ESSAY

On the road from classical to modern molecular biology

Tom Maniatis

I graduated from the University of Colorado in Boulder in 1965, and, while browsing through the university bookstore, I noticed a new book called *Molecular Biology of the Gene* by James Watson. When I read it, I was captivated by the elegance and power of an exciting new field that combined structure, genetics and biochemistry. Delving more deeply, I was fascinated by the classic paper of Francois Jacob and Jacque Monod on the operon theory and the subsequent identification of the Lac and λ phage repressors by Walter Gilbert and Mark Ptashne, respectively. These breakthroughs in our understanding of gene regulation in prokaryotes, which were based on a combination of ingenuity, genetics and biochemistry, defined classical molecular biology and inspired me to seek a PhD in the new field and, ultimately, to study the mechanisms of gene regulation in eukaryotes.

On the basis of my interest in molecular biology, Joseph Daniel, my undergraduate research advisor at Colorado, suggested that I contact Leonard Lerman, who had recently moved from the University of Colorado Medical School to the new Department of Molecular Biology at Vanderbilt University. I took Joe's advice and ultimately joined Leonard's lab, where I studied the structure of highly compacted DNA by small-angle X-ray scattering. Leonard was an excellent mentor who encouraged critical thinking, and I enjoyed learning a new field. Toward the end of my thesis research, Mark Ptashne visited Vanderbilt, and during his dinner with the graduate students I had the opportunity to discuss my interests in his work. That conversation ultimately led to an offer to join Mark's lab at Harvard for postdoctoral studies.

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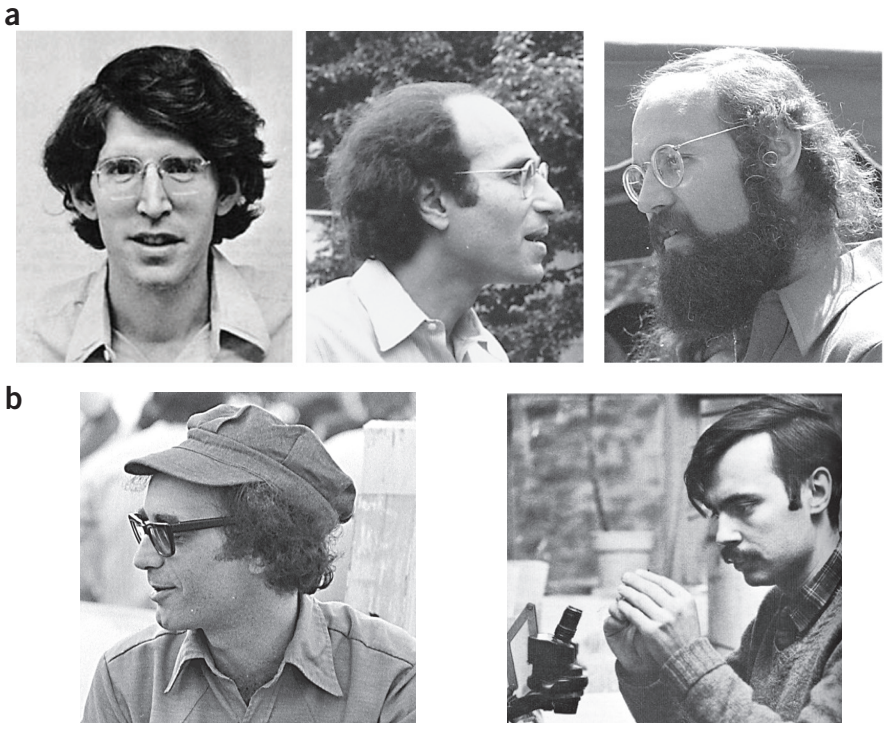

Figure 1 Photos of me and my colleagues taken circa 1976–1978. (**a**) Tom Maniatis, Richard Axel and Hal Weintraub. (**b**) Argiris Efstratiadis and Fotis Kafatos. Courtesy of the Cold Spring Harbor Archives.

My postdoctoral project was the characterization of the λ repressor/operator protein–DNA complex. I found that the repressor binds cooperatively to multiple adjacent DNA sequences in the λ operators and that mutations in these sites weakened DNA binding *in vitro* and increased expression *in vivo*. This early use of restriction enzymes and DNA binding assays presaged my later studies of eukaryotic promoters and enhancers. To determine the DNA sequence of the λ operators, Mark trapped Fred Sanger on a ski lift at Keystone and coerced him into hosting us in his lab at the UK Medical Research Council laboratory in Cambridge. We were initially the unwelcome American duo, but Fred quickly warmed to us and was a gracious host and mentor. Fred's quiet brilliance and modesty affected everyone fortunate to work with him. It was a particularly exciting time in Fred's lab, as he and Allen Coulson were developing the Sanger sequencing method (and back home, at Harvard, Allan Maxam and Walter were developing an alternative approach to sequencing). As these methods were still in development, Mark and I worked with Bart Barrell and John Donelson using an older, more cumbersome procedure to determine the DNA sequence of a 27–base-pair λ operator, which required nearly a full year to complete. Over the next several years, Mark and his lab went on to show how the organization of the operators has a fundamental role in the λ 'genetic switch'. I benefited

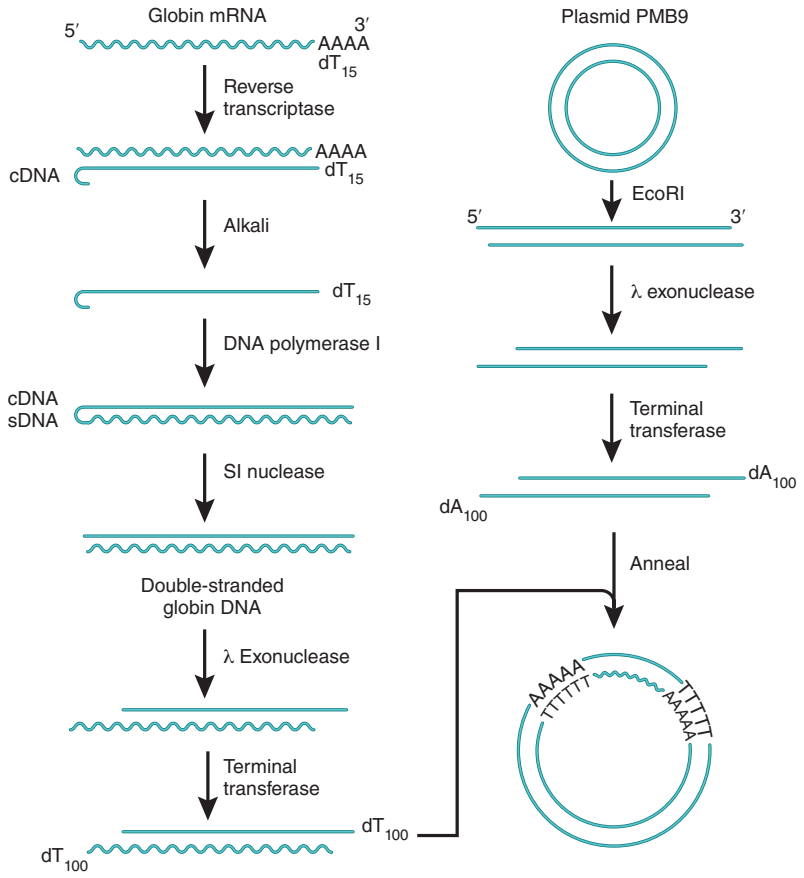

Figure 2 Schematic illustrating the synthesis and cloning of double-stranded cDNA from purified rabbit β-globin mRNA using the sequential activities of reverse transcriptase and DNA polymerase I. Reverse transcriptase generates a short hairpin that functions as a primer for second-strand synthesis. S1 nuclease cleaves the hairpin, the ends are prepared for the terminal transferase reaction by λ exonuclease treatment, and oligo-dT is added to the ends. The plasmid PM9 vector is cleaved by EcoRI and subjected to oligo-dA addition by terminal transferase. The cDNA is annealed to the plasmid DNA through dA:dT base pairing, and the hybrid DNA molecule is introduced into *E. coli*. Redrawn from ref. 2.

enormously from my time in Mark's lab. Mark was an excellent and intellectually demanding mentor, and he deeply influenced my thinking and writing. He is the best I have known at designing clever, incisive experiments that address deep, mechanistic questions.

While still a postdoc at Harvard, I attended the 1976 Cold Spring Harbor Symposium on chromatin. This meeting would profoundly influence the future directions of my science and lead to life-long friendships. During the annual lobster bake on the Cold Spring Harbor Laboratory (CSHL) beach, I met Richard Axel and Hal Weintraub (**Fig. 1a**). I felt an immediate intellectual and personal connection with them, and we remained close scientific colleagues and friends until Hal's untimely death at the age of 50 in 1995. We spoke every week on the phone, often late at night discussing the latest data, paper or idea. We often attended meetings together, including some in fancy locales overseas. Hal was always dressed in jeans, t-shirt and high-top basketball shoes, and only rarely could we accommodate Richard's expensive tastes like dining at a Michelin-rated restaurant. On one occasion, Hal appeared very much out of place in a spectacular restaurant when an elegant French gentlemen sitting at the next table turned to Hal and said "nice shoes." The gentleman was François Mitterrand, the President of France. Hal was the most imaginative and creative biologist I have known.

Upon returning to Harvard from the UK, I met Argiris (Arg) Efstratiadis, who was a graduate student (and MD) in Fotis Kafatos's lab at Harvard (**Fig. 1b**). Arg and I connected immediately, and I came to enjoy his humor, intellectual intensity and madness. An initial brief conversation led to a highly productive collaboration with Arg and Fotis, the ultimate goal of which was to clone full-length complementary DNAs (cDNAs). At that time, Herb Boyer and Stanley Cohen had shown that a eukaryotic ribosomal gene provided by Don Brown could be joined to a plasmid and propagated in bacteria, and Dale Kaiser and Paul Berg's labs had shown that double-stranded DNA molecules could be joined by dA:dT joining. David Hogness exploited this method to clone and characterize random fragments of *Drosophila* genomic DNA, and his laboratory went on to transform *Drosophila* developmental biology through the elegant application of genetics and genomic cloning methods.

Our strategy for cDNA cloning was to perfect a method for generating full-length double-stranded cDNA, which could then be inserted into a plasmid by dA:dT tailing. However, our goal of cloning cDNAs was derailed by the recombinant DNA controversy in Cambridge, Massachussetts, which led to a moratorium on recombinant DNA research in July 1976. The moratorium was the result of a complex mix of genuine concerns for safety, post-Vietnam social activism, historic town-and-gown antagonisms, city and scientific politics and intellectual dishonesty. The latter led to an infamous recital of a 'slow-death scenario' at a city council meeting by an opponent of the research in the guise of providing 'scientific' advice to the mayor. The end result was that we were unable to insert our *in vitro*–synthesized DNA into a bacterial plasmid.

Fortunately, however, we were rescued by Jim Watson, who offered me a place to work at the CSHL, where recombinant DNA research was allowed. CSHL was a beehive of activity when we arrived. Jim Watson had recruited an exceptional group of young investigators, including Joe Sambrook, Phil Sharp, Rich Roberts, Mike Botchan and many more. Restriction enzymes were being used to create genetic and physical maps of viral and eukaryotic genomic DNA in Joe Sambrook's lab, and these tools contributed to the discovery of RNA splicing by Rich Roberts and his colleagues, who were also producing dozens of new restriction enzymes (RNA splicing was independently discovered in Phil Sharp's lab at MIT). CSHL also provided an endless source of new information and interesting people and science through its meetings program. It was in this atmosphere that we succeeded in generating and thoroughly characterizing the first full-length cDNA clones of β-globin mRNA[1,2]. This required the purification of a number of enzymes that were not commercially available, the observation that reverse transcriptase leaves a short hairpin DNA at the 3′ end of the transcript and the establishment of conditions for cleaving the hairpin by the single-strand–specific S1 nuclease. (**Fig. 2**). The production and faithful propagation of full double-stranded DNA clones provided powerful tools for isolating genes from

genomic DNA and for the production of mammalian proteins (see ref. 1 for a more detailed discussion of cDNA cloning in the mid-1970s).

When the Cambridge moratorium on recombinant DNA was further extended in October 1976, it became clear that I could not establish a competitive research program in recombinant DNA at Harvard, and adequate space was not available at CSHL. I was contacted by the Chairman of Biology at Caltech, Robert Sinsheimer (a nationally recognized and highly respected opponent of recombinant DNA research at the time), who offered me a faculty position. This prompted an angry phone call to Sinsheimer from George Wald, one of the leaders of the anti–recombinant DNA group in Cambridge. Sinsheimer responded by saying that he did not see a conflict between his opposition to recombinant DNA as "a private citizen" and his responsibility as department chairman to recruit outstanding faculty. Sinsheimer later became supportive of recombinant DNA research and ultimately became an early proponent of the Human Genome Project, which relied on recombinant DNA technology.

At Caltech I was greeted by a highly collegial faculty, and thus began an intense and exciting period of activity that coincided with important advances in understanding gene regulation during development in Eric Davidson's and Norman Davidson's labs and immunoglobulin diversity in Lee Hood's lab, the birth of behavioral neurogenetics in Seymour Benzer's lab, and the discovery of homeotic genes in Ed Lewis' lab. Norman was an exceptional mentor who played an important part in the success of my lab with sage advice and critical input. Every scientific discussion with Norman started with a yellow pad on which he would carefully write down the key facts. I also enjoyed my brief interactions with Max Delbruck, but I was shocked by his aggressive questions in seminars.

My lab focused on the development of genomic DNA libraries. The promise of using specific cDNA hybridization probes and isolating individual genes by screening genomic libraries was realized with the isolation and determination of the genomic organization of the rabbit and human globin gene clusters[3,4]. However, the generation of a fully representative genomic library with the necessary physical and biological containment conditions required considerable effort and the use of highly efficient means of introducing the recombinant phage into bacteria[3] (**Fig. 3**). This advance made it possible to identify RNA splicing mutations that cause the inherited blood disease thalassemia[5], to establish the transient transfection method in collaboration with Yasha Gluzman at the CSHL[6] and to stably introduce cloned genes into cultured cells with Richard Axel and Michael Wigler[7]. These recombinant DNA tools not only made it possible to study eukaryotic gene regulation at a level comparable to that of the earlier prokaryotic studies but also played an important part in the emergence of the biotechnology industry.

It was at this time that Ed Fritsch, a postdoctoral fellow in my lab (who had a key role in genomic cloning), and I organized a successful CSHL summer course in molecular cloning. A remarkable byproduct of this summer course was the *Molecular Cloning Manual*[8], which we wrote with Joe Sambrook. At the time, none of us (perhaps with the exception of Jim Watson, who orchestrated the effort) could foresee the impact the cloning manual would have on the training and dissemination of recombinant DNA technology to a generation of emerging molecular biologists.

In the 1980s, our interest in globin genes continued after my return to Harvard, where I was surrounded by extraordinary colleagues and benefited enormously from the outstanding students and postdocs attracted to the Department of Biochemistry and Molecular Biology. I taught at Harvard College for nearly 30 years and was constantly stimulated and challenged by brilliant students. During this time, our interest in globin genes led to the development of an *in vitro* splicing system[9], the discovery of the lariat splicing intermediate[10] (also reported by Phil Sharp). We also discovered the serine/arginine splicing factor SC35 (SRSF2)[11] and found that exon sequences are required for accurate RNA splicing[12]. Finally, we identified and characterized splicing enhancers and their role in alternative splicing[13].

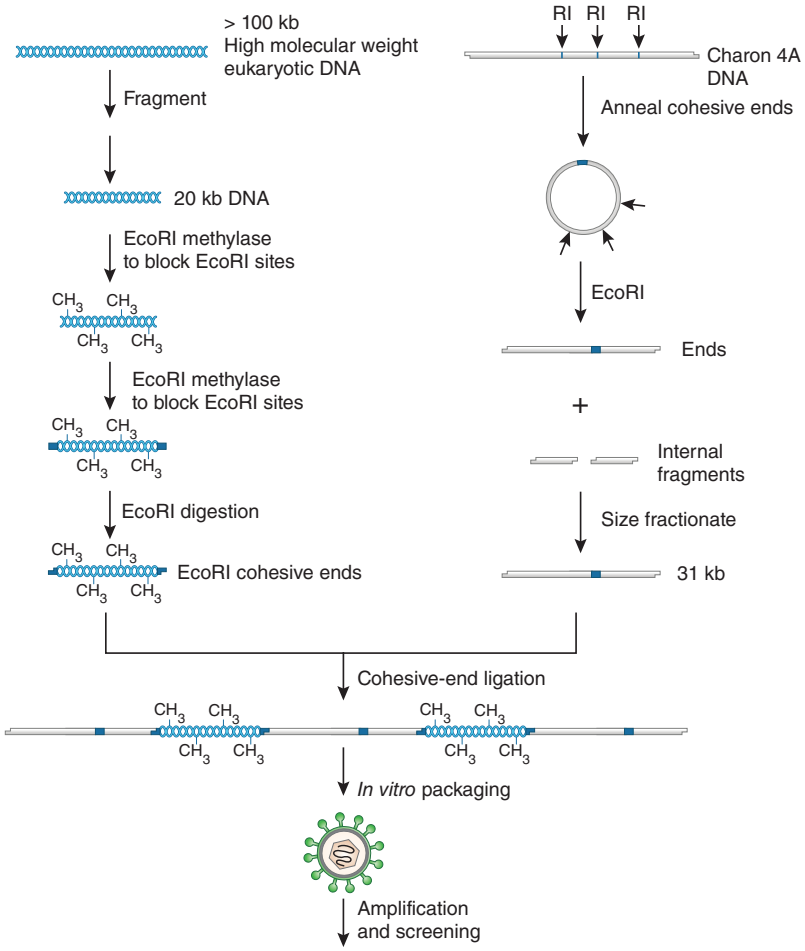

Figure 3 Schematic illustrating our strategy for the construction of a genomic DNA library. High molecular weight genomic DNA was fragmented, size fractionated to enrich for 20-kb fragments and modified by EcoRI methylase to block the EcoRI sites from cleavage. We then added EcoRI linkers by blunt-end ligation and generated EcoRI cohesive ends by EcoRI digestion of the linkers. Bacteriophage λ 'arms' were generated by EcoRI cleavage, size fractionated and then ligated to genomic DNA to form long concatemers, a substrate for efficient *in vitro* packaging of the recombinant DNA into phage heads. The library was then amplified in bacteria. Redrawn from ref. 3.

The transfection methods also made it possible to investigate mechanisms of globin and interferon gene regulation, leading to the identification and characterization of transcriptional promoters and enhancers and the discovery of the combinatorial nature of *cis*-regulatory elements and the assembly of multicomponent splicing enhancers (enhanceosomes[14]). Interest in the mechanism by which the enhanceosome is assembled led to studies of the signaling pathway required for the activation of interferon gene expression in response to viral infection. This, in turn, led to the finding that the processing of the p105 protein precursor of the p50 subunit of nuclear factor-κB is ATP dependent[15], to the first evidence for a role of the ubiquitin-proteasome pathway in signal transduction[16] and to the discovery of the ubiquitin-dependent IκB kinase with James Chen[17].

In 1980, Mark Ptashne and I cofounded Genetics Institute, which quickly established and improved gene cloning and expression methods directed toward the treatment of human diseases. We were fortunate to have assembled an incredible group of young scientists working at the forefront of technologies to produce protein drugs using recombinant DNA methods. Among the drugs developed and approved by the US Food and Drug Administration (FDA) at the Genetics Institute were the blood clotting factors VIII and IX, erythropoietin and bone morphogenic proteins. As chairman of the scientific board of the Genetics Institute for over 15 years, I was exposed to a broad spectrum of human biology and medicine and experienced first hand the translation of basic research into treatments of human diseases.

On the basis of our finding that the ubiquitin-proteasome pathway is required for the activation of nuclear factor-κB[16] in 1994, Fred Goldberg and I, along with Michael Rosenblatt and Kenneth Rock, founded the biotechnology company ProScript. The objective was to develop proteasome inhibitors to treat inflammatory diseases and cancer. Research at ProScript led to the development of a small-molecule inhibitor of the proteasome called Velcade. When ProScript was acquired by Millennium Pharmaceuticals, Julian Adams, the chief scientific officer of ProScript, moved to Millennium, where he championed the clinical development of Velcade and, along with Ken Anderson of the Dana Farber Cancer Institute, succeeded in gaining FDA approval of Velcade (bortezomid) for the treatment of multiple myeloma in 2003. Thus, in less than ten years, a basic research discovery (the role of the proteasome in signal transduction) was translated into an effective treatment of a fatal blood disease[18].

In 1990, my sister was diagnosed with amyotrophic lateral sclerosis (ALS) and died three years later. My exposure to this devastating neurological disorder had a transforming impact in my life. Initially, I chaired an ALS Association committee to search for new directions in ALS research, and later my laboratory became directly involved in the search for an understanding of ALS disease mechanisms. I am hopeful that my experience in innate immunity, RNA biology and gene regulation and my experiences in drug development will lead to a treatment or cure for ALS. Three years ago, I moved to Columbia University to join an incredible neuroscience program, where I interact closely with Richard Axel, Charles Zuker and Tom Jessell. In addition to studying ALS, my laboratory studies the role of cadherin-like genes in the generation and function of single-cell diversity in the nervous system. My move to Columbia has transformed my personal and scientific life, and I very much look forward to many more years of exciting science in the company of my wife, Rachel, and my close friends and colleagues.

I have not mentioned the extraordinary graduate students and postdoctoral fellows I have been fortunate to work with. Some of their names are found in the reference list, but all current and past members of my lab are listed on my website (http://www.maniatislab.columbia.edu/index.php.html). I am deeply indebted to them for enriching my intellectual life, for all of their work and accomplishments that made my laboratory a special place and for going on to make enormous contributions to research and teaching. On the surface, the work in my lab over the past 36 years seems unusually diverse, but the common thread is a deep interest in the fundamental mechanisms of gene expression and how these mechanisms relate to the underlying biology.

COMPETING FINANCIAL INTERESTS
The author declares competing financial interests: details are available at http://www.nature.com/doifinder/10.1038/nm.2931.

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