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OBITUARY

Obituary: John Sulston (1942-2018)

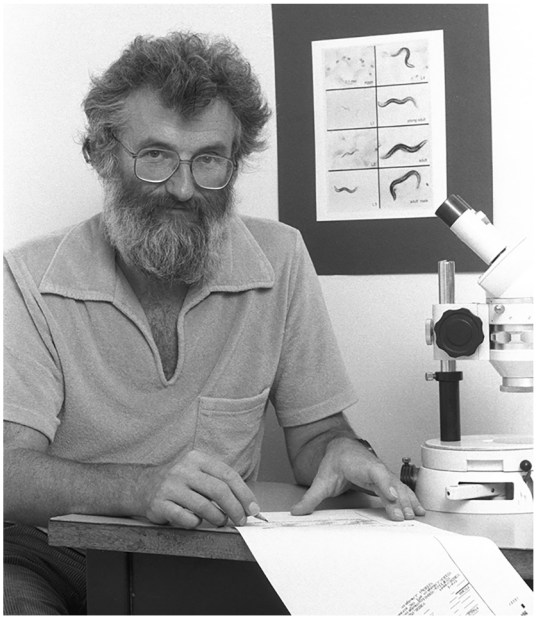
John White*

John Sulston, a pioneer in the developmental studies of the nematode *C. elegans* who went on to spearhead the sequencing of the genome of this organism and ultimately the human genome, died on 6th March 2018, shortly after being diagnosed with stomach cancer. Here, I reflect on John's life and work, with a particular focus on his time working on the developmental genetics and lineage of *C. elegans*.

In the late 1960s, the Medical Research Council backed an ambitious proposal by Sydney Brenner to establish a new model organism in which fundamental aspects of development and nervous system function could be investigated using the power of genetics. This organism was to be *Caenorhabditis elegans*, and John and I both joined Sydney's newly established operation at the Laboratory of Molecular Biology (LMB) in late 1969. John had done his PhD with Colin Reese on methods for the chemical synthesis of nucleic acids and had recently completed a postdoc with Leslie Orgel at the Salk Institute seeking pre-biotic organic molecules that can replicate. However, as new recruits to Sydney's *C. elegans* project we were both working on the nervous system of *C. elegans*: John was developing histochemical techniques to identify neurotransmitters while I was developing strategies for reconstructing the worm's neural circuitry from electron micrographs of serial sections. I first encountered John during the lunch breaks when Sydney used to regale us with his extraordinary monologues. John sat to one side not saying much and eating his lunch of crusty bread and cheese, brushing crumbs out of his beard.

John and I enjoyed a pint of beer on Friday evenings and so gradually got to know each other. His father was an ordained minister in the Church of England but John questioned Christianity as a teenager and became a devout atheist. However, Christian values were deeply engrained in him. He came over as very self-effacing and modest, but this hid an inner self-confidence and steely resolve. He lived frugally, eschewing material wealth and believing that one should work for the common good (a principle that extended to his scientific work: John was an early and strong proponent of the open data movement). He drove an ancient half-timbered Morris Traveller that he maintained himself. He was very fond of his garden (informal in style, with an appearance that somehow reminded me of his beard), where he and his wife Daphne grew vegetables. John had a great sense of fun and organised riotous punting trips and Guy Fawkes parties; I also remember him leading the conga at a Christmas party in the lab.

I discovered that we both liked making gadgets and, when we were young, used to undertake dangerous experiments in our bedrooms involving electric arcs between carbon rods fed with mains electricity. In the lab, John developed several gadgets and techniques, which all had an elegant simplicity and are still used in labs around the world. Early on, he developed a protocol for freezing worms for long-term storage, such that mutant strains could be maintained without continuous propagation, a tremendous advance for genetic studies. He later developed a simple method to mount worms, allowing live animals to be observed with the then newly available technique of Nomarski (differential interference contrast) microscopy. John solved the problem of picking up a small larva from a plate by using an eyelash attached by wax to a toothpick. He made microscope cross-hairs from gossamer so an observer could mark a point without obscuring the subject. John's ingenuity and resourcefulness continued to be deployed to good effect later on when developing methodology for large-scale sequencing.



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While developing histochemical techniques for identifying neurotransmitters, John made the surprising discovery that the number of cells in the ventral cord increased from 15 to 57 during the first larval stage. It was dogma at the time that nematodes did not undergo further cell divisions in non-gonadal tissues after hatching. Intrigued, John started to study newly hatched larvae using oil-immersion Nomarski optics. After much patient observation, he was able to determine that the extra cells were produced from ten blast cells by a stereotyped pattern of cell divisions. We were excited when we found we could assign lineage positions to classes of neuron that were being identified from the electron microscopy (EM) reconstructions we were undertaking, suggesting that lineage mechanisms might have a role in determining cell fate. Most

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surprisingly of all, some cells were seen to die soon after being born. The phenomenon of cell death was known to developmental biologists at the time, but was thought to be a method of sculpting structures such as the digits of a vertebrate appendage. John's observation showed that certain cells are destined to die soon after birth.

As part of his efforts to promote *C. elegans*, Sydney gave a series of inspirational lectures at universities in the USA that led to the recruitment of an extraordinarily talented collection of American postdocs to our laboratory. Several of these entered into highly productive collaborations with John. Notably, Bob Horvitz, a consummate geneticist, became fascinated with John's lineage studies and determined the postembryonic lineages of the muscle cells of the hermaphrodite while John tackled the technically challenging problem of lineaging the male tail. A few years later, Donna Albertson managed to do a partial EM reconstruction of the male tail and this enabled the assignment of cell types to positions on the termini of the lineage tree (Sulston et al., 1980). Judith Kimble lineaged the somatic gonad as a graduate student in David Hirsch's lab in Colorado, thereby finishing the postembryonic lineages. Soon after this work she came to Cambridge to become John's first postdoctoral fellow.

Bob and John began looking for lineage mutants, and were able to find 14 genes that gave altered lineages when mutated (Sulston and Horvitz, 1981). With Marty Chalfie, they discovered an interesting class of lineage mutant that gave reiterated lineages (Chalfie et al., 1981). Around this time, I became interested in the mechanisms that give rise to lineages – are they cell intrinsic or due to inductive signalling from external entities? Donna and I did an experiment in which we killed the cells of the gonad primordium. Much to our surprise, the vulva, which John had shown does not arise from the primordium, nevertheless did not form. This observation encouraged me to develop a laser microbeam system that could kill individual cells with minimal collateral damage. John and Judith used the system to good effect to show that some lineages appeared to be autonomous whereas others required the presence of an adjacent cell (Sulston and White, 1980; Kimble, 1981). Interestingly, some of the lineage changes seen on cell ablation were similar to those seen in certain mutants, leading to the startling insight that these mutations were probably in genes that were part of an intercellular signalling mechanism.

Some time later, Ed Hedgecock and John sought mutants that affected cell death. They found that most of the deaths were suicides rather than murders perpetrated by adjacent cells (Hedgecock et al., 1983). Bob Horvitz carried on this line of enquiry when he set up his own lab at the Massachusetts Institute of Technology (MIT), studies that ultimately led to the identification of the genes that act during cell death. Similar genes are found in vertebrates and serve crucial roles in eliminating immune cells with inappropriate affinities and cells with damaged DNA that could lead to a tumour if allowed to proliferate.

Marty Chalfie became interested in studying mechanotransduction and, with John, sought mutants that exhibited abnormalities in their response to mechanical stimulation. This work ultimately led to the identification of classes of neuron that mediated a touch response (Chalfie and Sulston, 1981). By this time, the nervous system reconstruction was proceeding apace and the circuitry of the touch neurons had been determined. John did some strategic cell ablations on developing larva, which allowed Marty to come up with a credible description of how the touch circuitry functions (Chalfie et al., 1985). This was an exciting time for us as it indicated that by using a combination of connectivity, mutants, cell ablation and behavioural observations it was possible to deduce how a sensory signal is transduced into a behavioural response.

Probably John's best-known work in *C. elegans* is his reconstruction of the embryonic lineage. Lineage studies of nematode embryos had been attempted at the turn of the 20th century, but these had not gone beyond a few divisions. Sydney was interested at the outset of the *C. elegans* project in the possibility of determining lineages from live specimens using Nomarski optics. Early attempts to do this by photographing optical sections and trying to piece together the sequence of cell divisions post hoc were not successful and the project languished. After John had published his work on post-embryonic lineages, other groups initiated tentative attempts to determine the lineage of the developing embryo. In 1977, a dispute arose around a study from another laboratory on the lineage of the gut cells in the embryo. John was asked to adjudicate and quickly found that the lineage, which was about to be published, was wrong. This prompted John, after much soul searching, to set out to determine the complete lineage of the embryo by direct observation. This was a monumental task because the cell cycle is short and the visibility within the developing embryo is very poor away from the surface. John focussed on this seemingly impossible task with great determination, shutting out all distractions. He would disappear into his small microscope room for two four-hour stretches each day. All we could hear was the slapping on the bench of the coloured pencils that John used to draw diagrams of the group of cells he was following (colours indicated depth). Fortunately, the embryonic lineage turned out to be invariant, allowing John to focus on a few cells at a time and build up the lineage piecemeal. After 18 months, John emerged and announced that he had completed the lineage. There was no way that this could be validated except by someone else repeating what John had done, a near-impossible task. But such was our faith in John's abilities that we were convinced that the lineage that he produced was correct, which indeed turned out to be the case. However, there was one final challenge: a lineaged embryo needed to be fixed, sectioned and reconstructed from electron micrographs in order to assign cell types to the terminal twigs on the lineage tree. This was a particularly difficult undertaking in that it required John to identify every single cell in a single embryo and then give this to Nichol Thomson, our electron microscopist, to fix and section. Embryos are notoriously difficult to prepare for EM because the eggshell presents a permeability barrier. We held our breath and crossed our fingers and eventually a pile of EM pictures was produced. They were murky, but good enough for us to reconstruct the embryo and identify cell types. The lineage was now complete (Sulston et al., 1983). Through these studies, John had opened the door to developmental studies of *C. elegans*, work for which he was awarded the Nobel Prize along with Sydney Brenner and Bob Horvitz in 2002.

At the time that John had finished his lineage, there was considerable effort being expended by the *C. elegans* community to identify the physical of location of genes defined by mapped mutations as a prelude to sequencing. Usually this was done by walking away from a known region of chromosome towards the region containing the gene of interest. John realised that a complete physical map of the *C. elegans* genome would considerably facilitate this task for the whole community. Bob Waterston, a postdoc at the LMB, shared John's enthusiasm for projects that benefitted the community. He had recently taken up a position at Washington University in St. Louis and agreed to collaborate with John in an ambitious project to map the whole *C. elegans* genome. Progress on the mapping project was relatively swift and enabled the

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rapidly expanding number of investigators studying *C. elegans* to identify their gene of interest on the physical map (Coulson et al., 1986).

John and Bob (Waterston) came to the conclusion that the logical extension of the mapping project was to sequence the whole genome, a radical step. To this end, they recruited a group of highly talented people, including Richard Durbin, a mathematician who had been studying the development of the nervous system in *C. elegans*, and Alan Coulson, who had worked with Fred Sanger developing DNA sequencing methods. At the time, sequences were starting to be obtained from some prokaryotes, but nothing like the ∼100 megabases of the *C. elegans* genome; despite the huge challenge, this was achieved by 1998 (C. elegans Sequencing Consortium, 1998). Furthermore, they felt that sequencing the worm could be a prelude to sequencing the human genome (which is 30 times larger), a glittering prize that could bring untold benefits to human health and welfare. The resources needed to sequence the human genome were huge and required the establishment of purpose-built centres. Bob Horvitz managed to convince his PhD supervisor, Jim Watson, that this was a feasible project and Jim put his weight behind the mounting effort to establish a multi-centre human genome sequencing initiative. John was at the centre of all this activity and was determined to make the project happen, even if it meant dressing up in suits to persuade politicians and senior science administrators that such an enterprise not only had enormous potential benefits but also was becoming feasible. The biotech industry was also becoming interested in investing in large sequencing projects, with the idea that any sequence data they obtained could be patented. This was anathema to John who passionately believed that all human sequence data should be in the public domain. Other groups suggested limiting sequencing to expressed regions of the chromosomes. John disliked this approach as he realised that non-coding regions contained all the crucial information of when and where genes are expressed, and so needed to be sequenced. Eventually, a successful international collaboration was established between the Wellcome foundation and the Medical Research Council in the UK (centred at the Sanger Centre, where John was director) and the National Institutes of Health in the USA. This large enterprise was a resounding success, and the completion of the human genome sequence was announced in February 2001 (Lander et al., 2001). All sequence data were made open access. This fascinating episode of scientific accomplishment is vividly described in a book that John wrote with Georgina Ferry (Ferry and Sulston, 2002).

Once the human genome sequence had been finished, John left the world of ‘big science’ and joined the faculty of the Institute for Science Ethics and Innovation at Manchester University, where he concerned himself with the ethical ramifications of our knowledge of our DNA sequence.

John died at home in sight of his beloved garden. He had a simple humanist burial and lies in a cardboard box in a grave in Cambridgeshire that has no enduring markings. I can think of no other scientist of his generation who has made more of a difference to our understanding of basic biological processes and to the wellbeing of the human race.

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