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DANIEL NATHANS
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A Biographical Memoir by

DANIEL DIMAIO

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DANIEL NATHANS

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BY DANIEL DIMAIO

DANIEL NATHANS, A SCIENTIST whose pioneering use of restriction endonucleases revolutionized virology and genetics and whose personal qualities had a profound impact on those who knew him, passed away in November 1999 at the age of 71. He was the University Professor of Molecular Biology and Genetics at the Johns Hopkins University School of Medicine, where he served on the faculty for 37 years, and a senior investigator of the Howard Hughes Medical Institute since 1982. Dan is survived by his wife, Joanne; three sons, Eli, Jeremy, and Benjamin; and seven grandchildren.

Dan was born and raised in Wilmington, Delaware, the youngest of eight children of Russian Jewish immigrants. He attended the University of Delaware, initially living at home and commuting by hitchhiking, and graduated with a degree in chemistry in 1950. He then entered medical school at Washington University in St. Louis, largely because, he claimed, his father saw him "as the last chance to have a doctor in the family." Dan began medical school with the intention of returning to Wilmington as a general practitioner, but a summer job in a local hospital bored him and made him rethink these plans and return early to St. Louis for a research position in Oliver Lowry's laboratory. While at

Washington University, Dan caught the attention of another of his professors, W. Barry Wood, who was later to move to Johns Hopkins and recruit Dan to join him in Baltimore.

After completing medical school in 1954 Dan did a medical internship at the Columbia-Presbyterian Hospital in New York City. He remembered this year as one of his most valuable because of the real-life problems he faced and the real responsibility he had for patients, but this was also a year that reinforced his decision to enter the laboratory. Dan then spent two years as a clinical associate at the National Cancer Institute, caring for patients and carrying out research on the synthesis of immunoglobulin by myeloma tumors. During this time, Dan met Joanne Gomberg and after a whirlwind courtship they were married. After returning to Presbyterian Hospital for two more years as a medical resident, Dan realized that his calling lay in medical research, and he finally dropped his plans to practice clinical medicine, much to his father's bewilderment.

Dan began his basic research career at the Rockefeller Institute in 1959 with Fritz Lippman. He initially enrolled in a Ph.D. program, which he abandoned because he decided he did not want to sit through any more lectures. Dan began by studying the mechanism of protein synthesis in myeloma cells, but he soon turned his attention to the more tractable *E. coli* system. These were the days before the discovery of messenger RNA and the elucidation of the genetic code, and Dan investigated the soluble factors that catalyzed the transfer of amino acids from amino acyl-tRNA to the growing polypeptide chain. He soon made his first major research contribution, the development of a bacterial cell-free system that supported protein synthesis. He then got wind of the discovery of RNA bacteriophage in Norton Zinder's laboratory and showed that phage RNA could support the synthesis of viral coat protein in a cell-free system. This was the first

example of a purified mRNA that directed the synthesis of a specific protein. Many laboratories extended these initial observations, leading to a number of fundamental insights into the mechanism of protein synthesis. This important early work already displayed the rigor, clarity of thought, and impact that characterized Dan's entire body of work.

Dan continued his studies on bacteriophage and protein synthesis after he was recruited to Johns Hopkins in 1962 by Barry Wood, who was by then chairman of the Department of Microbiology. During his early years on the Hopkins faculty Dan carried out important studies on the regulation of bacteriophage translation by the viral coat protein, and he demonstrated that puromycin inhibited protein synthesis by being incorporated into the growing polypeptide chain, resulting in premature termination of translation. In experiments that presaged his later studies with restriction enzymes, he showed that 5-fluorouracil-substituted phage MS2 RNA generated subgenomic viral RNA fragments that encoded specific viral proteins.

In the late 1960s Dan's animal virus colleagues, Bernard Roizman and Robert Wagner, left Hopkins, and Barry Wood asked Dan to teach medical students about these viruses. Dan accepted this assignment with trepidation, because he knew little about the topic, but he was soon struck by the dramatic effects that tumor viruses had on cells. At this time, molecular studies of animal viruses were in their infancy, but the basic tools of propagation of animal viruses in cultured cells, plaque assays, and in vitro transformation systems had been developed. Dan saw the parallels with the bacteriophage, the analysis of which had spawned molecular biology, and he realized that the study of simple animal viruses would provide important insights into carcinogenesis and the biology of animal cells. What was lacking were the powerful genetic techniques of bacterial systems; during the next decade, it

was Dan who provided the tools that allowed detailed molecular genetic analysis of mammalian viruses and cells.

Dan decided to redirect his research effort to the analysis of animal viruses, and after some thought he selected the simplest DNA tumor virus, SV40, as the object of his attention. Even though the SV40 genome was only about 5,000 base pairs of double-stranded circular DNA, a small size Dan found comfortable, this virus had the ability to grow lytically in monkey cells and to cause permanent tumorigenic transformation of rodent cells. To learn how to grow and handle SV40, Dan spent a sabbatical leave in 1969 with Leo Sachs and Ernest Winocour at the Weitzman Institute. While in Israel, in one of those wonderful moments we all dream about, Dan received a letter from his Hopkins colleague Hamilton Smith describing a new enzymatic activity from the bacterium *Hemophilus influenzae* that degraded DNA from foreign cells but did not degrade its own DNA. Ham also mentioned preliminary evidence suggesting that this enzyme cleaved DNA at specific nucleotide sequences. Perhaps with his studies of 5-fluorouracil-fragmented-phage RNA in mind, Dan immediately realized the implications of this discovery. "Well, that set me off thinking that we could use restriction enzymes to dissect the genome of a small papovavirus and learn something about how the virus works . . . and perhaps learn something about what genes are required for transformation."[1]

Dan returned to Hopkins with some radiolabeled SV40 DNA in his luggage, and he set about testing his ideas. With Stuart Adler, a medical student, he surveyed the ability of all known restriction enzymes to cleave SV40 DNA. Early attempts to use the *E. coli* B restriction enzyme were unsatisfactory because it did not cleave DNA at specific sites. But then Ham Smith and his postdoctoral fellow Thomas Kelly showed definitively that the *H. influenzae* restriction enzyme

cut DNA at specific recognition sites consisting of short defined nucleotide sequences.[2] (Actually, it turned out that the cleavage activity was due to a mixture of two restriction enzymes, HindII and HindIII.) In Dan's words, here were the "trypsins and chymotrypsins for DNA" that could be used to reduce an apparently featureless DNA molecule into homogeneous, manageable pieces derived from specific regions of the viral genome, onto which individual genetic activities could be mapped. Years later, Tom Kelly admitted that he and Ham Smith found the specific sites, but they had not fully appreciated the significance of the specific fragments.

Dan and his student Kathleen Danna focused on Ham Smith's enzyme, showing that it did in fact cleave SV40 DNA into 11 specific pieces. These results were published in 1971 in the *Proceedings of the National Academy of Sciences*, in a paper that ushered in the modern era of genetics. Figure 1 was standard fare and showed that cleavage altered the sedimentation profile of SV40 DNA. But Figure 2 crossed the divide: The DNA fragments were separated and revealed by polyacrylamide gel electrophoresis. In the discussion, with Dan's characteristic understatement, the New World came into view.

> The availability of pieces of SV40 DNA from specific sites in the molecule should be helpful for the analysis of the function of the SV40 genome. For example, when the order of fragments in the genome is known, it should be possible to map "early" and "late" genes and those genes that function in all transformed cells. It may also be possible to localize specific genes by testing for biological activity, e.g., T-antigen production or abortive transformation. If specific deletion mutants become available, the analysis of restriction enzyme digests may . . . [allow] mapping of such mutants. Comparison of restriction endonuclease digests by polyacrylamide gel electrophoresis has also provided a new method for detecting differences in DNA . . . It should [also] be possible to . . . obtain quite small, specific fragments useful for the determination of nucleotide sequence."[3]

This vision was soon transformed into reality in work that was often breathtaking in conception and elegance. Together with Kathy Danna and George Sack, a medical fellow, Dan determined the specific order of each fragment in relation to the others and constructed the first cleavage map of a viral genome. This map, which was to serve as a framework for localizing functional elements of SV40 DNA, was constructed by isolating overlapping partial digestion products and determining their constituent fragments—an approach developed in the analysis of proteins was applied to DNA with brilliant insight and success. Indeed, one of Dan's great strengths was his ability to adapt approaches developed in other fields to the study of genes. This is nicely illustrated by the identification of the origin of SV40 DNA replication, the first genetic signal to be positioned on a eucaryotic viral genome. Here, he designed a gradient-of-label experiment, modeled on the experiment of Howard Dintzis to map the direction of protein translation in vitro, to determine the temporal order of synthesis of specific viral DNA fragments in infected cells. In a figure that told a story of a thousand words, the results were displayed, mapping the origin and terminus of viral DNA replication and establishing that replication was bi-directional and proceeded symmetrically.

In the following years, Dan and his colleagues and collaborators devised a series of approaches to exploit the specific cleavage of DNA by restriction enzymes to dissect the genome of SV40. Restriction-fragment DNA fingerprinting was used to map sequence differences between different strains of SV40 DNA (and hence uniquely identify these strains) and to follow the genetic changes that occurred during virus evolution. A marker rescue approach using restriction fragments was developed and used in conjunction with the cleavage map to locate the position of mutations

that resulted in temperature-sensitive defects in virus replication. In collaboration with George Khoury and Malcolm Martin, viral transcription units were mapped to specific segments of the viral genome in lytically infected and transformed cells. This work established many important features of the SV40 genome and life cycle, including the identification of the early and late genes and the demonstration that they were transcribed in divergent directions.

The early work from Dan's laboratory used restriction enzymes to map various functions of the viral genome; however, there was soon a subtle shift from using these reagents to map viral RNA and DNA to using them to actually generate viral mutants and reconstruct the viral genome. The altered genomes were then reintroduced into cells and assayed for biological activity. Initially, specific restriction fragments were deleted to generate viral mutants lacking defined segments of the genome, leading to the identification of the large T antigen as the product of the viral A gene. Later, Dan's laboratory developed more sophisticated methods of site-directed deletion and point mutagenesis to analyze SV40 regulatory signals and proteins to infer the function of individual viral proteins and *cis*-acting signals. These studies led to the precise localization of the DNA replication origin (and even the identification of individual nucleotides in the origin that controlled the rate of viral DNA replication) and the demonstration that the SV40 large T antigen was a multifunctional protein with independently acting domains. Gone were the days of random mutagenesis followed by the laborious task of separating the interesting mutants from the chaff, replaced by a more directed approach of deliberately manipulating the genome to generate the desired mutant. In a particularly elegant demonstration of the power of combining directed mutagenesis techniques with more classical approaches, Dan and his students provided con-

vincing genetic evidence that the ability of the SV40 large T antigen to recognize the viral origin underlay the role these two elements played in the initiation of viral DNA replication. Although the techniques developed in Dan's laboratory were superseded by new methodology made possible by advances in oligonucleotide synthesis, these early experiments alerted the scientific community to the power of this "new genetics."

This work galvanized the scientific community, and soon many laboratories were exploiting restriction enzymes to analyze DNA. Of particular importance were Paul Berg's identification of Eco RI as an enzyme that cleaved SV40 DNA at a unique site, defined by Dan as ground zero on the Hind cleavage map, similar analysis of SV40 by Joseph Sambrook, and the application of these techniques to bacteriophage ϕX-174 DNA by Clyde Hutchinson. In addition, Dan was generous in distributing his reagents and information widely, a practice that bore early fruit in the determination of the complete nucleotide sequence of SV40 DNA by Walter Fiers and Sherman Weissman and their co-workers.

Dan freely admitted that he did not foresee the recombinant DNA revolution made possible by the in vitro manipulation of DNA. Nevertheless, many of the techniques developed by Dan's laboratory in the 1970s helped form the foundation of the nascent field of genetic engineering that was being developed by Paul Berg, Stanley Cohen, Herbert Boyer, and others. Indeed, in a certain sense, a main goal of genetic engineering was to amplify segments of cellular DNA in SV40-size packets so that they could be analyzed by the restriction enzyme-based methods developed to study SV40 itself. Dan was quick to recognize the uses and potential risks of this technology. He was a signatory of the letter calling for a moratorium of certain recombinant DNA experiments, but he was also among the first to use molecular

cloning to construct and to propagate replication-defective animal virus mutants in bacteria.

In the latter part of his scientific career Dan studied the cellular response to growth signals and isolated and characterized some of the first cellular genes whose expression was regulated by growth factor treatment. Dan was struck by the fact that the genes induced most rapidly by growth factors were often transcription factors, and he pointed out the parallel between these transcription factors and viral immediate-early proteins that orchestrated the sequential program of viral gene expression and genome replication. Dan was particularly intrigued by the ability of variant proteins, such as those produced by alternative splicing, to modulate the activity of the full-length form. Some of his final publications concerned the DNA binding specificity of these cellular transcription factors, work that mirrored his early interests in SV40 large T antigen and sequence-specific recognition of the viral replication origin.

This work brought Dan fame and great recognition, including election to the National Academy of Sciences, numerous honorary degrees, the National Medal of Science, and the 1978 Nobel Prize for physiology or medicine. He greeted the news of his Nobel Prize with characteristic skepticism, insisting on independent confirmation before he would comment on the award, and humility, paying tribute to his wife, Joanne, whose main job, he stated, was to make sure that his head didn't get too large for his hat. The day Dan learned of the Nobel Prize, he deferred departmental celebrations until he had led his regularly scheduled laboratory session for a small group of medical students (although reports had it that little instruction occurred that day). In what was undoubtedly a refreshing break from custom, Dan, at a celebratory university assembly, declined to answer a question from the audience about a matter of public policy by

gently reminding the questioner that he was not an instant expert on topics unrelated to his research simply because he had won a Nobel Prize! He was delighted to share the Nobel Prize with Ham Smith and Werner Arber, who carried out the early genetic analysis of restriction and modification in bacteria and who predicted the existence of restriction enzymes. The Nobel citation recognized the role this work played in the birth of modern genetics and predicted much of the genetic revolution that is still underway.

Dan's impact was not restricted to his published work. Because of his fairness and insight, his counsel was widely sought, first by students, colleagues, the staff who washed the petri dishes and swept the floors; later by presidents of the United States, when he was a member of the Presidential Council of Advisors on Science and Technology, and even by the Pope, when Dan was called to Vatican City to provide advice to the Holy See on scientific matters. In these duties, Dan was served well by his interests and knowledge in a wide range of areas, including history, politics, literature, and the arts, which he shared with his wife, Joanne.

Dan's gifts were also recognized at Johns Hopkins, where he was chairman of the Department of Microbiology for many years. He viewed the ideal department to be one the size of a small extended family, and his main role as chairman to be one of *paterfamilias*, fostering the careers of his junior faculty. Dan also served for one year as interim president of the Johns Hopkins University and led the university through a challenging time that saw the successful redefinition of the relationship between the School of Medicine and the Johns Hopkins Hospital. He compared the presidency to his year as a medical intern at Columbia-Presbyterian Hospital, which also forced him to make quick decisions based on limited data, but he tackled it with characteristic thoughtfulness, fairness, good sense, and grace. A genera-

tion of administrators learned what the scientists had known all along—that Dan was a man of few words who invariably saw the core of the problem and had the vision to find solutions. As aptly put by Jeremy Berg, chairman of biophysics at Johns Hopkins, Dan had the highest signal-to-noise ratio of anyone he had ever met. Most importantly, because he put the interests of the Johns Hopkins University and the biomedical research endeavor first and never sought self-aggrandizement, Dan was able to marshal the support of diverse constituencies. In short, he had the moral authority to lead.

Dan was also a wonderful teacher, particularly in a one-on-one setting, when he would wander through the laboratory, sit down, and ask, "What's new?" He was always intensely interested in the science and wanted to see the data, and weekend mornings would often find him patiently passaging monkey cells in the tissue culture room. But this interest carried with it a risk. If you hadn't thought through your results, Dan could solve your problem on the spot. We quickly learned to analyze our own results carefully before showing them to Dan, lest we be scooped by the boss! Dan also would not accept facile explanations or hand waving, and instead he would insist, "Well, what's your experiment?" He embraced young scientists, even those of us who did not immediately grasp how the analysis of DNA fragments, defined by the arbitrary cleavage specificities of bacterial enzymes, would revolutionize genetics. Dan taught us his approach to science, one that entailed reducing a problem to its essential features and then attacking it at its core, and we soon learned not to be satisfied with the surface nuggets along the streambed, but to dig a mine and find the mother lode.

As Dan approached his seventieth birthday he looked forward to completing his term as university president and

returning to the laboratory. He told me, "I'm ready to come back full-time to being a professor, to do some teaching, and to continue with my research. I'm thinking about what new areas of science I want to get into. I'm hoping that Howard Hughes Medical Institute will want to consider to support me for a little while, and I am looking forward to continuing what I consider a privileged life."[1]

The following year Dan was diagnosed with acute myelogenous leukemia. He continued to come to the laboratory between courses of chemotherapy and hospitalizations, and he particularly enjoyed long walks through his Mt. Washington neighborhood with Joanne. He was also enormously proud of the accomplishments of his family. For many years, Joanne was a lawyer serving in the City of Baltimore's Department of Legislative Reference. Benjamin is on the faculty of the University of Pennsylvania, Eli is a lawyer who has returned to graduate school at Hopkins to obtain a Ph.D. in history, and Jeremy is a neuroscientist who had joined Dan on the faculty in the Department of Molecular Biology and Genetics at Hopkins. On the night of November 16, 1999, Dan passed away at home.

Dan Nathans changed the way we viewed viruses and genes. When he began his studies dissecting the genome of SV40 in 1970, genes were *terra incognita*. The coastline had been roughly charted by classical genetic experiments, but the vast unbroken interior stretched on toward a distant horizon. Dan taught us how to draw lines of longitude and latitude on this map, and gave us the first lessons on how to fill in all the glorious detail. The true measure of this work is that today we can barely imagine how to analyze viruses and genes without using the approaches pioneered in Dan's laboratory. Challenge a student to design a molecular genetic experiment that doesn't entail the use of restriction enzymes

or molecular cloning. You might as well ask for bricks without straw.

Despite the enormous importance of his scientific contributions and administrative service, those who knew Dan will remember him chiefly for his personal qualities. He was gentle, soft-spoken, modest, scrupulously fair, and unswervingly honest. His success was leavened by his humility, and his intelligence by his wisdom. For as long as I can remember, conversations about Dan focused not on his scientific accomplishments but on these extraordinary human characteristics. To work closely with Dan Nathans was a privilege beyond measure, an experience that forever shaped our science and our lives.

I FIRST MET Dan Nathans when I arrived at Johns Hopkins as a medical student in 1974, and I got to know him well beginning in 1977, when I entered his laboratory to carry out Ph.D. dissertation research. This memoir is based largely on my personal recollections of Dan and his laboratory dating from this exciting time. I was greatly assisted by an audiotape interview that I conducted with Dan in 1996, in which he reflected at length on his background, scientific career, and administrative duties.[1] Another valuable source of information was the article he published on the occasion of his being awarded the 1978 Nobel Prize in medicine or physiology entitled, "Restriction Endonucleases, Simian Virus 40, and the New Genetics" (1979). I am also greatly indebted to Thomas Kelly, Thomas Shenk, and Steven Desiderio, who encouraged me to commit my memories and thoughts to paper; to my friends and colleagues who shared their recollections of Dan with me; and to Keith Peden and Charles Radding, who provided helpful suggestions on this manuscript.

NOTES

1. Audiotape interview of Daniel Nathans conducted in July 1996 in the series "Leaders of American Medicine," sponsored by Alpha Omega Alpha.

2. T. J. Kelly and H. O. Smith. A restriction endonuclease from *Hemophilus influenzae*. II. Base sequence of the recognition site. *J. Mol. Biol.* 53(1970):393-409.

3. K. Danna and D. Nathans. Specific cleavage of simian virus 40 DNA by restriction endonuclease of *Hemophilus influenzae. Proc. Natl. Acad. Sci. U. S. A.* 68(1971):2913-17.

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