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Memories of a Senior Scientist

Steps in the study of DNA methylation from ϕX174 to genomic imprinting

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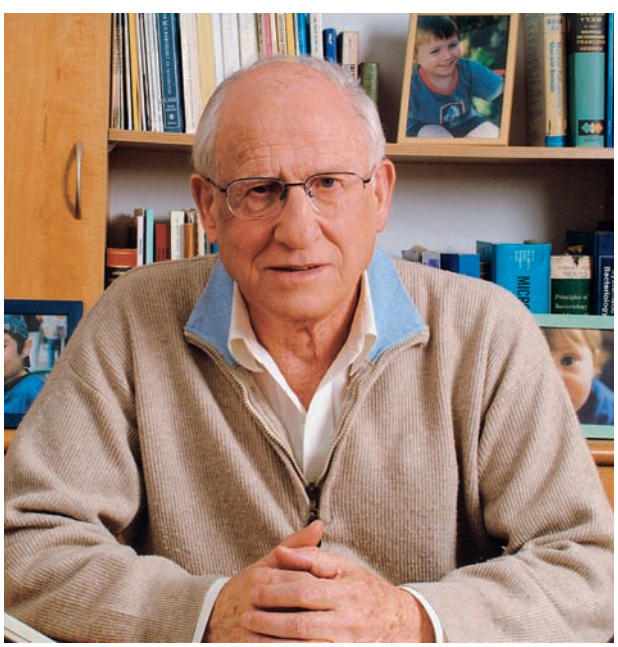

My first steps in research were taken in Jacob Mager's laboratory at the medical school of the Hebrew University of Jerusalem. I worked together with Avram Hershko (Nobel laureate in Chemistry 2004) on purine nucleotide metabolism in red blood cells under the supervision of Prof. Mager, a superb biochemist and outstanding mentor. Having finished our graduate studies, we both went for postdoctoral training. Avram to Gordon Tomkins's laboratory in San Francisco, where he initiated his studies on intracellular protein degradation, and I went to Robert Sinsheimer's laboratory in Caltech to study the process of DNA replication in the bacteriophage ϕX174.

In a joint effort with John Sedat, a very talented graduate student in Sinsheimer's laboratory, we discovered a single 5 methyl cytosine (5mCyt) residue in the ϕX174 genome. This observation had been published in the Journal of Molecular Biology in 1970 [1] without knowing its biological significance. The significance of the 5mCyt residue in the phage biology was subsequently revealed, upon my return to my home laboratory. We found that this methyl group serves as a signal for the binding of a specific protein to the phage DNA. This protein, the product of gene A of the virus, plays a critical role in the excision of one genome length during phage maturation [2]. At that time, it was the first demonstration of a specific protein that interacts with methylated DNA to carry out an important biological function. This prompted my interest in DNA methylation, which was at this time a deserted field in biological research (in the years 1970–1975).

John Sedat, my friend from the good days in Caltech, having finished in 1974 his postdoctoral training in Fred Sanger's laboratory in Cambridge, U.K., decided to join my laboratory in Jerusalem for one year before assuming a position at Yale. At that time, I was ready to channel my efforts from studying DNA methylation in prokaryotes to DNA methylation in eukaryotic systems. Looking for a suitable eukaryotic organism to study DNA methylation, we chose Drosophila that had advanced genetics and defined stages in development among other advantages. Yet, before embarking on this project we managed to develop several quantitative assay systems for the analysis of 5mCyt in DNA. Preliminary experiments with embryo, pupae, larvae and adult Drosophila DNA using mass spectrometry (one of the new analytical tools

that we developed) seemed to reveal 5mCyt in the DNA of the adult, less in pupae and none in the embryo, but the annoying thing was that the mass obtained by mass spectrometry was not quite that of 5mCyt. We kept these results on hold and did not publish them. In fact, several years later when we developed the nearest neighbor analysis of 5mCyt in DNA, we could claim that 5mCyt is undetectable in Drosophila DNA [3].

Although we did not publish our results, the rumor that we have data on DNA methylation in Drosophila spread fast. The rumor reached also Art Riggs in California who recently published a theoretical article on X inactivation, differentiation and DNA methylation [4]. In spite of the fact that I told Art Riggs that we were not sure about DNA methylation in Drosophila, he invited me to spend a sabbatical in his laboratory in the City of Hope Research Center in Duarte, California, where we could quietly assess the scarce data on DNA methylation in eukaryotes and discuss new ideas on how to advance the field further. Just before leaving for a sabbatical in California I started to collaborate with Howard Cedar, a newcomer to Israel, who was engaged in studying chromatin and gene expression. Our first joint project was the analysis of DNA methylation in chromatin by mass spectrometry. We found that methylcytosine in chromatin is enriched in nucleosomal DNA [5]. This observation drew attention of molecular biologists who were interested in chromatin structure and gene expression. When I went to spend a sabbatical with Art Riggs, Howard Cedar took a sabbatical in Richard Axel's laboratory in Columbia University in New York. We will resume our collaboration on our return to Jerusalem (vide infra).

Arriving in the City of Hope, I found Art Riggs deeply involved with Keiichi Itakura, in the chemical synthesis and cloning of Somatostatin and Insulin (this pioneering work, started the Gentech company). Yet Art was very interested in DNA methylation. I needed a short break from my work on DNA methylation, so I decided to take advantage of Itakura's unique ability to chemically synthesize oligonucleotides and try to repair a mutation in ϕX174. I developed a method that is known now as "site directed mutagenesis". We published our results under the title "Efficient correction of a mutation by use of a chemically synthesized DNA" [6]. Two weeks later a similar study that used oligonucleotides synthesized enzymatically was published by Michael Smith and colleagues in the Journal of Biological Chemistry [7]. While I regarded this achievement as a curiosity and gladly returned to my favorite field of interest, DNA methylation. Michael Smith continued in developing the site-directed mutagenesis method that earned him the Nobel Prize in Chemistry in 1993.

Art Riggs and myself had then time to discuss DNA methylation which eventually resulted in a review published in 1980 in Science [8]. This review became a landmark in methylation research. It introduced the concepts that gene specific and cell specific methylation patterns are established during gametogenesis or embryo development and that these patterns, once established, are clonally inherited and faithfully maintained. These concepts were later verified by a collaborative effort by Howard Cedar and myself. This collaboration became effective in the early 80's when we returned from our sabbaticals in the States. The Science review became a citation classic (over 1,000 citations) that clearly reflected the rapid growth of the DNA methylation field of research. Hundreds of laboratories joined the wagon. In 1984 the field grew so much that an entire book was required to accommodate all the new data [9].

A series of publications in the early 80's marked my fruitful collaboration with Howard Cedar. We demonstrated that gene specific methylation patterns in mammals are clonally inherited [10] and maintained by a maintenance methylase that prefers hemimethylated DNA as a substrate [11]. Having developed our nearest neighbor analysis method to study methylation in mammalian cells, we could show that methylation in mammals is specific to cytosine residues in CpG-containing sequences [12] and we also discovered that plant DNA is methylated in the symmetric palindromes CpG and CpNpG [13]. This observation was a milestone in plant DNA methylation research. Another important observation was that cell differentiation in the mouse is associated with genome-wide transient demethylation [14]. This demethylation seemed to be achieved by an active demethylation activity. While spending time in Giulio Cantoni's laboratory at the NIH as a Fogarty Scholar, I learned that direct removal of the methyl groups from DNA is inconceivable on thermodynamic grounds. Cantoni, the discoverer of the synthesis of the universal methyl donor S-adenosylmethionine, came to this conclusion having made thermodynamic calculations of methylation-demethylation years earlier. During my sabbatical in Cantoni's laboratory at the NIH, I demonstrated that demethylation could be achieved by replacement of 5mCyt with cytosine by a repair type mechanism [15]. Although several mechanisms have been proposed since then for active demethylation of DNA, the exact mechanism of DNA demethylation remains controversial. One most interesting mechanism that perhaps comes close to the bona fide demethylase is based on oxidative demethylation of 5mCyt to Cyt residues on the DNA [16]. This mechanism was first suggested by Moshe Szyf, a former graduate student in my laboratory, who is now professor of pharmacology in McGill University in Montreal.

Technological advances in molecular genetics allowed us, in the early 90's to study for the first time, methylation on the gene level in the mouse embryo. Methylation analysis of specific genes revealed dynamic changes in methylation patterns during the early stages of embryo-

genesis and gametogenesis [17]. We found that zygotic methylation of all genes is erased by the precavitation stage by active demethylation [18], and that global de novo methylation with the exception of CpG islands, follows at the pregastrula stage [19].

A small subset of genes are imprinted, expressed monoallelically in a parent specific manner and show differentially methylated regions (DMRs). The differentially methylated regions that are present in all imprinted genes are in general established during gametogenesis and maintained during embryo development [20]. This methylation plays a dual role in imprinting, first in establishing the imprint during gametogenesis and in propagation of the imprint during embryogenesis, and second in regulating monoallelic expression. To those working in the field of genomic imprinting it was clear that imprinting is controlled by imprinting boxes. We have discovered the first imprinting box in a differentially methylated region present in intron 2 of the Igf2r gene [21]. The box consists of two cis elements: a de novo methylation signal (DMS) and an allele discrimination signal (ADS). More recently, a bipartite imprinting box was discovered by us in an imprinted gene cluster [22]. The mechanism of action of this bipartite imprinting box is currently being investigated [23] and its implication in neurodevelopmental diseases is gradually being appreciated [24 in press.].

Now, when I sum up my fruitful and enjoyable long research career that is still ongoing, I must thank all my colleagues of the past and present and my wonderful students who have given me inspiration and joy throughout my long years of research.

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