My biochemical journey from a Cambridge undergraduate to the discovery of phosphotyrosine

The most notable moment in my career as a biochemist was the discovery of phosphotyrosine, a somewhat serendipitous finding that turned out to have some very important consequences, notably, in human cancer. My career as a biochemist which has spanned nearly 60 years, began when I was 16. At the time, I was in the sixth form at Felsted School, a boarding school in Essex England, and my biology master, David Sturdy, elected to teach me some extracurricular biochemistry, giving me one-on-one tutorials on glycolysis and the TCA cycle. These early biochemistry lessons turned out to be invaluable because I was able to regurgitate them to answer a question in the University of Cambridge scholarship exam in the autumn of 1960. As a result, I was lucky enough to be awarded an Exhibition at Gonville and Caius College, the college where my father had studied for a medical degree during World War II. When I arrived in Cambridge in October 1962 to read natural sciences (see Figure 1), it was a natural choice to take biochemistry as one of my three required first-year courses. The Part I biochemistry course was taught by a series of excellent lecturers, including Philip Randle (a prominent diabetes researcher who described the Randle Cycle), Brian Chappell (who discovered mitochondrial transporters) and Asher Korner (a pioneer of cell free systems to study protein synthesis). It quickly became clear that biochemistry was an exciting subject, and Brian Chappell, my biochemistry supervisor at Caius, made supervisions a lot of fun. I also took Part I courses in invertebrate zoology and, importantly, organic chemistry, which gave me insights into how the metabolites we were learning about in biochemistry worked as chemicals.

In the first week of the biochemistry course, we were introduced to the molecules of life, including the structures of the 20 amino acids including tyrosine – indeed, I see from my 1962 class notes that I first drew out the structure of tyrosine nearly 60 years ago (Figure 2). The structure of tyrosine, and those of the 19 other amino acids, became etched in my mind, and this knowledge proved pivotal to the discovery of tyrosine phosphorylation some 17 years later!

At the end of my second year at Cambridge, I applied to take the final-year honours biochemistry course. The Part II class size was limited to 40 students, and it was extremely competitive to get into (5 out the 40 students in our year were subsequently elected as Fellows of the Royal Society), but luckily I was accepted and started in October 1964. The course was taught in the old Biochemistry Department building on Tennis Court Road, which had been built in 1924, when Gowland Hopkins was chair of department. Lectures were given by the department faculty, including Asher Korner on protein synthesis and Alison Newton on nucleic acids, as well as by emeritus faculty, Dorothy Needham on muscle biochemistry and Rudolf Peters on fluorine chemistry and British anti-Lewisite. For me, however, the highlight of the course was the guest lectures by scientists from the Laboratory of Molecular Biology (LMB) two miles up Hills Road from the department, which included lectures by two Nobel Prize winners – Max Perutz, who described

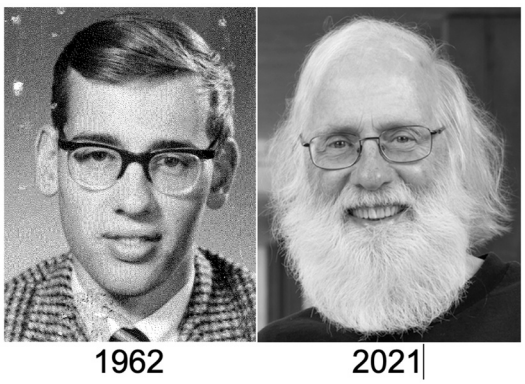

Figure 1. Then and now.

his new higher resolution X-ray crystallography structures of different forms of haemoglobin, and Fred Sanger, who lectured on the methods he had developed to determine the sequence of insulin and more generally the sequence of any purified protein (this was many years before he pioneered new DNA sequencing methods; Figure 3). Our lectures on the genetic code were also exciting, particularly the new evidence that it was a triplet code emerging from the bacteriophage work being done by Francis Crick and Sydney Brenner at the LMB, who gave us a guest lecture, with reports of new codon assignments being published regularly during the course of the year. However it was the lectures by Fred Sanger, Max Perutz and Asher Korner that cemented my fascination with proteins, a love affair that has now lasted for nearly 60 years! The Part II course also had laboratory classes three afternoons a week, with 2- week practical projects organized for each section of the course. Among the graduate student demonstrators for the protein synthesis section was Tim Hunt (later awarded the 2001 Nobel Prize for discoveries of the cyclin proteins involved in the cell cycle), who was a charismatic teacher, and persuaded me that Asher Korner's lab would be a great place to work as a graduate student.

I had not decided what I was going to do when I graduated from Cambridge in 1965, but sometime during the Easter Term, a member of the department suggested that I should apply to be a graduate student in the Department of Biochemistry, saying that if I got a first class degree I would be eligible for a Medical Research Council Graduate Student Scholarship, which would provide me with the princely salary of £500 a year. This seemed like a good way to defer having to make a decision about what to do with my life, and so when I graduated in June 1965 with a first class degree in biochemistry, I applied to the department. At the time, with the excitement about the completion of the genetic code, it was clear that molecular biology, then still in its infancy, was going to become the most important area in biology. The only faculty member in the department doing truly molecular studies was Asher Korner, who was investigating how growth hormone stimulates protein synthesis in the liver, using cell-free systems derived from livers of growth hormone-treated hypophysectomized rats (ironically, we learned many years later that growth hormone stimulates the liver to secrete IGF-1 that acts elsewhere in the body, and that both factors signal to cells by triggering activation of tyrosine kinases!). Luckily, Asher accepted me into his lab, and after an interlude involving an 8- week, 13,000- mile driving adventure from London and reaching Persepolis in southern Persia with three other Cambridge graduates, I started as a graduate student in the Department of Biochemistry at the beginning of October 1965. Together with two other new graduate students, Adrian Dunn and Russell Pemberton, I joined Tim Hunt, Brigid Hogan, John Kay, Tony Pegg, Julian Smith and Richard Jackson, who were second- and third-year graduate students already in Asher's group.

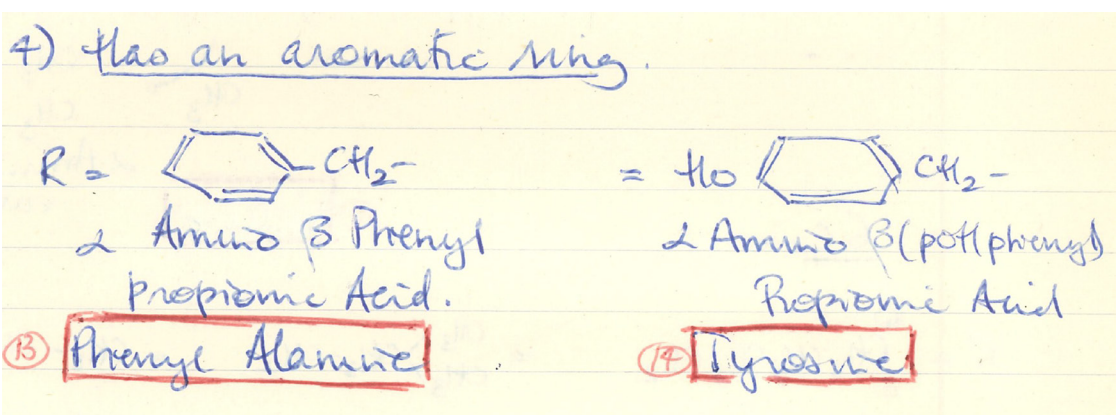

Figure 2. The structures of the R-groups of the two aromatic amino acids (from my Part I biochemistry class notes drawn in 1962).

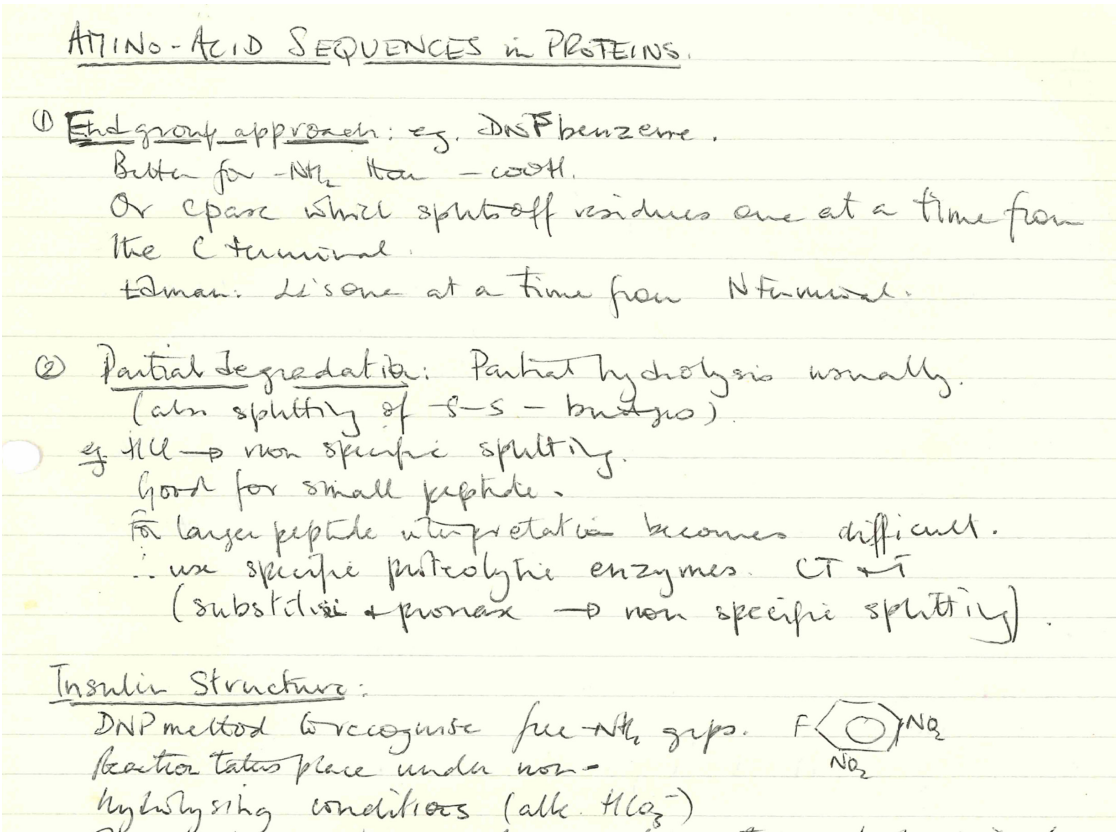

Figure 3. My notes on Fred Sanger's Part II lecture on 'Amino acid sequences in proteins', 9 February 1965.

Asher Korner was a very hands-off adviser, and we were largely left to our own devices, but with the more seasoned graduate students to provide advice and encouragement, this was not a problem. In fact, Asher left Cambridge in the middle of 1967 to become the inaugural Professor of Biochemistry at the newly opened University of Sussex near Brighton, leaving all of us in Cambridge to finish our degrees, being supervised by Alan Munro, who had trained with Asher and was now a Demonstrator (Assistant Professor) in the department. In the lab, I started working on various projects using the rat liver cell-free system to study protein synthesis, and my first talk at a scientific meeting was a short presentation at the 475th Meeting of the Biochemical Society held at the National Institute for Medical Research in Mill Hill, 20–21 October 1967, on work I had done demonstrating that rat liver 'cell sap' contains an exonuclease activity that degrades added ^{3}H-labelled RNA.[1 2] Luckily, I was 'rescued' from rat liver by Tim Hunt, who persuaded me that rabbit reticulocytes were a much better system for studying the mechanisms of protein synthesis, because reticulocytes actively synthesize only two major proteins, the α and β chains of haemoglobin, whose sequences were already known. Tim and I teamed up and published a series of papers together on the mechanisms of globin chain synthesis, before Tim graduated and left for postdoctoral studies in the USA in July 1968.

I defended my PhD in early 1969, and became a research fellow at Christ's College, while continuing to study haemoglobin synthesis in the Department of Biochemistry, now working together with my fellow graduate student Richard Jackson, who had become a research fellow at Pembroke College. In October 1971, I 'followed' my first wife Pippa Marrack (who had been a graduate student with Alan Munro, initially in the Department of Biochemistry and then at LMB) to La Jolla, California, where she started a postdoc in immunology with Dick Dutton in the Department of Biology at the University of California, San Diego, and, at the suggestion of Alan Munro, I joined Walter Eckhart's group at the newly founded Salk Institute for Biological Studies as a research associate. Here, I switched fields completely , investigating the polyomavirus DNA tumour virus being used as a model system for understanding human cancer. After 2 years studying how polyomavirus replicates its circular DNA genome in infected mouse cells, I returned to the Department of Biochemistry in Cambridge for the final year of my college fellowship, with the plan to find a faculty position in the UK. When no job materialized in the first few months, I accepted a prior offer from the Salk Institute to become an assistant

professor, and started my lab there in February 1975, continuing to work on polyomavirus, and then soon adding the Rous sarcoma virus (RSV) RNA tumour virus as a second model system to elucidate the molecular mechanisms underlying the transformation of normal cells into cancer cells.

As described elsewhere,[3] in the next few years my lab identified the virally encoded transforming proteins of both polyomavirus and RSV. Then, intrigued by Ray Erikson's 1978 finding that the RSV transforming protein v-Src had an associated protein kinase activity important for transformation, in the summer of 1979 I set out to test whether the polyomavirus middle T (mT) antigen, the virus' major transforming protein, had a protein kinase activity. To my delight, when tested in an *in vitro* kinase assay by incubating a mT immunoprecipitate with γ^{32}P-ATP, mT itself became phosphorylated by an associated kinase activity. The next step was the routine experiment of determining whether mT was being phosphorylated on serine or threonine, the only known phosphoamino acids at the time, which involved separating a partial acid hydrolysate of the isolated^{32} P-labelled mT protein band by thin-layer electrophoresis at pH 1.9. Perplexingly, I discovered that the ^{32}P-containing compound released from mT did not co-migrate with either phosphoserine or phosphothreonine. Nevertheless, I realized that whatever this compound was it was likely to contain a phosphate ester, because it was stable to heating at 110°C in 6 N HCl.

Here is where all the hours I had spent memorizing the structures of the amino acids as a student became important, because I knew that there was a third hydroxy amino acid, tyrosine, although no one had ever reported tyrosine to be phosphorylated in proteins. Undaunted, I decided to test whether this unknown compound generated by mT might possibly be phosphotyrosine by synthesizing some phosphotyrosine marker. The novel ^{32}P-labelled compound turned out to co-migrate with synthetic phosphotyrosine, and this unexpected discovery was quickly followed by my finding that the RSV v-Src kinase activity was also specific for tyrosine, and, importantly, that RSV-infected chick cells had elevated levels of phosphotyrosine in protein, thus establishing that v-Src acts as a tyrosine kinase *in vivo*.[3]

These early experiments gave us the initial clues that tyrosine phosphorylation might play a role in cancer, and within the first few months after we reported tyrosine phosphorylation in December 1979, three other RNA tumour viruses had been found to encode tyrosine kinase oncoproteins, and the EGF receptor was reported to be an EGF-stimulated tyrosine kinase, which immediately suggested a mechanism through which dysregulated oncogenic tyrosine kinases could stimulate cells to divide in an uncontrolled fashion.

Over the past 42 years, the field of tyrosine phosphorylation has grown exponentially (as of October 2021, 83,000 papers in PubMed contain the keyword 'tyrosine kinase'), and we have learnt that tyrosine phosphorylation plays important roles in many diverse processes in multicellular eukaryotic organisms, from proliferation to cell cycle control and neural transmission. The first human oncogene shown to encode an activated tyrosine kinase was *BCR-ABL*, the fusion gene product of the t(9:22) chromosomal translocation breakpoint that drives chronic myelogenous leukaemia (CML). Since that was reported in 1984, over half of the 58 human tyrosine kinases have been implicated in cancer through acquisition of activating mutations or gene fusions, or else as a result of gene amplification. Beginning in the mid-1990s, the realization that tyrosine kinases can play driver roles in human cancer spawned attempts in both academia and pharma to develop selective tyrosine kinase inhibitors (TKIs) that might be useful therapeutically. The first successful outcome of these efforts was imatinib/Gleevec^{TM}, a BCR-ABL TKI that was approved by the FDA in 2001 for treatment of CML. Since then, what seems like a never-ending succession of new small-molecule TKI drugs have been approved for targeted cancer therapy and other diseases. As of this writing, 61 TKIs have been approved for clinical use worldwide, with many more in the pipeline. This is indeed a gratifying outcome of some simple biochemical experiments done on the transforming proteins of model tumour viruses over 40 years ago.

In many ways, the discovery of tyrosine phosphorylation was serendipitous, but without my thorough biochemical training I would probably have ignored the aberrant spot generated by the hydrolysis of ^{32}P-labelled mT antigen. Now nearly 60 years later (Figure 1), I remain indebted to all my teachers who had the patience and skill to instil in me the basic tenets of biochemistry as an undergraduate. ■

References

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