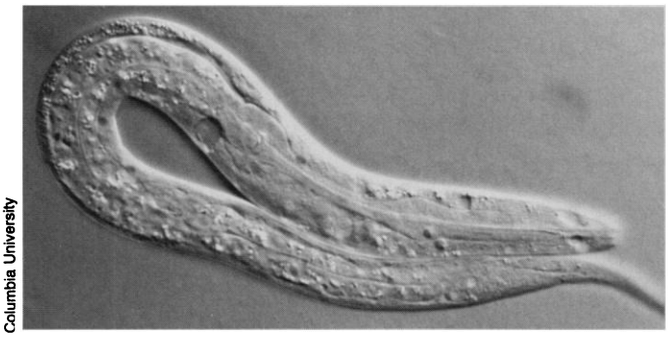
The Worm Project

An exhaustive study of the tiny roundworm C. elegans *has revealed a wealth of information about development and the brain. And now the effort to decipher the worm's genome is fast becoming the benchmark by which the human genome project will be measured*

IN 1963 SYDNEY BRENNER set out to learn everything there is to know about the nematode *Caenorhabditis elegans*, a tiny worm, a mere millimeter long. "I would like to tame a small metazoan," is how he put it in a letter to his boss, Max Perutz, head of the Medical Research Council's Laboratory of Molecular Biology in Cambridge, England. Brenner, who now laughs at his hubris, thought he could tame the worm fairly quickly and then move on to a more complicated organism, like an insect.

But now, 27 years later, the worm project, as this loosely knit collaboration is fondly known, is still going strong at the MRC and in nearly 100 labs around the world that are run by Brenner's "intellectual progeny." And as Brenner has said, what was once a joke has become one of the most exhaustively studied model organisms around.



Columbia University

The nematode *C. elegans*. *This tiny worm, a mere millimeter long, is one of the simplest differentiated organisms, but from its study have emerged countless insights into cellular development and the brain.*

Biologists have now traced the exact lineage of every one of the worm's cells—information known on no other organism. And in another first, they have a complete wiring diagram of the nervous system—all the neurons and the connections among them. From this detailed study has emerged a string of discoveries—in programmed cell death, heterochronic genes, sex determination, and neuronal guidance, to name a few. Indeed, a good portion of what is known about developmental genetics can be traced to Brenner's lab in Cambridge.

But all that can be seen as prelude, in a sense, to what comes next—the biggest research project yet on *C. elegans*, a 10-year effort to decipher its complete genetic instructions. Alan Coulson and John Sulston of the MRC and Robert Waterston of Washington University in St. Louis are just finishing a map of the entire genome of the worm. And now, in a chapter even Brenner could not have forseen—after all, DNA sequencing had not yet been invented—the three are embarking on a project to work out the full nucleotide sequence, all 100 million bases.

That's a remarkably ambitious goal, given that no one has ever sequenced a stretch of DNA anywhere near that long. And Coulson, Sulston, and Waterston are planning to do the sequencing faster than it's ever been done—and at a fraction of the usual cost. But James Watson of the genome center at the National Institutes of Health, which is funding the work along with the MRC, thinks they can pull it off. "These are people with really good track records. They have already thought big," says Watson, who adds that workers on the human genome project may well be able to pick up a few pointers from their counterparts in the *C. elegans* research.

This new genome effort is built directly on Brenner's vision of nearly 30 years ago: to use genetic analysis to probe the mysteries of development and the brain. The idea had its origins in a long series of conversations between Brenner and Francis Crick, now at the Salk Institute. Since the major questions of molecular biology, such as replication and transcription, had been solved or were about to be, they decided that the future lay in tackling the more complex problems of development and genetic control (*Science*, 22 June 1984, p. 1327).

To do so, Brenner wanted to find a new model system that could serve developmental biology the way *Escherichia coli* and phage had served molecular biology: an organism that could be handled with the ease of bacteria and viruses but that would provide clues into the control of complex processes in higher organisms.

After a brief flirtation with *C. briggsiae*, Brenner settled upon *C. elegans*. The beauty of the worm is that though it is extremely simple, it is a "real animal," as Waterston describes: "It has nerves, muscles, intestines; it reproduces. And if you hit it, it reacts." What's more, *C. elegans* is transparent, so investigators can actually watch the process of development unfold in a living animal under a microscope. At the same time, its entire life cycle is a mere 3 days, and 100,000 of them can live in a petri dish.

But while the worm's virtues may have been apparent to Brenner, biologists elsewhere were clearly underwhelmed. Says longtime collaborator Sulston: "It was seen as just Sydney's madness, and not to be taken seriously."

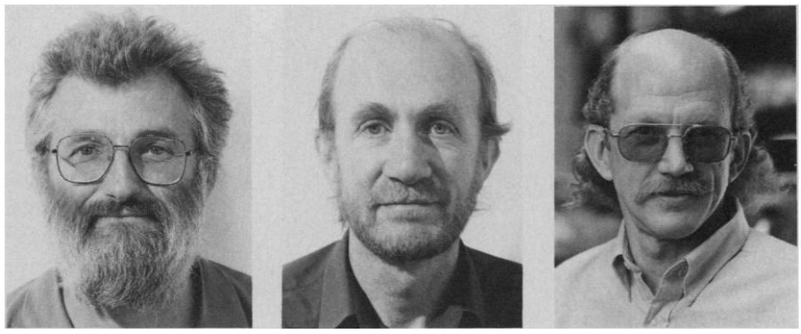
Brenner devoted the first 4 or 5 years to studying the genetics of the worm, estimating the size of its genome (100 million base pairs, as opposed to 3 billion in the human) and the number of essential genes (about 2000), and then mapping 100 of those genes into six linkage groups that corresponded to the worm's six chromosomes. After 5 years, Brenner's unconventional project had begun to attract what would eventually become a stream of graduate students and postdocs, including John White, John Sulston, Robert Waterston, Jonathan Hodgkin, and Robert Horvitz.

From the start, their aim was a complete understanding of the tiny beast. Explains Martin Chalfie of Columbia University: "The goal is not to understand just a nerve cell or a muscle cell but the entire organism, intact. We look at the total animal." And they have been nothing if not thorough.

In a project that spanned a decade, John White, Eileen Southgate, and Nichol Thomson of the MRC reconstructed the anatomy of the nervous system by painstakingly assembling thousands of serial section electron micrographs. "It is unprecedented," says Robert Horvitz of the Massachusetts Institute of Technology. "There are very few cells [302 neurons, as opposed to 100 billion or

so in man] but we know them all, and the connections between them." The wiring diagram, as it is called, was published as a single, 340-page issue of the *Philosophical Transactions of the Royal Society of London* in 1986.

Then, in another project that took most of the 1970s, Sulston and his collaborators worked out the cell lineage, starting with the zygote and tracking each cell division that gives rise to the 959 somatic cells in the adult hermaphrodite. Horvitz calls it "a technical tour de force," adding that Sulston "attained hero status" on that one. "Others had tried and failed. Sulston essentially locked himself in a small room for [the final] 2 years and emerged when the project was finished."



MRC

Sequencing crew. *John Sulston, Alan Coulson, and Robert Waterston's work on the worm may well pave the way for the human genome project.*

The payoff from the first two stages of the worm project has been enormous, says Jonathan Hodgkin, an MRC geneticist studying sex determination in the worm. The cell lineage brought an unprecedented precision to experimental manipulation. "Using a laser, you can ablate one cell and be absolutely confident of what cell has been killed and what it would normally give rise to," explains Hodgkin. And using the wiring diagram, "you can look at the complete neural circuit for a particular piece of behavior and get a complete and convincing description of the nature of that behavior," says Hodgkin, referring to Martin Chalfie's work on touch sensitivity. "You can look at it and say, 'that is all there is.' It is really Sydney Brenner's dream—that we would be able to predict behavior from a combination of neuroanatomy and genetics."

No sooner had Sulston finished the cell lineage in 1983 than he began his 7-year effort to develop a complete map of the worm genome—a goal dismissed as impractical, if not impossible, at the time. He got the idea for the map after he had been "staring at the lineage for several years and was beginning to despair of ever sorting it out. I decided we should try to understand every gene in the organism." And to do that, the worm community needed a new tool—a physical map.

Worm biologists had already constructed a genetic linkage map, which can be used to find the approximate location of a gene. But cloning the gene on the basis of that map was still a laborious and time-consuming task. Says Sulston: "Watching people clone genes the hard way made me feel we could make things easier by doing part of the cloning in advance." And that, in essence, is what a physical map is all about. It is a collection of cloned pieces of DNA, assembled in the correct order, that provides ready access to any piece of DNA.

Sulston's plan may sound commonplace today, with all the talk of mapping the human genome, but when he began, mapping on this scale was uncharted territory. Short stretches of DNA had been mapped this way, but as far as Sulston knew, no one had been ambitious—or perhaps foolhardy—enough to tackle the complete genome of any organism. Unbeknownst to him, however, Maynard Olson at Washington University in St. Louis had already embarked on a project to map the genome of the yeast *Saccharomyces cerevisiae*. The two groups struck up an informal collaboration, which Olson describes as a pleasure, that continues even now.

But was it feasible to map the worm genome, which is considerably larger than that of yeast? That was the question when several nematode biologists got together over beer one night during a meeting at Cold Spring Harbor Laboratory. "We decided it was possible to make a map of the entire genome," recalls Horvitz, who suspects that the beer played no small part in their optimistic assessment. "And we decided it was possible for two people to do it in a relatively short time." That second person was Alan Coulson, who had been working with Fred Sanger at the MRC on the development of DNA sequencing.

But then Coulson and Sulston, as well as Olson in St. Louis, had to figure out how in fact you do map an entire genome. Although their approaches varied, the basic idea they hit upon was to start with fragments and then reassemble the whole chromosome from those bits. As Waterston recalls, "it was not clear at the start how many fragments you would need, or how to generate them. And it was an open question whether it could be done at all."

They used restriction enzymes to chop the worm's chromosomes into fragments and then cloned them in cosmid vectors. Once they had thousands upon thousands of tiny pieces, the trick was to find a way to reassemble them. With help from Brenner and Jonathan Karn of the MRC, Coulson and Sulston came up with a "fingerprinting" strategy that involves identifying a distinctive pattern, or fingerprint, in each clone, and then, with the help of a computer, searching to see if any clones share that fingerprint and therefore overlap. Sulston credits Coulson with making the idea work. "Not until Alan came did the technique really become viable. He made it routine and reliable."

They fingerprinted about 17,500 clones in this way and then lined them up as well as they could in overlapping sets called contigs. But there were a couple of problems. First, they didn't know the proper order of the contigs or even which chromosome they belonged on. For that, they turned to the *C. elegans* community.

"From the start, this has been a community project," says Sulston, referring to the numerous investigators who have contributed the genes and other landmarks that helped orient the map in relation to the chromosomes. "Anytime anyone clones a gene the first thing they do is send it to John and Alan," explains Waterston. "Their names go together so often that a newcomer in my lab thought it was one person, John N. Allen." That gene then becomes a landmark on the map. And in return, the investigator gets back a clone containing the gene and the surrounding DNA.

As more genes were cloned and sent to Cambridge, the better the map became. "There is a kind of circularity to it," says Sulston. "The better the map is the easier it is to clone things and then the better the map becomes."

None of this would have been possible, say Sulston and others, were it not for the willingness of the *C. elegans* researchers to share their data far in advance of publication. "There is better cooperation [on the worm project] than in any field of biological research I am aware of," says Horvitz. "And there is a simple reason. The field is quite small, and it started with one person, Sydney Brenner. Many people who now head labs were friends 15 years ago in England. It is a community."

Indeed, when the worm biologists decided to collect all their data in one book, *The Nematode Caenorhabditis Elegans*, better known as the The Worm Book, published

by Cold Spring Harbor Laboratory in 1988, there was no question about how the title page should read: "Edited by William B. Wood and the Community of *C. elegans* Researchers."

After Brenner, people credit Sulston and Robert Edgar of the University of California at Santa Cruz for institutionalizing this spirit of cooperation. Edgar started a newsletter, *The Worm Breeders Gazette*, in which people contribute all their ideas—what they have found, what they are thinking of doing. Says Horvitz: "The philosophy is that everyone puts in everything they know. If you find it, you share it."

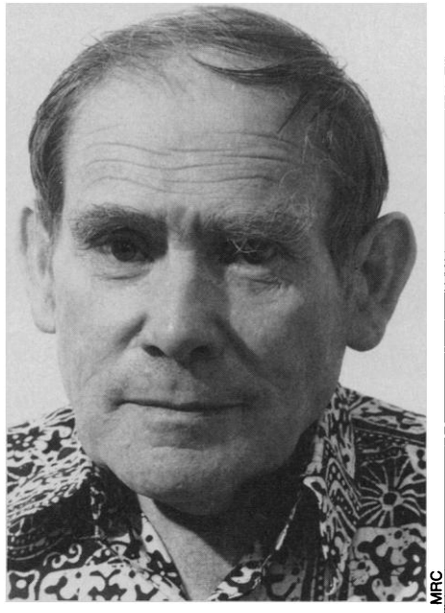
And Sulston set up the mapping database in a way that provides for "automatic sharing," as Horvitz describes it. As soon as a gene goes on the map, that information goes out on the electronic database. Says Chalfie: "The entire database of the map—which genes are mapped and cloned—is available. Anyone can call up, dial in, and look anywhere they want. It is all unpublished data, and it is available to all." And it works, he says—numerous collaborations have arisen as a result.

Through this communal effort, Coulson and Sulston had cloned about 90% of the genome and had mapped about 15% of that back to the chromosomes by 1986. And that is when the second problem with the map became readily apparent—namely, how to finish it.

Their map was in about 700 distinct pieces, or contigs, which meant there were about 700 gaps—places along the chromosomes where the DNA was proving tricky, if not impossible to clone. And that was after an exhaustive fingerprinting exercise, recalls Waterston, who spent his 1985–86 sabbatical with Coulson and Sulston and then signed on as a full collaborator on the genome project. Clearly, the original fingerprinting strategy had reached its practical limits. Waterston spent much of his sabbatical experimenting with various techniques for finishing the map.

The turning point came when Waterston returned to St. Louis. David Burke, a postdoc in Maynard Olson's lab down the hall was doing his first experiments with YACs, yeast artificial chromosomes. These are cloning vectors that can accept huge pieces of foreign DNA, 400,000 bases or so, at least ten times bigger than the cosmids the group had been working with. And there was good reason to believe that those elusive pieces of worm DNA that could not be cloned in cosmids could, in fact, be cloned in YACs. At last, it might be possible to finish the map.

It worked. Once they had cloned the nematode in YAC vectors, the group devised new strategies to look for overlaps among the YAC clones and between YACs and the original cosmids. Most of the gaps have now been closed; 95% of the genome has been cloned, and the group has gone from a map with about 700 separate pieces to one with just 150 much larger pieces. And many of the remaining gaps are trivial, says Waterston.



MRC

Worm patriarch. *Sydney Brenner's idea to study the nematode* C. elegans *spawned a three-decade effort involving nearly 100 investigators.*

Even in its partial state, the map has been a "godsend," says Chalfie. "The map revolutionized the way we do experiments," agrees Horvitz, who adds that in the old days, if you were going to clone a gene you had to first find some landmark nearby and then laboriously "walk" down the chromosome to find the gene. With the map, "you can literally walk to the freezer and pull out that piece of DNA," and then test for the gene. In the best case, a 1- or 2-year process has been collapsed into a few weeks.

In April the group reached a mapping milestone when they began sending out copies of their new grid, which is essentially the entire genome of the worm on a piece of filter paper no bigger than a postcard. Once they found YAC clones that spanned the entire genome, give or take a few holes, they transferred DNA from those clones onto this filter paper grid, starting at the left side with the leftmost piece of chromosome 1, and so on. Now anytime an investigator finds an interesting gene and wants to know where it resides on the physical map, "all you do is hybridize it to this piece of filter paper overnight," explains Waterston. "It is pretty neat."

Last summer, with the map almost complete, Coulson, Sulston, and Waterston finally decided to "go for it," says Waterston—"it" being the full 100-million base sequence. So far, the longest stretch of DNA completed to date is the 240-kilobase cytomegalovirus, sequenced by Bart Barrell and his colleagues at the MRC, though similar large-scale sequencing efforts are gearing up for *E. coli* and yeast.

The first 3 years are definitely a trial, says Sulston of the worm genome project. But if they can reach their goal of sequencing 1 million bases a year, in both the Cambridge and St. Louis labs, and if they can drop the cost from $3 or $5 to about 50 cents per base, they plan to seek enough money to knock off the entire sequence by the end of the decade. Both labs are scaling up to eight or ten people, making this the biggest worm project yet.

Their aim is to figure out how to sequence efficiently—something that clearly has to be done before anyone embarks on the human genome, which is 30 times larger than that of the nematode. And that is one of the reasons that James Watson keeps touting this project as a model.

The trio are the first to admit that they have no idea how they are going to do it, other than to rely on automation and robotics and some clever new ideas yet to be thought up. "Having the map in hand means we can go about this piecemeal," says Waterston, "We can change our minds in 3 years, and use new strategies for new segments."

"We would all be dubious if it were anyone but John [Sulston]," says Horvitz. "But he will figure it out. He has already done things that people categorically said would be impossible," adds Horvitz, referring to the cell lineage and the physical map.

Just last month, Coulson, Sulston, and Waterston learned that their money had come through in a novel funding arrangement that Watson, for one, hopes will be a forerunner for the rest of the human genome project. Although the exact cost is still being worked out—it will be in the range of $5 or $6 million for the first 3 years—NIH and the MRC have agreed to split the tab.

Coulson, Sulston, and Waterston like the arrangement for another reason. The path of least resistance would have been to split the project into a U.S. effort, funded by NIH, and an English effort funded by the MRC, says Sulston, "but we decided against that. That would allow people to drift apart, and we don't want that. We have gotten on very well for quite a long time."

Is the sequencing effort the final chapter in the worm project? Definitely not, says Sulston. "The idea is to bring it back to biology," agrees Waterston. Horvitz, who was in on the early planning for the project, views it as "an opportunity to obtain at a

level of resolution never before achieved an understanding of an animal that uses a nervous system to control behavior."

For worm biologists, the sequence will likely mean another revolution in how they do experiments. Says Chalfie: "It means that identification and cloning of genes—work that a lot of us spend a lot of time on—will already have been done. Instead of asking, Can we get the gene? we can ask, What is the function of that gene?"

To Sulston, the challenge is to learn how to read, or understand, the sequence. And biologists have a far greater chance of figuring it out in the well-studied worm, on which they can perform aggressive experiments, than they do in humans. Sulston and his colleagues are convinced that much of what they find in the worm will be relevant to human biology. Already, says Hodgkin, in the genes sequenced to date, worm biologists are encountering many "old friends"—conserved genes present in humans and other animals. He expects that soon the pattern will be reversed. "We will see them first in *C. elegans* and then go on to look for them in other animals."

Sulston agrees: "In a sense, one organism like this contains all of biology."

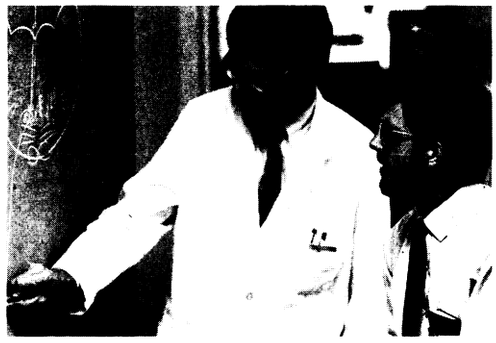
■ **LESLIE ROBERTS**

No Pain, No Gain?

Evolution has provided us with a nervous system that includes the endorphins, molecules that serve to reduce pain. Their evolutionary function is obvious (imagine a wounded hominid trying to escape a saber-toothed tiger). The evolutionary function of a system that enhances pain is less clear. Yet University of California at San Francisco researchers claim to have discovered just such a system, which, they propose, acts as a counterweight to the neurons that release endorphins.

These findings remain controversial, but if they are confirmed, they could have important implications for the treatment of drug addiction as well as for therapy in cases of chronic pain.

Howard Fields, leader of the research team, and his colleagues postulated the existence of the pain-enhancing system after studying the effects of morphine and induced morphine withdrawal on two sets of nerve cells in rats. Both are in the rostral ventromedial medulla, a brain region involved in pain modulation. One set is called "off cells," because their activity seems to shut off the experience of pain, possibly through the release of endorphins. The other set, which Fields dubbed "on cells," is active when rats respond to painful stimuli.



Painful discussion. *Howard Fields with Michael Rowbotham of the UCSF Pain Management Center.*

The San Francisco group reports in an upcoming issue of *Somatosensory and Motor Research* that in rats that had been lightly anesthetized, then injected with morphine, the soothing off cells were active but the pain-enhancing on cells were silent. In this state the rats did not respond to a mildly painful heat stimulus.

After morphine withdrawal was induced, however, the rats responded rapidly to the same type of stimulus, suggesting the presence of the hyperalgesia (increased sensitivity to pain) that is often reported in drug addicts going through withdrawal. During withdrawal, the soothing off cells were silent, while the on cells were active—and the higher the level of on cell activity, the faster the animal responded to the painful stimulus.

Fields and his colleagues were faced with the task of showing that the rapid response was due to on cell activity rather than simply to lack of activity on the part of the off cells. To do so, they inactivated the on cells that ordinarily responded during withdrawal. When those cells were put out of commission, the animals responded only slowly to the heat stimulus. According to Fields, this implies that the rapid reaction in withdrawal is due to "to some active process going on in the inactivated area," namely the firing of pain-enhancing on cells.

Some researchers think Fields has gone far beyond his data in extending his observations to propose a counterpart to the endorphin system. Several other labs have reported the existence of on cells, but just what role those cells play in the transmission of pain is the subject of a wide open debate.

"This stuff is so new you can't make a judgment about it," says Kenneth Casey, a neurophysiologist at the University of Michigan. Casey adds that the idea of a system that amplifies pain is a solid one, but "it is not clear that it is the on-off cells doing it."

Among those who have observed the on cells are Michael Behbehani, a neurophysiologist from the University of Cincinnati, and J. Peter Rosenfeld, a physiological psychologist from Northwestern University. Yet even they are not convinced that the existence of those cells alone explains pain-enhancing effects. "The story of the on-off cells is pretty clear," says Behbehani, "but there is some disagreement that it is as clear-cut as Howard Fields says it is."

Yet another reservation offered by some researchers is that Fields is observing a motor response rather than a sensory one. "He is looking at the spinal reflex," says Herbert Proudfit, a pharmacologist at the University of Illinois. "But I don't know what that says about the experience of pain in humans."

Undaunted, Fields has used his observations to propose a model of drug addiction in which morphine stimulates off cells, whose action is counterbalanced by increased on cell activity. On cell activity could lead to hyperresponsiveness to pain. As long as drug levels in the brain are high, the pain would be masked by the off cells. But as the drug levels decrease, sensitivity to pain would increase and the addict would need a new dose. Hence reducing the activity of on cells might reduce sensitivity to pain and help wean drug addicts from drugs. Chronic pain patients might also benefit if an on cell neurotransmitter could be identified and blocked.

It may be premature to ask whether there is an evolutionary rationale for a pain-enhancing system, but Fields offers a couple of "wild speculations." First, in an emergency, the body might need a way to override the pain-soothing effect of the endorphins. Second, "there is always going to be an evolutionary advantage to shortening reaction time," says Fields, giving the example of touching a hot skillet: pain enhancement could make it possible to pull a hand away before it gets burned.

■ **MARGUERITE HOLLOWAY**

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