Technological Tales: the Student's Story

Philip Board

The field of biochemistry and molecular biology has undergone constant change over the last 50 years and nowhere is this more evident than in the training of graduate students. The rate of change is increasing and the last 10-15 years has seen a quantum shift in the characteristics of typical PhD projects. As the result of developments in technology and instrumentation such as recombinant DNA, mass spectrometry or genome array analysis, the projects undertaken in the 1950s and 1960s that provided the basic building blocks of our present knowledge, do not even provide a starting point for the present generation of students. For example, let's compare the situation of a student in the late 1950s-early 1960s with one who is just starting in 2005.

Earl E. Bird was a student of the now legendary Professor Hans Metre who immigrated to Australia after World War II and is well known for his fundamental contributions to our understanding of The Career Academic (TCA) cycle. This pathway uses eager young scientists as a substrate and processes them through several enzymatic steps including MSc-PhD isomerase, PhD reductase, PhD kinase and finally PhD synthase to generate trained biochemists and molecular biologists. Although subject to feedback inhibition, some of the products recruit more substrate and perpetuate the cycle. Earl E. Bird was given the project of characterising PhD synthase from an Australian species as almost all previous work on this pathway had taken place overseas.

Bird started out by undertaking a literature review and spent several weeks in the library reading papers and making notes on an ingenious card index system. Bird was very proud of this novel system and was always pleased to demonstrate his capacity to retrieve references on a specific subject by passing a knitting needle through the holes punched in strategic places on the periphery of the cards. Unfortunately because delivery of scientific journals to Australia was by sea mail, the latest issues in the library were 6-12 months old. When he finally started in the lab, Bird had to screen a range of species to identify a good source of the enzyme and had to follow this by optimising the growth conditions for the best yields. While this was frustrating work, it was considered novel and ultimately gave rise to two chapters in Bird's thesis.

When Bird started to purify PhD synthase, he had a range of techniques available including ammonium sulphate precipitation, paper chromatography, ion exchange chromatography and starch gel electrophoresis. Towards the end of his time as a student, Bird was able to get hold of some Sephadex which allowed gel filtration and revolutionised protein chromatography. In order to measure synthase activity, Bird used a filter colorimeter and was required to record galvanometer deflections one sample at a time and construct a standard curve for each experiment. After purifying his enzyme, Bird determined its molecular size, its activity in a range of buffers and the effect of pH and ionic strength. Bird went on to study some reaction kinetics and to evaluate the effects of a range of inhibitors. A subsequent student in the lab used Bird's purification technique and used atomic absorbtion spectrometry to show that PhD synthase is a metalloenzyme and determined the amino acid composition by ion exchange chromatography. Initially, Bird under took most of his calculations with the aid of a slide rule and was very pleased when Professor Metre had a rush of blood to the head and decided to purchase a new mechanical calculator. After spending years chatting up Professor Metre's secretary, Bird convinced (bribed) her to type his thesis with the required triplicate carbon copies.

After graduating Bird received a glowing reference from Professor Metre and was appointed to an academic position in A New University (ANU). Although now retired, Bird continues to take an interest in the students in his former department, particularly in John E. Comelately who is currently following in his footsteps and investigating the TCA cycle. At the start of his project, Comelately has just undertaken a literature search. This was accomplished from Comelately's desk via his personal computer that is connected to the internet and PubMed. Comelately does not take notes on the papers he reads but simply files PDFs on his hard disk. He also subscribes to a free publication awareness service that provides daily email notification of new papers of interest.

Comelately's review revealed that there was already a lot known about the enzymes making up the TCA cycle. In the last few years their sequence had been determined

by cDNA cloning, their genes had been cloned and mapped and the structure of each protein had been determined by X-ray crystallography or NMR analysis of recombinant protein. However, Comelately is excited by other questions. He wants to know how the cycle is regulated and how it impacts on other cellular processes and how it varies in different individuals and different disease states. For these experiments, Comelately has a vast armory of techniques, kits, off-the-shelf reagents, services and instrumentation at his disposal including recombinant DNA technology (PCR, DNA sequencing, mutagenesis and 'Swiss army knife' cloning vectors). He could use mutagenesis to generate modified recombinant proteins, or label proteins and use confocal microscopy to track them in cells. He could also study the factors regulating gene expression and identify trace amounts of novel transcription factors by mass spectrometry and bioinformatics. If he chose to look at the impact of genetic defects in the TCA cycle he could selectively knock out genes, or he could use siRNA to knock down gene expression. In these manipulated systems, Comelately could study interactions and the effect on the expression of other genes by DNA array techniques.

While undertaking his project, any numerical data will be gathered by computers and downloaded for analysis onto Comelately's PC. Similarly, pictorial data will be stored as digital images. When he completes his research, Comelately will type his thesis himself using a word processor on his PC. His diagrams and figures will be expertly constructed using drawing and data analysis programs and will contain unlimited color. When Comelately presents his final seminar, it will probably be a PowerPoint extravaganza containing movie footage of the intracellular trafficking of PhD synthase tagged with GFP, a long way from Bird's talk and chalk presentations. Clearly Comelately is starting out in a golden age of research and discovery, with a range of tools and knowledge not even dreamed about by Bird and his student colleagues. However, as in Bird's day, PhD students are still required to make novel contributions to knowledge and understanding, and despite his apparent technological advantages, Comelately will still have to ask the right questions.

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