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Autobiographical sketch

DNA repair, DNA replication and human disorders: A personal journey

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I was born in 1946 and grew up in the industrial north-west of England close to the city of Manchester. My parents were German-Jewish refugees, who left Germany fairly early, in 1933. My father helped to establish and was one of the directors of a tannery, which made leather for shoes and handbags. This was part of a group of tanneries established first in Strasbourg by my great-grandfather Ferdinand Oppenheimer. I would describe my childhood and adolescent years as comfortable by general post-war standards. I went to a state primary school and obtained a scholarship to Manchester Grammar School (MGS), a fairly prestigious secondary school. As a child I was always interested in chemistry but had little interest in or knowledge of biology. The educational system in the UK at that time was such that one had to specialise very early and as a consequence I have had no formal biology education since the age of 12, something I have managed to hide reasonably successfully for the rest of my life! In my final two years at MGS I studied just physics, chemistry and mathematics and obtained a scholarship to Pembroke College, Cambridge (England) to study Natural Sciences, with the intention of becoming a chemist. In the second year at Cambridge, one of the options was a course on biochemistry. Having no real idea what this was, I read a book about it in the summer of 1965, and was truly astonished and excited to discover that the basis of life was just a bunch of rather complicated organic chemistry reactions. So I took the biochemistry course in my second year. By the end of that year, I was fed up with chemistry and for my final year I chose to do biochemistry rather than chemistry, a decision I have not regretted. The biochemistry lectures must have been pretty up-to-date, as we were told briefly about the discovery of DNA repair by Dick Setlow [1], a topic that seemed rather esoteric at the time.

1. PhD and postdoc: London, Tennessee and Sussex

In early 1967, in my final year at Cambridge, I saw an advertisement for a PhD position at the Chester Beatty Research Institute in London and went for an interview with Professor Peter Alexander, Christopher Dean and John Lett, a group of well-known radiobiologists. The work they proposed, to measure repair of double-strand breaks in mammalian cells, sounded interesting and I was, as it turned out erroneously, offered a place without any conditions. PhD positions are always offered on condition of obtaining a first or upper second class degree. Although I was expected to, and indeed went on to get a first-class degree, I decided that a bird in the hand was worth two in the bush and accepted the offer, another decision that I did not regret. I started my PhD studies in a new building in South London in October 1967 to discover that my proposed supervisor, John Lett, had taken a job in USA and his successor was a radiation physicist, Mike Ormerod. I was actually the only biochemist in the small department and this, together with my Cambridge degree, gave me a certain status above my station. Another important event that took place at the end of that year was my marriage, at what now seems like the ludicrously tender age of 21, to Judy Selbourne, a history graduate also from Manchester. This was a third decision I have not regretted and we have now been married for 43 years!

Measuring double-strand breaks was not easy. In 1967 McGrath and Williams had shown that single-strand breaks could be analysed by lysing bacterial cells on top of alkaline sucrose gradients and spinning out the denatured DNA strands in the ultracentrifuge [2]. The number of single-strand breaks could be measured from the rate of sedimentation of the DNA fragments. The Chester Beatty group had shown that this technique could also be applied to mammalian cells [3], and my job was to do similar measurements using neutral sucrose gradients to measure double-strand breaks. I was able to obtain a peak of fragmented DNA without difficulty. Unfortunately the position of the DNA remained the same, irrespective of the dose to which the cells were exposed, over a dose-range of 300–2000 Gy. In other words, it appeared that over a 10-fold dose range, there was no change in the apparent number of double-strand breaks. I struggled for a year trying to solve this mysterious phenomenon before temporarily abandoning the project.

1968 saw a great leap forward in the field of DNA replication with the discovery of Okazaki fragments in *Escherichia coli* [4], and this was followed by a similar claim for mammalian cells [5]. We realised however that the results obtained did not actually measure Okazaki fragments but were instead a consequence of a labelling artefact. This led to my first paper being a short note in Nature [6] (Fig. 1A) – the only first/last author paper I have managed to publish in Nature! It turned out of course that Okazaki fragments did indeed exist in mammalian cells, but the paper with the first claim was definitely wrong. We completed a mathematical analysis of this labelling artefact and were able to use it in a more positive way to measure the rate of replication fork progression in mouse cells [7]. At about that time, I came across a paper on centrifugation of high molecular weight DNA, showing that if DNA is centrifuged at high speeds, the rotor speed could affect sedimentation rates of DNA.

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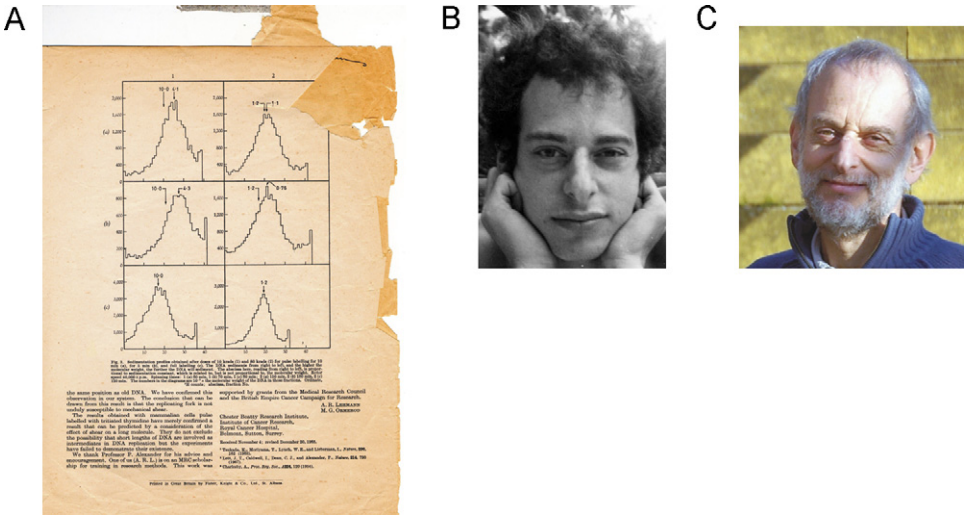

Fig. 1. (A) A page from my first paper, which, like the author, is showing signs of wear and tear forty years later (B, 1970; C, 2010).

I rushed back from the library, repeated my double-strand break experiments, but now ran the centrifuge at 10,000 rpm rather than 40,000 rpm and, look and behold, I obtained a nice dose–response for the induction of double-strand breaks and was able to show that this was linear for X-irradiation of mouse cells from 300–4000 Gy [8]. I did not detect any repair, most likely because I was using such high radiation doses. Unfortunately at lower doses, the DNA formed an aggregate and could not be analysed. My PhD work finished with an analysis of replicon sizes in mouse cells, again using alkaline sucrose gradients [9].

In the 1960–1970s getting one's BTA (Been To America) was de rigeur for a scientific career in the UK and Peter Alexander suggested I go to the lab of Dick Setlow, who had discovered excision-repair a few years earlier. Dick was kind enough to accept me for a postdoctoral position in his lab in Oak Ridge, Tennessee, in 1970. We arrived at Knoxville, Tennessee, airport in September 1970, laden with luggage and wearing our thick English overcoats so as not to fill up valuable luggage space. As the temperature was about 28 °C and the humidity close to 100%, Dick did not have too much trouble identifying us. Coming from London, arrival in Tennessee was something of a culture shock – we quickly learned that going more than 100 m on foot was considered by the locals to be close to insanity if you were able to travel by car. In the lab, things were also pretty different. I had been used to chatting to Mike Ormerod every day about my work during my PhD thesis, whereas Dick Setlow was very much hands-off. I floundered around for about 3 months searching for a decent project and getting a little despondent, before latching onto a topic that was to occupy much of my future career.

Dean Rupp and Paul Howard-Flanders had published their seminal work on postreplication repair in *E. coli* a couple of years earlier, in which they demonstrated daughter-strand gaps in UV-irradiated cells and showed that these gaps were subsequently sealed [10]. I thought it would be interesting to look if something similar happened in mammalian cells. Shortly after starting this project, I had the good fortune to attend the Biophysical Society meeting in New Orleans in February 1971, where I met Dean Rupp, Paul Howard-Flanders, Phil Hanawalt and other luminaries in the DNA repair field. I learned about the elegant experiments of Rupp and colleagues, in which they demonstrated that filling of daughter strand gaps in *E. coli* was effected by sister strand exchanges [11]. Dick Setlow had developed an elegant procedure for measuring gap sizes during nucleotide excision repair, by allowing the cells to incorporate bromodeoxyuridine (BrdU) into the gaps. BrdU-containing DNA is susceptible to breakage by UV light of 313 nm, so the filled-in gaps could be reconverted into breaks, and the size of the filled-in gap was inversely proportional to the dose of 313 nm irradiation needed to cleave the patch [12,13]. I realised that I could adapt this method to measure the size of daughter strand gaps during postreplication repair (PRR) and to determine whether the gaps were filled in by recombination with parental DNA, in which case they would not contain BrdU and would not be cleavable by 313 nm light. Alternatively if they were filled in by some kind of direct synthesis (that we now call translesion synthesis (TLS)) they would be cleavable. My results favoured the latter mechanism and argued against gap-filling by recombination in mammalian cells. To this day, I feel that this was the smartest experiment that I have done through my career, and I was excited to get it published in J. Mol. Biol. [14], at the time one of the top journals. (Even in those days, getting papers published in the best journals was important – I remember Mike Ormerod and I ranting about the stupid editor who had rejected one of our papers during my PhD. Le plus ça change!) Dick Setlow gave me the opportunity to present my data at the Gordon Conference on Nucleic Acids that he was chairing in the summer of 1971. I remember Paul Howard-Flanders giving me a hard time about interpretation of my data, but I got to meet another bunch of contemporary DNA repair stars such as Larry Grossman, and up and coming ones like Errol Friedberg and Priscilla Cooper.

My year in the US was highly formative and by the end of it, I had met almost everyone who was anyone in the DNA repair field and had generated data for two nice papers. That would certainly be extremely difficult nowadays. We returned to the UK in September 1971 and had to make cultural re-adjustments to small cars, public transport and gardens with roses. I had arranged a second postdoc at the University of Sussex with Sydney Shall. Sussex was one of a group of universities that were set up in the UK in the 1960s and it had established a good academic reputation in a few years as well as being regarded as pretty avant-garde. One reason I had chosen Sussex was that the professor of biochemistry, Asher Korner, had taught me about protein synthesis when I was an undergraduate at Cambridge and had particularly impressed me. I was distressed to discover on my arrival at Sussex that he had died very suddenly a few weeks earlier while only in his early 40s.

Sydney Shall was one of the first people to work on PARP, which at that time was a novel polymer of completely unknown function. For the next two years I dabbled a bit in studying PARP [15] as well as consolidating my work on PRR. I was singularly unimpressed and dismissive when Sydney suggested that PARP might be involved in