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Caenorhabditis elegans as a biological model

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Abstract
Caenorhabditis elegans is a free-living harmless transparent nematode. it has been extensively used as a biological model. It was the first multicellular organism to have genome completely sequenced and 40% of its genes have human matches. This soil nematode offered great potential for genetic analysis, partly because of its rapid (3-day) life cycle, small size (1.5-mm-long adult), and ease of laboratory cultivation. It is an important model system for biological research in many fields including genomics, cell biology, neuroscience and aging. Compared to the use of other model organisms, such as mice, the short life cycle of *C. elegans* reduces the experimental cycle and facilitates biological study. The mature "female," has precisely 959 somatic cells and the mature male, includes precisely 1031 somatic cells. As an invertebrate experimental system, it is now second only to *Drosophila melanogaster* in terms of convenience and popularity. Its transparency and the generation of multiple progeny have made the worm an attractive model for studies, including those on developmental and reproductive behavior. The properties of *C. elegans* and the research done using *C. elegans* provide a wealth of information and an attractive pool of resources for researchers.

Keywords: *C. elegans*, programme cell death, aging, biological model

Introduction
It is small (about 1 mm in length), transparent for ease of manipulation and observation, feeds on bacteria, such as *E. coli*, and it can be easily and cheaply housed and cultivated in large numbers (10,000 worms/petri dish) in the laboratory. The different *Caenorhabditis* species occupy various nutrient and bacteria rich environments. They feed on the bacteria that develop in decaying organic matter. Soil lacks enough organic matter to support self-sustaining populations.
C. elegans was the first multicellular organism to have its genome completely sequenced. Ever since its introduction by Brenner, *C. elegans* has been widely used in research laboratories (Wood, 1988) [23]. Due to its value as a research tool, a sophisticated knowledge infrastructure has developed, with freely disseminated research methods and protocols. Research on development and morphology can be done with a single cell within this multicellular organism (Donald, 1997; Horvitz, 2004) [2, 8]. *C. elegans* becomes an attractive organism in the study of human diseases. Brenner, Sulston and Robert Horvitz earned the Nobel Prize for PCD in Physiology or Medicine in 2002. For RNA interference Americans Craig Mello and Andrew Fire were awarded the Nobel Prize in Physiology or Medicine in 2006. The green fluorescent protein (GFP) reporter (Chalfie *et al*. 1994) [1] is now widely used for analysis of the timing and tissue specificity of transcription in living transgenic animals.
C. elegans has five pairs of autosomes and one pair of sex chromosome. It has two sexes, hermaphrodites and males. Hermaphrodites have two ovaries, oviducts, spermatheca, and a single uterus. Pure male is about 0.05% of the total population. When hermaphrodites mate with males, 50% of the progeny will be males and 50% will be hermaphrodites. In the laboratory, self-fertilization of hermaphrodites or crossing with males can be manipulated to produce progeny with desired genotypes that are especially useful for genetic study. A hermaphrodite can produce about 300 to 350 offspring under self-fertilization and more if it mates with males. These traits make it easy to produce numerous genotypes and phenotypes for genetic research. (Horvitz, 1997) [8].

Biology
The life cycle *is* rapid, temperature dependent and takes about 3.5 days (20 ^{0}C). Eggs are fertilized within the adult hermaphrodite and laid within a few hours afterward at about the 40 cell stage. Eggs hatch and animals proceed through 4 larval stages, each of which ends in a molt. In the period between hatching and the adult stage, they feeds and grows not only in size, but also in cell number.

When animals reach adulthood, they produce about 300 progeny each. Total life-span about 12 to 18 days at 20° (best growth condition).The life cycle is temperature-dependent. *C. elegans* goes through a reproductive life cycle (egg to egg-laying parent) in 5.5 days at 15 ^{0}C, 3.5 days at 20 ^{0}C, and 2.5 days at 25 ^{0}C. *C. elegans* can adopt an alternative life form, called the dauer larval stage, if plates are too crowded or if food is scarce. Dauer larvae are thin and can move but their mouths are plugged and they cannot eat. They appear to be non-aging and live about 15 more days.

Genome

The sequencing of the *C. elegans genome*, completed in 1998, provided researchers with the first complete DNA sequence of a multicellular organism. About 40% of *C. elegans* genes have human homologs. The results established a genome size of 97 Mb, and computer analysis predicted that there were about 19,000 genes in the genome, making the average gene density about 1 gene per 5 kb of DNA. Compared with the gene densities seen in larger animals, *C. elegans* genes are closely packed, in part because its introns are smaller on average than those of most other animals. However, the completed sequence has enabled researchers to make some generalizations about the *C. elegans* proteome: the complete set of proteins encoded by the 19,000 known and predicted *C. elegans* genes. In addition to the 19,000 known and predicted genes, there are several kinds of repetitive DNA sequences dispersed throughout the *C. elegans* genome. The Approximately 100 million base pair long and six chromosomes (named I, II, III, IV, V and X) and a mitochondrial genome. Two strains have historical importance. One strain, Bergerac, was collected from the soil in France by Victor Nigon of the Université de Lyon (Nigon 1949) [17] and the other strain, Bristol, was isolated by L.N. Staniland (National Agricultural Advisory Service, London) from mushroom compost near Bristol, England (Nicholas *et al* 1959) [16].

Recombinant DNA Technology to Study Development of *C. elegans*

DNA Transformation

Many aspects of *C. elegans* genetic and molecular analysis depend on DNA transformation, the ability to create transgenic animals by introducing cloned DNA sequences into the germ line. To transform *C. elegans*, experimenters generally microinject DNA through the cuticle into the syncytial distal gonad of the hermaphrodite. The injected DNA transgenes recombine with each other to form large extra chromosomal arrays that are incorporated into oocytes and retained in most cells during embryogenesis. The presence of centromere-like activity all along the chromosomes of *C. elegans* makes the maintenance of these linear fragments feasible. It is possible to demonstrate gene expression from such transgenes by the phenotypic rescue of a mutant phenotype, if the corresponding wild-type gene is present in the injected DNA. It is also possible to demonstrate expression visually if a suitable reporter gene is present in the injected DNA. One reporter gene is the *E. Coli β*-galactosidase gene *lacZ*, whose expression can be detected by X-Gal staining of fixed preparations. In *C. elegans*, the most widely used reporter gene is the jellyfish green fluorescent protein (GFP) gene, which has the advantage of being visible in living animals. Researchers use reporter genes to study the regulation of gene expression in *C. elegans* as they do in other organisms If an embryo from an injected hermaphrodite retains the transgenic array in its germ line, it can give rise to a *transmitting line* in which the array passes from one generation to the next. However, at a low frequency that seems to depend on the individual array, it will occasionally be lost at cell division. Loss can occur during meiosis, resulting in progeny that lack the array. Loss can also occur at mitosis during development to produce a clone of somatic cells that do not carry the array. This property can be useful for mosaic analysis of gene function. Irradiation of transmitting lines with X or γ rays can induce integration of a portion of the array at random into one of the chromosomes. Such integrated arrays are inherited in a completely stable fashion like any other chromosomal marker. However, integration of an injected transgene by homologous recombination at the site of the identical *endogenous gene* occurs only at extremely low frequencies.

RNA Interference

The phenomenon of RNAi, originally described by Fire *et al.* in 1998. RNA interference (RNAi) also called post transcriptional gene silencing (PTGS), is a biological process in which RNA molecules inhibit gene expression, typically by causing the destruction of specific m RNA molecules. Mechanism of post transcriptional gene silencing induced by exogenous dsRNA. Historically, it was known by other names, including co-suppression, post transcriptional gene silencing (PTGS), and quelling. Only after these apparently unrelated processes were fully understood did it become clear that they all described the RNAi phenomenon. In 2006, Andrew Fire and Craig C. Mello shared the Nobel Prize in Physiology or Medicine for their work on RNA interference in the nematode worm *C. elegans*, which they published in 1998. The use of RNA interference to inhibit the function of 86% of the 19,427 predicted genes of *C. elegans.* RNAi and miRNA pathway genes affect RNAi-induced transcriptional gene silencing (RNAi-TGS) in the soma of *C. elegans***.**

RNA-Mediated Interference (RNAi)

Researchers discovered that injection of double-stranded copies of a specific *C. elegans* mRNA (dsRNA) into adult hermaphrodites could cause silencing of the corresponding gene in the injected animal and its progeny. This surprising phenomenon has been called RNA-mediated interference (RNAi). When an injected dsRNA enters a worm cell, an enzyme complex, named "dicer," cleaves the incoming dsRNA into small segments of about 22 bp, called short interfering RNAs *(siRNAs)*, which appear to be the active agents conferring specificity. These siRNAs are transported from one cell to another, apparently through specialized membrane channels, so that the siRNAs can spread from the initial site of entry to all cells in the body except possibly neurons. Because of this transport, the initial entry site of the dsRNA is not important, and researchers have even found strong specific inhibitory effects on gene expression when a dsRNA is fed to the worms. Feeding can be conveniently accomplished using bacteria that express the ds RNA as the worms' food. A second group of proteins promotes unwinding of the siRNAs and complexing of their antisense strands with complementary sequences of the corresponding endogenous mRNA. This sense-antisense complex can meet either of two fates. In one, destruction of the mRNA accomplishes gene silencing. In the second, the bound antisense strand acts as a primer for reverse transcription to form a new dsRNA, which is then cleaved by the dicer

complex to produce a new generation of siRNAs, still specific for the same gene. This second alternative leads to amplification of the siRNA population. One consequence of amplification is that an RNAi effect can persist through two or even three generations after initial exposure to a dsRNA

Programmed cell death

Programmed cell death is an important cellular process that controls the development and homeostasis of multicellular organisms, including the nematode *Caenorhabditis elegans.* Genetic and molecular studies in *C. elegans* have led to the identification of many genes that function in different aspects of programmed cell death. These genes define a genetic pathway of programmed cell death in *C. elegans* that is conserved between nematodes and mammals. In this article we will review our current understanding of programmed cell death in *C. elegans* and the insights we learn from functional characterization of the *C. elegans* cell-death genes. About one-sixth of the cells that arise during *C. elegans* embryogenesis are eliminated by a process of cellular suicidecalled programmed cell death or apoptosis**.** During apoptosis, the affected cell shrinks, degrades its DNA, and dies, after which it is engulfed and digested by a neighboring cell. In *C. elegans,* the pattern of apoptosis, like most other aspects of development, is invariant: The identity of the cells that die and the timing of their deaths is the same from animal to animal. The function of programmed cell death seems to be the elimination of unneeded cells that could be detrimental to the organism.

***C. elegans* for the study of programmed cell death:**

C. elegans is transparent; therefore cell divisions and cell deaths can be observed and followed in living animals using high magnification Nomarski optics. This feature has facilitated the determination of the entire cell lineage of *C. elegans*. Of the 1090 somatic cells that are generated during the development of the *C. elegans* adult hermaphrodite, 131 undergo programmed cell death. When observed with Nomarski microscopy, cells undergoing programmed cell death adopt a refractile and raised button-like appearance. Genetic and phenotypic analyses of mutants that are defective in different aspects of programmed cell death have helped define a genetic pathway of programmed cell death in *C. elegans*. The combination of a detailed genetic map, a corresponding physical map and the information provided by the almost finished genome sequencing (only 1% of the *C. elegans* genome left to be determined; further facilitates the molecular studies of programmed cell death in *C. elegans*. Powerful molecular and genetic techniques such as germline transformation and genetic mosaic analysis have been developed. These techniques in combination with detailed knowledge of the anatomy and cell lineage of *C. elegans* have catalyzed rapid progress in our understanding of the mechanisms of programmed cell death.

The genetic pathway of programmed cell death in *C. elegans*

Over the past twenty years, genetic studies in *C. elegans* have led to the identification of more than a dozen genes that are involved in different aspects of programmed cell death. Three genes, *nuc-1* (nuclease-deficient), *ced-1* and *ced-2* (cell-death abnormal), were first identified as genes that are involved in the removal or degradation of cell corpses: mutations in the *nuc-1* gene block degradation of DNA from dead cells; mutations in *ced-1* or *ced-2* gene prevent the engulfment of many cell corpses, leading to the mutant phenotype of persistent cell corpses.

Subsequently, mutations in two additional genes, *ced-3* and *ced-4*, were isolated. Mutations in *ced-3* were isolated as suppressors of the *ced-1* mutants, whereas the first allele of *ced-4* was identified as a suppressor of an egg-laying defective mutant in which two hermaphrodite specific motor neurons (HSNs) important for hermaphrodite egg-laying control inappropriately undergo programmed cell death. Soon after that, more cell-death genes were identified in various genetic screens for new mutations that affect different aspects of programmed cell death. These include four additional genes that mediate cell-corpse engulfment (*ced-5, ced-6, ced-7,* and *ced-10;* 20), two genes (*ces-1* and *ces-2;* cell death specification) that control the death fate of a specific set of cells. The *ced-9* gene that generally protects cells from programmed cell death and finally, the *egl-1* gene that is also required for almost all programmed cell deaths. The *ced-9* gene was initially identified by a gain-of-function (*gf*) mutation that prevents most programmed cell deaths in *C. elegans* as mutations in *ced-3* or *ced-4* do. The normal function of *ced-9* was revealed by the phenotype of its loss-of-function (*lf*) mutants in which many cells that would normally live undergo programmed cell death, suggesting that *ced-9* acts to protect cells from programmed cell death. The *egl-1* gene was originally defined by several gain-of-function mutations that cause inappropriate death of HSN neurons in hermaphrodites. Subsequent isolation of an *egl-1* loss-of-function mutation as a *cis*-dominant suppressor of *egl-1*(*gf*) mutations and examination of the *egl-1*(*lf*) phenotype suggest that the *egl-1* gene is required for almost all programmed cell deaths rather than just playing a role in specifying the death of HSN neurons as its *gf* mutant phenotype implicates. Programmed cell death in *C. elegans* appears very similar to apoptosis in mammals. In both types of organisms, the process helps control the size of cell populations by eliminating excess cells. The investigation of genes that control apoptosis is an important aspect of current cancer research. Although tumor formation often involves the release of normal controls on cell proliferation, it can also result from the failure of apoptosis. Sequence similarity between the membrane associated protein product of the *C. elegans ced-9* gene and the mutant protein product of the *bcl-2* oncogene, which helps cause tumors by blocking apoptosis in humans, underscores the close relation between the control of programmed cell death in *C. elegans* and mammals. In fact, although nematodes and humans are separated by almost a billion years of evolution, their apoptosis-controlling genes are still functionally interchangeable: The normal human allele of the *bcl-2* gene can restore the normal process of cell death when injected into a *C. elegans ced-9* mutant.

Genomic Approaches to study aging in *C. elegans*

Aging is the most universal of biological process and Complex proceess driven by diverse molecular and biochemical events. Oxidative damage, replicative senescense, accumulated stress and metabolic rate specify life span. Aging, or organismal senescence, is defined as gradual changes in an organism that "adversely affect its vitality and function, but most importantly, increase the mortality rate as a function of time" (Finch, 1990) [3]. The etiology of the aging process can be viewed within the framework of evolutionary theory; natural selection cannot act on post-reproductive animals, hence there is no selection for mechanisms to maintain an organism past reproductive age. Aging can

therefore be regarded as a byproduct of this lack of maintenance. Numerous theories exist to explain the aging phenotype within this evolutionary context, two of the most influential being those of the "disposable soma" [13, 14] and of antagonistic pleiotropy (Williams, 1957) [22].*C. elegans* is a powerful model system for the study of aging, because of its genetics, relatively short life span, and ease of propagation of populations of synchronized individuals. Numerous single-gene mutations (AGE genes) have been identified that increase *C. elegans* life span (summarized in the Genes and Interventions Database; http:// sageke.sciencemag.org; Friedman and (Kenyon *et al*., 1993) [12]. The best characterized of these (*daf-2, age-1*) are in an insulin-like signaling pathway which culminates in altering the activity of the transcription factor *daf-16* (Ogg *et al*., 1997) [18]. This same signaling pathway controls the entry of developing nematodes into the alternate, dauer larval stage. The cost to fitness of these longevity mutants predicted by evolutionary theory was observed under stressful laboratory conditions (Walker *et al*., 2000). [21]. Aging is characterized by general physiological decline over time. A hallmark of human senescence is the onset of various age-related afflictions including neurodegeneration, cardiovascular disease and cancer. Although environmental and stochastic factors undoubtedly contribute to the increased incidence of disease with age, recent studies suggest that intrinsic genetic determinants govern both life span and overall health. Current aging research aims at achieving the 'longevity dividend', in which life span extension in humans is accomplished with a concomitant increase in the quality of life (Olshansky *et al*., 2007) [19]. Aging worms experience a decline in mobility (Herndon *et al*., 2002; Huang *et al*., 2004; Hsu *et al*., 2009) [7, 11, 9]. chemotaxis (Murakami and Murakami, 2005) [15] and reproductive capacity (Hughes *et al*., 2007) and become increasingly susceptible to lethal infections (Garigan *et al*., 2002; Garsin *et al*., 2003) [5, 6]. Although environmental and stochastic factors undoubtedly contribute to the increased incidence of disease with age, recent studies suggest that intrinsic genetic determinants govern both life span and overall health. Current aging research aims at achieving the 'longevity dividend', in which life span extension in humans is accomplished with a concomitant increase in the quality of life (Olshansky *et al*., 2007) [19]. Significant progress has been made using model organisms, especially the nematode worm *Caenorhabditis elegans*, to delineate the genetic and biochemical pathways involved in aging to identify strategies for therapeutic intervention in humans.

Conclusion

The special features of *C. elegans* make it an excellent model for the genetic analysis of development in multicellular animals and the good understanding of developmental mechanisms achieved in *C. elegans* will help to study the development of other nematodes. A number of genes have been identified and are in the process of being characterized. An even larger set of essential genes remains to be identified. A lack of molecular markers for various aspects of germ-line development has limited the analysis of mutant phenotypes and dependency relationships. The properties of *C. elegans* and the research done using *C. elegans* provide a wealth of information and an attractive pool of resources for researchers. This well-established model system provides educators a good resource in teaching biology.

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