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# DNA 50 Years After: A Personal Perspective

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50 years ago a trio of brief papers in *Nature* exposed the mechanism underlying Darwin and Wallace's theory of evolution and offered explanation for the experimental results of Mendel. Society has never looked back. These papers changed our understanding of our biology and of our relationship to the natural world. Although it is often said that these papers presented the discovery of the structure of our genetic material: DNA they in fact simply presented a culminating flash of insight that followed almost a century of investigation. I recently asked a third year undergraduate audience to name the discoverer of DNA. I was surprised when several among them assured me that (he) had in fact been Watson and Crick. Only one ventured the correct name of Meischer. Their embarrassment deepened when I asked them to name those involved in elucidating the structure of DNA. Several, who once again assured me that Watson and Crick had been alone, reddened when I rephrased the question and asked who had been awarded the Nobel prize for the discovery. They added the name Wilkins - of course they knew it - and some were even aware of the controversy surrounding the treatment given Rosalind Franklin - but only from the less than objective account given in Watson's "Double Helix". None were aware of the essential groundwork done by Nobel laureates Fischer, Kossel or the Braggs. Nor were any aware of the work of Levene, Astbury, Gulland or of others upon whose contributions Watson and Crick directly built their own - and who might with luck and added longevity have won that prize for themselves. Even Erwin Chargaff and Linus Pauling, the quasi and actual Nobel laureates whose contributions were fundamental and who were fixtures on the international stage until the



very recent past did not achieve a mention. The current generation has all but forgotten that Levene had worked out the structure of the DNA polynucleotide strand more than a decade before the *Nature* papers. Although he correctly established covalent linkage of base, sugar and phosphate he was trapped by his technology. With dogmatic determination he asserted the structure to be a simple tetranucleotide. Two generations before Levene's work Meischer had understood DNA to be very large and had even discussed the complexity that could be generated by combinatorial assortment of a limited number of monomer units. Although insightful, without the benefit of knowing Mendel's work or the results of the painstaking analysis by the organic

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chemists Meischer could not have made the next great leap. Griffiths and later Avery with McLoed established the ability of high molecular weight DNA to transfer a genetic trait and incidentally performed the first cloning experiments.

It was only one vitally important detail that was presented in the Nature papers – the helical paired antiparallel arrangement of the DNA stands. Even that was more a matter of conjecture than proven fact. The specific pairing of the bases and the antiparallel arrangement of strands were fortunate guesses but they provided a mechanism for both the replication of genetic information and the occasional variation that provides a basis for natural selection. The thin evidence of helicity and base stacking provided by the initial fibre diffraction patterns required almost two decades of refinement before the models could be considered finished. Almost as soon as this had been done Donohue, piqued by the failure of the community to grant adequate recognition to his role in informing Watson of the correct tautomeric forms of the bases, challenged the legitimacy of the interpretation and offered an alternative model. By that time the weight of accumulated evidence did strongly favour the revised Watson-Crick models – but the evidence of 1953 could as easily have been interpreted in terms of the straw man offered by Donohue.

I grew up in Cambridge, the son of a Canadian Don whose unlikely field of study - Chinese history and linguistics -brought our family into even more unlikely contact with the eminent sinologists: JD Bernal and Joseph Needham who had converted from biochemical science. The excitement surrounding the newly proposed model for DNA was inescapable. While still a high school student I attended the 1966 meeting of the British Association for the Advancement of Science where Watson and Crick each made an appearance for the benefit of interested members of the public. Crick and Brenner's work defining the triplet basis of the genetic code was still fresh and the code itself was still being worked on. From the audience an elderly professor of Zoology scolded the heroes of the moment for their mistaken belief that a molecule with the simplicity of DNA could possi-

bly encode life – only proteins had the needed complexity! Of course he was right. Sadly, it is unlikely that the gentleman lived to see the developing revolution in the byway of epigenetic inheritance. So easily do we forget, that while DNA may serve as library for genetic information, a library is not a society of learned readers. That same meeting provided opportunities to visit the Cavendish laboratory where I squinted at a homebuilt X-ray camera and a sensual 8A balsa model of myoglobin. Physically far more impressive was the Mullard Cambridge Observatory where the brightly coloured ink was still fresh on the skymaps of Quasars 3C48 and 3C273 and where the meaning of their redshifts were being actively debated. At that meeting Fred Hoyle was still confidently defending his steady state model of the Universe. The announcement of the first pulsar was only months away. To a high school student with the hope that science was a place where one might mark the world this was heady stuff. A visiting family friend later provided an opportunity to visit the nuclear accelerator at Harwell and even peek into the room where the Atlas computer – then one of the largest (and least reliable) in the world was – once again being given an overhaul. Its hand built core memory, knitted together from tiny ferrite rings, each capable of holding one bit of information, was out and being worked on.

I remember trying rather unsuccessfully to present the ongoing work on the genetic code to the members of my all boys high school science club. For them the Chemistry of Genetics was a matter of testosterone enforced practical concern rather than a subject for academic study. The subsequent year that I spent teaching at a village high school in India under the auspices of the British Voluntary Service Overseas organization brought home the international nature of the Scientific endeavour and of the pride that came with having a shared cultural background with a famous contributor. The name of Har Gobind Khorana was known to all the students and teachers at that unsophisticated village school, although none had any idea of the nature of his contribution. Little did I then know of the indirect but profound influence that H.-G. Khorana would soon play

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upon my own life. Upon completing my year I joined my family who had in the meantime returned to Canada, and enrolled in the Biochemistry program at the University of British Columbia. I chose the program because of the high excitement that surrounded the recent elucidation of ‘the code’. Two members of that Department, Michael Smith and Gordon Tener had trained directly under Khorana while he worked at the BC Fisheries Research Board. It was in my final year as 4<sup>th</sup> year project student that Khorana spent a sabbatical year at UBC and had the pleasure of being almost knocked over by myself, wine glass in hand, while celebrating some now forgotten success of one of the Dixon lab’s graduate students. Soon after, beginning graduate work in Edmonton I fell under the supervision of Richard Morgan who had been directly involved in the elucidation of “the code” while in Khorana’s lab in Wisconsin.

By 1970, despite the enormous progress that had been made in the analysis of nucleic acids there were still many unresolved issues concerning the finer points of DNA structure, replication and transcription. DNA Polymerase I had only just been knocked off its pedestal as THE putative replicative enzyme. Okazaki fragments were about to be announced and DNA polymerases II and III were about to be discovered. The problem of establishing the sequence of a particular high molecular weight DNA molecule seemed insurmountable. It was not until 1971 that the 12 residue sequences of the cohesive ends of lambda bacteriophage were reported: a result of three years work using methods derived from the earlier sequencing of t-RNA. Gel electrophoresis was still used only for the analysis of proteins. The methods of restriction analysis, blot hybridization and cloning that underpin modern methods of Molecular Biology were yet to be invented. My graduate years were spent performing enzyme preparations – DNA polymerase I and T4 ligase, were not yet commercially available – and manning the Department’s only analytical ultracentrifuge during graveyard hours whenever time became available. Closed circular DNA was not readily obtainable and the class of enzymes now

called topoisomerases were still to be described. Preparation of the closed circular replicative form of phiX174 DNA was an iffy month long affair involving first phage preparations and then repeated preparative cesium chloride ultracentrifuge runs. Often at the end of the prep residual viscous cell wall polysaccharide would prevent the supercoiled DNA band from being unloaded from the gradient

My thesis topic: arose out of a controversy that developed over the handedness and magnitude of natural DNA supercoiling. Ethidium, an antitrypanosomal drug with a large planar phenanthridinium ring was introduced to the study of DNA by Paoletti and Lepecq. Unlike the previously studied acridine dyes it bound DNA almost exclusively by an intercalative mode. One of the most spectacular effects upon binding was its dramatically enhanced and very pretty red fluorescence when excited by ultraviolet light. The supercoiling of circular viral DNA was first reported in 1965. Ethidium was used soon after to titrate those natural supercoils, but 1971 measurements of the fluorescence depolarization due to resonance transfer between Ethidium molecules when bound to DNA suggested that the earlier proposal that Ethidium unwound the helix had been incorrect and that overwinding actually occurred. The result was that both the magnitude and sense of the supercoiling of natural DNA circles remained controversial. By 1974 we had solved the issue by directly synthesizing negatively supercoiled DNA and in the process made the unexpected discovery that previous estimates of the numbers of superhelical turns present in all naturally occurring closed circular DNA had been too low by a factor of at least 2. As a bonus I was able to confirm the assignment of handedness of the supercoiling on the basis of the unexpected treble clef appearance of our DNA in some electron micrographs. There had also been one of those moments of private exhilaration. I had read that the ribosomal RNA’s had been successfully resolved by electrophoresis in an agarose gel and detected by staining with methylene blue. Agarose at that time was a rare and precious commodity used in bead form for gel exclusion chromatography but not yet as a

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support medium for electrophoresis of DNA. I scrounged an obsolete vertical mode starch gel electrophoresis apparatus and poured a 0.2% agarose gel – it was too expensive to make the gel stronger and I knew the supercoiled circles I was working with were far larger than the ribosomal RNA's that had been tested previously. The gel was too fragile to be run in the normal vertical position used for starch gels so I arranged paper wick electrodes and set the gel horizontally over the buffer chambers on the metal bench top of our cold room. Knowing the fluorescent property of ethidium when bound to DNA, I added a little to the buffer. Because of the risk that someone in authority might terminate my experiment for safety reasons – or worse, electrocute themselves – the run was started late one evening before the weekly graduate student trip to an evening of beer consumption at the Faculty club. In the wee small hours, half soused and with a hand held UV lamp I stared over the many brilliantly fluorescing bands that my supposedly pure DNA samples presented. It was both a thrilling and an appalling prospect. There was no way to record the result – the Department did not then have the kind of photographic facilities that later became standard. Without further study it was not possible to eliminate the possibility that the multiplicity of bands was some peculiar artefact. Had my supervisor been made aware of the many bands in my supposedly homogeneous samples there would certainly have been a delay before I could present my thesis. I switched the current back on and went home thinking that the morrow would resolve the matter. In a way it did. The gel had done what agarose gels always do if not adequately buffered. It had proceeded to migrate through itself to leave only a pile of compressed gel. With no more agarose and fearing the complications that had presented themselves I let the matter drop.

The following year, after starting my Post Doc at Cal Tech I took the matter up again. By then others had reported extraordinary resolving powers of agarose gels and of the sensitive detection of DNA afforded by ethidium. A whole new domain of DNA fine structure opened itself as we became

able to separate isomers that differed by a single topological linking number. We initially used tube gels with the slippery agarose gel held in place by a fragment of dialysis tubing and a rubber ring. To get a flat surface upon which to layer our samples it was necessary to slide the newly formed gels out past the end of their supporting glass tubes and slice off the concave end formed by capillary action as the gel set. During runs the gels would frequently collapse or disintegrate. Sometimes a retaining ring would fall off, dumping gel and upper buffer chamber. Despite the limitations the tube gel system did lend itself to rapid experimentation with different gel consistencies and buffers. The antimalarial drug: Chloroquine, was found to allow fine manipulation of the superhelical state of plasmids and to give superior electrophoretic resolution of the topological isomers. Eventually we acquired one of the new flat plate vertical apparatuses developed by the Cold Spring Harbour Laboratory. When the glass plates were roughened by sand-blasting agarose gels would sometimes remain in place long enough to allow exquisite resolution. The flat arrangement of bands also facilitated the quantitation needed to establish relationships among the various species. Upon presenting our results at a Gordon Conference we ran headlong into unanticipated controversy. Workers at Cold Spring Harbour suspected that a student from our laboratory had spied on them while taking one of their courses. It took the personal intervention of the respective resident Nobel Laureates, James Watson and Max Delbruck to restore a semblance of peace.

At that same 1975 Gordon conference the earliest versions of the modern DNA sequencing methods were announced. Chemistry dating from the 1940's had been applied to the creation of the base specific cleavage method of Maxam and Gilbert while the Sanger lab developed chain termination methods, using properties of DNA polymerase, discovered in the 1950's by Kornberg's lab. Both labs resolved their fragments using a denaturing electrophoretic technique that had been developed by Hans van de Sande and Tom Maniatis for the separation of oligonu-

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cleotides. The effect of these presentations was stunning. So was the report of the first relatively rapid method for oligonucleotide synthesis developed by Narang in Ottawa. Against this background, a spectacular row over who had the correctly calibrated fragment lengths that enveloped the discussion of newly discovered ladder of nucleosomal chromosome fragments now seems a sideshow.

Although DNA cloning methods had been reported almost two years earlier, a moratorium was in force that prevented newcomers from flooding into the field. Type II restriction enzymes were just beginning to be discovered and made commercially available. Southern Blot Hybridization was also about to appear. As confidence grew the moratorium on cloning was lifted. With almost no warning we launched headlong into the breathtaking new era of genomics. Apart from the sudden flood of new gene sequences the structure of DNA presented new phenomena. Anomalies in the migration rates of DNA fragments became apparent. Voltage dependence of the migration of high molecular weight DNA was eventually recognized to be a consequence of the DNA molecules aligning themselves with the electrical field as they progressed through the gel. In the laboratory of Charles Cantor these observations became the basis of the pulse field electrophoretic separation of whole chromosomes. Other anomalies in the migration of small fragments through acrylamide gels were eventually recognized to be a result of intrinsic bending of the DNA fragments. In turn these bends were recognized as contributing to the interaction of proteins that control the expression of many genes.

Despite the sudden and dramatic progress brought about by the introduction of gel electrophoresis and cloning the other key element of the new technology: oligonucleotide synthesis was still far from routine. It was not until 1979 that an unambiguous structural assignment based on analysis of diffraction by a single crystal of DNA oligonucleotide appeared. This structure was one that left the field breathless. The strange left handed “Z” structure with its alternating syn- and anti- bases and crooked backbone was

revealed. This novelty was so totally unlike the familiar A and B families that many wondered whether we did indeed know the structure of DNA. DNA was clearly far more versatile than had initially been assumed. Even the true “B” single crystal structure that quickly followed proved to contain a wealth of detail not anticipated from the earlier work using fibre diffraction. We now recognize the contributions that end effects and crystal packing forces play in distorting the structures of short oligonucleotides that form single crystals. The new reality: that naked DNA is not the simple object described in fibres under tension opened a new era in the study of the 3 dimensional structure of natural DNA sequences. In a brief period of new exploration it was found that torsion built into closed circular molecules could cause some DNA to form Z-structures and hairpins as well as other “excited” state structures that were quite distinct from the familiar A and B-DNA families. DNA triplexes and the G tetraplex had previously been described in simple DNA polymers, but it was only with their appearance as a result of strand disproportionation under environmental influence in ordinary DNA that serious attention was given to their possible roles in Biology. The G tetraplex is almost certainly involved in stabilizing the single stranded ends of eukaryotic telomeres while triplexes and Z-DNA may have transient roles accomodating superhelical torsion during replication and transcription. Even in such an apparently simple molecule as DNA, the complexities created by the competing effects of bending and twisting moments in the context of varying base sequence defy straightforward analysis. Crystal structures necessarily represents a ground state, but living systems are dynamic. Once proteins are added new deformations are imposed on DNA that wildly exceed those thus far observed in naked DNA.

Single stranded nucleic acids are far more conformationally mobile than their double stranded counterparts. We now have a growing family of well characterized RNA structures, many with special binding properties and in some cases catalytic activities. Thus far similar attributes have not been detected in naturally occurring DNA

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molecules but it is likely to be a simple matter of time before this happens. Although we still have few dramatic examples of how local DNA structure is biologically important there is a widely held suspicion that evolution cannot have failed to assign function to subtle conformational effects which would reveal themselves were we able to perform experiments on the time scale of generations. With the nearly complete sequencing of human and mouse genomes a new class of conserved sequences have been discovered which appear not to be transcribed and which certainly do not encode proteins in the usual way. At the present time there are no clues to the possible reasons for the conservation of these sequences. Apart from the knockout experiments there is not even an obvious experimental route to assigning them a function. A new generation of graduate students have an adventure before them.

Often we forget that key elements in the developing story of the nucleic acids were contributed by scientists working with very few resources. Among many important Canadian contributions Gordon Tener at UBC developed a key method for purifying single species of t-RNA that played an essential role in determination of their structure. Mike Smith participated in the development of the Sanger Sequencing technique and later earned his own Nobel prize by setting forth the first site directed mutagenesis method. Hans van DeSande, in collaboration with Tom Maniatis developed the denaturing gel technique that later became a basis for both Sanger and Maxam and Gilbert sequencing methods. Narang in Ottawa developed the first rapid method for DNA synthesis that pointed the way to the later development of the phosphoramidite method. Richard Morgan in Edmonton did important work on multistranded DNA structures. Large laboratories with million dollar budgets easily overwhelm the product of isolated small laboratories once a key technique has been developed but that key initial phase of scientific invention is almost always carried out by individuals. It is tragic that in our anxiety to emulate the big science being done elsewhere, that small Canadian science has largely disappeared. Small scale science, most particular-

ly that which is at the bleeding edge is always vulnerable to being eliminated in a bad year. Small science has given very good value for money and frequently has produced great insight.

This last 50 years coincided with the brief period in history that may mark the high point of human civilization. We careen towards an uncertain future of runaway population growth, resource depletion and global overheating. Despite the human catastrophes of the 20<sup>th</sup> century and early 21<sup>st</sup>, the wealth of knowledge handed us by past generations has for this brief period created a world society with the leisure to inquire into the full range of life's complexity and even approach the probable limits to knowledge of our Universe. In the flood of new information and in our rush to acquaint students with the latest results we frequently forget to tell the story of how it all came about. In the modern western world few children learn a trade or even wisdom at the feet of parents. They are instead left to pick up what they can at the hands of the overtaxed schools. Few of them have the patience or time needed to search the older literature buried in the deeper stacks of our libraries. The oral tradition, which our forbears used to educate their young is now largely displaced by the twin cacophonies of consumer advertising and popular mass culture. We rely upon storytelling by others, either through the medium of television or through the University lecture hall to provide the younger generation with basic elements of culture. It is the duty of those still working in the field to retell this story so that some of the coming generation of scientists can continue this tradition when their time comes. Without conscientious effort, collective amnesia otherwise assigns the results of creative endeavors of the preceding many to the singular heroes of the moment.