XO). Male population can be easily maintained through mating with hermaphrodites. A single hermaphrodite produces about 300 progeny by self-fertilization and more if it mates with males. Cross-fertilization of hermaphrodites with males allows the generation of double or multiple mutants, facilitating genetic analysis. Reproduction of *C. elegans* by self-fertilization allows the generation of genetically identical populations that do not undergo inbreeding depression.

Although *C. elegans* is a relatively modern addition to the arsenal of model organisms, its biology has already been investigated to an exceptional level. The simple body plan, the transparent egg and cuticle, and the nearly invariant developmental plan of this nematode have facilitated exceptionally detailed developmental and anatomical characterization of the animal (detailed information is available online at the WormAtlas website). The complete sequence of cell divisions (the cell lineage) and the normal pattern of programmed cell deaths that occur as the fertilized egg develops into the adult have been elaborated. Wild-type hermaphrodites consist of 959 somatic cells, 302 of which are neurons. The transparency of the animal body allows easy visualization and monitoring of cellular processes and has permitted recording and determination of the complete pattern of cell divisions that generate the 959 somatic cells of the adult (the cell lineage). Cells can be easily followed during development using differential interference contrast (DIC) light microscopy. Despite its apparent simplicity, there is a high degree of differentiation; worms have muscle cells, hypodermis, a nervous system, intestine, gonads, glands, and an excretory system. The nematode is one of the few multicellular organisms for which routine cryopreservation has been made possible. *C. elegans* strains can be stored indefinitely in liquid nitrogen, making the maintenance and distribution of large collections of mutants feasible and cost-effective.

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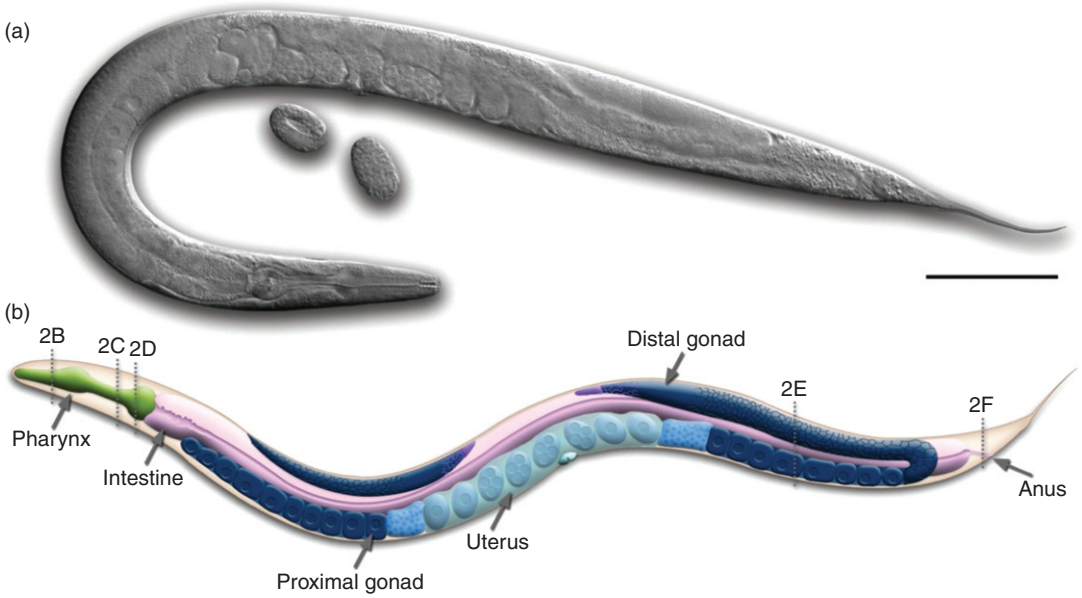

Figure 1 Anatomy of the adult *C. elegans* hermaphrodite. (a) DIC image of an adult *C. elegans* hermaphrodite. Scale = 100 µm. (b) Anatomical features of the *C. elegans* hermaphrodite. Dotted lines and number-letter combinations signify individual EM cross-sections shown in WormAtlas. Courtesy of WormAtlas (http://www.wormatlas.org/hermaphrodite/introduction/IMAGES/introfig1.jpg).

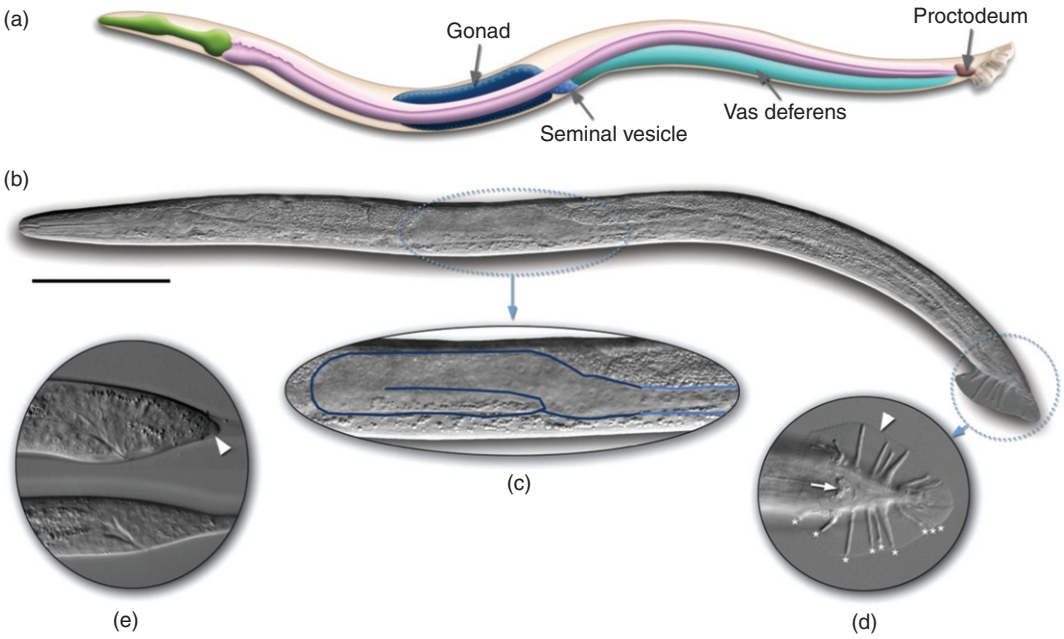

Figure 2 Anatomy of the adult male. (a) Anatomical features of *C. elegans* males. (b) DIC image of an adult *C. elegans* male. Scale = 100 µm. (c) Enlarged image of the unilobed distal gonad of the adult male that is shown in (b). (d) Tail of the adult *C. elegans* male. The cloaca and the fan are indicated by the arrow and the arrowhead, respectively. (e) The *C. elegans* male tail is starting to bugle at the L3 larva stage (bottom). At L4 larva stage, the tail hypodermis has retracted (arrowhead, top). Courtesy of WormAtlas (http://www.wormatlas.org/hermaphrodite/introduction/IMAGES/introfig5.jpg).

Life Cycle

C. elegans completes a reproductive life cycle in 2.5 days at 25 °C (or in 3.5 days at 20 °C), progressing from fertilized embryos through four larval stages (L1–L4), to become egg-laying adults, which then live for about 2–3 weeks (**Figure 3**). After fertilization of oocytes, embryonic development takes 14 h to complete. Eggs then hatch into L1 larvae. The next 50 h, larval development proceeds through three additional developmental stages L2, L3, and L4. All these juvenile stages are separated by a short phase of lethargus and molting. *C. elegans* develops and reproduces within a wide range of

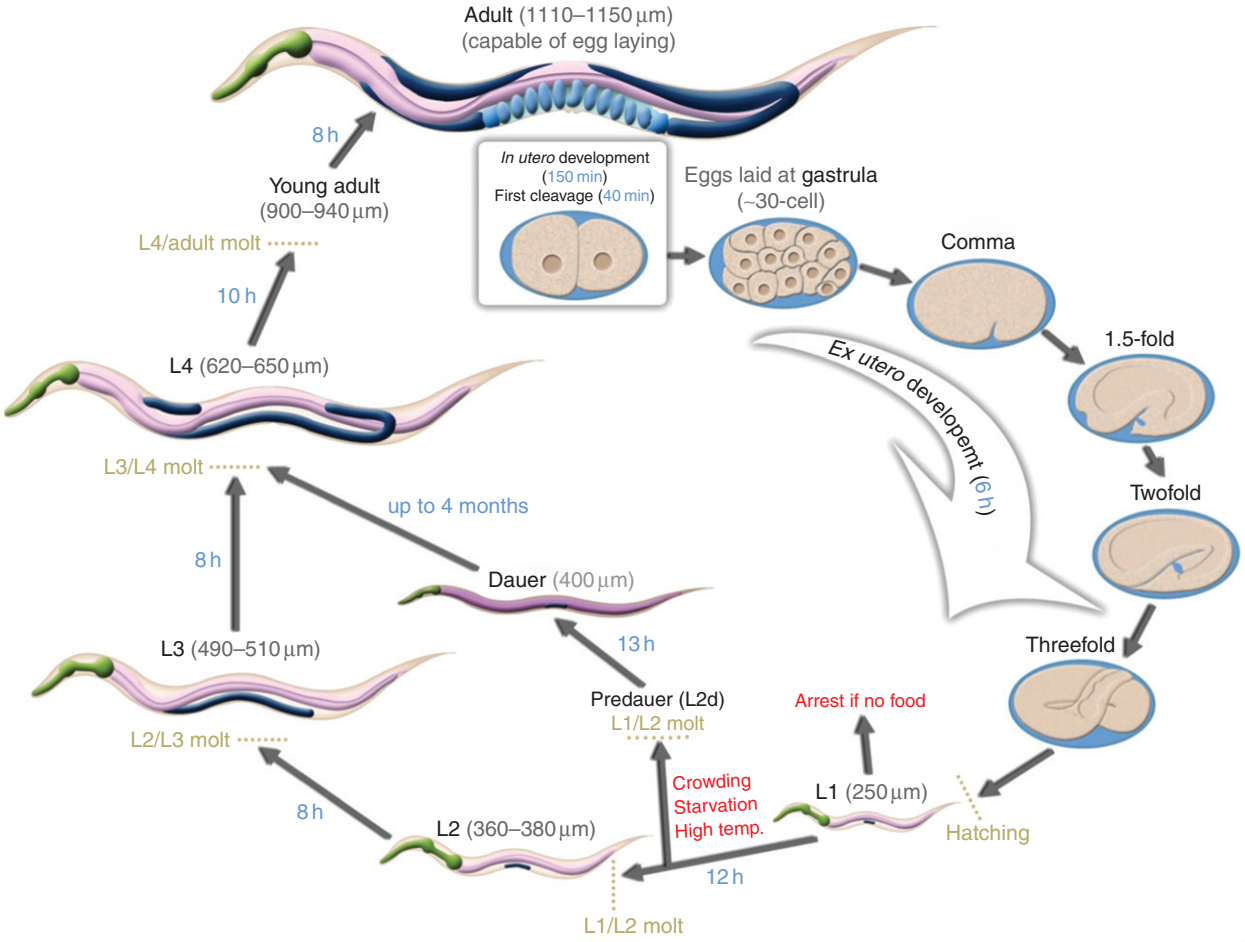

Figure 3 The life cycle of *C. elegans*. Numbers in blue indicate the approximate time spent at each developmental stage (22 °C). The first cleavage takes places at about 140 min postfertilization. Eggs are laid at about 150 min postfertilization. The length of the animal in micrometers at each developmental stage is shown in parentheses next to the stage name. Courtesy of WormAtlas (http://www.wormatlas.org/hermaphrodite/introduction/IMAGES/introfig6.jpg).

temperatures (usually between 15 and 25 °C). However, development pauses at temperatures below 8 °C and worms become sterile above 27 °C.

If, during development, animals encounter adverse conditions such as starvation, overcrowding, or high temperature, larvae can enter an alternative life stage of diapause called the dauer (German word meaning 'enduring') larva, during which animals move but do not feed. The dauer larva is a 'non-aging' developmental form that survives without food for weeks up to several months. Dauer larvae are constantly moving and they are extremely resistant to various stress conditions. A thick cuticle covers dauer animals and most tissues are reduced in volume. Dauer larvae can for several months and are able to remain in this stage for a long time. Nictation is a well-characterized dispersal behavior of the dauer larva. In natural habitats, animals stand on their tail, waving, which allows them to search and attach to a new over-passing carrier host, such as slugs, snails, or beetles that will transport them to a new niche. When a dauer larva encounters favorable environmental conditions, it reenters the life cycle at the fourth larval stage, progresses into adulthood to reproduce, and then completes the final week or so of its life span.

Genetics and Genomics

C. elegans is a well-established and powerful genetic system. When a hermaphrodite parent is subjected to a mutagenizing agent, the F1 progeny self-fertilize to produce F2 animals that are homozygous for recessive mutations. In this way, thousands of mutations that disrupt development or various behaviors have been identified and, after crossing with males and standard gene mapping, positioned on a detailed genetic map. Rapid and precise genetic mapping can be achieved by taking advantage of a dense single-nucleotide polymorphism map. The size of the *C. elegans* genome is about a hundred million base pairs and contains around 20 000 genes. Compared to mammalian genomes, the *C. elegans* genome shows relatively low complexity. *C. elegans* genes contain few and mostly short introns with an average of intergenic distance of about 5 kb. Among metazoans, the *C. elegans* genome exhibits a distinctive feature, whereby some genes are arranged in operons (polycistronic messages, which are transcribed and then *trans*-spliced into monocistronic messages before translation).

A physical map of the *C. elegans* genome, consisting of overlapping cosmid and YAC clones covering most of the six

chromosomes, has been constructed to facilitate cloning of genes that have been positioned on the genetic map. Sequencing and high-quality annotation of the complete *C. elegans* genome has been accomplished. A remarkable corollary of the *C. elegans* sequencing project is that more than 65% of human disease genes currently identified have a counterpart in the worm. All ~20 000 predicted open reading frames (ORFs) have been subjected to expression profiling under numerous conditions using microarray technology. In addition, expressed sequence tags (ESTs) and ORF sequence tags (OSTs) have been obtained for all *C. elegans* genes, providing an extensive collection of nematode complementary DNAs (cDNAs). Comprehensive information concerning gene structure, expression patterns, protein–protein interactions, mutant or RNA interference (RNAi) phenotypes, and microarray data is available in WormBase, the online resource for nematode-related information.

The Nervous System

The nervous system of *C. elegans* is made up of 302 neurons and 56 glial cells in hermaphrodites and 381 neurons and 92 glial cells in males. Neurons form two distinct and semi-independent networks: the large somatic nervous system (282 neurons) and a small pharyngeal nervous system (20 neurons). The pharynx is the feeding organ of the worm, constantly pumping and grinding food. These two nervous systems exhibit topological differences and are loosely linked through a single neuron. In the somatic nervous system, neurons are positioned between the hypodermis and the body wall muscles. In the pharyngeal nervous system, the neurons are embedded among pharyngeal muscles.

The nervous system is the organ with the highest cell number and variety (at least 118 different neuron classes). *C. elegans* neurons are divided in four main categories according to their functionality within the circuitry of the nervous system: (1) motor neurons, which make synaptic contacts onto muscle cells and drive muscle contraction; (2) sensory neurons, which have sensory specializations responsible for sensing various stimuli; (3) interneurons, which receive input from incoming synapses and send signals out to other neurons; and (4) polymodal neurons, which perform more than one of the above functions. The complete wiring diagram of *C. elegans* neurons and their connecting synapses has been elucidated by means of serial section electron micrographs. Despite the small number of neurons, the *C. elegans* nervous system mediates a rich variety of behaviors, such as forward and backward locomotion, obstacle avoidance, exploratory and foraging movements, response to a wide range of sensory cues, including mechanical, chemical, thermal, and light stimuli, and changes in the osmolarity of the environment. Sensory cues are particularly important for regulating entry and exit from the dauer stage and for feeding. Differences in the number and type of neurons between hermaphrodites and males are responsible for sex-specific behaviors, such as egg laying in hermaphrodites and the mating behavior in males.

Experimental Malleability

C. elegans is an established and broadly valued model organism for modern biomedical research that offers unparalleled experimental advantages, such as genetic and molecular tractability, a complete cell lineage, ease of laboratory culture and manipulation, and a completely sequenced genome. Advanced molecular biology and genetics methodologies pioneered in this organism are available that greatly facilitate high-throughput approaches for the generation and phenotypic analysis of mutants, comprehensive drug screenings, dissection of molecular interactions, and reconstruction of regulatory and metabolic cascades.

C. elegans is amenable to unbiased forward and reverse genetic screens and particularly susceptible to gene inactivation by RNAi. Indeed, RNAi was first described and used for whole-genome-scale functional genomics in *C. elegans*.

Mutagens that have been used to introduce mutations in *C. elegans* genome include specific chemicals such as ethylmethane sulfonate (EMS), diethyl sulfate (DES), and *N*-nitroso-*N*-ethylurea (ENU); X-ray, γ-ray, UV-ray, or ionizing particle ray irradiation; and transposable elements. DNA manipulated *in vitro* can be introduced back into animals for functional assays by either DNA microinjection or DNA-coated microparticle bombardment. Vectors are available for the transgenesis and identification of transgenic animals, for cell-specific expression, and for generation of fusions to marker genes such as *E. coli* β-galactosidase and the jellyfish green fluorescent protein (GFP) so that individual cells can be visualized in stained or living animals. To this end, advanced high-resolution fluorescence microscopy and imaging methodologies have been developed. Viable mutant strains, strains that overexpress a specific gene or lack a gene function, can be efficiently generated and the resulting phenotypes can be rapidly identified.

Primary culture methodologies are available for the analysis of specific groups of cells and neurons *ex vivo*. Electrophysiological study-specific nematode neurons and muscles are also possible. Microsurgery with a laser beam can be used to specifically ablate individual cells within live animals, and whole classes of cells can be rendered nonfunctional or killed by cell-specific expression of toxic genes.

In addition to these features, worms carrying mutations in a variety of highly conserved biochemical pathways display a wide variety of phenotypes that are easily scored. For this reason, *C. elegans* is especially attractive for investigating the biological effects of drugs and identifying their molecular targets. The small size, the rapid life cycle, and the high fecundity of *C. elegans* permit screening of thousands of animals over multiple generations in microtiter plates. This, coupled with effortless handling and a notable low cost of cultivation and maintenance, allows seamless implementation of high-throughput drug-screening approaches, as well as in-depth genetic and biochemical studies of the molecular pathways targeted by specific drugs. Furthermore, the transparency of the worm allows appropriate fluorescent markers to be readily followed *in vivo*. In addition, the detailed description of the worm nervous system provides a unique setting for studying the action of many drugs that act on the central nervous system (CNS) in humans. Given that essential neuronal

processes and functions (e.g., neurotransmitters and their neuronal receptors) are highly conserved between *C. elegans* and vertebrates, such endeavors are expected to yield information relevant to humans.

Areas of Research

C. elegans has been used as a model organism in major research areas including but not limited to developmental biology, neurobiology, cell biology, and aging. The worm has contributed decisively to our understanding of fundamental cellular and developmental processes including cell signaling, programmed cell death, neuronal and synaptic properties and function, muscle biology, and sex determination. Similarly, *C. elegans* has contributed enormously to our understanding of human neurodegenerative disorders, cancer, and aging. Indeed, *C. elegans* has also emerged as a powerful experimental system to study the molecular and cellular aspects of human disease *in vivo*. It has been estimated that about 42% of the human disease genes have an ortholog in the genome of *C. elegans* including those genes associated with Alzheimer's disease, juvenile Parkinson's disease, spinal muscular atrophy, hereditary non-polyposis colon cancer, and many other age-related disorders. Modeling a human disease in a simple invertebrate, such as *C. elegans*, allows the dissection of complex molecular pathways into their component parts and thus provides meaningful insight into the pathogenesis of a complex disease phenotype. Notable examples are the discovery of major factors involved in apoptosis, phagocytosis and necrosis, the discovery of key genes implicated in cancer, the study of genes involved in human pathologies (Alzheimer's disease, Kallmann syndrome, Duchenne muscular dystrophy, stroke, neurodegenerative disorders, etc.), and the discovery of genes regulating longevity (insulin/insulin-like growth factor 1 (IGF1) signaling). Thus, the nematode holds promise for deciphering disease pathogenesis and formulating educated and effective therapeutic interventions. The nematode is also becoming a popular platform that can be used to gain a deeper understanding of the mechanism of action of antiparasitic agents and various human drugs, as well as for the discovery of new bioactive compounds.

The important contributions of *C. elegans* to a wide range of biomedical research fields are underscored by the three Nobel prizes awarded to researchers for their seminal discoveries using the worm. In 2002, Sydney Brenner, Bob Horvitz, and John Sulston were awarded the Nobel Prize in Physiology or Medicine for their studies on genetics of organ development and programmed cell death. In 2006, Andy Fire and Craig Mello were awarded the Nobel Prize in Physiology or Medicine for the discovery of RNAi in *C. elegans*. In 2008, O. Shimomura, M. Chalfie, and R. Y. Tsien were awarded the Noble Prize in Chemistry for their work with GFP.

See also: Apoptosis; Behavioral Genetics; Brenner, Sydney; Cell Determination; Cell Differentiation; Cell Division Genetics; Cell Division in *Caenorhabditis elegans*; Cell Lineage; Cell Markers: Green Fluorescent Protein; Cell/Neuron Degeneration; Bacterial Chemotaxis; Developmental Genetics of *Caenorhabditis elegans*; Embryonic Development of the Nematode *Caenorhabditis elegans*; Epistasis; Gene Expression; Genetic Marker; Aging, Genetics of; Microinjection; Mitochondrial Mutants; Neurogenetics of Neurotransmitter Release in *Caenorhabditis elegans*; Oogenesis, in *Caenorhabditis elegans*; Polycistronic mRNA; Reporter Gene; RNA Interference; Spermatogenesis in *C. elegans*; Transgenic Animals; *Trans*-Splicing.

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