Opinion on *C. elegans* as a model organism

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Preface

Acadian Seaplants requested a third party opinion on the use of *Caenorhabditis elegans (C. elegans)* as a model for higher animal responses to extracts of seaweeds. Their interest is in using this model to screen for bioactive compounds and thereby reduce the need for seaweed product testing in mammalian models and humans.

Background

C. elegans is a small nematode (non-segmented roundworm) that has been used as a model organism for scientific investigation since the 1960s. This animal became widely used in genetics and developmental biology as a result of the pioneering work of Dr. Sydney Brenner who developed it as a model to study animal development, particularly that pertaining to the nervous system. Dr. Brenner chose *C. elegans* because of its simplicity; *C. elegans* is small in size (adults reach about 1mm in length), it is composed of only about 600 cells of which about half are neurons, and it is easy to grow in bulk populations, with a life cycle of about three and half days.[1, 2] In 1998, *C. elegans* became the first multicellular organism for which the whole genome was sequenced.[3] This genome sequence data made *C. elegans* a more widely relevant model in biological studies.

C. elegans is used extensively by researchers around the world as a tool to study a variety of fundamental questions in the biological sciences, including those related to development, neurobiology, innate immunity, cell death and aging. The use of *C. elegans* as a model organism to study numerous diseases, most particularly Alzheimer's disease, Parkinson's disease, and Duchenne muscular dystrophy, but also other conditions such as diabetes and obesity, has been the subject of a number of

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[1] Brenner, S. (1974) The genetics of Caenorhabditis elegans. Genetics. 77: 71-94.

[2] Brenner shared the 2002 Nobel Prize in Physiology or Medicine with Robert Horvitz and John Sulston. The title of his Nobel lecture "Nature's Gift to Science," in reference to c. elegans (http://www.nobelprize.org/mediaplayer/index.php?id=523).

[3] Sequencing Consortium (1998) {Genome sequence of the nematode $ C. elegans $: A platform for investigating biology}. Science. 282: 2012-2018.

comprehensive reviews.[4,5,6] *C. elegans* is also used as a model for biomedical and environmental toxicology studies.[7] Textpresso™, an information extracting and processing package for biological literature, has a section devoted to *C. elegans* with 28,347 full-length papers in its corpus.[8] Similarly, a search of PubMed using the search term "C. elegans" resulted in 24,594 hits.[9]

Opinion

It is undeniable that *C. elegans* is an excellent model for use to study fundamental biological questions and is a valid tool for biomedical research. It is part of a continuum of biological models that include *E. coli*, yeast, Drosophila, zebrafish, mammalian cell lines and rodent models. Indeed, *C. elegans* (and zebrafish) provide researchers a means of doing experiments that are normally done in cell culture, but in a whole organism. Thus, more advanced research questions may be addressed using *C. elegans* than could be addressed using cells in culture, which lack organismal complexity.

While *C. elegans* is certainly a distant relative of humans, more than 60% of human genes have counterparts in *C. elegans*, marking the organism a suitable model for a number of human diseases and biochemical processes.[10] Thus, many of the key components that regulate human metabolism have conserved roles in the worm. For example, *C. elegans* does not possess adipose tissue, yet many of the key regulators of

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[4] Kaletta, T. & M.O. Hengartner (2006) Finding function in novel targets: C. elegans as a model organism. Nat Rev Drug Discov. 5: 387-399. NOTE: Table 1 of this review provides an excellent summary of *C.elegans* disease models.

[5] Giacomotto, J. & L. Ségalat (2010) High-throughput screening and small animal models, where are we? British journal of pharmacology. 160: 204-216.

[6] O'Reilly, L.P. et al. (2014) C. elegans in high-throughput drug discovery. Advanced drug delivery reviews. 69: 247-253.

[7] Leung, M.C.K. et al. (2008) Caenorhabditis elegans: An Emerging Model in Biomedical and Environmental Toxicology. Toxicological Sciences. 106: 5-28.

[8] http://www.textpresso.org/celegans/

[9] http://www.ncbi.nlm.nih.gov/pubmed; The search was performed on June 30, 2015.

[10] O'Reilly, L.P. et al. (2014).

lipid metabolism in mammals are conserved including 5' adenosine monophosphate-activated protein kinase (AMPK), target of rapamycin (TOR) kinase, sterol response element binding protein (SREBP) and CCAAT/enhancer binding protein (C/EBP), where they play roles in the regulation of fat droplets.[11]

A major advantage of the *C. elegans* model is the ease with which various genetic approaches can be applied. For example, RNA interference (RNAi) can be used for any gene in the genome, which greatly facilitates the study of proteins involved in the mechanism of drug action and the pinpointing of drug targets. Transgenes and gene knockout models are used extensively, as are reporter genes such as green fluorescent protein.[12] For example, some of the typical models of Alzheimer's and Parkinson's diseases in *C. elegans* are based on the overexpression of human beta-amyloid (or tau proteins) and human α-synuclein, respectively.[13]

The attributes of *C. elegans* as a model organism make it a powerful and versatile model for early discovery and mechanistic-type research. It can deliver answers to research questions in a relatively fast and cost-effective manner. Because it is a whole organism (allowing an *in vivo* approach), *C. elegans* can be used to evaluate test inputs (e.g. extract, compound, drug) for efficacy and additionally for absorption, distribution, metabolism, excretion or toxicity (ADMET) characteristics at an early stage in the discovery pathway.[14] Such investigations may uncover undesirable effects or performance issues related to the test input that might otherwise only have been detected at a later stage. Thus, the use of *C. elegans* as a model system can help mitigate the risk of failing at a large scale, for example, at the stage of expensive toxicology testing in rodents.

Some of the limitations of *C. elegans* as a model system are specific to the organism, whereas others are shortcomings common to a number of model organisms such as zebrafish or Drosophila. First, *C. elegans* is typically cultured with live *E. coli* as a food source. This is problematic because it raises the possibility that the test input may directly affect the bacteria and then indirectly affect the worms. It is also an issue for dosing since the test input of interest may be assimilated and metabolized by the

[11] Jones, K.T. & K. Ashrafi (2009) Caenorhabditis elegans as an emerging model for studying the basic biology of obesity. Disease models & mechanisms. 2: 224-229.

[12] Transgenic lines of *C. elegans* can be frozen and recovered upon thawing much like cell lines.

[13] Teschendorf, D. & C.D. Link (2009) What have worm models told us about the mechanisms of neuronal dysfunction in human neurodegenerative diseases. Mol Neurodegener. 4: 1-13.

[14] Williams, C.H. & C.C. Hong (2011) Multi-step usage of in vivo models during rational drug design and discovery. International journal of molecular sciences. 12: 2262-2274.

bacteria. Thus, a typical approach is to use high doses of test input to offset bacterial degradation. Another approach is to use heat-killed bacteria, which appears to be an effective way of increasing drug uptake by the worms.[15] A related issue is the fact that *C. elegans* possesses a hard cuticle that may be a barrier to the uptake of non-water soluble substances whose uptake may also be impacted by selective intestinal absorption.[16] Thus, Burns and colleagues tested 1,000 commercially available drug-like small molecules and discovered that less than 10% of these molecules accumulated in *C. elegans* to concentration greater than 50% of that present in the worm's environment.[17] The authors proposed that the hit rate of future small-molecule screens with *C. elegans* would be improved by compound pre-selection using their approach to determine the likelihood of the test compounds reaching their *in vivo* targets.

A third limitation is the lack internal organs/tissues (e.g. heart, lungs, skeleton), which restricts investigation somewhat. Thus, for certain therapeutic areas such as cardiovascular disease, other whole animal models such as zebrafish are more suitable. In relation to this, more than 60% of human genes have a *C. elegans* counterpart but there are many human genes and disease targets that do not have a homolog in *C. elegans* so that other systems of study may be more appropriate. Conversely, a lack of a gene homolog may be used to advantage in some situations. For example, it is suggested that because *C. elegans* does not possess a counterpart to mammalian leptin, it may be a good model to study anti-obesity compounds independent of the hormone's inhibition of food intake.[18] Moreover, the expression of human transgenes in *C. elegans* has been a fruitful approach for many research questions. Thus, mutations in α1-antitrypsin that lead to diseases of lung and liver were successfully modeled in *C. elegans* through expression of a mutant transgene of α1-antitrypsin. Although the worm does not possess lungs or liver, the protein product of the transgene misfolds and accumulates in the worms' intestine, which can be monitored, and drug candidates are thereby evaluated for effects on protein accumulation.[19] In cases such as this one, there is the added advantage of having no endogenous homologous gene to interfere with assessment of the transgene's effects.

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[15] Zheng, S.-Q. et al. (2013) Drug absorption efficiency in Caenorhbditis elegans delivered by different methods. PloS one. 8:

[16] O'Reilly, L.P. et al. (2014).

[17] Burns, A.R. et al. (2010) A predictive model for drug bioaccumulation and bioactivity in Caenorhabditis elegans. Nature chemical biology. 6: 549-557.

[18] Kim, H.M. et al. (2010) Characterization of taurine as anti-obesity agent in C. elegans. J Biomed Sci. 17: S33.

[19] Gosai, S.J. et al. (2010) Automated high-content live animal drug screening using C. elegans expressing the aggregation prone serpin α1-antitrypsin Z. PloS one. 5: e15460.

Finally, the phenotypic outputs such as paralysis, uncoordinated locomotion and other movement defects, which are frequently measured in assays using *C. elegans*, are complex and have been cumbersome to record and quantify. The need for worm-tracking systems is being met by the advent of a number of different options for visual capture and data analysis.[20, 21] The lack of rigorous computational methods has likely contributed to the slow adoption of *C. elegans* for use in high-throughput screening,[22] a situation that is now changing.

In short, *C. elegans* has proven to be a versatile model organism that can be used to answer fundamental questions in biology as well as more applied research including biomedical research where it is become increasingly part of the pre-clinical landscape as whole animal model for a numerous human diseases and as a tool for drug discovery. There are a number of companies now using *C. elegans* in their discovery process (Acadian Seaplants is one of them) and there are contract research firms such as Nemametrix (Eugene, OR) that are now providing screening services using *C. elegans* disease models.[23] *C. elegans* can and is being used to screen for bioactives and can reduce the need for testing in mammalian models. The use of *C. elegans* is not a replacement for *in vivo* testing in mammalian models, but it can help guide such testing by helping researchers predict outcomes with more confidence when planning mammalian experiments.

C. elegans is one of a number of organisms that can be chosen as a model system to study complex biological processes. In his Nobel lecture, Sydney Brenner said that he "considered that having chosen the right organism turned out to be as important as having addressed the right problems.

[20] http://www.wormbook.org/chapters/www_tracking/tracking.html

[21] Mathew, M.D. et al. (2012) WormScan: A technique for high-throughput phenotypic analysis of Caenorhabditis elegans. PloS one. 7: 3.

[22] O'Reilly, L.P. et al. (2014).

[23] Website: http://nemametrix.com/pharmaceutical-drug-discovery/