

Accolade for *elegans*

Essay

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The Vision

In 1963, Sydney Brenner, one of the founders of molecular biology, had reached an intellectual impasse. He felt that there were few advances left in that field that would have the significance of the discovery of mRNA and the elucidation of the genetic code, both of which he had participated in, and in any case with so many Americans joining in, the chemical details of replication and so forth would all be worked out soon. Brenner thought large thoughts, and the questions that were left seemed too small.

Brenner worked at the Medical Research Council Laboratory of Molecular Biology in Cambridge, England, and starting a completely new research initiative required him to formulate ideas just well enough to convince the LMB chairman, Max Perutz, and the leadership of the MRC, that the ideas were good ones. He began by deciding that the big questions were animal development and behavior, and that genetics and biochemistry could be used to understand these processes. The question was how: molecular genetics was still firmly centered in prokaryotic cells at the time, and the classical developmental organisms like amphibians, beloved of embryologists, were monstrously large and complex. Brenner had learned the power of using the simple bacteriophage for understanding molecular genetics, and what he needed was a phage equivalent for developmental biology. The first letter Sydney wrote to Max suggested that he could start fresh:

I would like to tame a small metazoan organism to study development directly. My ideas on this are still fluid and I cannot specify this in greater detail at the present time.

A set of criteria was developed, a search was begun, and five months later, Brenner had settled on a hermaphrodite nematode worm. As he wrote in a proposal utterly devoid of the preliminary results and experimental methods expected today, it would be ideal because it could be grown in quantity, like a micro-organism; it had a short life cycle; it was superbly amenable to genetic analysis because it could reproduce by self-fertilization but also be cross-fertilized by males; and it had few cells, about a thousand, so detailed studies of cell lin-

eages and patterns would be possible. *Caenorhabditis elegans*, the creature finally chosen for taming, rose to the top of the short list because it also had superior optical qualities for light and electron microscopy, and understanding development requires an understanding of structure, at single-cell resolution and ultimately at subcellular resolution.

Brenner's vision marks the first step toward this year's Nobel Prize in Medicine or Physiology, shared by Sydney Brenner, John Sulston, and Bob Horvitz. They are cited for the progress they made in understanding organ development and cell death, with the assistance and collaboration of the hermaphrodite nematode *C. elegans*. Within the group of researchers who study *C. elegans*, it is seen as Our Prize, rightly awarded to the leaders who chose, developed, and exploited our organism to address major questions of biology.

Reading Brenner's two short 1963 letters to Perutz and the MRC today, it is striking how clear that vision was from its first conception. While Brenner's ideas were still fluid in that first letter, he suggested that the repressor/operator theory of Jacob and Monod would be the central clue for understanding development. Indeed, the principle of differential regulation of gene expression is so central to developmental biology that it is impossible now to see how it could have worked otherwise. And the whole point was to do things completely and entirely. His proposal to the MRC ended with the words:

We propose to identify every cell in the worm and trace lineages. We shall also investigate the constancy of development and study its genetic control.

This concept of completeness was foreign to biology at the time, but it presaged the genomic approach that dominates biology today. In fact, *C. elegans* was the first model animal to embrace genomics, because both the ideas and the nose-to-the-grindstone work ethic were natural in a community that wanted to know every cell, every gene, every synapse.

The Lineage

Brenner's next act of genius was to enlist colleagues who could extend his vision and actually carry it out. Outrageously brilliant, charismatic, and witty, Brenner was able to convince a talented group to join him at the MRC in the late 1960s and 1970s. Like *C. elegans* itself, the field is known for the prominence of lineage, and almost everyone who works on *C. elegans* can trace a path back to Brenner in a few steps. Among the first to join was the organic chemist John Sulston, in 1969, returning to Britain after postdoctoral work on prebiotic evolution at the Salk Institute. Bob Horvitz followed in 1974, fresh from graduate research at Harvard on phage T4.

Sulston began by determining the DNA content of the worm, but then moved on to the big question of identifying every cell and tracing the lineage. At the time, it was thought that all somatic nematode cells were generated in the embryo, but Sulston discovered that

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many somatic tissues continued to divide in post-embryonic nematodes. There were 556 somatic cells at hatching and 959 somatic cells in the adult hermaphrodite. Neurons, muscles, epithelial cells, and gonadal cells all increased in number. Sulston's first discovery was that you could see a lot just by looking: that cell divisions could be watched directly in the live worm, and therefore the cell lineage could be traced by examining it directly over the three days of postembryonic development. He watched the divisions that gave rise to postembryonic neurons, a total of about 80 cells. The divisions were patterned and reliable from animal to animal. Horvitz was persuaded to join the project, and the two of them completed all of the lineages outside the gonad, in a paper published in 1977.

What is the point of knowing the cell lineages? By itself, a lineage chart is gargantuan, impressive, and sterile. Sulston and Horvitz knew from the outset that the lineage was a tool for discovery and a tool for the generation of other projects (an example of how descriptive science is needed before hypothesis-driven science can emerge). The structure of the postembryonic lineage began to frame the questions that led to the Nobel Prize. Cells died in nematode development, many cells, and they died in a reproducible pattern. What determined which cells lived and died? In many cases, cell lineages were fixed. Cell divisions occurred at reproducible times. How could one cell give rise to different daughters in a precise pattern? For cells that formed the vulval structures, the exact pattern of cell lineage was not identical from animal to animal even though the final number of cells was identical. What allowed cells to coordinate their choices with one another? Why? How?

A typical scientist would have focused his efforts, chosen one of these problems, and pursued it. Sulston was atypical. He knew half the cell lineage, but not the other half, and he wanted closure. Embryonic development took just 12 hours and generated the first 556 somatic cells. Embryos were transparent and cell divisions could be observed starting right after fertilization. But the patterns of embryonic division rapidly become complicated and appallingly difficult to follow by simple observation. Nevertheless, Sulston became convinced that it was possible to describe the whole process. After further work on postembryonic development, he returned to focus on the embryo. For a long period he did nothing but concentrate fiercely at his microscope for hours on end in a darkened room, watching the embryonic cells divide, rearrange, migrate, and differentiate. Hundreds of embryos were tracked, and gradually he became able to piece together more and more of the lineage. In 1983, he emerged with the complete pattern of division from fertilization to hatching. The landmark 1983 paper marks the first time that the entire pattern of cell divisions that gave rise to an animal was defined, and it remains the only example. Together with the complete anatomy of the *C. elegans* nervous system, defined by John White and collaborators in 1986, these lineage charts remain the organizing principle for *C. elegans* developmental biology, and have inspired the further work of the entire field.

The Screens

Understanding the mechanism of development meant disrupting it and watching the consequences. Develop-

mental questions about autonomy and cell interactions can be posed by classical experimental embryology in animals like frogs and beetles, but the tiny worm was ill-suited to conventional dissection. A laser microbeam cell ablation technique developed by John White allowed cells to be killed from an aerial vantage point at the microscope. Using this technique, it became clear that some cells developed independently, whereas others chose their fates in concert with neighboring cells. These experiments defined the questions that would be pursued in molecular detail, but they were not meant to stand alone. A genetic attack was intended to be the special tool that would allow *C. elegans* to make contributions to developmental biology.

Brenner's first paper on *C. elegans*, published in *Genetics* in 1974, describes mutagenesis with the point mutagen EMS, mutant analysis, mapping to chromosomes, and complementation tests. Most of the standard mutants that are still used for mapping were described in that paper, but at that point the screens were open-ended explorations of the kinds of mutants that could arise. The determination of the lineage allowed a more focused set of screens to be developed. Horvitz and Sulston began to isolate mutants in which the cell lineages were altered. Mutants with missing or duplicated cells were found and shown to have defects in the patterns of cell divisions. Fortunately for all, the pattern changes in these *lin* (lineage) genes usually led to visible phenotypes in the worms that could be observed under the dissecting microscope.

Horvitz ultimately chose vulval development for his main efforts. The lineage had suggested cell communication, and Judith Kimble had shown that killing the gonad blocked vulval cell divisions. Starting with Sulston, and continuing in his own lab at MIT, Horvitz performed massive screens for *lin* mutants in which vulval cells were missing or duplicated. In the spirit of completeness, the screens were conducted to saturation, the point at which mutations could be assumed to arise in all possible genes. In retrospect, completeness was crucial. Many genes that affect development will be hard to find because of multiple functions that cause lethal phenotypes when mutated, or because of redundant functions with other genes. In vulval development, the screens were so extensive that it was possible to isolate rare, viable, vulval-specific alleles of essential genes. Moreover, because the extensive screens yielded animals whose vulval defects resulted from mutations in two different genes, synthetic or redundant pathways became accessible. To give a sense of the depth of the screens, the identical gain-of-function *ras* (*let-60*) mutation at residue 13 was isolated independently five times in direct screens. It's likely that every possible G to A transition in the genome that can be generated by EMS mutagenesis has been sampled for its effect on vulval development.

Genetic characterization after mutant isolation is where much of the work and artistry of genetic analysis occurs. Horvitz and his colleagues asked what a developmental control gene would look like, what a particular kind of mutant said about the normal function of the affected gene, and how you could use different classes of mutations to dissect functions of the genes. Performing laser ablations in mutant backgrounds refined the models for gene action and cell interactions. These

ideas echoed and inspired similar ideas in *Drosophila* developmental genetics, which was blossoming in the same period of the 1970s, and which was recognized by the recent Nobel Prize to Ed Lewis, Christiane Nusslein-Vollhard, and Eric Wieschaus. Where the *Drosophila* groups elucidated the earliest events of embryonic patterning, vulval analysis presented a first model for organogenesis. The gonad, epithelial cells of several types, muscles, and neurons assembled into the egg-laying structures. Work from Horvitz's group identified the genes and signaling pathways that allowed these cells to coalesce. The initial trigger was the signal from the gonad that induced vulval cell fates, but later signals between epithelial cells refined their choices. The muscles knew their fates on their own, but migrated to the gonad by directed chemotaxis. The neurons targeted their axons and branched at the vulval epithelium to complete the innervation of the organ.

Sulston and his collaborators initiated the genetic analysis of programmed developmental cell death. Sulston isolated the first cell death mutant, *nuc-1*, which has defects in a DNase that breaks down chromatin in dying cells. Ed Hedgecock, a postdoctoral fellow at MRC-LMB in the early 1980s, found the first two *ced* (cell death) genes that affect multiple aspects of cell death. In *ced-1* and *ced-2* mutants, cell corpses persisted long after they would usually be engulfed and destroyed. These mutants were unexpected, since they indicated that removing the debris after cell death is not a generic housekeeping function but an active process that is under specific genetic control. Hedgecock later switched his interests to neural development, and led the group that defined the first instructive axon guidance cue (UNC-6, which, like its vertebrate counterpart Netrin, patterns axons along the dorsal-ventral axis). Horvitz, long fascinated by the phenomenon of cell death in development, continued the *ced* screens. He and his student Hilary Ellis began to seek a different class of mutations, those that would prevent all cell deaths. The screen was facilitated by the original *ced-1* mutant, because suppression of *ced-1* was a much easier screen than a direct screen for cell number. Three central genes emerged over the next few years, *ced-3*, *ced-4*, and *ced-9*. Two genes, *ced-3* and *ced-4*, were required to promote all cell deaths. The third, *ced-9*, which was identified based on a rare gain-of-function mutation, prevented all cell deaths.

Cell death was known to occur in vertebrate development, and the notable work of Rita Levi-Montalcini had shown that it could be prevented by NGF (nerve growth factor). The principle of a protective factor for cell survival was established. But the idea of specific death genes like *ced-3* and *ced-4* was new. Those who studied developmental mammalian cell death had shown that it had specific morphological features that suggested something beyond starvation or explosion, and coined a term for that morphology of death, apoptosis. *ced-3* and *ced-4* mutants suggested that the apoptotic morphology corresponded to the functions of specific genes. Moreover, using genetic mosaic analysis, Junying Yuan and Horvitz showed that *ced-3* and *ced-4* carried out their functions in the dying cell. Here was another surprise: the cell committed suicide.

The Molecules

By the end of the 1980s, it was time to make the transition from genes to molecules. Each organism had to take its own path to molecular biology, and in *C. elegans*, as in many others, transposable elements were the sequence tags that make gene cloning possible. The first vulval gene to be isolated was *lin-12*, cloned by Iva Greenwald at the MRC-LMB, who reasoned that a wild strain with high copy number of the transposon Tc1 could give rise to spontaneous mutations with Tc1 insertions. *ced-3* and *ced-4* were cloned based on insertions of the transposons Tc3 and Tc4, a transposon discovered in part because of the insertion in the *ced* mutant. Yet transposons in *C. elegans* have never been as well-behaved as the *Drosophila* P element transposon, and something else was needed. Sulston emerged again as a leader, with another "large vague project"—he had a plan to create a complete physical map of *C. elegans* clones. With Brenner's support, the first step of the *C. elegans* genome project began. Like the lineage map, the physical genome map required someone with the patience and drive to complete a huge endeavor, qualities that Sulston had already demonstrated. Unlike the lineage map, one group, no matter how skilled, could not hope to do it alone. Here, the lineage relationship of *C. elegans* researchers became valuable, because their shared experiences as a community encouraged cooperativity between groups. Essentially all researchers agreed to contribute their mapping and cloning data to the assembly of the physical map, long before publication. The genes cloned by individual groups became the anchors for the assembling physical map, facilitating the cloning of additional genes.

Several groups including Horvitz, Greenwald, and Sternberg were important players in the cloning of the vulval *lin* genes; Horvitz's group dominated the cloning of the *ced* genes. The molecular identification of genes in the vulval pathway began with the identification of *lin-12*, and the almost simultaneous cloning of the *Notch* gene in *Drosophila*. Importantly, these genes were found to be similar—very similar. Together with the cloning of the Hox genes in *Drosophila* and the identification of mammalian homologs, these results revealed a profound conservation of developmental control genes across the animal kingdom. Conservation had always been the hope for those working on model systems, but the phenomenology of development varies so widely between animals that it was not assured. In the end, with the complete picture from the genomes in view, the degree of conservation has come as a surprise even to those that love the worm and the fly.

Specific insights emerged from the analysis of the vulval pathway as well. *lin-12/Notch* genes were shown to be the key players in lateral signaling, a special kind of developmental induction in which two cells compete to receive different cell fates. After the EGF pathway was found to be the inducing signal for vulval development, pathway analysis in *C. elegans* and in the fly eye demonstrated that the most important pathway for EGF/tyrosine kinase receptor signaling involves Ras activation and the MAP kinase cascade. EGF (and other factors that act through tyrosine kinases) and Ras have central roles in controlling vertebrate cell proliferation and development. Before the worm and fly work, cell biological approaches had found a wealth of signals downstream

of the EGF receptor, and downstream of Ras, but had been unable to prove how the central information flows through the cell.

For the cell death pathway, the cloning of *ced-3* and *ced-4* provided unique insight into mechanisms of cell suicide. When Yuan and Horvitz first sequenced the clones, they were dissimilar to any known molecules, but as they puzzled through the molecular biology, a mammalian protease with homology to the unpublished CED-3 appeared in the literature. The similarity between CED-3 and ICE led to the model that protease activity triggered cell suicide. Caspases, the family of proteases related to CED-3, are now known to play roles in most cell deaths throughout animals. CED-4, related to mammalian APAF, is an activator of CED-3. CED-9, the death-repressing activity, was found to be similar to Bcl2, a mammalian oncogene that stimulated lymphoma formation by preventing cell death in B cells. Genetic analysis showed that *ced-9* was upstream of *ced-3*, and indeed the conserved Bcl2-CED-9 family controls the initiation of caspase activity in diverse animals and cell types. The perceived significance of CED-3, CED-4, and CED-9 has increased in the scientific world with the increasing appreciation of the importance of cell death in biology. During development, every cell chooses whether to live or die in response to its own condition and signals from other cells. Tumor cells are resistant to conditions that trigger cell death in normal cells, contributing to their runaway growth. Neurodegenerative diseases trigger programmed cell death pathways aberrantly, and blocking the caspases can delay or decrease neuronal cell death in response to disease or insult. *C. elegans* was the right place to begin the analysis of cell death because the simple lineage and small number of cells made it possible to know exactly which cells lived and died at single-cell resolution. Once the principles were established in *C. elegans*, they could be confirmed and elaborated in more complicated systems.

All three laureates have made other contributions of great significance. Brenner went on to tame another novel organism, the pufferfish Fugu, whose loyal following is smaller than *C. elegans*—so far. Horvitz's studies of lineage and fate have extended to developmental and functional neurobiology. Sulston found another large, less-vague project in leading the sequencing of the *C. elegans* genome and has also played a major role in human genome sequencing.

Brenner had a vision, Sulston created the tools to address it, and Horvitz brought the vision to fruition in a series of dramatic examples. The different scientific personalities were all essential to the final successes, which grew from Brenner's creativity, Sulston's tenacity and patience, and Horvitz's focus. They also provided leadership by example and by setting standards of scientific quality and completeness. Brenner took ten years to publish his first *C. elegans* paper in 1974, and *C. elegans* researchers are still known for publishing the fewest papers with the most tables.

The idea of working on *C. elegans* is still the idea of the bacteriophage. You find the simplest, most amenable animal, and you pick the simplest, most amenable problem in development, and there it is. It seems straightforward now with the awarding of the Nobel Prize. Yet Horvitz's graduate advisor told him that this postdoc-

toral choice would end his career, and Brenner recalls that in early years, *C. elegans* was considered a joke organism, often confused with the notorious flatworm of memory transfers. Brenner, Sulston, and Horvitz took risks, and they did hard experiments that took a long time. The early experiments were not flashy: the landmark Brenner paper was in *Genetics*, the Sulston and Horvitz lineage papers were in *Developmental Biology*. But there is a joy in risk. Sulston remarked a few years ago at a *C. elegans* meeting that it was a little sad that the talks were so good these days, because when a field is at the edge, some of the ideas are so wrong. For Brenner, Sulston, and Horvitz, the ideas were unconventional but right.