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Minireview

# RNA interference in nematodes and the chance that favored Sydney Brenner

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## Abstract

The efficiency of RNA interference varies between different organisms, even among nematodes. A recent report of successful RNA interference in the nematode *Panagrolaimus superbus* in *BMC Molecular Biology* has implications for the comparative study of the functional genomics of nematode species, and prompts reflections on the choice of *Caenorhabditis elegans* as a model organism.

RNA interference (RNAi), the inactivation of gene expression by double-stranded (ds) RNA, has become a major method of gene inactivation in the past ten years. The fact that the trigger for RNAi is composed of dsRNA was discovered in the nematode worm *Caenorhabditis elegans* [1]. This gene-inactivation method is far from being applicable to all nematodes, however, especially in the external application mode used in *C. elegans*. A recent paper by Shannon *et al.* in *BMC Molecular Biology* [2] describes its successful use in two *Panagrolaimus* species that belong to a different nematode suborder from *C. elegans*. This increases the range of nematode species over which comparative functional genomics is in principle possible, and reinforces the accumulating evidence that susceptibility to RNAi is widely distributed over nematode species.

## Is RNA interference a universal phenomenon in eukaryotes?

RNAi was first described in plants and has now been found in a variety of unicellular and multicellular eukaryotes. The mechanism of inhibition entails the cleavage of the dsRNA trigger into smaller dsRNAs of 21-23 base pairs, called small interfering (si)RNAs, which recognize the target mRNA and lead to its destruction. Sensitivity to long endogenous dsRNAs may be maintained by selection against the spread of transposons or viruses or against the spurious expression of other repetitive sequences, all of which are likely to be transcribed in both directions to at least some degree, and thus to produce dsRNA.

It is a particularly convenient feature of *C. elegans* that it is sensitive to external dsRNAs provided very simply either by soaking the worms in a RNA preparation [3] or by feeding them with *Escherichia coli* bacteria expressing dsRNA from a plasmid [4], as outlined in Figure 1. Sensitivity to external dsRNAs is thought to be required for repression of viruses, although this remains to be directly proven in *C. elegans*, for which no natural viruses are known. It requires both a mechanism for uptake of the dsRNAs into intestinal cells, and the spread of the interference to other cells [5]. In *C. elegans*, this phenomenon has made possible systematic gene-inactivation screens using a library of bacteria expressing dsRNAs against most open reading frames. Similar gene inactivation by feeding with bacteria expressing dsRNAs is also possible in species of flatworms and cnidarians.

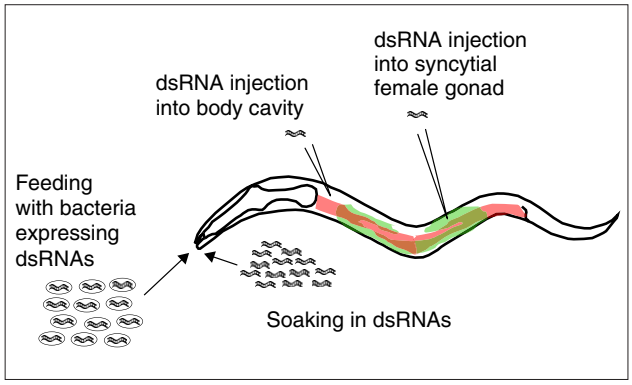


**Figure 1**
Different modes of administration of dsRNAs for RNA interference. External application of RNAs by soaking or feeding bacteria requires the dsRNAs to cross the intestinal barrier and the siRNA signal to spread systemically. In some species octopamine is required to induce feeding behavior or electroporation may be used. Injection into the body cavity only requires systemic spreading of the signal. Internal application by injection into the syncytial female gonad ensures transmission to the next generation, regardless of systemic spreading. Intestine is in red, gonad in green.

## Diversity in sensitivity to RNAi among nematodes

The efficiency of RNA interference is far from general, however, even in nematodes (Figure 2). Until recently, the use of bacteria expressing dsRNAs in nematodes was restricted to *C. elegans*. Even within the *Caenorhabditis* genus, the second most studied species, *Caenorhabditis briggsae*, was found to be insensitive to external application of dsRNAs. Interestingly, *C. briggsae* seems to be deficient in the uptake of dsRNAs in the intestine, a deficiency that can be complemented by expression of *C. elegans sid-2* (*systemic RNA interference defective 2*), a gene that was found in a genetic screen for mutants defective in systemic RNAi [5]. The *sid-2* gene encodes a putative transmembrane protein expressed in the intestine and probably involved directly in the uptake of dsRNAs from the intestinal lumen. Only one other tested *Caenorhabditis* species (*C.* sp. 1 SB341) - and none of the close relatives of *C. elegans* - was found to be sensitive to external RNAi. However, so far all species of the genus tested have been found to be sensitive to dsRNAs introduced by injection into the gonad [5].

Outside the *Caenorhabditis* genus, the prospects seemed even less bright. Despite extensive efforts, it has not been possible to implement RNAi (by injection, soaking or feeding) in the two laboratory 'satellite' model systems used for extensive genetic screens and comparative studies to *C. elegans* - *Oscheius tipulae* and *Pristionchus pacificus*. Morpholino oligonucleotides had to be used instead, a poor and expensive alternative (Figure 2).

The recent article by Shannon *et al.* [2] however encourages the hope that other culturable nematode species may turn out to be sensitive to RNAi. *Panagrolaimus* is a genus of free-living nematodes more distantly related to *Caenorhabditis* than are *Oscheius* and *Pristionchus*, and it encompasses many different ecologies and reproductive modes. Burnell and colleagues [2] show, using dsRNA-expressing bacteria, that *Panagrolaimus superbus* and, to a lesser degree, *Panagrolaimus* sp. PS1159 are sensitive to external RNAi. This study opens the way for functional genomic analysis in these *Panagrolaimus* species and suggests that other culturable 'free-living' nematodes may be sensitive to RNAi.

The situation is different for nematodes of medical or economic interest. So far, only a few key species have been tested, and we can now expect variation in RNAi efficiency even between closely related animals. Several plant-pathogenic nematodes are sensitive to external application (by soaking in dsRNAs, in the presence of octopamine to induce feeding behavior) and possibly also through expression of dsRNAs from a transgenic plant [6]. The insect-pathogenic nematode *Heterorhabditis bacteriophora* is also sensitive to dsRNAs administered by soaking [7]. RNAi has been unsuccessful or unreliable in nematodes that are vertebrate pathogens, however, including various evolutionary groups within nematodes (Figure 2) [8]. In the genomes of these nematodes, some of the genes encoding upstream components of long dsRNA processing seem to be lacking or unrecognizable.

In summary, the use of RNAi for functional genomics is so far restricted to a few nematode species. The work of Burnell and colleagues [2] suggests that the phylogenetic distribution of the dsRNA effect on nematodes is capricious and that researchers who find no effect in one species should keep trying in a variety of other species. The complex phylogenetic distribution also raises questions about the evolutionary pressures, perhaps from pathogens, acting on the mechanisms of response to various forms of internal or external dsRNAs. It also makes retrospectively remarkable the choice of *C. elegans* as the laboratory model system, 35 years before RNAi was discovered.

## The lucky choice of *C. elegans*

In the 1960s, Sydney Brenner, after his earlier work using phage genetics, chose *C. elegans* to study development and neurobiology of a multicellular organism. Brenner said: "Thus we want a multicellular organism which has a short life cycle, can be easily cultivated, and is small enough to be handled in large numbers, like a micro-organism. It should have relatively few cells, so that exhaustive studies of lineage and patterns can be made, and should be amenable to

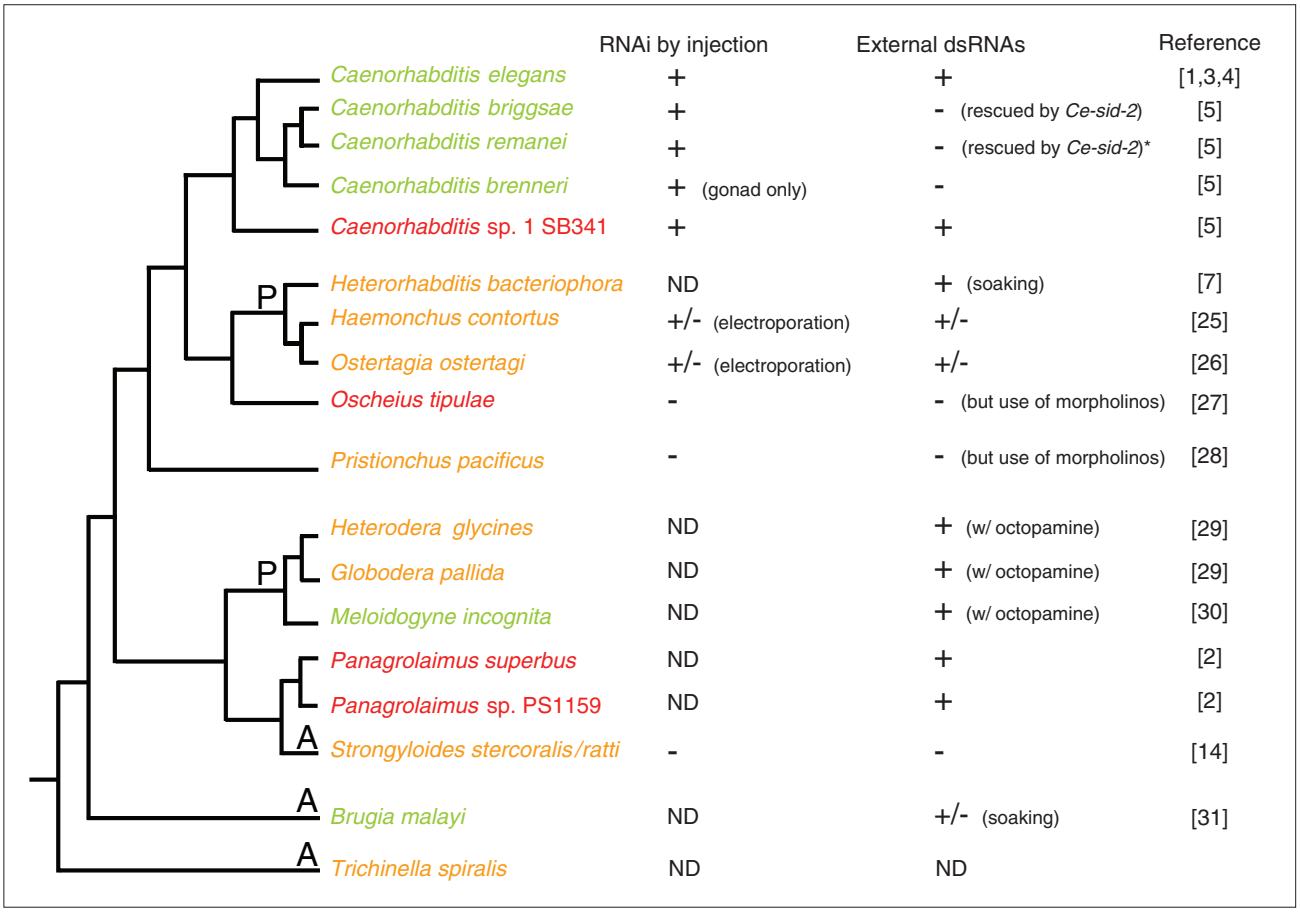


**Figure 2**
Phylogenetic relationships and RNAi susceptibility of nematode species. Phylogenetic relationships are redrawn from [32,33]. A indicates transition to animal parasitism. P indicates transition to plant parasitism. Susceptibility of each species to internal and external modes of RNAi administration is indicated on the right. A + sign indicates that RNAi has been successful, a - sign that it has not. A +/- sign indicates poor efficiency. ND, not determined. Colors of species name indicate the status of genome sequencing: green, published; orange, ongoing or planned; red, not planned. *I. Nuez and MAF, unpublished data.

genetic analysis." (Proposal to the Medical Research Council, October 1963). He later added: "Since a nervous system is essentially a cellular network, we had to be able to observe junctions between cells and their processes and this could only be achieved with the electron microscope, which has the necessary resolution." (Nobel Lecture, 2002).

After trying several other exotic organisms and isolating "nematodes from nature to find the best one" [9], Brenner chose *C. elegans* for several reasons: its easy culture in large numbers on two-dimensional surfaces of agar plates with *E. coli* and in defined liquid media; its fast generation time (3.5 days); its mode of reproduction with self-fertile hermaphrodites and facultative males for crosses; its transparency in light microscopy and good contrast in electron microscopy; the constancy of its neuronal composition (which was known at least for other nematodes such as *Ascaris*, though at the time not for *C. elegans*) [9].

Several common free-living nematode species could have met most of these criteria, for example in the *Oscheius*, *Rhabditis* or *Pristionchus* genera. Within the *Caenorhabditis* genus, another obvious candidate was *C. briggsae*. Brenner indeed first intended to work on *C. briggsae* (Proposal to the Medical Research Council, October 1963) which was the species that Ellsworth Dougherty, his nematode contact in Berkeley, was beginning to culture axenically. However, the Dougherty *C. briggsae* strain turned out to grow less well than a *C. elegans* strain isolated in Bristol (N2). So Brenner finally chose the latter (J. Hodgkin, personal communication). Brenner was surely a visionary when he turned to studies of development and behavior of *C. elegans*, but there

are several future developments he could not possibly have foreseen when he chose *C. elegans* over *C. briggsae* and other nematode genera. The first of these is that *C. elegans* is so far the only hermaphroditic free-living species in which external application of dsRNAs inactivates gene expression. *Panagrolaimus superbus* is a male-female species and *P.* sp. PS1159 is parthenogenetic. Furthermore, the N2 strain that Brenner chose as a reference is much more sensitive to RNAi in the germ line than other *C. elegans* isolates (such as the CB4856 strain commonly used for single nucleotide polymorphism (SNP)-based mutant mapping; [10]). Second, easy transgenesis of *C. elegans* is possible because of its syncytial germ line: any form of injected DNA recombines and forms an additional chromosome that is frequently passed onto later generations [11]. Transgenesis by injection has so far proved impossible in *Oscheius tipulae*, *Pristionchus pacificus* and *Panagrolaimus* species and is much more difficult in *C. briggsae* (the efficiency of establishing lines is lower and there is more silencing and mosaicism [12]). Transgenesis in parasitic nematodes has so far been restricted to transient expression [13,14]. Third, *C. elegans* can be frozen, as was successfully achieved by John Sulston in 1969 [15]. Freezing *Pristionchus pacificus* [16], some *Rhabditis* species or other *Caenorhabditis* species such as *C.* sp. 3 has proved much more difficult. And finally, the efficiency of chemical mutagenesis by ethane methyl sulfonate is a balance between toxicity and mutagenic effect; mutagenesis is less efficient in *Oscheius tipulae*, for example, than in *C. elegans* [17].

In the history of science, a model organism may remain successful because of unexpected turns of chance, and *C. elegans* might not have become so popular had some of the features mentioned above been lacking. A further retrospective bias is introduced by the fact that *C. elegans* has been studied more than other species. Methods have been optimized for *C. elegans* N2: for example, culture conditions have to be changed for optimal culture of other *C. elegans* or *C. briggsae* strains (higher agar concentration because of worm burrowing [18]); immunostaining has been optimized for *C. elegans*; and so on. On the other hand, other discoveries might have been made if another species had been chosen. The recent finding of *Caenorhabditis* sp. 9, a male-female species that can hybridize with the hermaphroditic *C. briggsae* (MAF, unpublished data) may make *C. briggsae* a popular species for some evolutionary studies. In contrast, a close relative of *C. elegans* that can hybridize with it is still missing.

In addition to these methodological advantages, it is starting to become clear that the choice of strain and species has also influenced the biological results. Taking the example of vulva development, the effect of gonad ablation is clear cut in *C. elegans*: vulval tissue does not form at all, because of the lack of induction by the gonadal anchor cell. Yet in *Panagrolaimus* sp. PS1159, the outcome of the same ablation is much less clear: some vulval tissue forms in a variable manner depending on the individual [19]. At a smaller evolutionary scale, Wnt pathway mutations were not found in the original screens for vulva mutants on the N2 strain, but have a stronger effect in the genetic background of other *C. elegans* wild isolates (J. Milloz, I. Nuez and MAF, unpublished data). Brenner's choice is therefore also important for the textbook picture that emerges from studying a model organism. Even given the overall universality of biological mechanisms and molecular pathways, the rapid evolution of partial redundancy between processes and molecular pathways means that key results of experimental manipulations (such as cell ablation and gene inactivation) are highly dependent on the species and strain chosen as the model organism.

## Nematode genomes

*C. elegans* was the first multicellular organism to have its genome fully sequenced [20]. With the increase in sequencing capabilities, genome sequencing of various nematode species is under way [21,22], regardless of the possibilities for functional studies. The successful use of RNAi in *Panagrolaimus superbus* makes it a 'superb' candidate for genome sequencing.

In the *Caenorhabditis* genus, the genome sequences of five species are now available, with the best assembly being that for *C. briggsae* [23]. Molecular divergence is high, which makes these genomes useful for the annotation of conserved noncoding regions of the *C. elegans* genome. Most other nematode genome and expressed sequence tag sequences (completed or planned) are for animal- or plant-parasitic nematodes, because of their medical or economic importance. Annotation of sequences from these parasites can make use of the good annotation of the *C. elegans* genome. The draft assembly of the genome of *Brugia malayi* (the cause of filariasis) suggests conservation of large-scale, but not small-scale, synteny and of many operons [24]. Functional characterization of gene function may be possible for plant parasites using RNAi interference, but direct characterization of gene function in the vertebrate parasites will be difficult because of the present lack of gene-inactivation techniques. Alternative methods for delivery of dsRNAs or siRNAs will be needed.

Besides their use for the development of nematicides, these nematode genomes will be of great interest for genome evolution studies. The nematodes form an abundant and highly diverse group of animals. Perhaps because of the

short generation time of many of them, genome evolution is rapid in nematodes. Large sequencing projects will provide tools to study genome evolution at different evolutionary scales: from intraspecific evolution to large-scale divergence within nematodes.

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