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Using *C. elegans* for aging research

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Over a century ago, the zoologist Emile Maupas first identified the nematode, *Rhabditis elegans,* in the soil in Algiers. Subsequent work and phylogenic studies renamed the species *Caenorhabditis elegans* or more commonly referred to as *C. elegans*; (*Caeno* meaning recent; *rhabditis* meaning rod; *elegans* meaning nice). However, it was not until 1963, when Sydney Brenner, already successful from his work on DNA, RNA, and the genetic code, suggested the future of biological research lay in model organisms. Brenner believed that biological research required a model system that could grow in vast quantities in the lab, were cheap to maintain and had a simple body plan, and he chose the nematode *C. elegans* to fulfill such a role. Since that time, *C. elegans* has emerged as one of the premiere model systems for aging research. This paper reviews some initial identification of mutants with altered lifespan with a focus on genetics and then discusses advantages and disadvantages for using *C. elegans* as a model system to understand human aging. This review focuses on molecular genetics aspects of this model organism.

Keywords: *C. elegans*; longevity; aging; insulin/IGF-1; dauer

Introduction

In 1974, a little more than a decade after his first thoughts about working on a model organism, Brenner published four manuscripts, including one entitled 'The genetics of *Caenorhabditis elegans*'(Brenner 1974) and a new field began. In this influential paper (Brenner 1974), Brenner outlined methodology for isolation, complementation, and mapping of worm mutants. Importantly, the publication also included the successful isolation of several hundred mutants affecting behavior and morphology, a discussion of the number of defined genes, and an estimation of mutation frequency. Since that time, many discoveries including dissection of programmed cell death (Coulson et al. 1986; Ellis et al. 1991), the systematic cloning of the genome (Coulson et al. 1986; Crawford 2001), the deciphering of the entire DNA sequence (Consortium 1998), microRNAs (Lee et al. 1993; Reinhart et al. 2000), RNA interference (Fire et al. 1998), and the use of GFP (Chalfie et al. 1994) have been done in *C. elegans* which has led to an expansion in the number of researchers working with *C. elegans*.

C. elegans for aging research

For research on aging, early studies in *C. elegans* focused on the feasibility of measuring lifespan and the use of 5-Fluoro-2′-deoxyuridine (FUDR) to maintain synchronous cultures of aged animals (Hosono 1978a, 1978b). In 1977, Klass (1977) published that *C. elegans* was a good system for aging studies as he established a method to consistently measure lifespan, and he concluded that this could lead to future detailed analysis combining genetics and biochemistry. In these early studies, Klass found that altering either temperature or the amount of food resulted in a change in lifespan. In addition, only small effects on lifespan were observed based on parental age or parental lifespan. Klass performed a clonal genetic screen for mutants with altered lifespan and identified five mutants (Klass 1983). Interestingly, later genetic work on these mutants in the laboratory of Tom Johnson, mapped all of them to a single genetic locus, named *age-1* (Friedman & Johnson 1988). This was the first breakthrough in aging research for studies based on *C. elegans* as this study revealed that it was possible to identify mutants that altered lifespan and more importantly, individual genes could modulate lifespan.

From the initial characterization of mutants that altered lifespan, the words lifespan and aging have often been used interchangeably. However, lifespan is a single measureable parameter that defines the amount of time an organism is alive but does not give any indication for how an animal is actually aging. Lifespan as a measurement gives little detail about the health of the animal. For this reason, healthspan, defined as the time that an individual is active, productive and free from age-associated disease, is starting to become the focus of aging research (reviewed in (Tissenbaum 2012)).

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Equally important for aging research is the use of the term regulation. Is aging regulated? Is lifespan regulated? A regulated process should indicate that this is a trait that would be selected for over time. However, fitness competitions between wild type and *daf-2* mutants, show that after four generations, none of the *daf-2* mutants remained primarily because of the early fertility defects in the *daf-2* mutants (Jenkins et al. 2004). Therefore, similar to other studies with long-lived mutants and consistent with the antagonistic pleiotropy theory of aging (Lakowski & Hekimi 1996; Gems et al. 1998; Chen, Pan et al. 2007; Chen, Senturk et al. 2007; Curran & Ruvkun 2007; Anderson et al. 2011), *daf-2* mutants exhibit a heavy fitness cost with lifespan extension (Jenkins et al. 2004). Taken together, lifespan and aging should not be used interchangeably and the use of the word regulation should be monitored (reviewed in (Lithgow 2006; Tissenbaum 2012)).

Several years after the *age-1* gene was identified, another gene was shown to modulate lifespan. Similar to mutation in *age-1*, *daf-2* mutants showed adult lifespan extension (Kenyon et al. 1993). Interestingly, previously, both *daf-2* and *age-1* had showed similarity based on a different phenotype. Under favorable growth conditions, *C. elegans* develop from an egg through four larval stages (L1-L4) each separated by a molt, and then a final molt into a reproductive self-fertilizing adult hermaphrodite. In response to unfavorable growth conditions, in particular, high levels of a secreted pheromone (i.e. crowding, low food), worms can enter an alternative developmental mode (at the L3 stage) forming dauer larvae (Riddle & Albert 1997). Dauer (German for enduring) larvae (alternate L3) maximize survival until conditions become more favorable, whereupon they will molt and form a reproductive adult. Genetic screens identified mutants affecting the ability to enter this dauer program. These mutants were named *daf* mutants indicating the dauer formation phenotype. Both *daf-2* and *age-1* were initially isolated in this type of screen because both *daf-2* and *age-1* (originally identified as *daf-23*) mutants show a dauer constitutive (*daf-c*) phenotype such that even under good growth conditions, mutants will enter the dauer stage (Albert et al. 1981). Genetic epistasis analysis placed these two genes in a similar genetic epistasis pathway for dauer formation that was distinct from the other *daf-c* mutants (Vowels & Thomas 1992). These studies also revealed that both *daf-2* and *age-1* mutants could be suppressed by a mutation in the *daf-16* gene (Albert et al. 1981; Riddle 1988; Vowels & Thomas 1992; Riddle & Albert 1997). *daf-16* (also known as *daf-17*) was also isolated in these early dauer formation genetic screens because *daf-16* mutants show a dauer defective (*daf-d*) phenotype such that even under poor growth conditions, mutants will not enter the dauer stage (Albert et al. 1981; Riddle 1988; Vowels & Thomas 1992; Riddle & Albert 1997).

Subsequent molecular cloning beginning in 1996, explained why these genes were separate and distinct from other pathways. The genes encoded for members of an insulin/IGF-1 signaling (IIS) pathway where *daf-2* encoded for an IIS receptor, *age-1* encoded for the catalytic subunit of the PI 3-kinase, and *daf-16* encoded for a forkhead box O (FOXO) transcription factor downstream of the PI 3-kinase signaling cascade. Since then, studies have shown that the IIS pathway is evolutionarily conserved such that mutations in this pathway in flies and mice are also linked to lifespan extension (Barbieri et al. 2003; Yen et al. 2011).

Molecular and genetic studies in *Drosophila* and *C. elegans* have identified FOXO as a central regulator of lifespan (Lin et al. 1997; Ogg et al. 1997; Giannakou et al. 2004; Hwangbo et al. 2004). Modulation of *Drosophila* FOXO (dFoxo) and *C. elegans* FOXO (*daf-16*) dosage can either decrease or increase the lifespan of the organism (Lin et al. 1997; Ogg et al. 1997; Giannakou et al. 2004; Hwangbo et al. 2004). Importantly, advances in genomic research have led to new findings in the area of genome-wide association studies in humans. Multiple human population studies have found an association between single nucleotide polymorphisms (SNPs) in human FOXO3 and human lifespan extension (Lunetta et al. 2007; Willcox et al. 2008; Anselmi et al. 2009; Flachsbart et al. 2009; Li et al. 2009; Soerensen et al. 2010; Zeng et al. 2010; Banasik et al. 2011; Malovini et al. 2011), and the strength of the association appears to increase with age (Flachsbart et al. 2009). Therefore, FOXO3 has emerged as a candidate longevity gene in humans. Taken together, just over a decade from the molecular identification of DAF-16 in *C. elegans*, multiple studies have linked SNPs associated with human DAF-16/FOXO3 and human lifespan extension.

Dauer and longevity connections

Early studies on dauer larvae showed that dauers were ‘ageless’; namely once a dauer recovers and develops into a reproductive hermaphrodite, the subsequent adult lifespan (post-dauer) is independent from the time spent as a dauer (Klass & Hirsh 1976). Therefore, it was thought that *daf-2* and *age-1* were long lived merely due to activation of part of the dauer program manifested in the adult. However, (Kenyon et al. 1993) addressed these concerns by performing lifespan analyses on several other *daf-c* mutants (later shown to be part of a TGF-β signaling cascade) and found that these mutants did not affect lifespan and the issue seemed resolved. However, approaches including genome- wide microarrays and unbiased LC/MS proteomics have shown that the profiles

of adult long-lived *daf-2* mutants are most similar to wild-type dauer larvae (McElwee et al. 2004; McElwee et al. 2006; Depuydt et al. 2014). Moreover, recent studies (Shaw et al. 2007) re-examined the TGF-β *daf-c* mutants and found in contrast to earlier studies, these mutants showed lifespan extension. Similarly, recent genetic data revealed that the connections between the IIS pathway and the TGF-β signaling pathway are intertwined to modulate both lifespan and dauer formation (Narasimhan et al. 2011). Taken together, multiple studies suggest that the longevity of *daf-2* mutants is due to activation of the dauer program in the adult. Despite the fact that a dauer program, an alternative hibernation state to delay reproduction until growth conditions are favorable, seems worm specific, the signaling pathways that were identified to regulate dauer formation modulate longevity from worms to mice, and are associated with human longevity.

Advantages of worms

Why has *C. elegans* been used so successfully for aging research? What would make an organism suitable for aging research? As suggested by Sydney Brenner in 1963, the ability to easily and cheaply grow large quantities of worms in the lab is very helpful for aging research, especially when identifying long-lived mutants. *C. elegans* also have a relatively short lifespan (average approximately 17 days at 20 °C), and the lifespan is largely invariant. The latter allows for identification of mutants that shorten or lengthen average lifespan by a little as 10–15% and still be of statistical significance. Additional benefits of using *C. elegans* include that the entire genome is sequenced and annotated, the availability of an RNAi library comprising approx. 80% of the genes in the genome, the ease of generating transgenic strains and the recent development of gene-targeting approaches. This has allowed for extensive forward and reverse genetic screens for genes that modulate lifespan. The RNAi library allows RNAi to be done by feeding worms bacteria that produce the desired dsRNA and then either the worm or their progeny are scored for a longevity phenotype (Ahringer 2006). Using genome-wide RNAi feeding libraries, the importance of the mitochondria, signal transduction, the response to stress, protein translation, gene expression, and metabolism were found to modulate lifespan (Dillin et al., 2002; Lee et al., 2003; Hamilton et al. 2005; Hansen, Hsu et al. 2005; Hansen, Taubert et al., 2007). Another advantage working with *C. elegans* for studying the aging process is that the lifespan assay is straightforward, which allows for large numbers of worms to be assayed in a single experiment. Therefore, statistical significance can be tested in addition to the analysis of mortality rates. Together, these techniques allow one to comprehensively survey the worm genome for genes that modulate lifespan. This has led to the identification of more than 200 genes and regimens that modulate lifespan in *C. elegans* and revealed evolutionarily conserved pathways that modulate lifespan. Therefore, the combination of the short, invariant lifespan, ease of assays, ample genetic, molecular and genomic tools, and evolutionary conservation has allowed *C. elegans* to develop into a premiere model system for aging research.

Disadvantages of worms

Despite all the excellent advantages of working with *C. elegans* for aging research, there are also several disadvantages for *C. elegans* as a model for human aging. First, *C. elegans* have a simple body plan, and lack many defined organs/tissues including a brain, blood, a defined fat cell, internal organs, and is evolutionarily distant from humans. Second, *C. elegans* are also only 1 mm in length which makes biochemistry more difficult. Typically, all biochemistry, microarray, immunoprecipitation, and chromatin immunoprecipitation is performed on whole worm extracts of either mixed-stage animals or animals at a similar growth stage. This may lead to limited understanding of any tissue-specific signaling such as whether a gene is expressed in the hypodermis or the intestine. Finally, *C. elegans* cell culture is limited with no system equivalent to *Drosophila* S2 cells.

Conclusion

C. elegans has proved to be an invaluable animal for aging research. Thus far, research has focused on the use of lifespan as a measurement of the aging process. These studies have led to the identification of hundreds of genes and regimens that modulate lifespan. Although the initial studies identified genes that altered lifespan and affected dauer diapause, these signaling pathways have nonetheless identified longevity-associated pathways across phylogeny. However, to truly use *C. elegans* for aging research, future studies should focus on understanding the connection between longevity and how an animal ages, with a focus on health. Aging is much more than a lifespan measurement. Aging involves the coordination of multiple systems in an organism and how they change as a function of time. We should strive to use model systems to reveal this systemic coordination on a molecular and genetic level, and how this leads to healthy aging rather than simply lifespan extension.

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