Sydney Brenner (1927–2019)

Those of us who started graduate school in any branch of life sciences in the 1990s and early 2000s are familiar with 'Uncle Syd' who wrote articulate, irreverent and funny columns, that gave us reprieve from the daily grind of the laboratory (https://www.cell.com/current-biology/libraries/loose-ends). They all had a point of view: about biology (the one on redundancy is not to be missed), career tracks in science and what promotions in academia do to take you away from doing research. Those letters came out in the beginning of each month as part of 'loose ends' in *Current Biology*, offering advice on all aspects of a scientific career. Today, almost 20 years after Sydney Brenner's last column, a lot of what he had to say still rings true and many are prescient, e.g. computational approaches to understand biology (https://www.cell.com/current-biology/libraries/loose-ends).

Early career and the triplet codons

Brenner was born in South Africa and after completing his Master's degree in Johannesburg, moved to Great Britain for his graduate study. He was a graduate student in chemistry at Oxford University during the time when Francis Crick, James Watson, Rosalind Franklin and Maurice Wilkins had unravelled the structure of the DNA[1–4]. After obtaining his doctorate in chemistry, Brenner became part of the Laboratory of Molecular Biology at Cambridge, where he spent the next 20 years of his illustrious career (https://www.salk.edu/scientist/sydney-brenner/).

The early 1960s saw scientists grappling with an important question in molecular biology. How could four nitrogenous bases code for 20 amino acids? This period saw fruitful collaborations between Brenner and Crick, who along with Nerenberg and Matthaei from National Institutes of Health, USA deciphered the triplet RNA/DNA code for amino acids. Brenner, Crick and colleagues were interested in understanding how four bases could code for 20 amino acids. They surmised that multiple bases would code for a single amino acid. Although techniques like sequencing and PCR were not available at that time, the scientists did some elegant experiments to find out the number of bases coding for an amino acid. Brenner and colleagues used proflavin to induce mutations in a bacteriophage gene and found that when proflavin caused insertion or deletion of bases, the gene would be rendered non-functional. However, if three bases were added or deleted, the gene remained functional. Further, when the DNA sequence of the gene had a single base-pair deletion that rendered the gene non-functional, functionality could be restored by inserting a base-pair in the general area of the deletion. These important experiments indicated that the genetic code used triplet codons. At the same time, Nirenberg and colleagues discovered the first of the 64 codons that code for amino acids. They ruptured *Escherichia coli* bacterial cells to release the contents of the cytoplasm. This allowed for a cell-free system where the scientists could add a specific RNA and find the protein that was synthesized by that RNA in a controlled manner. To this system, they added a poly-U RNA and went on to extract the protein that was synthesized by this RNA. This protein was entirely composed of the amino acid phenylalanine, indicating that the code for phenylalanine was UUU[5–11]. Together, these and other experiments showed that each amino acid was coded for by three bases or codons. Very soon the codons for each amino acid as well as the stop codons for ending a protein chain were found, thus allowing us to understand what is today known as the central dogma of molecular biology, i.e. DNA → RNA → protein.

An elegant geneticist

After working with bacteria to understand the triplet codons, Brenner brought to the forefront the study on *Caenorhabditis elegans*, a free-living soil nematode. He, like many at the time, felt that one of the frontier areas in biology was understanding development and the nervous system. His thoughts on how the organism was chosen are quoted below[12]:

'Part of the success of molecular genetics was due to the use of extremely simple organisms which could be handled in large numbers: bacteria and bacterial viruses. The processes of genetic replication and transcription, of genetic recombination and mutagenesis, and the synthesis of enzymes could be studied there in their most elementary form, and, having once been discovered, their applicability to the higher forms of life could be tested afterwards. We would like to attack the problem of cellular development in a similar fashion, choosing the simplest possible differentiated organism and subjecting it to the analytical methods of microbial genetics. Thus we want a multicellular organism which has a short life cycle, can be easily cultivated, and is small enough to be handled in large numbers, like a micro-organism. It should have relatively few cells, so that exhaustive studies of lineage and patterns can be made, and should be amenable to genetic analysis.'

Brenner describes the characteristics of a model organism, the description used by him is used even today during talks by worm biologists.

'We think we have a good candidate in the form of a small nematode worm, *Caenorhabditis briggsae*, which has the following properties. It is a self-fertilizing hermaphrodite, and sexual propagation is therefore independent of population size. Males are also found (0.1%), which can fertilize the hermaphrodites, allowing stocks to be constructed by genetic crosses. Each worm lays up to 200 eggs which hatch in buffer in twelve hours, producing larvae 80 microns in length. These larvae grow to a length of 1 mm in three and a half days, and reach sexual maturity. However, there is no increase in cell number, only in cell mass. The number of nuclei becomes constant at a late stage in development, and divisions occur only in the germ line. Although the total number of cells is only about a thousand, the organism is differentiated and has an epidermis, intestine, excretory system, nerve and muscle cells. Reports in the literature describe the approximate number of cells as follows: 200 cells in the gut, 200 epidermal cells, 60 muscle cells, 200 nerve cells. The organism normally feeds on bacteria, but can also be grown in large quantities in liver extract broth. It has not yet been grown in a defined synthetic medium.

To start with we propose to identify every cell in the worm and trace lineages.

We shall also investigate the constancy of development and study its control by looking for mutants.'

Although the above quote refers to *C. briggsae*, in due course *C. elegans* was selected as the preferred organism. Thus, this was almost a textbook case of an organism waiting to be chosen for genetic studies.

C. elegans had been previously studied in the 1940s by Victor Nigon and Ellsworth Dougherty[13]. However, it reached its current status of the model organism of choice for neurobiologists, geneticists and cell biologists thanks to Brenner. He embarked on the project of working out the genetics and other cell biological aspects of this small roundworm[14]. He led from the front in isolating mutants[15], being part of the reconstruction of the entire nervous system (still the only organism for which it has been achieved)[16–18], and finally being part of recognizing the value of a physical map[19].

Brenner along with a number of colleagues studied many aspects of neuronal development and propagated the use of this system in multiple parts of the world, including England, parts of Europe, many parts of USA and India. His work along with that of his colleagues John Sulston and Robert Horvitz, who worked on detailing the cell lineage of the organism and on programmed cell death during development of *C. elegans*, resulted in a Nobel Prize in Physiology or Medicine in 2002. During his Nobel lecture titled 'Nature's gift to science', Brenner spoke about how choosing the right organism for one's research is as important as the research question. He went on to pay tribute to *C. elegans* and spoke about how genetics together with cell-biological approaches in this nematode could allow scientists to study how genes allow for specification of complex structures[20].

Indian connections

Towards the early 1990s, Brenner became interested in the small genome size of the vertebrate *Fugu rubripes* (pufferfish). He was involved in sequencing the genome of this fish with colleagues in Singapore and Cambridge. Brenner became associated with the Institute of Molecular and Cell Biology (now IMCB-A*STAR) at Singapore in the mid 1990s, where he collaborated extensively with Byrappa Venkatesh (who also spent a few years as a postdoc with Brenner at Cambridge) to sequence parts of the pufferfish genome[21]. Brenner used to spend weeks visiting and talking to researchers, including students and postdocs in the corridors of IMCB. He became an honorary citizen of Singapore and started pursuing his interests in evolutionary studies there. He continued to visit and live in Singapore for the next two and a half decades until his demise. In fact, he co-authored a paper on lamprey evolution from Singapore at the age of 90 (ref. 22).

Apart from Venkatesh, three Indian academics worked with Brenner at the LMB. Anand Sarabhai did his Ph D under Brenner in the 1960s. He worked on termination of polypeptide chains with Dick Epstein and Sydney Brenner[23,24]. He also went on to define the properties of the region between the stop codon of one gene and the start codon of the next gene in operons[25]. Padmanabhan Babu spent three years with Brenner at LMB, during which time he isolated mutations in *C. elegans* using phosphorous-32 (ref. 26). One of the mutants he isolated, *lin-4*, was important in cell lineage studies in the worm[27]. Babu went on to study worms in the late seventies and early eighties at Tata Institute of Fundamental Research (TIFR), Mumbai. According to Babu, Brenner was an encouraging mentor and was happy to have him move with the worm to TIFR, where he already knew and was in constant touch with Obaid Siddiqi. After Babu, R. N. Singh from India went to work at Cambridge, with John White and John Sulston. Now there are about a dozen *C. elegans* laboratories in India that study different aspects of development, cell biology as well as neurobiology.

Brenner was in touch with a subset of leaders of the biology community in India and visited the country multiple times. Obaid Siddiqi (founder of the Molecular Biology Unit at TIFR, Mumbai), Anand Sarabhai, Pushpa M. Bhargava (founding Director of CSIR-Centre for Cellular and Molecular Biology, Hyderabad) and others met with Brenner often, and some of their correspondence can be found at the Cold Spring Harbor archives and The Wellcome Library archives. In the early 1970s Brenner visited TIFR, Mumbai and interacted with the scientists there (Figures 1 and 2). In fact, his greatest impact on Indian biology seems to have been at the small Molecular Biology Unit at TIFR, where a significant number of scientists turned their attention to neurobiology that continues to remain a strong area of research to this day. In 2002, Brenner visited biotech and other technology-oriented companies in India, sponsored by Singapore government organizations. He also visited research institutions like the Centre for Human Genetics, Bengaluru, TIFR, Mumbai and National Centre for Biological Sciences (NCBS-TIFR), Bengaluru. Sharat Chandra (Director, CHG) remembers hosting Brenner at CHG, where he delivered the David Hungerford Memorial Lecture in 2005 (Figure 3). The

Figure 1. Sydney Brenner with Obaid Siddiqi and Anand Sarabhai at TIFR, Bombay. Courtesy: P. Babu and K. VijayRaghavan.

past few decades saw Brenner becoming interested in theoretical biology, a subject on which he gave many talks. As recently as 2012, he delivered a lecture on the architecture of biological complexity at the International Centre for Theoretical Sciences (ICTS-TIFR), Bengaluru.

K. VijayRaghavan (Principal Scientific Advisor to the Government of India) shared his impressions on Brenner's influence on Indian science: 'Sydney Brenner's association with India goes back to a time well before his first visit to the Molecular Biology Unit of the Tata Institute of Fundamental Research. In the efforts to decipher the genetic code, Brenner (whose collaborators at the time included Anand Sarabhai from India) was competing with Alan Garen and Obaid Siddiqi. Garen commented, crtically but with effusive praise, on Brenner about the latter's use of the "Occam's broom"[28,29]. Siddiqi's stay in Alan Garen's laboratory after his Ph D with Guido Pontecorvo allowed him to get to know all the leaders of the new field of molecular biology. On returning to India to start the Molecular Biology Unit, Obaid invited them to teach course[29] in Bombay. Brenner visited at least once, perhaps twice in the 1960s–early 1970s. These visits by Brenner and others had a deep impact in shaping the science at the Molecular Biology Unit. Soon, most of the founders of molecular biology decided to move to "solve" the nervous system and understand how the brain works. Max Delbruck chose a fungus, *Phycomyces*, to understand behaviour, a choice whose time was yet to come. Gunter Stent chose the leech. Stent's school succeeded in making the leech an extraordinary preparation linking cellular physiology to behaviour. Seymour Benzer, seeing to link genes to behaviour, just as Morgan and his school had linked genes to visible phenotypes, used the fruit-fly as a model. Brenner, independent-minded as always, chose the worm. He turned the argument of the (supposedly) limited behavioural repertoire of the worm to argue that this was all the more reason to more readily unravel the links between genes and behaviour. The Molecular Biology Unit was also caught, willingly in the intellectual tsunami of change to neurogenetics. P. Babu, a physicist from the Tata Institute visiting Murray Gel-Mann at Caltech was invited to join the Molecular Biology Unit and then Brenner's group visited at the MRC Laboratory of Molecular Biology. He came back and introduced the worm to India. Babu was followed by R. N. Singh, who worked with John White and John Sulston, pioneering the use of electron microscopy to study the nervous system of the worm. Both Babu and Singh, however moved to study *Drosophila*. In this latter system, the interactions with the LMB grew with Brenner's help. I and then K. S. Krishan visited for several months each interacting with Brenner and his *Drosophila* colleagues. Much of the culture of the Molecular Biology Unit is owed to these interactions and those by visitors from the LMB, particularly Mike Wilcox, Peter Lawrence and Graeme Mitchison. Brenners later visits to India where to Bangalore. He was invited to the 100th Anniversary celebrations of IISc. There, in a 45-minute lecture, Obaid's introduction of Brenner took 40 min. Going up to the podium, Brenner impishly asked, "Any questions", as if his lecture had ended. He also visited NCBS in that visit, talking about the differences between genetics and genomics. Brenner helped grow the A*Star system in Singapore and visited NCBS to work on collaborations with India. Soon after the visit, he was awarded his Nobel Prize. Some years later, in helping invite him to India again, I wrote warning him that another visit to NCBS could lead to another Nobel. He wrote back immediately agreeing to visit. This was in 2012 (I had to skip that visit as I was hospitalised by an attack of dengue). Brenner's Turing lecture in ICTS was, as always, incisive. He pointed out that biology could not escape the onslaught of big-data and this onslaught was to be welcomed. However, data would not solve deep questions in biology, for which theory and a bottom-up approach, keeping cells as the centre of abstraction was needed. He regarded

Figure 2. Sydney Brenner with Pushpa M. Bhargava and colleagues having tea at TIFR, Bombay. Courtesy: P. Babu.

Figure 3. Sydney Brenner delivering the David Hungerford Memorial Lecture at CHG, Bangalore. Courtesy: Sharat Chandra.

escapes into "holistic" approaches which were not anchored in cells and genes, as pointless.'

Sydney Brenner's life and work are and will continue to be an inspiration for generations, for he was someone for whom science was both an adventure and a way of life.

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