Perspectives

Anecdotal, Historical and Critical Commentaries on Genetics

Edited by James F. Crow and William F. Dove

EARLY WORMS

JUST over 21 years ago, in October of 1967, SYDNEY BRENNER soaked a culture of hermaphroditic nematodes of the species *Caenorhabditis elegans* in a solution of ethyl methane sulfonate. A week later, examining their F_2 descendants, he noticed a short, "dumpy" animal among the long, thin wild-type worms. The dumpy animal was picked to a separate culture plate and allowed to produce self-progeny, which were also dumpy: it was a true-breeding mutant. The new strain was given the name E1. Crosses with the parental wild-type strain showed that the mutant phenotype was due to a single autosomal recessive mutation—in modern nomenclature, allele *e1* of the gene *dpy-1*.

BRENNER went on to analyze many other mutants, finding that they were easy to generate, assign to complementation groups and map genetically. The results of hundreds of mutagenesis experiments and crosses were meticulously recorded in rows of green files that came to occupy almost 20 feet of shelf space. This work culminated in the definitive paper he published in GENETICS 15 years ago. Thus, in a very real sense, the isolation of *e1* marks the birth of nematode genetics. Over the past two decades, research in *C. elegans* has expanded inexorably and with an increasing sense of excitement. Today, strain E1 is only the first of at least 10,000 genetic variants of *C. elegans* stored in some 70 different laboratories and studied in ever increasing detail by over 300 investigators. There seems to be no limit to the variety of problems that can be studied using *C. elegans*, and "the worm" now stands second only to Drosophila as a favorite invertebrate for geneticists.

Why should this have happened? As with so many other experimental systems, the success of *C. elegans* has been the result of both luck and design. The initial choice was dictated by certain useful properties of this species, but subsequent work revealed unforeseen advantages which have played a major role in its popularization.

The original reasons for choosing *C. elegans* were that it is small, anatomically simple and suitable for electron microscopy. It is also easy to grow and to manipulate genetically, being able to reproduce either by self-fertilization or by cross-fertilization. Despite its simplicity, it has the full range of differentiated cell types found in more complicated animals. In addition, *C. elegans* had been the subject of research by several nematologists (E. DOUGHERTY and V. NIGON in particular), so that some background knowledge was available.

Among the additional advantages was the extremely small size of the genome, about 80,000 kb (SULSTON AND BRENNER 1974). No other metazoon is known to have so little DNA. At the time the measurement was made, it was not appreciated just how useful this would prove to be. The consequences of a small genome are that the genes are correspondingly smaller, introns are fewer and shorter, gene families are smaller and repeated sequences are less numerous than in other animals. All of this makes molecular genetics—cloning, sequencing, hybridization, walking and so on—much easier and simpler than in organisms with large genomes. The small genome has also permitted a project that would be daunting in any other animal: constructing a complete physical map of the genome (COULSON *et al.* 1988). As of late 1988, this project has reached a point where 90% of the genome has been assembled into "contigs" (regions of overlapping DNA clones ranging from 50 to 4000 kb). The ultimate aim is to reduce the number of contigs (now about 250) to six, the haploid number of chromosomes.

A second fortuitous advantage has been the ability to store worms frozen in liquid nitrogen. Some nematode species do not survive freezing, or else require inconvenient freezing protocols. *C. elegans*, however, is easy to freeze (by slow cooling in dilute glycerol) and stocks that have been frozen for over 18 years can still be thawed to yield viable worms. In terms of human generations, this is the equivalent of resurrecting people from 40,000 BC. Freezing means that stocks can be maintained essentially forever without genetic alteration and at little cost. Parental lines for

any given mutant should always be available and stocks that might otherwise be abandoned can be preserved on the off chance that they may later become useful (for example, the transgenic lines now being generated in great numbers).

An advantage related to the ability to freeze worms, but more to the fact that *C. elegans* genetics was founded by one man, is the single defined and universally agreed wild type, which is homozygous, permanently preserved and stable. Almost all mutant lines have a defined pedigree that traces back to this single stock. Consequently, genetic background effects can be controlled, phenotypes can always be compared to the same wild type and alterations in the DNA sequence of mutants can be referred back to the original wild-type sequence. In contrast, most other standard genetic organisms (*Escherichia coli, Saccharomyces cerevisiae*, Arabidopsis, Drosophila) do not have a single, agreed wild-type strain.

A third benefit that only gradually became clear is the superior optical qualities of *C. elegans*, which permit the transparent living worms to be examined at high resolution using Nomarski microscopy. Some nematode species of comparable size contain excess refractile material which severely limits light microscopy. Without the good optical qualities of *C. elegans*, the complete description of the somatic cell lineages (by J. E. SULSTON, H. R. HORVITZ, J. E. KIMBLE and collaborators) would have been harder or impossible to obtain. Also, laser microsurgery (developed by J. G. WHITE) would be a less powerful technique.

A final advantage that has emerged over the years has been the establishment of a group of enthusiastic and dedicated workers. All of the early genetic work was due to BRENNER, but the appeal of the system was such that more and more disciples began to accumulate. As with the mutant strains, so with the scientists: almost every worker in the field has an intellectual pedigree that traces back to a single source. Moreover, most of the early workers had a common background in either bacterial or phage genetics—especially T4, like BRENNER himself. He remarked that switching from phage to nematodes was a comfortable transition because both T4 and *C. elegans* live by eating *E. coli*, the one from the inside and the other from the outside. Scientists who had worked only on prokaryotes usually were briefly disconcerted at having to deal with a diploid organism but thereafter took to worm genetics like ducks to water.

The "phage" influence colored a lot of the early work, for example, the emphasis on powerful selections and rare events, the usefulness of conditional mutants (both temperature sensitive and suppressible nonsense) and the need for long-term storage. Not all of these influences lasted, one instance being the use of wooden toothpicks for transfers and crosses. The average toothpick, fine for picking plaques, is too blunt for manipulating individual worms less than 1 mm long, Thus, early workers spent excessive amounts of time sharpening wooden picks to a fine point with a razor blade and autoclaving them to prevent the spread of fungal and bacterial contamination. Only after some years did R. K. HERMAN introduce the now universal platinum worm pick: a wire with a flattened scoop or loop at the end, ideal for picking up worms and instantly sterilized by flaming.

There also were unsuccessful attempts to turn the worm into an honorary microbe by making it a colonial organism. Some paralyzed mutants of *C. elegans* move so little on a lawn of bacterial food that each worm plus its descendants forms a small separate mound. In principle these could be treated as colonies and replica-plated to a different medium, but in practice the idea never really worked. Perhaps if there had been more interest in developing biochemical genetics (still a neglected area of *C. elegans* research), schemes like this might have been pursued further.

It is interesting to compare the original aims of research on *C. elegans* with the present state of the field. Initially there was a strong emphasis on behavioral genetics with the hope of using mutants as a means of decoding both how the neuroanatomy is specified genetically and in turn how this neuroanatomy dictates the behavior of the animal. Several investigators were particularly interested in sensory behavior such as chemotaxis (*e.g.*, WARD 1973). Here again the prokaryotic influence was felt, in this case as a result of the beautiful work of J. ADLER on bacterial chemotaxis. Both sensory and locomotory mutants were found and studied, but it became apparent that it was going to be hard to understand them without more information on the normal development of the animal and its complete structure.

Gradually it became clear that it was both feasible and desirable to study the biology and development of the whole animal. Many research groups have tried to concentrate on one single aspect of *C. elegans* biology, but it has been striking how frequently findings in one area have affected research in another and the field as a whole has adopted a rather holistic attitude. Information and techniques have continued to accumulate steadily, so that it now seems possible that some parts of the development of this animal will be completely understood in terms of genes and proteins, the ultimate goal of molecular developmental biology.

Another early goal was to use *C. elegans* to analyze the nature of eukaryotic genes, which appeared to be disproportionately larger than prokaryotic genes. Even in this animal, there was far more DNA than seemed to be necessary. This, of course, is a problem

that has been solved with the advent of molecular cloning. Introns, transposable elements, repeated sequences and large regulatory regions all contribute to the greater size of eukaryotic genomes, and the "C value paradox" has essentially vanished. However, there remain many questions about the large-scale organization and evolution of animal genomes, and the study of *C. elegans* is likely to contribute useful information on these questions. For example, it should soon be possible to use the physical map of the genome in order to ask if there is any significant long-range ordering of repeated sequences.

In some ways, the wheel has come full circle: attention is once more being focused on the construction and function of the nervous system because the tools for detailed molecular analysis of these problems are now available. At the same time, *C. elegans* will continue to be used for studying a host of other problems in genetics and in cellular, developmental and molecular biology (for a general review, see WOOD 1988). Given the appeal of the system and the fact that it is still largely virgin territory with respect to research areas such as evolutionary genetics, population biology and physiological and biochemical genetics, we can expect to see expansion and diversification in the years to come.

JONATHAN HODGKIN
MRC Laboratory of Molecular Biology
Hills Road, Cambridge CB2 2QH
England

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