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SHORT COMMUNICATION

Notes from Abroad on the Deciphering of Biology's Rosetta Stone

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I worked in Marshall Nirenberg's group at NIH from September 1962 to September 1964. My admiration and consideration for Marshall and his group's contribution as recognized recently by the American Chemical Society is enormous. I now draw the parallel between how the Rosetta Stone enabled our culture to learn of the past in Egypt and the decipherment of the Genetic Code, for example, comparing the hieroglyphics and the string of DNA bases.

The conformation of DNA was worked out by another hero of mine, Francis Crick, in collaboration with James Watson by glorified inspired guess work. My PhD supervisor, Dan Brown at Cambridge University, determined the chemical structures of DNA and RNA together with Nobel Prize winner Alexander Todd (later Lord Todd). At Dan's 70th birthday party, Lord Todd averred: "What we did, Dan was to solve the structure of nucleic acid including DNA. All that Watson and Crick did was to solve the conformation". As usual, the great man Todd spoke some basic truths bluntly. Actually Alexander Todd forged the makings of my career. When I was in my last year of Ph.D. studies, Todd acted as my supervisor whilst Dan Brown was on sabbatical leave. Todd asked me what I wanted to do. I replied: 'biochemistry'. He immediately arranged a Research Associate position at MIT's Department with Professor J.M. Buchanan, an expert in nucleic acid components' metabolism.

But first let me explain a little here on DNA. Working with Francis Crick was also inspiring as he had ideas about everything, and I was lucky that he spent 6 weeks in my Danish lab as he emigrated to the Salk Institute. When he was here in 1976 in Denmark, some scientists in New Zealand pointed out that the crude X-ray fibre diffraction data on DNA could also be explained by a side-by-side model for the 2 strands. Jim Watson reportedly said: 'rubbish'. Francis said: 'it could be', but he pointed out that he knew the answer because of the work I did with Aaron Klug on solving the 3D structure of tRNA because we showed RNA helices at atomic resolution. If there are helices in RNA then it was pretty certain that helices occurred in DNA. In point of fact, a small piece of double stranded DNA was not solved by X-crystallography until 1978 at Caltech by Dickersen and Drew.

I use this story to highlight the importance of Marshall's work which allowed all biologists and chemists to understand and control the various cellular molecular mechanisms that have had tremendously positive outcomes in molecular medicine for which NIH continues to be famous. The genetic code truly gives us insight into function which the DNA informational strands cannot.

How did I reach NIH? Essentially it was due to Marshall's friends and eminent scientist supporters who acted when Marshall's discovery in 1961 stimulated a host of competitors. The news of Marshall's talk at the IUB Congress in Moscow spread quickly. As a later President of the IUB, which included molecular biology in 1991 to become the IUBMB, I wished that I had presided at such a momentous occasion. In the Fall of 1961, Marshall gave a full house seminar at MIT on the synthetic mRNA work showing that polyU made polyPhe. Even Jim Watson who came with people from Harvard did not read his usual paper. The only untoward incident came in the question time when a post doc from NYU (Ochoa's group) tried to convince the audience that they were also as far ahead in the genetic code as NIH particularly because they discovered the enzyme polynucleotide phosphorylase which was

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used to synthesis the polynucleotides for studying the function of synthetic mRNA. This intrusion by NYU turned everyone, I believe, in the room to support the work of the young NIH researcher against the big New York group. Certainly, we have heard of the great support from NIH colleagues particularly for synthesizing the polynucleotide. Marshall had friends at NIH and MIT, who had become my friends also. In particular Dan Levin and the Schlessingers (Milt and Sondra) told me that Marshall was looking for people and thought my expertise in organic chemistry would be useful and so it turned out later to be true. I discussed the situation with Jack Buchanan who thought it was a wonderful opportunity and wanted to support NIH. Jack Buchanan helped by releasing me from a 3-year contract at MIT and so Marshall signed me up. Thanks to Dan Levin's Bethesda contacts my arrival at NIH was helped greatly. Indeed, Art Weissbach and his wife even stored our trunks whilst we took a touring holiday on the way from Boston to Bethesda to get the appointment date right. We obtained a beautifully, convenient apartment at Lakeview in the North Bethesda countryside in those days. It was a luxury after the dismal more expensive living conditions in Boston.

When I arrived in September 1962, Phil Leder and Sid Pestka had just joined Marshall and Bill Sly. Bill Jones and Heinrich Matthaei had just left. Marshall worked closely with his MD recruits and technicians Norma and Theresa. I had some more freedom since I had the job of trying new things especially since Marshall realized that the main competition would not be NYU but Madison in the form of Gobind Khorana's group that had taken over from Cambridge University as the chief synthesizers of oligonucleotides. Of course Gobind Khorana shared the Nobel Prize with Marshall for synthetic mRNA function. I was extremely impressed by Marshall's strategy of looking for quick methods especially when making an active cell free system and analysis of the polypeptide products were painstakingly long. Then came the triplet-binding assay developed with Phil, and we were home and dry as long as we could synthesise trinucleoside disphosphates (triplets).

All hands in the rapidly growing group were trying all sorts of methods even making degradative enzymes work backwards. Already by the Cold Spring Harbor Symposium in 1963, our group had made very significant progress in assigning triplets to amino acids (Nirenberg et al. 1963). By the time of the IUB Congress in New York in the summer of 1964 most of the genetic code was decoded. The group had had a blanket of secrecy so that Marshall's lecture was thrilling with all the new information. It was a great time for celebration. I felt extremely sorry for the shocked looks on Khorana's group faces after the lecture. However, they did well in the end.

The great positive thing about science is our fraternity and common friendships. I have naturally maintained close friendships with many of Marshall's group and later became close friends with many of the members of that time Khorana's group and Gobind himself. Also a few of the NYU groups including Severo Ochoa and Charles Weissmann became friends in later years.

My expertise in synthetic chemistry paid off for Marshall's group in that my synthesis (copying Khorana) of oligo dT was used by Phil, Bill Sly and Sid to make poly A for the new non U coding experiments where we showed AAA to be the triplet for Lys (Leder et al. 1963).

I am proud that Marshall wrote an acknowledgement on a poster for a Symposium in Honor of Marshall Nierenberg in 1987 at UMDNJ, Robert Wood Johnson Medical School, Piscataway, NJ, organized by Sid Pestka and colleagues. It was entitled 'The Genetic Code. Foundations of the New Biology'. I cite: 'To Brian Clark who did pioneering work on the synthesis of oligodeoxynucleotides and on the expression of a simple gene. With Best Wishes, Marshall Nirenberg'. Indeed that was the first time that it was shown that DNA could make mRNA which was translated into a polypeptide in the same test tube, rather an important step in functional genomics.

Marshall was also kind enough to suggest that I publish part of this work showing template specificity for RNA polymerase with a technical assistant Taysir Jaouni that Marshall had asked me to take care of. This is a common example of Marshall's generous care for members of his group to ensure that they received due recognition for their work (Clark and Jaouni 1965).

Why did I not stay at NIH? During 1964 I received an offer of a staff position by Francis Crick and Sydney Brenner at the British Medical Research Council's newly established laboratory of Molecular Biology (LMB). I really enjoyed working at NIH where financial support and human resources were not a problem. Space was the problem but we managed. However, I found that the Molecular Genetics Division of LMB was going to emphasise punctuation of the code. I did not find much evidence at NIH of going in this direction, perhaps because of the high magnesium concentration used in the decoding studies. Low magnesium concentrations were needed to identify more natural events. I also feel that I received a good job offer since I was working at the leading decoding laboratory with knowledge of new technology. Anyway the concentration of luminaries at the LMB in Cambridge with Nobel Prize winners Max Perutz, John Kendrew, Fred Sanger, and Francis Crick already there and Aaron Klug, Cesar Milstein, and Sydney Brenner working for Prizes to come. The connection with Stockholm has not dried up yet with ex post docs and students achieving the honour so that

15 have come to the laboratory which certainly makes it the premier laboratory in molecular and cell biology.

Although Sydney Brenner wanted me to work a chain termination and I did decode Tyrosine to help him identify termination codons, I was enthusiastically persuaded to collaborate by Kjeld Marcker, who had discovered formyl methionyl-(tRNA) with Fred Sanger. Our collaboration showing the role of the tRNA in initiation and decoding it of course leaned heavily on the expertise I learned at NIH. Then AUG became about the universal coding for initiation and shows where genes start in modern genomics. I'm proud of the fact, GUG, UUG and even CUG have been shown to be initator codons. A nice later friendship I can reveal is that the GpUpG I needed was sent to us by Gobind Khorana on a piece of chromatography paper (Clark and Marcker 1966).

I looked forward to calling on Marshall whenever I was in the Washington area, to hear of his current interests and to find out if they coincided with mine. He was a humble, modest, curious academic giant. At NIH, we found him in a windowless inside office stacked with books, but he was always welcoming and gave himself up to expressions of encouragement to show his interest for my work. By the way, in the photograph Marshall used for our leaving party, my wife Margaret is misnamed as Mrs. Marshall. To show Marshall's broad interest in helping his friends, I can relate how he gave me information on protein separation technology which became the basis for proteomics technology. On one of my visits he showed me a thesis by Pat O'Farrell on 2D gel protein separations. I took a copy back to Denmark and showed it to Julio Celis whom I had brought from Cambridge to work on suppressor tRNA but Julio right away started 2D gel work with his group. He was so successful that he has become the "father of proteomics" and Director of the Danish Cancer Institute. This was a very positive outcome of Marshall's friendship and help.

I think that the biggest tribute I can pay to Marshall as a person is that his character, curiosity, humility and generosity reminds of the arguably most lauded and most admired scientist, double Noble Prize Winner Fred Sanger. Fred worked at the bench all his life obviously with green fingers. His modesty and curiosity in others' work had no bounds. Marshall was in the same mould, a rare phenomenon this day. He was a gently, enquiring, friendly, generous man who was behind the most important biological discovery of the century rightly I think comparable to the Biological Rosetta Stone.

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