The Continuing Tale of a Small Worm

This last of three articles on the tiny nematode Caenorhabditis elegans *describes the first analysis of anatomy and wiring in a complete nervous system*

"We have a complete description of the whole nervous system in terms of all the neurons and the connections they make." This simple statement, by John White of the Medical Research Council's Laboratory of Molecular Biology, Cambridge, England, sums up a brilliantly conceived and doggedly implemented research effort of more than a decade's duration on the 1-mm-long nematode *Caenorhabditis elegans*. White is currently contemplating the eventual publication of what amounts to a detailed atlas of this modest worm's nervous system, an opus that threatens to exceed the capacity of even the most accommodating journal. A 500-page monograph is the likely compromise.

The assault on the complete neural anatomy of *C. elegans* is part of a massive project initiated by Sydney Brenner 20 years ago to achieve a complete understanding of the animal in terms of neural function and developmental biology (see *Science*, 22 June, p. 1327, and 6 July, p. 40). So, in addition to the detailed map of all 302 neurons in the adult worm, there is also an accurate tracking of lineage development from egg to the 959 somatic cells of the mature adult, a task that has been the prime responsibility of John Sulston. And genetic and molecular biological analysis has begun to probe the relationship between what is written in the genes and the means by which the organism assembles itself. This simple organism has become, in Brenner's words, the subject of serious science.

Learning about "the brain"—how it works and how it is wired—is frequently described as one of the great frontiers of biology, and of philosophy too for that matter. The 302 neurons of *C. elegans*, as against the 100 billion or so in *Homo sapiens*, offers a simplicity that should be experimentally tractable. But one should not be mislead by the simplicity, because it is more apparent than real: the 302 neurons of *C. elegans* can be divided into 118 types on various anatomical and histochemical criteria; and these neurons make some 8000 synapses throughout the animal, all of which presents no mean task for analysis. In any case, as stated by Robert Horvitz, of the Massachusetts Institute of Technology, "the diversity of neuron types makes the nervous system an excellent place to look, if you are interested in what makes one cell different from another."

The map of the adult nervous system has been cumulatively sketched out through the assembly of nearly 1000 serial section electron micrographs, a task in which Eileen Southgate and Nichol Thomson played major roles. The clear message from comparison of the maps of genetically identical individuals is that they are essentially the same, although there are minor differences in cell morphology, position, and connectivity. This is what the late British biologist Conrad Waddington would have called developmental noise. "The logic of the connectivity is the same between two individuals, but the details differ," comments Horvitz, who, like a number of U.S. researchers has spent several years in Brenner's laboratory. "The differences give you a framework for thinking about the level of noise."

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Combined with the simplicity of the *C. elegans* nervous system, the relative invariance is what makes it a good experimental model for studying the construction and functioning of a "brain." The basic questions are simple: How does the nervous system function to control behavior? How are the wiring patterns established? How are different neuron types generated?

The immense benefit of knowing the detailed anatomy and lineage of the nervous system in this little worm is already fully apparent in attempting to answer these questions. For example, it has been possible by various techniques—including ablation by laser microbeam and by genetic mutation—to assign specific neurons or entire reflex circuits to particular behaviors. And the conservation of neural anatomy between the tiny *C. elegans* and the much bigger nematode *Ascaris lumbricoides* has allowed Anthony Stretton and his colleagues at the University of Wisconsin, Madison, to carry out biochemical and physiological studies on *Ascaris* neurons that correspond to neurons of known connectivity in *C. elegans*.

Compared with neurons in higher animals, those in *C. elegans* are relatively simple in structure: most have a single, unbranched axon, or process. The cells are distributed among ganglia in the head, tail, and the ventral midline of the body. The neuronal processes run in parallel bundles along the ventral cord, the dorsal cord, and a nerve ring around the pharynx. Synaptic contacts are made *en passant* between processes in these bundles.

One way in which White approached the question of connectivity—why processes make the connections they do—was through neighborhood analysis. "We wanted to know how many neighbors a process has at any particular point and how many it chooses to synapse with," he says. "The surprising thing to me was the high frequency of contacts made: each process synapses with almost 50 percent of its neighbors."

The observation that any particular neuronal process inhabited very restricted neighborhoods yet made a high frequency of connections in those neighborhoods led White to suspect that connectivity may have more to do with where processes are located than with highly specific recognition of one cell by another. In other words, if a neuron were transplanted to a new location its processes would establish new and different connections. There is, nevertheless, clearly some degree of selectivity at play here, as, in any particular case, a process almost always makes contacts with the same subset of neighbors.

Although direct neuron transplantation is not yet feasible, there are several observations that support White's hypothesis that synaptic selectivity may involve surprisingly little specificity. First, slight differences in connectivity

between genetically identical individuals are correlated with differences in process location.

Additional evidence comes from a pair of apparently identical neurons, denoted AQR and PQR, one of which is located in the head and the other in the tail. Not surprisingly, the neighborhoods in these two locations are distinct, as too are the patterns of connections made by the processes of the two neurons. Similarly, in a mutant studied by Martin Chalfie of Columbia University, New York, a neuron, PVM, which is normally located in the tail finishes up instead in the head. This misplaced cell makes an entirely new set of synapses in its new location. "The implication," says White, "is that neurons have the potential to form more classes of synapse than they actually do."

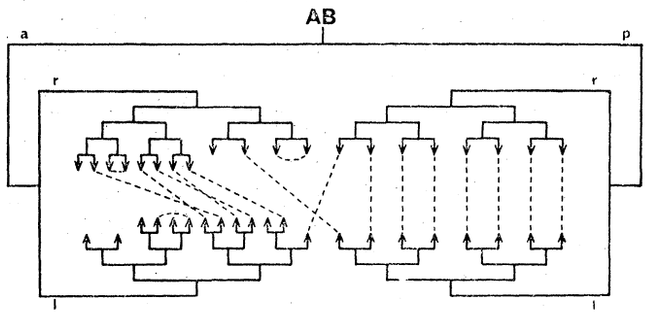
These observations tend to push the business of connectivity to questions of what makes the processes grow in the correct direction and along the correct paths. Both Chalfie and Edward Hedgecock, at the Roche Institute of Molecular Biology, Nutley, New Jersey, are tackling this difficult problem, with some success. They have found, for instance, that the length to which the processes of certain neurons grow depends not on their reaching a target but on some kind of built-in measure. A neuron of this class that is misplaced in the direction of a normal synaptic partner is likely to send its process too far, for example. What determines both the extent and direction of process growth, however, remains to be elucidated.

White is much impressed by the fact that an essentially invariant wiring pattern emerges from the interaction of a small number of disparate mechanisms: first, cell lineage specifies neuron cell type; second, particular cell types are produced in particular positions; third, particular cells send particular processes into particular neighborhoods; and fourth, within each neighborhood there is some selectivity as to which cells form synapses.

Basic information about development has come principally from elucidation of the cell lineages. Analysis of a series of mutants that affect the nervous system complements and extends this basic picture. This dual approach—of anatomy and genetics—has always been part of Brenner's strategy with *C. elegans*.

Several striking features about nervous system lineages have emerged. First, neurons are produced neither clonally nor from an orderly series of repeated cell divisions. Rather they are generated by patterns that are "unpredictably complex," in the words of Sulston. Second, cell determination is autonomous: in most cases a cell is what it is principally because of its history rather than as a result of interaction with other cells. Third, about 20 percent of neuronal cells die almost as soon as they are formed: these deaths appear to be intrinsically programmed suicides.

Bizarre symmetry



Bilateral symmetry in the AB *group of cells is generated in an orderly manner posteriorly* (p) *but not anteriorly* (a). *Analogous pairs of precursors, which generate approximately the same groups of cells on the left* (l) *and right* (r) *sides, are linked by dotted lines.*

A common feature of neurogenesis, says Sulston, is the repeated sublineage, in which "a pattern of cell division that is found at several points in the lineage gives rise to more or less the same array of cell types at each appearance." This type of pattern is much more apparent in postembryonic than in embryonic development, a difference, suggests Sulston, that might reflect the greater evolutionary antiquity of the embryonic phase. Repetitious elements that would once have been apparent in embryogenesis might have "been largely obscured by later modifications."

Sulston suspects that cell lineage has evolved by duplication, "much as have other elements of biological organization, from genes to limbs." When duplications occurred at termini of lineages, clones of identical cells would have been produced. Duplications earlier in a lineage would have led to repeated sublineages, such as are seen in the nervous system. "The resulting repetitious elements then seem to have further evolved by diversification." Sulston cites as support for this idea the high incidence of cell death, which appears to eliminate specific unwanted cells generated from duplicated sublineages, and certain mutants that seem to reveal normally repressed repetitions.

The overriding impression derived from the lineage work is that the principal function of the complex patterns of cell division is to produce the right cell in the right place at the right time. An alternative strategy might be for all cells of a given type to be produced by a given lineage and for cells then to migrate to their required positions. In fact, although cell migration occasionally occurs, long-range movement is not common. Instead, cells of a particular type can be produced by entirely different lineages in seemingly illogical ways. Whether the mixed-lineage/no-migration strategy is indeed evolutionarily easier to achieve or is simply the one that developed here is a moot point. Maybe, as Brenner is fond of saying, it's just the way the cookie crumbled.

But perhaps the most bizarre discovery from the lineage analysis has been the unexpected way in which some bilaterally symmetrical structures are assembled. "One would have expected that left and right sides would have been made by mirror image assembly," says Horvitz, "but that is often not true." "Bilateral symmetry is often put together very laboriously," observes Sulston. "Cells appear to be recruited because of their positions, not because of equivalent lineages."

This observation on symmetry highlights what is generally true for *C. elegans* development: someone starting from scratch and with a logical mind would not have designed a worm this way. "Yes, there are rules," says Sulston, "but they are not the sort that can be perceived from afar. They tell us something about the way the animal evolved." Sulston, White, Thomson, and E. Schierenberg recently wrote of the unruly rules: "On this view, the perverse assignments, the cell deaths, the long-range migrations—all the features which could, it seems, be eliminated from a more efficient design—are so many developmental fossils. These are places to look for clues both to the course of evolution and to the mechanisms by which the lineage is controlled today."

Just as the oddities of the normal system can help reveal the underlying mechanisms of control, so too can a more direct intervention through the generation and study of mutants. By now about 500 of the animal's genes have been defined and mapped. Thousands of mutants have been isolated over the years, many of which affect specific behaviors via the nervous system: for instance, in

chemotaxis, movement, thermotaxis, osmotic avoidance, egg-laying, touch sensitivity, and in the synthesis and degradation of neurotransmitters. Obtaining such mutants is a first step in trying to understand the molecular basis of the functioning and development of the nervous system.

The work on touch-insensitive mutants, which has been carried out by Chalfie and Sulston, illustrates very well the approach to genetic dissection of neural development and nerve cell differentiation. There are six touch receptor cells, which are located in the head, mid- and tail regions of the body and mediate the worm's response to gentle tactile stimulation. All six have long processes that run just below the cuticle and act as mechanoreceptors.

Chalfie and Sulston have been screening for specifically touch-insensitive mutants and have by now listed 250 mutations in 15 genes that affect this behavior. Mutations in eight of these genes result in visible defects in the touch neurons, whereas mutations in the other seven produce touch insensitivity in the absence of visible defects in the nerve cells.

Four of the eight genes affect ultrastructural features of the cells, including the organization and number of microtubules and the presence of a characteristic extracellular material known as mantle. A mutant defective in another gene has

The Other T-Cell Receptor Gene

During the past year or so immunologists have achieved a long-sought goal—the identification of the receptor molecules by which T cells recognize antigens. Knowledge of the structure of this receptor is essential for an understanding of how T cells perform their immune functions, which include both killing cells they recognize as foreign and controlling the activities of other immune cells, including the antibody-producing B cells.

The T-cell receptor is now known to consist of two different protein chains, both of which are structurally similar to antibody proteins. In addition, several months ago two groups reported that they had cloned receptor genes. One was cloned from mouse cells and the other from human cells, but both turned out to code for the same chain, which has been designated β (*Science*, 25 May, p. 859). The α chain remained elusive, until now that is. Susumu Tonegawa, Herman Eisen, and their colleagues at the Massachusetts Institute of Technology have reported the cloning of what appears to be an α-chain gene from a line of mouse T cells.* They have also cloned a β-chain gene from the same cell line.

The MIT workers used the subtraction method of cloning, which Mark Davis of Stanford University School of Medicine and Stephen Hedrick of the University of California at San Diego developed and used to clone the mouse β-chain gene. That gene may have turned up first, Tonegawa suggests, because there are five to ten times more copies of its messenger RNA (mRNA) than of α-chain mRNA's in T cells. The MIT workers may have found the α-chain clone because they screened a very large number of clones, 100,000 compared to the 5,000 screened by Davis and Hedrick.

The evidence that it is an α-chain gene is still indirect, Tonegawa notes. The gene is expressed specifically in T cells, as predicted for a T-cell receptor protein, and has undergone a rearrangement. Complete T-cell receptor genes, like those of antibody genes, are assembled during maturation of the cells by joining shorter DNA segments. In addition, determination of the nucleotide sequences of the proposed α and β clones has shown them to be different. "The predicted protein sequence of the α chain has homology to immunoglobulins [antibodies] as the β chain does, and to about the same extent," Tonegawa says, "but it is very different from the β chain." About 30 percent of the amino acids of the α and β chains are identical.

The overall organizations of the two chains are very similar to each other, however, and to those of antibody proteins. Both have molecular weights of about 33,000. They each contain a short region that extends into the cytoplasm of the cell, a membrane-spanning segment, and a large extracellular segment. Both have cysteine residues just outside the transmembrane region that might form the disulfide bond that holds the chains together. As in antibody chains, the extracellular segment consists of a constant region, which is the same for all chains of the same type, and a variable region, which differs from molecule to molecule and is involved in antigen recognition. The constant region of the MIT β-chain clone, which is from a cytotoxic cell line, and that of the Hedrick and Davis clone, which is of helper T-cell origin, are essentially identical, Tonegawa points out, but the two genes use different variable region coding segments.

At least five gene segments coding for β-chain variable regions have been identified and there may be many more. The repertoire of α-chain variable regions may be more limited. "There appears to be only a few for an entire population of cytotoxic T cells," Tonegawa explains. Some variability can be generated, however, in the joint between the variable region coding segment and that for the J (for joining) segment, which in antibody light chains lies between the variable and constant gene segments. Antibody heavy chains, and the β chain of the T-cell receptor, also contain a fourth coding segment, designated D for diversity. A D segment has not yet been identified for the α chain.

The T-cell receptor only recognizes a foreign antigen in conjunction with a histocompatibility molecule, which is a marker for self. Partly because of the apparently limited variability of the α chain and partly because there are indications that the variable regions of the two receptor proteins may interact less closely than the variable regions of light and heavy antibody chains, Tonegawa suggests that the α chain primarily recognizes the histocompatibility molecule, whereas the β chain primarily interacts with the foreign antigen, although he stresses that more work will be needed to confirm this suggestion. Analysis of the T-cell receptor, which was already moving rapidly should proceed even faster if both genes are now in hand.

—JEAN L. MARX

*H. Saito, D. M. Kranz, Y. Takagaki, A. C. Hayday, H. N. Eisen, S. Tonegawa, *Nature (London)* **309**, 757 (1984).

touch cells that lack processes. Mutations in a sixth gene cause touch cells to degenerate soon after they are formed. And mutations in the other two genes affect the lineages that generate the touch cells.

Chalfie believes that he and Sulston have detected most, if not all, of the genes that, when mutated, can have major deleterious effects on this set of neurons. Interestingly, no one has found a mutation that specifically affects the direction of growth of touch cell processes without having a similar effect on other neurons. Nor is there yet a mutation that specifically affects a subset of the six touch cells in any way.

Overall, then, the differentiation and positioning of the touch-sensitive neurons is brought about by a combination of the activities of a small group of genes specific to these cells and an unknown number of genes that affect these and other cells.

Using this type of genetic approach, genes and gene products known to control the development and functioning of the neurons involved in touch sensitivity, as well as in other behaviors, can be identified and perhaps isolated. "The *C. elegans* genome is 20 times bigger than that of *Escherichia coli* and half that of *Drosophila*," says Sulston. "There is currently a big push to clone the genes, to map the whole genome, and then we will be able to focus on specific bits." Although some of the genes involved in neurogenesis are likely to specify cell surface components, for instance, which may play a direct and clear role in assembly of the nervous system, others may well prove to have functions that are less obviously related to development. "We might find an enzyme that is involved in some aspect of biosynthesis, for instance, but as a practical matter it would represent a gene that is involved in the logic of development."

This comment illustrates the recognition by Brenner and his associates that development is unlikely to be the result of a discrete, sequential developmental "program," but instead is the outcome of a more holistic logic of molecular assembly (see *Science*, 22 June, p. 1327). In which case, one will need to know everything about the molecular genetics and biochemistry in order to understand how the animal is put together. "And what you will see," says Sulston, "is just one of many possible ways in which *C. elegans* can be made. Development has evolutionary history built into it, and the result is not always the most obvious or logical way of doing things."

Brenner and his colleagues are occasionally asked whether knowing everything about *C. elegans* reveals anything about the rest of the biological world, much of which appears at first sight to be a good deal more complicated. "This is the same question that was asked when phage genetics was started," comments Horvitz. "Although certain detailed aspects of molecular genetics have turned out to differ, many basic principles have proved to be universal. I expect the same thing will happen with *C. elegans*. We should learn a lot both about how it differs and how it is similar to other organisms. From these studies of *C. elegans* fundamental principles concerning both development and behavior may well emerge."

Caenorhabditis elegans may be a small organism, but it is by no means simple.—**ROGER LEWIN**

The Art of Learning from Experience

Statistician Bradley Efron tells what his field is about and how a new method, the bootstrap, exploits the power of large-scale computing

"Statistics," says Bradley Efron of Stanford University, "is quite underappreciated." Most people who think of statistics at all consider it as simply a tool—a way to tell if data are significant or to estimate confidence intervals. But statistics is a deeply philosophical subject that tries to get at how we learn from experience. It is a dynamic field, full of arguments and beginning to change its very nature as its practitioners exploit the power of large-scale computing.

Efron, 45, is one of the leaders in the new statistics. He has invented an extremely promising new statistical tool, called "the bootstrap" and which, he says, "substitutes computing for thinking."

Although statistics is often thought of as a branch of mathematics, it actually lies on the border between mathematics and philosophy. "Obviously," says Efron, "statistics has mathematical structure—that's the only way anyone has found to say things in statistics." But the subject matter of statistics does not concern itself with typical mathematical reasoning in which results are deduced from axioms. Its logic goes in the opposite direction. Statisticians start with examples of things that are and try to determine what axioms could have given rise to them. "To step backward from what you've seen to what might have given rise to it is logically, mathematically, and actually difficult," Efron remarks. "We statisticians think deduction is child's play. In a sense, statistics is the most ambitious intellectual attack."

For Efron, the decision to become a statistician came only gradually and after he realized what he believes are his limitations as a mathematician. He always wanted to be a mathematician, he says, but he had a problem. "I was a terrific 19th-century mathematician. Give me a calculus problem and I could knock it dead. But I was not a very good twentieth century mathematician. I like to compute things. Modern mathematicians don't compute. They organize their ideas to another level of abstraction beyond calculations. I was terrible at things like modern abstract algebra. I have no mind for it at all."

Efron grew up in St. Paul, Minnesota, the son of a truck driver who was also an amateur mathematician. He learned from his father how to do calculations in his head. Set on becoming a mathematician, he majored in math at the California Institute of Technology, where he graduated second in his class. Then he started graduate school at Stanford, still majoring in math. But he was suspended from Stanford when, as editor of the school humor magazine, he published an article poking fun at religion. When he returned to Stanford, he returned to the statistics department.

Efron jokes that his suspension from Stanford will haunt him to his grave. "I often say that if I cure cancer, the Stanford newspaper story will begin, 'Bradley Efron, who once was kicked out of Stanford, today discovered a cure for cancer.' " But his reentry into statistics proved providential. Here was a field after his own heart—a field where com-