This is a potted, subjective history of my scientific life

My background and expertise lies in DNA sequencing and that is what brought me to Cambridge. My first visit was a day trip, to pick up some sequencing tips from Bart Barrells' people at the LMB. At that time: in my first job since leaving university, I was very happily employed as an assistant in Bob Honess's lab at Mill Hill. These were my formative years in science. During this brief period, I entered a romance with Bob's PhD student, John Nicholas. I accompanied him to the USA for his postdoc and we worked on the same bench in Joe Nevins' lab at Duke University. For me, this was a very happy time.

At the end of John's postdoc, he wanted to return to Mill Hill, then stay if possible. I thought about settling in the USA. However, I decided in the end, to come back to the UK. I came to John Sulston's group at the LMB, to start the *C.elegans* genome sequencing project ***picture***

These were formative years too, but very different, and a watershed. Tragically, Bob Honess got cancer and was soon dead: by the age of 43 ***picture***

This event had repercussions which affected every aspect of my life. John Nicholas ended up back in the USA and has ended up working on KSHV ***picture***

I had the notion that I would be happier doing small group research, so when the time came, I did not follow John Sulston to the Sanger Centre, but stayed on at the LMB.

Back then in 1992, EST sequencing was, um, Hot Stuff ***Venter * - *picture***

- so - the plan cooked up, was for me to do an EST sequencing project on Rat brain, in order to find new genes important for brain development. In keeping with the legacy of Sydney Brenner, John Sulston handed me over to his colleague, Michel Goedert, who Sydney had originally hired to "solve the retina" but who was now making great strides in Alzheimers disease research.

The plan was for the EST sequencing project to be a formal collaboration between the LMB and the nascent Sanger Centre, but this was *before* Wellcome Trust funding had been secured. I started - then continued, single handed: library construction, sequencing, sequence analysis etc. Unfortunately, the work never did become a collaboration, with either one side or the other!

A bit later, when the Sanger Centre was up and running, Michel and I were incorporated into the new LMB division of Neurobiology, which Michel is now head of. Somehow, my work slipped under the radar. Michel was not interested and just left me to it. Now that I was securely employed, I decided to carry on and make the most of it: following my own interests and seeking wisdom. I tried to stay aligned, by concentrating on genes relevant to ongoing work in the division.

picture My work with Synaptotagmins began in the mid-90s when I identified a novel brain EST similar to synaptotagmin, a protein thought to be the calcium trigger for synaptic vesicle exocytosis. I obtained the full length sequence of the gene represented by this EST. By the time it was published, it was the 5th described member of a rapidly expanding gene family.

picture Synaptotagmin was originally identified by a method commonly used at the time: monoclonal antibodies raised against a tissue of interest, were used to pick out individual tissue components. Such antibodies labelled a synaptic vesicle protein. When the corresponding gene was cloned, it was called synaptotagmin.

Once the gene was cloned, it was possible to search for gene relatives by DNA hybridization. In this way, during the 10 years pre-genome, 13 different Synaptotagmin family members were identified in rodents. Synaptotagmin relatives were found in other model organisms too, demonstrating evolutionary conservation. Genetic experiments with the original Synaptotagmin, **Syt1**, in flies, worms and mouse, showed that this protein is required for SV exocytosis.

On finding **Syt5** among my ESTs, I dutifully mapped the human gene to chromosome 19 where it turned out to be a neighbour of **Syt3**. During this mapping exercise, I became aware of splice variants lacking the Transmembrane domain ***picture*** Since the Transmembrane domain is important in anchoring Synaptotagmins in membranes, I was curious to know whether the same thing might affect other Synaptotagmin genes.

I used RNase protection assays directed at this region in different Synaptotagmin genes, to tackle this question ***picture*** This showed that some Synaptotagmins **do** express variants altered in this region, and that sometimes, Rat and Human variants are different.

All of this work was done pre-genome, but with the turn of the century, whole genome sequences appeared. 2 group leaders in the Neurobiology division, are interested in how synaptotagmin functions as a molecular machine ***picture***

I figured I could contribute something, by looking to see what the new genome sequences could tell us about Synaptotagmin functions.

More and more genome sequences have appeared over the years, so my efforts to pull out the Synaptotagmins has been an ongoing thing. As a complement to my colleagues' efforts to understand **"how it works"** ie. what are the biophysical properties of synaptotagmin, my comparative evolutionary studies could consider **"how it works"** by asking *"what is the nature of a Synaptotagmin gene?"* and *"how many Synaptotagmin genes might an organism have - or need?"*

During the last 10 years, I have been searching the primary nucleotide sequence databases, with these questions in mind ***picture*** My paper, published this year, is a culmination of this work, including 46 genomes.

This work has revealed some surprises ***picture***

One is the presence in plants, of genes closely resembling animal Synaptotagmins. One of the plant proteins has been investigated so far: it is a plasma membrane protein with similar biochemical characteristics to synaptotagmin. Plants have no nervous system, so how do these proteins function in plants? Can this question inform the study of animal synaptotagmins?

In order to classify the plant genes, I had to consider ***what is it,*** that makes a Synaptotagmin gene a Synaptotagmin gene. By examining plant and animal genome sequences, it was possible to track separate gene lineages, by their individual, conserved intron patterns. This showed that the plant and animal proteins are not homologous. It also showed little evolutionary divergence among the plant genes. In stark contrast, the metazoan Synaptotagmin genes show enormous diversity in number and type ***picture***

While some of the simplest animals on the planet, have large numbers of Synaptotagmin genes, the functions of which are currently pure speculation, other more complex animals have only a limited repertoire.

The Calcium trigger function of certain Synaptotagmins, highlighted here in red, appears to be a specialization, retained by purifying selection. It appears to be a function which became important, before the acquisition of a nervous system, as suggested by the highly conserved **Syt1** gene of *Trichoplax*, a basal metazoan which lacks a nervous system.

The biological meaning of some striking patterns of sequence conservation exposed here, remain to be investigated. I hope that my gene collection will be useful to experimentalists trying to understand **"how these genes work"** in the biology of their multicellular hosts.

Now ... ***picture* ...** as became apparent almost straight away, my decision not to go forth with the Nobel prize-winning John, was a serious mistake. I chose security, but my last 15 years at the LMB were lonely and arduous. It was not in anyone's interests, to get involved with what I was doing. Eventually, I reached a brick wall, painfully.

picture My interests do not lie in a mechanistic understanding of protein function. I have always been interested in genes and how they work.

Now, I must find a happier place in which to pursue the next phase of my life. I would like to make a contribution, as part of a well-functioning team.

picture Making music **IS** an area in where my team playing abilities are consistently put to use: whether as a member of a piano trio, a pit orchestra or symphony orchestra.

Update July 2018

After two Cambridge University research contracts, lasting from 2010 to 2017, I decided to abandon science in favour of a potentially happier route forwards as a musician. So far, so good!