The Mind of a Worm

James H. Thomas

In 1967, Sydney Brenner isolated the first behavioral mutants of the nematode *Caenorhabditis elegans*, and in 1970, John White began the systematic reconstruction of its nervous system. This dual approach of genetics coupled with detailed morphological analysis, now enhanced by the tools of molecular biology and electrophysiology, still dominates the study of the function and development of the C. *elegans* nervous system. Although Brenner's vision of a comprehensive understanding of this simple animal has taken time to mature, findings of the past few years indicate that the tree is bearing fruit.

Caenorhabditis elegans offers an interesting combination of advantages and limitations for the study of behavior, a fact that often excites considerable feeling in discussions of its utility as a model for neurobiology. The miniature scale of the hermaphrodite's nervous system, both in number of neurons (302) and in overall size (1 mm), has allowed its complete reconstruction from serial-section electron micrographs (*1*, *2*). The neurons fall into 118 structural classes, and the number and positions of morphologically identifiable chemical synapses (5000), gap junctions (600), and neuromuscular junctions (2000) are very similar among individuals. The gross behavioral function of any set of neurons can be tested by killing them with a laser microbeam, a technique that almost miraculously erases the target neurons while leaving other cells unaffected (*3*, *4*). With this technique, a specific behavioral role has been identified for about 40 of the 118 classes of neurons, and the structural classes of neurons have been shown to correspond to functional classes (*4*–*6*). Finally, over 250 genes that regulate behavior have been identified by mutation. These genes are at various stages of being analyzed with the genetic and molecular tools available in C. *elegans* (*7*, *8*).

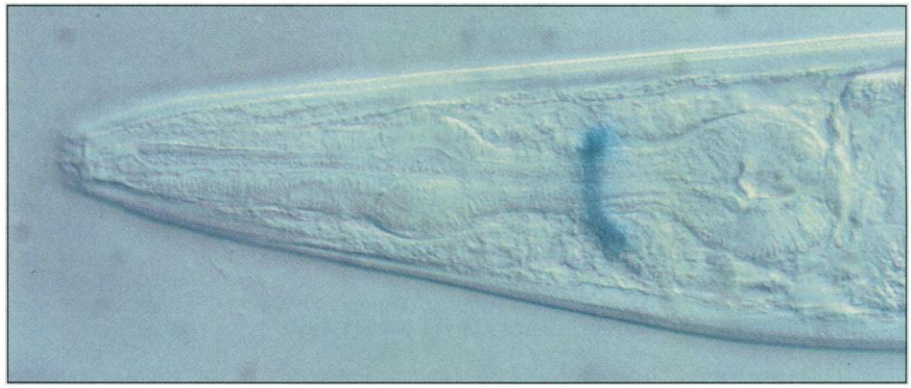
The main limitation in this endeavor is that electrophysiology is not easy in C. *elegans*, because the neurons are small, although extracellular recordings from muscle (*9*) and patch clamping and intracellular recordings from neurons (*10*) are now proving feasible. It is also sometimes argued that such a simple animal may lack the behavioral complexity and plasticity that typify larger animals. Although this is undeniable (indeed, this simplicity is often touted as an advantage), nematodes display a rich and varied array of behavioral patterns, two examples of which are described below. What's more, C. *elegans* is capable of several forms of nonassociative learning that can persist for more than a day (*11*), and there are hints that it can exhibit associative learning as well.

The C. *elegans* nervous system is not only small, it has particularly simple neurons, most having one or two unbranched processes that make and receive synaptic connections en passant rather than at specialized terminals (*1*). Despite these differences from vertebrates, many of the fundamental mechanisms of neuronal function are shared with other animals. The major small molecule neurotransmitters in C. *elegans* [for example, acetylcholine, γ-aminobutyric acid (GABA), serotonin, dopamine, and glutamate] and often even the pharmacology of their receptors are familiar to any neurobiologist. Familiar proteins also regulate synaptic release in these worms (for example, synaptotagmin) (*12*), transport small molecule transmitters into synaptic vesicles (*13*), and mediate axonal transport (for example, kinesin-like proteins) (*14*, *15*).

One behavior that illustrates the sophistication of nematode behavior is the sensory response to volatile odorants. *Caenorhabditis elegans* responds to odorants by directed movement up or down an airborne gradient of the stimulant (*16*). These responses themselves are not surprising, but the range of odorants detected is astonishing: Worms are attracted to about half of the 121 volatile compounds that have been tested. *Caenorhabditis elegans* can distinguish gradients of many odorants, even in the presence of saturating amounts of others. Such competition assays reveal that the worms distinguish at least seven classes of odorants (*16*)—implying that these animals have a large number of odorant receptors linked to at least seven independently adapting transducers. Remarkably, all of these responses, including those to odorants that escape detection by my own 10 million olfactory neurons, appear to be mediated by just two classes of sensory neurons, called AWA and AWC (*16*). These results indicate a richness of sensory perception that is belied by a simple count of the worm's 15 classes of chemosensory neurons.

More than 20 genes have been identified by mutations that perturb response to one or more odorants (*16*, *17*). Some mutations disrupt responses to all tested odorants (*16*); many of these cause specific morphological defects in the odorant sensory neurons (*18*). Other mutations show some odorant specificity. For example, mutations in five genes—*odr-1*, *odr-2*, *odr-5*, *daf-11*, and *daf-21*—perturb response to benzaldehyde and isoamyl alcohol, representatives of two odorant classes, but have little effect on response to other odorant classes (*16*, *17*). This specificity for the odorant classes is the same as that caused by the killing of the AWC neurons (*16*), suggesting that these genes are required for AWC function. Other mutations cause other, often complex, patterns of odorant response defects (*16*). Molecular analysis of these genes will reveal how C. *elegans* senses, distinguishes, and responds to such a diversity of odorants.

For another example of a sophisticated behavior, one would hardly turn first to defecation, and yet, even this lowly activity is surprisingly complex in C. *elegans*. Defecation consists of three separate motor steps that are coordinated spatially and temporally. These steps, named for the muscles involved, are referred to as pBoc (posterior body muscle contraction), aBoc



The central nervous system of *C. elegans*. The head of *C. elegans* with the nerve ring—the central nervous system—highlighted by a promoter fusion expressing lacZ (blue). [Courtesy of I. Hope]

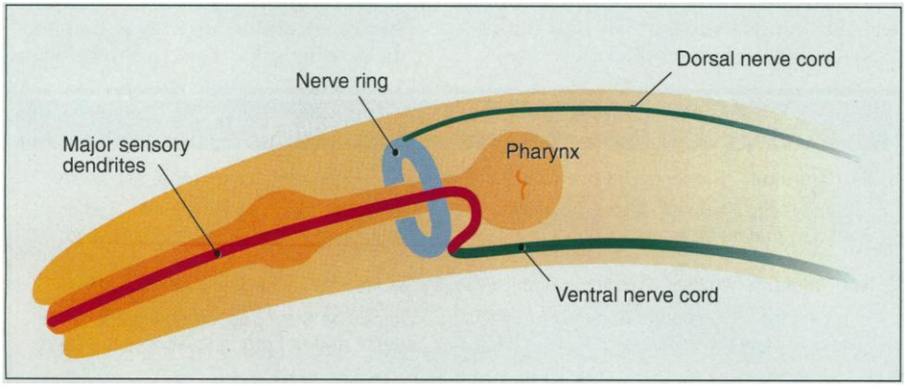
The author is in the Department of Genetics, University of Washington, Seattle, WA 98195, USA.

(anterior body muscle contraction), and Exp (expulsion, or enteric, muscle contraction). Each step of this defecation motor program (DMP) is readily observed and occurs frequently, facilitating the identification and analysis of mutations that perturb the process. Over a dozen genes are defined by mutations that individually eliminate either the pBoc, aBoc, or Exp steps of the DMP without perturbing the remaining steps (*19*). For example, *unc-16* mutants lack the aBoc contraction, yet the presence, relative timing, and strength of the pBoc and Exp contractions are unaffected (*19*). These highly specific mutant defects imply that each motor step is partly under the control of distinct genes. In contrast, mutations in seven other genes affect both the aBoc and Exp steps (*19*), suggesting that these two steps of the DMP share an underlying genetic program. The pattern of defects displayed by these mutants provides a particularly clear example of the dissection of a behavior into its genetic parts. The first steps have also been made toward cellular and molecular analysis of the DMP. Two partially redundant GABA-containing neurons, AVL and DVB, appear to be motor neurons for the Exp step of the DMP (*6*). Mutations in *unc-25* eliminate the synthesis of GABA and cause an Exp defect, suggesting that AVL and DVB use GABA to excite their enteric muscle targets (*19*, *20*). Another identified gene, *exp-1*, may mediate this unusual role of GABA as an excitatory transmitter (*19*). Interestingly, GABA is also used in *C. elegans* in its familiar role as an inhibitory transmitter, probably acting on GABA-A receptors (*20*).

A particularly interesting feature of the defecation behavior is its temporal regulation. Under standard growth conditions, a DMP is activated every 45 s with a standard deviation of about 4 s. Ethological studies (*21*) suggest that the periodicity is controlled by an endogenous clock: (i) The periodicity is not well correlated with any obvious trait, such as feeding rate or gut distention; (ii) the clock continues running in the extended absence of motor program expression; (iii) the clock can be reset by a well-defined mechanosensory stimulus; and (iv) the clock is temperature-compensated, running at nearly the same speed between 20° and 30°C.

Defecation periodicity is subject to diverse sensory regulation, ranging from mechanosensory resetting of the clock to graded lengthening of the period when less food is available. However, when each motor step of the DMP is eliminated individually by mutation, the remaining motor steps can occur with nearly normal periodicity, suggesting that the clock runs independently of its motor output (*19*, *21*).

Several mutations have been identified that affect the periodicity of activation of the DMP without any apparent effects on the DMP itself. For example, partial loss-of-function mutations in the gene *cha-1*, which is required for the synthesis of acetylcholine, cause a profoundly lengthened defecation cycle (*19*). However, this result is difficult to interpret as specific involvement of acetylcholine in defecation because acetylcholine has many functions in the *C. elegans* nervous system (*13*). In contrast, mutations in several other genes cause defects more specific to defecation, including long or short cycles (*19*, *21*). An intriguing example is *dec-8*, a mutant that activates a second "echo" DMP 13 s after the principal DMP (*21*). These mutants show that defecation periodicity is at least partly under the control of genes distinct from those that mediate the individual motor steps. Molecular analysis of these genes promises to identify mechanisms that generate and regulate short period neuronal oscillators.



Some of the major neuronal bundles in the head. The nerve ring (blue) encircles the pharnyx. Projecting from the nerve ring region are sensory dendrites (the major bundle is shown in red) and the dorsal and ventral nerve cords (green), which extend the length of the body. Anterior, left; ventral, down.

Here it is possible only to scratch the surface of *C. elegans* behaviors that are under intensive genetic study. Others include locomotion (*22*), mechanosensory responses (*23*, *24*), egg laying (*25*), feeding (*26*), and sensory response to a pheromone (*27*, *28*). For all of these behaviors, researchers are in the midst of the genetic analysis and have just begun the molecular studies that follow from the genetic raw material. The long-term promise of studying *C. elegans* for neurobiology lies principally in two areas. First, the genetic approach has the power to identify and define in vivo functions of a wide variety of both known and currently unimagined molecular components common to neuronal development and function in all organisms. Second, the simple nervous system may permit a comprehensive understanding of how all the parts of a nervous system, from gene products to neurons, are integrated to produce the complex patterns characteristic of animal behavior.

References and Notes

1. J. G. White, E. Southgate, J. N. Thomson, S. Brenner, *Philos. Trans. R. Soc. London Ser. B* **314**, 1 (1986).
2. D. G. Albertson and J. N. Thomson, *ibid.* **275**, 299 (1976).
3. M. Chalfie and J. Sulston, *Dev. Biol.* **82**, 358 (1981).
4. L. Avery and H. R. Horvitz, *Neuron* **3**, 473 (1989).
5. M. Chalfie *et al.*, *J. Neurosci.* **5**, 956 (1985).
6. S. L. McIntire, E. Jorgensen, J. Kaplan, H. R. Horvitz, *Nature* **364**, 337 (1993).
7. S. Brenner, *Genetics* **77**, 71 (1974).
8. R. Wilson *et al.*, *Nature* **368**, 32 (1994).
9. D. M. Raizen and L. Avery, *Neuron* **12**, 483 (1994).
10. L. Avery, D. M. Raizen, S. Lockery, "*C. elegans*, Modern Biological Analysis of an Organism," H. Epstein and D. Shakes, Eds., *Methods Cell Biol.*, in press.
11. C. H. Rankin, C. D. Beck, C. M. Chiba, *Behav. Brain Res.* **37**, 89 (1990).
12. M. L. Nonet, K. Grundahl, B. J. Meyer, J. B. Rand, *Cell* **73**, 1291 (1993).
13. A. Alfonso, K. Grundahl, J. S. Duerr, H. P. Han, J. B. Rand, *Science* **261**, 617 (1993).
14. A. J. Otsuka *et al.*, *Neuron* **6**, 113 (1991).
15. N. Patel, D. Thierry-Mieg, J. R. Mancillas, *Proc. Natl. Acad. Sci. U.S.A.* **90**, 9181 (1993).
16. C. I. Bargmann, E. Hartwieg, H. R. Horvitz, *Cell* **74**, 515 (1993).
17. J. J. Vowels and J. H. Thomas, *Genetics*, in press.
18. L. A. Perkins, E. M. Hedgecock, J. N. Thomson, J. G. Culotti, *Dev. Biol.* **117**, 456 (1986).
19. J. H. Thomas, *Genetics* **124**, 855 (1990).
20. S. L. McIntire, E. Jorgensen, H. R. Horvitz, *Nature* **364**, 334 (1993).
21. D. W. C. Liu and J. H. Thomas, *J. Neurosci.* **14**, 1953 (1994).
22. T. A. Starich, R. K. Herman, J. E. Shaw, *Genetics* **133**, 527 (1993).
23. M. Chalfie and M. Au, *Science* **243**, 1027 (1989).
24. J. M. Kaplan and H. R. Horvitz, *Proc. Natl. Acad. Sci. U.S.A.* **90**, 2227 (1993).
25. C. Desai and H. R. Horvitz, *Genetics* **121**, 703 (1989).
26. L. Avery, *ibid.* **133**, 897 (1993).
27. J. H. Thomas, D. A. Birnby, J. J. Vowels, *ibid.* **134**, 1105 (1993).
28. M. Estevez *et al.*, *Nature* **365**, 644 (1993).

29. I thank L. Avery, B. Hille, J. Hurley, C. Rankin, and members of my lab and the Avery lab for comments on this manuscript.